CLINICAL CHEMISTRY - THEORY, ANALYSIS, CORRELATION



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Clinical Chemistry - Theory, Analysis, Correlation

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Licensing

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1.1: Introductory Principles

1. A stock solution of NaOH is exactly 12.0 M. How many ml of the stock solution would you use to prepare one liter of a 3% weight/volume solution? (MW NaOH = 40 gm/mmol)?
   a. 833 mL
   b. 625 mL
   c. 62.5 mL
   d. 6.25 mL
   e. 83.3 mL
2. How many ml of concentrated HC1 (sp. gr. = 1.20; 30% purity) would be needed to prepare one liter of a 4 M solution (atomic weight H = 1; Cl = 35)?
   a. 72 mL
   b. 86.4 mL
   c. 144 mL
   d. 200 mL
   e. 400 mL
3. The pH of a 0.1 M solution of HCl is
   a. 1
   b. 3.5
   c. 4
   d. 6
   e. 7
4. If a patient has a serum calcium concentration of 5.0 mEq/L, what is this value in mg/dL (atomic weight of Ca = 40.08)?
   a. 1 mg/dL
   b. 10 mg/dL
   c. 20 mg/dL
   d. 100 mg/dL
   e. 200 mg/dL
5. How would you prepare 3 liters of 6N H_2SO_4 solution from the concentrated acid available? (Sp. Gr. = 1.84, 100% purity Mol. Weight H_2SO_4 = 98)
   a. take 239 mL acid and dilute to 3 liters
   b. take 441 mL acid and dilute to 3 liters
   c. take 479 mL acid and dilute to 3 liters
   d. take 882 mL acid and dilute to 3 liters
   e. take 958 mL acid and dilute to 3 liters
6. You will be performing a calcium/ magnesium analysis by the atomic absorption spectrophotometer. The procedure does not specify the type of water to be used for preparing reagents. What would be the best choice of water?
   a. double distilled
   b. type I water
   c. type III water
   d. type II water
   e. filtered with a 0.22 μpore filter
7. A buffer can be defined as:
   a. a strong acid plus a strong base
   b. a weak acid plus the salt of the acid
   c. a strong acid plus the salt of the acid
   d. a salt solution
8. The pH of a solution composed of 0.4 M HA (Ka dissoc. = 2.0×10^{-6}) and 0.2 M NaA is:
   a. 2.7
   b. 3.0

c. 5.4
d. 6.0
e. 6.8

9. The total concentration of a buffer ($HA + A^-$) is 0.275 mol/L. The concentration of the weak acid is 0.025 mol/L, and its dissociation constant is 10-6 mol/L. What is the pH of this solution?
   a. 4
   b. 5
   c. 6
   d. 7
   e. 8
10. The pH of a buffer solution is 6.5. The pK_a of the buffer is 7.5. If the total concentration of the buffer ($HA + A^-$) is 10 mmol/L, what is the concentration of the conjugate base?
   a. 0.0909 mmol/L
   b. 0.909 mmol/L
   c. 9 mmol/L
   d. 90.9 mmol/L
   e. 4.45 mmol/L
11. In a 1 to 5 dilution of 2.4 M NaOH, the final concentration is:
   a. 0.40 M
   b. 0.040 M
   c. 0.48 M
   d. 0.048 M
   e. 0.50 M
12. The molecular weight of glucose is 180 g/mol. A 3600 mg/L solution
   a. 40 mM
   b. 20 mM
   c. 400 mM
   d. 200 mM
   e. 2 mM
13. 10.0 mL of 0.5 M HC1 was used to titrate 5.0 mL of NaOH to its equivalence point. The molarity of the NaOH is:
   a. 0.5
   b. 2.0
   c. 1.0
   d. 0.1
   e. 0.2
14. 100 mg of $CaCl_2$ was made up to 25 mL. The calcium concentration of the solution is:
   a. 100 mg/dL
   b. 400 mg/dL
   c. 40 mg/dL
   d. 10 mg/dL
   e. 50 mg/dL
15. A sodium concentration is reported as 326 mg/dL. The concentration expressed in mEq/L is: (atomic weight Na is 23):
   a. 100
   b. 128
   c. 142
   d. 150
   e. 185
16. In a total protein analysis where the color follows Beer's Law, a patient's serum had an absorbance of 0.300 at 540nm; a 5.0 g/L standard had an absorbance of 0.250 at 540 nm, the patient's protein concentration is:

a. 4.2 g/L
b. 6.0 g/L
c. 3.0 g/L
d. 9.0 g/L
e. 3.6 g/L

17. 100 mg of $CaCl_2 \cdot 2H_2O$ was made up to 500 mL. The atomic weight of Ca = 40, Cl = 35.5, H = 1, O = 16. The calcium concentration of the solution is:
    a. 100 mg/dL
    b. 5.4 mg/dL
    c. 27 mg/dL
    d. 54 mg/dL
    e. 10 mg/dL
18. The atomic weight of magnesium is 24 g/mol. A 360 mg/L solution of magnesium is:
    a. 1.5 mM
    b. 2.0 mM
    c. 0.67 mM
    d. 0.15 mM
    e. 15 mM
19. In a four-fold serial dilution, the undiluted specimen is in tube #1. What is the dilution in tube #5?
    a. 1:16
    b. 1:32
    c. 1:64
    d. 1:128
    e. 1:256
20. How much sodium sulfate must be weighed out to prepare 300 mL of a 25% w/v solution? (Mol. Weight: Na_2SO_4 = 142).
    a. 19.9 g
    b. 25 g
    c. 75 g
    d. 166 g
    e. 498 g
21. How many mL of a 5 M solution would be needed to make 250 mL of a 2 M solution?
    a. 20 mL
    b. 40 mL
    c. 62.5 mL
    d. 100 mL
    e. 625 mL
22. You need to prepare 500 mL of a dilute glucose standard (200 mg/L) from a stock standard (2500 mg/L). How much of the stock standard would you need?
    a. 5 mL
    b. 10 mL
    c. 20 mL
    d. 40 mL
    e. 80 mL
23. How much $CaSO_4$ (Ca = 40; S=32; O =16) should be weighed out to prepare one liter of a 0.5 M solution of $CaSO_4$?
    a. 6.8 g
    b. 13 g
    c. 27.2 g
    d. 34 g
    e. 68 g
24. A tube contains the following: Serum 0.25 mL, Saline 0.75 mL. Antigen 1 mL. What is the serum dilution?

a. 1:2
b. 1:4
c. 1:7
d. 1:8
e. 1:16

25. Find the weight of $Na_2SO_4 \cdot 7H_2O$ that must be used to prepare 200 mL of a 25% solution of Na_2SO_4. Atomic weight: H = 1, Na = 23, S = 32, O = 16).

a. 62 g
b. 94 g
c. 116 g
d. 256 g
e. 173 g

Use the following key to answer questions 26 through 31:

a. 1,2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

26. Which of the following statements about laboratory water are true?:

1. Reagent grade water is obtained from Type III water which has been polished with activated carbon, ion exchange resins, and a bacterial filter
2. Laboratory grade water is obtained by pumping tap water through a selectively permeable membrane
3. Distillation removes organic impurities while deionization removes inorganic impurities
4. Steam from boiled water is cooled and collected to make Type I water.

27. Volumetric pipets are used for which of the following situations?

1. to pipet 2 mL saline for serial dilutions
2. to pipet 5 mL of stock standard solution into a volumetric flask
3. to pipet 2 mL of blood for a chromatographic extraction
4. to pipet 0.5 mL solution from a 1 mL pipet

28. Volumetric flasks are:

1. used for approximate quantities
2. not to be used for quantities below 100 mL
3. used with refrigerated or non-refrigerated solutions
4. never used as storage bottles

29. TC on a pipet means “TO CONTAIN”. This pipet should be:

1. blown out only
2. drained only
3. used only to deliver solution between two calibration marks on the pipet
4. rinsed after delivery

30. Pyrex laboratory glassware:

1. is six times stronger than regular borosilicate
2. has alkaline metals for strength
3. is known for its heat and thermal shock resistance
4. is made of over 96% silica giving it quartz-like qualities

31. You are asked to make reagents for and run a new procedure. You have decided to use a reagent that is labeled PG or practical grade that is on the Chemistry Lab’s stock shelves. When you have completed the test, the control values are too high. What should you do?:

1. run the test again with a reagent blank vs. a water blank
2. look for a chemical that is labeled “Technical Grade” and remake the reagent
3. send out the results in spite of the control values since many times controls do not match hen new reagents are used
4. remake the reagent with USP or NBS grade chemicals

32. Safety regulations in the clinical laboratory fall under which government agency?
    a. Centers for Disease Control
    b. Food and Drug Administration
    c. National Institute of Laboratory Sciences
    d. Occupational Safety and Health Administration
    e. College of American Pathologists
33. Gas Cylinders are securely fastened (chained, etc.) because:
    a. they are valuable and may be stolen.
    b. this prevents them from tipping over.
    c. this prevents the valves from shaking loose.
    d. this secures them in a known place
    e. the gas can be better controlled
34. Universal precautions require that:
    a. all infectious material be labeled.
    b. all potentially infectious material be labeled.
    c. all body fluids be treated with equal precautions.
    d. serum and blood products must be separated.
    e. all body fluids must be labeled carefully.
35. A vaccine is not available for which of the following infectious agents commonly encountered by laboratory personnel?
    a. hepatitis B
    b. human immunodeficiency virus
    c. polio virus
    d. influenza virus
    e. pneumonia virus

Use the following key to answer questions 36 and 37.

a. 1,2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

36. Mercury (Hg) used in the laboratory can be toxic by:
    1. contact of liquid Hg
    2. ingestion of liquid Hg
    3. breathing of volatile Hg fumes
    4. reaction with atmospheric O_2 to form combustible material
37. The following data should be obtained from a centrifuge for quality control of the instrument’s maintenance.
    1. nomogram RCF
    2. nominal RCF
    3. maximum g force
    4. revolutions per minute

Use the same key to answer questions as in questions 36 and 37.

38. Elements of the Chemical Hygiene Plan include:
    1. Maintaining health records for 30 years after an employee terminates employment
    2. Maintaining copies of MSDSs in the laboratory director’s office

3. The provision of personal protective equipment by the employer
4. Storing chemicals in a single area, regardless of the toxicity

39. Safety practices in the laboratory include:
    1. The lack of need to use safety glasses if corrective lenses are worn
    2. Not wearing contact lenses in the laboratory since they prevent proper washing of the eyes in the event of a spill
    3. Allowing sandals to be worn in the summer
    4. Flushing the eyes for 15 minutes in the event of contact with chemicals
40. When considering latex allergy:
    1. There is no concern regarding the development of respiratory symptoms in some cases
    2. Powder-free latex gloves are safe to use for workers with latex allergy
    3. The allergy comes from the latex itself and never from the chemicals added to the latex during harvesting
    4. Latex proteins can be adsorbed onto the powder that is used on many gloves
41. The requirements for operation of an electronic balance include:
    1. Placement in a vibration-free location
    2. Placement by a sunny window to ensure good lighting
    3. Verification of the optical zero
    4. Placement under an air conditioning vent to ensure a cool temperature
42. Volumetric flasks:
    1. Should not be used as storage containers
    2. Contain an exact volume when filled to indicator line
    3. Are calibrated to be used at the temperature specified on the flask
    4. Can be used as transfer devices to deliver an exact amount of fluid

Answer

1. c (p. 35-36)
2. e (p. 36-37)
3. a (p. 39)
4. b (p. 35-36)
5. c (p. 34-36)
6. b (p. 7)
7. b (p. 38-39)
8. b (p. 39)
9. d (p. 39)
10. b (p. 39)
11. c (p. 34-35)
12. b (p. 36-37)
13. c (p. 35)
14. b (p. 35-36)
15. c (p. 35-36)
16. b (p. 37-38)
17. b (p. 36-37)
18. e (p. 36-37)
19. e (p. 34-35)
20. c (p. 35)
21. d (p. 34)
22. d (p. 34)
23. e (p. 35-37)
24. d (p. 34)
25. b (p. 35-37)
26. a (p. 5-6)
27. a (p. 14,17)

28. d (p. 13)
29. d (p. 14)
30. d (p. 10)
31. d (p. 9)
32. d (p. 26)
33. c (p. 28)
34. c (p. 31-33)
35. b (p.33)
36. a (p. 30)
37. d (p. 24-25)
38. b (p. 27-29)
39. d (p. 28)
40. d (p. 33-34)
41. b (p. 21-22)
42. a (p. 13)

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1.2: Laboratory Management

1. One of the major aspects of the Health Insurance Portability & Accountability Act of 1996 is:
    a. Setting guidelines for the retention of laboratory records
    b. Determining the availability of health insurance for all Americans
    c. Determining the education requirements of health-care workers
    d. Establishing security and privacy standards
    e. Determining accounting guidelines for calculating profit from Medicare
2. All outpatient tests are reimbursed according to a code number. These numbers, are called?
    a. Clinical Laboratory Improvement Amendments (CLIA '88) codes
    b. Common Procedure Terminology (CPT) codes
    c. Health Care Financing Administration (HCFA) codes
    d. Department of Health and Human Services (HHS) codes
    e. Joint Commission on Accreditation of Healthcare Organizations (JCAHO) codes
3. An organization chart should exist for which of the following?
    a. Hospital
    b. Department of Pathology
    c. Each clinical laboratory
    d. All of the above
    e. None of the above
4. A good working laboratory requires decisions to be based on information for which of the following:
    a. Laboratory Director
    b. Laboratory general supervisors
    c. Testing personnel
    d. All of the above
    e. None of the above
5. Determining the competency of testing personnel is a CLIA '88 responsibility of which of the following:
    a. General supervisors
    b. Laboratory Director
    c. Manager of the Department of Pathology
    d. Technical Supervisor
    e. Director of a hospital's Human Resources
6. The time needed for the retention of laboratory records is not defined by regulation and law, but is dependent upon the needs of each laboratory.
    a. True
    b. False
7. Which of the following is NOT an example of a laboratory management report?
    a. Total work load
    b. Work load by shift
    c. Turn-around time
    d. Frequency of use of test billing codes
    e. Routine quality control
8. Measuring the overall productivity of the laboratory and of each laboratory section should be part of any operational performance system developed. Which of the following should NOT be included as indicators in the review of productivity?
    a. Number of performed tests/total FTE
    b. Number of performed tests/worked hour
    c. Worked hours as a percentage of paid hours
    d. CAP work-load units
9. The types of laboratory tests specified in CLIA '88 are:

a. Waived tests
b. Moderate complexity tests
c. High-complexity tests
d. All the above
e. None of the above

Answer

1. d (p. 46)
2. b (p. 59)
3. d (p. 49)
4. d (p. 52-53)
5. b (p. 54)
6. b (p. 48)
7. d (p. 60)
8. d (p. 60)
9. d (p. 53)

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1.3: Sources and Control of Preanalytical Variation

Use the following key to answer questions 1 through 4.

a. 1,2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

1. Moderate levels of hemoglobin can cause methodological interference in which of the following analytical procedures?
    1. cholesterol
    2. albumin
    3. triglycerides
    4. iron
2. The information on a chain of custody document includes:
    1. subject name
    2. person sending specimen
    3. person receiving specimen
    4. condition of seals
3. The purpose of a chain of custody document is to:
    1. ensure that no tampering of the sample occurred
    2. ensure that the specimen is derived from the appropriate individual
    3. ensure that the reported results are correct for the appropriate individual
    4. ensure that quality control was performed
4. Which of the following patient variables can affect measured analyte concentrations?
    1. diurnal variation
    2. posture
    3. stasis
    4. height
5. All of the following are higher in the morning than the afternoon or evening (in persons with a normal wake:sleep cycle) EXCEPT:
    a. ACTH
    b. Cortisol
    c. Iron
    d. Prolactin
    e. Renin/aldosterone
6. Which of the following is typically decreased after meals?
    a. Chloride
    b. Gastrin
    c. Growth hormone
    d. Insulin
    e. Triglycerides
7. EDTA contamination will decrease all of the following analytes EXCEPT:
    a. Alkaline phosphatase
    b. Calcium (spectrophotometric methods)
    c. Creatine kinase
    d. Magnesium (spectrophotometric methods)
    e. Potassium

8. The mechanism of interference in hemolyzed samples is correctly matched with the test name in all of the following combinations EXCEPT:
    a. Bilirubin – release from red blood cells
    b. Cholesterol – interference in assay
    c. Creatine kinase – interference in assay
    d. Lactate dehydrogenase – release from red blood cells
    e. Magnesium – release from red blood cells.
9. Appropriate tests to use for delta checks, since they show low intra-individual variation, little day to day variation, but higher inter-individual variation, include:
    a. Alkaline phosphatase
    b. Creatine kinase
    c. Glucose
    d. Lactate dehydrogenase
    e. Phosphate
10. The percent intraindividual variation is highest for:
    a. Albumin
    b. Calcium
    c. Creatinine
    d. Sodium
    e. Triglycerides
11. Regular aerobic exercise is associated with:
    a. Decreased glucose
    b. Decreased bilirubin
    c. Decreased creatine kinase
    d. Increased aspartate aminotransferase
    e. Increased alanine aminotransferase
12. Short term, intensive exercise, such as running a marathon, is associated with:
    a. Decreased potassium
    b. Decreased uric acid
    c. Decreased glucose
    d. Decreased creatine kinase
    e. Increased phosphate
13. All of the following increase after a meal EXCEPT:
    a. Glucose
    b. Insulin
    c. Iron
    d. Potassium
    e. Triglycerides
14. Stress is associated with which of the following?
    a. Decreased cortisol
    b. Decreased catecholamines
    c. Decreased cholesterol
    d. Increased high density lipoprotein cholesterol
    e. Increased TSH
15. All of the following are increased by upright posture EXCEPT:
    a. Albumin
    b. Calcium (total)
    c. Cholesterol
    d. Iron
    e. Potassium

16. Which of the following statements regarding differences between arterial and venous blood is INCORRECT?
    a. Arterial blood has higher concentrations of metabolites extracted by tissue, such as oxygen and glucose.
    b. Arterial blood has lower concentrations of waste products, such as carbon dioxide and ammonia.
    c. Capillary blood is more similar to arterial than to venous blood.
    d. The difference between arterial and venous blood is increased with poor tissue perfusion.
    e. The difference between arterial and venous blood glucose is similar in fasting and postprandial collections.

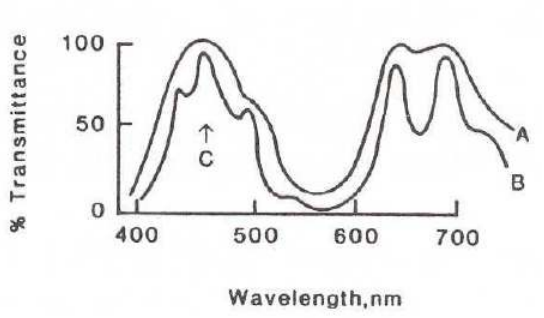
Answer

1. b (p. 73)
2. e (p. 73-74)
3. a (p. 74)
4. a (p. 66-68)
5. d (p. 69)
6. a (p. 69)
7. e (p. 71)
8. a (p. 73)
9. a (p. 80)
10. e (p. 67)
11. c (p. 67-68)
12. c (p. 67)
13. d (p. 69)
14. c (p. 68)
15. d (p. 68)
16. a (p. 70)

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1.4: Spectral Techniques

1. If one switched from a large cuvette to a smaller one, the %T readings on the spectrophotometer would:
   a. decrease
   b. increase
   c. fluctuate
   d. remain unchanged
   e. have equal absorbance readings
2. A solution is measured in a spectrophotometer with a resulting %T of 10%. Calculate the absorbance.
   a. 0
   b. 0.5
   c. 1.0
   d. 1.5
   e. 2.0
3. Compound Y has been determined to have a molar absorptivity (extinction coefficient) of 1000 liters/ mole • cm at 390 nm. An unknown solution of this compound has an absorption of 0.750 when placed in a cell with a light path of 1 cm. The concentration of this compound is:
   a. 375 mole/L
   b. 750 mole/L
   c. 750 μmole/L
   d. 0.375 mmole/L
   e. 750 mmole/L
4. The %T scale on a spectrophotometer is a logarithmic one while the absorbance scale is a linear scale. Because of this relationship, very high absorbance readings are inaccurate.
   a. true
   b. false
5. A standard solution of Compound X at 10 mg/L gives A_{450} = 1.200 when used in a specific colorimetric assay. A serum sample analyzed for Compound X gives the following results: run undiluted: A_{450} = 2.400, run diluted 1:10: A_{450} = 0.300. The concentration of Compound X in serum is:
   a. 2.5 mg/L
   b. 2.0 mg/L
   c. 20 mg/L
   d. 25 mg/L
   e. 200 mg/L
6. Compounds A and B both have a chemical formula of $C_9H_{18}N_2O$; therefore, both compounds must have the same molar absorptivity coefficient (ϵ or a) and the same spectral curve:
   a. true
   b. false
7. Refer to the following spectral-transmission curve made on the same colored solution using two different instruments. All of the following statements are true except: (Note:one instrument had a bandpass of 35 nm, the other 5 nm)
   a. A is the curve which was obtained with the 35 nm instrument
   b. B is the curve obtained with the 5 nm instrument
   c. reading the color intensity at point C would yield the greatest sensitivity
   d. 550 nm would be an appropriate wavelength at which to read the reaction
   e. a and b



8. To detect transmitted light, either barrier tubes or photomultiplier tubes can be used. Both work on the principle that incident photons of light interact with a thin metallic layer resulting in the generation of an electric current.
    a. true
    b. false

Instruments - Spectrophotometers

Match each of the following instrument components with its most accurately described function — Questions: 53 thru 55

a. slit(s)
b. barrier layer cell
c. interference filter
d. photomultiplier
e. potentiometer

9. Converts electromagnetic energy to electricity
    a.
    b.
    c.
    d.
    e.
10. Renders light rays parallel
    a.
    b.
    c.
    d.
    e.
11. Converts light to electricity and amplifies the electron flow
    a.
    b.
    c.
    d.
    e.
12. Which of the following combinations are not consistent?:
    a. tungsten lamp, diffraction grating, glass cuvette, barrier layer cell
    b. Xenon arc lamp, diffraction grating, quartz cuvette, photomultiplier tube
    c. Deuterium lamp, quartz prism, quartz cuvette, photomultiplier tube
    d. Deuterium lamp, glass prism, glass cuvette, photomultiplier tube
    e. B and D
13. The principle of atomic absorption spectrophotometry is best stated as:
    a. ground state (unexcited) atoms absorb light energy of a characteristic wavelength

b. excited atoms emit light of a characteristic wavelength after absorbing energy
c. excited atoms emit light of a longer wavelength after absorption of energy of a shorter wavelength
d. ground state (unexcited) atoms absorb light energy of a characteristic wavelength, become ionized, and emit light
e. excited atoms absorb light

14. The most common light source in atomic absorption instruments is a:
    a. Xenon arc
    b. flame
    c. hollow cathode lamp
    d. grating
    e. deuterium lamp
15. In atomic absorption spectrophotometry, the purpose of the light source is:
    a. to provide a chamber to house the flame
    b. to produce a wavelength of light characteristic for the metal in the cathode
    c. to act as a detector of light energy transmitted by the flame
    d. to transmit argon or neon gas into the flame as a source of fuel
    e. to act as a detector of light energy allowed to be transmitted by the chopper
16. If one desired to measure calcium by atomic absorption spectrophotometry, the light source would have to be made of which component(s)?:
    a. magnesium
    b. deuterium
    c. tungsten and quartz
    d. calcium
    e. zinc
17. The heat of the atomic absorption (A.A.) flame is not critical for accurate and precise measurement of Ca^{++} since the procedure uses a calibration curve.
    a. True
    b. False
18. A single source for the hollow-cathode lamp is used for Ca^{++}, Mg^{++}, and Li^{+}, while a different lamp must be used to measure Cu^{++}.
    a. True
    b. False
19. Most atomic absorption instruments use filters for their monochromometers.
    a. True
    b. False
20. The purpose of the flame in atomic absorption is to excite the atoms so that they can absorb light from the hollow cathode lamp.
    a. True
    b. False
21. Nephelometers measure light at:
    a. a different wavelength than the input light
    b. the wavelength of maximum
    c. the wavelength of maximum fluorescence
    d. the same wavelength as the input light
    e. the point of longest scatter
22. Turbidity measurement is equivalent to:
    a. fluorescence
    b. nephelometry
    c. absorbance
    d. phosphorescence

e. quenching

23. Most spectrophotometric analyses use absorbance measurements because there is no direct relationship between %T and concentration.
    a. True
    b. False

Match the principle which best describes the effect of each of the following (questions 24 thru 28):

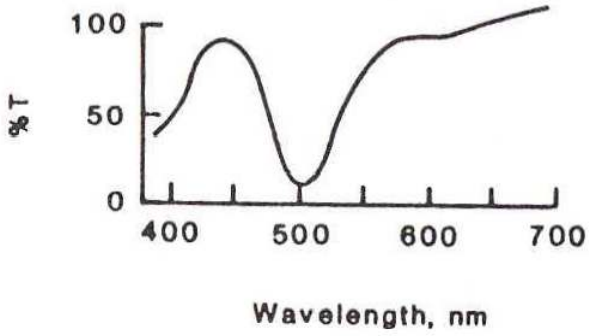
Principle

a. spectrophotometry
b. fluorometry
c. turbidity
d. nephelometry

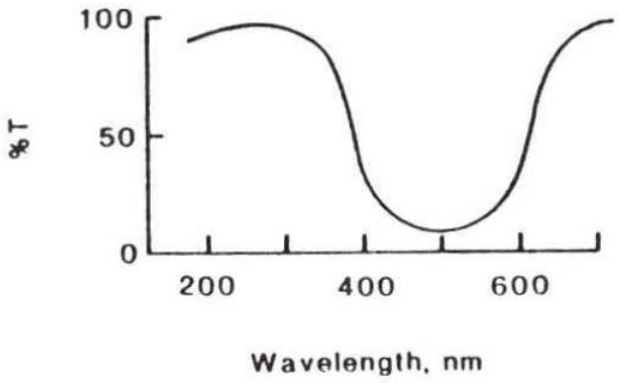
Effect

24. Increase in light absorption caused by particulate interaction with absorbent compound.
    a.
    b.
    c.
    d.
25. Absorption and emission of light at different wavelengths.
    a.
    b.
    c.
    d.
26. Decrease in observed light intensity caused by light scattering.
    a.
    b.
    c.
    d.
27. Increase in observed light intensity caused by light scattering.
    a.
    b.
    c.
    d.
28. Absorption of incident light by compound resulting in a decrease in observed light intensity
    a.
    b.
    c.
    d.
29. Which of these reactions can occur in a flame? (M = metal)
    a. M^+ + electron → M°
    b. M* → M° + photon
    c. M° + photon → M*
    d. a and c
    e. all of the above
30. A spectral transmittance curve for compound X is shown below. The wavelength at which measurement should be made is:
    a. 400
    b. 450
    c. 500

d. 550
e. 600



31. When an atom, ion or molecule absorbs a photon of light, this is termed:
    a. atomic transition
    b. fluorescence
    c. radiationless transition
    d. electronic transition
    e. Beer's Law
32. A beam of white light is passed through a green filter, and the transmitted light is passed into a green solution. The transmitted light from this solution was measured and the %T and absorbance recorded. This experiment is repeated. The filter remains green but now a red solution is used. Which of the following responses for %T and absorbance would be expected?
    a. %T and absorbance both increase
    b. %T increases, absorbance decreases
    c. %T decreases, absorbance increases
    d. %T and absorbance both decrease
    e. %T and absorbance values remain unchanged
33. Both atomic absorption and flame photometers use filters as the monochrometers:
    a. true
    b. false
34. In sunlight, what color is the solution of the compound whose spectral curve is shown below:
    a. blue
    b. green
    c. yellow
    d. purple
    e. colorless



35. 10 mL of a solution is read spectrophotometrically in a 2 cm cuvette. The absorbance obtained after correcting for dilution is 30. The molar absorptivity of this compound, whose M.W. is 1000, is 150. What is the concentration of this compound in mg/mL?
    a. 0.1 mg/mL
    b. 1 mg/mL
    c. 10 mg/mL
    d. 100 mg/mL
    e. 1000 mg/mL
36. A 0.01 M solution of a compound is prepared and its absorption at 260 nm is measured in a 20 mm cell and found to be 0.500. What is molar absorptivity of this compound?
    a. 50
    b. 25
    c. 12.5
    d. 2.5
    e. none of the above
37. You are performing a manual procedure in which you need 14 10 mm cuvettes. Only 13 10 m cuvettes are available so you read one of the standards in a 20 mm cuvette. Upon calculating the concentration of this solution, you find the standard appears:
    a. more concentrated than it should be
    b. less concentrated than it should be
    c. the same concentration as expected
38. A linearity check is performed on the spectrophotometer using a solution that follows Beer's Law up to a concentration of 2.5. The following values are obtained:

Concentration (mol/L)	Absorbance
1.01	1.60
0.75	1.35
0.50	0.90
0.25	0.45

You would conclude:

a. the instrument is linear
b. the instrument is non-linear

Use the following code to answer questions 39 thru 41:

1. If A is greater than B
2. B is greater than A
3. A and B are about equal

39. A. U.V. light absorbed by glass
    B. U.V. light absorbed by fused silica
40. A. linearity of spectrum produced by a prism
    B. linearity of spectrum produced by a diffraction grating
41. A. transmission of blue light by a cuvette filled with a red solution
    B. transmission of yellow light by a cuvette filled with a red solution

Use the following Key to answer questions 42 through 56:

a. 1,2 and 3 are correct

b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

42. The following can affect the linear response of concentration vs. absorbancy and thus result in a deviation from Beer's Law:
    1. stray light
    2. use of photometer instead of a spectrophotometer
    3. very high analyte concentration
    4. Using only one standard for calculation
43. On linear graph paper, a straight line through zero with three plotted standards indicates which of the following?:
    1. the concentration of the standard vs. the absorbance is linear
    2. the test is in compliance with Beer's Law'
    3. the concentration of the standard vs. the log %T is linear
    4. the test is not in compliance with Beer's Law.
44. Deviations from Beer's Law are caused by
    1. stray light
    2. polychromatic light
    3. very high concentrations of substance being measured in a colorimetric reaction
    4. very low concentration of absorbing material
45. Which of the following are preferred for checking the wavelength calibration of a spectrophotometer?:
    1. cobalt sulfate
    2. didymium jilter
    3. potassium dichromate
    4. helium oxide filter
46. The concept of thermal excitation as used in emission photometry involves:
    1. the absorption of light by certain alkali metals
    2. characteristic line spectra being given off by excited orbital electrons as they return to the ground state
    3. orbital electrons absorbing radiant energy which is converted to electrical energy as measured by a gal manometer
    4. the heating of alkali metal salts to produce a colored light of characteristic wavelength
47. In flame photometry the measured light comes from:
    1. burning fuel
    2. light produced when atoms go from ground state (M°) to excited state (M*)
    3. light from a tungsten bulb
    4. light produced when atoms go from excited state (M*) to ground state (M°)
48. The purpose of the nebulizer in atomic absorption is to:
    1. break chemical bonds
    2. excite atoms
    3. measure the sample flow rate
    4. disperse aqueous sample into fine droplets
49. Lanthanum diluents is used in atomic absorption measurements to:
    1. precipitate interfering proteins
    2. bind Mg^{++} so it will not interfere with Ca^{++} measurements
    3. release Ca^{++}
    4. release Ca^{++} complexes
50. Which of the following statement(s) concerning the use of cesium (Cs) as an internal standard for flame photometry is (are) true? Cesium:
    1. is normally present in very low concentrations in body fluids
    2. has an emission line easily distinguished from those of Na and K
    3. is used to correct for minor variations of reaction conditions

4. can be replaced by other elements such as magnesium

51. Turbidimetry and nephelometry measure:
    1. fluorescence in turbid solution
    2. light absorbance by particles in suspension
    3. light absorbance by a colored substance
    4. light scatter by particles in suspension
52. Components of a nephelometer include:
    1. light source
    2. detector
    3. filter
    4. chopper
53. In fluorometry:
    1. the compound measured absorbs light of a shorter wavelength and emits light of a longer wavelength
    2. quenching occurs when a substance other than the one being measured emits fluorescence
    3. fluorometery is a relative measurement of light signal
    4. the compound measured absorbs light of a longer wavelength and emits light of a shorter wavelength
54. Which of the following types of lamps would be most appropriate for the entire UV range?
    1. mercury vapor
    2. tungsten iodide with quartz envelope
    3. tungsten with glass envelope
    4. deuterium
55. An acceptable means of achieving spectral isolation in the UV range is (are):
    1. reflectance diffraction grating
    2. glass filter
    3. quartz prism
    4. glass prism
56. The correct wavelength for reading a sample on a spectrophotometer is:
    1. the wavelength of the highest absorbance maxima
    2. the wavelength at which the most light passes through the solution
    3. the wavelength at which a straight line (percent transmittance vs. concentration) is obtained on a semi-log paper
    4. the wavelength at which the photo detector has optimum response.
57. When a fluorescent molecule absorbs polarized excitation light, the emitted light loses polarization when:
    a. the the light intensity decreases
    b. light intensity increases
    c. the excited state changes
    d. the fluorescent molecule is held rigid
    e. the fluorescent molecule
58. To retain a linear relationship between fluorescence intensity and the amount of light absorbed by a solution, the absorbance of the solution should not exceed:
    a. 0.001
    b. 0.01
    c. 0.1
    d. 1.0
    e. 2.0
59. Fluorescence polarization assays are different from those that measure only fluorescence intensity because:
    a. those which measure intensity can tolerate unstable light sources.
    b. those which measure intensity can tolerate less intense light.
    c. the light intensity is greater.
    d. polarization measurements are less affected by variations in fluorescence intensity.

e. polarization measurements are more affected by variations in fluorescence intensity.

60. Florescence attenuation assays are used in dedicated fluorometric instruments such as the Abbott TDx. In these assays, the fluorescent dye concentration is:
    a. Constant
    b. linearly proportional to analyte Concentration
    c. logarithmically proportional to analyte concentration
    d. proportional to analyte formation
    e. inversely proportional to analyte formation
61. Chemiluminescent reactions are those in which one of the products is
    a. Luminol
    b. oxidized luminol
    c. reduced luminol
    d. heat
    e. light
62. Time delayed fluorescence is a term that refers to:
    a. fluorescence which starts to occur only after one millisecond following exposure to exciting light
    b. fluorescence which is delayed by solution chemistry
    c. fluorescence which is measured after a time delay
    d. the time it takes for a photon to jump from a ground state to an excited state
    e. the delayed time required before the incident light excites the fluor
63. Atomic absorption can be considered the opposite of flame emission because in atomic absorption:
    a. thermal energy applied to the sample causes formation of excited atoms
    b. a specific light source is necessary for the determination of each element
    c. electron's movement to a higher energy level is measured.
    d. the amount of light absorbed by an electron moving to a higher energy level is measured.
    e. light is given off by excited atoms.
64. In a fluorometer, when the primary excitation source produces fluorescence, the secondary light emission by the sample has:
    a. a longer wavelength than the primary light
    b. a shorter wavelength than the primary light
    c. the same wavelength as the primary light
    d. no specific wavelength
    e. any of the above
65. Which of the following describes a physical property that distinguishes luminescence from fluorescence?
    a. fluorescence is at longer wavelengths.
    b. in luminescence a photon goes from an excited state to a ground state.
    c. in fluorescence a photon goes from an excited state to a ground state.
    d. luminescence has a longer transition time to the ground state.
    e. fluorescent light is always polarized.
66. Chemiluminescence can be induced by
    a. light
    b. flame ionization
    c. a chemical reaction
    d. firefly luciferase
    e. atomic absorption

Answer

1. b (p. 88)
2. c (p. 88,38)
3. c (p. 88,38)
4. b (p. 88)
5. d (p. 88,38)

6. b (p. 86-88)
7. c (p. 89,90,92)
8. a (p. 91)
9. b (p. 91)
10. a (p. 91)
11. d (p. 91)
12. d (p. 91-93)
13. a (p. 95)
14. c (p. 95)
15. b (p. 95)
16. d (p. 95-96)
17. b (p. 97)
18. a (p. 96)
19. b (p. 96)
20. b (p. 95)
21. d (p. 101-103)
22. c (p. 101-102)
23. a (p. 88)
24. c (p. 101-102)
25. b (p. 97-98)
26. c (p. 101-102)
27. d (p. 101-103)
28. a (p. 88-89)
29. e (p. 97)
30. c (p. 92-93)
31. d (p. 86)
32. c (p. 86-88)
33. b (p. 95-97)
34. d (p. 86)
35. d (p. 88-89)
36. b (p. 88-89)
37. a (p. 88-89)
38. b (p. 88-89)
39. 1 (p. 91)
40. 2 (p. 90)
41. 3 (p. 88)
42. b (p. 88-89)
43. a (p. 88-89)
44. a (p. 88-89)
45. c (p. 93-94)
46. c (p. 87, 97)
47. d (p. 97)
48. d (p. 96)
49. d (p. 96)
50. a (p. 97)
51. d (p. 101-102)
52. a (p. 102)
53. b (p. 97-98)
54. d (p. 89-90)
55. b (p. 90)
56. b (p. 92-93)
57. e (p. 100-101)

58. c (p. 99)
59. d (p. 100-101)
60. a (p. 99)
61. e (p. 99-100)
62. c (p. 99)
63. d (p. 99)
64. a (p. 98)
65. d (p. 98-100)
66. c (p. 99-100)

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1.5: Chromatography

1. Choose an appropriate definition from Column B for each phrase in Column A:

A	B
i. equilibrium concentration distribution coefficient ii. theoretical plate iii. height equivalent to theoretical plate iv. retention time v. R_f vi. phase	a. the length of a column in millimeters b. a column height related to separating ability c. ratio at equilibrium of the amounts of a substance dissolved in two immiscible solvents which are in contact d. separation of solute between two phases or immiscible solvents e. gas chromatography f. the ratio of solute mobility/solvent mobility g. duration of solute on a column h. gas, liquid, solid i. two solvents immiscible in each other

2. Chromatography does not separate compounds if:
    a. they are not colored
    b. there is no chemical relationship between them
    c. the capacity factors are identical
    d. the number of theoretical plates is small
    e. the Rf value is large
3. Gas chromatography:
    a. uses immiscible liquids
    b. has two phases
    c. uses air
    d. separates large molecules
    e. has gas-gas phase separation
4. A substance with a greater R_f would travel a _____ distance than a substance with a lesser R_f and a method that decreases resolution will _____ the separation of the peaks:
    a. shorter/decrease
    b. shorter/increase
    c. greater/decrease
    d. greater/increase
5. Which substances cannot be separated by GLC?:
    a. amino acids
    b. fatty acids
    c. alcohols
    d. steroids
    e. proteins
6. If you have a GLC with a relatively polar stationary phase and are using helium as the carrier gas, you would expect n-octanol to have a shorten longer retention time than n-butanol; and, increasing the temperature of the oven would increase/decrease the retention times of both substances:
    a. shorter/increase
    b. shorter/decrease
    c. decrease/increase
    d. longer/decrease
7. In GLC, derivitization is often done to increase/decrease the volatility and increase/decrease the stability of compounds of interest:
    a. increase/increase

b. increase/decrease
c. decrease/increase
d. decrease/decrease

8. The GLC detector which unequivocally identifies compounds of interest is:
   a. MS (mass spectrometer)
   b. TC (thermal conductivity)
   c. FID (flame ionization detector)
   d. EC (electron capture)
   e. NPD (nitrogen phosphorous detector)
9. In an HPLC with a nonpolar stationary phase and a polar mobile phase, you would expect n-octanol/n-butanol to have the shorter retention time, and decreasing the size of the beads would increase/decrease the retention time of both substances:
   a. n-octanol/increase
   b. n-octanol /decrease
   c. n-butanol/increase
   d. n-butanol/decrease
10. Reversed phase means a stationary phase with a mobile phase:
   a. nonpolar/nonpolar
   b. nonpolar/polar
   c. polar/polar
   d. polar/nonpolar
11. If the peaks of your standards come out too soon on a GLC recording, this could be the result of:
   a. increased oven temperature
   b. too large a sample volume
   c. too small a sample volume
   d. increased injector temperature
   e. high detector voltage
12. In paper chromatography, the following standards had these R_f values:

Cystine	0.20
Lysine	0.43
Arginine	0.67
Glycine	0.85

The solvent front traveled 8 cm, and the spot representing the unknown traveled 1.6 cm. What is the identification of the unknown?

a. cystine
b. lysine
c. arginine
d. glycine
e. none of the above

Use the following Key to answer questions 13 through 15:

a. 1, 2 and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

13. Retention time in a GLC is a function of:
   1. detector temperature
   2. oven temperature

3. solubility in the solvent
4. length of the column

14. Retention time is a qualitative entity obtained when utilizing HPLC and GC. For certain HPLC and GC procedures, the retention times may be unacceptably lengthy. Name the parameters which can be adjusted to decrease the retention time for HPLC
    1. mobile phase flow rate
    2. detector temperature
    3. column temperature
    4. size of sample injection
15. Name the parameters which can be adjusted to decrease the retention time for GC:
    1. size of sample injection
    2. detector volume
    3. injector temperature
    4. column oven temperature

Answer

1.
    i. c (p. 110)
    ii. d (p. 114)
    iii. b (p. 114)
    iv. g (p. 112)
    v. f (p. 116)
    vi. h (p. 110)
2. c (p. 115)
3. b (p. 110,156)
4. c (p. 116)
5. e (p. 156-157, 169)
6. b (p. 157-158, 163-164)
7. a (p. 162)
8. a (p. 173)
9. c (p. 138-139)
10. b (p. 137-138)
11. a (p. 159-161)
12. a (p. 116)
13. c (p. 158-159)
14. b (p. 132-133)
15. d (p. 162-164)

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1.6: Mass Spectrometry

1. Mass spectrometry operates on the principle that:
    a. Compound specific ion fragments can be measured and quanitified
    b. Charged particles moving through a magnetic field can be separated from other charges particles according to their mass-to-charge ratios
    c. Molecules being analyzed by mass spectrometry must be in the gaseous state
    d. Bombardment of molecules by high energy electrons causes ions to be formed
    e. All of the above
2. The molecular ion provides useful information about:
    a. The amount of energy required for ionization
    b. The molecular weight and information about the possible structure of the molecule
    c. The relative intensities of the fragments formed during full scan analysis
    d. The analytical purity as well as the intensity of the parent ion
    e. All of the above
3. The main advantage of Electron Ionization is that:
    a. The resulting fragmentation is extensive and provides significant structural information about the molecule
    b. It is a much faster ionization process than other ionization techniques such as chemical ionization
    c. It is a soft ionization process that maximizes the ions life time
    d. All of the above
    e. None of the above
4. Chemical Ionization is a process that:
    a. Generates full scan spectra characteristic of the molecule being analyzed
    b. Is only used to identify large molecules such as proteins and peptides
    c. Generates relatively stable ions of high intensity due to its gentle ionization
    d. All of the above
    e. None of the above
5. The main advantage of LC/MS over GC/MS is:
    a. It has a significant cost advantage
    b. It is simpler and faster to use
    c. It may be more suitable for compounds that are less volatile or thermally labile
    d. All of the above
    e. None of the above
6. The main disadvantage of LC/MS over GC/MS is:
    a. The difficulty of converting molecules of interest that are solvated in liquid to the gaseous form
    b. Interfacing helium gas to serve as the mobile phase carrier gas
    c. Bombarding of the liquid phase by high energy electrons to generate positive ions
    d. All of the above
    e. None of the above

Answer

1. e (p. 173)
2. b (p. 177)
3. a (p. 176,179)
4. c (p. 176)
5. c (p. 182-183)
6. a (p. 182-183)

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1.7: Radioisotopes

1. Which of the following subatomic particle ratios is important to maintain nuclear stability?
    a. neutron/proton
    b. proton/electron
    c. negatron/neutron
    d. positron/electron
    e. neutron/alpha
2. Characteristic X-rays are emitted during the nuclear reaction involving:
    a. gamma decay
    b. positron decay
    c. electron capture
    d. negatron decay
    e. none of the above
3. If decay factors for a radionuclide 8 days and 16 days after the calibration date are 0.9 and 0.8 respectively, what will be the radioactivity of 1 millicurie of this radionuclide 24 days from now?
    a. 0.72 microcurie
    b. 0.072 millicurie
    c. 0.170 millicurie
    d. 0.89 millicurie
    e. 0.72 millicurie
4. A crystal scintillation detector is primarily used for detecting radionuclides emitting:
    a. positrons
    b. gamma rays
    c. negatrons
    d. X-rays
    e. ultraviolet light
5. Beta emission is the emission of:
    a. a proton
    b. an electron
    c. a neutron
    d. a ray of light of very short wavelength
    e. an X-ray
6. Specific activity is the radioactivity as expressed in disintegrations/second per mole of a compound or atom. Which of the following has the highest specific activity?
    a. ^{125}I ($t_{1/2}$ = 60 days)
    b. ^{35}S ($t_{1/2}$ = 62 days)
    c. ^{3}H ($t_{1/2}$ = 12 years)
    d. ^{14}C ($t_{1/2}$ = 5000 years)
    e. ^{131}I ($t_{1/2}$ = 8 days)
7. Gamma emission is the emission of:
    a. a proton
    b. an electron
    c. a neutron
    d. a ray of light of very short wavelength
    e. an X-ray
8. In the radioactive decay of ^{3}H atom to a ^{3}He atom, the subatomic particle emitted from the nucleus is a (an):
    a. alpha particle
    b. electron (negatron)
    c. positron

d. gamma ray
e. neutron

9. A secondary scintiliator (e.g., POPOP) is used in liquid scintillation counting:
   a. to facilitate dissolution of the sample
   b. to obtain a homogenous scintillation mixture
   c. to enhance the counting efficiency by “wavelength shifting”
   d. as the internal standard
   e. to reduce chemiluminescence
10. ^{125}I is used as a label because:
   a. it has high specific activity
   b. it is a high-energy emitter
   c. it has a long half-life
   d. all of the above
   e. none of the above

Use the following Key to answer Questions 11 through 17

a. 1,2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

11. ^{125}I-labeled ligands are preferable to ^{3}H-labeled ligands in radioimmunoassays because of:
    1. longer shelf life
    2. higher specific activity
    3. lower cost
    4. gamma-emission
12. During the course of radioactive decay, a proton is converted to a neutron in:
    1. beta decay
    2. alpha decay
    3. electron capture
    4. positron decay
13. Scintillation counters measure light pulses. These light pulses are related to:
    1. ionizing radiation
    2. high-energy gamma rays
    3. high-energy beta particles
    4. random electrons
14. The most commonly encountered radioactive emissions in the hospital laboratory are:
    1. alpha -1 and gamma
    2. alpha -1 and beta
    3. beta + and gamma
    4. beta - and gamma
15. The most commonly used radioisotope(s) in the chemistry laboratory is/are:
    1. ^{125}I
    2. ^{35}S
    3. ^{3}H
    4. ^{14}C
16. Radioisotopes are most commonly used to measure in antibody-based competitive assays:
    1. metals
    2. antibodies
    3. electrolytes

4. antigens

17. Instrumentation used for detecting and recording beta-emitting isotopes differs from that used for gamma emitters because a beta scintillation counter requires:
    1. a photomultiplier tube
    2. a hollow cathode lamp
    3. a sodium iodide crystal
    4. presence of a fluor

Answer

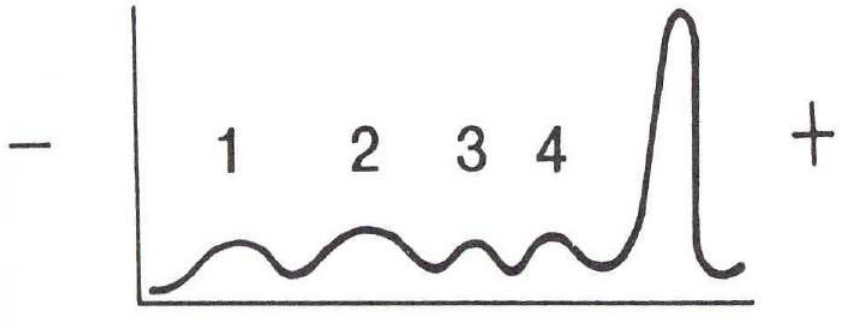
1. a (p. 190)
2. c (p. 190-191)
3. e (p. 191-192)
4. b (p. 194)
5. b (p. 190)
6. e (p. 192)
7. d (p. 191)
8. b (p. 190)
9. c (p. 196)
10. a (p. 192)
11. c (p. 192)
12. d (p. 190)
13. c (p. 194)
14. d (p. 192)
15. e (p. 192)
16. c (p. 189)
17. d (p. 194-197)

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1.8: Electrophoresis

1. Given: Electrophoretic buffer pH: 8.6, protein A: isoelectric point: 7.2, protein B: isoelectric point: 5.1, protein C: isoelectric point: 8.6. The order of mobility of proteins A, B and C toward the positive electrode (slowest to fastest) will be:
    a. A, B, C
    b. B, C, A
    c. C, A, B
    d. B, A, C
    e. C, B, A

Examine the tracing and use the following key to answer questions 2-5:



Key:

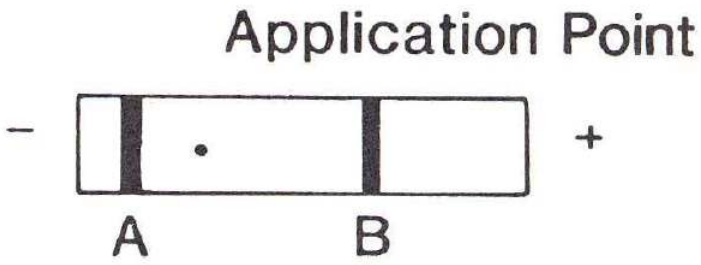
a. alpha 1 globulin
b. beta globulin
c. gamma globulin
d. albumin
e. alpha 2 globulin

2. The area depicted by #1 is _____
3. The area depicted by #2 is _____
4. The area depicted by #3 is _____
5. The area depicted by #4 is _____
6. The electrophoretic pattern above represents a:
    a. normal pattern
    b. monoclonal peak
    c. polyclonal peak
    d. relative hypoalbuminemia
    e. b and d

Use the following Key to answer questions 7-9:

a. 1, 2 and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

7. Below is a strip demonstrating serum protein electrophoretic bands. Which of the following is (are) true?



1. band A has a higher isoelectric point than band B
2. band A is demonstrating a phenomenon called wick flow
3. band A is demonstrating a phenomenon called electroendosmosis
4. band A has taken on a + charge in the buffer since it is traveling toward the cathode

8. The main difference in procedures among the various applications for electrophoresis in the clinical lab is the:
    1. support medium
    2. pH
    3. stain
    4. voltage
9. Which of the following support media is/are used when separation based on molecular size is desired?
    1. starch gel
    2. agarose
    3. acrylamide gel
    4. cellulose acetate
10. The most important limiting factor in capillary electrophoresis is:
    a. Heat generated
    b. Absence of a physical support
    c. Sensitivity of detection
    d. Run times
11. What is the basis of the superiority of capillary electrophoresis methods compared to other electrophoretic techniques?
    a. Absence of a physical support
    b. Faster heat removal
    c. Small sample volume
    d. Easy visualization of separated analytes

Answer

1. c (p. 203-204,208)
2. c (p. 213-214)
3. b (p. 213)
4. e (p. 213)
5. a (p. 213)
6. a (p. 213, 214)
7. b (p. 204-208)
8. b (p. 207, 211)
9. a (p. 206, 207)
10. c (p. 209-210)
11. b (p. 209-210)

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1.9: Immunology and Immunochemistry

1. Which of the following describes the chemical characteristic of an antibody?
    a. carbohydrate
    b. lipid
    c. protein
    d. electrolyte
    e. ligand
2. In quantitative immunoassays the single most important factor responsible for success or failure is:
    a. specificity
    b. affinity
    c. titer
    d. monoclonal property
    e. antibody absorption
3. Immunonephelometry is based upon the principle that:
    a. antigen and antibody form complexes in antigen excess
    b. antigen and antibody react to form precipitin line
    c. antigen and antibody form small complexes in antibody excess
    d. antigen and antibody form large complexes which precipitate
    e. antigen and antibody form large lattice structures which precipitate
4. Which of the following describes a sandwich fluoroimmunoassay?:
    a. reagent antibody (solid phase) + antigen in patient sample, incubate, + luoroscein labeled anti-antigen
    b. reagent antibody (solid phase) + antigen in patient sample, incubate, + fluoroscein labeled anti—antibody
    c. reagent antibody (solid phase) + antigen in patient sample, incubate, + fluoroscein labeled indicator antigen
    d. reagent antibody (solid phase) + antigen in patient sample + fluoroscein labeled antiantigen, incubate, + indicator antigen
    e. reagent antibody (solid phase) + indicator antigen, incubate, + fluoroscein labeled anti—antigen
5. In double diffusion, immune precipitation reactions occur when two lines cross each other: The reaction is one of:
    a. antigen identity
    b. identity
    c. partial identity
    d. antibody identity
    e. non-identity

Use the following Key to answer Questions 6 – 24.

a. 1,2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

6. Which of these are precipitin based assays?
    1. Ouchterlony
    2. ELISA
    3. immunoelectrophoresis
    4. complement fixation
7. Which of the following factors affects antigenicity?
    1. chemical nature
    2. size
    3. conformation
    4. genetics
8. Which of the following describe the properties of an antibody?

1. has H & L chain
2. can be degraded into Fab and Fc fragments
3. has V and C regions
4. has J chain

9. Antibody affinity describes:
    1. heterogeneity of antibody
    2. closeness of fit
    3. diversity of antibody
    4. the association constant
10. Cross-reactivity
    1. usually involves low affinity antibodies
    2. is often found in crossed immunoelectrophoresis gels
    3. is often observed between similar antigenic determinants
    4. is the most important reaction in nephelometry
11. Antigen-antibody precipitation reactions occur because:
    1. both molecules are multivalent in nature
    2. large complex lattices are formed
    3. antibody and antigen are at equivalence
    4. antigen is present in excess
12. Radial imunodiffusion is a technique:
    1. which quantifies antigens
    2. which requires a mono-specific antibody solution
    3. which results in the formation of precipitin rings
    4. which measures antigen equivalence
13. Antigens present in biological fluids are subject to degradation depending upon:
    1. nature of the antigen
    2. concentration
    3. stability to storage conditions
    4. susceptibility to enzymes
14. Agglutination reactions are:
    1. dependent upon antigen bridging between antibody molecules
    2. dependent upon formation of antibody bridges between antigen particles
    3. dependent upon the positive charge of the latex bead
    4. usually best done using 1gM reactions
15. Complement fixation assays:
    1. are more efficient with IgM than with IgG
    2. do not work with all classes of IgG
    3. make holes in the membranes of target cells
    4. results are read from aggregates of red cells
16. Which of the following are indicator molecules for immunoassays?:
    1. enzymes
    2. fluorescing molecules
    3. radionuclide
    4. absorbing molecules
17. Microtiter or ELISA readers:
    1. are spectrophotometers adapted to read small volumes
    2. can rapidly read 96 samples
    3. are spectrophotometers adapted to read titers
    4. are optical character readers for microtiter plates

18. The Western Blot technique involves:
    1. electrophoresis of proteins in polyacrylamide or agarose
    2. transfer of separated proteins to nitrocellulose
    3. detection of individual species of proteins by immunoassay
    4. detection of very low levels of individual proteins
19. The sandwich technique with labeled antibody that is used for antibody measurement:
    1. has three layers
    2. has two layers
    3. has antigen (1st layer), antibody (second layer), and second antibody with enzyme label (third layer)
    4. has antibody (1st layer) and second antibody with enzymelabel (second layer)
20. Substances that are known to interfere with immunoassays are
    1. compounds that are chemically similar to the antigen
    2. sample matrix
    3. heterologous antibodies that react with the reagent antibody
    4. hemoglobin
21. An heterogeneous, competitive binding immunoassay requires
    1. labeled antigen
    2. separation of the bound label
    3. excess antibody
    4. a large antigen with multiple binding sites
22. A non-competitive immunoassay is characterized by
    1. sandwich formed between antigen and antibody
    2. possible high dose hook at high antigen concentrations
    3. not applicable to small molecules
    4. excess antibody reaction
23. Which of the following are associated with nephelometric immunoassays?
    1. small immune complexes are formed by the unknown antigen and reagent antibody
    2. polyethylene glycol prevents complex formation
    3. light scatter is proportional to antigen concentration
    4. antigen excess
24. Measures that can be instituted to improve specificity of an assay include
    1. purify the antiserum by absorption techniques
    2. use sandwich technology
    3. select an antibody which binds an unique epitope on the antigen
    4. use chemiluminescent molecules as label

Answer

1. c (p. 219)
2. a (p. 230)
3. c (p. 232-233)
4. a (p. 239-241)
5. e (p. 225)
6. b (p. 225,231)
7. e (p. 217-218)
8. a (p. 219-220)
9. c (p. 222)
10. b (p. 222-223)
11. a (p. 223-224)
12. a (p. 225)
13. e (p. 230-231)

14. c (p. 234)
15. a (p. 236-237)
16. a (p. 238)
17. b (p. 239-240)
18. e (p. 231-232)
19. b (p. 239)
20. a (p. 229-231)
21. a (p. 240)
22. e (p. 239)
23. b (p. 232-233)
24. a (p. 229-231)
25.

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1.10: Competitive Binding Assays

1. The equation Ab + L ↔ AbL has which one of the following mathematical relationships?:

   a. $$K_a = \frac{[L][AbL]}{[AbL]} \tag{1.10.1}$$

   b. $$K_a = \frac{[Ab][AbL]}{[L]} \tag{1.10.2}$$

   c. $$K_a = \frac{[Ab][L]}{[Ab][AbL]} \tag{1.10.3}$$

   d. $$K_a = \frac{[AbL]}{[Ab][K]} \tag{1.10.4}$$

   e. $$K_a = \frac{[Ab][L]}{[L][AbL]} \tag{1.10.5}$$

2. The binding constant K_a for most saturation or competitive type reactions is on the order of:
   a. $K_a = 10^{+1}$ L/mol
   b. $K_a = 10^{+3}$ L/mol
   c. $K_a = 10^{+5}$ L/mol
   d. $K_a = 10^{+9}$ L/mol
   e. $K_a = 10^{+15}$ L/mol

3. Indicators (labels) for competitive reactions do not include which one of the following?:
   a. fluorescent dyes
   b. enzymes
   c. hormones
   d. radioisotopes
   e. fluorescent labels

4. For a good competitive binding assay, the proportion of antibody to labeled ligand should be:
   a. more Ab than labeled ligand
   b. in the same molar ratio
   c. less Ab than labeled ligand
   d. greater in free ligand
   e. less in free ligand

5. In order to determine the amount bound and unbound in a radioisotope competitive assay, what is necessary?
   a. the two forms must be separated from each other
   b. the two forms must be derivatized
   c. the two forms must be analyzed by different counters
   d. a high specific activity must be chosen
   e. the ratio must be determined in the same assay tube

6. Which of the following compounds are not often monitored by competitive binding assays?
   a. therapeutic drugs
   b. protein hormones
   c. estrogens
   d. antibiotics
   e. electrolytes

7. Given that at equilibrium a solution contains 1 x 10^{-7} M unbound antibody, 1 x 10^{-6} M unbound ligand, and 1 x 10^{-7} M bound antibody, what is the approximate association constant K_a?
   a. 10^5

b. 10^{6}
c. 10^{7}
d. 10^{8}
e. 10^{13}

8. What is the function of the radioisotope in a competitive binding assay?:
   a. to affect the equilibrium
   b. to make the ligand bind better
   c. to make the antibody bind
   d. to determine the amount of free or bound material
   e. to determine the effect on the antibody binding curve
9. One limiting physical property of a competitive binding assay is the _____ of antigen and antibody:
   a. affinity constant
   b. acidity constant
   c. alkaline constant
   d. radioactivity constant
   e. enzyme constant
10. In the competitive binding assays which of the following is/are kept constant?:
    a. ligand
    b. labeled ligand
    c. antibody (total)
    d. ligand and antibody (total)
    e. labeled ligand and antibody (total)
11. What type of relationship exists between % of labeled ligand bound and concentration of added ligand in a competitive binding displacement reaction?:
    a. linear
    b. logarithmic
    c. non linear
    d. variable dose response
    e. non-variable dose response
12. Radioisotopes are used in competitive binding assays to label ligands. They require one step in the procedure that:
    a. separates antibody and labeled ligand
    b. separates labeled ligand from unlabeled ligand
    c. separates labeled antibody from unlabeled ligand
    d. separates antibody-bound ligand from free ligand
    e. separates unbound antibody from ligand
13. If an antibody has an association constant of 108 M then what is the most likely limit of sensitivity of an immunoassay using it as reagent?:
    a. 10^{-4} M
    b. 10^{-6} M
    c. 10^{-8} M
    d. 10^{-10} M
    e. 10^{-16} M
14. Competitive binding reactions are (best answer/answers):
    a. reversible
    b. non-reversible
    c. involve a binding protein and a ligand
    d. (a) and (c)
    e. (b) and (c)
15. Characteristics of competitive binding reactions include:

a. specificity
b. affinity
c. typically involves the binding of large molecules to binding proteins
d. use only antibodies as binding proteins

16. To generate antibodies to small molecules, it is necessary for the latter to be:
    a. aromatic
    b. contain long aliphatic chains
    c. greater than a 1000 dalton molecular weight
    d. coupled to large macromolecules
17. A competitive binding reaction is described by which equation:
    a. Ligand + Binding Protein ↔ Ligand:Binding Protein Complex
    b. Ligand + Labeled Ligand ↔ Ligand:Labeled Ligand Complex
    c. Ligand + Labeled Ligand + Binding Protein ↔ Ligand:Binding Protein Complex + Labeled Ligand:Binding Protein Complex + Ligand + Labeled Ligand
    d. Labeled Ligand + Binding Protein ↔ Labeled Ligand: Binding Protein Complex
18. As the concentration of ligand increases in a competitive binding reaction, the amount of labeled ligand bound to the binding protein:
    a. increases
    b. decreases
    c. stays the same
19. Heterogeneous competitive binding formats require that:
    a. the binding protein modulates the activity of the label
    b. the bound labeled ligand be physically separated from the free, labeled ligand
    c. a radioactive element is used as the label
    d. none of the above
20. Labels for homogeneous assay formats include enzymes, radioisotopes, and substrates
    a. True
    b. False
21. Cross-reactivity is when molecules that are structurally similar to the ligand competitively bind to the binding protein
    a. True
    b. False
22. The dose response curve for a competitive binding assay (best answer):
    a. increases when measuring the free (unbound) labeled ligand
    b. decreases when measuring the bound labeled ligand
    c. decreases when measuring the free (unbound) labeled ligand
    d. increases when measuring the bound labeled ligand
    e. (a) and (b)
    f. (c) and (d)
23. CEDIA and EMIT are examples of:
    a. heterogeneous competitive binding assays
    b. homogeneous competitive binding assays competitive
    c. immunometric assay
    d. fluroimmunoassay
    e. point-of-care testing methods

Answer

1. d (p. 248)
2. d (p. 248)
3. c (p. 250)

4. b (p. 148)
5. a (p. 249,250)
6. e (p. 247)
7. b (p. 248)
8. d (p. 249, 250)
9. a (p. 248, 252)
10. e (p. 248, 254)
11. c (p. 248)
12. d (p. 249, 250)
13. c (p. 248, 251)
14. d (p. 247)
15. a (p. 247, 248., 250)
16. d (p. 247)
17. c (p. 248)
18. b (p. 248)
19. b (p. 249)
20. b (p. 249-251)
21. a (p. 252-253)
22. e (p.248)
23. b (p. 259)

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1.11: Colligative Properties

1. A solution contains 75 mmol/L of NaCl and 100 mmol/L of glucose. What would be its freezing point?:
   a. -0.326°C
   b. -0.465°C
   c. -0.512°C
   d. -0.651°C
   e. -0.930°C
2. A urine sample has a freezing point of -0.83°C. What is its osmolarity?:
   a. 224.1 mosmoles/L
   b. 224.1 osmoles/100 ml
   c. 446.1 mosmoles/L
   d. 446.1 osmoles/100 ml
   e. 892.4 osmoles/100 ml
3. Choose the solution that has a freezing point closest to that a 300 milliosmolar solution of glucose:
   a. 100 millimolar $ZnCl_2$
   b. 150 millimolar maltose
   c. 300 millimolar NaCl
   d. 100 millimolar $ZnSO_4$
   e. 150 millimolar $Ba(OH)_2$
4. A mole of which of the following substances equals 1 osmole of the same substance?
   a. $NaHCO_3$
   b. NaCl
   c. K_2HPO_4
   d. glucose
   e. $Ba(OH)_2$
5. The calculated osmolality of serum with Na = 139 mmol/L, Cl = 101 mmol/L, K = 4 mmol/L, glucose =1300 mg/L, and BUN = 140 mg/L,
   a. 256
   b. 270
   c. 280
   d. 290
   e. 300

Use the following Key to answer Questions 6-9:

a. 1, 2 and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

6. The measurement of osmolarity is based upon what principle(s)?:
   1. vapor pressure decrease
   2. viscosity
   3. freezing point depression
   4. specific gravity
7. As the osmolality of a solution increases, which of the following occur(s)?:
   1. osmotic pressure increases
   2. vapor pressure decreases
   3. freezing point decreases
   4. boiling point decreases

8. Osmotic pressure is:
    1. not measurable by how much the vapor pressure changes
    2. equal to number of solvent molecules times a factor
    3. measurable by how much it raises the freezing point of water
    4. proportional to concentration of solute particles
9. The presence of unmeasured solutes in serum can be determined by:
    1. increased osmolality
    2. decreased osmolality
    3. measured osmolality significantly less than calculated osmolality
    4. measured osmolality significantly greater than the calculated osmolality

Answer

1. b (p. 267,269)
2. c (p. 269)
3. a (p. 267, 269)
4. d (p. 267)
5. d (p. 267)
6. b (p. 269)
7. a (p. 269)
8. d (p. 267-269)
9. d (p. 267)

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1.12: Electrochemical Measurements

1. The antibiotic Valinomycin has a high affinity for:
    a. sodium
    b. lithium
    c. potassium
    d. calcium
    e. magnesium
2. In the Nernst equation, E^0 represents:
    a. measured volume
    b. gas constant
    c. electrons transferred
    d. Faraday constant
    e. standard half cell potential
3. Match the items in Column B with the definitions in Column A:

B	A
a. voltometry of a dropping Hg electrode b. the measurement of current passing through a solution at a given charge c. measurement of charge d. measurement of current at a constant potential e. measurement of current as a function of potential	i. amperometry ii. coulometry iii. conductometry iv. polarography v. voltammetry

4. The K ion electrode has an absolute specificity for K and therefore has interferences from no other cations.
    a. true
    b. false
5. The PCO_2 electrode reduces O_2 to H_2O and measures the resulting decrease in current
    a. true
    b. false
6. The statement which most correctly describes the measurement of pH with a glass electrode is:
    a. hydrogen ions pass through a special kind of permeable glass
    b. the internal and external half-cells have the same potential
    c. a special kind of glass sensitive to hydrogen ions is used
    d. the temperature knob on the pH meter compensates for the influence of temperature
    e. temperature is not involved with pH measurements
7. In potentiometric measurements, the voltage difference between the indicator and reference electrodes is proportional to:
    a. ion concentration (mmol/L)
    b. ion activity
    c. log of ion concentratioin (mmol/L)
    d. log of ion activity
    e. ion concentration (mg/L)
8. In the PCO_2 electrode, all of the following are true except:
    a. carbon dioxide from the sample chamber diffuses through a membrane and dissolves in electrolyte solution
    b. the PCO_2 electrode measures pH change caused by the production of carbonic acid
    c. the measuring electrode is a CO_2 specific electrode
    d. the measuring and reference electrodes are combined into one unit
    e. the CO_2 measurement can be related to total CO_2

Use the following Key to answer Questions 9-15:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

9. The CO_2 electrode works by:
    1. measuring a potentiometric change
    2. measuring a change in current
    3. measuring a change in pH
    4. using an enzymatic reaction with CO_2
10. Ion selective electrodes:
    1. allow ion exchange on surface resulting in a change of potential
    2. are pH dependent
    3. are temperature dependent
    4. do not require a reference electrode
11. Potentiometry:
    1. is the measurement of the electrical potential difference between two electrodes in an electrochemical cell
    2. measures voltage
    3. applications include pH and PCO_2 measurements
    4. principles are used for measuring potassium using valinomycin membrane
12. Which of the following devices are considered to be of the amperometric—coulometric type?
    1. PO_2 electrode
    2. pH electrode
    3. Chloridometer (Cotlove)
    4. PCO_2 electrode
13. A KCl salt bridge, widely utilized in electrochemical cells, has all of the following functions except:
    1. lowers liquid—junction potentials
    2. conducts electricity
    3. allows ions to migrate
    4. measures the potential between two solutions
14. The PCO_2 electrode of a blood gas instrument measures:
    1. reduction of CO_2 at the cathode
    2. change in pH of the electrode solution
    3. the time required for CO_2 to pass through a special membrane
    4. partial pressure of CO_2
15. Which statement(s) is/are false concerning the chloridometer?
    1. generating anode generates silver ions
    2. electrons from the generating electrodes combine with hydrogen ions to form hydrogen gas
    3. automatic timer is initiated at the start of the silver ion generation
    4. AgCl is sensed by the detector electrode

Answer

1. c (p. 277)
2. e (p. 274)
3.
    i. d (p. 282)
    ii. c (p. 285)
    iii. b (p. 283)
    iv. a (p. 282)

v. a (p. 282)

4. b (p. 277,280)
5. b (p. 279-282)
6. c (p. 278-279)
7. d (p. 274,276)
8. c (p. 279)
9. b (p. 279)
10. a (p. 276-277)
11. e (p. 273-274)
12. b (p. 282-285)
13. d (p. 273)
14. c(p. 279)
15. c (p. 285)

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1.13: Automation

Use the following Key to answer Questions 1 - 3:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

1. Which of the following is a/are general characteristic(s) of discrete multichannel analyzers?
    1. each specimen is added into a separate compartment along with the reagents
    2. the absorbance of the reaction mixture is read at a steady-state plateau
    3. carryover between samples is minimized
    4. all of the tests done by the analyzer are performed on each specimen
2. For random access analyzers, it is true that:
    1. all of the available tests done by the analyzer are performed on each specimen
    2. reagent and sample volumes are dependent upon the inner diameter of pump tubing
    3. the integrity of individual samples is maintained through the segmentation of the sample stream with air bubbles
    4. the analyses that are performed for a sample are only the ones that were ordered
3. The dry—chemical reagent technique has the following features:
    1. the dry reagent is impregnated on a solid phase
    2. can be prepared by most laboratories using common reagents
    3. the measuring reaction begins when sample comes in contact with reagent
    4. usually cheaper than techniques
    5. using bulk reagents
4. An instrument has 17 reagents available for analysis and can perform 3 tests at a time. The immediate test repertoire of this instrument is:
    a. 3
    b. 14
    c. 17
    d. 20
    e. 51
5. Stat. Instruments should have high test throughput:
    a. true
    b. false
6. An instrument can process 40 samples an hour and perform 6 tests on each sample simultaneously. The test throughput of this analyzer is:
    a. 6
    b. 40
    c. 46
    d. 240
    e. 280
7. Which of the following is not usually considered to be a function of an automated analyzer?
    a. computation
    b. proportioning of reagent
    c. auto-verification
    d. incubation
    e. mixing

Use the following Key to answer Questions 8 through 11:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

8. Automated Front-End specimen processing includes:
    1. specimen identification
    2. specimen sorting
    3. specimen decapping
    4. specimen aliquoting
9. The goals of laboratory automation are:
    1. to reduce cost
    2. to decrease laboratory testing
    3. to reduce laboratory errors
    4. to increase workstations
10. The major drawbacks of Total Laboratory Automation are:
    1. Substantial financial investment
    2. Inability to sort and decap specimens
    3. Limited availability of laboratory space
    4. Lack of clot and aliquot capabilities
11. In some laboratories, manual Front-End Specimen Processing accounts for:
    1. Is needed for workstation consolidation
    2. 60% of the testing cost
    3. Is required for archiving of specimens
    4. Is responsible for most of the lost specimens
12. Autoverification of laboratory results is the automated reporting of selected test results without verification by the technologist or laboratory supervisor.
    a. True
    b. False
13. Archiving and retrieval of specimens is a time consuming process that can be automated using stand-alone systems.
    a. True
    b. False

Answer

1. b (p. 293-294)
2. d (p. 288, 296)
3. b (p. 293-294)
4. a (p. 295-296)
5. b (p. 296-297)
6. d (p. 296)
7. c (p. 290)
8. e (p. 291-292)
9. b (p. 289-290)
10. b (p. 291-292)
11. c (p. 292)
12. a (p. 290-291)
13. a (p. 292)

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1.14: Point-of-care (Near-patient)

Use the following Key to answer Questions 1 through 6:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

1. Which of the following statements is not true regarding the use of quality control (QC) for single-use, point-of-care devices?
    1. QC material and testing is often incorporated into the device.
    2. Liquid QC material must be run at least every 24 hours.
    3. Electronic QC checks can be performed to validate testing devices.
    4. Central laboratory personnel are required to perform QC testing.
2. Once point-of-care devices, such as glucose meters, are placed into service which of the following must be routinely performed on the devices?
    1. Linearity
    2. Proficiency testing
    3. Demonstration of accuracy vs. another routine laboratory device
    4. Clinical utility
3. Which of the following are synonyms for ‘point-of-care’ testing?
    1. Decentralized testing
    2. Bedside testing
    3. Waived testing
    4. Alternate site testing
4. Types of technologies employed for point-of-care testing devices include:
    1. Small testing devices requiring manually read endpoints.
    2. The use of simple, portable instruments.
    3. Instruments remaining attached or fixed to the patient.
    4. Devices that use chromatographic methods.
5. Which of the following are proven or potential benefits of ‘point-of-care’ testing?
    1. Less blood is needed for analysis.
    2. Precision and accuracy are as good as central laboratory testing
    3. Results are more rapidly available to physicians.
    4. Always results in improved patient care (decreased morbidity and mortality).
6. Implementation of available point-of-care testing programs requires the active participation of:
    1. Nursing personnel
    2. Central laboratory personnel
    3. Physicians
    4. Administration personnel
7. Which of the following statements is not true about ‘point-of-care’ testing?
    a. Employs technology as advanced as that used in the central laboratory
    b. Is mostly performed by non-laboratorians.
    c. Always requires a small amount of blood to be drawn from patients.
    d. Is not always subject to regulatory control.
    e. Can provide results to physicians much faster than the central laboratory.
8. All of the following tests but one are now proven to have clinical utility as a ‘point-of-care’ test. This test is:
    a. Troponin I (or T)
    b. ACT
    c. Potassium

d. Prothromin time (PT)
e. Glucose

9. Institutional responsibility for point-of-care testing almost always falls to:
   a. Nursing personnel
   b. Central laboratory personnel
   c. Central supply personnel
   d. Senior hospital administration
   e. Medical board members
10. When available, proficiency testing is not required for all point-of-care testing?
   a. True
   b. False
11. Which of the following statements most accurately reflects the flow of data from point-of-care testing (POCT) devices?
   a. Can always be electronically transmitted to the LIS.
   b. Is not legally valid if not sent electronically to the LIS.
   c. Can use infrared beams to transfer data to the LIS.
   d. If electronic connectivity is available, each device must be directly linked to the LIS.
   e. Data from POCT is always available in the patient's electronic record.

Answer

1. a (p. 315)
2. a (p. 306)
3. e (p. 304)
4. e (p. 311-314)
5. b (p. 305)
6. e (p. 308)
7. c (p. 304)
8. a (p. 305)
9. b (p. 306)
10. b(p. 309)
11. c (p. 316)

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1.15: Laboratory Information Systems

Use the following Key to answer Questions 1 through 8:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

1. The laboratory information system (LIS) is designed to provide information to:
    1. the laboratories
    2. the physician
    3. administrative support services
    4. the patient
2. Which of the following information concerning a specific patient is entered into the LIS?
    1. specific test request
    2. specimen collection time
    3. patient demographics
    4. laboratory data.
3. Which of the following describes the function(s) of the LIS?
    1. collection of patient information
    2. generation of specimen test results
    3. assembling of data output
    4. producing ancillary reports
4. The term “interfacing” refers to which of the following?
    1. the display of computer-generated information.
    2. the degree of effort that the system makes to be accurate.
    3. the information of a video screen device.
    4. the exchange of information between the computer and operator.
5. The technologist usually interfaces with the LIS to accomplish the following:
    1. obtain billing information
    2. enter patient test data
    3. obtain inventory data
    4. verify patient data
6. The software of the LIS is usually:
    1. easily changed
    2. purchased from a commercial supplier
    3. available from help
    4. a complex series of instructions
7. Patient identification in a LIS may be accomplished in the following way(s):
    1. hospital patient number
    2. social security number
    3. encounter number
    4. billing number
8. What function(s) is (are) provided by an laboratory information system?
    1. Results reporting
    2. Specimen tracking
    3. Phlebotomy labels
    4. Management reports

9. Which of the following would not make a satisfactory LIS specimen number?
    a. The patient medical record number (e.g., 123456 where 123456 is the patient's medical record number).
    b. A number generated by the LIS starting from 1 and incrementing by 1 for each new specimen (e.g., 1, 2, 3 and so on).
    c. A number consisting of the year followed by the number of days past January 1st followed by the number of specimens received since midnight by the system (e.g., 20011801234 is the 1234th specimen received on the 180th day past January 1st in year 2001).
    d. A number started by an unique letter for the each day of the week followed by a serial number generated by the LIS (e.g., specimens on Monday start with M1, M2, M3, and so on).
    e. A number starting at 99999999 and decremented by 1 for each specimen received.
10. What is the order of events occur between an LIS and a multichannel chemistry analyzer in a two-way instrument interface?
    1. Instrument transmits specimen number to LIS
    2. Instrument transmits analytical results to LIS
    3. LIS transmits test requests to instrument
    4. Bar code containing the specimen number is read by instrument
    5. Tech reviews results
        a. 1, 2, 3, 4, 5
        b. 5, 4, 3, 2, 1
        c. 4, 1, 3, 2, 5
        d. 3, 1, 4, 2, 5
        e. 2, 4, 1, 3, 5
11. Which of the following is not required in a test report?
    a. Time and date of report
    b. Test result with normal ranges
    c. Date and time test was collected and/or performed
    d. Technologist performing test
    e. Performing laboratory
12. Total laboratory automation refers to:
    a. A laboratory with a large number of automated analyzers.
    b. A totally robotic laboratory operating without humans.
    c. A laboratory travelling on wheels.
    d. Automation which eliminates much of the manual tasks associated with centrifugation, making aliquots, racking, pipetting, specimen storage, etc. and integrates different instruments.
    e. A computer controlled laboratory.
13. If a laboratory has a situation where a patient's privacy has violated, it most likely results from:
    a. A computer hacker coming through the Internet.
    b. An employee who does not understand the meaning of patient privacy.
    c. A newspaper reporter looking through trash.
    d. A badly managed computer system allowing easy access.
    e. Telephone taps.
14. Which of the following statement is wrong?
    a. Computerized order entry is easy to implement.
    b. The laboratory has responsibility for the accuracy of reports generated by interfaced computers.
    c. Paper is the primary mode for patient reports in most institutions.
    d. A major source of errors is the manual transcription of information.
    e. Computerization of laboratory ordering and reporting can be highly cost effective.
15. What is HIPAA?
    a. An acronym used by computer programmers for a program which behaves like a hippopotamus.
    b. A new federal law which deals with insurance, insurance billing, and patient privacy.
    c. A new federal law regulating the laboratory and the way it handles patient results.
    d. A new laboratory procedure regarding the handling of laboratory instrument interfaces.

e. A bill proposed by the Bush administration on health care reform.

Answer

1. a (p. 322-323)
2. e (p. 323)
3. e (p. 323)
4. d (p. 325-326)
5. c (p. 326-328)
6. c (p. 332)
7. a (p. 323-333)
8. e (p. 323)
9. a (p. 324)
10. c (p. 325-326)
11. d (p. 328)
12. d (p. 326)
13. b (p. 334-335)
14. c (p. 328)
15. b (p. 335)

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1.16: Statistics and Reference Ranges

1. Presuming there is a healthy and a diseased population, which fact is not true?:
    a. healthy and disease values overlap
    b. healthy and disease cut off points will determine the number of false positives
    c. healthy and disease values are always clearly separated
    d. healthy and disease cut off points will determine false negatives
    e. healthy and disease values are a continuum
2. What is a minimum number of samples to obtain a good reference range?:
    a. 20
    b. 40
    c. 120
    d. 200
    e. 500
3. The mean of a series of results is 100 mg/L and the standard deviation is 2 mg/L. The percent coefficient of variation of this analysis is:
    a. 0.5
    b. 1
    c. 2
    d. 4
    e. 8
4. Test A correctly identified 17/100 true positives while correctly identifying 90/100 true negatives. Test B correctly identified 55/100 true positives while correctly identifying 80/100 true negatives. Which of the following statements is true?:
    a. Test A has a better sensitivity and specificity
    b. Test B has a better sensitivity and specificity
    c. Test A has a better sensitivity but a worse specificity
    d. Test B has a better sensitivity but a worse specificity
    e. Tests A and B have equal sensitivities and specificities
5. The 95% confidence limits for Ca^{++} in control sera were established as 9.2-10.2 mg/L. The standard deviation is:
    a. 0.1 mg/L
    b. 0.2 mg/L
    c. 0.25 mg/L
    d. 0.5 mg/L
    e. 1.0 mg/L
6. What percentage of tests should fall within ± one standard deviation if the distribution for the population is considered normal?:
    a. 28
    b. 35
    c. 68
    d. 45
    e. 95
7. Precision applied to chemistry determination means the same as:
    a. accuracy
    b. reproducibility
    c. recovery
    d. conformance with Beer’s Law
8. The following set of values was obtained for K+ concentration for a quality control sample: 4.7, 3.9, 4.0, 4.1, 4.3, 4.5, 3.9, 4.2, 4.2, 4.6, 4.0, 3.9, 4.6, 3.8, 4.2, 4.5, 4.1, 4.7, 3.9, 4.8. The mean for this population of data is:
    a. 4.0
    b. 4.1

c. 4.2
d. 4.4
e. 4.6

9. A 95% confidence limit for cholesterol precision was found to be 1300-1500 mg/L. The coefficient of variation for this methodology is:
   a. 1.23%
   b. 3.57%
   c. 7.14%
   d. 14.28%
   e. 28.56%

Use the following Key to answer Questions 10-14:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. 4 only is correct
e. all are correct

10. A normal value is usually defined as:
    1. a result from a healthy person
    2. a gaussian distribution
    3. a usual laboratory value
    4. the results of a t-test
11. Reference populations should be defined in terms of:
    1. age
    2. sex
    3. race
    4. genetic background
12. The positive predictive value (accuracy) of a test varies with:
    1. test sensitivity
    2. disease prevalence
    3. test specificity
    4. test linear range
13. The means and standard deviations of methods A and B are $X_A = 80$, $SD_A = 6$; $X_B = 40$, $SD_B = 1.5$. The following statements are true about these 2 methods:
    1. there is no apparent bias between the 2 methods
    2. method A is less precise than method B
    3. there is no difference in precision between the two methods
    4. there appears to be a bias between the two methods
14. An indication of the spread of the distribution of a set of measurements is given by the:
    1. range
    2. variance
    3. standard deviation
    4. mean
15. A test has a sensitivity of 95% and a specificity of 80%, and a positive predictive value of 50%. If a result of this test was positive (i.e., abnormal), then the likelihood that disease was present is:
    a. 95 out of 100
    b. 80 out of 100
    c. 50 out of 100
    d. 20 out of 100
    e. none of the above

16. A useful test to determine a statistical difference between the means of two different populations is the:
    a. F-test
    b. t-test
    c. chi-square test
    d. linear regression test
    e. variance test
17. A receiver-operating characteristic (ROC) curve is used to graphically display:
    a. Linearity of a new method
    b. Differences between variances of two methods
    c. Correlation (regression) between methods
    d. Ability of a test to discriminate between disease and non-disease
    e. The distributions of diseased and non-diseased populations

Answer

1. c (p. 364-365)
2. c (p. 369)
3. c (p. 345)
4. d (p. 374)
5. c (p. 346)
6. c (p. 346)
7. b (p. 348)
8. c (p. 344)
9. b (p. 346-347)
10. b (p. 342, 364)
11. e (p. 366)
12. a (p. 372-373)
13. c (p. 348-349)
14. a (p. 345)
15. c (p. 373)
16. b (p. 349)

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1.17: Quality Control

1. In establishing the 95% confidence limits for a particular method for a quality control program, one would expect how many test results to fall beyond the limits of allowable variation by chance?:
   a. one in 95
   b. one in 5
   c. one in 10
   d. one in 20
   e. one in 30
2. A laboratory test has ± 2 SD control limits of ± 5. The mean, target value is 15. How many days was the method out of control during the two week period shown?:
   a. 1
   b. 2
   c. 3
   d. 4
   e. 5

Day	1	2	3	4	5	6	7	8	9	10
Value	11	13	18	7	22	28	15	14	26	14

3. The 68% confidence limits of a glucose control are 750-850 mg/L. A control and a series of patient specimens are run. The control reads 750 mg/L. If control results must be within ± 2 SD, what action should you take?:
   a. report out patient results, since control results are within acceptable limits
   b. repeat the control, since control results are out of control; if repeat results are in control, report out patient results
   c. repeat both the control and all patient specimens using same methodology
   d. repeat both the control and all patient specimens using different methodology
   e. run second control if within range, report patient results
4. A medical technologist should never use the pattern of patient results, i.e. abnormal values, as an indicator of instrument performance.
   a. true
   b. false

Use the following Key to answer Questions 5-7:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. 4 only is correct
e. all are correct

5. Which of the following is/are acceptable quality control pool material?
   1. frozen
   2. lyophilized
   3. low temperature liquid
   4. fresh patient samples
6. When performing an analysis on a stat. analyzer, a quality control sample should be run:
   1. during the first shift
   2. depending upon the proven stability of the test system
   3. by each technologist
   4. usually at least once every 8 hour shift
7. If the quality control sample is out of range (>3 SD), the technologist should:
   1. recalibrate the instrument

2. troubleshoot the instrument
3. record the out-of-range result on the QC chart
4. notify the supervisor if the problem cannot be solved within 30 minutes

8. Calibrators may be used as quality control pools if:
   a. the usual quality control pools are expired
   b. the usual quality control pools just won't 'come in range'
   c. there is a temporary shortage of the usual quality control pools
   d. there are no quality control materials that contains the analyte
   e. never

Answer

1. d (p. 390)
2. b (p. 391)
3. a (p. 346, 391)
4. b (p. 392)
5. a (p. 388)
6. c (p. 387-388, 399)
7. e (p. 393)
8. d (p. 396)

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1.18: Evaluation of Methods

Use the following Key to answer Questions 1- 3:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

1. The key areas for a method evaluation study include:
    1. Evaluation of the method(s)
    2. Validation of the method's functionality
    3. Verification of the method's functionality
    4. Demonstration that a method can fulfill a manufacturer's specifications
2. Important criteria for choosing a method to evaluate include:
    1. Costs
    2. Home town of the company/manufacturer
    3. Recommendations by colleagues
    4. Functional accuracy of the method
3. Basic method-evaluation experiments include:
    1. Repeatedly performing all required preventative maintenance
    2. Determining assay linearity
    3. Determining the reference interval of the method
    4. Determining random and systematic error
4. Constant systematic error is usually caused by mis-calibration.
    a. true
    b. false

Answer

1. e (p. 405)
2. b (p. 408)
3. c (p. 410, 411)
4. b (p. 411)

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1.19: Interferences

Use the following Key to answer Questions 1- 8:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

1. Basic types of analytical interferences include those that arise from:
    1. limitation of detectors
    2. chemical substances in the sample
    3. disease states
    4. sample processing
2. Spectrophatometer errors are the greatest:
    1. where the absorbance is the smallest
    2. at an absorbance near 0.20
    3. where the absorbance is the greatest
    4. where absorbance is about 0.5 to 0.2
3. Spectral interference can be caused by:
    1. absorbance by compounds with similar spectral properties
    2. turbidity
    3. fluorescence
    4. sample blanks with spectral properties similar to the absorbance cell
4. Correction of spectral interference includes use of the following:
    1. kinetic measurements
    2. sample blank
    3. bichromatic analysis
    4. sampling of reagent
5. Correction of chemical interferences can be achieved by:
    1. dilution of the interferent
    2. increasing reaction specificity
    3. removing the interferent
    4. using bichromatic analysis
6. In vivo interferences affect interpretation of results. These include:
    1. age
    2. gender
    3. drugs
    4. diet
7. The effect of in vivo interferences can be overcome in the majority of cases by:
    1. resting the patient
    2. avoidency stasis
    3. checking patient’s weight
    4. a good clinical history
8. Interferences common to immunoassays include:
    1. incomplete saturation of solid-phase binding sites for antigen or antibody
    2. use of radioactive labels
    3. rheumatoid factor
    4. icterus and hemolysis

9. HAMAs are:
    a. endogenous autoantibodies
    b. iatrogenic antibodies
    c. endogenous, cross-reacting compounds, e.g., DLIFs
    d. heterophilic anti-immunoglobulin antibodies
    e. homogenous active mouse anti-reaction antibody

Answer

1. e (p. 428)
2. b (p. 428-429)
3. a (p. 430-431)
4. a (p. 430-433)
5. e (p. 434)
6. e (p. 436)
7. d (p. 436-437)
8. b (p. 435-436)
9. d (p. 436)

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1.20: Body Water and Electrolytes

1. The plasma volume of a 3200 g neonate is approximately:
    a. 150 mL
    b. 250 mL
    c. 350 mL
    d. 500 mL
    e. 1000 mL
2. The cation with the highest extracellular concentration is
    a. potassium
    b. sodium
    c. calcium
    d. magnesium
    e. lithium
3. The anion with the highest extracellular concentration is:
    a. protein
    b. bicarbonate
    c. chloride
    d. phosphate
    e. lactate
4. The cation with the lowest intracellular concentration is:
    a. potassium
    b. sodium
    c. calcium
    d. magnesium
    e. chloride
5. The cation with the highest intracellular concentration is:
    a. potassium
    b. sodium
    c. calcium
    d. magnesium
    e. lithium
6. Total body water represents about what percent of body weight in adults?
    a. 80
    b. 70
    c. 60
    d. 50
    e. 40
7. The osmolarity of extracellular fluid is mostly dependent on:
    a. potassium
    b. sodium
    c. calcium
    d. magnesium
    e. lithium
8. What is the largest fluid compartment?
    a. plasma water
    b. interstitial fluid
    c. intracellular fluid
    d. transcellular water

e. intercellular water

9. The plasma volume in 70 kg adults is approximately:
    a. 2000 mL
    b. 2500 mL
    c. 3000 mL
    d. 3500 mL
    e. 4000mL
10. Intracellular water accounts for about what percent of an adult's body weight?
    a. 60
    b. 50
    c. 40
    d. 30
    e. 20
11. The anion with the highest intra-cellular concentration is:
    a. protein
    b. bicarbonate
    c. chloride
    d. lactate
    e. none of the above
12. Total body water represents about what percent of body weight in neonates?
    a. 40
    b. 50
    c. 60
    d. 70
    e. 80
13. Low serum potassium levels are usually associated with all of the following EXCEPT:
    a. low total body potassium
    b. low intracellular potassium
    c. intracellular sodium increase
    d. extracellular alkalosis
    e. increased urinary potassium excretion
14. Extracellular fluid volume is normally largely governed by:
    a. sodium
    b. urea
    c. glucose
    d. phosphate
    e. magnesium
15. The significant electrolyte changes observed in metabolic alkalosis include:
    a. sodium unchanged/chloride increased
    b. sodium unchanged/chloride decreased
    c. sodium increased/chloride increased
    d. sodium decreased/chloride decreased
    e. sodium increased/chloride decreased
16. Edema is the result of:
    a. increased extracellular sodium
    b. decreased extracellular sodium
    c. increased plasma volume
    d. decreased plasma volume
    e. none of the above

Use the following Key to answer questions 17 through 19

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

17. Deficits of body sodium are associated with:
    1. renal disease
    2. gastrointestinal losses
    3. hypoaldosteronism
    4. congestive heart failure
18. Hyponatremia can be caused by:
    1. equal losses of sodium and water
    2. water retention in excess of sodium retention
    3. sodium retention in excess of water retention
    4. sodium loss in excess of water loss
19. Which of the following are important regulators of extracellular water volume?
    1. hypothalamic control of thirst
    2. renin angiotensin—aldosterone system
    3. pituitary release of anti-diuretic hormone (ADH)
    4. atrial naturetic peptide (ANP)

Answer

1. a (p. 445)
2. b(p. 446)
3. c (p. 446)
4. c (p. 446)
5. a (p. 446)
6. c (p. 444)
7. b (p. 446)
8. c (p. 444)
9. d (p. 444-445)
10. c (p. 444)
11. a (p. 446)
12. e (p. 445)
13. c (p. 459)
14. a (p. 450)
15. b (p. 460)
16. a (p. 455)
17. a (p. 456)
18. c (p. 456)
19. e (p. 449-450)

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1.21: Acid-base Control

1. The major buffer of blood is hemoglobin because:
   a. it has a heme group
   b. it binds O_2
   c. it has large numbers of histidine residues with an effective pK_a of 7.3
   d. it binds CO_2
   e. it catalyzes the reaction between CO_2 and H_2O
2. The primary buffer of plasma is:
   a. albumin
   b. phosphate
   c. bicarbonate
   d. hemoglobin
   e. all of the above
3. Respiratory alkalosis can be seen in patients with:
   a. severe diarrhea
   b. pneumonia
   c. hysterical hyperventilation
   d. hyperkalemia
   e. morphine poisoning
4. The usual, primary, compensatory response to a metabolic acid-base disorder is renal.
   a. true
   b. false
5. The major waste metabolite of cells is CO2, which is carried in RBC's to the lungs for removal.
   a. true
   b. false
6. A patient's pH, HCO_3, PCO_2 are 7.42, 24, and 40 respectively. How would you expect these values to change, if at all, if the patient subsequently had a serum lactate of 31.5 mEq/l?
   a. pH increases, HCO_3 increases
   b. pH increases, HCO_3 decreases
   c. pH and HCO_3 remain the same
   d. pH decreases, HCO_3 increases
   e. pH decreases, HCO_3 decreases
7. A three year old boy was brought unconscious into the emergency room. Blood gases were performed. This patient demonstrates:
   a. metabolic alkalosis
   b. metabolic acidosis
   c. respiratory acidosis
   d. respiratory alkalosis
   e. compensated metabolic acidosis

pH	7.26
PCO_2	54 mm Hg
HCO_3	38 mEq/L
Base excess	+14 mEq/L

8. The red cell contains more Cl^- in:
   a. arterial blood than venous blood

b. venous blood than arterial blood
c. no difference between a and b

9. Given: pH = 7.2, PCO_2 = 83 mm Hg, PO_2 = 53 mm Hg, $[HCO_3]$ = 31 mEq/L. The most probable diagnosis is:
   a. uncompensated respiratory acidosis
   b. partially compensated metabolic acidosis
   c. uncompensated respiratory alkalosis
   d. totally compensated metabolic alkalosis
   e. laboratory error
10. Given: pH = 7.47, PCO_2 = 75, PO_2 = 93, $[HCO_3]$ = 37 mEq/L. The most probable diagnosis is:
   a. uncompensated respiratory acidosis
   b. partially compensated metabolic acidosis
   c. uncompensated respiratory alkalosis
   d. partially compensated metabolic alkalosis
   e. laboratory error
11. Given: pH = 7.4, PCO_2 = 85 mm Hg, PO_2 = 85 mm Hg, $[HCO_3]$ = 67 mEq/L. The most probable diagnosis is:
   a. uncompensated respiratory acidosis
   b. partially compensated metabolic acidosis
   c. uncompensated respiratory alkalosis
   d. totally compensated metabolic alkalosis
   e. laboratory error

FOR QUESTIONS 12 - 15:
Given the following laboratory information on patient:

- pH = 7.1
- PO_2 = 100 mm Hg
- pK_a' = 6.1
- PCO_2 = 20 mm Hg
- a = 0.031
- BUN = 20 mm Hg
- Creat. = 16 mg/L
- Glucose = 6450 mg/L
- Protein = 86 g/L

12. What is the $[HCO_3^-]$ in mEq/L in this patient?
   a. 36.8
   b. 26.8
   c. 16.8
   d. 6.3
   e. 0.68
13. What is the total CO_2 content (in mEq/L) of this patient's serum?
   a. 17.5
   b. 6.9
   c. 27.5
   d. 37.5
   e. 20.7
14. This person is most likely in a:
   a. metabolic alkalosis
   b. respiratory alkalosis
   c. metabolic acidosis
   d. respiratory acidosis
   e. cannot tell with information given

15. Which of the following terms can be used to further describe this patient's acid-base condition?
    a. uncompensated
    b. totally compensated
16. A 34 year old woman entered the emergency room comatose. She was suspected of taking an overdose of an unknown drug. Her blood gas results are below. This patient demonstrates:
    a. metabolic alkalosis
    b. metabolic acidosis
    c. respiratory alkalosis
    d. respiratory acidosis
    e. compensated metabolic acidosis

pH	7.15
PCO_2	80 mm Hg
HCO_3	28 mEq/L
Base excess	-5 mEq/L
PO_2	60 mm Hg
Oxygen saturation	80%

17. If a sample had a total CO_2 of 40 mmol/L and a PCO_2 of 28 mm Hg, the blood pH would probably:
    a. indicate an acidemia
    b. indicate an alkalemia
    c. indicate a normal value
    d. not be able to be calculated from the available data
    e. indicate lab error
18. A person's blood buffering capacity is not dependent upon hemoglobin levels, but upon the plasma ratio of $HCO_3^-/\alpha PCO_2$:
    a. true
    b. false

Use the following Key to answer Questions 19 - 27:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

19. On a blood gas instrument, which of the following are directly measured values?
    1. H_2CO_3
    2. total CO_2
    3. $[HCO_3]$
    4. pH
20. Which of the following statements is (are) true concerning measurement of oxygen saturation?:
    1. can be estimated by a blood-gas instrument
    2. can be measured directly by a blood-gas instrument
    3. can be measured spectrophotometrically
    4. can be measured by enzymatic analysis
21. Which of the following can cause a "shift to the right" of the hemoglobin-oxygen dissociation curve?:
    1. decreased pH
    2. increased CO_2
    3. increased 2,3 diphosphoglycerate (2,3 DPG)

4. increased temperature

22. Ventilation is controlled by:
    1. pH sensitive receptors of the kidney
    2. pH chemoreceptors in the carotid artery
    3. pH chemoreceptors in the lung
    4. pH sensitive receptors of the brain
23. CO_2 can be transported in the following forms:
    1. covalently bound to protein
    2. as dissolved CO_2
    3. as bicarbonate
    4. as a hemoglobin-bicarbonate complex
24. The major difference(s) between expired air and atmospheric air is (are):
    1. expired air has lower PO_2
    2. expired air has higher PO_2
    3. expired air has higher CO_2
    4. expired air has lower PCO_2
25. The renal compensatory responses to a metabolic acidosis are:
    1. increased sodium excretion
    2. increased bicarbonate reabsorption
    3. to produce an alkaline urine
    4. increased hydrogen ion excretion
26. Which of the following can lead to an acidosis?
    1. ingestion of methanol
    2. renal dysfunction
    3. diarrhea
    4. hypochloremia
27. An elevated anion gap is associated with which of the following disorders:
    1. Diabetic ketoacidosis
    2. Lactic acidosis
    3. Dehydration
    4. Asthma
28. Which of the following is not a cause of asthma?:
    a. cockroaches
    b. dogs
    c. cats
    d. plastic ware
    e. plant pollen
29. Allergic response to latex products can be a problem for laboratory workers using disposable gloves?
    a. True
    b. False

Answer

1. c (p. 465)
2. c (p. 464)
3. c (p. 475)
4. b (p. 473)
5. b (p. 465)
6. e (p. 471, 473)
7. c (p. 473-474)

8. b (p. 465, 468)
9. a (p. 473-474)
10. d (p. 474-475)
11. e (p. 473)
12. d (p. 464, 471)
13. b (p. 464, 467)
14. c (p. 471, 473)
15. a (p. 473)
16. d (p. 473-474)
17. e (p. 473)
18. b (p. 464, 467)
19. d (p. 471)
20. b (p. 469-470)
21. e (p. 468)
22. c (p. 466)
23. a (p. 465, 467)
24. b (p. 466)
25. c (p. 469, 473)
26. a (p. 471-473)
27. a (p. 470)
28. d (p. 473-474)
29. a (p. 474, 33-34)

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1.22: Renal Function

1. To monitor a patient with impaired function, the most useful serial serum determination would be:
    a. creatine
    b. creatinine
    c. BUN
    d. Uric acid
    e. Total nonprotein nitrogen
2. The end product of protein metabolism in man is:
    a. creatinine
    b. urea
    c. uric acid
    d. ammonia
    e. amino acids
3. Clearance tests for urea and creatinine measure:
    a. tubular excretion
    b. early loss of kidney function
    c. glomerular filtration rate
    d. concentrating ability
    e. blood flow and pressure
4. Determination of serum uric acid is useful in the diagnosis of:
    a. thyroid disease
    b. pancreatic disease
    c. viral hepatitis
    d. rheumatoid arthritis
    e. gout
5. The function unit of the kidney is the:
    a. cortex
    b. medulla
    c. nephron
    d. distal tubule
    e. collecting duct
6. Glomerular filtration describes:
    a. formation of a protein free plasma-like fluid from blood
    b. formation of urine
    c. formation of plasma-like fluid from blood
    d. formation of serum-like fluid from blood
    e. formation of electrolyte solution from plasma
7. The proximal tubule functions to:
    a. reabsorb 80% of salt and water
    b. concentrate salts
    c. form the renal plasma threshhold
    d. reabsorb urea
    e. reabsorb phosphate
8. The loop of Henle:
    a. responds to antidiuretic hormone
    b. has a high Tm for glucose
    c. filters depending upon GFR
    d. has a counter current mechanism

e. filters, depending upon glomerular pressure

9. The waste product of muscle metabolism which is excreted into the urine is:
    a. creatine
    b. creatinine
    c. pseudocreatinine
    d. creatine phosphate
    e. all of the above
10. Creatinine is a good substance to use for a clearance test because:
    a. it is exogenous
    b. it is reabsorbed
    c. its blood values are stable
    d. it is affected by fluid intake
    e. all of the above
11. A patient's BUN value is 150 mg/L and his creatinine is 50 mg/L. What conclusion would you draw from these results?
    a. patient is normal
    b. patient is in early stage of renal failure
    c. patient protein intake is quite low
    d. patient has suffered muscle deterioration
    e. one of the values is in error
12. Uric acid:
    a. is a waste product of protein metabolism
    b. is further metabolized to allantoin in anthropoid apes and Dalmations
    c. is filtered by the glomerulus and subsequently excreted
    d. is often elevated in hemolytic anemia
    e. . all of the above
13. Normal creatinine clearance is approximately (mL/min):
    a. 60-80 mL/min
    b. 70-100 mL/min
    c. 90-120 mL/min
    d. 120-150 mL/min
    e. 130-160 mL/min
14. Which of the following serum analytes is most affected by diet?:
    a. amonia
    b. creatine
    c. creatinine
    d. urea
    e. sodium
15. The final concentration step in urine formation occurs in the:
    a. proximal tubule
    b. loop of Henle
    c. distal tubule
    d. collecting tubules
    e. glomerulus
16. You measure electrolytes on a patient and get the following results (mmol/L):

Na	140
K	3.8
Cl	106

CO_2	11

In addition, the BUN is 720 mg/L, and the creatinine is 99 mg/L. The glucose is 750 mg/L. You should:

a. report the patient's results because the anion gap is normal
b. repeat because the anion gap is too small
c. repeat because the anion gap is too large
d. report the patient's results. The anion gap is abnormal, but it is explained by evidence of renal failure.
e. report the patient's results. Anion gap is abnormal, but is explained by evidence of diabetes

Use the following KEY to answer Questions 17—26:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

17. Which of the following is a function of the proximal tubule?:
    1. reabsorption of amino acids and protein
    2. reabsorption of glucose
    3. reabsorption of uric acid and salts
    4. acidification of urine
18. The renal response to a decreased blood pH is to:
    1. decrease the secretion of H^+
    2. increase the secretion of H^+
    3. decrease the reabsorption of HCO_3^-
    4. increase the reabsorption of HCO_3^-
19. Which of the following describes one or more property of the hormone aldosterone?:
    1. acts on the distal tubule
    2. changes distal tubule water permeability
    3. acts on $Na^+/K^+(H^+)$ pump
    4. cannot be affected by certain diuretics
20. Which of the following is/are a function of the nephron complex?:
    1. control of body electrolytes
    2. control of body pH
    3. elimination of body wastes
    4. metabolism of drugs by microsomal oxidative system
21. A serum creatinine has been measured, and the result is 50 mg/L. Which of the following serum determinations would most likely correspond with this creatinine value?:
    1. uric acid = 40 mg/L
    2. uric acid = 15 mg/L
    3. BUN = 120 mg/L
    4. BUN = 520 mg/L
22. Which of the following would you expect to be elevated in renal failure?:
    1. BUN
    2. uric acid
    3. creatinine
    4. sodium
23. The presumptive urine fluid leaving the proximal tubule is:
    1. enriched in urea compared to plasma
    2. hyperosmolar compared to serum

3. 80-90% reduced in volume from the original filtrate
4. 20% reduced in volume from the original filtrate

24. Urinary ammonium ions are:
    1. synthesized by the liver
    2. synthesized by renal tubular cells
    3. excreted to form an alkaline urine
    4. excreted in response to an acidosis
25. Urinary phosphate is:
    1. excreted under the influence of parathyroid hormone
    2. the major buffer of urine
    3. increased in chronic renal failure
    4. excreted by the proximal tubule
26. Which of the following are physiological functions of the kidney?
    1. excretory
    2. electrolyte and water balance
    3. acid-base balance
    4. protein conservation

Answer

1. b (p. 483,490)
2. b (p. 483)
3. c (p. 487)
4. e (p. 484,490)
5. c (p. 479)
6. a (p. 480)
7. a (p. 480, 481)
8. d (p. 481,482)
9. b (p. 483-484)
10. c (p. 484, 487)
11. e (p. 488, 490)
12. c (p. 484)
13. c (p. 487)
14. d (p. 483)
15. d (p. 482)
16. d (p. 489 - 490)
17. a (p. 480, 481)
18. c (p. 483)
19. b (p. 482)
20. a (p. 479)
21. d (p. 488, 490)
22. a (p. 488-490)
23. b (p. 480)
24. c (p. 483)
25. a (p. 482 - 483)
26. e (p. 479)

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1.23: Liver Disease

1. Liver function tests can vary depending on a number of factors, but which of the following would be LEAST likely to occur after obstruction of the common bile duct?:
    a. elevation of serum conjugated bilirubin
    b. elevation of serum unconjugated bilirubin
    c. elevation of serum alkaline phosphatase
    d. appearance of bilirubin in urine
    e. decrease of urine urobilinogen
2. A patient presents with elevation of unconjugated bilirubin, normal serum alkaline phosphatase, normal “liver” enzyme levels and no bilirubin in the urine. This combination would suggest:
    a. viral infection of liver
    b. chemical damage to liver
    c. increased rate of hemolysis
    d. obstruction of common bile duct
    e. all of the above are equally likely
3. The BSP function test:
    a. can distinguish between hepatic and obstructive liver disease
    b. can distinguish between prehepatic jaundice and intravascular hemolysis
    c. Is no longer used because of adverse affects to the dye.
    d. is not effective because BSP is excreted in urine
    e. can distinguish between viral and alcoholic hepatitis
4. The following results were obtained on a 73 year old black male:

- alk. phos. = 431 IU/L (ULN = 100)
- acid phos. = 5 IU/L (ULN = 6)
- GT = 13 IU/L (ULN = 29)

Which of the following diseases does this man most likely have?:

a. bone disease
b. diffuse liver disease
c. prostatic cancer
d. liver cancer
e. renal disease

Use the following KEY to answer Questions 5-8:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

5. Important function(s) of the liver is/are:
    1. detoxification of drugs
    2. conjugation and excretion of bilirubin
    3. synthesis of albumin
    4. conservation of bicarbonate
6. Liver is involved in which of the following metabolic functions?:
    1. synthesis of cholesterol
    2. synthesis of bile acids

3. storage of vitamin A
4. synthesis of plasma immunoglobulins

7. Liver function can be assessed by which of these parameters?:
    1. blood hemoglobin levels
    2. serum bile acid concentration
    3. serum glucose levels
    4. serum ALT and AST levels
8. Fecal urobilinogen levels may be markedly decreased because of:
    1. hemolysis
    2. decreased intestinal reabsorption of urobilinogen
    3. increased serum bilirubin
    4. biliary obstruction

Answer

1. b (p. 497-500, 504)
2. c (p. 502-504)
3. c (p. 501-502)
4. a (p. 502, 503)
5. a (p. 493-497)
6. a (p. 494-497)
7. c (p. 502-504)
8. d (p. 504)

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1.24: Bone Disease

1. The most active form of Vitamin D is 1, 25 dihydroxycholecalciferol, and this final hydroxylation takes place in:
    a. skin
    b. liver
    c. kidney
    d. skin and liver
    e. liver and kidney
2. Which of the following changes in serum analyte concentration are associated with hypercalcemia and hyperparathyroidism?:
    a. low phosphorous, and low Vitamin D_3
    b. high phosphorous, and low Vitamin D_3
    c. low phosphorous, and high Vitamin D_3
    d. high phosphorous, and high Vitamin D_3
    e. normal phosphorous and Vitamin D_3
3. Which of the following changes in serum analytes are associated with rickets and Vitamin D deficiency?:
    a. low calcium, and low phosphorous
    b. low calcium, and high phosphorous
    c. high calcium, and low phosphorous
    d. high calcium, and high phosphorous
    e. calcium and phosphorous normal
4. Osteoporosis can be differentiated from osteomalacia by which of the following combinations of serum analyte levels?:
    a. low Ca, normal P, high alkaline phosphatase
    b. high Ca, normal P, low alkaline phosphatase
    c. high Ca, high P, normal alkaline phosphatase
    d. normal Ca, normal P, high alkaline phosphatase
    e. normal Ca, normal P, normal alkaline phosphatase
5. The best biochemical marker of bone turnover is:
    a. Urine hydroxyproline
    b. Serum osteocalcin
    c. Serum bone alkaline phosphatase
    d. Urine γ-carboxyglutamic acid
    e. Urine N-terminal teleopeptides
6. Peak bone mass is the:
    a. Maximum bone mass attained by a fetus
    b. Maximum bone mass attained by an individual
    c. Not affected by genetics of environmental factors
    d. Maximum bone mass attained by postmenopausal women
    e. Maximum bone quality achieved in puberty
7. Which of the following treatments does not increase the risk for secondary osteoporosis?
    a. Chronic glucocorticoid therapy
    b. Methotrexate
    c. Insulin
    d. Cyclosporin A
    e. Long-term treatment with phenobarbital or phenytoin.

Use the following Key to answer Questions 8-13:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct

e. all are correct

8. Which of the following are causes of hypermagnesemia?:
    1. magnesium sulfate therapy
    2. magnesium-continuing antacids
    3. renal failure
    4. thyrotoxicosis
9. The primary target organs for Vitamin D_3 action are:
    1. kidney
    2. intestine
    3. bone
    4. parathyroid gland
10. Which of the following changes are associated with renal parenchymal disease?:
    1. decreased serum calcium
    2. increased parathyroid hormone
    3. decreased calcitriol synthesis
    4. decreased serum phosphorous concentration
11. Which of the following are important in regulating plasma calcium levels?
    1. parathyroid hormone (PTH)
    2. calcitonin
    3. 1, 25, dihydroxyvitamin D_3
    4. aldosterone
12. Which of the following factors are determinants of bone health?
    1. Nutritional
    2. Gonadal steroids
    3. Exercise
    4. Environmental lifestyle factors
13. Osteoperosis is primarily a disease of:
    1. Infants
    2. Postmenopausal women
    3. Former athletes
    4. Men and women older than 70 years

Answer

1. c (p. 513)
2. c (p. 525, 527)
3. a (p. 522-523)
4. e (p. 517)
5. e (p. 510-511)
6. b (p. 508-509)
7. a (p. 524)
8. a (p. 513,514, 516)
9. e (p. 511-512)
10. c (p. 518, 520)

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1.25: The Pancreas- Function and Chemical Pathology

1. All of the following hormones are produced primarily in pancreatic islets EXCEPT:
    a. Gastrin
    b. Glucagon
    c. Insulin
    d. Pancreatic polypeptide
    e. Somatostatin
2. Which of the following statements concerning islet cell function is correct?
    a. Insulin is the initial protein product of pancreatic beta cells
    b. Insulin is co-secreted with glucagon
    c. Insulin and C-peptide are produced in equimolar amounts
    d. Alpha cells are the major source of somatostatin in the pancreas
    e. Delta cells produce pancreatic polypeptide and vasoactive intestinal peptide
3. All of the following enzymes are produced in their active forms by the pancreas EXCEPT:
    a. Amylase
    b. Cholesterol esterase
    c. Lipase
    d. Ribonuclease
    e. Trypsin
4. Which of the following statements concerning pancreatic exocrine function is correct?
    a. Amylase is activated in the duodenum by the action of gastric acid and trypsin
    b. Exocrine secretion produces 8-10 liters of pancreatic juice daily
    c. Gastric acid is critical in inactivation of pancreatic enzymes
    d. Pancreatic bicarbonate production is critical to the digestive process
    e. The amount of amylase and lipase in pancreatic secretions is about 100 times plasma levels
5. Which of the following statements concerning hypoglycemia and insulinoma is correct?
    a. Hypoglycemia is most commonly due to insulinoma.
    b. Insulinoma is typically a highly aggressive tumor, with early metastasis to the liver and lungs.
    c. Insulinoma can be distinguished from sulfonylurea ingestion because C-peptide is increased only in the former
    d. Insulin injection is not associated with elevated C-peptide.
    e. Ratio of insulin to glucose > 20 suggests that excess insulin is the cause of hypoglycemia.
6. Which of the following statements concerning islet cell tumors is INCORRECT?
    a. Gastrin production in excess (Zollinger-Ellison syndrome) is usually caused by an islet cell tumor of the pancreas
    b. Glucagonoma usually presents with hyperglycemia, weight loss, and skin rash
    c. PPomas usually present with watery diarrhea and weight loss
    d. Somatostatinomas are usually large, malingant tumors with minimal or non-specific syndromes
    e. VIPomas are associated with watery diarrhea, hypokalemia, and achlorhydria
7. The most efficient marker for the diagnosis of acute pancreatitis is
    a. Amylase in serum
    b. Amylase in urine
    c. Lipase
    d. Pancreatic amylase isoenzyme
    e. Trypsinogen (immunoreactive trypsin)
8. Which of the following statements concerning tests of acute pancreatic injury is correct?
    a. Amylase and lipase are highly specific for pancreatitis, but not very sensitive
    b. Elevation of both amylase and lipase to more than 5 times upper reference limits with rise and fall over 48 hours is highly suggestive of pancreatitis

c. Lipase will generally return to normal earlier than amylase because of its shorter half-life
d. Renal insufficiency is associated with reductions in both amylase and lipase
e. With optimized lipase assays, lipase activity is typically about 1/4th that of amylase

9. Factors associated with a poor prognosis in acute pancreatitis include all of the following EXCEPT:
   a. Amylase over 10 times the upper reference limit
   b. Calcium decrease to less than 80 mg/L (2 mmol/L) over the first 48 hours
   c. Glucose over 2000 mg/dL at the time of admission
   d. Hematocrit decrease over 10% over the first 48 hours
   e. Partial pressure of oxygen < 50 mm Hg over the first 48 hours
10. Causes of increased salivary amylase include all of the following EXCEPT:
   a. Coronary artery bypass surgery
   b. Diabetic ketoacidosis
   c. Ectopic pregnancy
   d. Intestinal ischemia
   e. Mumps
11. The most sensitive test of pancreatic insufficiency is
   a. Amylase
   b. Bicarbonate level in duodenal fluid after secretin administration (secretin test)
   c. Fecal fat excretion
   d. Immunoreactive trypsin in serum
   e. Trypsin catalytic activity in stool

Answer

1. a (p. 539)
2. c (p. 538)
3. e (p. 540)
4. d (p. 539)
5. d (p. 541)
6. c (p. 541-542)
7. e (p. 543)
8. b (p. 543)
9. a (p. 544)
10. d (p. 544-545)
11. b (p. 546)

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1.26: Gastrointestinal Function and Digestive Disease

1. Which of the following statements concerning gastric function is INCORRECT
    a. Acid is produced by the chief cells of the stomach
    b. Gastrin is produced by G-cells, primarily in the antrum
    c. Intrinsic factor is produced by parietal cells, found throughout the stomach
    d. Mucous cells are important in protection of the stomach from acid digestion
    e. Pepsinogen, produced by chief cells, begins the process of protein digestion
2. Fat soluble vitamins include all of the following EXCEPT:
    a. Vitamin A
    b. Vitamin C
    c. Vitamin D
    d. Vitamin E
    e. Vitamin K

For questions 3 and 4, use the following Answer Key:

a. 1,2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

3. *Helicobacter pylori* has been implicated in the pathogenesis of
    1. Chronic gastritis
    2. Gastric cancer
    3. Peptic ulcer
    4. Gastric lymphoma
4. Which of the following statements concerning tests for *H. pylori* is correct?
    1. Antibody tests for H. pylori are both sensitive and specific
    2. The CLO test measures urease activity in gastric biopsies and detects H. pylori indirectly by detecting a color change in a pH indicator
    3. Urease tests are more sensitive in patients taking proton pump inhibitors than in untreated patients
    4. Stool antigen tests detect current infection, and become negative with successful eradication of infection
5. All of the following statements concerning Zollinger-Ellison syndrome are true EXCEPT:
    a. Associated with recurrent gastric ulcers, often involving the duodenum
    b. Basal acid output (BAO) is usually over half of the maximal acid output (MAO)
    c. Diarrhea is often present and may be accompanied by steatorrhea
    d. Gastrin production by G-cell hyperplasia is the most common cause
    e. Tumors are often small, but metastatic tumor occurs in over half of cases
6. Which of the following statements concerning Multiple Endocrine Neoplasia (MEN), type 1, is correct?
    a. Associated with tumors of the ovary and pheochromocytoma
    b. Chromosome mutations are found on chromosome 11
    c. Due to a mutation in the Ret oncogene
    d. Occurs in women much more frequently than in men ((10:1)
    e. Occurs sporadically with no inheritance pattern
7. Which of the following statements concerning laboratory diagnosis of Zollinger-Ellison (Z-E) syndrome is INCORRECT?
    a. Gastrin level over 4 times the upper reference limit is strongly suggestive of the disease
    b. It is important to document lack of achlorhydria before making the diagnosis of Z-E syndrome
    c. Provocative tests of gastrin production are more reliable than basal gastrin in establishing the diagnosis.
    d. Secretin is the most sensitive and specific secretory agent for the diagnosis of Z-E syndrome
    e. When secretin is given, gastrin should be checked at 1 and 2 hours after administration

8. Steps involved in the absorption of Vitamin B12 include all of the following EXCEPT
    a. Release of B12 from food by the action of gastric acid
    b. Binding of B12 to non-specific (R-binders) in the stomach
    c. Release of B12 from R-binders by the action of pancreatic bicarbonate
    d. Binding of B12 to intrinsic factor, produced by gastric parietal cells
    e. Absorption of B12 in the ileum when bound to intrinsic factor
9. All of the following might be abnormal in both malabsorption and maldigestion EXCEPT:
    a. Beta carotene level in plasma
    b. D-xylose absorption
    c. Fecal fat excretion
    d. Serum albumin
    e. Vitamin B12 absorption
10. The most sensitive test for malabsorption or maldigestion is:
    a. Beta carotene level in plasma
    b. D-xylose absorption
    c. Fecal fat excretion
    d. Serum albumin
    e. Vitamin B12 absorption
11. All of the following statements concerning celiac disease are correct EXCEPT:
    a. Antibodies to tissue transglutaminase are the most sensitive and specific marker of diseae
    b. Antibodies to gliadin are present in most affected individuals
    c. Gluten, found in wheat and some other grain products, is the trigger for celiac disease
    d. Individuals with celiac disease are at high risk for developing carcinomas of the small bowel
    e. Use of a gluten free diet may lead to disappearance of circulating antibodies
12. Which of the following statements concerning lactose intolerance is correct?
    a. Associated with symptoms when beef or pork is ingested
    b. Causes malabsorption syndrome in 50% of affected individuals
    c. Diarrhea after ingestion of lactose-containing foods is a common mode of presentation
    d. Most commonly seen in persons of European ancestry
    e. Usually requires lactose tolerance testing to establish the diagnosis
13. All of the following statements regarding carcinoid syndrome are true EXCEPT:
    a. Associated with flushing and diarrhea
    b. Caused by carcinoid tumors, usually found in the distal ileum or appendix
    c. Markedly increased urine excretion of 5-hydroxyindole acetic acid is seen in most cases of carcinoid syndrome
    d. Metastasis to the liver is usually required before symptoms of carcinoid syndrome occur
    e. Primary carcinoid tumors of the appendix are the single most common cause of carcinoid syndrome
14. Which of the following statements concerning inflammatory bowel disease is correct?
    a. Antibodies to neutrophil cytoplasmic antigens are often associated with Crohn's disease
    b. Antibodies to Saccharomyces cerevisiae are commonly found in ulcerative colitis
    c. Autoimmune markers are found in about half of cases
    d. Both Crohn's disease and ulcerative colitis typically affect the colon and rectum exclusively
    e. Specificity of these autoimmune markers is only about 50-60%
15. All of the following statements concerning tests for occult blood in stool is correct EXCEPT:
    a. Assays usually rely on the peroxidase activity of hemoglobin
    b. Drying of stool samples results in reduced sensitivity, so rehydration of cards before testing is usually recommended to reduce both false positive and false negative results
    c. Immunochemical methods for hemoglobin reduce the likelihood of false positive results due to plant peroxidases
    d. Immunochemical tests may be falsely negative is stool samples are contaminated by toilet bowl sanitizers.
    e. Restriction of meat in the diet is recommended to prevent false positive results

16. Correct statements concerning CEA and colorectal cancer include all of the following EXCEPT:
    a. CEA is a family of glycoprotein molecules rather than a single antigen
    b. CEA levels are usually highly reproducible between assays from different manufacturers
    c. Early colon cancer is associated with increased CEA in only about 25% of cases
    d. Increased CEA may also be due to smoking, liver disease, or inflammatory bowel diseae
    e. The most important use of CEA is not in screening for colon cancer, but in follow-up of patients after surgery or other form of treatment

Answer

1. a (p. 552)
2. b (p. 554)
3. e (p. 556)
4. c (p. 556)
5. d (p. 556-557)
6. b (p. 557)
7. e (p. 556-557)
8. c (p. 557)
9. b (p. 560-561)
10. c (p. 561)
11. d (p. 558)
12. c (p. 558)
13. e (p. 558-559)
14. c (p. 559)
15. b (p. 563)
16. b (p. 563)

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1.27: Cardiac and Muscle Disease

1. CK-MB activity and troponin I levels peak at 18-24 hrs. post-MI and are usually normal by 24-36 hrs.
    a. True
    b. False
2. LD "flip" ($LD_1 > LD_2$) or LD total activity is a recommended marker for myocardial infarction.
    a. True
    b. False
3. The best system for the collection of blood samples to rule out AMI is:
    a. 0,2,4,6,8 and 24 hrs. post-admission
    b. 0,6,12 and 12 hrs. post-admission
    c. 0,24,48 and 72 hrs. post-admission
    d. 0, and each day post-admission
    e. any random combination
4. CK-MB measurement for ruling out MI is:
    a. highly sensitive, but not very specific
    b. highly specific, but no very sensitive
    c. is both highly sensitive and specific
    d. is neither highly sensitive nor specific

Use the following Key to answer Questions 5-12:

a. 1,2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

5. Which of the following are types of arrhythmias?
    1. tachycardia
    2. cardiomyopathy
    3. bradycardia
    4. myocarditis
6. Measurement of serum myoglobin is not very useful for ruling out MI because:
    1. it is present in too low concentrations
    2. levels rise and fall too rapidly (<12 hrs.) and is not useful for individuals who appear late.
    3. there are no specific assays to measure myoglobin
    4. it is not specific for cardiac disease
7. Which of the following drugs are used for the treatment of arrythmias?
    1. beta-blockers
    2. digoxin
    3. diuretics
    4. quinidine
8. Pathological disorders of muscle can be categorized as resulting form:
    1. degeneration of activating neurons
    2. degeneration of muscle cells
    3. damage by trauma
    4. genetic abnormalities
9. Which of the following is (are) true about congestive heart failure (CHF)?
    1. CHF is caused by a failure of the heart to pump blood.
    2. CHF can be diagnosed by physicians on clinical grounds and by the use of measurements of brain natriuretic peptide.

3. There is an increasing prevalence of CHF.
4. CHF is usually caused by cardiac rhythm abnormalities.

10. Acute coronary syndromes include:
    1. Congestive heart failure
    2. Unstable angina
    3. Cardiomyopathy
    4. Myocardial infarction
11. Which of the following statements is (are) true?
    1. CK-MB and Troponin I have the same clinical sensitivity for detecting myocardial infarction.
    2. ALT and LD are currently valuable markers for detecting myocardial infarction.
    3. Troponin I is more specific for myocardial infarction. Than are CK-MB or myoglobin.
    4. Troponin I should be used in preference to Troponin T.
12. CK-MB is increasingly be replaced by [fill in one of the following], a more specific marker for myocardial infarction.
    a. Troponin I or T
    b. Bone natriuretic peptide
    c. C-reactive protein
    d. Tropomyosin
    e. Titin

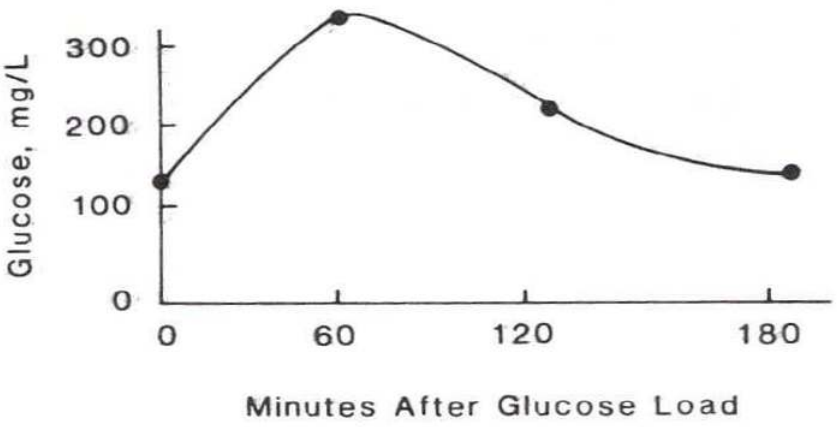
Answer

1. a (p. 576)
2. b (p. 577)
3. b (p. 576)
4. a (p. 576-577)
5. b (p. 574)
6. c (p. 578)
7. c (p. 575)
8. e (p. 574-575)
9. a (p. 574)
10. c (p. 573)
11. b (p. 576-577)
12. a (p. 573)

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1.28: Diabetes

1. Type 1 diabetes is defined/described as:
   a. secondary to certain conditions and syndromes
   b. insulin dependent
   c. impaired glucose tolerance test
   d. glucose intolerance during pregnancy
   e. non-insulin dependent
2. In performing a glucose tolerance test on a patient, the peak glucose level occurred after 3 hours. What is the tentative diagnosis?
   a. normal
   b. questionable diabetic
   c. diabetic
   d. impaired tolerance
   e. child diabetic
3. In the fasting state, diabetes may be tentatively considered as the differential diagnosis if the glucose level is greater than:
   a. 1100 mg/L
   b. 1260 mg/L
   c. 1600 mg/L
   d. 2000 mg/L
   e. 2500 mg/L
4. The liver is the major organ which can release glucose into the circulation during fasting because its cells contain:
   a. glucose oxidase
   b. glucose-6-isomerase
   c. glucose-6-convertase
   d. hexokinase
   e. glucose.-6-phosphatase
5. The following glucose tolerance curve is representative of:
   *PLEASE NOTE, THE VALUES FOR THE Y-AXIS NEED TO BE MULTIPLIED BY 10
   a. hyperinsulinism
   b. hypoglycemia
   c. mild diabetes
   d. impaired glucose tolerance
   e. normal



6. Type II diabetes is defined/described as:

a. secondary to certain conditions and syndromes
b. insulin dependent
c. impaired glucose tolerance test
d. glucose intolerance during pregnancy
e. non-insulin dependent

7. The glucose value of a normal 2 hour post-glucose tolerance test is:
   a. markedly elevated above fasting levels
   b. markedly below fasting levels
   c. within fasting normal limits
   d. slightly elevated above normal levels
8. What is the minimum serum glucose levels at which urine would be positive for the presence of glucose?:
   a. 1000 mg/L
   b. 1400 mg/L
   c. 1800 mg/L
   d. 2200 mg/L
   e. 2600 mg/L
9. Increased levels of insulin cause the glucose intake of cells to:
   a. increase
   b. decrease
   c. be blocked
   d. be maintained on a constant level
   e. remain unchanged
10. The most useful analyte for monitoring long-term (6 to 8 weeks) stability of blood glucose is:
    a. lactic acid
    b. urinary ketone bodies
    c. insulin
    d. blood pH
    e. glycosylated hemoglobin
11.

Column A	Column B
i. tumor in islet cell of pancreas	a. hyperglycemia
ii. epinephrine produced by a pheochromocytoma	b. hypoglycemia
iii. Cushing's disease	c. normal

Use the following Key to answer Questions 12-17:

a. 1,2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

12. Which of the following are hyperlycemic agents?
    1. glucagen
    2. epinephrine
    3. cortisol
    4. thyroxine
13. What is/are the most likely presentation/s of a type I diabetic in an out-of-control situation?:
    1. ketoacidosis
    2. hyperosmolar coma

3. hypoinsulinemic
4. non-ketotic

14. What is/are the most likely presentation/s of a type II diabetic in an out-of-control situation?:
    1. ketoacidosis
    2. hyperosmolar coma
    3. hypoinsulinemic
    4. non-ketotic
15. Which of the following are most useful for monitoring glucose control in a known diabetic?:
    1. growth hormone
    2. serum insulin
    3. urinary ketones
    4. urinary glucose
16. The most frequent complications of chronic diabetes are:
    1. retinopathy
    2. nephropathy
    3. neuropathy
    4. microangiopathy
17. Which of the following are the major complications of diabetes?:
    1. angiopathy
    2. male impotence
    3. nephropathy
    4. pheochromocytoma

Answer

1. b (p. 590)
2. c (p. 596, 597)
3. b (p. 596)
4. e (p. 587)
5. c (p. 590, 596)
6. e (p. 590-591)
7. c (p. 596-597)
8. c (p. 598)
9. a (p. 589)
10. e (p. 598)
11.
    i. b (p. 595)
    ii. a (p. 589)
    iii. a (p. 590)
12. e (p. 589)
13. b (p. 590, 594)
14. c (p. 590-594)
15. d (p. 597)
16. e (p. 593-595)
17. a (p. 593-595)

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1.29: Lipid Metabolism

1. Which is the main pathway of triglyceride digestion?
    a. triglycerides to 1, 3 diglycerides to 1-monoglyceride and fatty acids
    b. triglyceride to free fatty acids and glycerol
    c. triglycerides to 1, 2 diglycerides to 1-monoglycerides and fatty acids
    d. triglycerides to 1, 2 diglycerides to 2-monoglycerides and fatty acids
    e. triglycerides to 2, 3 diglycerides to 2-monoglycerides and fatty acids
2. After a meal, the bloodstream transports chylomicrons and VLDL to all tissues of the body. The principal site of uptake is:
    a. liver
    b. intestine
    c. kidney
    d. brain
    e. adipose tissue
3. The appearance of the plasma of a patient with markedly increased pre—beta lipoprotein, after it stands overnight at 4°C would be:
    a. clear
    b. turbid underneath a thick cream layer
    c. clear underneath a thick cream layer
    d. turbid with no cream layer
    e. clear to slightly cloudy
4. Triglycerides are hydrolyzed by the enzyme:
    a. glycerokinase
    b. amylase
    c. lipase
    d. hydrolase
    e. LCAT
5. On agarose gel lipoprotein electrophoresis, chylomicrons:
    a. migrate in the region of beta proteins
    b. migrate in the region of alpha$_2$ proteins
    c. remain at the origin
    d. migrate in the region of albumin
    e. migrate in the region of alpha$_1$ proteins
6. The drug “lovastatin” is used to lower serum LDL-cholesterol by:
    a. binding dietary cholesterol
    b. binding bile acids
    c. inhibiting LDL-uptake by cells
    d. inhibiting cellular cholesterol synthesis
    e. all of the above
7. Major risk factors for coronary artery disease include:
    a. genetic predisposition
    b. elevated serum cholesterol
    c. hypertension
    d. cigarette smoking
    e. all of the above
8. The currently accepted cut-off for classification of a high risk for coronary heart disease (CHD) is associated with which of the following sets of total cholesterol (IC) and LDL—cholesterol (LDL—C) values?
    a. TC>1500 mg/L, LDL-C>1000 mg/L
    b. TC>2000 mg/L, LDL-C>1300 mg/L

c. TC>2000-2390 mg/L, LDL-C>1300- 1590 mg / L
d. TC>2400 mg/L, LDL-C>1600 mg/L
e. TC>2000 mg/L, LDL-C>1600 mg/L

Use the following Key to answer Questions 9-20:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

9. A lipoprotein electrophoretogram shows a heavily stained beta region. Which of the following chemistries should show a corresponding increase?:
    1. cholesterol
    2. fatty acids
    3. phospholipids
    4. triglycerides
10. The role of the liver in nutrition is to metabolize:
    1. cholesterol
    2. fatty acids
    3. phospholipids
    4. triglycerides
11. Effective way(s) of achieving a lowering of serum lipids are/is:
    1. diet
    2. exercise
    3. drugs
    4. vitamins
12. Cholesterol is essential for the normal functioning of an organism because it is:
    1. an essential component of all cell membranes
    2. the major component of chylomicrons
    3. a precursor of all steroid hormones
    4. the major gluconeogenic substrate of the liver
13. Very low density lipoproteins (VLDL) are distinguished from high density lipoproteins (HDL) by the following differences:
    1. VLDL have a density of less than 1.006; HDL have a density of greater than 1.063
    2. VLDL have a density of less than 1.0; HDL have a density greater than 1.0
    3. VLDL contain mostly triglycerides; HDL contain mostly phospholipids
    4. VLDL contain Apo A-I; HDL contain Apo B
14. Patients with hereditary hyperlipoproteinemia have an elevation of serum lipoprotein concentrations. Which of the following lipoprotein families serve as determinants of the abnormal 1ipoprotein patterns?:
    1. chylomicrons
    2. VLDL
    3. LDL
    4. HDL
15. Bile salts:
    1. are produced by the liver
    2. are strongly hydrophylic and hydrophobic
    3. emulsify dietary triglyceride
    4. have amphipathic properties
16. Which of the following statements are true concerning the low— density lipoprotein molecule? It:
    1. protects against heart disease

2. is the primary vehicle for delivering cholesterol to peripheral tissues
3. is the primary vehicle for removing cholesterol from peripheral tissues
4. is greatly elevated in Type IIb familial hypercholesterolemia

17. Chylomicrons are elevated in plasma because of:
    1. Type I hyperlipoproteinemia
    2. intake of certain drugs that cause a secondary hypertriglyceridemia
    3. Type V hyperlipoproteinemia
    4. the sample being drawn following a meal
18. The major source(s) of blood cholesterol is (are):
    1. liver
    2. adipose cells
    3. intestine
    4. muscle
19. The primary factors that influence plasma cholesterol levels are:
    1. diet
    2. genetics
    3. gender
    4. plasma triglyceride levels
20. Lipoprotein particles are classified primarily according to their:
    1. density
    2. protein composition (% by weight)
    3. migration pattern in agarose
    4. site of synthesis

Answer

1. d (p. 605)
2. e (p. 605)
3. d (p. 619, 622)
4. c (p. 605, 612)
5. c (p. 613, 614)
6. d (p. 621)
7. e (p. 628)
8. e (p. 633)
9. b (p. 613)
10. e (p. 606)
11. a (p. 621, 635)
12. b (p. 606)
13. b (p. 613)
14. a (p. 619)
15. e (p. 605)
16. c (p. 616, 619)
17. e (p. 614, 619, 620)
18. b (p. 608)
19. a (p. 610-611)
20. b (p. 613)

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1.30: Alcoholism

Use the following Key to answer Questions 1-6:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

1. Diagnosis of alcoholism can best be achieved by:
    1. smelling the patient's breath
    2. elevated serum gammaglutamyl transferase levels
    3. obtaining an alcohol level at the time of interview
    4. history of drinking with spousal verification
2. Ethanol is metabolized by following pathway(s); the
    1. alcohol dehydrogenase
    2. microsomal ethanol oxidizing system
    3. catalase
    4. gamma glutamyl transferase
3. Nutritional alterations associated with alcoholism include:
    1. protein calorie malnutrition
    2. vitamin B1 (thiamin) deficiency
    3. iron accumulation
    4. magnesium deficiency
4. Which of the following organ system(s) is/are affected by alcoholism?
    1. muscle
    2. liver
    3. spleen
    4. pancreas
5. The usual cause of death by alcoholism is by:
    1. splenomegaly
    2. cardiomyopathy
    3. cancer
    4. cirrhosis
6. Which of the following analytes found in serum are increased in alcoholism?
    1. albumin
    2. serum globulins
    3. hemoglobin
    4. bilirubin
7. The laboratory tests that best correlate with the severity of alcoholic hepatitis are:
    a. Alkaline phosphatase, total bilirubin
    b. Albumin, aspartate aminotransferase (AST)
    c. Total bilirubin, prothrombin time (PT)
    d. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase
    e. Globulins, prealbumin
8. A blood alcohol concentration (BAC) of >100 mg/dL is diagnostic for alcoholism?
    a. True
    b. False

9. There is no direct relationship between the blood alcohol concentration (BAC) and increased frequency of physical signs and symptoms of ethanol intoxication.
    a. True
    b. False

Use the following Key to answer Questions 10-11:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

10. Which of the following statements are true?
    1. Alcohol is a social beverage that can be legally consumed.
    2. Alcohol is a potent drug, similar to other narcotics.
    3. Disease related to alcohol consumption is among the 10 leading causes of death in the United States.
    4. The usual legal limit for a blood alcohol concentration (BAC) is 80 to 100 mg/dL
11. Which of the following nutrient classes are often deficient in alcoholics?
    1. Protein
    2. Minerals
    3. Vitamins
    4. Phospholipids

Answer

1. d (p. 641)
2. a (p. 642, 644)
3. e (p. 644-645)
4. c (p. 645-649)
5. d (p. 647)
6. c (p. 649-651)
7. b (p. 651)
8. b (p. 641-642)
9. b (p. 642)
10. e (p. 640-641)
11. e (p. 644-646)

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1.31: Iron, Bilirubin, Porphyrin

FOR QUESTIONS 1 - 3 use the following KEY:

a. formation of amino levulinic acid (ALA) by ALA synthase
b. conversion of ALA to porphobilinogen (PB) by ALA dehydratase
c. conversion of PB to Uroporphyrin I
d. conversion of PB to Uroporphyrin III
e. conversion of coproporphyrin to protoporphyrinogen IX

1. Normally the feed—back inhibition by Heme operates at which one of the above steps?:
2. In acute intermittent porphyria this rate-limiting step is defective:
3. This step is severely inhibited by lead:

Use the following Key to answer Questions 4-9:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

4. Most of the iron in the body is found in:
    1. hemoglobin
    2. haptoglobin
    3. ferritin
    4. peroxides
5. The transport protein(s) of iron is/are:
    1. ferritin
    2. haptoglobin
    3. hemosiderin
    4. transferrin
6. The total iron binding capacity (TIBC) calculated from:
    1. transferrin
    2. ferritin
    3. serum iron
    4. hemosiderin
7. Heme is degraded to form the following compounds:
    1. a linear tetraphrrole
    2. biliverdin
    3. bilirubin
    4. protoporphyrin
8. Bilirubin is bound by which of the following compound(s)?
    1. bile acids
    2. haptoglobin
    3. hemoglobin
    4. albumin
9. Heme synthesis is an integral part of which of the following body systems?
    1. respiratory
    2. bone marrow
    3. genitourinary
    4. hepatic
10. Delta bilirubin is:

a. free, unconjugated bilirubin
b. unconjugated bilirubin, covalently bound to albumin
c. bilirubin monoglucuronide
d. conjugated bilirubin that does not react in direct reaction
e. none of the above

11. Measurement of urinary urobilinogen is:

a. useful in detection of renal disease
b. useful in detection of inborn errors of heme synthesis
c. useful in detection of liver disease
d. useful for screening for porphobilinogens
e. not particularly useful for any of the above

Answer

1. a (p. 664)
2. d (p. 665-667)
3. b (p. 669)
4. b (p. 659)
5. d (p. 660)
6. b (p. 661-662)
7. a (p. 671)
8. d (p. 671)
9. c (p. 664)
10. b (p. 671)
11. e (p. 673)

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1.32: Hemoglobin

1. The primary structure of the hemoglobin molecule is:
    a. the heme group
    b. two α and β two chains
    c. the folding sequence
    d. the amino acid sequence
    e. the globin portion
2. The globin portion of the hemoglobin A molecule is comprised of:
    a. four polypeptide chains
    b. a porphyrin group and polypeptide chains
    c. the Γ chain (Γ globin)
    d. the α helical structure
    e. heme and α plus β chains
3. Each of the heme molecules in hemoglobin can bind ____molecule (s) of oxygen?
    a. one
    b. two
    c. three
    d. four
    e. five
4. The affinity of oxygen for hemoglobin does NOT depend on:
    a. oxygen content (saturation) of hemoglobin
    b. partial pressure of oxygen
    c. 2, 3 diphosphoglycerate concentration in the red blood cell
    d. temperature
    e. number of polypeptide chains
5. The oxygen dissociation curve is graphed by plotting which parameters?
    a. O_2 Hb saturation vs. pH
    b. O_2 Hb saturation vs. arterial blood pCO_2
    c. O_2 Hb saturation versus oxygen tension (PO_2)
    d. O_2 Hb unsaturation (dissociation) versus pH
    e. O_2 Hb unsaturation (dissociation) versus arterial blood pCO_2
6. The Bohr effect:
    a. is conventionally indexed by the P50 value
    b. reflects 2, 3 diphosphoglycerate concentration
    c. is plotted as a function of temperature versus pH
    d. expresses the fact that the oxygen affinity of hemoglobin varies with pH
    e. is usually determined by measuring the temperature and the 2, 3 diphosphoglycerate ratio
7. Methemoglobin is:
    a. hemoglobin combined with carbon monoxide
    b. hemoglobin combined with methionine
    c. hemoglobin combined with heavy metals
    d. oxidized hemoglobin
    e. un-oxidized hemoglobin
8. Match the hemoglobin name and structure:

1. adult Hb A	a. $\zeta_2\epsilon_2$
2. adult Hb A2	b. $\alpha_2\epsilon_2$
3. fetal Hb F	c. $\alpha_2\gamma_2$
4. embryonic Grower I	d. $\alpha_2\beta_2$
5. embryonic Grower II	e. $\alpha_2\delta_2$

9. At birth the major circulating hemoglobin is usually:
    a. Adult Hb A
    b. Methemoglobin
    c. Embryonic hemoglobin
    d. Adult Hb A2
    e. Fetal hemoglobin
10. Which of the following is not a molecular mechanism responsible for structural hemoglobin variants?
    a. amino acid substitution
    b. deletions and insertions of DNA
    c. unequal crossing over of DNA
    d. chain elongation
    e. denaturation of polypeptide chains
11. Sickle cell hemoglobin is the result of:
    a. substitution at position 6 (valine for glutamine)
    b. substitution at position 6 (lysine for glutamine)
    c. denaturation of the hemoglobin outside the cell to form red blood cell sickles
    d. puncturing of the plasma cell membrane (pop sickle)
    e. variation in chain elongation of the Hb A molecule (Hb A to Hb F)
12. Sickle cells cause pathophysiologic problems because:
    a. they block microcirculation
    b. they gel and precipitate
    c. they cause acidosis
    d. they are larger than normal red cells
    e. they are double membraned cells
13. Thalassemias are inherited hemoglobinopathies characterized as:
    a. specific amino acid substitutions
    b. decreased production of globin chain(s)
    c. abnormal structure of specific chains
    d. elongation of the chain polypeptide
    e. deletion of several amino acids
14. Methemoglobinemia can be caused by all but one of the following:
    a. drugs
    b. chemicals
    c. amino acid substitutions
    d. deficiency of methemoglobin reducing ability of red cells
    e. white cell amino acid substitution
15. Which of the following does NOT affect blood levels of hemoglobin?
    a. age
    b. gender
    c. physical exercise
    d. posture
    e. race

16. In which of the following groups is the carboxyhemoglobin elevated to abnormal levels of 4% to 20% compared to the healthier 0.2 to 0.8%?
    a. residents of Los Angeles
    b. individuals whose heme is degraded to bilirubin
    c. factory workers
    d. smokers
    e. laboratory workers
17. Oxygen saturation is used clinically to determine:
    a. tissue hypoxia or hyperoxia
    b. oxygen affinity
    c. anemia
    d. erythopoietin stimulating effect of hypoxia
    e. polycythemia

Answer

1. d (p. 677-678)
2. a (p. 677)
3. a (p. 679)
4. e (p. 680-681)
5. c (p. 680)
6. d (p. 680-681)
7. d (p. 682)
8. 1-d, 2-e, 3-c (p. 677-679), 4-a , 5-b
9. b (p. 688-689)
10. e (p. 682, 690)
11. e (p. 690-691)
12. d (p. 691)
13. b (p. 688-689)
14. e (p. 682, 690)
15. e (p. 690-691)
16. d (p. 691)
17. a (p. 691)

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1.33: Human Nutrition

1. The suggested amount of fat in the American diet should be:
    a. <50% of total calories
    b. <40% of total calories
    c. <35% of total calories
    d. <10% of total calories
    e. less than an individual's plasma cholesterol.
2. The form of malnutrition in which there is a deficiency of protein but not total calories is called:
    a. marasmus
    b. kwashiorkor
    c. essential malnutrition
    d. based malnutrition
    e. none of the above
3. Which of the following might not be considered an essential nutrient?
    a. Lysine and methionine
    b. Vitamin D
    c. Linoleic and linolenic acids
    d. Vitamin C
    e. Vitamin A

Use the following Key to answer Questions 4-7

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

4. The Recommended Dietary Allowance (RDA) defines:
    1. The amount of money that an American family should spend in order to maintain an average diet.
    2. The maximum amount of total calories that an individual should consume to maintain a healthy state.
    3. The minimum amount of essential amino acids in a diet to maintain a healthy state.
    4. Suggested dietary intake of a nutrient required to meet the needs of most healthy persons.
5. The following patients can be associated with an increased risk for malnutrition:
    1. very-low birth weight
    2. cancer patients
    3. abdominal surgery patients
    4. geriatric patients
6. Which of the following serum proteins are most useful as markers for malnutrition?
    1. prealbumin
    2. thyroxine-binding globulin (TBG)
    3. albumin
    4. gamma globulins
7. Chronic alcoholism is associated with a high risk for:
    1. hypocalcemia
    2. calorie malnutrition
    3. hypercalcemia
    4. protein malnutrition
8. Consequences of protein-energy malnutrition (PEM) include all of the following EXCEPT:
    a. Increased length of stay (LOS) in intensive care units of hospitals
    b. Increased rate of infections

c. Increased use of medical resources
d. Increased death rates
e. Increased incidence of AIDS

9. The *hypermetabolic* state can be associated with all the following except:
    a. Major burns
    b. Peritonitis
    c. Bone fractures
    d. Diabetes mellitus
    e. Major head trauma

Use the following Key to answer Questions 10-11

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

10. Under-nutrition and poor nutrition affect:
    1. Children in developing countries
    2. Adults in developing countries
    3. Obese individuals
    4. Institutionalized individuals

11. The hypermetabolic state can be associated with which of the following biochemical finding:
    1. Negative nitrogen balance
    2. Increased basal metabolic rate
    3. Synthesis of acute phase proteins
    4. Hypoglycemia

Answer

1. c (p. 699)
2. b (p. 698)
3. b (p. 696)
4. d (p. 696)
5. e (p. 699-701)
6. e. (p. 696-701)
7. a. (p. 697)

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1.34: Trace Elements

1. The primary organ that is adversely affected by chronic toxic cadmium exposure is:
    a. muscle
    b. lung
    c. kidney
    d. pancreas
    e. testis
2. Copper is a cofactor for a large number of enzymes called oxidases.
    a. True
    b. False

Use the following Key to answer Questions 3- 6

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

3. Which of the following statements about chromium are true?
    1. It is an essential element.
    2. It is an important glucose tolerance factor.
    3. It is a co—factor in iron metabolism.
    4. It is found in urine mostly bound to peptides.
4. Serum aluminum measurements may be requested for which of the following situations?
    1. monitoring end—stage renal dialysis patients.
    2. monitoring Al levels in dialysate water.
    3. patients receiving long term parenteral nutrition
    4. monitor liver transplant patients.
5. Which of the following trace elements inhibit essential enzymes by reacting with the protein's sulfhydryl groups?
    1. Mercury
    2. Lead
    3. Arsenic
    4. Aluminum
6. Fluoride
    1. at concentrations of about 1 mg/L can promote caries resistance
    2. is not known to inhibit enzymes
    3. at concentrations greater than 10 mg/L can weaken teeth
    4. impairs thyroid gland function

Answer

1. c (p. 716)
2. a (p. 710-711)
3. a (p. 709)
4. a (p. 715)
5. b (p. 716,717)
6. b (p. 712)

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1.35: Vitamins

Use the following Key to answer Questions 1- 7

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

1. Determination of person's vitamin status can be made by chemical measurement of:
    1. he active vitamin in fluids or cells
    2. urinary metabolites of the vitamin
    3. enzymatic activity requiring the vitamin
    4. urinary metabolites of a substance whose production is dependent upon the vitamin
2. Absorption of dietary vitamin B12 requires:
    1. pancreatic proteases
    2. active phosphorylation
    3. intrinsic factor
    4. intestinal esterases
3. A deficiency of vitamins A, E, and D can be associated with which of the following clinical problems:
    1. pancreatic insufficiency
    2. alcoholic liver disease
    3. biliary tract disease
    4. chronic calorie malnutrition
4. Vitamins:
    1. comprise a group of small molecular weight compounds
    2. serve as cofactors in a number of enzyme reactions
    3. are obtained from diet (food sources)
    4. are a special group of amine compounds found in herbs
5. Vitamin deficiencies are the result of
    1. inadequate concentration in the diet
    2. inadequate enzyme intake
    3. inadequate intestinal absorption
    4. inadequate conversion to molecular forms
6. The fat soluble vitamins include:
    1. Vitamin A
    2. Vitamin E
    3. Vitamin K
    4. Thiamine
7. The water soluble vitamins include:
    1. Vitamin C
    2. Riboflavin
    3. Pyridoxine
    4. Vitamin B
8. Which of the following groups of vitamins can be synthesized by the human liver?:
    a. fat soluble vitamins
    b. water soluble vitamins
    c. carotenoids
    d. all of the above
    e. none of the above

9. Which of the following vitamins is necessary for the synthesis of active coagulation factors?
    a. Vitamin A
    b. Niacin
    c. Vitamin D
    d. Biotin
    e. Vitamin K
10. Which of the following vitamins is believed to function as an antioxidant?
    a. Vitamin A
    b. Vitamin D
    c. Vitamin E
    d. Vitamin K
    e. Vitamin B_{12}
11. MATCH the precursor with the biologically active vitamin.

Presursor	Active vitamin
a. riboflavin b. pyridoxal c. beta-carotene d. ascorbic acid e. pantothenic acid f. biotin g. niacin h. citric acid	i. — retinol (Vitamin A) ii. — vitamin B6 iii. — nicotinamide adenine dinucleotide (NAD) iv. — coenzyme A v. — flavin adenine dinucleotide

12. While vitamins A and E are stored for relatively long/short time periods, vitamins K, B_6, and C are stored for relatively long/short time periods:
    a. long/short
    b. long/long
    c. short/long
    d. short/short
    e. none of the above for these combinations of vitamins
13. The dietary sources of the fat soluble vitamins are animal organ meats (i.e., liver) while the dietary sources of the water soluble vitamins are fruits and vegetables.
    a. True
    b. False

Answer

1. e (p. 726)
2. b (p. 744-746)
3. e (p. 726, 727)
4. a (p. 723)
5. b (p. 723, 726)
6. a (p. 726)
7. e (p. 733, 734)
8. e (p. 723)
9. e (p. 733)
10. c (p. 731)
11. i. c (p. 727), ii b (737), iii g (737), iv e (743), v a (735)
12. a (p. 727, 734)
13. b (p. 727-750)

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1.36: Pregnancy and Fetal Function

1. Fetal lung maturity can be assessed from amniotic fluid because the surfactant is primarily composed of:
    a. protein
    b. cholesterol
    c. glycerol
    d. triglycerides
    e. phosphatidylcholine and other phospholipids

Use the following key for questions 2 and 3

a. creatinine
b. nile blue sulfate staining cells
c. absorbancy at 450 nm
d. functional test, such as the fluorescence polarization assay
e. “shake test”

2. In diabetic pregnancies, large-for-gestational-age fetuses are not uncommon. Which amniotic fluid test would most accurately predict fetal pulmonary maturity at 38 weeks’ gestation?
3. Which *rapid* amniotic fluid test would most accurately predict fetal pulmonary maturity at 38 weeks’ gestation?
4. Fetal hemolytic disorders (Rh problems) are best diagnosed by the following test of amniotic fluid:
    a. estriol
    b. phospholipids
    c. bilirubin
    d. surface tension
    e. human chorionic gonadotropin
5. Which of the following tests is useful to predict ancephaly or meningomyelocele?:
    a. serum human placental lactogen
    b. amniotic fluid surface tension
    c. amniotic fluid cortisol levels
    d. amniotic fluid alpha-fetoprotein
    e. amniotic fluid
6. A sample is received for fetal maturity studies, but the laboratory is not sure if the sample is a proper amniotic fluid specimen; that is one not heavily contaminated with maternal urine. Which of the following analytes can be used to differentiate between urine and amniotic fluids:
    a. creatinine
    b. protein
    c. sodium
    d. bilirubin
    e. alpha-fetoprotein
7. Which of the following sets of results from amniotic fluid is likely to be associated with fetal immaturity?
    a. L/S = 2.9, PG = negative
    b. L/S = 1.8, PG = positive
    c. L/S = 1.5, PG = negative
    d. L/S = 2.1, PG = positive
    e. L/S = 2.0, PG = negative
8. The best screening test for neural tube defects is measurement of serum:
    a. placental lactogen
    b. bilirubin
    c. estriol
    d. alpha-fetoprotein
    e. chronic gondatrophin

9. The primary pathway for removal of amniotic fluid during the third trimester of pregnancy is fetal swallowing.
    a. True
    b. False
10. At what time period do the following biochemical pathways mature or reach maximal levels? Match the time in column A with the pathway in Column B.

Column A	Column B
i. 8-9 weeks gestation	a. fetal hemoglobin production
ii. Parturition at birth	b. adult hemoglobin production
iii. 4 weeks post-partum	c. high levels of lung surfactant
iv. 38 weeks gestation	d. maternal serum chorionic gonadotropin levels
v. 16 weeks gestation	e. maternal plasma unconjugated estriol production

Question 11 - 17

a. 1,2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

11. Amniocentesis is performed for which of the following conditions?:
    1. management of Rh incompatibility
    2. identification of fetal maturity
    3. identification of potential genetic disorders
    4. identification of fetal sex
12. The primary source (s) of amniotic fluid in the third trimester of pregnancy is (are):
    1. skin
    2. lung
    3. intestine
    4. kidney
13. Serial measurements of maternal serum estriol demonstrate a pattern of slightly decreasing levels of estriol. The most likely clinical condition (s) associated with this pattern is (are):
    1. fetal growth retardation
    2. imminent fetal birth
    3. fetal death
    4. toxemia of pregnancy
14. Preeclampsia is associated with which of the following clinical symptom(s):
    1. hypertension
    2. edema
    3. proteinuria
    4. glucosuria
15. The beta chain of human chorionic gonadotrophin (hCG) is closely related to the beta chains of which of the following proteins?
    1. follicle-stimulating hormone (FSH)
    2. thyroid stimulating hormone (TSH)
    3. lututrinizing hormone (LH)
    4. all of the above
    5. none of the above
16. Premature labor:
    1. Can be prevented by treatment with high-dose MgSO4.

2. Is a leading cause of perinatal death.
3. Can be caused by infections and maternal drug use.
4. A cause of toxemia of pregnancy

17. Premature labor can be detected by:
    1. Leaking amniotic fluid
    2. Maternal AFP levels
    3. Fetal fibronectin
    4. Low maternal unconjugated estriol levels

Answer

1. e (p. 762)
2. d (p. 772)
3. e (p. 772)
4. c (p. 773)
5. d (p. 768, 775)
6. a (p. 772)
7. c (p. 771-772)
8. d (p. 768)
9. a (p. 755-756)
10.
    i. d (p. 758)
    ii. e (p. 759)
    iii. b (p. 765)
    iv. c (p. 763)
    v. a (p. 765)
11. a (p. 769)
12. c (p. 756)
13. b (p. 757-759, 770)
14. a (p. 766)
15. e (p. 757)
16. a (p. 766)
17. b (p. 766)

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1.37: Extravascular Fluids

1. Which of the following is not a serous body fluid?
    a. pleural
    b. pericardial
    c. peritoneal
    d. ascitic
    e. cerebrospinal
2. Serous fluids are formed by:
    a. derivation of body fluids from plasma
    b. derivation of body fluids by glomerular filtration
    c. derivation of body fluids by ultrafiltration
    d. derivation of serum from plasma
    e. membranes of the joints
3. Serous effusions can be designated as transudates or exudates based on the following except:
    a. glucose content
    b. specific gravity
    c. colloid osmotic pressure
    d. protein content
    e. enzyme content
4. Septic synovial fluid usually has all but one of the following characteristics:
    a. low viscosity
    b. leucocytes >10,000
    c. culture positive
    d. glucose lower than serum
    e. red-brown color
5. Synovial fluid is a body fluid found in which of the following body spaces?
    a. lung
    b. pericardium
    c. small intestines
    d. bone joint
    e. pleural cavity
6. Glucose levels are decreased in exudative processes because of:
    a. membrane impermeability
    b. glucose reaction with membranes
    c. bacterial infection
    d. glucose autooxidation catalyzed by exudate specific enzymes
    e. diurnal variation
7. Synovial fluid does NOT perform which of these functions?
    a. acts as a lubricant between bones of a joint
    b. acts as the glucose supplier to joint cells
    c. inhibits uric acid formation
    d. acts as electrolyte balancing media
    e. acts to carry nutrients to cartilage
8. The amount of fluid volume in a normal knee joint is:
    a. 0.1 to 3.5 mL
    b. 1.0 to 6.0 mL
    c. 5.0 to 15 mL
    d. 10 to 25 mL

e. more than 15 mL

9. Which of the following is true of exudates?
    a. specific gravity less than 1.015
    b. specific gravity less than 1.005
    c. total protein less than 30 g/L
    d. exudate protein/serum protein ratio greater than 0.5
    e. exudate glucose/serum glucose ratio greater than 0.5

Answer

1. e (p. 780, 781)
2. a (p. 780-781)
3. c (p. 782)
4. e (p. 785)
5. d (p. 784)
6. c (p. 782-783)
7. c (p. 784)
8. a (p. 784)
9. d (p. 782)

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1.38: Central Nervous System

Use the following key to answer Questions 1-16.

a. 1,2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

1. The Central nervous system is comprised of:
    1. brain
    2. sympathetic nerves
    3. spinal chord
    4. parasympathetic nerves
2. The cerebrospinal fluid:
    1. is about 150 mL
    2. is produced and reabsorbed at a rate of about 150 mL/day
    3. is replaced every 4 to 6 hours
    4. is constant in volume
3. The blood brain barrier:
    1. is a physiological barrier separating brain and CSF fluid from substances carried in the blood
    2. allows brain and CSF composition to be different
    3. is determined at cell surfaces of the choroid plexus
    4. is permeable to water
4. The blood brain barrier is impermeable to:
    1. proteins
    2. carbon monoxide
    3. aniline dyes
    4. alcohol
5. In the case of drugs, the ability to cross the blood brain barrier:
    1. is greater if it is lipid soluble
    2. is less if it is ionized
    3. is dependent upon disease status
    4. is dependent upon amino acid transport
6. In normal CSF:
    1. protein levels are less than 800 mg/L
    2. protein levels are equal to serum
    3. glucose levels are 60-80% of serum
    4. glucose levels are equal to serum
7. The brain’s energy reserve:
    1. is similar to that of the liver
    2. is primarily the lipid of adipose tissue
    3. is in the grey matter
    4. is very small
8. The primary energy source(s) of the brain is/are:
    1. fatty acids
    2. triglycerides
    3. cholesterol
    4. glucose

9. Factors that determine whether a molecule will be excluded from the brain by the blood-brain barrier include:
    1. molecular weight
    2. molecular polarity
    3. molecular ionization
    4. capillary permeability
10. In the norepinephrine neuron the precursor amino acid tyrosine is taken up and converted to:
    1. levodopa
    2. dopamine
    3. phenylalanine
    4. norepinephrine
11. Serotonin neurons within the control nervous system use which amino acid precursor(s) to form the neurotransmitter?
    1. tyrosine
    2. phenylalanine
    3. norepinephrine
    4. tryptophan
12. Which of the following drugs is (are) used to treat psychiatric disorders:
    1. tricyclic antidepressants
    2. lithium
    3. monoamine oxidase inhibitors
    4. phenytoin
13. When monitoring drugs such as amitriptyline and imipramine
    1. the type of illness should be considered
    2. dexamethasone depression test results should be consulted
    3. care must be taken to keep specimens refrigerated
    4. the metabolites should also be monitored
14. Which of the following can act as neurotransmitters?
    1. acetylcholine
    2. gamma-amino-butyric acid
    3. dopamine
    4. norepinephrine
15. Lithium is most useful for the treatment of:
    1. unipolar depression
    2. classical schizophrenia
    3. psychosis
    4. bipolar depression
16. The dexamethasone suppression test of cortisol is used to confirm the diagnosis of:
    a. unipolar depression
    b. bipolar depression
    c. psychoses
    d. classic schizophrenia
    e. none of the above
17. Antipsychotic drugs vary very little in their potency and greatly in their metabolism
    a. True
    b. False

Answer

1. b (p. 789)
2. e (p. 789)
3. e (p. 790)

4. b (p. 790-791)
5. a (p. 790)
6. b (p. 791, 801-803)
7. d (p. 792)
8. d (p. 792)
9. e (p. 790)
10. c (p. 793)
11. d (p. 793)
12. a (p. 800)
13. d (p. 803-804)
14. e (p. 792-793)
15. d (p. 800, 807)
16. a (p. 801)
17. b (p. 798-799)

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1.39: Endocrinology

1. Increased urinary 17-hydroxycorticosteroids are observed in:
    a. Cushing's syndrome
    b. Addison's disease
    c. hypopituitarism
    d. nephrosis
    e. decreased function of hypothalamus
2. A person having an abnormally low serum cortisol baseline level is given ACTH. A subsequent serum cortisol measurement taken 1 hour after ACTH shows no change in the serum cortisol level. The most probable diagnosis of the patient
    a. Cushing's syndrome
    b. Addison's disease
    c. adrenal tumor
    d. hypopituitansm
    e. hypoactive hypothalamus
3. In chronic thyroiditis, circulating antibodies against the storage protein for T_3 and T_4 are frequently found. This storage protein is:
    a. triiodothyronine
    b. thyroid binding globulin
    c. thyroglobulin
    d. thyroxine binding pre-albumin
    e. thyrotropin
4. Progesterone is secreted:
    a. by the endometrium during the last 2 weeks of the menstrual cycle
    b. by the anterior pituitary during the last 2 weeks of the menstrual cycle
    c. by the corpus luteum during 'the last 2 weeks of the menstrual cycle
    d. by the graafian follicle during the first week of the menstrual cycle
    e. by the hypothalamus during the entire menstrual cycle
5. Aldosterone, a steroid hormone synthesized in the adrenal medulla, controls which of the following?:
    a. electrolyte reabsorption in the proximal tubule
    b. active Cl^- reabsorption in the loop of Henle
    c. $Na^+/K^+(H^+)$ pump in the distal tubule (Na+ being reabsorbed
    d. water reabsorption in collecting ducts
    e. urinary calcium excretion
6. Anti-diuretic hormone (arginine vasopressin), a polypeptide hormone synthesized in the pituitary gland, controls which of the following?:
    a. electrolyte reabsorption in the proximal tubule
    b. active Cl^- reabsorption in the loop of Henle
    c. $Na^+/K^+(H^+)$ pump in the distal tubule (Na+ being reabsorbed)
    d. water reabsorption in the collecting ducts of the nephron
    e. blood pressure
7. Gastrin is a hormone which controls production of:
    a. renin
    b. mucus
    c. HCl
    d. lipase
    e. amylase
8. Ovulation can be detected by immunoassay of which of the following hormones?:
    a. LH

b. FSH
c. estrogen
d. progesterone
e. testosterone

9. The most important metabolite of epinephrine and norepinephrine is:
   a. homovanillic acid
   b. vanillylmandelic acid
   c. dopamine
   d. 5-hydroxyindole acetic acid
   e. serotonin
10. Which of the following is not used to assist the reproduction desires of infertile couples?
   a. LH-RH
   b. IVF
   c. GIFT
   d. ZIFT
   e. ICSI

Use the following key for Questions 11-14:

a. increased
b. decreased
c. no change

With polycystic ovarian disease, the ovary produces excessive quantities of androgens. Indicate what changes in hormone production would occur for the hormones listed below:

11. progesterone
12. testosterone
13. estradiol
14. FSH
15. In hyperparathyroidism, plasma immunoreactive PTH will be:
   a. normal
   b. absent
   c. elevated
   d. present at a low level
   e. fluctuating over a wide range, from very low to very high
16. There is no endocrine hormone from which of the following chemical classes?:
   a. proteins
   b. amino acids
   c. steroids
   d. polypeptides
   e. carbohydrates
17. Hormones are carried in blood by which of the following mechanisms?
   a. in chylomicrons
   b. non-covalently bound to protein
   c. covalently bound to protein
   d. covalently bound to carbohydrates
   e. non-covalently lipids
18. Which of the following hormones act by way of the “second” messenger, cyclic AMP?
   a. cortisol
   b. testosterone
   c. insulin
   d. thyroxin

e. progesterone

19. The hypothalamic-pituitary axis cannot be stimulated by:
    a. positive feedback loop
    b. negative feedback loop
    c. direct CNS stimulation
    d. direct CNS inhibition
    e. insulin
20. A physician want to order thyroid function tests on an acutely ill, hospitalized patient suspected of being hypothyroid. The best advice would be to:
    a. Order a 3rd generation TSH test
    b. Order measurements of free T_3
    c. Order a TRH challenge test
    d. Wait until the illness is over before performing the thyroid investigation
    e. Immediately treat with thyroid replacement therapy
21. The precursor molecule of all estrogens and androgens is:
    a. androstenedione
    b. progesterone
    c. dehydroepiendrosterone
    d. 20,22 despregnenolone
    e. cholesterol
22. In the male, increased FSH and LH and decreased testosterone is most likely associated with:
    a. primary hypergonadism
    b. secondary hypergonadism
    c. primary hypogonadism
    d. secondary hypogonadism
    e. normal function
23. Elevated T_4 and lowered T_3 uptake together suggest the likelihood of:
    a. hyperthyroidism
    b. decreased TBG
    c. hypothyroidism
    d. decreased TBG
    e. hyperthyroidism of pregnancy
24. Decreased T_4 and decreased T_3 uptake together suggest the likelihood of:
    a. hyperthyroidism
    b. increased TBG
    c. hypothyroidism
    d. decreased TBG
    e. secondary hyperthyroidism
25. TSH is secreted by the:
    a. anterior pituitary
    b. posterior pituitary
    c. hypothalamus
    d. adrenal cortex
    e. thyroid gland
26. Pregnancy, estrogen therapy, and oral contraceptives characteristically:
    a. cause hyperthyroidism
    b. cause hypothyroidism
    c. have no effect on circulating T_4 concentration
    d. increase circulating TBG

e. change levels of circulating free T_4

27. Most of the circulating T_4 and T_3 is bound to:
    a. thyroxine-binding prealbumin
    b. thyroxine-binding globulin
    c. albumin
    d. thyroglobulin
    e. none of the above

28. A serum TSH level five times upper limit of normal in the presence of a low T_4 and low T_3 uptake:
    a. establishes the thyroid as the cause of hypothyroidism
    b. rules out the thyroid as the cause of hypothyroidism
    c. establishes the pituitary as the cause of hypothyroidism
    d. establishes the diagnosis of secondary hyperthyroidism
    e. fails to do any of the above

Use the following Key to answer Questions 29—38:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. 4 only is correct
e. all are correct

29. Screening for thyroid disease may be appropriate for which of the following populations?
    1. Newborns
    2. First trimester pregnant women
    3. Individuals older than 60 years
    4. All individuals of all ages

30. Which of the following hormone(s) is/are from the adenohypophysis?:
    1. thyrocalcitonin
    2. serotonin
    3. glucagon
    4. ACTH

31. Which of the following hormones are produced by the pituitary gland?:
    1. FSH
    2. growth hormone
    3. thyroid stimulating hormone
    4. adrenocorticotrophic hormone

32. Which of the following describe the hypothalamic hormones? They:
    1. are small peptides
    2. are not affected by direct CNS stimulation
    3. act as releasing factors or inhibitors of pituitary hormone release
    4. act directly on all endocrine glands

33. Steroid hormones are transported in blood:
    1. covalently bound to protein
    2. as free hormones
    3. in red blood cells
    4. non-covalently bound to specific proteins

34. Hirsutism can be associated with:
    1. normal endocrine function
    2. menopause
    3. the polycystic ovary syndrome

4. feminizing tumors

35. Which of the following is (are) considered the active form of steroid hormones in plasma?
    1. Hormones bound only to specific transport proteins
    2. Hormones weakly bound to albumin
    3. Free hormone that has been glucuronated
    4. Hormone not bound to any protein
36. Increases in which of the following are associated with a pheochromocytoma?:
    1. urinary metanephrines
    2. plasma aldosterone
    3. urinary VMA
    4. urinary 17-hydroxysteroids
37. The production of T_3 and T_4 is normally influenced by which of the following?:
    1. plasma T_3 concentration
    2. plasma T_4 concentration
    3. TSH
    4. plasma thyroglobulin
38. The hormone TSH performs which of the following actions?
    1. stimulates production of thyroid hormones (T_4 and T_3)
    2. promotes iodide organification by the thyroid gland
    3. promotes release. of T_4 and T_3 from the thyroid
    4. increases when TRH levels increase
39. Aldosterone is secreted by which of the following parts of the adrenal gland
    a. the chromaffin cells
    b. the zona glomerulosa
    c. the zona fasciculata
    d. the zona reticularis
    e. the adrenal medulla
40. Which one of the factors listed below does not directly stimulate aldosterone release?
    a. hypokalemia
    b. ACTH
    c. angiotensin II
    d. angiotensin III
    e. ACTH
41. Which of the following androgens is secreted by the adrenal cortex in approximately equal quantities to cortisol?
    a. pregnenolone
    b. DHEA sulfate
    c. androstenedione
    d. testosterone
    e. dihydrotestosterone
42. Which one of the following steroids is the immediate precursor to testosterone in the steroid hormone synthetic pathway?
    a. DHEA
    b. DHEA sulfate
    c. Androstenedione
    d. Pregnenolone
    e. dihydrotestosterone
43. Which of the following is not a physiological property of cortisol?
    a. increases gluconeogenesis
    b. increases lipolysis
    c. increases protein catabolism

d. stimulates leukocyte migration to sites of injury
e. inhibits phagocytosis

44. Which of the following regarding the steroid synthetic pathway is not correct?
    a. cholesterol is converted to pregnenolone
    b. 11 deoxycortisol is converted to cortisol
    c. pregnenolone is converted to progesterone
    d. androstenedione is converted to testosterone
    e. 17 hydroxyprogesterone is converted to aldosterone
45. Cortisol has only weak mineralocorticoid activity in the kidney because
    a. it has low affinity for the aldosterone receptor
    b. it is rapidly converted by an enzyme in the renal cell to inactive cortisone
    c. it can not enter the renal cell
    d. it is in too low concentration
    e. it is very rapidly filtered through the glomerulus and is therefore ineffective.
46. Which of the following does not stimulate cortisol release?
    a. hyperglycemia
    b. severe illness
    c. trauma
    d. major depression
    e. interleukin-1
47. The most common cause of Addison's disease in North America is
    a. tuberculosis
    b. hemochromatosis
    c. infarction
    d. adrenoleukodystrophy
    e. autoimmune destruction (adrenalitis)
48. A patient presents to the emergency department with profound postural hypotension, hyponatremia, hyperkalemia, and his skin is darker than usual. Which of the following diagnostic tests should be carried out as soon as possible?
    a. baseline serum cortisol measurement
    b. plasma ACTH measurement
    c. urine free cortisol
    d. short ACTH stimulation test
    e. dexamethasone suppression test
49. Which one of the following observations are not consistent with the diagnosis of Conn's sydrome?
    a. low serum potassium
    b. high urine potassium excretion
    c. polyuria and polydipsia
    d. hypertension
    e. high plasma renin activity
50. The most specific procedure for localizing aldosterone secreting adenomas (Conn's syndrome) is
    a. bilateral adrenal venous catheterization and measuring aldosterone
    b. nuclear medicine imaging using radioactively labelled iodocholesterol
    c. aldosterone response to postural change
    d. abdominal CAT scan
    e. abdominal palpation

The following statement concerns questions 51 and 52. A new born female child is born with ambiguous genitalia and develops hyponatremia, hyperkalemia, and hypotension shortly after birth.

51. Which of the following is the most likely cause of her problem?
    a. autoimmune destruction of the adrenal gland

b. infarction of the adrenal gland
c. 21 hydroxylase deficiency
d. 11 hydroxylase deficiency
e. 17 hydroxylase deficiency

52. Which of the following diagnostic tests will confirm the diagnosis in the case described above?
    a. short ACTH stimulation test
    b. cortisol measurement
    c. testosterone measurement
    d. aldosterone measurement
    e. 17- hydroxyprogesterone
53. Which of the following causes of congenital adrenal hyperplasia is associated with hypertension, ambiguous genitalia in females and precocious puberty in males.
    a. 21-hydroxylase deficiency
    b. 11-hydroxylase deficiency
    c. 17 hydroxylase deficiency
    d. 3 beta-hydroxysteroid dehydrogenase deficiency
54. Which one of the following hormones can not be expressed ectopically by tumours?
    a. ACTH
    b. Antidiuretic hormone (ADH)
    c. Growth hormone releasing hormone (GHRH)
    d. Corticotrophin releasing hormone (CRH)
    e. Cortisol
55. A patient presents with hypertension, central obesity, abdominal striae, muscle weakness, and moon face. Which of the following tests would be most useful to perform first?
    a. ACTH
    b. aldosterone
    c. random serum cortisol
    d. 1 mg overnight dexamethasone suppression test
    e. CRH stimulation test
56. A patient with confirmed Cushing's syndrome has the following results. The urine free cortisol and serum cortisol measurements are elevated but do not suppress when given a high dose (8 mg) of dexamethasone. The ACTH is however unmeasurable. These results are suggestive of which of the following causes of Cushing's syndrome?
    a. adrenal adenoma
    b. pituitary adenoma
    c. bronchial carcinoma
    d. bronchial carcinoid
    e. pheochromocytoma
57. A patient with confirmed Cushing's sydrome has the following results. The urine free cortisol and serum cortisol measurements are elevated but suppress by more than 90% after being given 8mg of dexamethasone. The plasma ACTH concentration is slightly higher than the upper limit of normal. These results are suggestive of which cause of Cushing's syndrome?
    a. adrenal adenoma
    b. pituitary adenoma
    c. bronchial carcinoid
    d. bronchial carcinoma
    e. ectopic secretion of ACTH
58. Which of the following is the most specific procedure to differentiate between an ectopic source and pituitary source of ACTH in a patient with Cushing's syndrome?
    a. high dose dexamethasone suppression test
    b. ACTH concentration
    c. radiological imaging of the pituitary

d. MRI imaging of the pituitary
e. petrosal sinus sampling of ACTH concentration

59. The best test to assess adrenal reserve in a patient withdrawn from long term glucocorticoid therapy is which of the following?
    a. morning serum cortisol
    b. urine free cortisol
    c. insulin hypoglycemia stimulation test
    d. ACTH stimulation test
    e. aldosterone

60. Which of the following statements regarding cortisol is false?
    a. Cortisol levels vary throughout the day, usually highest in the morning and lowest in the evening
    b. Normally 90% of cortisol is bound to cortisol binding globulin
    c. Long term exposure to high levels of cortisol can lead to osteoporosis
    d. Cortisol reduces the rate of elimination of free water by decreasing the GFR.
    e. Cortisol levels are increased by psychological stress

61. The rate limiting step in the biosynthesis of cortisol which is stimulated by ACTH is which of the following?
    a. the conversion of cholesterol to pregnenolone
    b. the conversion of pregnenolone to progesterone
    c. the conversion of progesterone to 17 hydroxyprogesterone
    d. the conversion of 17 hydroxyprogesterone to 11 deoxycortisol
    e. the conversion of 11 deoxycortisol to cortisol

62. Which one of the following findings is not common to both primary and secondary adrenal insufficiency?
    a. low cortisol
    b. possible hypoglycemia
    c. weakness
    d. anorexia
    e. hyperpigmentation

63. Which of the following endocrine abnormalities is not commonly associated with multiple endocrine neoplasia (MEN) type 1?
    a. hyperparathyroidism
    b. pituitary tumours
    c. pancreatic islet cell tumours
    d. pheochromocytoma

64. Which of the following endocrine abnormalities is not commonly associated with multiple endocrine neoplasia (MEN) type 2A?
    a. pituitary tumours
    b. medullary carcinoma of the thyroid
    c. pheochromocytoma
    d. hyperparathyroidism

65. Which of the following endocrine abnormalities is commonly associated with multiple endocrine neoplasia (MEN) type 2B?
    a. pituitary tumours
    b. pheochromocytoma
    c. pancreatic islet tumours
    d. hyperparathyroidism

66. The most important symptom associated with pheochromocytoma is
    a. cardiac palpitations
    b. increased sweating
    c. headache
    d. increased anxiety
    e. sustained or episodic hypertension

67. A patient presents with increased sweating, episodic hypertension, palpitations, headache, and anxiety. Physical examination and thyroid testing have ruled out hyperthyroidism as a possible cause. Which one of the following approaches would be best at this point.
    a. measure plasma epinephrine and norepinephrine
    b. collect a 24 hour urine specimen and measure vanillylmandelic acid (VMA)
    c. collect a 24 hour urine specimen and measure urine catecholamines or metanephrines
    d. perform a clonidine suppression test
    e. collect a 24 hour urine and measure dopamine
68. The enzyme which converts norepinephrine to epinephrine in the adrenal medulla is a/an
    a. hydroxylase
    b. decarboxylase
    c. oxidase
    d. methyltransferase

Use the following key for questions 69-73

a. increased
b. decreased
c. no change

Indicate the changes in hormone level that would occur in male primary hypogonadism for the hormones listed below.

69. FSH
70. total testosterone
71. SHBG
72. free T4
73. LH

Use the following key to answer question 74 and 75,

a. 1, 2 and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. 4 only is correct
e. all are correct

74. An elevated serum prolactin level can be associated with:
    1. a pituitary tumor
    2. some drugs
    3. PCOS
    4. hyperthyroidism
75. SHBG concentrations are increased by:
    1. obesity
    2. pregnancy
    3. androgens
    4. hyperthyroidism
    5. hyperprolactinemia

Which of the following statements is true/false:

76. Measurement of serum FSH levels is a reliable indicator of the perimenopausal state.
    a. True
    b. False
77. In females, inhibin A and inhibin B play an important role in the regulation of FSH secretion.
    a. True

b. False

78. In testicular feminization syndrome the karotype is that of a normal female.
    a. True
    b. False
79. In a male patient with low testosterone levels, FSH and LH levels within the reference range are not consistent with secondary hypogonadism.
    a. True
    b. False

Answer

1. a (p. 891)
2. b (p. 897)
3. c (p. 830-831, 839)
4. c (p. 856-857)
5. c (p. 879-880)
6. d (p. 821)
7. c (p. 812)
8. a (p. 856)
9. b (p. 883)
10. a (p. 874)
11. c (p. 683)
12. a (p. 683)
13. c (p. 683)
14. b (p. 683)
15. c (p. 527, 814-815)
16. e (p. 811)
17. b (p. 817-818)
18. c (p. 813-814)
19. e (p. 814-815)
20. d (p. 837, 846)
21. e (p. 854)
22. c (p. 868)
23. e (p. 840)
24. c (p. 841)
25. c (p. 812)
26. d (p. 836)
27. b (p. 832)
28. a (p. 841)
29. a (p. 838, 840, 841)
30. d (p. 812)
31. e (p. 812)
32. b (p. 812, 823)
33. d (p. 817)
34. b (p. 863)
35. c (p. 816-818, 820, 832)
36. b (p. 890)
37. a (p. 835)
38. e (p. 835)
39. b (p.812, 879)
40. a (p. 459, 885)
41. a (p. 881)
42. c (p. 882)

43. d (p. 880)
44. e (p. 882)
45. b (p. 880)
46. a (p. 879, 890)
47. e (p. 898)
48. d (p. 897)
49. e (p. 895-896)
50. a (p. 896)
51. c (p. 888-889)
52. e (p. 888)
53. b (p. 889)
54. e (p. 882)
55. d (p. 891)
56. a (p. 886)
57. b (p. 894)
58. e (p. 894-895)
59. c (p. 897-898)
60. d (p. 880)
61. a (p. 881)
62. e (p. 889-890)
63. d (p. 890)
64. a (p. 890)
65. b (p. 890)
66. e (p. 890-891)
67. c (p. 890-891, 898-899)
68. d (p. 883)
69. a (p. 867, 868)
70. b (p. 867, 868)
71. a (p. 866-867, 868)
72. c (p. 867, 868)
73. a (p. 867, 868)
74. a (p. 860-864)
75. b (p. 855)
76. b (p. 858)
77. a (p. 855)
78. b (p. 869)
79. b (p. 868)

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1.40: Diseases of Genetic Origin

1. A certain trait is expressed independent of gender and even though each person is not homozygous for the gene. This type of inheritance is best termed as:
   a. autosomal recessive
   b. autosomal dominant
   c. X-linked dominant
   d. X-linked recessive
   e. multifactorial
2. “Lyonization” is a process by
   a. Barr body analysis of cells is made
   b. chromosomes are displayed in metaphase cells
   c. phenylalanine is detected in urine
   d. amino acidurias are screened in newborns
   e. X-chromosome in each female’s cells is randomly inactivated
3. Organic acidurias occur as a result of:
   a. glycogen storage diseases
   b. aminoacidopathies
   c. errors of urea metabolism
   d. defects of copper metabolism
   e. LDL receptor defects
4. The Lesch-Nyhan syndrome is associated with a defect in the metabolism of:
   a. amino acids
   b. minerals
   c. glycogen
   d. purines
   e. pyridines
5. Match the following tests used to detect carriers of inborn errors with the disorder that they are designed to’ detect

a. hemoglobin electrophoresis	
b. hexosaminidase	i. Tay-Sachs disease
c. creatine kinase	ii. Duchenne muscular dystrophy
d. phenylalamine	iii. Sickle cell anemia
e. orotic acid	iv. Hemophilia A
f. Factor VIII assay	v. Hurler’s syndrome
g. α-L-iduronidase	

Use the following KEY to answer Questions 6-14:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

6. The basic premise(s) of the role of genes include the following:
   1. one structural gene encodes a polypeptide with a specific function
   2. one structural gene is paired with a regulatory one defining a structural protein
   3. a mutation of a structural gene results in a structurally altered polypeptide
   4. structural genes are limited in length to specific chromosomes
7. Testing for phenylketonuria and galactosemia is important because:
   1. the fetus can be terminated

2. the fetus can be given enzymes to prevent defects
3. the urinary excretion of amino acids and sugars can be reduced with consequent salvage of the kidney
4. these diseases can be controlled by diet

8. Alpha-fetoprotein:
    1. is a plasma protein made by the fetal liver
    2. is virtually non-detectable in non-pregnant females
    3. if present in elevated amounts (2.5 times MOM) indicates fetal or pregnancy complications
    4. is present in equivalent amounts in maternal serum and amniotic fluid
9. Measurements of serum alpha-fetoprotein levels may be requested for:
    1. diagnosis of acute hepatitis
    2. screening for fetal neural tube defects
    3. diagnosis of spina bifida
    4. monitoring progress of certain cancers
10. Which of the following are considered inborn errors of metabolism:
    1. amino acidopathies
    2. organic acidurias
    3. glycogen storage diseases
    4. Wilson's disease
11. A Structural gene locus can have a series of alleles. Which of the following are alleles?
    1. Duffey blood groups a and b
    2. hemoglobin A and hemoglobin S
    3. thalassemia
    4. hemoglobin A and C
12. Which of the following describe lysosomal storage diseases?
    1. recessively inherited disorders
    2. deficiency of specific acid hydrolases
    3. deficiency of hydrolases located within lysozomes
    4. autosomal dominant disorders
13. Inherited diseases can result in enzyme deficiencies. These can be the biochemical basis for a disease because of:
    1. lack of production of a product
    2. accumulation of substrate
    3. shunting of substrate to alternate pathway with production of a toxic compound
    4. urinary loss of metabolites
14. Inherited diseases of intermediary metabolism often produce:
    1. reduced serum levels of normal metabolites
    2. elevated urinary excretion of normal metabolites
    3. normal to lower excretion of normal metabolites
    4. urinary excretion of an abnormal metabolite

Answer

1. b (p. 910-912)
2. e (p. 911)
3. b (p. 926)
4. d (p. 931)
5.
    i. b (p. 921)
    ii. c (p. 912)
    iii. a (p. 915, 932)
    iv. f (p. 912, 934)
    v. g (p. 920-922)

6. b (p. 910)
7. d (p. 924)
8. a (p. 934)
9. c (p. 934)
10. e (p. 919-932)
11. a (p. 910)
12. a (p. 919)
13. a (p. 923)
14. c (p. 923)

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1.41: Molecular Biology in the Clinical Laboratory

1. Complementary DNA (cDNA) is best defined as:
    a. two DNA strands, each strand consisting of one type of purine base
    b. two DNA strands, each strand consisting of one type of pyrimidine base
    c. two DNA strands, covalently linked to each other
    d. a strand of DNA in which the order of pyrimidine and purine bases complement the opposite base order on a strand of RNA
    e. a strand of DNA in which the order of pyrimidine and purine bases complement the opposite base order on a strand of DNA
2. The polymerase chain reaction (PCR) is a technique used to:
    a. increase the sensitivity of enzyme reactions
    b. enhance the sensitivity of DNA detection methods
    c. cross-ink bonded stationary phases for HPLC
    d. measure polymerized collagen in urine
    e. degrade DNA to smaller fragments
3. Analysis of DNA fragments by complementary DNA hybridization is performed by:
    a. Northern blot technique
    b. Southern blot technique
    c. Western blot technique
    d. Sandwich technique
    e. ELISA technique

Use the following Key to answer Questions 4-5.

a. 1, 2,and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

4. Genetic defects can be detected in vitro by:
    1. analysis of DNA
    2. measuring amniotic fluid lecithin and sphingomyelin
    3. measurement of specific enzyme levels
    4. measurement of amniotic fluid bilirubin
5. Restriction enzymes are:
    1. a group of bacterial enzymes
    2. enzymes that inhibit DNA replication
    3. enzymes that “cut” (hydrolyze) at specific base sequences
    4. a group of enzymes that are involved with keeping specific ions outside of the cell.

Answer

1. e (p. 938)
2. b (p. 940-941)
3. b (p. 940941)
4. b (p. 939)
5. b (p. 939)

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1.42: Neoplasia

1. Elevations of which enzyme are usually associated with prostate cancer:
    a. alkaline phosphatase
    b. creatine kinase
    c. lactic dehydrogenase
    d. prostate-specific antigen
    e. terminal deoxynucleotidyl transferase
2. Prognosis of patients with breast cancer is often correlated with:
    a. liver enzyme levels
    b. prostate-specific antigen levels
    c. terminal deoxynucleotidyl transferase levels
    d. estrogen and progesterone receptor tissue levels
    e. phenotypic heterogeneity
3. Estrogen receptors are useful to assess prognosis of which types of cancer?
    a. breast
    b. liver
    c. colon
    d. lymphocytes
    e. germ cell
4. The leading cause of cancer deaths for both males and females is lung cancer?
    a. True
    b. False

Use the following Key to answer Questions 5-10.

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. only 4 is correct
e. all are correct

5. Cancer is a long-term process and goes through obligatory phases; these include:
    1. induction phase
    2. In Situ phase
    3. invasion phase
    4. dissemination phase
6. Screening tests for cancer include:
    1. cytological analysis of urine
    2. mammography
    3. Papanicolaou smear
    4. testing of stool for occult blood
7. The antigens associated with monitoring of cancer include:
    1. carcenoembryonic antigen
    2. alpha-fetoprotein
    3. prostate specific antigen
    4. breast cancer antigen
8. Measurement of tumor antigens is most useful for:
    1. screening for cancers
    2. classifying cancers
    3. diagnosis
    4. monitoring cancer

9. Oncogenes (protooncogenes) are:
    1. normal cell genes
    2. tumor antigen genes
    3. genes associated with cancer
    4. related to risk for tumor metastasis
10. For which of the following populations might it be reasonable to screen for breast cancer genes??
    1. All women
    2. Women of Ashkenazi-Jewish background
    3. Women with elevated CEA levels
    4. Women who are members of high-risk families with history of breast cancer.

Answer

1. d (p. 968)
2. d (p. 969)
3. a (p. 969)
4. a (p. 962)
5. e (p. 963)
6. e (p. 964)
7. e (p. 966)
8. d (p. 965-966)
9. b (p. 962)
10. d (p. 970)

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1.43: Laboratory Evaluation of the Transplant Recipient and Donor

1. The most frequently performed transplants in the United States is:
    a. Heart
    b. Kidney
    c. Liver
    d. Lung
    e. Pancreas
2. Which of the following techniques is NOT used for immunological monitoring of the transplant recipient?
    a. Mixed lymphocyte cultures
    b. Human leukocyte antigen testing
    c. Cyclosporin measurements
    d. Panel reactive antibody testing
    e. Lymphocyte crossmatch
3. Which of the following drugs are NOT monitored in the transplant recipient?
    a. Cyclosporin
    b. Tacrolimus
    c. OKT3 levels
    d. Corticosteroids
    e. White blood cells counts
4. Laboratory evaluation of the transplant donor includes testing for HIV?
    a. True
    b. False
5. Laboratory evaluation of the pancreas transplant recipient includes all of the following except:
    a. Serum creatinine
    b. Serum amylase
    c. Serum creatine kinase
    d. Urine amylase
    e. Glucose tolerance test

Answer

1. b (p. 974)
2. a (p. 986)
3. d (p. 981-986)
4. a (p. 975)
5. c (p. 979)

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1.44: Toxicology

1. The dose-response relationship for a toxicant is expressed as:
   a. the level at which all the treated group will die
   b. the level at which several of a treated group will die
   c. the level at which 1% of a treated group will die
   d. a semilogrithmic relationship between effect and level
   e. a linear relationship between effect and level
2. The following mechanisms in for the questions listed in Column A are associated with toxic agents. Match the mechanism with the agent in Column B.

Column A	Column B
i. Irreversible enzyme inhibition ii. Reversible enzyme inhibition iii. Hepatic poisoning iv. Blockage of oxygen transport v. Depression of the central nervous system vi. Damage to DNA and RNA	a. cyanide b. acetaminophen c. Carcinogens or teratogens d. benzodiazepines e. salicylates f. tylenol g. arsenates h. opiates i. organophosphates

3. Match the drug in Column A (questions) with its antidote in Column B.

Column A	Column B
i. Acetominophen ii. Opiates iii. Organophosphates	a. chlorpromazine b. methionine c. physostigmine d. oxygen e. sodium nitrate f. naloxone g. atropine

Use the following Key to answer Questions 4-5:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. 4 only is correct
e. all are correct

4. Which of the following statements about poisons are true?
   1. all substances are poisons
   2. poisons differ qualitatively from other compounds
   3. substances taken in sufficient quantities will be poisons
   4. most poisons have antidotes
5. Which of the following factors can influence toxicity of an agent?
   1. chemical properties
   2. route of administration
   3. duration of exposure
   4. age

Answer

1. d (p. 991)
2.
    i. i (p. 999)
    ii. g (p. 999)
    iii. b (p. 998, 1007)
    iv. a (p. 999)
    v. h (p. 997-998)
    vi. c (p. 999)
3.
    i. b (p. 1000,1007)
    ii. f (p. 1000)
    iii. g (p. 1000)
4. b (p. 991)
5. e (p. 991-994)

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1.45: Addiction and Substance Abuse

1. The drug most frequently abused for males between the age of 18-25 years is:
    a. Alcohol
    b. Tobacco
    c. Marijuana
    d. Cocaine
    e. PCP
2. The addictive process includes all but the following factors:
    a. Tolerance
    b. Negative reinforcement
    c. Lower socio-economic group
    d. Denial
    e. Craving

Use the following Key to answer Questions 3-4:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. 4 only is correct
e. all are correct

3. Diagnostic criteria of addiction include:
    1. Tolerance
    2. Loss of control
    3. Withdrawal symptoms
    4. Use despite acknowledged reasons not to use
4. Which of the factors regarding a drug screen are true?
    1. Results should be reviewed by a medical review officer.
    2. Are always randomly performed.
    3. Are used as part of a contingency contract.
    4. Never need confirmation testing of the results.

Answer

1. b (p. 1019)
2. b (p. 1017-1018)
3. e (p. 1020)
4. b (p. 1022)

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1.46: Enzymes and Isoenzymes

1. Enzymes accelerate the reaction rate by:
    a. decreasing the amount of free energy of activation necessary for the reaction
    b. shifting the equilibrium position of a reaction
    c. causing thermodynamically incompatible reactions to occur
    d. increasing the rate of the reaction in one direction only
    e. c and d
2. Enzyme nomenclature is used to describe:
    a. the reaction taking place
    b. the physical conditions of the assay
    c. the conversion of products to substrates
    d. only two point reactions e. the buffer in the assay
3. A substance which when added to the enzyme attaches to a site removed from the active site so that the enzyme cannot bind its natural substrate is a (an):
    a. competitive inhibitor
    b. non-competitive inhibitor
    c. substrate analog
    d. enzyme cofactor
    e. coenzyme
4. To assure that zero order kinetics is maintained in an enzyme reaction, the substrate concentration should be:
    a. equal to the K_m
    b. less than the K_m
    c. at least 10 times greater than the K_m
    d. equal to $1/K_m$
    e. equal to the enzyme concentration
5. For which order of reaction is the rate dependent only on the enzyme concentration?:
    a. zero order
    b. first order
    c. second order
    d. mixed order
    e. none of the above
6. The point at which an enzyme reaction is proceeding at the greatest rate is:
    a. the Michaelius constant (K_m)
    b. zero order kinetics
    c. first order kinetics
    d. point where the rate of the reaction is dependent on the substrate concentration
    e. $[S] = K_m$
7. Enzymes of metabolism:
    a. are present in all cells
    b. are plasma specific enzymes
    c. have a known function in serum
    d. have a known function in plasma
    e. are produced in large quantities after eating
8. Isoenzymes are:
    a. multiple molecular forms of an enzyme family that catalyze the same reaction
    b. different enzymes which exhibit the same enzymatic specificity
    c. multiple molecular forms of different enzymes which catalyze the same reaction
    d. different enzymes which exhibit the same electrophoretic mobility

e. enzymes with the same tertiary structure which catalyze the same reaction

9. CK isoenzymes are diagnostically important because:
   a. erythrocyte and cardiac sources of elevation can be distinguished
   b. cardiac and hepatic sources of elevation can be distinguished.
   c. statistical analyses of the patterns of the 5 isoenzymes give a diagnostic classification of the various liver diseases
   d. the isoenzyme pattern indicates the specific tissue involved in a malignancy
   e. they are absolutely tissue specific
10. The distribution of isoenzymes:
   a. is the same throughout the body
   b. varies greatly during adult life
   c. is dependent on physiological needs
   d. varies by individual
   e. varies by organ
11. Serum from adults contain which isoenzyme of alkaline phosphatase?:
   a. heart, kidney, liver
   b. liver, kidney, bone
   c. kidney, red cells, liver
   d. red cells, liver, brain
   e. placenta, lungs, brain
12. In coupled enzyme systems, the enzyme in the last reaction:
   a. is employed as an activator
   b. must exhibit the same kinetic properties as the enzyme in the primary reaction
   c. is the one whose product is measured
   d. is of no significance
   e. forms the substrate for the enzyme being measured
13. The plot of 1/velocity vs. 1/substrate concentration is known as the:
   a. Arrhenius plot
   b. initial rate
   c. Michaelis-Menten plot
   d. maximal velocity
   e. Lineweaver-Burk plot
14. Situation: You are running an assay of enzyme “X” which utilizes the coenzyme NAD. Its activity is measured in terms of NADH produced. You have obtained the following data:

    Micromolar extinction coefficient (ϵ) of NADH (at 340 nm) = 6.22 x 10^{-3} L $\cdot$ μmol^{-1} $\cdot$ c^{-1}; light path (b) = 1 cm total volume of assay = 3.0 mL sample volume = 0.5 mL absorbance change 7 minutes = 0.350

    Calculate the enzyme activity in IU/liter:
   a. 0.048
   b. 1.30
   c. 48
   d. 336
   e. 4.8
15. If zero order kinetics are followed, allowing the reaction to run twice the time will:
   a. cause the product to be denatured
   b. halve the amount of product formed
   c. have no effect on the amount of product formed
   d. double the amount of product formed
   e. double the absorbance change per minute
16. Skeletal muscle tissue contains which of the following CK isoenzymes?

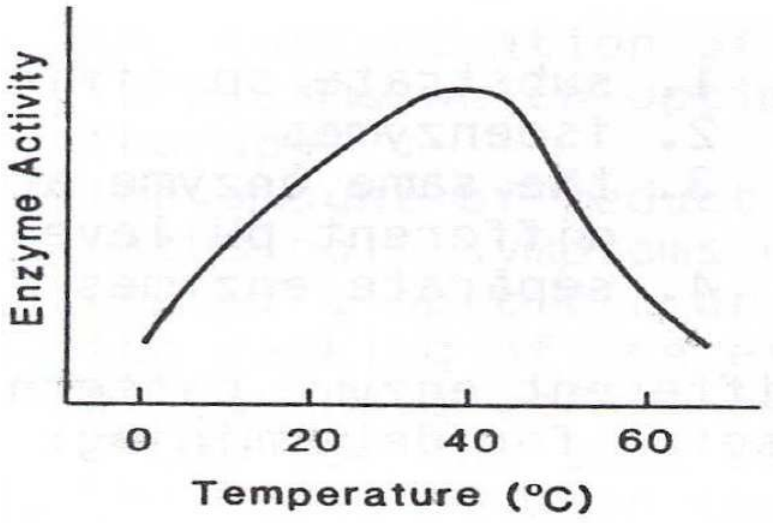
a. MM only
b. MB only
c. BB only
d. MM + MB
e. MM + BB

17. Creatine kinase (CK) isoforms are:
    a. degradative forms of individual CK isoenzymes
    b. polymerized forms of individual CK isoenzymes
    c. intracellular form of individual CK isoenzymes
    d. imunoglobulin-CK complex of individual isoenzymes
    e. none of the above.
18. If the sample volume is halved in an enzymatic reaction, the final calculated enzyme activity will:
    a. increase four-fold
    b. decrease four—fold
    c. increase two—fold
    d. decrease two-fold
    e. remain the same
19. According to IUB classification, creatine kinase is a/an:
    a. oxidoreductase
    b. transferase
    c. hydrolase
    d. lyase
    e. ligase

Use the following Key to answer Questions 20-27:

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. 4 only is correct
e. all are correct

20. An enzyme is:
    1. a protein
    2. highly specific
    3. not consumed in a reaction
    4. a catalyst
21. The type of curve below shows the effect of enzyme activity in relation to:
    1. substrate concentration
    2. temperature
    3. activator concentration
    4. pH



22. An advantage of kinetic methods of measuring enzyme activity is that:
    1. temperature is not critical
    2. pH is not critical
    3. there is elimination of the lag phase
    4. linearity is demonstrable
23. Which of the following statements concerning the measurement of serum enzymes is/are true?:
    1. enzymes are increased in serum following cell destruction and release of cellular constituents.
    2. enzymes are totally organ specific. A rise in an enzyme tells the physician exactly which organ is diseased.
    3. enzymes can rise to significant levels over background and help indicate the nature of the disease
    4. enzymes are depressed after stimulation of the exocrine glands
24. Acid phosphatase and alkaline phosphatase are:
    1. substrate specific
    2. isoenzymes
    3. the same enzyme acting at different pH levels
    4. separate enzymes
25. Different isoenzyme patterns are useful for determining:
    1. the age of the sample
    2. the severity of the disease process
    3. the condition under which the sample was stored
    4. which tissue was involved in the disease process
26. Isoenzymes can be measured by which of the following procedures?
    1. chromatography
    2. electrophoresis
    3. immunoassay
    4. heat stability
27. Which of the following compound(s) is (are) an enzyme cofactor?
    1. chloride
    2. pyridoxyl-5-phosphate
    3. magnesium
    4. thiamine-pyrophosphate
28. Enzyme measurements are usually performed in the:
    a. lag phase
    b. linear phase
    c. substrate depletion phase

d. none of the above
e. doesn't matter which phase is used

29. An international unit of enzyme activity is defined as the number of:
    a. μmoles product formed/second
    b. μmoles product formed/minute
    c. mmoles substrate consumed/liter
    d. mmol substrate consumed/second
    e. none of the above
30. Nicotinamide adenine dinucleotide (NAD) is best considered a enzyme:
    a. cofactor
    b. activator
    c. substrate
    d. inhibitor
    e. holoenzyme
31. An organ's isoenzyme composition never changes except as a result of disease.
    a. True
    b. False

Answer

1. a (p. 1047)
2. a (p. 1050-1051)
3. b (p. 1057)
4. c (p. 1054)
5. a (p. 1053-1054)
6. b (p. 1053-1054)
7. a (p. 1061)
8. a (p. 1065)
9. b (p. 1067-1070)
10. e (p. 1067)
11. b (p. 1068)
12. c (p. 1060)
13. e (p. 1055)
14. c (p. 1055)
15. d (p. 1054)
16. d (p. 1067)
17. a (p. 1066)
18. e (p. 1055)
19. b (p. 1051)
20. e (p. 1045, 1046, 1048)
21. c (p. 1056, 1059)
22. c (p. 1052-1053)
23. b (p. 1060-1061)
24. d (p. 1051)
25. d (p. 1068-1069)
26. e (p. 1071)
27. c (p. 1057)
28. b (p. 1054)
29. b (p. 1055)
30. c (p. 1044, 1057)
31. b (p. 1068)

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1.47: Therapeutic Drug Monitoring

1. At the end of __ half lives a steady state level of drug is reached with multiple dosing. At the end of this period therapeutic monitoring for maintenance therapy can be initiated:
    a. one
    b. two
    c. three
    d. four
    e. five

Use the following Key to answer Questions 2-16.

a. 1, 2, and 3 are correct
b. 1 and 3 are correct
c. 2 and 4 are correct
d. 4 only is correct
e. all are correct

2. Pharmacokinetics is the quantitative study of drug distribution in the body. It permits the user:
    1. to mathematically describe the fate of a drug after administration
    2. to compare drugs and dosage forms
    3. to predict blood levels
    4. to mathematically describe the kinetic (movement) of pharmacological agents through tissue
3. The principle(s) which underlie monitoring of drugs in patients is/are:
    1. increasing drug concentrations are more effective
    2. the blood levels are inversely related to the clinical effects
    3. drug receptors have a variable response to drug and the ratio of drug/ receptor must be measured
    4. there is a relationship between clinical effects and drug concentration in plasma
4. The therapeutic index is defined as:
    1. the concentration of drug in plasma which optimizes therapy
    2. the amount of reduction of pathologic symptoms obtained by use of a specific drug
    3. the ranking of the effect of the drug versus other drugs for the same disease
    4. the ratio between the average toxic dose and the average therapeutic dose
5. The LADME system to describe drug disposition includes consideration and measurement of which of the following processing:
    1. absorption
    2. metabolism
    3. liberation
    4. elimination
6. Absorption of drug takes place when the drug is given:
    1. extravascularly
    2. intravascularly
    3. orally
    4. intravenously
7. Mechanisms of absorption include:
    1. passive diffusion
    2. active transport
    3. facilitated transport
    4. convective transport
8. Once drug molecules are absorbed, they distribute within the bloodstream and can:
    1. be confined to the blood space
    2. leave the bloodstream to enter other extravascular fluids

3. migrate into various tissues or organs
4. semilogarithmically be related to dose

9. Biotransformation is the process of converting the parent drug to one or more metabolites. Which of the following, statements is/are true?
    1. the metabolites are usually more polar
    2. usually metabolism takes place in the liver and kidney
    3. metabolites are usually less active than parent drug
    4. usually metabolites can form prodrug and be effective at receptors
10. The most prominent physiologic factor(s) which influence drug disposition include(s):
    1. body weight
    2. age
    3. temperature
    4. gastric emptying time
11. The most important pathological factors which influence drug disposition are:
    1. renal impairment
    2. liver impairment
    3. gastrointestinal diseases
    4. cancer metastasis
12. Which liberation factor(s) affect(s) the blood level curves after oral dosage of a drug:
    1. size of liberation
    2. rate of liberation
    3. gastric emptying
    4. extent of liberation
13. High levels of drug in serum or plasma are usually associated with:
    1. shock
    2. intensity of clinical effectiveness
    3. low therapeutic index
    4. toxicity
14. The rationale for using blood levels as indicators of clinical response is based on the following assumptions:
    1. For those receptors interacting with drugs, the concentration at the receptors determines pharmacologic effect.
    2. It is not possible to sample at the site of action (biophase).
    3. Blood and serum are the fluids closest in equilibrium to the biophase.
    4. Blood, serum and plasma are the transport systems by which drug reaches the receptor.
15. Therapeutic drug monitoring is usually NOT performed when:
    1. blood level is not related directly to clinical response
    2. liver impairment is present and the patient is on drugs eliminated by hepatic metabolism
    3. clinical or pharmacological response is easily and accurately measured
    4. the drug is used as a prophylactic
16. Most drugs are administered:
    1. as a single dose
    2. in a series of doses
    3. at the time of diagnosis
    4. at specified interval

Answer

1. d (p. 1079, 1085)
2. a (p. 1081)
3. d (p. 1087-1088)
4. d (p. 1074, 1086)

5. e (p. 1075-1076)
6. b (p. 1076)
7. e (p. 1076)
8. a (p. 1076)
9. a (p. 1076)
10. e (p. 1076-1077)
11. a (p. 1077)
12. c (p. 1077)
13. d (p. 1079)
14. a (p. 1087-1089)
15. b (p. 1079)
16. c (p. 1079)

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1.48: Urinalysis

1. Oval fat bodies are:
    a. white cells with fat inclusions
    b. degenerated tubular epithelium containing fat
    c. globules of free fat
    d. fat-filled casts
    e. fertilized ova
2. If a precipitate of amorphous phosphates obscures the sediment in urine, the precipitate can be removed by adding:
    a. dilute sodium hydroxide
    b. ethanol
    c. concentrated nitric acid
    d. dilute acetic acid
    e. acetone
3. The action of the Clinitest tablet is based on:
    a. the reduction of ferric ions
    b. the reduction of cupric ions
    c. an enzyme specific for glucose
    d. an enzyme specific for reducing sugars
    e. the reaction of glucose with o-toluidine
4. Which of the following analytes react(s) with para-dimethylaminobenzaldehyde to form a cherry red compound?
    a. creatinine
    b. ketones
    c. protein
    d. glucose
    e. urobilinogin
5. A healthy individual may occasionally have __ RBC/high powered field in a random urine specimen:
    a. zero
    b. 2-4
    c. 8-10
    d. 15-25
    e. 25-30
6. The specific gravity of normal urine is:
    a. 1.000 - 1.005
    b. 1.005 - 1.020
    c. 1.005 - 1.030
    d. 1.010 - 1.040
    e. 1.05 - 1.07
7. The nitroprusside test used on dipsticks detects:
    a. beta-hydroxybutyric acid
    b. creatinine
    c. glucose
    d. acetoacetic acid
    e. blood
8. The protein test on the Multistix is based on the principle that:
    a. protein changes the pH of the test area and therefore the color of the indicator
    b. proteins are zwitterions and change the charge on the buffer
    c. the buffer changes the charge on the proteins causing them to form colored complexes
    d. at a fixed pH certain indicators will have one color in the presence of protein and another in the absence of protein

e. proteins precipitate at high pH

Answer

1. b (p. 1104)
2. d (p. 1097 see table)
3. b (p. 1099-1100)
4. e (p. 1100)
5. b (p. 1013)
6. c (p. 1097)
7. d (p. 1100)
8. a (p. 1099)

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CHAPTER OVERVIEW

2: Laboratory Exercises

The student should complete each experiment as a study guide. Some exercises call for experimental results. The information called for, such as the appearance of serum in various phlebotomy tubes under different storage conditions, should now be part of the body of knowledge of a student who has completed the course and is preparing for the examination. Care is taken to include all common calculations, essential for the medical technologist, such as dilutions, in addition to examples of calculations for important techniques such as the use of Beer's law and chromatographic data.

Finally, the Questions for Discussion should be answered. Any student unable to complete data, calculations, and questions for discussion should read the pages assigned for laboratory preparation. In this case, the pages will serve a remedial function, and should be reviewed only when necessary.

2.1: Basic Spectrophotometry
2.2: Absorbance Spectra
2.3: Standard Curve
2.4: Buffer Preparation
2.5: Buffer Titration/buffering Capacity
2.6: Flame Photometer
2.7: Chloride Determination (Coulometric Method)
2.8: Electrolytes/anion Gap
2.9: Serum Osmolality
2.10: Serum Glucose- Trinder Method
2.11: Glucose Tolerance Test
2.12: Total Serum Protein
2.13: Serum Albumin (Bromcresol Green Method)
2.14: Serum Creatinine (Jaffe Method)
2.15: Bilirubin (Waters and Gerande – DMSO Method)
2.16: Cholesterol (Total and HDL)
2.17: Triglycerides
2.18: Lipoprotein Electrophoresis
2.19: Kinetic Enzyme Analysis
2.20: Enzymes Rate, Creatine Kinase (CK)
2.21: Amylase (Modified Caraway Method)
2.22: Creatine Kinase (CK) Isoenzyme Electrophoresis
2.23: Blood Gas Analysis
2.24: Phlebotomy
2.25: Exercise 25; Quality Control
2.26: Extraction Technique
2.27: Thin Layer Chromatography (TLC) of Drugs
2.28: High Performance Liquid Chromatography (HPLC)
2.29: Gas Liquid Chromatography (GLC)
2.30: Radioimmunoassay (RIA)

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2.1: Basic Spectrophotometry

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related readings, the student will be able to:

1. Locate the power switch, zero adjustment (where applicable), fine adjustment, coarse adjustment, sample chamber, wavelength adjustment, and readout device on a spectrophotometer.
2. Check a spectrophotometer for proper wavelength calibration and adjust if necessary.
3. Properly adjust (set) wavelength, zero (dark current) and 100% transmittance (zero absorbance) of a spectrophotometer.
4. Measure the absorbance of several solutions.
5. Calculate the value of an unknown solution by using it's absorbance and that of a known standard.

glossary

- **Blank:** the solution placed in the sample compartment of a spectrophotometer when adjusting the transmittance to 100%
- **T:** The absorbances of all other test solutions are then measured against the blank. Often the word is used as verb, "to blank a spectrophotometer".
- **Dark current adjustment**: adjusts current through detector to "zero" when no light is present.
- **Fine and coarse adjustments:** similar to "zero adjustments' on Certain instruments, allows finer control of 100% transmittance settings.
- **Wavelength adjustment**: device which adjusts angle of grating, prism, or mirror to achieve a desired nominal wavelength setting.
- **Zero adjustment**: sets instrument's current reading to 100% transmittance (zero absorbance) when a blank sample is present in the sample compartment.

MATERIALS:

- Spectrophotometer(s) Didymium Filter
- Test solutions Cuvettes
- Wavelength verification solutions

PROCEDURE:

Spectrophotometer Components

1. Locate the power switch on the spectrophotometer and turn the instrument on.
2. While the instrument warms up (10 minute minimum), locate the following: fine adjustment, coarse adjustment, zero (dark current) adjustment, sample chamber, wavelength adjustment, and readout device.
3. Move the fine, coarse, zero, and wavelength controls to familiarize yourself with their operation.

Wavelength Calibration

There are several methods that can be used to verify proper wavelength adjustment. Your instructor will demonstrate the use of a Didymium Glass Filter and a Color Calibrator. Specific instructions will vary depending on the materials that are used. Following the instructions given by your instructor, check the wavelength calibration of your spectrophotometer and adjust if necessary.

Adjustment

Before using a spectrophotometer, there are several simple adjustments that must be made. These are:

1. Adjust wavelength adjuster to the desired wavelength
2. Adjust the zero (dark current) if instrument is so equipped.
3. Adjust for 100% transmittance (zero absorbance) using a blank solution (distilled water or other material as instructed)
4. Recheck the zero and 100% T, adjust if necessary.

These adjustments should be made before each use of a spectrophotometer. Step #3 must always be performed following any adjustment or change in the wavelength.

Unknown Readings

Your instructor will demonstrate the proper use of a cuvette in a spectrophotometer. Using this information and steps 1 through 4 from above, measure the absorbance of the standard and unknown solutions provided at a wavelength of 540 nm. Use distilled water as your blank solution. Record your results on the data sheet for this exercise.

Determining Concentration

There are three methods commonly used to determine concentration. They are instrument calculation, manual calculation, and use of a standard curve. Instrument calculation and standard curves will not be dealt with in this exercise.

Manual Calculation

To calculate an unknown concentration manually, the absorbance of the unknown is compared to the absorbance of a standard using the following formula.

A = Absorbance, [ ] = concentration

$$\frac{A_{unknown}}{A_{standard}} \times [standard] = [unknown] \tag{2.1.1}$$

Calculate the values for solutions 1 and 2 using the reading obtained earlier. The value for the standard is 200 g/L. Record your results on the data sheet for this exercise.

OPTIONAL EXERCISE:

Effect of incorrect instrument settings

A. Incorrect dark current setting

1. Adjust the zero (dark current) so that the instrument setting is not on infinity, but on 2.0 absorbancy reading at 540 nm.
2. Now measure the absorbance of the standard and unknown solutions provided. Record results on the data sheet.
3. Calculate the concentration of the unknown.

B. Incorrect blank setting

1. Adjust the dark current back to its proper setting.
2. Place blank solution in instrument and set the 100% T (zero absorbance) to read 90%T.
3. Repeat steps A2 and A3 above.

C. Comparison of different spectrophotometers.

1. Follow the above Procedure.
2. Calculate concentration of unknowns.
3. Record results on data sheet.

Data Sheet, Exercise #1	NAME:_____ DATE: _____

Absorbance and Concentration of Solutions

Material	Absorbance	Concentration
Standard	_____	200 g/L
Unknown #	_____	_____
Unknown #	_____	_____
OPTIONAL EXERCISES	**Absorbance**	**Concentration**
Standard	_____	200 g/L
Unknown #	_____	_____
Unknown #	_____	_____

Absorbance and Concentration of Solutions		
B	**Absorbance**	**Concentration**
Standard	_____	200 g/L
Unknown #	_____	_____
Unknown #	_____	_____
C	**Absorbance**	**Concentration**
Standard	_____	200 g/L
Unknown #	_____	_____
Unknown #	_____	_____

DISCUSSION QUESTIONS:

1. What is the advantage of using a calculation method to determine an unknown value?
2. Why must the 100% T be adjusted after the wavelength has been changed?
3. Why will the zero setting not change significantly as the wavelength is changed?

OPTIONAL QUESTIONS:

4. How does an incorrect zero (dark current) setting affect the calculated unknown concentration?
5. How does an incorrect zero absorbance setting affect the calculated unknown concentration?
6. Do two different spectrophotometers give the same values for unknowns after proper calibration?

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2.2: Absorbance Spectra

RELATED READING: Pages 92-94

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related readings, the student will be able to:

1. Measure absorbance values for a solution at a variety of wavelengths.
2. Construct a graph plotting wavelengths vs. absorbance values.
3. Identify the wavelength of maximum and minimum absorbance for each solution used.
4. Explain the relationship between solution color, spectrophotometer wavelength, and absorbance.

GLOSSARY

Absorbance maxima and minima: those wavelengths at which compounds in solution exhibit minimal or maximal absorbance of light. These minima and maxima are fixed for a stated set of conditions (pH, solvent, and temperature), but may vary if these conditions vary.

Rectilinear (linear) graph paper: most comonly used graph paper, in which the X (horizontal) and Y (vertical) axes are divided into equally spaced segments.

MATERIALS

- Spectrophotometer
- Sample Solutions
- Cuvettes

PROCEDURE

1. Fill one cuvette approximately 1/2 full with distilled water (water blank).
2. Fill additional cuvettes approximately 1/2 full with each of the five colored solutions (sample).
3. Select a spectrophotometer and adjust the wavelength to 375 nm.
4. Adjust the zero control.
5. Insert the water blank and adjust for zero absorbance (100% transmittance).
6. Remove the blank and insert the sample cuvette containing the red solution.
7. Read the absorbance and record it on the data sheet.
8. Read and record the absorbance of each solution at this wavelength.
9. Repeat steps 3 through 7 for each wavelength listed on the data sheet.
10. Empty the sample cuvettes and rinse them with water.
11. Adjust the spectrophotometer to 420 nm and blank it with distilled water.
12. Insert a strip of white paper into an empty cuvette and position the cuvette in the sample well.
13. Look down into the sample well and observe the color of the light that is visible on the paper strip. Record your observation on the data sheet.
14. Repeat steps 11, 12, and 13 at 540 nm and 610 nm.
15. Using the graph paper provided at the end of this exercise, construct a graph plotting wavelength on the X axis and absorbance on the Y axis. Plot the absorbance values for each different colored solution on the same graph., using a different colored ink or symbol for each curve.

DATA SHEET, EXERCISE #2	NAME: _____ DATE: _____

TEST SolutionS

Wavelength	RED	BLUE	GREEN	YELLOW	VIOLET
375					

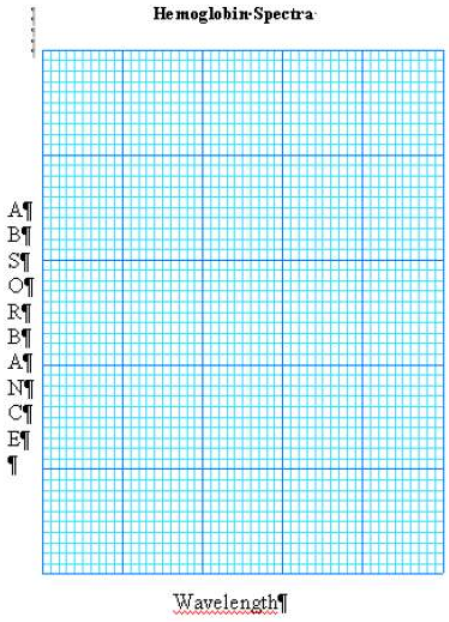
Wavelength	RED	BLUE	GREEN	YELLOW	VIOLET
400					
425					
450					
475					
500					
525					
550					
575					
600					
625					
650					

Solution Color	Absorbance Maximum	Absorbance Minimum
Red		
Blue		
Green		
Yellow		
Violet		

Color Observation	
Wavelength	**Color Observed**
420 nm	
540 nm	
610 nm	

Discussion Questions

1. Why were two absorbance peaks seen with the violet and green solutions?
2. Are there instrument factors that will cause variance from the curves you plotted?
3. Would you expect to see identical curves produced by different spectrophotometers?



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2.3: Standard Curve

RELATED READING: Pages 40, 87-89

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related readings, the student will be able to:

1. Collect data appropriate for construction of a standard curve.
2. Use this data to construct a standard curve.
3. Use a standard curve to determine the values for unknown solutions.

GLOSSARY

Semi-logarithmic graph: a graph in which one axis is a linear scale while the other axis is a logarithmic (log, to base 10) scale. The log scale may be 1, 2, or more cycles (factors of 10).

Stock solution: a primary solution of a compound or buffer, usually prepared in a concentrated form with pure solid or liquid compound. This solution is used to prepare more dilute, "working" solutions.

MATERIALS

- 16x 100 mm test tubes
- Stock $KMnO_4$
- Pipets
- Distilled Water
- Spectrophotometer
- Unknown solutions
- Graph paper

PROCEDURES

1. Label 16 x 100 mm test tubes one through five.
2. Using a 5 mL serological or Mohr pipet, pipet stock solution into tubes one through four in the amounts shown below.
3. Using another 5 mL serological or Mohr pipet, deliver distilled water into tubes 2, 3, 4, and 5 in the quantities shown.

DILUTION TABLE

Tube	Stock Solution	Distilled Water	Final Concentration
1	5.0 mL		50 mg/L
2	4.0 mL	1.0 mL	40 mg/L
3	3.0 mL	2.0 mL	30 mg/L
4	2.0 mL	3.0 mL	20 mg/L
5	1.0 mL	4.0 mL	10 mg/L

4. Mix each solution well.
5. Adjust the spectrophotometer to 540 nm and zero it with a distilled water blank.
6. Pour each solution Into a cuvette and read the absorbance and transmittance of each.
7. Record your results on the data sheet.
8. Read the absorbance and transmittance of the assigned unknown solutions and record them on the data sheet.
9. Using the values for your stock dilutions, plot absorbance vs. concentration on the linear graph paper and transmittance vs. concentration on semi-logarithmic graph paper; both graph papers can be found at the end of this exercise.
10. Using the graphs you have constructed, determine the concentration of each of the unknowns.

DATA SHEET, EXERCISE #3

NAME: _____
DATE: _____

STANDARDS

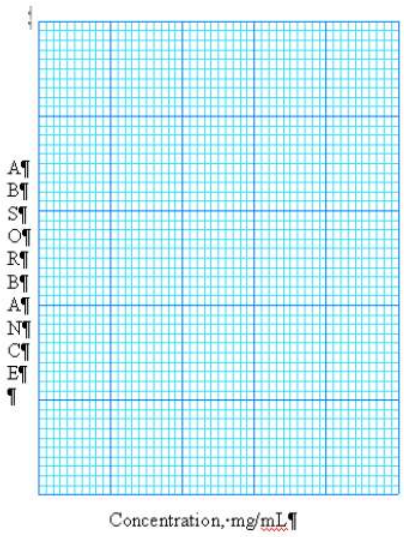
CONCENTRATION		
(mg/L)	Absorbance	Transmittance
5.0		
4.0		
3.0		
2.0		
1.0		

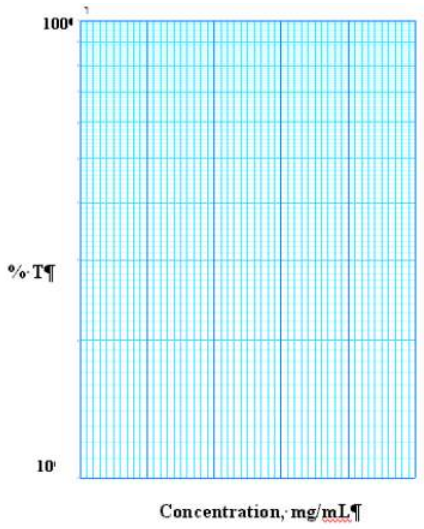
UNKNOWNS

	Absorbance	Transmittance	Concentration
Unknown #			
Unknown #			
Unknown #			

Discussion Questions

1. Why were different types of graph paper used?
2. Is the absorbance of $KMnO_4$ linear over the concentration range used for this lab?
3. Why was a wavelength of 540 nm used?
4. Compare the calculation method of determining concentration used in exercise 1 with the standard curve method you have just performed. What are the advantages and disadvantages of each? Which will yield a more accurate answer? Why?





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2.4: Buffer Preparation

RELATED READINGS: Pages 13-15, 17-19, 278-279, (Appendix B)

OBJECTIVES

Upon completion of this exercise, appropriate discussions, and related reading, the student will be able to:

1. Prepare buffer solutions from aqueous standards.
2. Measure pH using a pH meter.
3. Calculate pH values using the Henderson-Hasselbach equation.

PRINCIPLE

Buffers are used in almost all biochemical reactions to maintain optimum pH conditions. The buffer, composed of a weak acid (HA) and its conjugate base (A-), is chosen to provide maximal buffering capacity at a given pH. The calculation of the quantity of each buffer component needed to achieve the desired pH is determined by use of the Henderson-Hasselbach equation:

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \tag{2.4.1}$$

where pKa is the dissociation constant for the weak acid. The maximum buffering capacity occurs when the pH of the solution is equal to the pKa of the weak acid. Knowledge of how to prepare a buffered solution is critical for accurate biochemical analysis.

Materials

- pH calibrating solution
- 50 mL Volumetric Flasks
- pH meter Pipets
- O.2M Na_2HPO_4
- Distilled Water
- O.2M NaH_2PO_4 (pKa = 6.8)

pH METER CALIBRATION (Optional)

1. Remove electrode from storage solution.
2. Check that the internal KCl solution is filled to the proper level.
3. If the KCl level is incorrect, check with you instructor.
4. Carefully blot the electrode dry (do not wipe the electrode).
5. Immerse the tip of the electrode in the first calibration buffer (usually pH 7.00).
6. Turn the instrument to “measure”.
7. After allowing for equilibration (approx. 30 seconds), adjust pH reading to 7.00 according to your instructor’s directions.
8. Turn the instrument to “standby”.
9. Remove the electrode from the calibrating solution.
10. Rinse the electrode with distilled water and blot dry.
11. Repeat steps 5 and 6 using a second calibration buffer. This is usually pH 4.00 for a pH measurement <7.0, or pH 10.0 for a pH measurement >7.0.
12. After calibration is completed turn the instrument to “standby”.
13. Leave the electrode standing in neutral buffer or distilled water.

PROCEDURE

1. Carefully add the volumes of stock Na2HPO4 solution shown on the data sheet into clean labeled 50 mL volumetric flasks.
2. Bring to volume with NaH2PO4 and water as indicated on data sheet.
3. Mix each combination thoroughly.
4. Following the directions given by your instructor, measure the pH of each buffer solution and record the value on the data sheet.
5. Calculate the expected pH of each buffer using the Henderson-Hasselbach equation and record the value on the data sheet.

6. Seal each flask with parafilm and store as indicated by your instructor. These buffers will be used in the titration exercise (Exercise #5).

DATA SHEET, EXERCISE #4	NAME: _____ DATE: _____

Solution	Stock Solution	Stock Solution	H_2O	Measured pH	Calculated pH
	0.2 M Na_2HPO_4	0.2 M Na_2HPO_4	H_2O mL		
A	4.0 mL	46.0 mL	0		
B	12.3 mL	37.7 mL	0		
C	30.5 mL	19.5 mL	0		
D	43.6 mL	6.4 mL	0		
E	46.0 mL	4.0 mL	0		
F	2.0 mL	23.0 mL	25 mL		

CALCULATIONS

Calculate the theoretical pH of each buffer solution using the Henderson-Hasselbach equation

$$pH = pK_a + \log \frac{[A^-]}{[HA]} \tag{2.4.2}$$

Think! Which of the stock buffer solutions contains the conjugate base (A-) and which the weak acid (HA)?

Discussion Questions

1. Describe how a pH electrode measures pH.
2. Will buffer “E” that you prepared, be more effective against acid or alkaline changes? Why?
3. How much 0.2 M sodium acetate and 0.2 M acetic acid would be required to make 100 mL of pH 4.7 acetate buffer? (pKa = 4.75).

0.2 M	sodium acetate	_____mL
0.2 M	acetic acid	_____mL

4. Solution F has half the concentration of conjugate base and weak acid as solution A but has the same pH. How can you explain this?
5. How do the measured and calculated pH of solutions B & 0 compare to the pHs expected from Appendix B, in Kaplan and Pesce?

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2.5: Buffer Titration/buffering Capacity

RELATED READINGS: Pages 5-7, 13, 34-35, 278-281.

OBJECTIVES

Upon completion of this exercise, appropriate discussions, and related readings, the student will be able to:

1. Prepare standard dilutions of a stock buffer.
2. Select a pH Indicator based on reaction range and the pH of the solution to be titrated.
3. Perform a titration.

PRINCIPLE

The capacity of a buffer solution to maintain a constant pH depends on the specific components of the buffer, it's concentration and the amounts of acid or base challenging the buffer. Changing any of these variables will have an impact on the buffering capacity of a solution.

GLOSSARY

Buffer capacity: the ability of a buffered solution to accept added acid or alkali and maintain the initial pH of the solution.

Titration: the process by which known amounts of dilute acid or alkali are added to a solution to achieve a neutral pH and determine the amount of acid or base in the solution.

deionized water
0.2M NaH_2PO_4 24.0 grams NaH_2PO_4 q.s. to 1L with
deionized water

Comments: In order to prevent mishandling of your pH meter, carefully go over and demonstrate proper use of the instrument with the students. You may find it useful to show how easy it is to contaminate materials by not properly cleaning the electrode between samples.

Note that these phosphate solutions employ anhydrous salts. Weight will need to be adjusted if hydrated salts are used.

MATERIALS

- Flasks
- pH indicators
- Burettes
- Volumetric pipets
- 0.01 N NaOH solution
- 100 mL volumetric flasks
- Distilled water
- Buffer Solutions from Exercise # 4

PROCEDURE

1. Prepare a 1:10 and a 1:100 dilution from each of the stock buffers prepared in the previous exercise as indicated by your Instructor.
2. Measure the pH of the original buffer and each dilution, and record the values on the data sheet.
3. Pipet 5.0 mL of the 1:100 dilution of buffer into a small Erlenmeyer flask.
4. Add 3 drops of the *appropriate pH indicator to the flask; mix gently.
5. Using a buret or pipet, slowly titrate the 5 mL aliquot of buffer with 0.01 M NaOH solution. Mix continuously by swirling the flask during the titration.
6. Continue titrating until the pH indicator changes to the expected color.
7. Record the volume of NaOH solution required for the titration on the data sheet.
8. Repeat steps 3 through 7 for the 1:10 dilution and the undiluted (stock) buffer solutions. You may find It desirable to adjust the amount of stock buffer used for the titration based on the results of the 1:100 and 1:10 titrations.

9. Correct the volume of NaOH used for the 1:10 and undiluted buffers to compensate for sample size and record these values on the data sheet.

* Choose a pH indicator from the list shown below. When performing this type of procedure, it is desirable to use an indicator that, at the initial pH of the solution being titrated, shows one of it's characteristic colors and has not begun to change color. For example, if the buffer you are titrating has an initial pH of 4.6, you would use an indicator that shows one color at a pH below 5.5-6 and a different color at 7—8. The specific range is not critical in this exercise, but you must be able to visualize an end point.

Indicator	Acid Color	pH Range	Basic Color
Methyl Violet	Yellow	0.15-3.2	Violet
Congo Red	Blue	3.0-5.0	Red
Methyl Orange	Red	3.2-4.5	Yellow
Methyl Red	Red	4.4-6.2	Yellow
Phenol Red	Yellow	6.4-8.2	Red
Phenolphthalein	Colorless	8.2-10	Pink

DATA SHEET, EXERCISE #5	NAME: _____ DATE: _____

Buffers Being Titrated

	Undiluted	1:10	1:100
Initial pH			
Titration Sample Volume (mL)			
Volume of NaOH used (mL)			
Corrected NaOH Volume (mL)			

CALCULATIONS

$$\text{Corrected NaOH volume} = \frac{\text{Volume of NaOH used}}{\frac{x}{5}} \tag{2.5.1}$$

Where x is the volume of buffer being titrated.

Discussion Questions

1. What observation can be made regarding the pH of a buffer as it is diluted? How can this be explained on the basis of the Henderson-Hasselbach equation?
2. How did you expect the volumes of NaOH needed for the titrations to compare? Did your results support this expectation?
3. What observation can be made regarding the buffering capacity of a buffer as it is diluted?

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2.6: Flame Photometer

RELATED READING: Pages 5-7, 9-11, 97. See Methods in CD-ROM for Sodium and Potassium

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related reading, the student will be able to:

1. Identify the aspirator, burner, and photodetectors and explain the function of each.
2. Properly “start up” the flame photometer.
3. Dilute samples using a semi-automated diluter.
4. Calibrate the flame photometer by adjusting flow rate, Li sensitivity, Li blank, and Na/K standard.
5. Determine Na and K values on 4 unknowns with 90% accuracy.
6. Properly shut down the instrument.

PRINCIPLE

When Na and K are atomized into a flame, their valence electrons are excited to a higher energy level. As these electrons return to their normal (ground state) energy level, they emit photons of light energy. Each element emits light of a characteristic wavelength, the intensity of which is directly proportional to it’s concentration. Photodetectors connected to readout devices are used to detect the emitted light and quantitate Na and K levels. This same principle can be applied to other elements if appropriate filters and detectors are used. This type of instrument is no longer used for routine measurement of sodium and potassium, however induced plasma arc devices, which are used for measuring a wide variety of a variety of metals, use the same principles of excitation but at much higher temperatures.. This experiment is designed to demonstrate the principles of this technology. To minimize the variance of the procedure an internal standard, lithium, is used. Similarly for the induced plasma arc technique, internal standards are used to reduce the variance.

GLOSSARY

Aspirator: device that draws material, usually a liquid, up into an instrument for additional processing.

Flame ignitor: an electronic device that produces a spark to ignite the gases in a flame photometer.

Sample cups: small plastic cups used with many automated instruments, into which small volumes of sample are placed. The instruments usually aspirate sample directly from the sample cups.

MATERIALS

- Flame Photometer
- Diluter-dispenser
- Distilled water
- Na/K standard unknown samples
- Controls
- Lithium internal
- Sample cups
- Standard

DILUTION PROCEDURE

1. Run the diluter through several cycles of fill and dispense, ending with a dispense cycle,. Set the diluter for a 1:100 dilution.
2. Press down on the diluter control knob and allow the diluter to aspirate air.
3. Place a clean disposable sample cup labeled “Blank” under the diluter tip and press the control knob again. Allow the diluent to flow down the side of the sample cup to minimize splashing. Set this cup aside to be used later.
4. Put the stock Na/K standard under the diluter probe tip and press the control knob to aspirate an aliquot of standard.
5. Wipe the tip off gently.
6. Place a clean sample cup labeled “Standard” under the tip and dispense the diluted (1:100) standard into the cup.
7. Wipe the tip gently.
8. Repeat steps #3 through #6 to make a duplicate dilution of the stock standard.

9. Proceed to make duplicate dilutions of the controls and each unknown sample you were assigned.

FLAME IGNITION

1. Open propane shut-off valve located on top of the propane cylinder by turning the valve two turns counterclockwise (or as indicated on the instrument).
2. Turn on the air supply unit or open air valve fully.
3. Turn instrument switch to the ON position.
4. A faint clicking sound should be heard and should cease within a few seconds. The clicking sound is from the electronic flame ignitor. The “Flame On” indicator should then come on.
   If ignition does not take place within fifteen seconds, turn the Instrument off and repeat the ignition procedure.

ASPIRATION RATE

1. Pipet 1.0 mL of distilled water into a sample cup.
2. Put the aspirator tip Into the 1.0 mL water sample and measure the time it takes the Instrument to aspirate the 1 mL completely.
3. If this time is not 45 seconds (±5 seconds) the aspiration rate must be adjusted. Ask the laboratory instructor for assistance.
4. Observe the inside of the atomizer bowl for the formation of fine droplets.

CALIBRATION/OPERATION

1. Aspirate lithium blank (diluent) for approximately fifteen seconds.
2. Adjust the sensitivity control (Li Response) for maximum sensitivity (Maximum = center of the arrow). Note: If the sensitivity drifts significantly from this point during testing, the source of the problem must be identified and corrected.
3. Adjust the zero controls for Na and K to obtain a reading of 0.0 on each scale.
4. Aspirate diluted Na/K standard for approximately 15 seconds.
5. Adjust the balance (standard) control to achieve the Na and K values for the standard being used.
6. Check these values with those of the second dilution of the standard. If the values do not repeat (±2 Na, ±0.05 K), an additional set of dilutions should be made using the same diluter. If reproducible results are still not obtained, the diluter and flame photometer should be evaluated for malfunction.
7. Aspirate each of the dilutions to be tested and record each result on the data sheet. For any sample that does not give consistent results (Na ± 2, K ± 0.05), make additional dilutions until acceptable values are obtained.
8. After all samples have been tested, aspirate distilled water for a minimum of 1 minute to flush the aspirator and burner.

OPTIONAL ACTIVITIES

A. Under the guidance of the instructor, observe the color of the burner flame while aspirating distilled water, lithium diluent and separate solutions of sodium and potassium salts. Record your results on the data sheet.
B. To demonstrate the effect of contamination on flame photometry, place a lithium blank under the aspirator and zero the instrument. Remove the blank and “contaminate” it by touching it with your finger tip, stirring it with an applicator stick or some other method approved by the instructor. Sample the “contaminated” blank(s) and observe for changes from the zero values.
C. Re-adjust the aspiration rate so that it is approximately 55 sec. Reanalyze one of the samples for Na and K. Repeat, but after adjusting the aspiration rate to approximately 35 sec. Record Na and K values and actual aspiration rates on data sheet.

SHUT DOWN

1. Close the propane valve by turning in a clockwise direction.
2. When the “Flame On” indicator goes off, turn off the air supply, then the instrument power switch.

DATA SHEET, EXERCISE #6	NAME: _____ DATE: _____

RESULTS:

	Sodium (Na), mmol/L		Potassium (K), mmol/L	
Lithium Blank	Dilution 1	Dilution 2	Dilution 1	Dilution 2

	Sodium (Na), mmol/L		Potassium (K), mmol/L	
Na/K Standard				
Normal Control				
Abnormal Control				
Unknown 1				
Unknown 2				
Unknown 3				
Unknown 4				

OPTIONAL ACTIVITY

A. Flame Color:

Water	_____
Lithium Diluent	_____
Na	_____
K	_____

B. Lithium blank after contamination

Na	_____mmol/L
K	_____mmol/L

C.

Aspiration rate (mL/min)	Na, mmol/L	K, mmol/L

Discussion Questions

1. If the Lithium diluent is changed during a run, would the instrument need to be recalibrated? Why or why not?
2. How are variances in flame “quality” corrected for in flame photometry?
3. How are variances In aspiration rate corrected for in flame photometry?
4. What is the advantage to running samples in duplicate?
5. What are two reasons, other than to obtain a Li blank, for running the diluter through a cycle before making your first dilution?
6. Why Is It important to note the formation of droplets in the atomizer bowl?

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2.7: Chloride Determination (Coulometric Method)

RELATED READINGS: Page 285, See Methods in CD-ROM for Chloride

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related reading, the student will be able to:

1. Clean a chloridometer and prepare it for operation.
2. Blank the instrument according to manufacturer's instructions.
3. Check and adjust calibration of the instrument with the chloride (Cl^-) standard.
4. Prepare serum samples for Cl^- determination.
5. Determine the Cl^- concentration of unknown samples with 90% accuracy.
6. List the common causes of error in coulometric Cl^- determinations.

PRINCIPLE

In the coulometric measurement of chloride ions, silver ions (Ag^+) are generated at a constant rate by an electrode and released into a dilute acid solution. There they combine with Cl^- ions in the test sample to form an insoluble precipitate, AgCl. When all Cl^- is removed from solution, an excess of Ag^+ ions is detected by a sensor and the titration is stopped. Because the rate of Ag^+ ion generation is constant, the amount of time required to remove all Cl^- ions is directly proportional to concentration as shown in the following:

$$\frac{[Unknown]}{time_{Unknown}} = \frac{[Standard]}{time_{Standard}} \tag{2.7.1}$$

OR

$$[Unknown] = \frac{time_{Unknown}[Standard]}{time_{Standard}} \tag{2.7.2}$$

Some instruments report a titration time in seconds from which the Cl^- concentration can be calculated. Others perform an internal calculation and directly show a Cl^- value in mmol/L.

GLOSSARY

Spike: a known amount or volume of a substance that is added to a biological sample.

MATERIALS

- Buchler-Cotlove Chloridometer And/or Corning Chloridometer
- Sample beakers
- Acid Reagent
- Silver polish
- Acid Buffer
- Distilled Water
- Standard
- Controls
- Gelatin Reagent (indicator)
- Serum Samples

PROCEDURE

BUCHLER METHOD

1. Clean electrode with silver polish and rinse well with distilled water. This should be repeated approximately every 10 specimens (or whenever results become erratic) to insure reproducible results.
2. Blanking instrument
    a. Set DIRECT READER to BLANK

b. Set TITRATION RANGE to HIGH
c. Set D.C. MICROAMPERES to +10
d. Set DIGITAL READOUT to 0000
e. Set ADJUST TITRATE to 2
f. With a pipet or Oxford Pipettor, dispense 4 mL of Nitric-Acetic Acid into chloride titration cups.
g. Add 4 drops of gelatin indicator.
h. Place cup under stirrer and turn ADJUST TITRATE control to 1.
i. When the MICROAMPERES needle stops falling, set the adjustable microampere needle to 10 Microampere above this value.
j. Turn ADJUST TITRATE control to 2.
k. Run duplicate blanks to determine if readout is stable. Run additional blanks, if necessary, until stability is obtained.
l. Adjust the Time Delay Dial to the number appearing on the digital readout. This interval will be automatically subtracted from the final mmol/L.

3. Titrating Samples
   a. Set DIRECT READER to TITRATE.
   b. Set DIGITAL READOUT to 0000.
   c. With a pipet or Oxford Pipettor dispense 4 mL of Nitric—Acetic Reagent into chloride titration cups.
   d. Transfer 0.05 mL of standard, specimen, or control into reagent with pipettor.
   e. Add 4 drops of indicator.
   f. Place cup under stirrer and turn ADJUST TITRATE to 1.
   g. When MICROAMPERES needle moves to near 0, turn ADJUST TITRATE control to 2.
   h. Multiply the number appearing on the digital read-out after titration by 2 to determine the chloride concentration in mmol/liter.
   i. Repeat steps c through h for each additional sample to be tested.
   j. When all samples have been tested, leave the titration electrode immersed in distilled H_2O.

CORNING METHOD

Make certain all electrodes are clean and have been rinsed with distilled water. Use silver polish if necessary.

Conditioning

1. Place approximately 15 mL (exact volume is not critical) of Acid Buffer into a sample cup.
2. Place cup on platform.
3. Squeeze release clips on the electrode head and gently lower electrodes into buffer solution.
4. Press the “Condition" switch down. The instrument should operate and stop at <200. If the instrument continues beyond a reading of 300, turn the power switch off and consult with your instructor.

Calibration

1. Using the same sample cup and solution that were conditioned, pipet 100 uL of standard into the acid buffer solution.
2. Press the titrate switch down. When titration stops, the value displayed is the Cl- concentration in mmol/L.
3. If the observed reading for the standard is ±2 mmol/L from the expected value, proceed with the titration procedure. If the instrument is out of calibration, consult with your instructor and then proceed as follows.
4. Turn the appropriate calibration screw on the back of the instrument clockwise to increase the value or counter clockwise to decrease the value. One half turn will increase/decrease the value approximately 2 mmol/L.
5. Continue testing and adjusting the standard until stable calibration is achieved.

Titration

1. Pipet 100 uL of sample (patient, control) into the sample cup*.
2. Press the titrate control down.
3. When titration stops, read and record your value on the data sheet.
4. Repeat steps 1, 2, and 3 for each sample to be tested.
5. When all titrations are complete, place the electrodes in a sample cup containing 15-20 ml of distilled water.

*The conditioned acid buffer solution may be used for multiple samples, and should be changed within 3 samples after the change solution indicator comes on. When changing the acid buffer solution repeat steps 1 through 4 of the conditioning procedure. It is

not necessary to re- calibrate when changing the buffer solution.

OPTIONAL EXERCISE

1. Add 0.05 mL of a 1 M KBr solution to one of the previously analyzed unknowns or controls.
2. Vortex-mix the sample.
3. Assay the sample for Cl^- as per the above directions.
4. Record Cl^- result on the data sheet.

DATA SHEET, EXERCISE #7	NAME: _____ DATE: _____

RESULTS

	Cl^-, (mmol/L)	
	Buchler	**Corning**
Standard		
Normal Control		
Abnormal Control		
Unknown 1		
Unknown 2		

OPTIONAL EXERCISE

$$\text{Unspiked } [Cl^-] = \text{original result for sample} \times (0.95) = \_\_\_\_\ mEq/L \tag{2.7.3}$$

$$\text{Sample spiked with } Br^- = \_\_\_\_\ mEq/L \tag{2.7.4}$$

$$\%\text{ reactivity of Br} = \frac{\text{Spiked result} - \text{unspiked result}}{50\ mEq/L} \times 100\% \tag{2.7.5}$$

Discussion Questions

1. If Br^- reacts with Ag^+ in the same manner as Cl^-, what error would be introduced to a sample if it contained unusually high levels of Br^- ions?
2. Do you think that iodide (I^-) will interfere in this assay?
3. Why is it necessary to blank or condition only the first titration?

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2.8: Electrolytes/anion Gap

RELATED READING: Chapter 24

See Methods in CD-ROM for Anion Gap, Chloride, Carbon Dioxide, Sodium and Potassium

OBJECTIVES

Following completion of this exercise, appropriate discussion, and related reading, the student will be able to:

1. Determine Na+, K+, and Cl- levels in serum samples with 90% accuracy.
2. Given the CO2 values for the samples tested, calculate the anion gap on these samples using the measured values.
3. List the causes of an increased and a decreased anion gap.

PRINCIPLE

Routine "electrolytes" usually consist of Na^+, K^+, Cl^-, and HCO_3^-(CO_2) determinations. This allows for the calculation of an anion gap. This value is used to evaluate the relationship between anions and cations. There are several ways this calculation can be performed. Two of the more common methods are:

Reference Intervals

Anion Gap = (Na^+, K^+) - (Cl^- + HCO_3^-)	11-18 mmol/L
Anion Gap = Na^+ - (Cl^- + HCO_3^-)	7-14 mmol/L

The reference intervals are instrument specific. The bias between instruments will determine the reference interval for an institution.

We have used a "contamination" exercise for chlorides similar to the one suggested for flame photometry. Care should be exercised in using the acid reagent.

*Acid reagent can be prepared from stock reagents in the following proportions:

- 900 mL deionized water
- 6.4 mL conc. nitric acid
- 100 mL conc. glacial acetic acid

MATERIALS

Serum Samples Instruments may be any of these:

- Controls
- Flame photometer, Chloridometer
- Na/K Standard
- ISE chemistry instrument
- Cl Standard
- Blood gas instrument
- Acid Buffer
- Point of care device
- Sample cups
- Lithium Diluent if flame

PROCEDURE

Perform Na^+, K^+, and Cl^- (and CO_2 if available) determinations on the serum samples provided. Using the CO_2 values provided by your instructor, determine the Anion Gap for each sample. For the samples with abnormal Anion Gap values, consult with your instructor to determine what other test results are known.

DATA SHEET, EXERCISE #8	NAME: _____ DATE: _____

RESULTS:

	Na^+	K^+	Cl^-	CO_2	ANION GAP
	Analyte Concentrations, mmol/L				
Normal Control					
Abnormal Control					
Sample #					
Sample #					
Sample #					
Sample #					
Sample #					
Sample #					

Discussion Questions

1. What are two common diseases or conditions that cause an elevated anion gap?
2. Which of the additional test results provided by your instructor would have a direct affect on the anion gap.
3. Which of the additional test results are associated with diseases that produce abnormal anion gaps?
4. An abnormally low anion gap has been suggested as a quality control indicator for false laboratory results. For which analyte? What is the interferent? By which method? Why is a low anion gap a useful indicator?

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2.9: Serum Osmolality

RELATED READINGS: Pages 265-271. See Methods in CD-ROM for Osmolality

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related reading, the student will be able to:

1. Determine serum osmolatlity using a vapor pressure osmometer.
2. Determine serum osmolality using a freezing point osmometer.
3. Estimate the osmolality by calculation, given the concentrations of several components of a sample.
4. Discuss possible explanations for discrepancies seen in the results obtained using the three different methods of testing.

PRINCIPLE

Osmolality is an expression of the concentration of disolved particles (solute) in a specific amount of solution (solvent). This determination can be made directly or indirectly. The direct methods are usually based on the freezing point or the vapor pressure of a solution. The indirect method involves measuring the concentrations of major solutes and then calculating the osmolality. Each method has certain advantages and shortcomings. The reference range for serum osmolality is in the 280-300 mOsm/kg of water range.

MATERIALS

- Standards
- Vapor Pressure Osmometer (Wescor)
- Freezing Point Osmometer (Advanced or Precision Type)
- Serum Samples
- Controls

Comments

Students can perform serum CO_2 determinations prior to or as part of this exercise. Alternatively, you can provide CO_2 values for each of the samples used. This exercise provides an opportunity to illustrate how “other factors” can corroborate an abnormal anion gap. Students will first need to determine that the anion gap is abnormal and then, using a serum chemistry profile or “case history” can evaluate the validity of the abnormal results.

PROCEDURE

1. For each control or sample that you were assigned, determine the osmolality using both types of instruments that are available. Refer to the operating instructions dealing with the specific instrument you will be using. Record your results on the data sheet. Make a notation of the appearance of the serum samples you tested.
2. Your instructor will give you the concentrations of Na, K, BUN, and glucose of one or more of your samples. Using equations 14-2 (p.267) and ones from the Osmometry method in the CD-ROM and calculate the serum osmolalities. Record these values on the data sheet.

OPTIONAL EXERCISES:

A. 1. Your instructor will provide you with the following solutions: 100 mmol/L NaC1 and 100 mmol/L urea.
   2. Measure the osmolalitity of each solution and record on the data sheet.

B. Prepare a hemolysate of red blood cells, as described in Exercise #15, Bilirubin. Add a very small volume of this hemolysate; for example 10 L, to 1 mL of a sample whose osmolality had previously been measured. Record on data sheet.

C. For those laboratories who have access to both freezing point depression and vapor pressure osmometers, this experiment can demonstrate the effect of a volatile solute on osmolarity.
   1. Prepare an ethanol stock solution (75000 μg/mL, 1630 mmol/L) by adding 10 mL of 95% ethanol to 100 mL of distilled water.
   2. Place 1 mL of a serum whose osmolality had previously been measured and calculated into each of 2 small glass test tubes.
   3. To one labeled “control”, add 10 μL of distilled water. To the other labled “spike” slowly add 10 μL of the ethanol stock solution while gently vortex-mixing the sample. Mix both solutions.

4. Measure the osmolality by both freezing point and vapor pressure osmometers.
5. Record on data sheet.
6. Calculate the osmolal gap, as described by Equation 14-3, p. 267 of Kaplan and Pesce.

DATA SHEET, EXERCISE #9	NAME: _____ DATE: _____

RESULTS

	Appearance	Vapor Pressure	Freezing Point	Calculated
	Osmolarity, mmol/L			
Normal Control				
Abnormal Control				
Sample #				
Sample #				

OPTIONAL EXERCISES

A.	Osmolality, mmol/L
0.1 M NaC1	_____
0.1 M urea	_____

B. The hemolyzed sample was (circle one) mildly, moderately, grossly hemolyzed.

Previously measured osmolality (sample #____) =	_____mmol/L
Hemolyzed sample's osmolality =	_____mmol/L

C.	Vapor Pressure	Freezing Point
	Osmolality, mmol/L	
Unspiked Sample		
Ethanol spiked sample		
Osmolality gap		

CALCULATIONS

A.
$$\text{calculated mOsm/L} = 2 \cdot \text{Na (mmol/L)} + \frac{glucose\ mg/L}{180\ mg/mol} + \frac{BUN\ mg/L}{28\ mg/mmol} \quad (2.9.1)$$

B.
$$\text{calculated mOsm/L} = 1.86 \cdot \text{Na (mmol/L)} + \frac{glucose\ mg/L}{180\ mg/mol} + \frac{BUN\ mg/L}{28\ mg/mmol} \quad (2.9.2)$$

Discussion Questions

1. Does hemolysis appear to interfere with either instrumental method?
2. Provide a plausible explanation of any discrepancy seen in the results, using the different methods.
3. What appears to be the major advantage/disadvantage of each method?
4. How does the presence of a volatile solute affect the three methods of determining osmolality? What is the effect of a volatile solute on the osmolality gap?
5. Can you explain any difference in the osmolalities of the 0.1 M NaC1 and 0.1 M urea solutions?

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2.10: Serum Glucose- Trinder Method

RELATED READING: Chapter 32. See Methods in CD-ROM for Glucose.

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related reading, the student will be able to:

1. Determine serum glucose values with 90% accuracy using a colorimetric method.
2. Identify and explain specific differences between glucose oxidase and hexokinase methods.

PRINCIPLE

The Trinder method for glucose analysis utilizes the coupled enzyme reactions shown below:

$$Glucose + O_2 \xrightarrow{\text{glucose oxidase}} glucuronic\ acid + H_2O_2 \tag{2.10.1}$$

$$H_2O_2 + reduced\ dye \xrightarrow{\text{horseradish peroxidase}} oxidized\ dye + H_2O \tag{2.10.2}$$

The reaction is linear to approximately 10,000 mg/L (1000 mg/dL) and reaches its endpoint in 15-20 minutes at 37°C.

GLOSSARY

End-point - refers to the final state to be attained. In the case of chemical assays, this term refers to the point when the reaction is essentially completed and there is no net production of reaction product.

Water-blank -when the absorbance of a solution is measured vs pure water; water is used to “zero” the instrument (100% T).

MATERIALS

- Glucose standard
- Glucose Oxidase Reagent
- Controls
- Test Tubes (13 x 100 mm)
- Pipets Incubator
- Spectrophotometer

PROCEDURE

1. Label sufficient 13 x 100 mm test tubes for each standard, control, and patient sample to be tested.
2. Pipet 2.0 mL of glucose reagent into each tube.
3. Add 10 μL of standard, control, or serum to each appropriate tube.
4. Mix well.
5. Incubate each tube at 37°C for 20 minutes.
6. Using appropriate cuvettes, measure the absorbance of each reaction mixture at 525 nm against a water blank.
7. Calculate each glucose concentration using the absorbance and concentration of the standard by the proportion method (see Exercise #1).
8. Record your results on the data sheet.
9. Record the appearance of the serum samples on the data sheet.

OPTIONAL EXERCISE

1. Prepare a hemolysate as described in Exercise #15 Bilirubin
2. Add approximately 10 μL of the hemolysate to 1 mL of a sample whose glucose was previously measured. Remeasure the glucose concentration.
3. Record the value on the data sheet.

DATA SHEET, EXERCISE #10

NAME: _____
DATE: _____

RESULTS

Glucose, mg/L	A525	Measured Value	Target Value
Standard			
Normal Control			
Abnormal Control			
Sample #			
Sample #			
Sample #			

OPTIONAL EXERCISE

Sample #____:

Previously measured glucose ____x 0.99 = _____mg/L

Hemolyzed sample's glucose concentration = _____mg/L

Discussion Questions

1. What is (would be) the effect of hemolysis on the measurement of glucose by this method? How could you correct for the presence of hemolysis in a sample when using this method?
2. Is this reaction more or less specific than a glucose oxidase/oxygen electrode method? Why?
3. Bilirubin will cause interference in this method. Why can this interference not be corrected by the procedure discussed in question 1?
4. What are the major advantages and disadvantages of the glucose oxidase/peroxidase method of determining glucose?

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2.11: Glucose Tolerance Test

RELATED READING: Chapter 32. See Methods in CD-ROM for Glucose.

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related reading, the student will be able to:

1. Explain how to perform a Glucose Tolerance Test (GTT) for Gestational Diabetes.
2. Construct a graph showing the relationship of time to serum glucose concentration.
3. Perform calculations that allow evaluation of a glucose tolerance test.
4. Discuss the possible physiological status of the patient that would produce the observed results.
5. Determine urine glucose concentrations (Optional).

PROCEDURE

1. Perform glucose determinations on the patient samples provided using the procedure given in the previous exercise (#10). Each student will have GTT samples from one patient drawn at the following intervals: fasting, 60 min., 120 min., 180 min. The determination of urine glucose levels is an optional activity. Your instructor will give you specific instructions dealing with that procedure.
2. Perform glucose determinations on the urine samples provided with your GTT samples and record the results on the data sheet (optional).
3. Plot the results of serum glucose (mg/L, Y or vertical axis) vs. time (hours, X or horizontal axis) to establish a Glucose Tolerance Curve. Using the discussion of the O'Sullivan-Mahan test (as modified by Carpenter-Coustan) in Chapter 32 determine if the patient has gestational diabetes. The test uses a 100-g glucose load. Cut-off criteria for a normal response to this test are: fasting 950 mg/L, 1-hour 1800 mg/L, 2-hour 1550 mg/L, 3-hour 1400 mg/L. Two or more of the plasma glucose values must be equaled or exceeded for a diagnosis of gestational diabetes.

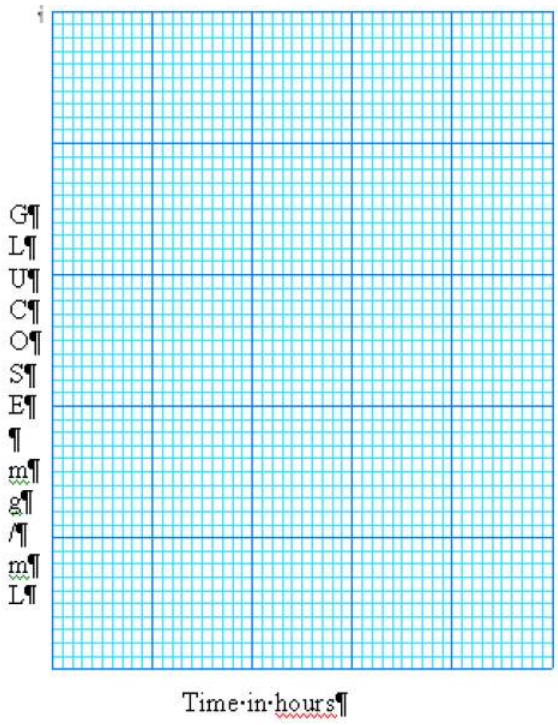
DATA SHEET, EXERCISE #11

NAME: _____
DATE: _____

	Absorb. 525 nm	Calculated Glucose Value (mg/L)	Target Glucose Value (mg/L)	Urine Glucose (Optional) (mg/L)	Exceeds limits?
Standard					
Normal Control					
Abnormal Control					
Fasting					
1 Hr.					
2 Hr.					
3 Hr.					

Discussion Questions

1. What do the results of your glucose values, the O'Sullivan-Mahan test and graph suggest regarding this patient?
2. Do the urine glucose values you obtained correspond to the serum values in the expected manner? Explain.
3. What are the advantages and disadvantages of using a 3 hr. post-prandial evaluation instead of a 4 or 5 hour tolerance test?



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2.12: Total Serum Protein

RELATED READINGS: Chapter 27. See Methods in CD-ROM for Total Serum Protein

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related reading, the student will be able to:

1. Measure serum total protein with 90% accuracy using the biuret and refractive index methods.
2. Compare the values obtained from colorimetric and refractive index measurement of protein.
3. Identify any significant difference in values determined by each method.
4. List the substance(s) that will interfere with each method.

PRINCIPLE

Biuret — Cupric ions in an alkaline solution will bind to the peptide bonds of protein molecules and form a blue-violet color. This complex can be measured photometrically and used to determine protein concentrations.

Refractive Index - Serum or other fluids containing dissolved solids will refract incident light. The amount of refraction is proportional to the quantity of dissolved solids. If corrections are made for non-protein substances, this method provides a reliable estimation of protein concentration.

glossary

TS meter - another name for a refractometer. TS stands for Total Solids, which is what the refractometer measures.

Normal saline -concentration of NaC1 (saline) approximating physiological concentrations, usually 0.15 M, 0.85%, or 0.9%.

Reagent blank -a solution consisting of reagent and saline (instead of sample) in the proportions employed in the reactions used to “zero” a spectrophotometer (100% T).

MATERIALS

- Standard
- Biuret reagent
- Test Tubes
- Controls
- Distilled Water
- Pipets
- Patient samples
- Spectrophotometers
- 3% NAOH
- Normal Saline TS (Total Solids) Meter

PROCEDURE

Biuret Method

1. Label sufficient 13 x 100 mm test tubes for a reagent blank, controls, and the serum samples to be tested.
2. Using a 5 mL serological pipet, pipet 4.9 mL of 3% NaOH into each tube.
3. Pipet 100 μL of distilled water into the reagent blank.
4. Pipet 100 μL of control, standard, or serum into each appropriately labeled test tube.
5. Pipet 1.0 mL of biuret reagent into all tubes.
6. Mix with a vortex mixer or by inversion.
7. Incubate at room temperature for 20 minutes.
8. Adjust the spectrophotometer to 520 nm and adjust for zero absorbance using the reagent blank. Transfer the reaction mixtures into an appropriate cuvette and measure the absorbance of each tube at 520 nm.
9. Record your results on the data sheet and calculate the total protein values for each sample using the proportional calculation method.

10. Record any unusual characteristics of the samples.

Refractometer Method

1. Clean the refractometer carefully with distilled water and blot dry.
2. Place a drop of sample (standard, control, or serum) on the “stage” of the refractometer.
3. Using sufficient light, look into the instrument and determine the total protein value from the appropriate scale and record it on the data sheet.
4. Clean the “stage area” thoroughly with distilled water and blot dry.
5. Repeat steps 2 through 4 for each additional sample to be measured.

OPTIONAL ACTIVITY

Measure the refractive index of distilled water, 0.85% NaCl, and any other material your instructor has prepared for you. Record your results on the data sheet.

DATA SHEET, EXERCISE #12	NAME: _____ DATE: _____

RESULTS

Total Protein, g/L	Absorbance at 520 nm	Biuret	TS meter	Sample Comments
Standard				
Normal Control				
Abnormal Control				
Sample #				
Sample #				
Sample #				
Sample #				
Distilled water				
0.85% NaCl				
Other				

Discussion Questions

1. Were there any significant differences in the values determined by the two methods? If there were significant differences, explain the possible causes.
2. Do you think that refractive index could be used as a regular method for serum protein determinations? If yes, what limitiations does it have. If not, why not?
3. What are the major interferences in the biuret method? Refractometry method?

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2.13: Serum Albumin (Bromcresol Green Method)

RELATED READING: Chapter 27. See Methods in CD-ROM for Albumin.

OBJECTIVES

Upon completion of this exercise, discussion, and appropriate reading, the student will be able to:

1. Perform serum albumin determinations on 4 samples with 90% accuracy.
2. Explain the principle behind the Bromcresol Green method for albumin.
3. List the factors that will cause interference with this method.

PRINCIPLE

Albumin is known for its ability to bind many types of organic compounds, including organic dyes. When albumin binds with Bromcresol Green (BCG) it causes a change in the absorbance maximum of BCG. This change can be measured spectrophotometrically and used to determine albumin concentration.

GLOSSARY

Reagent blank -a solution used to zero a spectrophotometer (set 100% T); usually contains all diluent and reagent in the reaction solution, but no sample. Some reagent blanks do contain the sample as well, but they lack one crucial reagent component needed to produce a color-yielding reaction.

MATERIALS

- Calibrator
- Serum samples
- Controls
- Bromcresol green reagent
- Spectrophotometer
- Pipets
- 13 x 100 mm test tubes

PROCEDURE

1. Label sufficient 13 x 100 mm test tubes for all samples, controls, calibrators, and a reagent blank.
2. Using a 5 mL serological pipet, pipet 2.5 mL of BCG reagent into each tube.
3. Pipet 10 μL of each sample, control or calibrator into the appropriate tubes. Add 10 μL of distilled water to the reagent blank tube.
4. Mix all tubes by inversion.
5. Read the absorbance of each tube at 628 nm against the reagent blank and record your results on the data sheet.
6. Calculate the albumin concentration of each sample in g/L using the absorbance/concentration proportion method (see Exercise #1 Basic Spectrophotometry).

OPTIONAL EXERCISE: FAST KINETIC MEASUREMENT

1. Follow steps 1 and 2 in the procedure.
2. Add 10 μL of sample or calibrator and mix rapidly.
3. At 10 seconds after mixing, measure the absorbance at 628 nm.
4. Read absorbance again at 1 minute and record on data sheet.
5. Calculate the μA (60 second reading minus the 10 second reading) for each specimen.
6. Calculate the albumin concentrations of the unknowns by methods of proportions (see Exercise #1).

$$\text{Concentration albumin} = \frac{\Delta A_{stand} \times [Alb]_{stand}}{\Delta A_{unknown}} \tag{2.13.1}$$

DATA SHEET, EXERCISE #13

NAME: _____
DATE: _____

RESULTS

	Absorbance	Albumin g/L (Target Value)
Calibrator		
Normal Control		
Abnormal Control		
Sample #		
Sample #		

OPTIONAL EXERCISE

	Absorbance at 628 nm		Albumin, g/L
	10 sec.	60 sec.	ΔA
Calibrator			
Normal Control			
Abnormal Control			
Sample #			
Sample #			

Discussion Questions

1. A hemolyzed sample is brought to the laboratory for albumin analysis. Can the sample be used? Discuss.
2. Why is it not desirable to incubate the reaction before measuring the absorbance?
3. Some analyzers can measure the absorbance of the BCG reaction within 30 seconds after adding sample. Does this tend to increase the specificity of the reaction?

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2.14: Serum Creatinine (Jaffe Method)

RELATED READING: Chapter 26. See Methods in CD-ROM for Creatinine.

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related readings, the student will be able to:

1. Explain the principle of protein-free filtrate preparation.
2. Prepare a protein free filtrate of serum.
3. Explain why a filtrate preparation is required in procedures of this type.
4. Determine the creatinine value of 2 unknown samples with 90% accuracy.
5. List substances that cause interferance with the alkaline picrate method of creatinine analysis.

PRINCIPLE

Creatinine reacts with picric acid in an alkaline solution to form a reddish colored complex. The reaction is commonly known as the Jaffe reaction and the red colored product as the Janovski complex.

MATERIALS

- 16 x 100 mm Test Tubes
- Creatinine Standard
- Picric Acid Reagent
- Controls
- Sodium tungstate
- Patient Samples
- Sulfuric Acid
- Spectrophotometer
- Distilled Water
- Sodium Hydroxide
- Table Top Centrifuge

PROCEDURE

1. Label 16 x 100 mm test tubes for each control and patient sample to be tested.
2. To each control and patient sample tube, add 1 mL sodium tungstate reagent, 1mL sulfuric acid reagent, and imL of distilled water. Mix well.
3. Add 1 mL of each control or patient serum to the appropriate tube.
4. Mix well and centrifuge for 5 minutes at 1500 RPM.
5. Label clean 16 x 100 mm test tubes for a blank, standard, and each control or sample for which a “filtrate” was prepared.
6. Add reagents/samples to each tube according to the following chart. It is acceptable to add picric acid reagent and NaOH sequentially to all tubes after all other materials have been added. Each test tube should contain the same final volume.

Volume of Reagent (mL)

	Distilled Water	Working Standard	Filtrate	Picric Acid	NaOH
Reagent Blank	4	0	0	1	1
Standard	3.5	0.5	0	1	1
Control (s)	2	0	2	1	1
Patient Sample	2	0	2	1	1

7. Mix well and allow to stand for 15 minutes at room temperature.
8. Transfer the contents of test tube to an appropriate cuvette and read the absorbance at 510 nm against the blank solution.

9. Record the results on the data sheet.
10. Determine the creatinine values for the control and patient samples by proportional calculation (see Exercise #1) using the concentration of the standard and it's absorbance.

OPTIONAL EXERCISE

Add a 0.10 mL of a solution of acetone (80 g/L) to 2.0 mL of a control or patient sample while mixing. Test solution for ketones by dipsticks. Assay unspiked and spiked samples for creatinine. Record results on data sheet.

DATA SHEET, EXERCISE #14	NAME: _____ DATE: _____

RESULTS

	Absorbance	Creatintine Value (mg/L)	Target Value
Creatinine Standard			
Normal Control			
Abnormal Control			
Pt #			
Pt #			

Optional Exercise

Unspiked sample		
Acetone-spiked sample		

Discussion Questions

1. What substances will cause interference in the alkaline picrate method?
2. What substances will interfere when using a protein free filtrate?
3. What problems will arise if the procedure is carried out at a temperature above room temperature?
4. Do you suspect that any of the samples you tested may have shown positive interference? How could this be dealt with?
5. In the optional exercise, was there any interference by the added acetone? Might there have been an interference if the reaction had been performed in the kinetic or endpoint mode without first preparing a protein-free filtrate?
6. Was the method in this experiment an end—point or kinetic procedure?

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2.15: Bilirubin (Waters and Gerande – DMSO Method)

RELATED READING: Chapters 27, 36. See Methods in CD-ROM for Bilirubin.

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related reading, the student will be able to:

1. Perform direct and total bilirubin analysis on 2 samples with 90% accuracy.
2. Explain the effect of free hemoglobin on bilirubin determinations in this method.

PRINCIPLE

Bilirubin will react with diazotized sulfanilic acid to yield a purple compound, azobilirubin. This product can be quantitated spectrophotometrically at 560 nm. This procedure is used to measure the soluble conjugated (direct-reacting) bilirubin alone or in combination with the less soluble unconjugated (indirect-reacting) bilirubin. To measure total bilirubin, a suitable solvent that allows for the measurement of both direct and indirect—reacting bilirubin is used. The unconjugated bilirubin concentration is the difference between direct-reacting and total bilirubin values. Care should be taken to protect samples from strong or prolonged light because bilirubin pigments are light sensitive.

MATERIALS

- Total bilirubin reagent
- Spectrophotometer
- Direct-reacting bilirubin
- Cuvettes
- Sodium nitrite
- Pipets
- Standard
- 13 x 100 mm
- Test Tubes
- Controls
- Samples

PROCEDURE

Total Bilirubin

1. Label a sufficient number of 13 x 100 mm test tubes as “Test” and “Blank” for each standard, control, and serum sample to be tested.
2. Add 3 mL of total bilirubin reagent to all “Blank” and “Test” tubes.
3. Using a Pasteur pipet, add 1 drop of sodium nitrite reagent to each “Test” tube.
4. At precise one minute intervals, add 200 μL of the appropriate standard/control/sample to each “Test” and “Blank” tube.
5. Mix well.
6. Incubate at room temperature for exactly 5 minutes.
7. Read the absorbance of the first sample against it’s own blank at 560 nm; repeat for each subsequent sample at 1 minute intervals.
8. Record your results on the data sheet.

Direct Bilirubin

1. Label a sufficient number of 13 x 100 mm test tubes as “Blank” and “Test” for the patient samples only.
2. Add 3 mL of direct bilirubin reagent to each “Blank” and “Test” tube.
3. Using a Pasteur pipet, add 1 drop of sodium nitrite reagent to each “Test” tube.
4. At precise one minute intervals, add 200 μL of serum to the “Blank” and “Test” tube for each sample and mix.
5. Incubate at room temperature for exactly 5 minutes.
6. Read the absorbance of the first sample against it’s own blank at 560 nm; repeat for each subsequent sample at 1 minute intervals.

7. Record your results on the data sheet.

OPTIONAL EXERCISE

1. Prepare red blood cells washed free of plasma. Resuspend the cells with distilled water to 5 times the original blood volume.
2. Add 0.05 mL of this hemolysate to one of the controls or samples run in the above procedure described for total bilirubin.
3. Record the results on the data sheet.

DATA SHEET, EXERCISE #15	NAME: _____ DATE: _____

RESULTS

	Absorbance (total)	Total Bilirubin (mg/L)	Absorbance Direct	Direct (Conjugated) Bilirubin (mg/L)	Indirect (Unconjugated Bilirubin (mg/L)
Standard					
Normal Control					
Abnormal Control					
Sample #					
Sample #					

CALCULATIONS

Calculate the total bilirubin for the controls and serum samples. Calculate the direct and indirect bilirubin for the patient serum samples. Use the absorbance/concentration proportion method.

$$\begin{gathered}\text{Total bilirubin} \\ = \text{conjugated (“direct”) bilirubin} - \text{unconjugated (“indirect”) bilirubin}\end{gathered} \tag{2.15.1}$$

$$\text{Unconjugated bilirubin} = \text{Total} - \text{Direct bilirubin} \tag{2.15.2}$$

Optional Exercise

	Absorbance	Total Bilirubin (mg/L)
Hemoglobin-spiked sample		
Unspiked sample (#)		

Discussion Questions

1. What substances are known to cause interference with this method?
2. Why is it necessary to run a serum blank with each sample?
3. What is the purpose of the sodium nitrite in the reaction?
4. Is the type of blank employed in this exercise a reagent or sample blank?
5. What compound was employed in the “total” reagent to solubilize the unconjugated bilirubin? What other solubilizers were also used?

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2.16: Cholesterol (Total and HDL)

RELATED READING: Chapter 33. See Methods on CD-ROM for Cholesterol.

OBJECTIVES

Upon completion of this exercise, discussion, and appropriate readings, the student will be able to:

1. Determine serum cholesterol values with 90% accuracy.
2. Separate HDL from other lipoprotein particles.
3. Determine HDL cholesterol values with 90% accuracy.
4. Using triglyceride, HDL—cholesterol, and total cholesterol values, calculate LDL-cholesterol concentrations.

PRINCIPLE

Cholesterol can be measured by a variety of methods. The most common of those currently in use utilizes a series of enzymatic pathways to produce a measurable product. The following series of reactions are widely used for point of care, automated and manual methods.

a. $$\text{Cholesterol - esters} \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{Fatty acids} \tag{2.16.1}$$

b. $$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest -4 - en -3-one} + H_2O_2 \tag{2.16.2}$$

c. $$H_2O_2 + 4 - \text{aminophenazone (or some other dye)} \xrightarrow{\text{Peroxidase}} \text{Oxidized dye } (A_{max}, 500nm) + H_2O \tag{2.16.3}$$

This method can be used to determine both total cholesterol and HDL cholesterol values.

MATERIALS

- 10 x 75 mm test tubes
- Serum Samples
- Cholesterol reagent
- Pipets
- Spectrophotometer
- Controls
- Refrigerated Centrifuge
- Precipitating Reagent

If available a point of care analyzer may replace the cholesterol reagents, and the spectrophotometer.

CHOLESTEROL ANALYSES PROCEDURE

(This may be replaced with a point of care instrument.)

1. Label 13 x 100 mm test tubes for a total cholesterol standard, Level 2 control, and total cholesterol for each serum sample.
2. Pipet 2.5 mL of cholesterol reagent into each tube.
3. Add 30 μL of sample to appropriate tubes for total cholesterol standard, Level 2 control, and each serum sample total cholesterol tube.
4. Mix well and Incubate at 37°C for 15 minutes.
5. Adjust a spectrophotometer to 500 nm and blank it with distilled water.
6. Following incubation, read the absorbance of each standard, control, and serum sample using appropriate cuvettes. Record your results on the data sheet.
7. Calculate the total cholesterol values for the control and each serum sample using the absorbance proportion method.

HDL CHOLESTEROL SAMPLE PREPARATION

PRINCIPLE

In the presence of Mn^{2+} and heparin, chylomicrons, VLDL, and LDL are precipitated, leaving only HDL in solution. The precipitated materials are sedimented by centrifugation, and the HDLcontaining supernatant can be removed.

PROCEDURE

1. Pipet 1 mL of each control and sample into labeled 10 x 75 mm test tubes.
2. Add 0.1 mL of precipitating reagent (1.06 M $MnCl_2$ with sodium heparin added) to each tube and mix well using a vortex type mixer.
3. Centrifuge at 2000 g for 30 minutes at 4°C.
4. Carefully remove the clear supernate into labeled 10 x 75 mm test tubes with Pasteur pipets and save it for subsequent analysis.

HDL CHOLESTEROL PROCEDURE

1. Follow steps 1 and 2 of total cholesterol procedure.
2. Add 50 μL of supernate (from precipitation procedure) to HDL control and HDL serum sample tubes, add 50 μL of HDL standard to appropriate tube.
3. Follow steps 4 through 7 of total cholesterol procedure.
4. Multiply the final value (from Step 7) by 1.1 to correct for the dilution made in the precipitation step.

DATA SHEET, EXERCISE #16	NAME: _____ DATE: _____

RESULTS

	ABSORBANCE (500 nm)	CHOLESTEROL LEVEL mg/L	CORRECTED HDL-C mg/L
Cholesterol Std			
HDL Cholesterol Std			(target value)
Level II Control			
HDL Control			(target value)
Sample # (Cholesterol)			
Sample # (HDL-C Cholesterol)			
Sample # (HDL-C)			

CALCULATIONS

Calculate LDL and VLDL values for each serum sample and the controls using the following equations.

$$\text{VLDL cholesterol, mg/L} = \frac{\text{Triglyceride level (mg/L)}}{5} \tag{2.16.4}$$

$$\text{LDL cholesterol} = \text{total cholesterol} - (\text{VLDL cholesterol} + \text{HDL-C}) \tag{2.16.5}$$

Your instructor will provide you with the measured triglyceride level for your sample.

DISCUSSION QUESTIONS

1. What additional lipid calculations can be made that are clinically useful?
2. If chylomicrons are present, what steps are necessary to determine HDL cholesterol?
3. What substance(s) will interfere with cholesterol determinations using this method?
4. If the triglyceride level of your sample was >4000 mg/L, could you calculate the LDL cholesterol?

5. For each of the serum samples tested, briefly discuss the lipid picture illustrated by your results. See Chapter 33 in Kaplan and Pesce
6. Why were two cholesterol standards employed in this exercise?

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2.17: Triglycerides

RELATED READING: Chapter 33. See Methods in CD-ROM for Triglycerides.

OBJECTIVES

Upon completion of this exercise, appropriate discussions, and related readings, the student will be able to:

1. Determine the triglyceride value of serum with 90% accuracy using an endpoint, coupled enzyme reaction.
2. Explain what steps need to be taken to deal with specimens with unusual characteristics.
3. Describe the appearance of samples with markedly elevated triglycerides.
4. List secondary causes of elevated triglycerides.

PRINCIPLES

Triglycerides are completely hydrolyzed to free glycerol and free fatty acids by the enzyme lipase. The liberated free glycerol content is then determined enzymatically as shown by the following coupled sequenced reactions.

$$\text{Triglycerides} \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Free Fatty Acids} \tag{2.17.1}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol}} \text{Glycerol-1-Phosphate} + \text{ADP Kinase} \tag{2.17.2}$$

$$\text{ADP} + \text{Phosphoenolpyruvate} \xrightarrow{\text{Pyruvate}} \text{ATP} + \text{Pyruvate Kinase} \tag{2.17.3}$$

$$\text{Pyruvate} + NADH + H^{+} \xrightarrow{\text{LD}} \text{Lactate} + NAD^{+} \tag{2.17.4}$$

The resulting decrease in absorbance at 340nm (decrease in NADH) is a stoichiometric measure of the glycerol present and is directly related to the triglyceride content of the sample.

MATERIALS

- Triglyceride reagent
- Patient samples
- Standard
- Control serum
- UV Spectrophotometer
- Cuvettes
- Pipets 37°C incubator

PROCEDURE

1. For each standard, control, and sample to be tested, pipet 3.0 mL of reagent into a clean labeled cuvette.
2. Incubate each cuvette for 3 minutes at 37°C.
3. Adjust a spectrophotometer to 340 nm and set zero absorbance (100% T) with distilled water.
4. Add 50 μL of standard to the warmed reagent.
5. Mix gently by inversion and read the absorbance immediately (within 15 seconds) and record.
6. Start a stopwatch and continue incubation.
7. Repeat steps 4 & 5 for each control serum to be tested; you will find it useful to space each timing at 30 second or 1 minute intervals from the standard.
8. After exactly 10 minutes of incubation take a second absorbance reading or each mixture and record your results on the data sheet.
9. Calculate the total $Delta$A for each standard, control, and serum sample and determine the triglyceride value using the following formula:

$$\frac{\Delta A_{Unknown}}{\Delta A_{Standard}} \times \text{conc. std} = \text{conc. mg/L} \tag{2.17.5}$$

Note

This procedure is linear to 4000 mg/L. Samples with higher values should be diluted with normal saline and retested.

OPTIONAL EXERCISE

1. Your instructor will provide a series of plasma samples that have been placed in a refrigerator (4-8 °C) overnight.
2. Observe these samples and indicate whether they are cloudy (turbid) or clear, exhibit a cream layer on the top, or some combination of these.
3. Record your observations on the data sheet.
4. From Chapter 33, what type, if any, of hyperlipidemia may be associated with each sample?

DATA SHEET, EXERCISE #17

NAME: _____
DATE: _____

RESULTS

	Initial	Absorbance (10 Minutes)	ΔA (Total)	Triglycerides (mg/L)
Standard				(Target Value)
Control				
Serum #				
Serum #				

OPTIONAL EXERCISE

Check one or more of these observations for each sample

Sample #	Clear	Slightly Cloudy	Very Cloudy	Creamy Layer	Type of Hyperlipidemia
1					
2					
3					
4					
5					
6					

Discussion Questions

1. What is the expected appearance of a serum sample containing elevated triglycerides?
2. What type (classification) of lipid abnormality will show marked elevation of triglycerides and the presence of chylomicrons?
3. What disease or condition can be a secondary cause of elevated triglycerides?

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2.18: Lipoprotein Electrophoresis

RELATED READING: Pages 201-214, Chapter 33.

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related reading, the student will be able to:

1. Perform an agarose electrophoresis procedure for lipoprotein.
2. Discuss the lipid profile illustrated by the results of the unknown serum sample used in this exercise.

PRINCIPLE

Using the Corning agarose electrophoresis system, four lipoprotein bands may be identified. They are in order of increasing electrophoretic mobility, chylomicrons, beta-lipoproteins (LDL), pre-beta lipoproteins (VLDL), and alpha-lipoproteins (HDL). Following electrophoresis, the separated fractions are stained with Fat Red 7B. Fat Red 7B stains unsaturated fatty acids in the lipoprotein complexes.

Plasma separated from blood anticoagulated with EDTA or serum are the recommended samples for this procedure.

MATERIALS

- Universal Buffer pH 8.6
- Methanol-water
- Agarose Gel
- Control Serum
- Fat Red 7B stain
- Staining trays
- Electrophoresis Chamber
- 55°C drying oven

PROCEDURE

1. Fill the electrophoresis cell base with 190 mL of Universal PHAB Buffer (95 mL in each chamber). Do not reuse buffer.
2. Gently peel the Agarose Universal Electrophoresis Film from its plastic cover, being careful to handle the agarose film only by it edges.
3. Fill the sample wells of the agarose film with 1.0 mL of plasma or serum.
4. Insert the loaded agarose film into the cassette holder of the Electrophoresis Cell Cover, agarose side facing out, matching the anode (+) side of the agarose film with the anode (+) side of the cell cover.
5. Place the cell cover on the electrophoresis cell base. (The power supply will automatically switch on.)
6. Allow the sample to migrate for 35 minutes at 90 volts.
7. Following migration, remove the cell cover from the electrophoresis cell base. Drain the excess buffer from the cell cover without inverting the cover. Grasp the agarose film by its edges and remove it from the cassette holder.
8. Wipe the moisture from the back of the agarose film, then place the film on a shelf of a drying chamber or appropriate incubator/oven. Dry at 55° C ± 5° for 15-20 minutes or until dry.
9. Remove the agarose film from the oven and allow to cool to room temperature.
10. Place the dried agarose film, agarose side up, on moistened filter-paper placed in the bottom of a staining dish.
11. Using a clean 10 mL glass pipet, dispense 10 mL of Fat Red 7B working stain solution evenly over the surface of the agarose film. Do not touch the pipet to the agarose surface.
12. Stain for four minutes. When the stain turns dark blue and begins to precipitate, the staining is complete.
13. Transfer the agarose film to the Methanol-Water clearing solution. Gently agitate for approximately 60 seconds or until the background is clear.
14. Wipe moisture from back of agarose film and dry at 55°C ± 5° for 15—20 minutes or until dry.
15. Interpret visually or quantitate using the appropriate densitometric equipment at 520 nm.

DATA SHEET, EXERCISE #18	NAME: _____ DATE: _____

Place electrophoretogram or a copy in the space below.

INTERPRETATION

Check the most appropriate interpretation for each control or unknown analyzed.

Sample #	Normal	Type I	Type II	Type III	Type IV	Type V

Discussion Questions

1. Why is it important that the buffer solution in the chamber not be reused?
2. In what way is interpretation of an electrophoretic pattern with a densitometer better than a visual interpretation?
3. What is the most common cause of poor results when performing electrophoresis procedures?

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2.19: Kinetic Enzyme Analysis

RELATED READINGS: Chapters 54 and 55. See Methods Aspartate aminotransferase, or Amylase, or Gamma-glutamyl transferase.

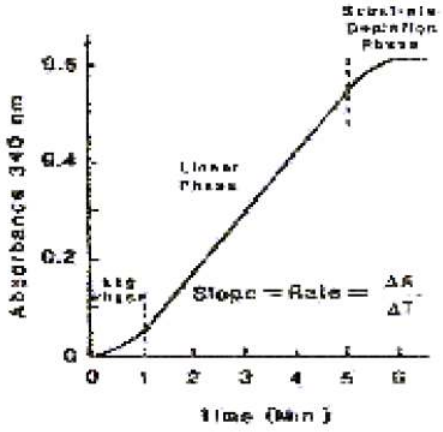
OBJECTIVES

Upon completion of this exercise, appropriate readings, and discussions, the student will be able to:

1. Perform a kinetic enzyme procedure.
2. Construct a graph to illustrate the relationship between time and absorbance change during an enzymatic reaction.
3. Calculate the amount of enzyme activity present in the sample.

PRINCIPLE

For most enzyme assays, if the activity is plotted against time, a curve with 3 general phases would usually be generated. A typical curve would appear as shown below.



The lag phase is the period during which temperature and kinetic equilibrium is established. The linear portion is the phase of zero order kinetics and is the phase during which analysis is usually performed. The substrate depletion phase occurs when one or more of the substrates is depleted.

MATERIALS

- Enzyme Reagent
- Graph paper
- Serum Sample Incubators
- Spectrophotometer (UV)
- 37°C incubator

PROCEDURE

1. Pipet 2.0 mL of enzyme reagent into a clean 12mm x 75mm cuvette.
2. Add 100 μL of sample to the enzyme reagent.
3. Mix well.
4. Incubate at 37°C for 1 minute.
5. Adjust the wavelength of a spectrophotometer to 340 nm (or any other wavelength necessary for the reaction to be monitored).
6. Blank the Instrument (set 100% T, zero absorbance) with distilled water.
7. Measure and record the absorbance of the solution after the initial 1 minute and each minute thereafter for a total time of 15 minutes. Return the cuvette to the incubator between readings.
8. Plot the absorbance readings on the vertical axis versus time on the horizontal axis using linear graph paper. Connect your points to form a smooth curve.

9. Plot the $Delta$A values on the vertical axis of the second graph versus time on the horizontal axis. Connect the points to form a smooth curve.

Discussion Questions

1. Propose an explanation for the shape of the absorbance vs time graph. Mark the lag, linear, and substrate depletion areas on your graph.
2. What are the relationships between the two graphs (A versus time and ΔA versus time)?
3. Why did the calculated enzyme concentrations differ at the different time points?
4. What would the effect on the calculated enzyme activity be if the sample volume in the experiment were halved?

DATA SHEET, EXERCISE #19	NAME: _____ DATE: _____

RESULTS

Time (min)	Absorbance	ΔA	Calculated enzyme activity
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			

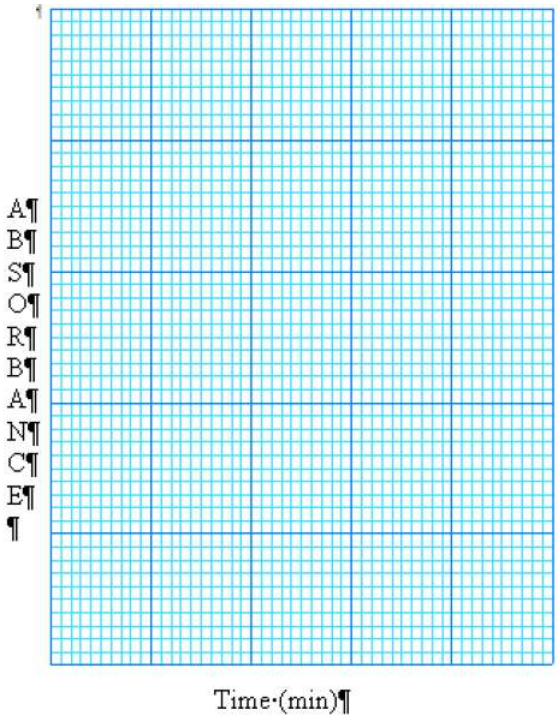
Calculate the amount of enzyme activity at the time points indicated above using the following formula:

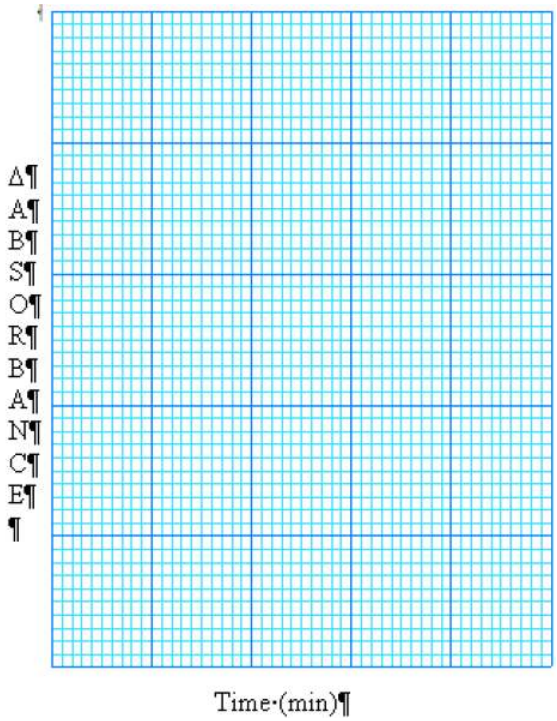
$$\text{U/L specimen} = \frac{\Delta A/min \times \text{total assay volume (mL)} \times 1000\ mL/L}{\text{abs. coeff.} \times \text{lightpath (cm)} \times \text{sample vol.(mL)}} \quad (2.19.1)$$

ΔA = change in absorbance

1000 mL/L = factor to convert units/mL to U/L

abs.coeff. = Absorbance coefficient = 6.22 cm^{-1} x L x $mmole^{-1}$ for NADH at 340nm





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2.20: Enzymes Rate, Creatine Kinase (CK)

RELATED READING: Chapter 31. See Methods on CD-ROM for Creatine kinase.

OBJECTIVES

Upon completion of this exercise and appropriate discussion and related reading, the student will be able to:

1. Perform a multipoint enzyme rate reaction for CK.
2. Calculate CK values using reaction rate and molar absortivity constant.
3. Explain the effect of time on enzyme activity.
4. Explain the effect of temperature on enzyme activity.
5. Explain the effect of pH on enzyme activity.

PRINCIPLE

Creatine phosphate and ADP are converted to creatine and ATP by the enzyme Creatine Kinase. This conversion can be measured by utilizing two additional enzymatic reactions that result in the conversion of NAD to NADH with a resulting increase in absorbance.

$$\text{creatine phosphate} + \text{ADP} \rightarrow \text{creatine} + \text{ATP} \tag{2.20.1}$$

$$\text{glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{glucose-6-phosphate} + \text{ADP} \tag{2.20.2}$$

$$\begin{aligned}\text{glucose-6-phosphate} + NAD^{+} &\xrightarrow{\text{glucose-6-phosphate dehydrogenase}} \\ &\rightarrow \text{6-phosphogluconate} + H^{+} + \text{NADH}\end{aligned} \tag{2.20.3}$$

Enzyme activity can be calculated using the following formula:

$$F = \frac{V_r}{V_s} + \frac{1}{\epsilon} + \frac{1}{PL} + 10^6 \tag{2.20.4}$$

where F = Enzyme factor, V_r = Total reaction volume (mL), V_s = Sample volume (mL), ϵ = Molar absorptivity constant, PL = Path length of cuvette in cm

For CK measurement by the following procedure:

$$F = \frac{2.1}{0.1} + \frac{1}{6.22 \times 10^3} + \frac{1}{1} + 10^6 = 3375 \tag{2.20.5}$$

Activity is expressed in International Units/liter (U/L) Further, ΔA/min X F = CK activity in Units/Liter.

MATERIALS

- UV Spectrophotometer
- Pipets
- Cuvettes
- 37°C Water bath
- CK Reagent

PROCEDURE

1. Pipet 2.0 mL of CK reagent into a clean dry cuvette for each control and serum sample to be tested.
2. Incubate the reagent at 37°C for 5 minutes to prewarm.
3. Adjust your spectrophotometer wavelength to 340 nm and set zero absorbance (100% T) with distilled water.
4. Add 100 μL of sample (control or serum) to the warned reagent, mix by inversion, and incubate for 2 mm at 37°C.
5. Read the absorbance every 2 min. for a total of 10 min., incubating between readings. Record your results on the data sheet.
6. Repeat steps 4 and 5 for each additional material to be tested. With practice, multiple samples can be spaced at 1 minute intervals.
7. Calculate the average ΔA/min. during the maximum reaction rate and record your answer on the data sheet.
8. Calculate the CK for each sample and control activity using the formula given previously.

9. Cover your sample tubes with parafilm and save as instructed for further testing.

OPTIONAL EXERCISES

A. Effect of Temperature

1. Repeat the CK analysis on one of the samples or controls, but perform the incubation at room temperature.
2. Calculate the CK activity, measure the room temperature and record results on the data sheet.

B. Effect of pH

1. Using a pH meter, measure the pH of the CK reagent and then adjust the pH of the reagent to approximately pH 6.0 with 0.1 M HC1 or pH 7.5 with 0.1 M NaOH.
2. Repeat the CK analysis of one of the samples or controls at 37°C.
3. Calculate the CK activity and record it and the pH of the reagent on the data sheet.

DATA SHEET, EXERCISE #20	NAME: _____ DATE: _____

RESULTS

	Time (Minutes)					Average ΔA/min.	CK (U/L)
	2	**4**	**6**	**8**	**10**		
Normal Control							
Abnormal Control							
Sample # 1							
Sample # 2							

Note

Dilute samples with isotonic saline if calculated CK value exceeds the assay's linear range as determined by your instructor.

OPTIONAL EXERCISES

A. Room temperature_____°C
CK activity of sample #_____at room temperature was____U/L.

B. pH of unadjusted CK reagent = _____
pH of adjusted CK reagent = _____
CK activity of sample #_____using the adjusted reagent was _____U/L.

Discussion Questions

1. Under what conditions would you expect the ΔA for each time interval to be approximately equal?
2. If a sample had a high CK value, what might you observe in your results in addition to the high ΔA value?
3. What is the effect of decreasing the incubation temperature on CK activity? What do you think might be the affect of increasing the incubation temperature on the activity of CK?
4. What was the effect of changing the pH of the CK reagent on the measured CK activity? Why does this occur.

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2.21: Amylase (Modified Caraway Method)

RELATED READING: Chapter 29. See Methods on CD-ROM for Amylase.

OBJECTIVES

Upon completion of this exercise, appropriate discussion and related reading, the student will be able to:

1. Describe the physiological action of amylase.
2. List four types of amylase methodologies.
3. Perform a serum amylase determination.
4. Identify the unique color of the iodide-starch complex.

PRINCIPLE

The iodometric (amyloclastic) method is based on the ability of iodide to form a vivid blue color in combination with starch. The starch is hydrolyzed by amylase in the sample to liberate smaller molecules such as dextran, maltose, and some glucose molecules. The smaller sugar molecules do not form a complex with iodide and do not give a blue color. As more starch is broken down, the intensity of the blue color decreases. Substrate (starch) and sample (amylase) are mixed together and incubated for a fixed time. Iodide color solution is added and the starch/iodide color complex is formed. A spectrophotometric measurement is made to determine color intensity (absorbance). The lower the final absorbance (the greater the difference between the blank and test solutions), the higher the serum amylase activity.

MATERIALS

- 13 x 100 mm test tubes
- Heating block or Water bath
- 16 x 100 mm test tubes
- Spectrophotometer
- 19 x 150 mm test tubes
- Normal control
- Starch Substrate
- Serum samples
- Iodide Reagent
- Pipets
- Distilled Water
- 0.9% saline

PROCEDURE

1. Place approximately 4 mL of starch substrate into a labeled 16 x 100 mm test tube. Place the tube in a 37°C heat block or water-bath for a minimum of 5 minutes to prewarm. Continue to warm at 37°C until needed for step 7.
2. Label two 19 x 150 mm test tubes for each control and sample to be tested. Label one of each pair of tubes “blank” and the other “test”.
3. Pipet 21.0 mL of distilled water into each of the 19 x 150 mm test tubes (“blank” and “test”). Set these tubes aside for future use.
4. Label two 13 x 100 mm test tubes for each control and sample to be tested.
5. Pipet 1.0 mL of 0.9% saline into each 13 x 100 mm tube.
6. Pipet 30 μL of the appropriate control or sample into each 13 x 100 mm tube labeled “test”. Mix each by inversion and incubate all 13 x 100 mm tubes (“test” and “blank”) at 37°C for 5 minutes to prewam.
7. Following the 5 minute prewarming, while continuing to incubate, add 0.5 mL of warmed starch substrate (from step 1) to all 13 x 100 mm tubes (“blank” and “test”). Incubate each tube for exactly 7 minutes.

Note

You may find it desirable to add the starch substrate to the tubes at 30 second intervals. This will allow for more exact timing.

8. After exactly 7 minutes of incubation, add 2.0 mL of iodide solution to each tube.
9. Add 30 μL of appropriate control or sample to each “blank” tube.
10. Transfer the contents of each 13 x 100 mm tube to the corresponding 19 x 150 mm test tube from step 3. Mix by inversion.
11. Measure the absorbance of the mixture in each tube (“test” and “blank”) at 660 nm against a distilled water blank. Record the absorbance on the data sheet.
12. Calculate the amylase value for each material tested using the formula shown below:

$$\frac{A_{blank} - A_{test}}{A_{blank}} \times 5000 = \text{Amylase units/L} \tag{2.21.1}$$

Record the values on the data sheet.

DATA SHEET, EXERCISE #21	NAME: _____ DATE: _____

RESULTS

	Absorbance 660 nm		Amylase units/L
	Blank	**Test**	
Normal control			
Sample #			
Sample #			

DISCUSSION QUESTION

1. Is the blank used in this experiment a reagent or sample blank?
2. Why is it necessary to use a “blank” for each sample?
3. Why is it necessary to know the method used to determine an amylase value to discuss a reference range?

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2.22: Creatine Kinase (CK) Isoenzyme Electrophoresis

RELATED READING: Chapter 55. See Methods on CD-ROM for Creatine kinase isoenzymes.

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related readings, the student will be able to:

1. Perform an electrophoresis and develop the electrophoretogram with an enzymic stain.
2. Identify each CK isoenzyme on the electrophoretogram.
3. Interpret the CK isoenzyme pattern, relating it to a possible disease state.

METHOD

Corning electrophoresis with P-L Biochemicals colorimetric development or other appropriate reagent.

PRINCIPLE

CK isoenzymes are separated electrophoretically on agarose plates at 90 V, pH 8.6. A substrate that can be converted to a fluorescent product by CK can then be applied and the isoenzyme bands observed under UV light.

GLOSSARY

Application - a term used to describe the process of placing a small volume of solution onto a stationary phase (thin layer plate, gel, paper, or column) for chromatographic or electrophoretic separation.

MATERIALS

- Barbital buffer (pH 8.6)
- Agarose gel
- CK substrate (Corning #0
- 39°C incubator
- Electrophoresis chamber
- Drying oven
- Sample applicator
- UV light source
- Serum samples
- CK control

PROCEDURE

1. Fill each chamber of electrophoresis cell base with 95 mL barbital buffer, pH 8.6 (good for one use only).
2. Remove plastic backing from agarose film.
3. Apply 1.0 μL aliquots of each serum to a sample well, allowing sample to absorb between applications. Place a control in position 3 or 4 (use 2 applications for control).
4. Load the agarose film into the top cassette so that negative side corresponds to negative side of the bottom cassette.
5. Electrophorese at 90 V for 20 minutes.
6. Near the end of electrophoresis, reconstitute one vial of Cardiotrac CK Fluorometric reagent with 1.0 mL buffer solution. Swirl to dissolve.
7. Prepare Incubator tray with moistened blotter pad and preincubate tray at 39°C.
8. Immediately following electrophoresis, remove film from cassette. Place film on flat surface with positive (+) side near you and blot edges. Pour entire contents of substrate vial along one edge. With a 5 mL serological pipet, gently spread the liquid from positive to negative side by lying pipet on its side in the solution and using it as a “spreader”.
9. Place film gel side up on prewarmed incubator tray, dry and replace cover, and incubate for 20-30 minutes at 39°C. (Increased incubation enhances sensitivity.)
10. Remove the agarose film from the incubation tray and dry at 55°C. Dry in oven for 15-20 minutes or until dry.
11. Inspect with UV light or scan on a fluorescent densitometer.

12. Attach a copy of densitometric tracing or part of the agarose gel to bottom of the data sheet.

DATA SHEET, EXERCISE #22	NAME: _____ DATE: _____

RESULTS

Observe bands as follows: (cathode to anode)

- CK (BB) - migrates closely with albumin.
- CK (MB) - migrates between CK-BB and CK-MM
- CK (MM) - remains near the origin.

CLINICAL INTERPRETATION

1. *Normal* Normal CK total with small amounts of enzymatic activity in CK (MM) fraction. No detectable MB or BB.
2. *Abnormal* CK (MB) present. Indicative of heart damage or necrosis. Usually accompanied by elevated MM and elevated total CK.
3. *Abnormal* Increased CK (MM). Reflects injury to skeletal muscle due to intramuscular (IM) injection, surgery, etc. Occurs in muscular dystrophy; may be accompanied by elevated MB.
4. *Abnormal* CK (BB) present. Reflects the release of brain tissue due to cerebrovascular accident, necrosis, or other brain trauma or damage to large bowel or pulmonary tissues.

Attach copy of densitometric scan or part of agarose gel below.

Discussion Questions

1. What bands were visible on your sample? What does this suggest?
2. Compare the findings from this exercise with the total CK enzymatic activity on the same sample from the CK exercise. Do the results support each other?
3. How might it be possible to convert the fluorescent stain into a colored stain visible in normal room lighting?

2.22: Creatine Kinase (CK) Isoenzyme Electrophoresis is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by LibreTexts.

2.23: Blood Gas Analysis

RELATED READING: Chapter 25. See Methods on CD-ROM for Blood Gas Analysis.

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related readings, the student will be able to:

1. Verify calibration of a blood gas analyzer and adjust calibration if needed. (For many instruments these values are preset and it is not possible to adjust calibration.)
2. Perform blood gas analysis as instructed.
3. Discuss the effect of improper collection or handling of arterial samples on blood gas values.

METHOD

Whatever automated blood-gas instrument is available.

MATERIALS

- Arterial blood samples
- Blood Gas Analyzer
- Controls

GLOSSARY

Calibrator -the initial of the two standards used for calibration of pH, PCO_2, and PO_2 measurements on a blood-gas instrument.

Sloping reagent - the second of the two standards used for calibration of the pH, PCO_2, and PO_2 measurements on a blood-gas instrument. This sets the slope of the calibration line.

PROCEDURE

1. Following the instructions given by your instructor, check the calibration of the blood gas analyzer and adjust the calibrator and slope setting as needed.
2. Verify calibration by running appropriate controls. Record your results on the data sheet.
3. Determine the gas values for the samples provided by your instructor. Record your results on the data sheet.
4. Following the instructions given by your instructor, alter the sample in some way.
5. Repeat the blood gas analysis on the altered sample and record the results on the data sheet.
6. When all samples have been tested, leave the instrument in stand-by mode.

OPTIONAL EXERCISES

Sample Alteration: The following is a list of suggested activities that can be used to illustrate the error that can be induced by the improper handling of arterial samples when performing blood gas analysis.

1. Introduce a small air bubble into the sample, recap, mix and return the sample to ice bath storage for 10 minutes. Retest the sample. Allow the sample to remain at room temperature for an additional 10 minutes, then retest.
2. Introduce a moderate air bubble into the sample. Recap, mix and allow the sample to remain at room temperature for 10 minutes. Retest the sample.
3. Uncap the syringe and place it in a 37°C incubator for 10 minutes. Retest the sample. (This would be similar to placing the sample on top of a warm instrument).
4. Draw 0.5 mL of heparin into the sample. Recap, mix and retest the sample. Allow the sample to stand at room temperature for 10 minutes. Retest the sample.
5. Allow the capped sample to remain at room temperature. Retest after 5, 10, and 15 minutes.
6. Allow the capped sample to stand on ice in a vertical position for 10 minutes, allowing the plasma and cells to separate. Retest the sample without mixing.

DATA SHEET, EXERCISE #23

NAME: _____
DATE: _____

RESULTS

	pH	PCO_2 (mm Hg)	PO_2 (mm Hg)	TCO_2 (mmol/L)	Base Excess (mmol/L)	O_2 Sat. (%)
Low Control						
Medium Control						
High Control						
Sample 1						
Sample 2						
Sample 3						

OPTIONAL EXERCISES

	Initial Sample Values	Altered Sample Values		
		Sample 1	**Sample 2**	**Sample 3**
pH				
PCO_2 (mm Hg)				
PO_2 (mm Hg)				
TCO_2 (mmol/L)				
Base Excess (mmol/L)				
O_2 Saturation (%)				

Note below how each sample was altered.

Discussion Questions

1. What was done to alter the samples you tested?
2. What blood-gas parameters were changed as a result of each sample manipulation?
3. Briefly discuss why these changes occurred.
4. Can you think of any circumstances in which a blood-gas sample received at room temperature might still be an acceptable specimen?
5. Can you think of any circumstance in which a blood-gas sample containing a large air bubble might still be an acceptable specimen?

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2.24: Phlebotomy

RELATED READING: Chapter 3, Pages 69-73

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related readings, the student will be able to:
1. Understand the proper method of processing phlebotomy tubes.
2. Understand the mechanisms of action and the uses of various anticoagulants.
3. Relate the color of a hemolyzed sample to the degree of hemolysis and the amount of free hemoglobin.

PRINCIPLE

Depending upon the type of test to be performed, there are many ways to collect blood, urine, and other body fluids. Usually a specific type of collection vessel is used to produce a specimen that is suitable for a particular type of analysis. For example, iodoacetate is placed in phlebotomy tubes to prevent glycolysis. To collect unclotted blood a chemical such as oxalate, EDTA, or heparin must be added to prevent coagulation. Other types of phlebotomy tubes, such as those used for trace metal analysis, are designed to reduce possible contamination of the sample from exogenous metals.

GLOSSARY

Red top -a term indicating a phlebotomy tube containing no anticoagulant or preservative used to collect serum. Term refers to the color of the rubber stopper on the tube. Also- “green top”, “purple top”, etc.

MATERIALS

- Phlebotomy tubes:
  - no anticoagulant (red top)
  - two heparin (green tops)
  - citrate (blue top)
  - EDTA (lavender top)
  - oxalate (black top)
  - iodoacetate (gray top)
  - fluoride (gray top)
- Centrifuge
- Refrigerator/freezer
- 16 x 100 mm glass test tubes

PROCEDURE

1. Students can perform this exercise either as individuals or in small groups.
2. A single set of phlebotomy tubes should be drawn from a single Individual. Standard phlebotomy procedures, as described by the Instructor, should be employed during this exercise. After the set of phlebotomy tubes are filled, they should be processed as described below.
3. After collection, the red top, one heparin, EDTA, oxalate, and citrate tubes should be centrifuged for 15 minutes.
4. The serum or plasma should be carefully removed from the cellular sediment with Pasteur pipets and placed in appropriately labeled 16 x 100 mm test tubes.
5. Cover and refrigerate the tubes if they will be used within 4 days. Freeze (-20°C) if they will need to be stored beyond 4 days. Note: Never freeze unseparated whole blood because the red cells will lyse.
6. The second heparin and the gray topped tubes, iodoacetate and fluoride, should be allowed to stand at room temperature overnight. The next day, they should be processed as described above.
7. Observe the appearance (including the color, degree of tubidity, etc.) of each set of specimens, and record in the Results Section of the Data Sheet. During class discussion, compare the results for the different sets of samples.
8. Have an aliquot of each plasma and serum measured for glucose. Record the glucose values for each sample on the data sheet.

OPTIONAL EXERCISE: HEMOLYSIS

MATERIALS

- One tube of heparinized blood
- micropipettes (green top) for entire class
- 16 x 100 mm test tubes

PROCEDURE

1. Place 20 mL of distilled water in each tube of a series of 5 test tubes, labeled 1 through 5.
2. Starting with tube #1, add either 0.01, 0.025, 0.05, 0.1 or 0.2 mL of well mixed, heparinized whole blood into the appropriate tube.
3. Mix the test tubes by covering with Parafilm and inverting.
4. By visual inspection, note and record the degree of red color imparted to each solution (score 0 to +4 degree of color).
5. Calculate the hemoglobin concentration in each solution, assuming an average hemoglobin concentration for the original specimen of 150 g/L.
6. Have an aliquot of each sample saved for analysis of potassium and lactate dehydrogenase (LD). Record results on data sheet.

DATA SHEET, EXERCISE # 24	NAME: _____ DATE: _____

RESULTS PART A:

Color of Phlebotomy Tube	Type of Specimen (plasma or serum)	Specimen Color	Presence of Turbidity	Glucose mg/L
Gray-overnight				
Gray-overnight				

RESULTS PART B

Solution #	Degree of Red Color (0-4+)	Approximate Concentration Hemoglobin (g/L)*	Lactate Dehydrogenase U/L	K^+ mmol/L
1				
2				
3				
4				
5				

*Use equation on following page.

Calculation of approximate hemoglobin (Hb) concentrations in the hemolysates:

Use basic dilution equation as follows:

$$(conc_i\ Hb)(vol.\ dil.) = (conc_f\ Hb)(final\ dil.\ vol.) \tag{2.24.1}$$

where $conc_i$ Hb = concentration of Hb in blood (150 g/L)

where $conc_f$ Hb = concentration of Hb in diluted solutions (#1-#5)

vol. dil. = volume of blood that is diluted

final dil. vol. = final volume that blood is diluted out to (mL)

This equation can be rearranged to read:

$$conc_f\ Hb = \frac{(conc_i\ Hb)(vol.\ dil.)}{(final\ dil.\ vol.)} \tag{2.24.2}$$

DISCUSSION QUESTIONS

1. What constituents of blood would be responsible for the different colors of the serum or plasma specimens observed?
2. What would cause the presence of turbidity in a serum or plasma specimen?
3. For each different type of phlebotomy tube, list several tests which might appropriately utilize that specific tube.
4. What tests are affected by the disruption of blood cells (i.e. mixing intracellular material with plasma or serum)?
5. What tests may be affected by the color (i.e., absorbance) of hemoglobin?
6. Should the phlebotomy tubes be drawn in any specific order?
7. Can you explain any differences between the glucose levels for the various samples? For the samples stored at 4°C and those with a preservative?
8. Which seems to be a better indicator of hemolysis, color, LD levels, or K+ levels?

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2.25: Exercise 25; Quality Control

RELATED READING: Chapter 21

OBJECTIVE

Upon completion of this exercise, appropriate discussion, and related readings, the student will be able to:

1. Gain an understanding and working knowledge of the principles of quality control (QC) in clinical chemistry.
2. Gain experience in plotting quality control data.
3. Perform statistical calculations and interpret QC data.

PRINCIPLE

In general, quality control of laboratory analyses is performed by assaying quality control pool samples on a daily basis and maintaining a record of these values. After a sufficient number of measurements (i.e., >20 obtained on consecutive days with the same control), an expected target range of acceptable results can be established. A common way of doing this is to determine the mean and standard deviation (S.D.) of the data, then set the acceptable range as the mean ±2 S.D. This will include 95% of the data, and we would expect a value outside of this range to occur by chance in only 1 of 20 subsequent analyses. When a result for a quality control pool falls outside the target range, the medical technologist must question the validity of the run and respond in some appropriate fashion, either by repeating the control analysis or by repeating the entire run after trouble-shooting the analyzer.

GLOSSARY

±2 S.D. limits - also called the 4 S.D. target interval, defines the range that includes all data between + 2 S.D. to - 2 S.D. about a mean value.

Out of control - a term indicating that the results from one or more quality control analyses falls outside of the “accepted” target range (± 2 S.D., for example).

MATERIALS

- Calculator

PROCEDURE

1. Calculate the mean, standard deviation, and coefficient of variation for the following results (mg/L) obtained on a quality control pool for calcium analysis. Use the table provided on the data sheet for calculation.

101, 102, 100, 98, 99, 101, 103, 100, 95, 98, 98, 100, 101, 99, 102, 103, 102, 104, 102, 104, 105, 105, 99, 95, 97, 99, 104, 95, 103, 96.

2. Consider the following data obtained from the analysis of a quality control pool on 20 consecutive days for five common laboratory analytes. Plot the graph on the Levy-Jennings plotsprovided on the data sheet. On each graph fill in the calculated mean (target value) and the + and - 2 S.D. range values.

glucose (mg/L): 940, 990, 860, 910, 900, 840, 980, 980, 1180, 780, 1020, 930, 910, 780, 950, 1050, 800, 850, 950, 820; 4 S.D. target interval 900-1100 mg/L.

chloride (mmol/L): 104, 101, 100, 104, 103, 102, 105, 104, 102, 105, 103, 102, 105, 104, 105, 106, 106, 107, 109, 108; 4 S.D. target interval 98-108 mmol/L.

potassium (mmol/L): 4.2, 4.4, 4.3, 4.9, 4.4, 4.5, 4.2, 4.4, 4.9, 4.5, 4.5, 5.1, 4.9, 4.7, 5.2, 4.9, 4.5, 4.7, 4.9, 4.7; 4 S.D. target interval 3.5-5.3 mmol/L.

calcium (mg/L): 86, 98, 99, 91, 109, 100, 93, 97, 95, 95, 110, 100, 90, 101, 89, 93, 103, 106, 91, 87; 4 S.D. target interval 85-105 mg/L.

sodium (mmol/L): 144, 139, 146, 142, 155, 139, 151, 143, 146, 132, 142, 147, 143, 144, 134, 139, 145, 148, 136, 142; 4 S.D. target interval 136-148 mmol/L.

3. Identify trends, shifts, and out of control data. Circle all out of range data, that is, those results >±2 S.D. from the mean or target value.

Discussion Questions

Examine each of the Levy-Jennings plots, and answer the following questions.

1. Which of the methods appear to be in overall good control?
2. Which of the methods may have accuracy and/or precision problems?
3. What decisions do you think might be made on day 20 for each of the analytes?
4. These Levy-Jennings plots review only one quality control pool, although 2 pools are usually analyzed each day. What would you do if the low ("normal") control pool was within the target range but the abnormal control pool was outside the established limits?

DATA SHEET, EXERCISE #25	NAME: _____ DATE: _____

RESULTS

Calcium value (X_i), mg/L	$X_i - X$	$(X_i - X)^2$

CALCULATIONS

N = 31

ΣX_i = _____

$\Sigma(X_I - X)^2$ = _____

Mean = $\frac{X+\Sigma X_i}{N}$ = _____mg/L

$S^2 = \frac{\Sigma(X_i - X)^2}{N-1}$ = _____ S.D. = standard deviation = _____mg/L

%coefficient of variation = % C.V. = S.D. $\frac{(100\%)}{mean}$ = _____%

95% interval = _____mg/L

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2.26: Extraction Technique

RELATED READINGS: Pages 125-126.

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related readings the student will be able to:

1. Understand the techniques of liquid—liquid extraction.
2. Purify and concentrate an analyte.

PRINCIPLE

There are a number of reasons why some type of extraction step is needed for an analytical method. These include: removing sample material that can destroy a component of the analytical system, (such as protein, that can “foul” an HPLC column), increasing the specificity of the assay by removing interfering compounds, placing the analyte in a medium that will allow a derivatization reaction, and concentrating the analyte for enhanced sensitivity.

Thus, in all cases, extraction techniques remove the analyte from its initial sample matrix. Extraction techniuqes can include: protein precipitation, column chromatography (ion-exchange, neutral resins, etc.), and liquid-liquid extraction. The latter technique is the most frequently used because of its simplicity. The analyte is typically extracted into a volatile organic solvent, allowing the analyte to be concentrated by evaporation of the organic solvent.

In this exercise, bilirubin in a serum sample will be extracted into chloroform and the amount extracted determined by spectrophotometric analysis. This extraction technique is often used to measure bilirubin in amniotic fluid because the colorimetric methods (Malloy-Evelyn or Jedrassik-Grof) lack sensitivity and direct spectrophotometric analysis of the amniotic fluid is subject to interference and lacks specificity.

MATERIALS

- Serum pool containing a known elevated amount of bilirubin
- Spectral-grade chloroform
- Spectrophotometer
- Normal saline
- Glass-topped, 50 mL centrifuge tubes (aluminum foil-wrapped)
- Centrifuge

PROCEDURE

1. Dilute 3 mL of the serum pool 1:5 with normal saline (0.85%, 0.15M NaCl).
2. In duplicate, pipet 5 mL of the diluted serum into 50 mL glass stoppered centrifuge tubes.
3. Add 10 mL of chloroform to each centrifuge tube.
4. Stopper the centrifuge tubes, and extract by vortexing or shaking the tubes for 30 seconds. Centrifuge at low speeds for 3-5 minutes to separate the two phases. If a protein mesh persists in the chloroform (lower) layer after centrifugation, break it up with a glass stirring rod, and recentrifuge.
5. Remove as much of the upper aqueous layer as possible with a Pasteur pipet and discard.
6. Use another Pasteur pipet to carefully transfer the chloroform layers to two aluminum foil covered 16 mm x 150 mm test tubes. Avoid contamination of the remaining aqueous phase by passing the tip of the pipet into the lower layer before aspirating the fluid. Cover, and place in dark.
7. Take one of the test tubes, and evaporate the sample to dryness by passing a stream of air or N2 gas over the surface of the liquid. The evaporation can be hastened by placing the test tube in a 37°C water bath. The evaporation must be performed in a hood or ventilated work space, since chloroform is toxic.
8. Reconstitute the residue in 1 mL of chloroform. Measure the absorbance spectra of both chloroform solutions from 350 nm to 550 nm at 25 nm increments. Record the absorbance readings on the data sheet.
9. Measure the absorbance spectrum of the diluted serum at the same wavelengths used in step #8. Record on the data sheet.
10. Plot all the spectra on the graph paper provided on the data sheet.

Discussion Questions

1. Why are the centrifuge tubes and glass test tubes covered with aluminum foil?
2. Are the spectra of the 3 solutions (diluted serum, and 2 chloroform extracts) the same? If not, can you explain the differences?
3. Which solution gave the highest absorbance at 450 nm? Do these absorbancies acurately reflect the actual bilirubin concentration in each solution? If not, why not?
4. Which of the solutions yielded a spectra most closely resembling that depicted in the figure below?

Amniotic fluid scan showing the method for determining absorbance at 450 nm. Arbitrary (base) line drawn from 375 to 525 nm shows where scan would have traced if no bilirubin pigment were present. Broken line indicates the absorbance recorded (O.D. when ordinate is in optical density units).

DATA SHEET, EXERCISE #26	NAME: _____ DATE: _____

RESULTS

Absorbance Measurements:

Wavelength (nm)	Diluted Serum	10 mL Chloroform	1 mL Chloroform
350			
375			
400			
425			
450			
475			
500			
525			
550			

Plot each spectral curve on the semi-logarithmic graph paper provided on the next page. Use a different colored pen for each sample.

Using the tangent method described on page 433, determine the absorbance at 450 nm, corrected for baseline.

Use Beer's Law, the absorbance at 450 nm by the tangent method, the molecular weight of bilirubin (569 g/mole), and the extinction coefficient for bilirubin of 60,700 L •mo1^{-1} • cm^{-1} to calculate the concentration of bilirubin in each sample.

CALCULATIONS

$$A = \epsilon c \ell \tag{2.26.1}$$

Sample	A_{450} from Tangent Method	Concentration of Bilirubin by Absorbance Calculation		Calculated Bilirubin in Diluted Serum	
		umol/L	**mg/L**	**umol/L**	**mg/L**
Diluted serum					
10 mL Chloroform					
1 mL Chloroform					

The actual bilirubin concentration in the control sample is _____mg/L

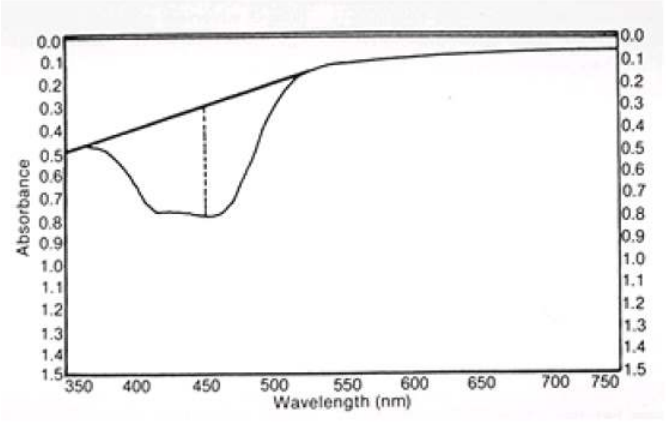
Assuming 100% extraction of the serum bilirubin into chloroform, calculate the
concentration of bilirubin in the diluted serum sample from the concentration of bilirubin in the chloroform extracts using the following formula:

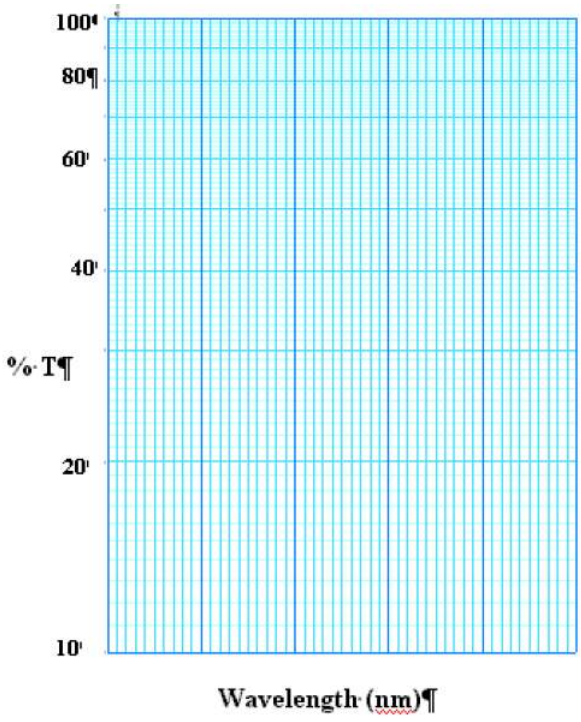
$$[Bili]serum(vol.\ extracted) = [Bili]CHCl_3\ (vol.\ of\ CHCl_3) \tag{2.26.2}$$

where: [Bili]serum = calculated bilirubin concentration in diluted serum

$[Bili]CHCl_3$ = measured bilirubin concentration in chloroform extracts

Record results above, and compare to the expected value based on a five-fold dilution of the quality control sample's known value.





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2.27: Thin Layer Chromatography (TLC) of Drugs

RELATED READINGS: Pages 110-115.

OBJECTIVES

Upon completion of this exercise, appropriate discussion and related readings, the student will be able to:

1. Understand and perform one-dimensional TLC.
2. Stain a thin layer chromatograph.
3. Calculate relative mobilities (Rf).

PRINCIPLE

Thin layer chromatography (TLC) is used in specialty areas of the clinical laboratory. Tests include 1) screening for drug overdoses, 2) screening for aminoacidurias, 3) determination of L/S ratios, and 4) screening for galactosuria or fructosuria. In the present exercise, we are interested in detection of the drug pseudoephedrine, a CNS stimulant used as a nasal decongestant.

Drugs can be separated by silica TLC. The mobility of each drug is a function of its relative affinity for each of the two phases (the solid support and the mobile solvent) which is related to the chemical nature of each drug. In general, the more polar the drug the more mobile it will be in a polar solvent. The mobile phase is manipulated by changing the polarity of its components and the pH. Changing the pH will, in turn, change the mobility of each drug since organic acid groups become more ionized while amino groups become less ionized as pH is increased.

MATERIALS

- Urine unknown (spiked with Drug standard a single drug)
- Silica gel plates
- Solvent prepared fresh (60 F-254, EM for each exercise Science)
- Applicator capillary tubes TLC developing solution: ethylacetate:methanol:NH_4OH, 85:10:5, V/V
- Hair dryer
- Developing tank capable of holding 20 x 20 glass plates

GLOSSARY

Application point - the place on the thin layer plate (or other stationary phase) where the sample is applied.

Developing chamber - the chromatography vessel in which the thin layer plate is placed until the solvent front reaches a desired place (is “developed”).

V/V - an abbreviation for the ratio of volumes of solvents or solutions used to prepare a solution.

PROCEDURE

1. Obtain an unknown and a standard from your instructor.
2. Obtain a silica plate that has been “preconditioned” by heating at 70°C for one hour.
3. Prepare solvent chambers by placing 100 mL of the chromatography solvent in the bottom of the developing tank that has been lined with sheets of filter paper.
4. Draw a line with a pencil 2 cm from the bottom of each chromatographic plate; label sample application positions as control, standard, or sample. Draw a line 15 cm from the application line.
5. Apply a small amount (3 μL) of standard, control, or sample to the silica gel at each application point. Do not allow the wet area to exceed 5 m in diameter. Dry the sample with the hair dryer. Repeat the application and drying steps. Apply a total of 20-30 μL of each sample (diameter $\leq$5 mm).
6. Transfer the plate, application end at bottom, to the developing tank and allow the solvent to migrate to the 15 cm line marked on the plate (approximately 45 mm.).
7. Remove the plate from the chamber and lay it face-up on a paper towel to air dry. Mark the end of the solvent front with a pencil before the plate dries. A gentle flow of warm air from the hair dryer directed against the plate will increase the rate of drying.

8. Spray the dried chromatogram ninhydrin solution. Blot the strip face up onto a paper towel to drain excess solution. Apply heat, using the hair dryer, to dry.
9. Outline the spots with a pencil, and record the color and appearance on the data sheet.
10. Record the distance the solvent front and the pseudoephedrine migrated (in mm) on the data sheet.

DATA SHEET, EXERCISE 27	NAME: _____ DATE: _____

RESULTS

Chromatography time: _____ minutes.

DRUG	Color	Distance Migrated (mm)	R_f
1			
2			
3			
4			
5			
Unknown			

The unknown drug is: _____

CALCULATIONS

Calculate the Rf of drug and unknown using the formula:

$$R_f = \frac{\text{distance spot migrated (mm)}}{\text{Distance solvent front migrated (mm)}} \tag{2.27.1}$$

Discussion Questions

1. If the solvent front migrated only half-way up the strip rather than 2/3 of the way, would the R_f values change?
2. If the solvent front migrated only half-way up the strip rather than 2/3 of the way, how would the resolution of the drug be affected?
3. What is the advantage to keeping the application spot very small? What would happen to the R_f values and resolution if the application spots were much larger?
4. If you detected the drug psudoephedrine by a TLC drug screen, would you report the result as positive?

Attach photocopy of your TLC plate here.

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2.28: High Performance Liquid Chromatography (HPLC)

RELATED READINGS: Pages 139-135.

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related readings, the student will be able to:

1. Interpret the pattern of a high performance liquid chromatography chromatogram.
2. Calculate certain parameters of a chromatogram including resolution and capacity factor.
3. Calculate the concentration of an unknown by the external standardization technique.

PRINCIPLE

Standardization of an HPLC assay is usually performed by proportional analysis, comparing the peak height (PH) of the analyte in the unknown to the peak height of a known amount (C) of standard:

$$\frac{C_{standard}}{PH_{standard}} = \frac{C_{unknown}}{PH_{unknown}} \tag{2.28.1}$$

This technique is also called external standardization. However, when a sample purification step is used, the recovery of the extracted analyte from the sample is usually variable. The above proportional calculation is only valid if one assumes virtually complete recovery. Since this is not often true, two techniques are used to correct for variable recovery. These are the method of standard additions and the method of internal standardization (the latter technique will be discussed in Experiment # 29).

The method of standard addition allows one to estimate the recovery of the analyte during sample processing. This method requires 2 aliquots of the specimen. One aliquot is analyzed without any modifications while the second aliquot is analyzed following the addition of a known amount (spike) of the standard. The pure standard is analyzed by direct HPLC analysis, that is, without any extraction. The first step in the calculation is to determine the recovery of the standard added to the second aliquot.

PH_S = peak height (mm) of standard

PH_x = peak height (mm) of analyte in unspiked aliquot of unknown

PH_{sp} = peak height (mm) of analyte in spiked aliquot of unknown

C_s = concentration of standard

C_x = concentration of analyte in unknown

C_{sp} = final concentration of standard added to second aliquot of unknown. The measured C_{sp} is compared to the actual estimate % recovery.

$$\frac{PH_{sp} - PH_x}{PH_s} = \frac{(C_{sp} + C_x) - C_x}{C_s} = \frac{C_{sp}\ (measured)}{C_s} \tag{2.28.2}$$

$$C_{sp}\ (measured) = \frac{(PH_{sp} - PH_x) \times C_s}{PH_s} \tag{2.28.3}$$

$$\frac{C_{sp}\ (measured)}{C_{sp}\ (actual)} \times 100\% = \%\ \text{recovery of spike} \tag{2.28.4}$$

Once the % recovery from the matrix of the sample is determined, the concentration of the analyte in the unknown is calculated by proportional analysis and corrected for the % recovery:

$$\frac{C_x}{C_s} = \frac{PH_x}{PH_s} \tag{2.28.5}$$

$$C_x = \frac{PH_x(C_s)}{PH_s} \times \frac{100\%}{\%\ recovery} \tag{2.28.6}$$

When developing an HPLC method it is often useful to determine parameters defining the chromatographic system. These parameters are also useful in monitoring system performance. Frequently calculated parameters include the peak resolution (R) and the capacity factor (k). The equations for calculating these parameters, using just the elution times (t) or elution volumes (v) are described on pp. 112-117.

MATERIALS

Sample chromatograms are found at the end of this exercise. The student will need a millimeter ruler and calculator. The sample chromatogram resulted from an
assay for serum theophylline performed as follows.

PROCEDURE

1. Pipet two 0.10 mL aliquots of pooled serum into two 12 x 75 mm glass test tubes, labeled “spike” and “unspiked”, respectively.
2. To the test tube labeled “unspiked”, add 0.10 mL of acetonitrile. To the test tube labeled “spike”, add 0.10 mL of theophylline standard.
3. Mix by vortexing, and centrifuge for 2-5 minutes at 800 x g to pellet precipitated protein. Save supernatants for HPLC analysis.
4. During centrifugation step, prepare working theophylline standard by mixing 0.10 mL of distilled water with 0.10 mL of 10 mg/L theophylline solution.
5. Inject 10 mL of each supernatant and 10 mL of working theophylline standard into HPLC system for analysis. The chromatograms are shown on page
6. Record time of elution of theophylline peak for standard and each supernatant. Measure the peak height (in mm) with a ruler.

DATA SHEET, EXERCISE #28	NAME: _____ DATE: _____

RESULTS

	Elution time (min) Theophylline	Peak Height (mm)	Final Theophylline Concentration
Working standard			
Unspiked aliquot			
Spiked aliquot			

CALCULATIONS

Calculation of Percent Recovery.

C_s = 10 mg/L,

C_{sp} (measured) = _____mg/L

% recovery = [C_{sp} (measured)]/10mg/L x 100% = $\frac{\text{_____}}{10\ mg/L}$ x 100% = _____%

Calculation of theophylline concentration in unknown.

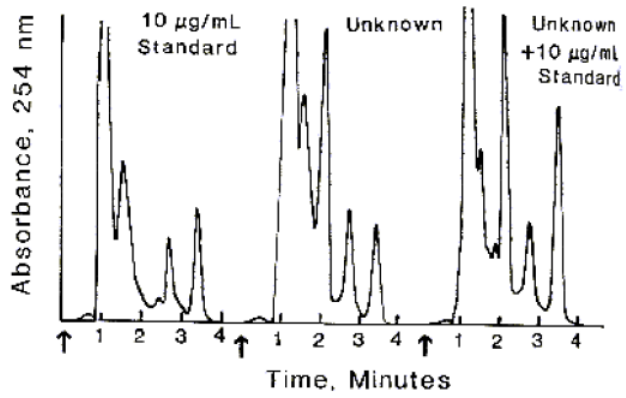
C_x = _____ x 100% = _____mg/L

Calculation of Capacity Factor (k').

Calculation of Peak Resolution (peaks at approximately 2.7 and 3.5 min.)

Discussion Questions

1. Why is it essential to measure the retention time for each peak?
2. What relationship does the elution time of an analyte have with the elution volume?
3. Would the results differ if peak areas were used rather than peak heights?



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2.29: Gas Liquid Chromatography (GLC)

RELATED READINGS: Pages 154-165.

OBJECTIVES

Upon completion of this exercise, appropriate discussion and related readings the student will be able to:

1. Interpret a gas chromatography (GC) pattern
2. Calculate the concentration of an unknown by the method of internal standardization.

PRINCIPLE

In GLC the electronic signals associated with the detectors are such that the area under a given peak is proportional to the concentration (C) of the substance being detected. In this case

$$\frac{C_u}{A_u} = \frac{C_{std}}{A_{std}} \tag{2.29.1}$$

where A is the area under the peak for the standard (std) or unknown (u). If a GLC system lacks an integrator and digital printout, you can cut the peak out and weigh it. If the detector does have a digital readout, a peak area number in arbitrary units will be printed out corresponding to each peak. The peaks are identified by their retention times.

There are many variables in the GLC (or HPLC) assay that can lead to inaccurate and imprecise analysis. These include variations in extraction efficiency, amount of sample injected, processing losses, and chromatographic errors. To minimize these errors, many chromatographic techniques employ an internal standard (I.S.). In this case, the concentration of the analyte (C_u) is related to the ratio of the peak area (or peak height) of the analyte (A_u) to the peak area of the internal standard (A_{IS}) as follows:

$$\frac{C_u}{\frac{A_u}{A_{IS}}} = \frac{C_{std}}{\frac{A_{std}}{A_{IS}}} \tag{2.29.2}$$

Any variation that occurs in the analysis will affect the I.S. to the same extent as it affects the analyte. So although variations in injecting the sample into the GLC system might lead to an increase or decrease in the A_u, the same change would occur to the A_{IS}, leaving ratio A_u/A_{IS} essentially unchanged. Thus, the internal standard technique is widely used whenever a suitable I.S. can be found.

MATERIALS

Sample chromatograms are found at the end of this exercise. These chromatograms resulted from an assay for alcohols using the following procedure. The student will need a balance and calculator to perform this exercise.

PROCEDURE

1. Sample preparation. Pipet 200 mL of internal standard into a 12 mm x 75 mm glass tube. Add 200 mL of serum, and mix.
2. Chromatographic parameters. Injection temperature, 210°C; column temperature, 100°C; detector temperature, 260°C; gas flow at 60 mL/min, with the proportions of nitrogen/air/hydrogen at 40:10:20.
3. Inject 0.5 mL of sample or standard into chromatograph (don't bend or blunt injection needle; avoid air bubbles in sample). Chromatographic run time is approximately 6 minutes.
4. Determine the retention time of each of the standards, and record in the Results section on the Data Sheet.
5. Identify your unknown by comparing its retention time to that of the standards.
6. Cut out the peaks of the unknown and the appropriate standard and weigh them.
7. Determine the concentration of the unknown and record in the Results section on the Data Sheet.

OPTIONAL EXERCISE

Repeat the calculation described on the data sheet, but use the peak heights in mm, rather than peak area. Is there any significant difference?

DATA SHEET, EXERCISE #29

NAME: _____
DATE: _____

RESULTS

Sample	Retention Time (min)	Peak Area (mm^2)	Peak Weight (mg)	Peak Area Ratio (A/A_{IS})
Standard Methanol				
Standard Ethanol				
Standard Isopropanol				
Internal Standard				
Unknown Alcohol (s)				
Unknown Alcohol (s)				
Internal Standard				

From the retention time, what alcohol (s) is/ (are) present in the unknown?

_____

CALCULATIONS

Calculate the concentration of the unknown alcohol as follows:

C_u = concentration of unknown alcohol,

C_{std} = concentration of corresponding alcohol standard

A_u = peak area of unknown alcohol

A_{std} = peak area of standard

A_{IS} = peak area of corresponding internal standard

$$\frac{C_u}{\frac{A_u}{A_{IS}}} = \frac{C_{std}}{\frac{A_{std}}{A_{IS}}} \tag{2.29.3}$$

$$C_u = C_{std} \times \frac{\frac{A_u}{A_{IS}}}{\frac{A_{std}}{A_{IS}}} \tag{2.29.4}$$

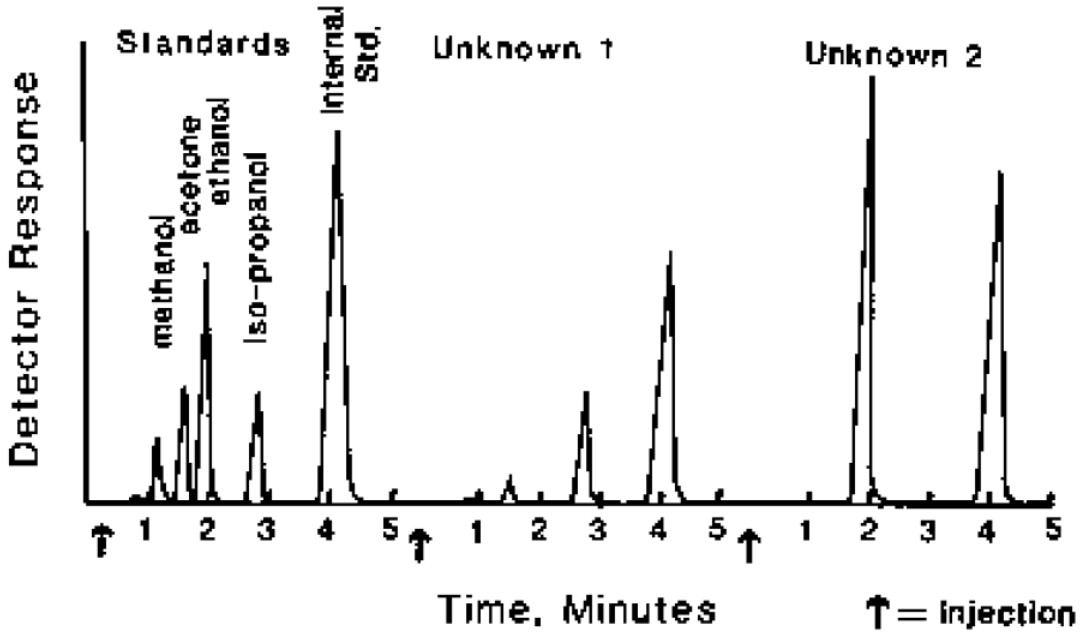
The concentrations of the unknown alcohol(s) is/are:

_____ μg/mL _____ μg/mL

OPTIONAL EXERCISE

Using peak heights, the concentration of the unknown alcohol(s) are:
_____μg/mL

The following chromatograms can be used for the above calculations. To weigh peaks, photocopy this page in order to have a copy to cut out.



Discussion Questions

1. If the polarity of the stationary phase for this analysis were increased, how would the retention times of the alcohol's change?
2. This assay does not require any extraction of the analyte from the sample. Might this fact affect column longevity?
3. How would you measure the levels of n-propanol in a sample?
4. What is the chemical difference between the alcohol's analyzed by this exercise? How does this difference relate to the observed elution pattern?
5. What assumptions are made concerning the use of a particular compound as an internal standard?

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2.30: Radioimmunoassay (RIA)

RELATED READINGS: Pages 187-200, 249-250, 254-256.

OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related readings, the student will be able to:

1. Explain the basic principles of RIAs.
2. Use the data presented to construct a standard curve.

PRINCIPLE

Many immunoassays are based upon the competition between labeled and unlabeled antigen (Ag) for a limited number of binding sites on antibody (Ab) molecules. Radioimmunoassays (RIAs) use an unstable isotope to label the antigen. Commonly used isotopes are tritium (^{3}H) and ^{125}I.

All RIAs are heterogeneous irnrnunoassays, that is, they require the separation of Ab-bound antigen from free, labeled antigen. Commonly used techniques for separating bound from free label include:naffixing the antibody to a solid phase (test tubes, Sephadex particles, or glass beads), precipitation of Ab-Ag complexes by a second Ab or polyethylene glycol, and absorption of free Ag by charcoal. Either the free or bound label (usually the latter) can be counted, and the amount of radioactivity (counts or disintegrations/minute) is related to the antigen concentration. This relationship is complex, and several mathematical equations have been used to “linearize” the data. One common technique is to plot the $\%B/B_0$ on a linear or logarithmic scale vs. the analyte concentration on a logarithmic scale. Another common technique is to use the logit plot of the standards (usually _5) which has the following relationship:

$$\text{logit}\frac{B}{B_0} = \ln\frac{\frac{B}{B_0}}{1-\frac{B}{B_0}} \tag{2.30.1}$$

where: B = bound counts in the presence of unlabeled antigen, B_0 = bound counts in the absence of unlabeled antigen, ln = natural logarithm

The logit calculation is usually plotted on a linear scale vs. the logarithm of the analyte concentration. It is important to remember that radioisotopes are considered hazardous, and there are strict regulations regarding their purchase, use, handling, and disposal. Before performing any radioisotope procedure, you must read the safety manual for your institution. Your instructor will review this information with you.

MATERIALS

Calculator or statistics table capable of performing natural logarithm transformations

PROCEDURE

1. Using the information provided on the data sheet average the bound counts for the “0” standard. This is B_0.
2. Average the bound counts (this is B) for each sample and standard.
3. Calculate B/B_0, $\%B/B_0$, $1\text{-}B/B_0$, and then logit B/B_0 for each sample.
4. Plot $\%B/B_0$ (vertical axis) vs T4 concentration of the standards on the log scale (X, or horizontal axis) of the first graph provided.
5. Plot logit B/B_0 (vertical axis) vs T4 concentration of the standards on the log scale (X-axis) of the second graph provided.
6. Determine the value of the T4 concentration in the unknown(s) by interpolation from both of the standard curves. Record on the Data Sheet.

Discussion Questions

1. If the counts listed in the data sheet for the “0” standard (B_0) were counted in one minute, what would the counting precision be? If these counts were accumulated during a 10 minute counting period, would the precision change?
2. Why is the logit conversion frequently used for data reduction in competitive binding assays?
3. What portions of the $\%B/B_0$ curve is most affected by the logit transformation? Does this improve the quality of the result?

4. A vial of ^{125}I labeled T4 is purchased with an activity of 10,000 cpm/μg (half-life = 2 months). The assay was not performed until 8 months later. What is the activity at that time?

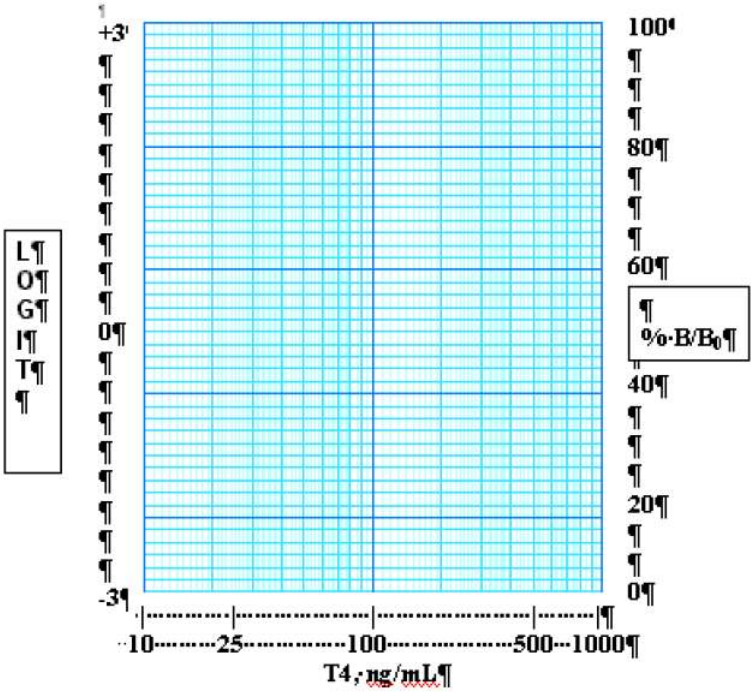
DATA SHEET, EXERCISE #30	NAME: _____ DATE: _____

RESULTS

Data from experiment:

Sample	Cpm	Average cpm	B/B_0	$1-B/B_0$	Logit B/B_0
T4 Standard (μg/L)					
0(B_0)	43285 41003				
25	37449 33274				
50	33107 30010				
100	23662 21976				
150	19583 19227				
300	14164 12808				

Unknowns						Calculated T4, μg/L
1	37448 35754					
2	26867 24047					
3	34174 33970					
4	24427 24025					
5	19331 17790					



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CHAPTER OVERVIEW

3: Urinalysis

objectives

- To show the range of linearity and to relate this to the commonly observed results.
- To show the correlation between visual and objective instrument quantitation and give confidence in their clinical recording of urinalysis data.
- To show that, for a number of these tests, timing of the test observation is very important.
- To show that the range of strip response to analyte concentration is very limited and that disease processes can result in values far greater than the maximum allowed response.

The purpose of these urinalysis experiments is to teach students laboratory principles by using one of the most common laboratory procedures. We have designed these experiments so that they do not require an instrument, although if one is available, it can add to the experience of the student. The experiments are designed to demonstrate basic analytical principles that are directly applicable to their broader, real world experience.

3.1: Protein in urine
3.2: Glucose in urine
3.3: Specific Gravity of Urine
3.4: Red Cells or Hemoglobin in urine
3.5: Nitrite in urine
3.6: Leukocytes or Esterase in urine

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3.1: Protein in urine

RELATED READING: Chapter 26, 32. See Methods in CD-ROM forTotal protein, urine.

Background to test

Small amounts of protein are found in the urine of healthy individuals. These proteins include albumin and Tamm-Horsfall mucoprotein. Individuals with glomerular or renal tubular damage excrete larger amounts of protein into final urine than do healthy individuals. As in the case of glucose, the cells of the proximal tubule in healthy individuals reabsorb most of the albumin present in the glomerular filtrate. If the reabsorptive capacity of the tubular cells is exceeded by either increased glomerular permeability to albumin or by a loss of proximal tubular reabsoptive capacity, increased amounts of albumin appears in final urine. Loss of the glomerular barrier can result in far greater protein excretion than the loss of tubular reabsorptive capacity. Other serum proteins, such as beta-2-microglobulin, are also present in urine, however their concentration is much lower, therefore albumin by far is the protein most commonly monitored in routine urinalysis. Proteinuria, or more commonly albuminuria, is commonly observed in diabetics, since this disease is found in 5-6% of the U.S. population. Similarly, hypertension can lead to proteinuria. The dipstick is a rapid method of determining the presence of urinary protein above a predetermined concentration.

Principle of the Method

When albumin binds to a dye embedded in the dipstick, there is a release of H+ and a local change in pH. This pH change results in a change in the color of a dye. The amount of color change in the dye is proportional to the concentration of albumin in the urine.

The reactions are as follows:

$$\text{albumin} + \text{dye} \rightarrow \text{albumin-dye complex} + H^+ \tag{3.1.1}$$

$$H^+ \text{ dye} \rightarrow \text{color change} \tag{3.1.2}$$

The dye requires a minimum pH change before a color change is visible. For many dipsticks this is in the range of 30-100 mg/L of urine. There is also a maximum color change that can be obtained with the strip reagent. This is commonly set at 2000 mg/L.

Reagents

All the reagents for the reaction are embedded in the pad of the dipstick. As with all reagents stored in a dried form, their stability is affected by moisture. Therefore the reagents must be protected from moisture. In addition, careful attention must be given to the manufacturer's shelf life, which should appear on the label of every dipstick container.

Specimen

Freshly voided urine is the preferred specimen. The first urine specimen of the day is considered the most desirable, because it is the most concentrated. Urine specimens are acceptable up to four hours after voiding. Refrigerated specimens are acceptable up to 24 hours after excretion.

Procedure

Collect the urine in an appropriate specimen container. After verifying that the strips are working (see quality control) quickly dip the strip in the urine, removing excess liquid by moving the edge of the strip against the rim of the container as you remove the strip from the container. After this initial pass to remove excess liquid, remove any remaining liquid by touching the entire edge of the strip to a gauze pad or a paper towel There should be no visible liquid on the strip except for that on the pad.

Results

Start timing the reaction as soon as the strip is placed in the urine. After 60 seconds read the strip visually. Match the observed color of the pad with the chart color on the bottle of strips or with a color chart, if that is available. Record your observation. If a Clinitek or other instrument is available, place the strip in the device as soon as you dip the strip and record the printed result. If reading visually, continue reading the results after 60 seconds, 120 seconds, and 300 seconds.

Calculations

No calculations are necessary for the visual readings. If instrument readings are made, the calibrated instrument calculates the protein concentration. If an instrument is used, correlate the visual reading with that of the instrument.

Quality Control

When visual readings are taken, be certain that the reader is not color blind. Before testing a test sample, take two strips and test a positive and a negative control sample. These results should be within accepted values. Ideally, a positive and negative control should be tested along with each batch of patient specimens tested.

Expected values

Urine from healthy individuals should give negative results. Urine from known diabetics may give positive results, depending upon the extent of glomerular damage caused by their diabetes.

STUDENT REPORT

Solution	Visual Color Score			Instrument Value at Set Times
Time	**30 sec.**	**60 sec.**	**300 sec.**	
QC negative				
QC positive				
1 g/L				
500 mg/L				
250 mg/L				
125 mg/L				
62 mg/L				
31 mg/L				

Test protein solution 1	
Test protein solution 2	
Test protein solution 3	

Discussion Questions

1. What is the range of linearity of the visual method?
2. What is the range of linearity of the instrument?
3. Does the test result change with time?
4. If the result does change with time, Why?
5. Does the range of linearity include all healthy and disease conditions?

Instrument notes

The thermal paper print out from many of these instruments is not acceptable as a permanent copy. The reason is that this type of paper is unstable, and becomes unreadable with time. Results must either be hand copied to the patient chart, or made permanent in some other manner.

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3.2: Glucose in urine

RELATED READING: Chapter 32 SEE URINALYSIS INFOBASE.

Glucose does not appear in the urine of healthy individuals. In diabetics, plasma glucose concentrations can be several-fold higher than that of healthy individuals. The kidney recovers glucose filtered through the glomerulus by active reabsorbtion by the cells of the proximal tubule. However, when the glucose concentration in the glomerular filtrate exceeds about 180 mg/dL, this reabsorptive capacity is exceeded. As a consequence, some of the filtered glucose is present in final urine. In general, the higher the plasma glucose, the greater the concentration in the final urine. However, urine output is a consequence of fluid intake, thus there is a considerable variance of glucose concentration (or many other analytes) in the urine of the same individual during a 24-hour period, or between individuals. The dipstick is a rapid method of determining the presence of urinary glucose, which can be used to screen for diabetes or monitor the therapy of diabetics.

Principle of the Method

The dipstick uses two coupled enzymatic reactions to measure the presence of glucose. The first reaction uses the enzyme glucose oxidase to oxidize the glucose, forming gluconic acid and hydrogen peroxide. The second reaction uses the hydrogen peroxide to oxidize a dye to form a colored product, or chromogen. The colored product can be visually observed or measured by an instrument. The amount of colored product formed is proportional to the amount of glucose present in the urine.

The reactions are as follows:

$$\text{Glucose} + O_2 \rightarrow \text{Gluconic acid} + H_2O_2 \tag{3.2.1}$$

$$H_2O_2 + \text{dye} \rightarrow \text{color change} \tag{3.2.2}$$

The chromogen formation is visible after a minimal amount is converted. For many dipsticks this is in the range of 75-125 mg/dL of urine. There is also a maximum color change that can be obtained with the strip reagent. This is commonly set at 2000 mg/dL.

Reagents

All the reagents for the reaction are embedded in the pad of the dipstick. As with all reagents stored in a dried form, their stability is affected by moisture. Therefore the reagents must be protected from moisture. In addition, careful attention must be given to the manufacturer’s shelf life, which should appear on the label of every dipstick container.

Specimen

Freshly voided urine is the preferred specimen. The first urine specimen of the day is considered the most desirable, because it is the most concentrated. Urine specimens are acceptable up to four hours after voiding. Refrigerated specimens are acceptable up to 24 hours after excretion.

Procedure

Collect the urine in an appropriate specimen container. After verifying that the strips are working (see quality control) quickly dip the strip in the urine, removing excess liquid by moving the edge of the strip against the rim of the container as you remove the strip from the container. After this initial pass to remove excess liquid, remove any remaining liquid by touching the entire edge of the strip to a gauze pad or a paper towel. There should be no visible liquid on the strip except for that on the pad.

Results

Start timing the reaction as soon as the strip is placed in the urine. After 30 seconds read the strip visually. Match the observed color of the pad with the chart color on the bottle of strips or with a color chart, if that is available. Record your observation. If a Clinitek or other instrument is available, place the strip in the device as soon as you dip the strip and record the printed result. If reading visually, continue reading the results after 60 seconds, 120 seconds, and 300 seconds.

Calculations

No calculations are necessary for the visual readings. If instrument readings are made, the calibrated instrument calculates the urinary glucose concentration. If an instrument is used, correlate the visual reading with that of the instrument.

Quality Control

When visual readings are taken, be certain that the reader is not color blind. Before testing a test sample, take two strips and test a positive and a negative control sample. These results should be within accepted values. Ideally, a positive and negative control should be tested along with each batch of patient specimens tested.

Expected values

Urine from healthy individuals should give negative results. Urine from known diabetics may give positive results depending upon the extent to which their glucose is controlled and their fluid intake.

STUDENT REPORT

Solution	Visual Color Score			Instrument Value at Set Times
Time	**30 sec.**	**60 sec.**	**300 sec.**	
QC negative				
QC positive				
1 g/L				
500 mg/L				
250 mg/L				
125 mg/L				
62 mg/L				
31 mg/L				

Test sugar solution 1	
Test sugar solution 2	
Test sugar solution 3	

Discussion Questions

1. What is the range of linearity of the visual method?
2. What is the range of linearity of the instrument?
3. Does the test result change with time?
4. If the result does change with time, Why?
5. Does the range of linearity include all healthy and disease conditions?

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3.3: Specific Gravity of Urine

RELATED READING: Chapter 26. SEE URINALYSIS INFOBASE.

Specific gravity is a property of urine that is dependent upon the amount of solids dissolved in urine. The specific gravity is primarily a measure of the salts excreted in final urine. Most commonly these salts include the ions sodium, potassium and chloride. Other commonly excreted metabolic products such as urea, and creatinine also make up a portion of the dissolved solids. All urine including that from healthy individuals contains dissolved solids. However, urine output is a consequence of fluid intake, thus there is a considerable variance of specific gravity in urine in the same individual, or between individuals. The dipstick is a rapid method of determining the specific gravity.

Principle of the Method

The dipstick employs a specially treated polyelectrolyte, whose pKa changes in response to increased concentration of ions. The changed pKa results in a changed pH in the environment of the pad, which causes a dye, also impregnated in the pad, to change color. The amount of change in dye color is proportional to the amount of dissolved ions present in the urine.

The reactions are as follows:

$$\text{Polyelectrolyte} + \text{changing ionic strength} \rightarrow \text{change pKa} + \Delta H^{+} \tag{3.3.1}$$

$$\Delta H^{+} + \text{indicator dye} \rightarrow \text{color change} \tag{3.3.2}$$

The dye requires a minimum pH change before a color change is visible; for many dipsticks this is in the range of 1.000. There is also a maximum color change that can be obtained with the strip reagent. This is commonly set at 1.030.

Reagents

All the reagents for the reaction are embedded in the pad of the dipstick. As with all reagents stored in a dried form, their stability is affected by moisture. Therefore the reagents must be protected from moisture. In addition, careful attention must be given to the manufacturer's shelf life, which should appear on the label of every dipstick container.

Specimen

Freshly voided urine is the preferred specimen. The first urine specimen of the day is considered the most desirable, because it is the most concentrated. Urine specimens are acceptable up to four hours after voiding. Refrigerated specimens are acceptable up to 24 hours after excretion.

Procedure

Collect the urine in an appropriate specimen container. After verifying that the strips are working (see quality control) quickly dip the strip in the urine, removing excess liquid by moving the edge of the strip against the rim of the container as you remove the strip from the container. After this initial pass to remove excess liquid, remove any remaining liquid by touching the entire edge of the strip to a gauze pad or a paper towel. There should be no visible liquid on the strip except for that on the pad.

Results

Start timing the reaction as soon as the strip is placed in the urine. After 45 seconds read the strip visually. Match the observed color of the pad with the chart color on the bottle of strips or with a color chart, if that is available. Record your observation. If a Clinitek or other instrument is available, place the strip in the device as soon as you dip the strip and record the printed result. If reading visually, continue reading the results after 60 seconds, 120 seconds, and 300 seconds.

Calculations

No calculations are necessary for the visual readings. If instrument readings are made, the calibrated instrument calculates the specific gravity. If an instrument is available, correlate the visual reading with that of the instrument.

Quality Control

When visual readings are taken, be certain that the reader is not color blind. Before testing a test sample, take two strips and test a positive and a negative control sample. These results should be within accepted values. Ideally, a positive and negative control

should be tested along with each batch of patient specimens tested.

Expected values

Urine from healthy individuals should give positive results within the range specified on the bottle. The most common abnormal results are from diluted urine.

STUDENT REPORT

Solution	Visual Color Score			Instrument Value at Set Times
Time	**30 sec.**	**60 sec.**	**300 sec.**	
QC negative				
QC positive				
1 g/L				
500 mg/L				
250 mg/L				
125 mg/L				
62 mg/L				
31 mg/L				

Test sugar solution 1	
Test sugar solution 2	
Test sugar solution 3	

Discussion Questions

1. What is the range of linearity of the visual method?
2. What is the range of linearity of the instrument?
3. Does the test result change with time?
4. If the result does change with time, Why?
5. Does the range of linearity include all healthy and disease conditions?

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3.4: Red Cells or Hemoglobin in urine

RELATED READING: Chapter 26 SEE URINALYSIS INFOBASE.

Red cells are not found in the urine of healthy individuals. Individuals with some forms of glomerular damage allow red cells to pass through the glomerular barrier. The dipstick is a rapid method of determining the presence of red cells in urine. If positive, microscopic evaluation of the urine sediment is usually performed.

Principle of the Method

The dipstick uses the peroxidase-like activity of hemoglobin (in the red cells or free) to convert a benzidine dye into a chromogen. The amount of color change in the dye is proportional to the amount of red cells present on the dipstick pad.

The reactions are as follows:

Intact red blood cells are lysed on the pad to release hemoglobin.

$$\text{Hemoglobin} + \text{complexed peroxide} \rightarrow \text{free } H_2O_2 \tag{3.4.1}$$

$$H_2O_2 + \text{benzidine dye} \rightarrow H_2O + \text{oxidized chromogen (color change)} \tag{3.4.2}$$

The dye reaction requires time to become visible. Each red cell on the pad gives a visible reaction. If only a few cells are present, the reaction gives a speckled appearance on the pad. There is also a maximum color change that can be obtained with the strip reagent. This is commonly set at 0.0150.062 mg/dl of hemoglobin, equivalent to 5-20 intact red blood cells per high powered field.

Reagents

All the reagents for the reaction are embedded in the pad of the dipstick. As with all reagents stored in a dried form, their stability is affected by moisture. Therefore the reagents must be protected from moisture. In addition, careful attention must be given to the manufacturer's shelf life, which should appear on the label of every dipstick container.

Specimen

Freshly voided urine is the preferred specimen. The first urine specimen of the day is considered the most desirable, because it is the most concentrated. Urine specimens are acceptable up to four hours after voiding. Refrigerated specimens are acceptable up to 24 hours after excretion.

Procedure

Collect the urine in an appropriate specimen container. After verifying that the strips are working (see quality control) quickly dip the strip in the urine, removing excess liquid by moving the edge of the strip against the rim of the container as you remove the strip from the container. After this initial pass to remove excess liquid, remove any remaining liquid by touching the entire edge of the strip to a gauze pad or a paper towel. There should be no visible liquid on the strip except for that on the pad.

Results

Start timing the reaction as soon as the strip is placed in the urine. After 60 seconds read the strip visually. Match the observed color of the pad with the chart color on the bottle of strips or with a color chart, if that is available. Record your observation. If a Clinitek or other instrument is available, place the strip in the device as soon as you dip the strip and record the printed result. If reading visually, continue reading the results after 60 seconds, 120 seconds, and 300 seconds.

Calculations

No calculations are necessary for the visual readings. If instrument readings are made, the calibrated instrument calculates the hemoglobin concentration. If an instrument is used, correlate the visual reading with that of the instrument.

Quality Control

When visual readings are taken, be certain that the reader is not color blind. Before testing a test sample, take two strips and test a positive and a negative control sample. These results should be within accepted values. Ideally, a positive and negative control should be tested along with each batch of patient specimens tested.

Expected values

Urine from healthy individuals should give negative results. Urine from known patients with kidney disease may give positive results depending upon the extent of glomerular damage present.

STUDENT REPORT

Solution	Visual Color Score			Instrument Value at Set Times
Time	**30 sec.**	**60 sec.**	**300 sec.**	
QC negative				
QC positive				
1 g/L				
500 mg/L				
250 mg/L				
125 mg/L				
62 mg/L				
31 mg/L				

Test solution 1	
Test solution 2	
Test solution 3	

Discussion Questions

1. What is the range of linearity of the visual method?
2. What is the range of linearity of the instrument?
3. Does the test result change with time?
4. If the result does change with time, Why?
5. Does the range of linearity include all healthy and disease conditions?

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3.5: Nitrite in urine

RELATED READING: Chapter 26. SEE URINALYSIS INFOBASE.

Bacteria are not found in the urine of healthy individuals. Individuals with some forms of kidney or bladder infection will pass bacteria into final urine. The dipstick is a rapid method of determining the presence of Gram-negative bacteria in urine, which have the ability of converting dietary nitrate into nitrite. If a positive nitrite result is obtained, a microscopic evaluation of the urine sediment or a urine culture is usually performed.

Principle of the Method

In the acid environment of the pad, urinary nitrate reacts with dye, converting it into a chromogen. The amount of color change in the dye is proportional to the concentration of nitrite in the urine and, indirectly, of bacteria present on the dipstick pad.

The reaction is as follows:

$$\text{Nitrite} + \text{dye} \rightarrow \text{coupled dye (chromogen)} \tag{3.5.1}$$

The dye reaction requires time to become visible. There is also a maximum color change that can be obtained with the strip reagent. This is commonly set at about 0.06 mg/dL of nitrite.

Reagents

All the reagents for the reaction are embedded in the pad of the dipstick. As with all reagents stored in a dried form, their stability is affected by moisture. Therefore the reagents must be protected from moisture. In addition, careful attention must be given to the manufacturer's shelf life, which should appear on the label of every dipstick container.

Specimen

Freshly voided urine is the preferred specimen. The first urine specimen of the day is considered the most desirable, because it is the most concentrated. Urine specimens are acceptable up to four hours after voiding. Refrigerated specimens are acceptable up to 24 hours after excretion.

Procedure

Collect the urine in an appropriate specimen container. After verifying that the strips are working (see quality control) quickly dip the strip in the urine, removing excess liquid by moving the edge of the strip against the rim of the container as you remove the strip from the container. After this initial pass to remove excess liquid, remove any remaining liquid by touching the entire edge of the strip to a gauze pad or a paper towel. There should be no visible liquid on the strip except for that on the pad.

Results

Start timing the reaction as soon as the strip is placed in the urine. After 60 seconds read the strip visually. Match the observed color of the pad with the chart color on the bottle of strips or with a color chart, if that is available. Record your observation. If a Clinitek or other instrument is available, place the strip in the device as soon as you dip the strip and record the printed result. If reading visually, continue reading the results after 60 seconds, 120 seconds, and 300 seconds.

Calculations

No calculations are necessary for the visual readings. If instrument readings are made, the calibrated instrument calculates the nitrite concentration. If an instrument is available, correlate the visual reading with that of the instrument.

Quality Control

When visual readings are taken, be certain that the reader is not color blind. Before testing a test sample, take two strips and test a positive and a negative control sample. These results should be within accepted values. Ideally, a positive and negative control should be tested along with each batch of patient specimens tested.

Expected values

Urine from healthy individuals should give negative results. Urine from known patients with kidney or bladder infections may give positive results depending upon the extent and type of bacteria present. A limitation of this screening procedure is the amount of time the urine has remained in the bladder prior to collection. A short time may limit the amount of nitrite that can be produced by any Gram negative bacteria and produce false negative results.

STUDENT REPORT

Solution	Visual Color Score			Instrument Value at Set Times
Time	**30 sec.**	**60 sec.**	**300 sec.**	
QC negative				
QC positive				
1 g/L				
500 mg/L				
250 mg/L				
125 mg/L				
62 mg/L				
31 mg/L				

Test solution 1	
Test solution 2	
Test solution 3	

Discussion Questions

1. What is the range of linearity of the visual method?
2. What is the range of linearity of the instrument?
3. Does the test result change with time?
4. If the result does change with time, Why?
5. Does the range of linearity include all healthy and disease conditions?

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3.6: Leukocytes or Esterase in urine

RELATED READING: Chapter 26. SEE URINALYSIS INFOBASE.

White cells are not found in the urine of healthy individuals. Individuals with some forms of kidney or bladder infection will pass white cells into final urine. The dipstick is a rapid method of determining the presence of certain types of white cells in urine, called granulocytes. If positive, microscopic evaluation of the urine sediment or a urine culture is usually performed.

Principle of the Method

The dipstick method detects the nonspecific esterases present in granulocytic leukocytes, which hydrolyzs an acid ester to release a alcohol. The alcohol reacts with a daizonium salt to form a purple product. The amount of color change in the dye is proportional to the number of white cells present on the dipstick pad.

The reactions are as follows:

$$\text{Esterase} + \text{acid ester} \rightarrow \text{acid} + \text{active alcohol} \tag{3.6.1}$$

$$\text{Active alcohol} + \text{diazonium salt} \rightarrow \text{purple color} \tag{3.6.2}$$

The dye reaction requires time to become visible. There is also a maximum color change that can be obtained with the strip reagent. This is commonly set at approximately 5-15 cells/hpf.

Reagents

All the reagents for the reaction are embedded in the pad of the dipstick. As with all reagents stored in a dried form, their stability is affected by moisture. Therefore the reagents must be protected from moisture. In addition, careful attention must be given to the manufacturer's shelf life, which should appear on the label of every dipstick container.

Specimen

Freshly voided urine is the preferred specimen. The first urine specimen of the day is considered the most desirable, because it is the most concentrated. Urine specimens are acceptable up to four hours after voiding. Refrigerated specimens are acceptable up to 24 hours after excretion.

Procedure

Collect the urine in an appropriate specimen container. After verifying that the strips are working (see quality control) quickly dip the strip in the urine, removing excess liquid by moving the edge of the strip against the rim of the container as you remove the strip from the container. After this initial pass to remove excess liquid, remove any remaining liquid by touching the entire edge of the strip to a gauze pad or a paper towel. There should be no visible liquid on the strip except for that on the pad.

Results

Start timing the reaction as soon as the strip is placed in the urine. After 2 minutes read the strip visually. Match the observed color of the pad with the chart color on the bottle of strips or with a color chart, if that is available. Record your observation. If a Clinitek or other instrument is available, place the strip in the device as soon as you dip the strip and record the printed result. If reading visually, continue reading the results after 60 seconds, 120 seconds, and 300 seconds.

Calculations

No calculations are necessary for the visual readings. If instrument readings are made, the calibrated instrument calculates the esterase concentration. If an instrument is used, correlate the visual reading with that of the instrument.

Quality Control

When visual readings are taken, be certain that the reader is not color blind. Before testing a test sample, take two strips and test a positive and a negative control sample. These results should be within accepted values. Ideally, a positive and negative control should be tested along with each batch of patient specimens tested.

Expected values

Urine from healthy individuals should give negative results. Urine from known patients with kidney or bladder infections may give positive results depending upon the extent and type of infection present.

STUDENT REPORT

Solution	Visual Color Score			Instrument Value at Set Times
Time	**30 sec.**	**60 sec.**	**300 sec.**	
QC negative				
QC positive				
4400 units/dL				
2200 units/dL				
1100 units/dL				
550 units/dL				
275 units/dL				
137 units/dL				

Test solution 1	
Test solution 2	
Test solution 3	

Discussion Questions

1. What is the range of linearity of the visual method?
2. What is the range of linearity of the instrument?
3. Does the test result change with time?
4. If the result does change with time, Why?
5. Does the range of linearity include all healthy and disease conditions?

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4.1: Calculations

A medical technologist is performing the following assay for a drug whose molecular weight is 318.5 daltons. A 2 mL aliquot of serum is extracted into 5 mL of diethyl ether. It has been established that the extraction efficiency of the analyte into ether is 85%. Four mL of the ether layer is transferred to a test tube and evaporated to dryness at 37°C under a stream of nitrogen gas. The residue is taken up in 2 mL of a reagent that shifts the absorbance maxima of the analyte to 310 nm. After 10 minutes incubation at 37°C, the absorbance of the solution is measured at 310 nm using a spectrophotometer with a 1 cm pathlength. The recorded absorbance is 2.475. Since this absorbance exceeds the linearity of the instrument, the absorbance is measured on a 1:10 dilution of the sample. The absorbance of the diluted sample is recorded as 0.475. The molar extinction coefficient of the analyte at 310 nm is 18.7 x 10^3 L•mol^{-1}•cm^{-1}.

QUESTION

What is the concentration of the drug in the original serum sample in mg/L.

Answer

The serum concentration of this drug is:_____ mg/L.

Answer

a. First calculate the concentration of the analyte in the final solution using Beer's law as follows (p. 88): A = ϵcb; where ϵ = 18.7 x 10^3 L • mol^{-1} • cm^{-1}, c = unknown, b = 1 cm, and A = 0.475.

$$\begin{aligned} 0.475 &= 18.7 \times 10^3\ L \cdot mol^{-1} \cdot cm^{-1}\ (1\ cm) \times c \\ c &= 0.0254 \times 10^{-3}\ mol/L \\ &or \\ c &= 0.0254\ mmol/L \end{aligned} \tag{4.1.1}$$

Since this absorbance was obtained on a 1:10 dilution, the actual concentration in the 2 mL sample is:

$$c = 0.0254\ mmol/L \times 10 = 0.254\ mmol/L \tag{4.1.2}$$

b. Now calculate the amount of material extracted (p. 34-37). The concentration is 0.254 mmol/L in the 2 mL sample, and the total amount of analyte extracted is:

$$\text{concentration} \times \text{sample volume} = \text{amount} \tag{4.1.3}$$

c. The micromoles of analyte extracted in part b were originally in a 4 mL aliquot of ether which represents 4/5 of the total volume into which the analyte was extracted. Thus, the actual total amount extracted from the 2 mL serum aliquot is:

$$0.508\ \mu mol/\ 4\ mL \times 5\ mL = 0.635\ \mu mol\ extracted\ from\ 2\ mL\ of\ serum \tag{4.1.4}$$

d. Since the extraction efficiency is only 85%, the total analyte extracted must be corrected for the 15% of the analyte that was not extracted. This is calculated as follows:

$$\frac{\text{amount extracted}}{\text{efficiency of extraction}} = \text{actual amount present in sample} = \frac{0.635}{0.85} = 0.747\ \mu moles \tag{4.1.5}$$

Thus, the analyte concentration in the 2 mL of serum is:

$$\frac{0.747\ \mu moles\ extracted}{2\ mL\ of\ sample} = 0.37\ \mu mol/mL \tag{4.1.6}$$

e. To calculate (pp. 35-37) the analyte concentration in the serum sample in mg/L use the following conversion formula:

$$\frac{\mu mol}{mL} \times \frac{1000\ mL}{L} \times mol.\ wt.\ (\mu g/mol) \times \frac{1\ mg}{1000\ \mu g} = mg/L \tag{4.1.7}$$

Thus

$$\frac{0.37\ \mu mol}{mL} \times \frac{1000\ mL}{L} \times 318.5\ \mu g/\mu mol \times \frac{1\ mg}{1000\ \mu g} = 118\ mg/L \tag{4.1.8}$$

The serum concentration of the drug is 118 mg/L (0.37 mol/L).

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4.2: Laboratory Equipment

A technologist is asked to weigh 80 g of sodium chloride (with an error of ± 1%) to prepare a 2M sodium chloride solution. Ten mL of this solution will be diluted to a final volume of 100 ml. Fifty-three mL of the diluted salt solution will be used to dissolve 10 mg (+ 1%) of albumin. It will take an overnight mixing to achieve solution of the albumin.

QUESTION

Which laboratory equipment discussed in Chapter 1 or shown In Figure 1-1 should be used for this preparation?

Questions to Consider

1. What are the different types of balances available, and how do they differ in their precision of weighing and use?
2. How are different types of glassware used to deliver different volumes of fluid?

Answer

Which laboratory equipment should be used for this experiment?

To weigh 80 g NaCl use:

To weigh 10 mg albumin use:

To deliver 10 mL of saline use:

Deliver 10 mL of saline for dilution into:

To deliver 53 mL of diluted saline use:

Dissolve albumin in a:

Answer

Answers to Questions to Consider

1. Balances differ in their useful range of operation, their achievable precision, and their ease of use (Table 1-11). Thus, precision mechanical balances are used to weigh gram quantities or greater when the reproducibility required is only in the 1-1,000 mg range. Mechanical trip balances are very easy to use for rapid, relatively precise weighings of 10-100 gm weights. Electronic microbalances, on the other hand, are only useful when weighing quantities <10 mg, but have reproducibility in the microgram range. The analytical balances have intermediary use and precision.
2. For delivery of an exact volume of fluid, volumetric pipets give the best accuracy and precision. However, not all pipet sizes are usually available in the laboratory. Mohr type graduated pipets are useful for delivery of volumes less than 25 mL. For volumes larger than 25 mL, one would need to use graduated cylinders. One should always use the smallest size of Mohr or graduated cylinder that will contain the desired volume. This will result in the smallest error of measurement (see Tables 1-6 and 1-7).

Answer

For weighing 80 g of NaCl, it would be time consuming and unnecessary to use the microbalances. A mechanical precision balance, would allow one to weigh the salt rapidly with an acceptable error of less than 1%. On the other hand, only the electronic microbalances would be useful for weighing as little as 10 mg of albumin with the desired accuracy.

To deliver exactly 10 mL of saline solution, one should use a 10 mL volumetric pipet. This should be delivered into a 100 mL volumetric flask for accurate. dilution. To deliver 53 mL of diluted saline, a 100 mL graduated cylinder should be used, since it is the smallest graduated cylinder that will contain the desired volume.

The albumin can be placed in either a 100 mL Erlenmeyer flask or a 100 mL beaker. For overnight stirring, an Erlenmeyer flask would be easier to cover to prevent evaporation and contamination and thus might be the most convenient flask to use.

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4.3: Sample Preparation

A laboratory is introducing serum separator (“barrier”) phlebotomy tubes. The 10 mL tubes containing the gel will be centrifuged for 10 minutes in a 52° angle rotor in a bench-top centrifuge. The centrifuge has a listed top speed of 2200 rpm.

The medical technologists in this laboratory soon note that often the tubes have clots that are incompletely covered by the barrier gel. This usually occurs when the centrifuge is filled to capacity. This problem is causing significant contamination of the serum with blood cells as well as reducing the amount of serum that can be poured off free of cellular contamination.

A technologist looks up a reference (p. 23, 24) on the use of these barrier tubes and notes that a force of 1000 x g for 10 minutes is recommended for proper use of the tubes. The technologist finds out that the fixed angle rotor in use in the laboratory has a radius of 5.75 inches from the bottom of the tube holder to the drive shaft.

QUESTIONS

1. What is the most likely cause of the problem?
2. How can the technologist use these data to allow the laboratory to use the barrier tubes properly?

Questions to Consider

1. What is the principle of action of the “barrier” phlebotomy tubes?
2. Is the centrifugal force being employed sufficient to use the barrier tubes properly?
3. What other factors affecting centrifuge performance can result in a relative centrifugal force that is insufficient for proper use of the barrier tubes?

Answer

1. The most likely problem is insufficient centrifugal force. By calculating the centrifugal force, one can establish if it is adequate to meet the manufacturer’s specifications.
2. The laboratory can take the following steps to solve the problem: One can centrifuge these samples longer. However, this could result in an inordinate delay in sample processing and cause heating and deterioration of the sample as well (p. 75-76). One can change rotors or centrifuges to have a larger radius during the centrifugation procedure, or increase the rpm, or both. One can also use a swinging-bucket rotor to allow the gel to be spread as a thinner, horizontal layer over the separated clot. Optimally, the laboratory might do all of these, that is, use a swinging-bucket rotor with a larger effective radius that can be spun at higher speeds.

Answers to Question to Consider

1. The barrier tubes contain a silicone-type gel that works on the following principle. The gel has a specific gravity (approximately 1.04 g/cc) which is less than that of the clot and greater than that of the serum. During centrifugation, the applied relative centrifugal force (RCF) pellets the clot beneath the less dense gel while the serum floats on top of the gel. To use the integrated gel-tubes, sufficient centrifugal force must be applied to pellet the clot beneath the gel (p. 24, 71-72).
2. Using the formula on page 25:

$$RCF = 1.12 \times 10^{-5}\ (R)(rpm)^2 \tag{4.3.1}$$

where R = radius (cm), rpm = revolutions per minute, g = 9.8 m/s^2, RCF = relative centrifical force

$$\begin{aligned} RCF &= 1.12 \times 10^{-5}\ (15\ cm)(2200\ rpm)^2 \\ &= 813 \times g \end{aligned} \tag{4.3.2}$$

(Using the nomogram on p. 26, one can estimate the RCF as 825 x g.) This RCF is the maximum centrifugal force that can be generated at the bottom portion of the tube, i.e. at the point furthest from the center of the rotor. Towards the center of the tube, the applied g force will be less. This centrifugal force is not within the manufacturer’s suggested limits and is probably the reason for the separation problems.

3. The RCF calculated above is a theoretical determination of the maximum g force that can be applied. As the number of tubes being centrifuged increases, the actual g force at a given speed setting will decrease. This happens because the speed setting on the centrifuge only adjusts the amperage (current) to the motor driving the rotor. The actual rpm generated varies with total load in the rotor. That is why the problem of insufficient sample separation occurred more often with full rotors; under these conditions, the g force will be even less than the maximum calculated. The actual rotor speed (rpm) under

working conditions should be determined by use of a suitable tachometer (p. 23-26). Another factor to consider is the angle of the tube during centrifugation (p. 24, 71-72). Because of the 52° angle, the gel layer is dispersed over a larger, slanted area. This slant increases the chance that a portion of the clot will remain uncovered by the barrier gel, especially when the centrifugation is being performed under less than optimal conditions, as was the case in this laboratory.

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4.4: Spectrophotometry

The concentration of a pure compound is to be determined by its absorbance at 290 nm and its known molar absorptivity at this wavelength. You have a choice of four different spectrophotometers available in the laboratory, employing either a tungsten, xenon, mercury or deuterium light source.

QUESTIONS

1. Which lamp might be the best light source for this experiment?
2. You subsequently learn that the spectrophotometer with the deuterium lamp has a glass cuvette, while the xenon spectrophotometer has a quartz cell. Does this affect your decision about which spectrophotometer to use? Why?

Questions to Consider

1. Do all these light sources emit equal amounts of light energy throughout the ultraviolet and visible spectra?
2. Does increased energy output of a light source have any advantage in a spectrophotometric assay?
3. Do all optical materials transmit light with equal efficiency?

Answer

1. Usually the deuterium lamp is preferred because it gives uniform light of good intensity in the ultraviolet region. (see p. 90).
2. Yes. The xenon spectrophotometer with a quartz cell will probably have overall better performance than will the instrument with regular glass. (Remember that sensitivity = L x M x R.) Thus, the instrument with the deuterium lamp would not be as useful as the spectrophotometer with the xenon light source.

Answers to Questions to Consider

1. The energy emitted from a light source is the result of heating a material to incandescence. The energy released during the emission process depends upon the nature of the material being used. Thus, most of the energy of the tungsten lamp is emitted in the far-red (>700 nm) as heat, with very little light emitted at wavelengths less than 300 nm. Xenon lamps produce a fair amount of light throughout the UV and visible spectra, while most of the light emitted by deuterium lamps falls in the UV spectrum. Mercury lamps produce a discontinuous light spectrum, with light being emitted only at discrete wavelengths. This is shown in Figures 4-7 and 4-8 (p. 90). Thus, only the xenon or deuterium lamp would be useful for experiments at about 290 nm.
2. As described on p. 92, the sensitivity of a spectrophotometric analysis is dependent upon the lamp output at a given wavelength. At a given photomultiplier tube response, the greater the light output, the higher the sensitivity. At a given concentration of material, the greater the input (incident) light, the greater the amount of light that will be transmitted (although the percent transmission remains constant). Thus, the more light transmitted, the greater the current output of the photomultiplier tube and the more precise and sensitive the assay.
3. As depicted in Figure 4-10 (p. 91), the transmission characteristics of optical materials vary greatly. Quartz glass is much more efficient at transmitting light at 290 nm than regular glass or many plastics.

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4.5: Refractometry

A laboratory is using a refractometer to measure serum protein in the STAT lab area. A technologist measures the total protein in one specimen as 89 g/L. When the technologist enters this value in the laboratory's information system (LIS), a delta check flag warns that this value is significantly different from the previously reported value of 78 g/L.

QUESTION

1. How can this difference be explained?
2. The technologist retrieves the previous sample and checks to ensure that the patient name and identification number are the same. Next, the technologist looks at both serum samples and then performs a "renal" profile on the current specimen (glucose, BUN, creatinine and electrolytes). The results of this analysis are glucose = 10,340 mg/L, BUN = 1050 mg/L, creatinine = 51 mg/L, and electrolyte levels within normal range. The technologist checks in the LIS and determines that the initial sample had a protein determination performed by a biuret assay as part of a routine chemistry profile. How do these test results explain the differences between the refractive index values for the two specimens?

Questions to Consider

1. What is the principle of measuring total serum protein by refractometry?
2. What other method is commonly used to quantitate total serum protein?
3. What are the major interferences for each method?
4. Why did the technologist look at the specimen, and what do you think was seen?

Answer

1. The results of the "renal" profile explain the difference between the total protein results because: Hemolysis and turbidity caused by hyperlipidemia are also causes of false positive results (see p 105). The refractive index difference was probably caused by a change in the concentration of one or more of the non-protein analytes.
2. The very large amount of glucose and serum urea nitrogen present in the current sample (reported total protein, 89 g/L) was the cause of the falsely elevated results obtained by refractometry. There is an additional 11.8 g/L of material (glucose + BUN + creatinine) in the sample. Since the initial sample was measured by the more specific biuret procedure, there was no false positive interference.

Answers to Questions to Consider

1. The refractometer is not specific for protein but measures the total mass of dissolved solids (page 104). The greater the mass of solids, the greater the angle of refraction of the incident light passing through the solution. The angle of refraction is indicated on a scale as mass/volume. The assumptions are that almost all the mass in serum is protein and that the concentration of the other serum components remains essentially constant from patient to patient (p. 104-105).
2. The most commonly used method for measuring serum total protein is the biuret reaction (see Total Protein on CD-ROM). The reaction is based on the color formed when copper complexes with the peptide bond. The refractometer is an infrequently used method for measuring serum total protein.
3. The biuret reaction has very few interferences. Since the refractometer is only a non-specific measure of total dissolved solids, any substantial increase of non-protein solutes will give rise to false positive increases in protein determination. Large increases in glucose, BUN, or bilirubin (in order of importance) can affect the refractive index.
4. The technologist examined the specimen to determine if the specimen was either lipemic or hemolyzed, both possible causes of false positive results. Since the technologist went on to do a renal profile, one can assume that the specimen did not appear lipemic or hemolyzed.

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4.6: Chromatography I

A laboratory is using the GLC chromatography system described in Methods to separate a series of alcohols. The chromatogram has the following appearance: Solvent front, 0.11 min.; Width of elution peaks at baseline absorbance: methanol, 0.1 min.; ethanol, 0.15 min.; isopropanol, 0.20 min.; n-propanol, 0.25 min.; acetone, 0.17 min. The elution times of the major peaks are listed in the figure legend. The laboratory decides to determine if acetone is sufficiently well separated from ethanol.

QUESTIONS

1. Is the separation (i.e. resolution) of acetone and ethanol adequate?
2. For quality control purposes, the HETP of the column is calculated weekly and compared to the initial value. When the HETP decreases to 70% of the initial value, the column is taken out of service. This column's initial HETP was 0.083 cm/theoretical plate. Is the current HETP acceptable?

Questions to Consider

1. What parameters are needed to calculate resolution?
2. Calculate k, the capacity factor, for each of the alcohols using Equation 5-6 on p. 117.
   Calculation:

$$k = \frac{V_R - V_O}{V_O} = \frac{t_R - t_O}{t_O} \tag{4.6.1}$$

3. Calculate the alpha factor for ethanol and acetone. (Equation 5-7, p. 117)
   Calculation:

$$\text{Alpha} = \frac{k_2}{k_1} \tag{4.6.2}$$

4. Calculate the number of theoretical plates for the internal standard (Equation 5-3, p. 114).
5. Using the calculated and measured parameters, now calculate the resolution, R, using equations 5-2 and 5-10.
6. Presume that the column is 200 cm long. What is the HETP? Use Equation 5-4 for this calculation.

Answer

1. Since an R greater than 1.25 (p. 112) is considered adequate, by either calculation the resolution between acetone and ethanol is sufficient.
2. Since the column's efficiency has only decreased by 13%, (0.083/0.094), the HETP is acceptable, and the column can remain in service.

Answers to Questions to Consider

1. Resolution between two compounds can be calculated by either Equation 5-2 (p.112) or Equation 5-10 (p. 116). Equation 5-2 is a general resolution equation which can be used to calculate resolution directly from the chromatogram using the distances (or time) between the peaks and the peak width. In equation 5-10, resolution is calculated using the theoretical plates of the column, a selectivity factor, and a capacity factor.
2. The capacity factor is calculated from the relationship:

$$\begin{aligned} k' &= \frac{t_R - t_O}{t_O} \\ &= \frac{0.98 - 0.11}{0.11} = 7.90\ methanol \\ &= \frac{1.33 - 0.11}{0.11} = 11.1\ ethanol \\ &= \frac{2.12 - 0.11}{0.11} = 18.3\ isopropanol \\ &= \frac{2.88 - 0.11}{0.11} = 25.2\ n-propanol \end{aligned} \tag{4.6.3}$$

3.

$$\begin{aligned} Acetone\ k' &= \frac{1.55 - 0.11}{0.11} = 13.1 \\ Alpha &= \frac{k'_2}{k'_1} = \frac{13.1}{11.1} = 1.19 \end{aligned} \tag{4.6.4}$$

4. Number of theoretical plates

$$N = 16\left(\frac{t_R}{W}\right)^2 = 16\left(\frac{2.88}{0.25}\right)^2 = 2123 \tag{4.6.5}$$

5. Using Equation 5-2:

$$\begin{aligned} R &= \frac{d_2 - d_1}{0.59(W_2 + W_1)} \\ &= \frac{1.55 - 1.33}{0.5(0.15 + 0.17)} \\ &= \frac{0.22}{0.16} \\ &= 1.37 \end{aligned} \tag{4.6.6}$$

or 5-10

$$\begin{aligned} R &= \left(\frac{N^{0.5}}{4}\right)\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2'}{(1 + k_2')^{2/3}}\right) \\ &= \left(\frac{2123^{0.5}}{4}\right)\left(\frac{1.19 - 1}{1.19}\right)\left(\frac{13.1}{(1 + 11.0)^{2/3}}\right) \\ &= 2.02 \end{aligned} \tag{4.6.7}$$

6. For a 200 cm column:

$$HETP = \frac{200}{2123} = 0.094\ cm/theoretical\ plate \tag{4.6.8}$$

HETP = total length of stationary phase/# of theoretical plates

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4.7: Radioisotopes

A medical technologist performs a competitive binding radioimmunoassay (RIA) with an ^{125}I tracer. The controls for this assay are not within established limits. While reviewing the data, the technologist notes that the raw counts per minute (cpm) for standards and controls are considerably lower (30%) than those obtained when the assay was run eight days before. The technologist is concerned that the natural decay of the ^{125}I has caused the amount of radioactivity to fall below the acceptable limit for running the RIA.

QUESTIONS

1. Has the cpm of the ^{125}I label decreased more than can be expected from natural decay?
2. The technologist reviews the assay with a laboratory supervisor who agrees that the total counts per assay tube, 7,000 counts, is 30% lower than the counts available the previous week. When the technologist tells the supervisor the amount of radioactive decay that should have occurred, the supervisor asks the technologist to check out the accuracy and precison of the micropipettes and the scintillation counter used in the assay. How can the technologist determine the accuracy and precision of the scintillation counter and micropipettes?

Questions to be Considered

1. What is the half-life of a radioactive isotope?
2. What is the decay factor for a radioactive isotope?
3. What percent of the ^{125}I should decay in eight days?
4. What is the expected precision for counting 7,000 counts? What is the expected precision and accuracy for micropipettes?

Answer

1. The counts available have decreased more than theoretically expected. The reduction in counts should have been 8.8%, not the 30% observed. Since there cannot be a loss of radioactivity greater than expected from natural decay, there must be something wrong with the scintillation counter or with the delivery of the radioactive component of the reaction into the reaction mixture.
2. The technologist can assess the accuracy and precision of the scintillation counter by counting a known standard that is usually available with each instrument. By comparing the cpm and the precision for the counts to what is listed, the technologist will be able to determine if the instrument is working properly.

Answers to Questions to be Considered

1. The half-life of a radioisotope is that time required for half of the radioactive atoms in a sample to decay into nonradioactive atoms (p 191). Thus, after this time passes, half of the radioactivity (disintegrations per minute, dpm) will remain .
2. The decay factor is the fraction of radioactive atoms remaining after a certain amount of time has elapsed. The decay factor is related to the number of half-lives that have elapsed for an isotope.. If the number of half-lives that have elapsed is known, then the decay factor for any isotope can be determined (see p 191-192,). Table 9-1 gives the decay factors for ^{125}I.
3. According to Table 9-1 (p 192), 91.2% of the ^{125}I. radioactivity should remain eight days after the initial experiment.
4. The expected precision (as percent coefficient of variation,%CV) can be calculated from the equation found on p.198. The %CV for 7,000 counts is 1.2%. A check on the accuracy and precision of the micropipettes employed in the assay can be accomplished using gravimetric or spectrophotometric techniques (see p 19). In this case, the technologist found that the micropipette used to deliver ^{125}I. tracer was inaccurate by 15% and very imprecise (%CV for replicate analyses of 20%). The micropipette was replaced and the assay again worked properly.

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4.8: Liquid Scintillation Counting

A competitive binding immunoassay for the measurement of cyclosporine is introduced in which tritiated (^{3}H) cyclosporine is used as the labeled ligand. The reaction mixture is incubated with charcoal to remove free ligand. The supernatant, containing ligand bound to antibody, is solubilized to allow mixture of the sample with the scintillation fluid and counting of the bound radioactivity. The medical technologist prepares the solutions for counting and counts them immediately. However, over the weekend, some of the results of the counting experiment are lost. As the samples were not thrown away, however, the medical technologist recounts them the following Monday. On the second counting, significantly lower counts per vial are obtained. The technologist's concern causes her to repeat the entire experiment including a second counting 48 hours after the first. The same pattern of lower cpm on the second counting is obtained, and the technologist is puzzled.

QUESTIONS

1. Why is there a difference in the results obtained 48 hours apart?
2. Which set of counts, initial or 48 hour results, should be used?

Questions to Consider

1. Are there any mechanisms other than radioactive decay processes that can give rise to photons in a liquid scintillation mixture?
2. What are some important characteristics of chemiluminescence reactions?
3. How can one detect the presence of chemiluminescence interference during a counting procedure?
4. How can one use the characteristics of this process noted in #2 to minimize chemiluminescence interference?
5. Will chemiluminescence reactions affect both standards and patient samples equally?

Answer

1. The difference in the results obtained 48 hours apart was caused by the presence of chemiluminescence reactions. The results were lower at the second counting because of the decay of the chemiluminescence reactions as discussed in answer #4 below.
2. The results from the 48 hour counting are probably the more accurate since the chemiluminescence interference has been minimized. Only if the standards are in the same matrix as the samples and all are counted within a very short time frame, could the initial results be used.

Answers to Questions to Consider

1. The non-radioactive process that can act as a source of photons in liquid scintillation counting is chemiluminescence, which is caused by chemical reactions that give rise to photons (p. 198). In liquid scintillation counting, chemiluminescence reactions can occur between the sample and the solubilizer material, between the sample and the solute ("fluor"), or between different components of the sample itself.
2. Like most chemical reactions, chemiluminescence reactions are time and temperature dependent. After a period of time, the chemiluminescence reaction becomes complete (p. 198) and the production of photons rapidly decreases. Chemiluminescence reactions occur more slowly at lower temperatures, although at low temperatures it takes longer for the chemical reactions to come to completion and cease making photons.
3. One can detect the presence of chemiluminescence reactions exactly as the medical technologist discovered it; that is, by repeated counting of the samples with time. If the counts per minute significantly decrease with time, the presence of decaying chemiluminescence reactions can be assumed.
4. By repeated counting of the samples and plotting the decay curve, one could determine how long one needs to wait before the curve flattens out, indicating a cessation of the chemiluminescence reactions. This process can be hastened by preincubating the samples at room temperature in the dark to allow the chemiluminescence reactions to be completed faster. The samples should then be transferred to a cold scintillation counter, cooled, and then counted. This process allows for rapid completion of the chemiluminescence reaction and the inhibition of any remaining reactions by the use of cold temperature counting.
5. The chemiluminescence reactions are very dependent upon the sample matrix. If the standards and the samples are not in the same matrix, they will be affected to different extents.

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4.9: Colligative Properties

A patient is suspected of having dilutional hyponatremia, that is a low serum sodium concentration caused by excess water retention leading to dilution of the serum sodium. The emergency room resident asks for a renal profile (sodium, potassium, chloride, total CO_2, glucose, and BUN) and a measured osmolality. The results of this analysis are as follows: Na = 127 mmol/L; K = 3.5 mmol/L; chloride = 107 mmol/L (all of these three analytes by dilutional ion selective electrodes); CO_2 = 19 mmol/L (by enzymatic analysis); glucose = 740 mg/L (by rate glucose oxidase); BUN = 130 mg/L (by enzymatic conductimetric analysis), and measured osmolality = 365 mOsm/L (by vapor pressure osmometry).

The resident questions the results and asks for a repeat analysis of the osmolality. Repeat analysis is reported out as 367 mOsm/L. The resident, still believing that the patient has dilutional hyponatremia and wanting to treat the patient, asks if the medical technologist can check the osmolality in some other way.

QUESTIONS

1. How can the technologist verify that the measured osmolality is accurate?
2. After looking at the specimen, the technologist decides to calculate the estimated osmolality by using the data from the renal profile and obtains a result of 266 mOsm/L. Why does this result help in the estimation of the accuracy of the measured osmolality?
3. The clinical chemist suggests that the physician accept the calculated osmolality as the more valid result and, in addition, that the physician order an alcohol quantitation — not a screen - because the chemist, based on the osmolality of 318 mOsm/L obtained by freezing point depression in the laboratory, calculates that the patient's alcohol level is very high. Is this information helpful for explaining the initial discrepancy? Why?

Questions to Consider

1. What property of solutions does osmolality measure?
2. What is the principle of measuring osmolality by vapor pressure? By freezing point depression? By calculation?
3. How did the medical technologist arrive at the value of 266 mOsm/L?
4. Why is the calculated osmolality so different from the osmolality measured by vapor pressure depression?
5. Would a similar difference be obtained if a freezing point depression osmometer were used?
6. How can the alcohol (e.g., ethanol) concentration in serum be estimated from the osmolality derived from the freezing point depression osmometry?
7. How can the presence of an alcohol be confirmed?

Answer

1. The technologist can verify the osmolality value by repeating the assay with a freezing point depression osmometer or by calculating the expected osmolality. In addition, the technologist must look at the specimen to see if significant hypertriglyceridemia exists since very lipemic samples can result in pseudohyponatremia (see SODIUM measurement on CD-ROM).
2. The difference between the calculated osmolality and the osmolality measured by vapor point depression suggests an interferent in one of the results, most likely the vapor point assay. The results suggest that there may be an unexpected substance in the serum.
3. Yes. The presence of alcohol in high quantity would explain the discrepancy of the results and indicate that the resident's assessment of dilutional hyponatremia is probably correct.

Answers to Questions to Consider

1. Osmolality is a measure of the number of moles of dissolved solute per kilogram of water. Osmolality does not distinguish the types of solute present, just the number of moles of solute (p. 267).
2. Vapor pressure osmometry is based on the fact that the greater the number of particles dissolved in a solvent (or moles of solute), the less solvent vapor will exist in the atmosphere over the solution. The decrease in vapor pressure is directly related to the osmolality of the solution, and is monitored by a thermocouple above the solution (pp. 270).

   Freezing point osmometry is based on the ability of the dissolved solutes to lower the point at which the solution will freeze.

The more solutes dissolved, the lower the temperature at which the solution will freeze. The actual freezing point is determined from the heat released upon freezing; the heat is measured by a sensitive thermocouple (pp. 269).

3. The equation used to estimate serum osmolality is equation 14-1 (p. 267) as follows:

$$\text{Calculated osmolality} = 2\ Na\ (mmol/L) + \frac{\text{glucose}\ (mg/L)}{180\ mg/mol} + \frac{BUN\ (mg/L)}{28\ mg/mmol} \tag{4.9.1}$$

Thus

$$\begin{aligned} \text{the calculated osmolality} &= 2(127) + 740/180 + 180/28 \\ &= 254 + 4.11 + 6.4 = 265\ mOsm/L. \end{aligned} \tag{4.9.2}$$

This osmolality is more consistent with the physician's diagnosis of dilutional hyponatremia.

4. The very large difference between the calculated and measured osmolality is probably because caused by the presence of some volatile substance in the patient's blood. Such a volatile substance would naturally increase the mass of vapor or the vapor pressure above the solution and falsely increase the vapor pressure and measured osmolality. A common type of volatile positive interferent would be an alcohol. (pp. 268).
5. No. Freezing point osmometry measures the presence of all solutes directly. Therefore, It would measure the volatile component accurately. There is no interference resulting from the vapor phase of a volatile component.
6. One can estimate the alcohol levels by assuming that only ethanol is present (the usual volitile) and using the calculated the OSMOLAR GAP, which is the difference between the measured osmolality by freezing-point depression. One could use Table 14-1 (p 268) or the equation described in the Alcohol chapter on the Methods CD-ROM to estimate the concentration of ethanol. Using the equation, one calculates as follows:

$$\begin{aligned} [\text{Measured osmolality} - 290] \times 46.07\ g/mol \times 0.92 &= \text{estimated concentration of alcohol} \\ [318 - 290] \times 46.07\ g/mol \times 0.92 &= [alcohol] \\ 1187\ mg/L &= [alcohol] \end{aligned} \tag{4.9.3}$$

The term 290 mol/L is the normal serum osmolality.

7. The presence of alcohol should be confirmed by either an enzyme based assay or by gas chromatography. The latter would be preferable since it would also indicate which alcohol was present.

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4.10: Ion Selective Electrodes

During a routine analysis of electrolytes, a technologist notes that one set of results is unusually low: sodium = 118 mmol/L; potassium = 3.8 mmolL. Upon repeat analysis, similar results are obtained. Both cations are measured by dilutional ion selective electrodes. A check in the laboratory information system ("computer") shows that a previous sample taken after an overnight fast had sodium and potassium concentrations within the laboratory reference ranges.

QUESTIONS

1. How can the technologist determine if the analysis is correct?
2. The medical technologist repeats the analysis on a flame emission photometer and gets essentially the same results, Na = 118 mmol/L, K = 3.9 mmol/L. Should an alternative method for sodium analysis be employed for this sample?

Questions to Consider

1. What are the principles of the most commonly used techniques for measuring Na and K?
2. What is the difference between "activity" and mass concentration measurements?
3. What is the most likely cause of such low and, most probably factitious, sodium results?
4. How can one quickly determine if one of the causes of factitious hyponatremia is present?
5. Are the results from the flame emission analysis unexpected? Why?
6. Why is the potassium result not as proportionally low as the result for sodium?
7. What are the alternatives available for a proper analysis of this sample?

Answer

1. The technologist should immediately look at the sample to determine if lipemia is present. Typically, the sample will be very turbid because of the high concentration of large lipoprotein molecules. If the sample is not lipemic, a total protein measurement (such as by refractometry) can be performed to see if the specimen has a large protein concentration.
2. Yes. An alternative method for sodium analysis which does not employ a dilution step should be used, such as an instrument with a non-dilution, ion-selective electrode. If such an instrument is not available, reject the sample.

Answers to Questions to Consider

1. Flame photometry (FAES) measures the light emitted from gaseous Na and K atoms after they have been raised to excited states in a propane-air flame (p. 97). Ion selective electrodes (ISE) measure the *activity* (p 274) of Na, K, and Cl, which is related to concentration. The activity measurement is based on the voltage difference established on the ISE in the presence of the specific ion (see pp. 273-279). The ISEs perform the analysis directly on the sample (non-dilutional ISE) or following a dilution of the sample (dilutional ISE); FAES measurements are performed on diluted sample.
2. Mass measurements by FAES are dependent upon sampling an exact volume, and therefore mass of Na or K, which is introduced into the flame. Usually the mass of Na or K will be evenly dispersed throughout the sample and dilutions will be accurate. In activity measurements, only the amount of material that is physiologically and chemically available - i.e., active - is measured (p. 274). If the sample is prediluted before being brought into contact with the ISE, that dilution must also be accurately performed.
3. One of the most likely causes of these very abnormal results is the presence of large amounts of lipid particles (very low density lipoproteins or chylomicrons) in the serum. The lipid expands the volume of the plasma although the concentration of sodium and potassium in the plasma water remains constant, as the cations do not readily dissolve in the lipid fraction of blood. The ion selective electrodes (ISE) measure the activity of sodium and potassium and therefore would respond only to their concentration in the water fraction of plasma. However, the instruments used in this laboratory dilute the sample prior to the analysis by the ISEs. The calculation of the concentration of sodium and potassium and plasma water depends on an accurate dilution of the plasma water . Since a significant percentage of this highly lipemic sample was lipid and not plasma, the instrument is actually sampling and diluting less plasma water, and less sodium and potassium, than expected from the volume aspirated. The calculation thus produces erroneously low values. This result is known as "pseudohyponatremia" (See p 280 and also Methods Sodium and Potassium in CD-ROM). Very elevated concentrations of serum protein may also be a cause of pseudohyponat:remia.
4. If pseudohyponatremia is caused by hyperlipidemia, the technologist can simply look at the specimen. If it is extremely milky, one can assume pseudohyponatremia is the cause of the very low sodium result. If it is clear, one should measure

serum protein concentration; the quickest way is by refractometry. This will determine if the sodium result is caused by pseudohyponatremia resulting from hyperproteinemia.

5. No. Flame photometers also predilute the specimen prior to analysis and thus are prone to the same dilutional error with turbid samples.
6. The erroneously low values of sodium and potassium concentrations are dependent upon the proportional error of the volume of lipid taken up with the sample. If 10% of the sample were lipid and contained 140 mmol/L of Na in the plasma water and no Na in the lipid fraction, a 10% error would be introduced yielding an erroneously low result of 126 mmol/L. Because the physiological concentration of sodium is restricted to a narrow range (approximately 7% range of concentration), the magnitude of the analytical error becomes medically significant, i.e. outside the typically encountered physiological concentration of serum sodium. For potassium, serum concentrations in normal individuals extend over a 33% range. Thus the 10% error will yield a lower result, but not the grossly abnormal result that is found for sodium (See Methods Sodium and Potassium in CD-ROM).
7. Analyzers employing ion selective electrodes that do not perform a dilution on the sample prior to analysis (direct or nondilutional ISEs) will yield accurate results. By measuring the activity of the ions directly in the sample, these instruments report the true plasma water concentrations of sodium and potassium. Alternatively, one can use a high speed centrifuge, such as the Beckman Airfuge, to separate chylomicrons from the plasma and allow sampling of the plasma free of large lipid particles. If neither of these alternatives is available, the sample should be rejected as inappropriate for analysis (Link to Methods CD Sodium and Potassium)

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4.11: Reference Intervals

A 49 year old black male is brought to an emergency room following several hours of chest pain and shortness of breath while working. He is a large, muscular, 230-pound, 6'1" man who works as a construction worker. Following admission to the CCU, a series of "cardiac" enzyme values are obtained to rule out the possibility of a myocardial infarction.

Time (hours)	CK (U/L)	LD (U/L)
Admission (0)	105	98
6	91	105
12	112	92
24	104	101
48	99	90

The laboratory's reference intervals for CK and LD are 10-80 and 0-100 U/L, respectively. The physician in charge of the patient is concerned that since the total CK values are significantly greater than the reference interval, the patient might have had a myocardial infarction.

QUESTION

Can we be certain that the reference intervals in use by the laboratory are valid in this case for helping the physician determine whether his patient had an M.I.? Why?

Questions to Consider

1. What biological parameters should be considered when a reference interval is defined?
2. How can the laboratory determine if a reference interval for a test should differentiate test values by the parameters listed in 1.
3. Why do you think this laboratory established the reference intervals using its own personnel?

Answer

This laboratory's reference interval was based on values obtained from young Caucasian (in this case) females and might be inappropriate for this patient. This is true for CK since most serum CK is derived from muscle, and this very muscular male might be expected to have a higher serum CK concentration, especially when compared to young females. The physician should be told that the values for his patient are probably very normal.

Answers to Questions to Consider

1. The most important factors are age, sex, and race (page 366). Other factors may also be very important depending on the specific analyte in question. Preanalytical variables must be properly controlled (se Chapter 3) when obtaining samples for a reference range study. For example posture during phlebotomy, is important for properly defining the reference interval for serum catecholamines, and many reference intervals must be determined in healthy individuals not taking certain foods or medications that might interfere with the analysis.
2. When establishing a reference interval, or validating a company's reference interval for a test, the laboratory should first review the appropriate journals (i.e., Clinical Chemistry) or textbooks (such as Clinical Chemistry, by Kaplan and Pesce) to see if age, sex or race differences have been reported for the method to be used. If they have, the laboratory should immediately begin to determine if such a difference exists for their method. If there is no such report, the laboratory may decide to evaluate these differences themselves.

   Laboratory data should be divided on the basis of sex and race (usually Caucasian and Black). With 30-40 values in each group, one can test for significant differences between the means of each population, i.e. male vs. female or Caucasian vs. Black (see Chapter 19). One can usually do this by the t-test (pp. 350-353). (One should also prepare histograms or distribution plots of the data [p. 343-346] to determine whether the data is normally distributed.) If there is no difference between the groups, the data should then be pooled into a single population. If there is a difference, one has then to determine if this difference is clinically significant (p. 370-371). If it is, two separate reference intervals must be reported for

the analyte. For these analytes, differences in the reference interval based on sex and race have been reported (See Method Creatine Kinase in CD-ROM).

3. The supervisor and clinical chemist of the laboratory go back to the data used to establish the reference interval for CK and find that the laboratory personnel were used. Many laboratories typically use their personnel as healthy subjects for reference interval studies because of the ready accessibility and professional cooperation of the medical technologists. However, the majority of medical technologists are young females. Thus, if age or gender would normally affect the data, the reference intervals might be inappropriate for older people or males. In addition, if the race of most of the workers differs from that of the typical hospital patient, an additional bias might be introduced.

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4.12: Quality Control

For Chemistry Laboratory tests, laboratory protocol requires the use of two different quality control samples, QC-A and QC-B, during an analytical run. The control limits are set at 95% confidence limits. At the completion of one set of measurements, a technologist notes that one control falls within the specified limits, but the second control is out of control, falling between +2 SD and +3 SD on the Levy-Jennings chart.

QUESTION

What is the technologist's best course of action?

Questions to Consider

1. What is the probability that analysis of the quality control specimen will yield a result of ± 1 SD about the mean, if the instrument is working perfectly? What is the probability of a result falling within ± 2 SD of the mean under the same conditions? What is the probability of a result being greater than ± 2 SD under the same conditions?
2. If one repeats the analysis immediately, what is the probability that the result will be within the ± 2 SD range if the instrument is working within specifications?
3. If an instrument has, in fact, become mis-standardized because of electronic or reagent drift, and a quality control specimen result is outside the ± 2 SD range, what would you expect the result of an immediate repeat analysis of the same quality control sample to be?

Answer

Given one result within the reference range and the other between two and three SDs from the mean, the technologist knows that the result greater than 2 SD could be a random chance event (2.5%) and that the instrument may b eworking perfectly. The analysis of the QC specimen that was out of range should be repeated. If the result falls within ± 2 SD, it can be assumed that the out-of-range result was a random chance event and that the instrument is operating properly. The results should be sent out.

If both QC specimens are now within range, i.e. QC-A and the repeat QC-B, but both are near the upper or lower 2 SD limits, the technologist may want to recalibrate the instrument anyway since these results may reflect a small, but definite, shift. The technologist would probably send most of the patient results out, repeating only those results with borderline values.

If, on the other hand, the repeat analysis of the out-of-range quality control pool yields a result that is still out of range, there is a very high likelihood that there is a real shift in the calibration and that this QC result reflects the new, biased population. The technologist should recalibrate and re-analyze all the patient samples up to the last within-range quality control result.

The above quality control decisions are based on the multirule Shewhart rules, described on in Chapter 21.

Answers to Questions to Consider

1. The target range for quality control specimens of ± 2 SD (95% confidence limits), assumes a normal (i.e. Gaussian or symmetrical) distribution of results about the mean value. Thus, 66.6% of all results should fall within the ± 1 SD limits about the mean and approximately 95% should fall within ± 2 SD (see Figure 19-5, Page 346) if the instrument is working perfectly. On the other hand, use of a 95% confidence interval accepts that 5% of all analyses will fall outside of the allowable limits for a perfectly working instrument. Thus, 25/1,000 results will be greater than + 2 SD from the mean and 25/1,000 will be less than - 2 SD from the mean.
2. For a single analysis, the probability of results falling within the current 95% target distribution remains the same. Thus, there is a 95% chance that a repeat analysis of the quality control sample, analyzed immediately following a random value which is outside the 2 SD limits, will yield a result falling within the ± 2 SD range if the instrument is working within proper specifications.
3. If the instrument is really miscalibrated, there will be a shift (bias) of the results towards a different population of results, i.e. one with a higher or lower mean value than that of the true target population for a properly calibrated instrument. Thus, an immediate repeat analysis of a quality control pool sample will have a 95% chance of falling within the population of results reflected by the new calibration, i.e. higher or lower than the normally accepted range (see Figure 21-1, Page 391).

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4.13: Electrolytes

A medical technologist obtained the following results on a routine renal profile:

- Sodium: 139 mmol/L
- CO_2: 20 mmol/L
- Potassium: 4.1 mmol/L
- BUN: 190 mg/L
- Chloride: 118 mmol/L
- Creatinine: 9 mg/L
- Glucose: 2500 mg/L

The sodium, potassium, and CO_2 are measured by ion selective electrodes. The chloride is measured by the ferric thiocyanate method. Upon reviewing the results, the medical technologist performs a quick written calculation and decides that the specimen should be reanalyzed, believing that the results are unusual.

QUESTIONS

1. What simple calculation can be used to check the validity of the electrolyte results? What can the technologist conclude from these results?
2. The technologist repeats the analysis. Essentially the same results are found. The technologist then examines the sample, and upon looking at it, repeats the chloride analysis by an alternative method. What do you think was the positive interference in this sample?

Questions to Consider

1. Which of the above results seem unusual?
2. What other result(s) can be calculated from these data?
3. The technologist calculated the anion gap for these results. Calculate the anion gap.
4. What are some possible causes of the calculated anion gap?
5. Can the anion gap be used to evaluate the validity of electrolyte analysis?
6. What are possible positive interferences in the ferric thiocyanate method for chloride?
7. What alternative method for chloride analysis reduces the effect of these positive interferent was probably?

Answer

1. To check the validity of the chloride result, the technologist calculated the anion gap. As discussed above in #5, the most likely cause of a very low anion gap is analytical error. The very low anion gap for these results suggested to the technologist that the chloride result was probably not accurate.
2. Upon examining the sample, the technologist probably saw a highly lipemic specimen. Knowing that lipemia can lead to falsely elevated chloride results, the technologist repeated the chloride analysis by a method less affected by lipemia, i.e. the chioridometer. If the specimen was clear and the result by the chioridometer was still elevated, bromism would have to be considered as the cause of the elevated chloride result.

Answers to Questions to Consider

1. The chloride result is in the high abnormal range (See Chloride on CD-ROM), and seems unusual since the other electrolyte results are within the normal range. The glucose result is elevated, but not strikingly so.
2. These data can be used to calculate the serum osmolality (p.267) and the anion gap (See p 446 and Method Anion Gap in CD-ROM).
3. The anion gap can be calculated from these results as:

$$\begin{aligned} \text{Anion gap} &= [Na^+] - [Cl^-] - [CO_2] \\ &= 139 - 118 - 20 \\ &= +1 \end{aligned} \tag{4.13.1}$$

4. The causes of a low anion gap are listed in the Anion Gap chapter in the Methods CD-ROM. In addition to those listed, lipemia can often cause a positive interference in the ferric thiocyanate procedure for chloride (See Method Chloride in CD-ROM).

5. Yes! A low anion gap has been suggested as a quality control check on electrolyte results (See Method Anion Gap on CD-ROM). Although there are physiological causes of low anion gaps, these are uncommon. Very low anion gaps should therefore suggest to the technologist that an analytical error may be present. The most frequent analytical cause of a low anion gap is a falsely elevated chloride result.
6. Lipemia of the sample is a common source of interference. In addition to lipemia, the presence of bromide ions (bromism) is an important source of positive interference. Present in many drugs, bromide causes a positive interference not only by reacting in the ferric thiocyanate reaction, but by producing more color on a molar basis than do chloride ions (See Method Chloride on CD-ROM).
7. The chloridometer would be a good alternative. Although bromism still causes a positive interference, it does so on an equal molar basis. The interference by lipemia is minimal in the chloridometer method.

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4.14: Blood Gases

A medical technologist obtained the following blood-gas results from a well-iced arterial specimen: pH = 7.14, PCO_2 = 51 mm Hg, PO_2 = 83 mm Hg, and HCO_3 = 10 mmol/L.

QUESTIONS

1. Are these results acceptable? Why?
2. Because of the above discussion, the specimen is re-examined, and small clots are noted. A repeat blood-gas specimen is obtained, and analysis yields the following data: pH = 7.23, PCO_2 = 22 mm Hg, PO_2 = 85 mm Hg, and HCO_3 = 10 mmol/L. Are these results now consistent with any acid-base disorder?
3. A half hour later a request for a stat lactic acid is received. The result is 21 mmol/L. Is this result consistent with all results listed above?

Questions to Consider

1. Are each of the individual results consistent with an acidosis or alkalosis?
2. Does any individual result indicate the type of acidosis or alkalosis likely to be present in this patient, i.e., metabolic or respiratory?
3. In combination, are these results consistent with a metabolic or respiratory alkalosis or acidosis?
4. What type of acid-base disorder does this patient have?
5. What other laboratory results do you think may be abnormal in a patient with these blood-gas results?

Answer

1. Because the results do not coincide with the combination of results expected for acid-base disorders, these results are likely to be erroneous and should be repeated. Erroneous results can be caused by a clot over one of the electrolytes or bubbles in the sample or improper sample processing. If blood gas results do not overtly fall into a recognizable pattern indicative of a metabolic or respiratory acidosis or alkalosis, the results must be questioned. A call to the attending physician might be useful in resolving the problem.
2. Yes. These results are consistent with a metabolic acidosis.
3. Yes (p. 473). A lactic acidosis is often associated with tissue hypoxia (low PO_2 in peripheral tissues) either caused by poor circulatory perfusion or poor pulmonary function (p. 473). In this situation, the latter seems to be the cause.

Answers to Questions to Consider

1. The pH of 7.14 obviously indicates acidosis (pH < 7.4). The pCO_2 of 51 mm Hg can indicate a respiratory acidosis or a metabolic alkalosis. The bicarbonate of 10 mmol/L could indicate a metabolic acidosis or a compensated respiratory alkalosis (Table 25-4, p. 473).
2. No. A single acid-base parameter cannot indicate whether an acid-base disorder is of metabolic or respiratory origin and, except for the pH, can not even indicate whether it is an acidosis or an alkalosis (p. 469-475).
3. The pH and bicarbonate results could indicate a metabolic acidosis; however, the pCO_2 is not consistent with this combination. A pH of 7.14 and pCO_2 of 51 mm Hg could be associated with severe respiratory acidosis, but the bicarbonate values would not be as low as reported, unless there were a mixed repiratory-metabolic acidosis, such as a severe lactic acidosis combined with a respiratory acidosis. However, in combination, these data do not obviously fit any acid-base disorder.
4. These results are usually associated with a metabolic acidosis, with partial compensation (p. 471-473).
5. Frequently seen causes of metabolic acidosis and the associated abnormal lab values are (pp. 471-473 [Table 25-5]):
    - **ketoacidosis**: increased glucose, ketones, anion gap, hematocrit, and BUN
    - **renal acidosis**: increased anion gap, BUN, and creatinine diarrhea: normal anion gap, increased chloride
    - **lactic acidosis**: increased anion gap and lactic acid
    - **drug intoxication**: some positive result on a drug screen, such as salicylate, and an increased anion gap
    - **D-lactic acidosis (very rare)**: acidosis, normal L-lactic acid levels, and an increased anion gap.

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4.15: Renal Disease, Creatinine Analysis

A 48 year old black male was brought to the emergency room, semicomatose. His initial blood chemistries were as follows: pH 7.25, PCO_2 21, PO_2 94, bicarbonate 18, base deficit 17 mmol/L, glucose 6857 mg/L (glucose oxidase), BUN 190 mg/L (enzymatic, urease/GLDH), creatinine (alkaline picrate) 21 mg/L (check upon repeat), ketones large (> 1600 mg/L), electrolytes within normal range.

The patient was treated intravenously with isotonic saline, insulin, potassium, chloride, and 50 g/L dextrose. The patient improved rapidly, and his blood glucose decreased. Twenty-four hours after entering the hospital, the following results were obtained on a renal profile: glucose 2050 mg/L, BUN 180 mg/L, creatinine 9.5 mg/L, electrolytes within normal range, ketones negative.

The physician in charge of the patient does not believe the patient was in renal failure. Since the creatinine result is now only slightly elevated while the BUN result is within the reference range, the physician questions the initial creatinine value.

QUESTION

What is the reason for the discrepancy in creatinine values?

Questions to Consider

1. How do serum urea and creatinine concentrations change in renal failure? In dehydration?
2. What methods are available for the measurement of creatinine?
3. Are there any advantages or disadvantages for the measurement of creatinine by the alkaline picrate reaction versus the enzymatic methods?
4. How is BUN measured? Are there any major interferants of BUN assays?

Answer

The reason for the apparently discrepant results for creatinine was the presence of large amounts of ketones which give a strong positive interference in the end-point alkaline picrate reaction. The second specimen, which was free of ketones following therapy, did not have interference from ketones and reflected true renal status as did the BUN results in both specimens.

Answers to Questions to Consider

1. In renal failure, both serum urea and creatinine become elevated usually in a parallel manner (Chapter 26). In dehydration, the urea may be elevated without proportional changes in creatinine (Chapter 26).
2. The most popular method of measuring creatinine is the alkaline picrate method. When performed without prior adsorption to, and elution from, fuller's earth, this method is not very specific; ketones, proteins, and many other compounds also react with the reagent (See Method Creatinine CD-ROM). The alkaline picrate method can be performed as an end-point or kinetic procedure.

   Some methods for the routine measurement of creatinine (See Method Creatinine CD-ROM) employ enzyme reagents. The creatinine amidohydrolase reaction uses three additional coupled reactions, eventually measuring the conversion of NADH to NAD^+. The creatinine iminohydrolase reaction generates NH_3 which is monitored by employing the GLDH reaction and the conversion of NADPH to $NADP^+$. Both of these enzymatic assays require enzyme reagents of high purity. The latter coupled reaction sequence is especially sensitive to the presence of endogenous ammonia.
3. The reasons for the widespread use of the alkaline picrate method have been its low cost and the lack of a suitable alternative. The alkaline picrate method has relatively poor specificity, because of positive interferences from ketones and protein, and negative interferences from bilirubin and hemoglobin (See Method Creatinine CD-ROM). The use of fuller's earth to remove these interferences is not practical when employing automated analyses.

   The more recently developed enzymatic methods have better specificity than the alkaline picrate procedure. The only major interferent is ammonia in the creatinine iminohydrolase method. The primary disadvantages of the enzymatic methods have been their high cost and the difficulty of securing pure enzyme reagents.
4. BUN can be measured by diacetyl monoxime or, more frequently, by a urease reaction. The urease reaction can be monitored by conductimetric means or by measuring the NH3 formed by a GLDH reaction (See Method Urea CD-ROM). None of these methods have significant interference by protein or ketones.

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4.16: Liver Disease, Alkaline Phosphatase

A liver profile (albumin, total bilirubin, the transaminases, and alkaline phosphatase) and an ammonia assay are performed on a random access analyzer on an aliquot poured off from a phlebotomy tube. The alkaline phosphatase results are zero or negative. Thinking that perhaps the sample has a highly elevated enzyme activity, and the result could be an artifact caused by substrate exhaustion, the technologist repeats the analysis on a tenfold dilution but again obtains very low results; 0 to 5 U/L. The sample is brought to special chemistry for an alkaline phosphatase isoenzyme analysis, and a typical alkaline phosphatase electrophoretic isoenzyme pattern is obtained. The isoenzyme analysis results are inconsistent with the results obtained on the previous analysis for the total alkaline phosphatase activity.

QUESTION

1. What can the technologist do to verify and possibly explain these results?
2. Do these additional observations help resolve the problem?

Questions to Consider

1. What are the reaction conditions for determination of total alkaline phosphatase activity?
2. What are the principles of the electrophoretic measurement of alkaline phosphatase isoenzymes?
3. What are the differences between the methods described in question 1 and 2?
4. Are there any special sample phlebotomy or sample storage conditions required when assaying for alkaline phosphatase?
5. What anticoagulants can be used to collect whole blood for ammonia analysis?
6. How does an increased temperature affect the activity of the alkaline phosphatase reaction?

Answer

1. After considering the above questions, the technologist determines that the blood was not clotted in the phlebotomy tube. Upon checking in the laboratory information system for previous results for this patient, the ‘technologist determines that previous results for alkaline phosphatase ranged from 120 mU/ml to 155 mU/ml. The phiebotomist reports that the sample was kept on ice when delivered to the laboratory. In addition, the technologist performing analysis for the total alkaline phosphatase activity notes that the temperature of the analyzer was two degrees too high.

   The technologist should verify the results by going back to the initial phlebotomy tube and determining the patient’s identification. The technologist should check in the laboratory’s information system (computer) for previous alkaline phosphatase results on the patient. Reanalysis on another aliquot from the phlebotomy tube would also be appropriate.

2. Yes. These observations suggest that the sample was a plasma specimen drawn for the ammonia determination. The most likely explanation for the results, therefore, is that the phlebotomy tube contained EDTA or oxalate as the anticoagulant. EDTA and oxalate are strong chelators and would effectively sequester magnesium ions, inhibiting the alkaline phosphatase reaction. Electrophoresis of the sample would separate the chelator from the isoenzymes and allow the activity to be expressed. The use of the proper phlebotomy tube for ammonia (that is, heparin, see #5 above) would have avoided the problem.

   The inappropriately elevated temperature of the analyzer had no bearing on the inhibition of the alkaline phosphatase activity; if anything, it would tend to increase the enzymatic activity.

Answers to Questions to Consider

1. Alkaline phosphatase has a pH optimum of about 9.5-10.5 and has an absolute requirement for Mg++ as a cofactor (See Method Alkaline Phosphatase on CD-ROM). Many molecules can act as a phosphate donor (substrate), although para-nitrophenyl phosphate is the most frequently used substrate (See Method Alkaline Phosphatase on CD-ROM).
2. Alkaline phosphatase isoenzymes carry different net charges and can be separated by polyacrylamide gel electrophoresis (see p 206-207) and identified as discrete bands of enzyme activity (See Method Alkaline Phosphatase on CD-ROM). The enzyme activity of the separated fractions is determined by addition of substrate reagent to the gels.
3. The electrophoretic method physically separates the isoenzymes from each other and from the serum matrix on the basis of charge differences. The method for measuring total akaline phosphatase activity is a kinetic enzymatic assay performed on the diluted serum matrix. The primary difference between the methods is the presence of the sample matrix in the total enzyme activity assay.

4. A red top serum phlebotomy tube is preferred, although the analysis can be performed on heparinized plasma. The isoenzymes are stable for a week at 4°C and longer when frozen (See Method Alkaline Phosphatase on CD-ROM).
5. Plasma samples for ammonia can use heparin, citrate, EDTA or oxalate in the collection tubes. The preferred anticoagulant is sodium heparin.
6. An increase in temperature of 2° over the standard temperature would serve to increase enzymatic activity (See Chapter 54). This temperature difference is not great enough to heat denature the enzyme.

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4.17: Bone Disease, Alkaline Phosphatase Isoenzymes

A “bone profile” (calcium, inorganic phosphate, and alkaline phosphatase) has been performed on a hospital patient who is being treated for pneumonia. The alkaline phosphatase result is 375 U/L (upper limit of normal for an adult is approximately 100 U/L). The calcium and inorganic phosphorus results are within accepted normal ranges. The patient’s physician would like the laboratory to determine the source of the elevated alkaline phosphatase.

QUESTIONS

1. What would be the best procedure(s) to perform to differentiate the source of the elevated alkaline phosphatase?
2. This laboratory uses polyacrylamide gel electrophoresis to determine the source of increased alkaline phosphatase. However, since it is Friday afternoon, the electrophoresis will not be performed until the following week. Since it is presumed that the serum alkaline phosphatase is elevated because of some tissue disease, an alternative approach is to measure some other analyte that would reflect liver or bone dysfunction. What other commonly available tests might be used to establish the source of the alkaline phosphatase?
3. The laboratory performs a hepatic profile consisting of AST, ALT, gama-glutamyl transferase, and total bilirubin. The results for all these analytes are within the accepted normal range. If the results of these tests are within the reference range, one assumes, with 95% confidence, that bone is the source of the increased serum alkaline phosphatase activity. If the enzymes are significantly elevated (>3 times upper limit), liver is assumed to be the source. Therefore, the most likely source of the increased serum alkaline phosphatase is presumed to be bone.

   The results of the hepatic profile are communicated to the physician who remains concerned about the source of the elevated alkaline phosphatase. Some information about the patient is requested, and the laboratory is told that the patient is a 14 year old male who was brought into the hospital three days ago for a severe upper respiratory tract infection. How does this additional information help resolve the problem of the elevated alkaline phosphatase activity?

Questions to Consider

1. Which tissue sources contribute the majority of the alkaline phosphatase activity found in serum?
2. What procedures are available to measure alkaline phosphatase isoenzymes?
3. Are there sensitive tests to determine whether liver is the source of elevated serum alkaline phosphatase?
4. Since calcium concentration, the marker for bone disease, is normal, does this rule out bone disease?
5. Are there any non-disease states that can cause an elevation in bone alkaline phosphatase activity?

Answer

1. The best procedure for estimating the source of alkaline phosphatase isoenzymes is polyacrylamide gel electrophoresis.
2. Since good alkaline phosphatase isoenzyme procedures are costly, the approach used here is reasonable. The enzymes gammaglutamyl transferase, AST, and ALT are quick and inexpensive ways to rule out liver as the source of an increased alkaline phosphatase (Chapter 27). Large elevations (2-3 times the upper limit of the reference ranges) of these enzymes, especially gamma glutamyl transferase, 5' nucleotidase, would strongly suggest that liver is the source of the elevated alkaline phosphatase. Relatively normal enzyme activities would point to bone as the source of the elevated alkaline phosphatase.
3. This additional information clearly supports the previous laboratory data. Both the physician and laboratory agree that the source of the elevated alkaline phosphatase is normal bone growth activity in this actively growing patient. The reference range for serum alkaline phosphatase for pre-adult males and females can be 2-3 times higher than the adult values (See Method Alkaline Phosphatase in CD-ROM).

Answers to Questions to Consider

1. The most usual tissue sources of alkaline phosphatase found in serum are liver, bone, and intestine (See Method Alkaline Phosphatase on CD-ROM).
2. The most commonly used methods for estimating the amounts of individual alkaline phosphatase isoenzymes are heat or chemical inactivation, and physical separation by polyacrylamide gel electrophoresis (See Method Alkaline Phosphatase on CD-ROM).
3. Gamma glutamyl transferase, 5' nucleotidase, and the transaminases, ALT and AST, are all used as markers of liver dysfunction (Chapter 27). Because the transaminases are relatively non-specific for liver function, many laboratories use

gamma glutamyl transferase or 5' nucleotidase, which are more specific for the liver and are not present in bone, to help differentiate between bone and liver disease. An elevation in serum alkaline phosphatase accompanied by normal levels of gamma glutamyl transferase or 5' nucleotidase would strongly suggest bone as the source of the alkaline phosphatase.

4. No. Such bone disease as osteoporosis, osteotitis fibrosa, and vitamin D resistant rickets can be associated with normal serum calcium levels (Chapter 28).
5. Increases in bone alkaline phosphatase activity can be seen in patients mending broken bones, patients being treated with lithium, and in pre-pubital, growing children (See Chapter 28 and Method Alkaline Phosphatase in CD-ROM).

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4.18: Diabetes, Ketone Analysis

A resident calls the laboratory, concerned about some apparently discordant laboratory results obtained for a 65 year old white female who came into the emergency room dehydrated and in moderate diabetic ketoacidosis. Laboratory results obtained following admission to the emergency room were as follows: pH, 7.17; PCO_2, 9 mmHg; glucose, 7832 mg/L; serum ketones (by dipstick), trace to small; potassium, 2.8 mmol/L; and sodium, 131 mol/L.

The patient was treated with fluids and insulin and she responded well, her glucose dropping to 1958 mg/L at 48 hours. What confuses the resident is that her serum ketone levels, after 48 hours of treatment, are now reported as moderate, although her glucose has decreased considerably. The resident questions whether the laboratory has mixed up or misanalyzed the specimen.

QUESTIONS

1. What can the laboratory do to verify the results?
2. The technologist retrieves the specimens in question and validates the patient's identification number on the specimen tube. The technologist repeats the glucose and ketone body analysis, obtaining results very similar to the original ones. The technologist also notes that the earlier specimen was fairly turbid, while the later specimen was not. What explanation would you give to the resident for the increase in serum ketones following treatment?

Questions to Consider

1. What are "ketone bodies?"
2. Why are ketone bodies produced during diabetic ketoacidosis?
3. What are the pathways involved for the production of ketone bodies?
4. How are ketones measured?

Answer

1. If the laboratory has alternative methods for ketone analysis, the levels of acetoacetic acid and acetone can be verified. The results are verified if alternative tests are not available if the repeat analysis agrees with the initial results.

   First, check the patient identification on each phlebotomy tube for this patient to make sure that each was most likely drawn from the correct patient. Then, the dipstick analysis for each specimen is repeated to ensure that there was no analytical error.
2. The resident should not be concerned with the results. Early in severe DKA, the reactions described above are shifted strongly toward the production of NAD^+ and β-hydroxybutyrate, with the measureable ketone, acetoacetic acid, assuming a realtively small proportion of the total 'ketone bodies'. (See Chapter 32 and Method Ketones on CD-ROM). Thus, the total amount of increased ketone bodies is greatly underestimated when measured by the nitroprusside reaction. Later, as the DKA is treated and resolved, the body begins to consume the β-hydroxybutyric acid and to metabolize it completely. In doing so, the reactions leading up to the production of beta-hydroxybuterate (Figure 32-9) are reversed, thus forming more acetoacetate in the process. Therefore, during the recovery phase (i.e. the second specimen in question) the apparent amount of ketones present increases, when in reality the total amount of ketones is decreasing; only a transient increase in acetoacetic acid is being detected. The turbidity of the specimen plays no significant part in the explanation of the ketone results.

Answers to Questions to Consider

1. "Ketone bodies" is a name given to a group of metabolites of fatty acid oxidation. They are acetoacetic acid, acetone, and β-hydroxybutyric acid. Only the first two are true ketones (See Chapter 32 and Method Ketones on CD-ROM).
2. During starvation or diabetic ketoacidosis (DKA) (Chapter 32), low insulin levels and relatively high hyerglycemic hormones (such as, cortisol) cause fat to be mobilized from adipose tissue, resulting in the release of free fatty acids. The fatty acids are oxidized by the liver to acetyl CoA and NADH. However, the large amounts of fatty acids being metabolized in ketoacidosis soon exceed the liver cells' capacity to recycle coenzyme A (CoA) and NAD^+. This prevents additional metabolism of fatty acids. To allow continued fatty acid metabolism, the acetyl CoA reacts to form the ketone bodies and free CoA and NAD^+ (Chapter 32).
3. The pathways of ketone body production are shown in Figure 32-9 page 594. Thus, the end-point of ketone body formation is β-hydroxybutyric acid and NAD+, which raises the NAD^+/NADH ratio and allows continued fatty acid oxidation.

4. Routinely, serum or urine ketones are detected by dry reagent strip techniques which employ the nitroprusside reaction (See Method Ketones on CD-ROM). However, this reaction only detects acetoacetic acid and, to a much lesser extent, acetone. β-hydroxybutyric acid does not reactat all. Acetoacetic acid and β-hydroxybutyric acid can be quantitated by specific enzymatic reactions employing β-hydroxybutyrate dehydrogenase and measuring either the production or consumption of NADH (See Method Ketones on CD-ROM). The enzymatic assays are rarely available in a routine laboratory.

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4.19: Alcohol

Three samples are sent to the laboratory for alcohol analysis. The patients are reported to be in varying states of coma; severe coma, mild coma, and sleepy drunk.

An alcohol screen by enzyme assay for each patient is reported as 3700 μg/mL, 2400 μg/mL, and negative, respectively. However, the patient with the negative result has a reliable history of drinking in the 24 hours before admission to the emergency room. The stat lab is requested to perform a repeat alcohol analysis on the serum sample. The same result is obtained.

QUESTIONS

1. Is it possible to predict the blood alcohol levels of each patient from their clinical appearance?
2. The lab is requested to repeat the analysis a third time. In addition, all the samples are cross-checked using a semiquantitative diffusion method. The diffusion test gives an extremely positive result for all three samples. What could explain the difference between the quantitative and qualitative results?

Questions to Consider

1. Are the acute effects of alcohol dose related?
2. Do these effects vary from individual to individual? Is it possible to predict the effect of a specific blood alcohol level on a single individual? Why?
3. What blood levels of ethanol are often associated with coma? Will the serum alcohol values help in treatment?
4. What other agents could exacerbate the clinical effects of alcohol, causing, for example, a deeper coma than might otherwise be expected?
5. What are the procedures available to screen for alcohol? To quantitate ethanol?
6. How specific for the presence of ethanol are the assays employed by this laboratory?
7. What other method would be useful in resolving the discrepancies between the results?

Answer

1. It is very difficult to predict, within a narrow range, the concentration of a blood alcohol level from the clinical appearance of the patient. The blood levels can vary greatly from individual to individual with the same symptoms.
2. The difference between the results obtained for the third patient by the qualitative and quantitative tests can be explained by the presence of some volatile alcohol other than ethanol. This alcohol would not be detected by the specific enzymatic assay but would be detected by the non-specific diffusion assay or by gas chromatographic analysis.

Answers to Questions to Consider

1. The effects of a toxicant such as alcohol are generally dose related, as shown on p 642 in Chapter 34 and in the chapter on Alcohol on the CD-ROM. In a general population, the greater the dosage, the greater the severity of the clinical response, i.e. coma, etc.
2. There is a great variation in individual response to the presence of ethanol. Ethanol is metabolized by a series of inducible liver enzymes, especially alcohol dehydrogenase. Individuals who are chronic abusers of alcohol have higher levels of activity of these enzymes and thus can metabolize ethanol more rapidly than the occasional drinker. In addition, a chronic ethanol abuser develops a physiological tolerance to this drug and thus will have fewer clinical symptoms. Thus, it is difficult to predict the effect of the same dose on different individuals (p 641).
3. Values above 3000 μg/mL are generally associated with coma (see p 642). Serum alcohol values will help in treatment only if they are very high--usually 4000 to 6000 μg/mL. At these levels, aggressive intervention such as dialysis may be used.
4. A very deep grade IV coma associated with ingestion of alcohol would tend to make the physician think of drugs and other non-therapeutic agents as well as other pathologic causes, e.g. hypoglycemia. Usually a drug screen would be ordered in this circumstance.
5. Procedures available for screening for alcohol in most laboratories include enzymatic and diffusion assays (See Method forAlcohol on CD-ROM). The enzymatic assays are based on the enzyme alcohol dehydrogenase. The rarely employed diffusion assays measure any volatile, reducing substance.
6. The enzyme method is fairly specific for ethanol and would not record the presence of substances such as methanol, isopropanol and ethylene glycol. In contrast, the diffusion method is sensitive to the presence of any volatile alcohol and

would be positive for methanol and isopropanol. In this case, a more specific method should be used to determine the toxic agent.

7. Gas chromatography would probably show that some other alcohol, such as methanol, was present.

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4.20: Toxicology

The Toxicology Laboratory is asked to measure acetaminophen on a stat basis for a suspected overdose. The result of the analysis is reported back as 200 μg/mL. The Emergency Room resident calls the laboratory for interpretation of the result, i.e., does the result indicate toxicity or not? The medical technologist asks the resident how soon after the suspected overdose the specimen was drawn. The resident, unable to obtain a history, does not know. The technologist asks the resident to obtain a second specimen in four hours to evaluate the patient's status.

QUESTIONS

1. Why was a second specimen requested?
2. The result of the second blood analysis for acetaminophen is 150 μg/mL. Does this second value help the laboratory to evaluate the toxicity of the ingested acetaminophen?

Questions to Consider

1. Is there a relationship between blood levels of acetaminophen and toxicity?
2. Why can't the laboratory advise the physician about the toxicity of the 200 μg/mL acetaminophen value?
3. Are there other reasons why a second specimen, several hours after the first one, might be important in determining the care of the patient?

Answer

1. By determining whether the acetominophen level of the second specimen is higher or lower than that of the initial one, the Toxicology Laboratory will enable the physician to decide whether the drug is in the absorption or elimination phase.
2. Although the second value is lower than the first, it is still quite high, suggesting that the patient is in the early part of the elimination phase. With these blood levels of acetaminophen, the physician would probably prescribe an antidote and treat the patient. The antidote for acetaminophen poisoning is N-acetylcystamine (See Table 51-7, p 1000).

Answers to Questions to Consider

1. There is a definite relationship between blood levels of acetaminophen and toxicity as shown in the in the Acetaminophen Chapter on the Methods CD-ROM and Figure 51-6 of the 4th Edition (p 1007).
2. As Fig. 51-6 demonstrates, the toxicity associated with a specific acetaminophen blood level is very dependent upon the time after drug ingestion. A 200 μg/mL level six hours after ingestion will be associated with mild to severe liver damage. However, that same level at 12 hours is considered extremely toxic, while at 3 hours after overdose the level would be considered clinically insignificant. Thus a single blood level without a good history is of limited use unless the level of acetaminophen is very high (i.e. greater than 250 μg/mL), or very low, i.e less than 100 μg/mL.
3. Two specimens drawn several hours apart can be used to determine whether the patient is still absorbing the drug or is in the elimination stage (Fig. 51-6). The patient is at greater risk for liver damage if the sample is drawn in the absorption phase, since the blood levels will increase. In that situation, usually more aggressive intervention measures will be taken. If the patient is in the elimination phase, the risk for additional toxicity is reduced.

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4.21: Sample Analysis I

During a day's work, using several types of chemical analyzers, the laboratory's technologists have prepared a number of dilutions on many different analytes, for a variety of reasons.

QUESTION

Which of the following dilutions might be considered acceptable or unacceptable? Why?

1. Alkaline phosphatase exceeded the reportable range (assay linearity) and a ten-fold dilution with saline was prepared.
2. Albumin exceeded the linear range of the assay. A ten-fold dilution was prepared with saline.
3. Lactate dehydrogenase (LD) showed very early substrate exhaustion, and a two-fold dilution was prepared with saline.
4. A sample to be analyzed for creatine kinase was highly lipemic, and a two-fold dilution was prepared for analysis.
5. Chloride in the specimen exceeded the linear range of the ferric thiocyanate method being used for analysis. A two-fold dilution was prepared for this specimen.
6. For a gas chromatography method for barbiturates, the peak for barbiturate went slightly off scale when a 50 microliter injection was used. The technologist prepared a 1:3 dilution of the sample for further analysis.
7. A sample is diluted two-fold for an analyte measured by a radioimmunoassay procedure because the initial result exceeded the highest standard employed.

Questions to Consider

1. What are the two most common reasons for preparing a dilution?
2. What are the major limitations on the extent to which an analyte should be diluted?

Before answering the problems, the student should read the appropriate analyte chapters in the CD-ROM and Chapter 23.

Answer

1. Acceptable. Most enzymatic methods have linear ranges wide enough so that a ten-fold dilution will usually bring the measured activity well into the acceptable range for measurement.
2. Unacceptable. Dilution should be made to bring the concentration of the analyte into the linear range but the concentration must be kept high enough that the analyte has measurable and reproducible activity. A ten-fold dilution of albumin will result in an albumin concentration so low that its measurement will likely be extraordinarily inaccurate.
3. May be unacceptable. LD assays tend to have relatively narrow linear ranges and thus a two-fold dilution might not be great enough to get the LD concentration within range. A five- or ten- fold dilution probably would have been better.
4. Probably unacceptable. Multiple dilutions would be more appropriate. Each result is multiplied by the dilution factor and the final results compared. When the results begin to repeat, one can assume that the lipemia effect has been diluted out and the results and valid. If the enzyme activity is too low for dilutions, the sample may have to be rejected for analysis or the lipid particles removed by ultracentrifugation.
5. Unacceptable. The ferric thiocyanate method is not linear below 70-75 mmol/L. Therefore, dilution of the specimen would most likely have reduced the concentration of Cl below the linear range. The analysis should be performed on another instrument, e.g. a chloridometer.
6. Unacceptable. There is no reason for introducing a possible dilution error for this type of analysis. Instead, the technologist should have re-injected 20-25 uL of sample.
7. May be acceptable. The technologist must look carefully at the dynamic range of the RIA used. Some standard curves extend over a 10-fold (1-log) range of concentrations while others may extend over a 1000-fold (3-log) range. For a 10-fold range standard curve, a single two-fold dilution may be sufficient. However, for a 1000-fold standard curve, a 10-fold dilution may be more appropriate. In either case, multiple dilution steps of the sample should be used to ensure that a result will be obtained, since many RIA's are not performed on a daily basis. If a single inadequate dilution is analyzed and the result obtained is still not within range, the analysis will have to be repeated a third time, which may result in an unacceptable delay.

Answers to Questions to Consider

1. Most patient samples are diluted: 1) to remove a possible inhibitor to the analysis (such as lipemia) or 2) because the analyte concentration exceeds the reportable range of the assay.

2. The major limitations are the reference (normal) range of the analyte, the analytical reportable (linear range. see p 410) of the method being employed, and the lower limit of detection of the assay. Dilutions an make the final concentration of the analyte fall within the upper portion of the reference interval of the analyte. This will usually be well within the linear range of the assay and well above the limit of detection.

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4.22: Sample Analysis II

A technologist is working alone on the third shift in a hospital's chemistry stat ('rapid response') laboratory. The technologist receives, simultaneously from the emergency room, five specimens for analysis. They are: a) a serum sample for a renal profile (Na, K, Cl, CO2, glucose, BUN, creatinine), b) a blood gas, c) a serum sample for potassium and glucose, d) a serum beta-hCG ("pregnancy" test), e) a sample for urinalysis (dipstick plus microscopic).

QUESTIONS

1. What should be the order in which these samples are analyzed?
2. Ten minutes after the first five samples arrived, another blood gas specimen arrives in the laboratory. Will this specimen affect the order of the analysis of the remaining specimens? Why?

Questions to Consider

1. What clinical and laboratory factors should the technologist consider in deciding upon the order of analysis?
2. What, if any, acute emergency situations might be most comonly associated with each of these types of specimens?
3. For which of the situations discussed in question #2 would the physician's potentially life—saving medical decision-making be most likely to depend on the laboratory results?

Answer

1. The best order of work would most likely be: a) Run the blood gas specimen immediately. b) While the blood-gas analysis is being performed (approximately 2 minutes), set up blood samples (specimens a, c, d) to centrifuge if they are clotted. While the samples are centrifuging (approximately 8-10 minutes), the technologist should get the reagents ready for the pregnancy test and, if necessary, calibrate the instrument that will perform the renal profile. If time is still available, the dipstick analysis can be performed on the urine sample before it is centrifuged. c) When the blood-gas analysis is complete, enter and verify the results. Then set up the pregnancy test, which requires 7-10 minutes to obtain a negative result, and it is probably best to get the incubation period going since this test's clinical priority is very high. Next analyze the serum specimens in the order of d) Glucose and potassium and e) Renal profile. Finally, while the serum samples are being analyzed (10-15 minutes), prepare to do f) Urinalysis.
2. The logic for prioritizing sample analysis does not change; one must assume a blood-gas specimen is needed for immediate medical decision making. The second blood-gas specimen should be analyzed immediately upon receipt before resuming the analysis of the remaining specimens.

Answers to Questions to Consider

1. The most important factor in making a decision on sample priority is the emergency nature of the patient's condition. The more acute the situation, the faster the laboratory result is required by the physician. An "emergency" can be defined as a potentially life-threatening situation requiring medical intervention within a relatively short period of time - ranging from minutes to several hours.

   Many hospitals encourage physicians to communicate with the laboratory and provide information on the medical problem of a patient, drug history, etc. Although this information can be invaluable to the technologist in prioritizing sample processing or interpreting results, it is rarely provided. In addition, in an attempt to get results faster (whether clinicly needed or not), many physicians order tests "stat.", although that might not be justified on clinical grounds.

   The major laboratory factors which must be considered in deciding upon sample priority include sample processing and analysis times. The time for sample processing prior to analysis includes time for allowing serum to clot and centrifugation time. Analysis time includes time for actual measurement and any lengthy incubation times. A lengthy incubation may often need to be started before analyzing the higher priority analyses just to get a lengthier procedure initiated.
2. a) A renal profile does not suggest any acute medical situation. A convenience for physician ordering, this profile might be ordered for both non-emergency and emergency situations. Unfortunately, when such a profile is ordered without an accompanying patient history or presumptive diagnosis, the technologist does not know which situation the physician has encountered. (See Chapters 24, 26.)

   b) A blood-gas specimen is difficult to draw and is usually obtained for critical situations involving many types of acute

acid-base disorders ranging from pulmonary arrest to ketoacidosis to poisonings. (See Chapter 25.)

c) Glucose and potassium are typically ordered together for some disorder of diabetes: diabetic ketoacidosis (DKA), hypoglycemia, etc. A patient presenting with DKA or hypoglycemia can often be in a critical situation. (See Chapter 32.)

d) A serum (or urine) beta-hCG will be ordered in cases of females of childbearing age presenting with lower abdominal pain. The physician needs to differentiate an ectopic pregnancy as the source of the pain from other sources of pain not associated with pregnancy. Other situations requiring an hCG analysis include those cases of young females requiring some other emergency intervention (surgical, x-rays, etc.). In these cases, the clinician needs to determine whether the patient is pregnant before beginning an intervention that could endanger a fetus. In all cases, some impending intervention by the physician will depend upon the hCG resuls from the laboratory. (Chapter 40.)

e) A urine sample may be ordered in cases of renal failure, possible kidney infection, etc. Rarely are these acute emergency situations. (See Chapter 26.)

3. For specimens a and e, the technologist can have no idea if the physician really requires immediate results to make a medical decision. Results for specimens b, c, and d very likely are needed for acute patient care. Although an ectopic pregnancy is a potentially life-threatening condition, the physician generally does not require immediate results. Therefore, specimens b and c are presumed to require the most rapid analysis.

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4.23: Phlebotomy

PROBLEM

During a third shift, the laboratory receives a request for a stat. renal profile on a serum sample. The patient is a 35 year old white male. The following values are obtained:

Specimen #1		Reference interval:
Sodium:	81 mmol/L	(133-145 mmol/L)
Potassium	2.0 mmol/L	(3.5-5.5 mmol/L)
Chloride	63 mmol/L	(95-100 mmol/L)
Total CO_2	18 mmol/L	(21-33 mmol /L)
Glucose	1150 mg/L	(600-1000 mg/L)
BUN	140 mg/L	(50-200 mg/L)
Creatinine	15 mg/L	(7-14 mg/L)
Anion gap	3 mmol/L	(15-25 mmol/L)

QUESTION

What should the technologist's response be to these data?

The technologist reviews the results and recognizes that the observed electrolyte levels are not usually compatible with life. The analyses are repeated along with quality control specimens; similar results are obtained.

The technologist calls the attending physician to discuss the results and obtains the following information. This patient had no serious problems other than hypertension and had been treated with diuretics and placed on a low sodium diet. In addition, he had been drinking a lot of water (amount not stated). He was admitted to the hospital after becoming disoriented and was placed on intravenous fluids. The specimen on which the above values were obtained was clear and nonhemolyzed.

The technologist reviews the patient's recent laboratory results in the laboratory information system (LIS). The technologist notes that the results of a renal profile ordered 5 hours earlier had totally normal results. However, a hematology profile drawn at the same time as the current renal profile had highly abnormal results, a hematocrit of 27% and a hemoglobin of 78 g/L.

The technologist calls the patient's physician and suggests that a third sample be obtained. The physician agrees.

The following results are obtained on the third specimen:

Sodium:	139 mmol/L	
Potassium	3.0 mmol/L	
Chloride	106 mmol/L	
Total CO_2	26 mmol/L	
Glucose	1180 mg/L	
BUN	240 mg/L	
Creatinine	25 mg/L	
Calculated osmolality	294 mOsm/L	(Reference range: 280-305)
Anion gap	10 mmol/L	
Measured osmolality	336 mOsm/L	

QUESTION

Based on this history and the above observations about the original specimen, which of the possible reasons for the results arrived at in Question to Consider #1 are now more/less likely?

Questions to Consider

1. What kinds of instrumentation, methodology, or phlebotomy problems could yield highly abnormal results like these?
2. What might a significant difference between a measured and calculated osmolality indicate?

Answer

1. First, the specimen should be visually inspected to determine if an obvious matrix problem could be the cause of these results (see chapter 3; lipemia, hemolysis, or fibrin clots). Next, the technologist must verify the original results. If possible, another instrument or alternative methods of analysis should be employed. Running qualtity control pools will help verify that the *instrument* is functioning properly.

   While the repeat analyses are running, the technologist should check in the laboratory information system for previous results obtained on this patient to see if earlier results were similar to the current ones (Chapter 21). If the results are verified, the technologist should immediately call the attending physician since these are critical values, even if they are unlikely to truely reflect the patient's current status.
2. Upon seeing such radically different results over a short period of time, both specimens were rerun. The results were unchanged. Upon talking to the nurse, it was discovered that the patient was receiving intravenous (IV) mannitol (consisting of mannitol in a solution of water into which a minute amount of sodium bicarbonate is sometimes added as a buffer) in the same arm from which the hyponatremic specimen was drawn. The IV site was in the posterior portion of the forearm. Although the second specimen was taken from the antecubital area (which was proximal to the IV site), the nurse felt there should be no dilution of blood taken from the antecubital area. The third specimen was drawn from a hand vein (distal) to the IV site. The first specimen was drawn before the patient was given IV fluids.

   The problem was caused by the dilution of the venous blood sample by IV fluid before it reached the venipuncture site. This is a commonly enountered problem (see p 73) problem, one that technologists should always be concerned about. The analysis was confirmed by the much higher measured osmolality. The mannitol in the sample caused the elevation in the measured osmolality.

Answers to Questions to Consider

1. With this combination of resutls a technologist ususally thinks "preanalytical error". See chapter 3 for an extensive review of preanalytical errors, especially pages 69-73. Possible phlebotomy or laboratory problems that could have caused these results are:
   - interference from extreme lipemia (See Sodium and Potassium Method on CD-ROM).
   - fibrin clots in the sample, which can cause a “short-sampling” by an instrument’s sample probe
   - dilution of the sample by intravenous fluids that were delivered into the arm above the site used for phlebotomy.
2. The difference between the calculated and measured osmolality is striking, indicating the presence of a large amount of an unmeasured substance. This substance might have been introduced into the patient from the intravenous line.

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4.24: Chain Of Custody

A patient is taken from the scene of an accident directly to the emergency room of a hospital. The physician notes that the patient is not responding well to stimuli. In order to differentiate between a possible neurologic trauma and alcohol-induced CNS depression, a blood alcohol measurement is ordered. A phletobomist draws the blood, initials the slip, and brings It to the laboratory where the specimen is logged in by a technologist. The alcohol level is recorded as 1500 mg/mL. Levels above 800-1000 mg/mL (0.1%) are defined as intoxicated by most state laws (see p 641, Chapter 34). A policeman comes into the laboratory with a subpoena for the laboratory's results. The patient is accused of driving while intoxicated, causing a fatal accident. A week later, a defense attorney calls and requests information about the specimen results.

After giving both the policeman and the defense attorney appropriate information, as cleared by the hospital's risk management team, the technologist is notified that she will be placed on the witness stand in the upcoming trial for this case. The phlebotomist is also notified of a subpoena to appear in court.

QUESTION

What information did the policeman and defense attorney request?

Questions to Consider

1. What is the difference between the specimen labeling and handling procedures for forensic/legal cases and those used in routine patient cases?
2. What is a chain of custody?
3. What legal question will be debated in the court which involves both the phlebotomist and the technologist?

Answer

The policeman asked for the laboratory result on the patient's alcohol level in order to establish that the patient was intoxicated. He will try to prove beyond a reasonable doubt that indeed the facts are correct.

The defense attorney wanted information regarding the alcohol analysis, including any QC performed at the time, etc. In addition, the attorney wanted to know who logged in the specimen, who did the actual analysis, and how the sample was stored, before and after analysis. In addition, the attorney wanted documentation on all these points. The defense attorney will try to establish that the data could be invalid or that it was possible that the sample could have been switched, tampered with, or altered.

Answers to Questions to Consider

1. The difference between specimen labeling and handling for legal cases and those used for routine patient care is that the physical condition, knowledge of each person who was in possession of the specimen, and limited access to the specimen must be documented in forensic cases (p. 73-74).
2. Chain of custody is a legal document, usually in written form, which describes everything which occurred during the transportation, analyses and storage of the specimen (p. 73-74). This includes the name of the person drawing the specimen and the names of each person who received the specimen, the condition of the specimen at time of transfer from one individual to another, and the condition of the specimen at the time of analysis. Also, if the sample is stored, the storage area must be secure, that is, locked with access available only to a limited number of authorized personnel. The special form used in these cases is called a *Chain of Custody* form. The special form used in these cases is called a Chain *of Custody* form.
3. The legal question which will be debated is whether or not the specimen was a legally valid one, even though a chain of custody form was not filled out. If the specimen was drawn by the phlebotomist and given directly to the technologist who performed the analysis, this may well be accepted as a proper chain of custody. If, however, other individuals were involved with the specimen who could not document their custody, then most often the laboratory data is not acceptable to the court.

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4.25: Toxicology - Cyanide Poisoning

A patient was admitted to University Hospital as a result of a gunshot wound. After intensive surgery and care for one month, the patient remained in a coma. At that point, the patient's condition started to worsen gradually. Most noticeably, there was a large amount of lactic acidosis. Because of the severity of the acidosis and the suspicious nature of the initial cause of the illness, the surgical team orders a stat blood cyanide.

Stat cyanide analyses are difficult to do and require about 4 hours to perform. The technologist in the stat area checks the record of the patient and notes that the patient has been in the hospital for at least one month. The technologist calls the surgical team and asks why a stat cyanide level is being requested. The response is that the patient had received visitors and seemed to worsen after the visits; the team wants to rule out a suspected cyanide poisoning. The lactic acid acidosis could be the result of cyanide poisoning.

QUESTION

What should the technologist's response be to a request for a cyanide measurement to rule out possible poisoning of a hospital patient?

Questions to Consider

1. Is cyanide an acute or chronic poison?
2. If an acute cyanide poisoning were present, would a stat cyanide request be appropriate? Would the results of the CN^- analysis be crucial for medical decision-making?
3. What other hospital personnel do you think should be contacted?

Answer

The medical technologist brings the request for a stat cyanide level to the attention of the clinical chemist. After discussion, the physicians are notified that if an acute cyanide (CN) poisoning is suspected, it is more accurate and convenient to detect CN in intravenous IV fluids (the suspected route of delivery) than in blood. The team physician explains that this is not possible since the IV fluids suspected of causing the problem were discarded the day before.

Since it was obvious that an acute poisoning was not present, because it was at least 24 hours after the fact, the clinical chemist suggests to the team that a stat cyanide level is probably unnecessary. The team insists on the analysis being made. The chemist suggests that additional hospital personnel should be involved.

Poisoning of patients in a hospital can and does occur. Because of the medicolegal aspects of a suspected poisoning, the technologist should immediately bring the problem to a supervisor.

Answers to Questions to Consider

1. Increased blood cyanide levels are most often seen clinically in cases of acute poisoning usually in industrial settings; see p 998-999 and 1005. Also see Web sites: www.gaiaguys.net/Cyanide_poisoning.htm, chemdef.apgea.army.mil/textbook/Ch-10.pdf (requires Adobe)
2. If an acute cyanide poisoning were taking place, a stat cyanide measurement would be appropriate. However, because of the slow turnaround time of the test compared to the acute effects of cyanide, the antidote (sodium nitrite or oxygen, see page 1005 and the Web site http://www.hse.gov.uk/pubns/misc076.htm) should be given immediately without waiting for the test result. The rationale for nitrite therapy is that the Nitrites cause formation of Methemoglobin by combining with the hemoglobin. Methemoglobin has a higher affinity for cyanide than does cytochrome oxidase and thus promotes its dissociation from this enzyme.
3. Hospital risk management must also be consulted if cyanide poisoning is suspected. The risk management team can then take proper steps to maintain confidentiality and take preventive measures. If a medical consultant is available to the laboratory, this person should be contacted by the technologist.

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4.26: Cardiac Isoenzymes

A physician called the laboratory concerned about the results of a cardiac profile (total CK and LD) obtained on his patient. While the specimen had normal levels of lactate dehydrogenase (LD) activity, the total creatine kinase (CK) levels were significantly elevated: 159 U/L (upper limit of normal for a Caucasian male, 100 U/L). Since the laboratory's policy is to perform isoenzyme analysis only for cardiac profiles with an elevated total CK result, CK isoenzyme analysis had already been performed. The result, obtained by an immuno-inhibition assay, was 9 ng/mL (upper limit of normal, 6 ng/mL). The sample was non-icteric and non-hemolyzed.

Although previously there had not been any concern about the patient's cardiac status, the physician is now worried about a possible myocardial infarct (MI), and is asking the laboratory to perform isoenzyme analysis on a number of serum samples drawn subsequently to the one processed for a cardiac profile.

QUESTION

What advice should the laboratory give to the physician regarding the need for CK isoenzyme analysis?

Questions to Consider

1. What is the principle of the "sandwich" immunoassay for CK-MB? What are the major sources of interference of the assay?
2. What alternatives to the sandwich immunoassay are there for CK-MB analysis? What are the relative advantages and disadvantages of each?
3. What is the distribution of the 3 CK isoenzymes in prostate gland tissue?

Answer

Upon further discussion with the laboratory's clinical chemist and supervisor, the physician reveals that the initial sample, for which a cardiac profile was ordered, was drawn immediately after a surgical procedure for resection of a prostate gland. Postoperatively, the patient experienced no EKG changes, chest pains, or arrhythmias.

The laboratory should advise the physician that further CK isoenzyme analyses are not needed and that the patient most likely did not have an MI. Since the prostate gland is rich in CK-BB, prostatic surgery will result in relatively large amounts of CK-BB being released into blood. Because this laboratory's immunoinhibition method does not blank out CK-BB activity, CK-BB causes a positive interference. The degree of interference is actually twice as large as the actual level of CK-BB present because the procedure calculates the final CK-MB activity by doubling the B activity to estimate the entire MB activity. Thus, CK-BB activity from both B chains is multiplied by 2.

The extent of such interference can be seen in the original data: total CK, 159 U/L, CK-MB activity, 66 U/L: that is 42% MB. Usually the % CK-MB in most known MIs is between 5-15%. The 42% MB with low total CK activity is a result of the presence of unblanked CK-BB activity.

Answers to Questions to Consider

1. The immunoinhibition technique was once in wide use, but it rarely employed today (See Methods of Creatine Kinase Isoenzymes on the CD-ROM). This assay worked by adding to the sample an antibody directed towards the "M" subunit of CK. The binding of this antibody would inhibit the enzyme activity of the "M" subunits, from CK-MM amd any CK-MB, but would not inhibit the "B" unit activity in CK-MB. After the addition of the antibody, the remaining CK activity of the solution would be measured. Since it was assumed that there is no CK-BB activity present, the measured "B" activity would be multiplied by a factor of two to give the full CK-MB activity.

   The primary interference with the assay would be the presence of CK-BB, which can come from a number of tissue sources. The presence of HAMA antibodies may cause falsely high results by binding to the anti-B antibody and preventing inhibition.
2. Immunometric CK-MB measurements work by adding to the sample a primary antibody (Ab) preparation that is directed to either the M or B subunit of CK. These antibodies, linked to some solid phase, can be monoclonal (MAb) or polycloncal (See see chapter 11 and 12 and Methods of Creatine Kinase Isoenzymes on the CD-ROM). After binding of any CK to the 'capture' antibody, there is a wash step of the solid phase to remove any interfering substances. A second, labeled antibody is added to the solid phase. The second antibody can be labeled with radioactive, fluorescent, cehmiluminescent, or enzyme

tags (see chapter 12 and 13). The secondary antibody is also either a monoclononal or polyclonal antibody, but is always the opposite of the first; that is, if the primary antibody was directed toward epitopes on the B subunit, than the secondary antibody is directed towards sites on the M subunit. This gives specificty to only the MB isoenzyme form of CK. In addiiton, most current assays use a monoclonal antibody for one of the steps (primary or secondary) and a polyclonal antibody preparation for the other step. The use of a monoclonal antibody increases the specificity of the assay.

The are very few interferences with the assay itself. The most frequent is the presence of HAMA antibodies, which can cause either falsely elevated or falsely low results, depending on the assay configuration.

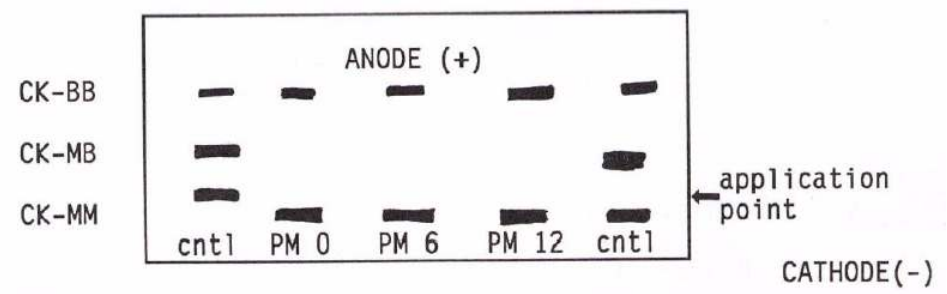
Measurement of CK isoenzymes by separation techniques can also be performed by agarose gel electrophoresis (See Creatine Kinase Isoenzymes on CD-ROM.). With this method one can detect other CK isoenzymes as well as rare, atypical CK forms. This method is relatively inexpensive, difficult to automate, rather slow, and infrequently used today.

3. The tissue distribution of CK isoenzymes is shown in Table 55-1 of the 4th Edition. Of interest is the very high percentage of CK-BB in prostate gland tissue.

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4.27: Creatine Kinase (CK) Isoenzymes

A series of three samples is sent to the Chemistry Laboratory with a request for a “cardiac profile”. All three samples are analyzed for total CK and have results >8000 U/L. It is noted that the samples are colored orange-green. The laboratory routinely analyzes all samples with elevated CK values for CK-MB, but the technologist is not quite sure if the immunoinhibition assay technique in use will work on samples with such elevated levels of CK or on ones which are so highly colored. The patient is a 44-year-old Caucasian female who was transferred to the hospital two days before from a community hospital. The patient was placed in the Medical Intensive Care Unit (MICU). After the technologist discusses the problem with the laboratory’s clinical chemist, the decision is made to perform CK isoenzyme analysis by electrophoresis rather than by the immunoinhibition assay. The results are described schematically below:



Where PM 0, 6, and 12 are the samples drawn at 0, 6, and 12 hours after admission. The technologist, concerned that this unusual pattern might be the result of technical or specimen collection errors, reviews the data with the laboratory’s clinical chemist.

QUESTION

What clinical problems could cause this CK isoenzyme profile?

In order to determine the nature of the patient’s disease, the clinical chemist and the technologist review other laboratory data on the patient.

Some other laboratory values obtained that day on that patient included:

Serum	
Na	134 mmol/L
K	7.0 mmol/L
BUN	570 mg/L
Creatinine	24 mg/L
Glucose	1260 mg/L
Anion gap	24 mmol/L
Lactic acid	25.2 mmol/L
Calcium	91 mg/L
Inorganic phosphorous	101 mg/L
Total bilirubin	229 mg/L
Direct bilirubin	110 mg/L
ALT (SGPT)	2270 U/L
AST (SGOT)	9710 U/L
Alk phosphatase (AP)	3950 U/L
5' nucleotidase	130 U/L

Urine Analysis

Protein	3000 mg/L
Bili	4+
Blood	4+
Nitrite	pos
WBC/RBC	TNTC
Bacteria	3+

(Reference intervals for many of these analyses are found inside the front and back covers of the 4th Edition and in the CD-ROM.)

In addition, the patient had a raised WBC and a markedly low RBC; Hb = 104 g/L. Platelet count was abnormally low; PT/PTT were 23 and 55 seconds, both markedly elevated; with FDP (fibrin degradation products) almost 20-fold elevated (256 mg/L). Serology for hepatitis were all negative; the patient had never been infected with hepatitis virus.

QUESTION

Which disease would be most likely to cause these laboratory results and the CK isoenzyme pattern?

Questions to Consider

1. What organs are major sources of CK-MM? CK-MB? CK-BB?
2. Are there any other electrophoretic variant forms of CK? If so, what are they?
3. Are there any common laboratory or sample collection errors that could cause such a CK pattern?
4. Which of the laboratory results are most unusual? What type of diseases is suggested by these results?
5. What are some causes of an increased serum lactic acid?
6. Why was the sample so unusually colored?
7. The original request was for a 'cardiac profile'; what other tests can be ordered to determine if this patient had cardiac damage?

Answer

1. Heart has the highest percentage and levels of CK-MB, although certain types of muscle fiber can also have high levels of CK-MB. The brain and colon have the highest levels of CK-BB activity, although lung tissue can often be a source of serum CK-BB.
2. The troponin I assay was run on all the samples and was not detected in all cases.

   The most likely explanation for the atypical CK electrophoretogram is some cancer, either lung or colon, which has metastasized to the liver. Since certain types of very aggressive lung tumors (oat cell carcinomas) often produce large amounts of CK-BB, lung cancer is most likely. Extensive metastasizing of the primary tumor to the liver would destroy the liver and would explain the large increases in AP, LD, 5’-nucleotidase, bilirubin, and bile acids (Chapter 27). The severe lactic acidemia is most likely caused by the rapid metabolism of the anoxic tumor, made worse by the diminished capacity of the liver to remove lactic acid from the blood.

   It should be noted that the most likely explanation for a severely atypical CK electrophoretogram is cancer.

> **Note**
>
> This patient died within 72 hours of hospitalization of severe liver metastasis from a primary oat cell carcinoma of the lung.)

Answers to Questions to Consider

1. Skeletal muscle is the major source of CK-MM in terms of percentage of total CK and absolute units of activity per gram of tissue (Table 55-1, p 1067).
2. There are two types of rarely seen isoenzyme forms of CK. They are the mitochondrial CK (mCK) and macro-CK forms. The latter may be complexes between CK and antibodies to CK (see Methods on CD-ROM CK isoenzymes). While the

mCK activity is found at a slightly cathodic position on agarose electrophoresis, the macro-CK activities can be found in unusual positions anywhere in the electrophoretogram.

SEE Web sites: www.labcorp.com/datasets/labc...o/sc005800.htm
puma.protein.bio.msu.su/biokh...f/bcm_1098.pdf
www.gpnotebook.co.uk/cache/1322254395.htm
www.ncbi.nlm.nih.gov/htbin-po...=r&uid=8013099

3. There are no known sample collection problems or technical errors that would result in such a pattern. Large amounts of total CK, with increased amounts of CK-BB and the presence of an atypical anodic band of mCK activity might be associated with disease of some organ containing large amounts of CK-BB, that is severe disease of the brain, colon, lung, or in men, prostate. In addition, one would have to consider the presence of some cancer since large amounts of CK-BB may be produced by some cancers (Chapter 55).

   Large amounts of CK-BB might prompt an acute immunological response by the patient with the resulting formation of Ig-CK-BB complexes that might migrate cathodically. The alternate explanation for the atypical cathodic band is mCK, which is often found in very acute terminal illnesses.
4. Certainly the most striking of these results are the extraordinarily large increases of LD, CK, and AST. The elevated levels of creatinine and BUN and the results of the urine analysis certainly demonstrate the presence of a protein-losing nephropathy (Tables 26-2 and 26-3 of the 4th Edition,). Elevated levels of the transaminases, bilirubin, and alkaline phosphatase are indications of a fairly severe liver disease.
5. Lactic acid is produced in large amounts by anoxic tissues, that is, tissues with very low oxygen levels (p. 471). This can be the result of general circulatory collapse with diminished oxygen delivery, or the result of increased oxygen consumption by tissues (fever, prolonged exercise, or rapidly metabolizing tumors). Further increases in serum lactic acid can be caused by a diseased liver which can no longer clear lactic acid from the blood.
6. The highly colored serum is most likely caused by large amounts of bile acids; probably the result of the severe liver dysfunction (p 497).
7. The best test to rule out cardiac damage would be a troponin (I or T) assay (see p. 576-577). These assays are now routinely available in most hospitals.

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4.28: Tumor Markers

A laboratory performs analyses for prostate specific antigen (PSA) by enzyme immunometric assays. In order to minimize costs, the laboratory performs these analyses twice a week: Tuesday, the day after an oncology clinic and Friday, the day after a urology clinic. On a Wednesday, an irate oncology physician calls the laboratory demanding to know where the result for the PSA test is for his patient. The technologist responsible for this assay looks up the result in the laboratory information system (LIS) and finds that the result are “pending.” The levels of PSA obtained that Tuesday were greater than the highest standard of the assay, and so the samples will be diluted and reanalyzed on Friday. The physician, not especially happy with the delay, agrees to wait until Friday for the results.

On Friday, the technologist in the PSA area performs the analysis on a 1:3 dilution of the sample. The results, once again, exceed the standard curves of the PSA assay and requires still further dilution. The laboratory supervisor, knowing that waiting for the next routine PSA assay would result in an unacceptable delay, asks the technologist to rerun the assays immediately.

QUESTIONS

1. What dilutions should the technologist prepare for the assays this time?
2. The technologist looks up the patient’s previous result PSA. It was 25 times the upper limit of normal. The technologist, who works 4 hours overtime, reports results on the sample that was diluted 1:50. Can you recommend a policy for the analysis of PAP and PSA in this laboratory that will avoid the above situation?

Questions to Consider

1. What analytical factors do you think should be considered when setting up dilutions of samples for subsequent analysis?
2. What clinical factors should be considered when setting up sample dilutions for analysis?

Answer

1. If a technologist is not sure of which dilution to make, then the technologist should set up more than one dilution. Hopefully, this will enable at least one of the dilutions to fit the analytical criteria listed in question number one. If more than one dilution falls within the linear range, a check on the validity of the results can be made by comparing the answers. Probably 1:5, 1:10 and 1:50 dilutions would be advised for this sample. This sample was drawn from a patient attending an oncology (cancer) clinic so one might predict very elevated levels of these tumor markers.
2. The laboratory should actively use the capability of most laboratory information system (LIS, Chapter 18) to review the previous results for tests that may have very high and that are infrequently performed. Knowing the previous result of a patient who attends a disease treatment clinic can provide information that can be used when choosing dilutions for a current specimen. If an LIS does not have this capability, a manual “delta” check file on index cards can be uesed.

   To minimize delays when samples are drawn from patients attending a clinic for the first time, the laboratory’s policy might require that the technologists perform the PSA analysis on an undiluted sample, a sample diluted two times, and a sample diluted ten times. Although this may appear to consume unnecessary resources, it will reduce turnaround time, repeat analyses, and perhaps the need for overtime laboratory work.

> **Note**
>
> More modern immunometric assays for PSA have larger reportable ranges and many modern instruments have the capability to automatically re-run the analysis for samples whose results are greater than the upper limit of the reportable range. The second analysis can be performed on an actual dilution or simply on a smaller volume of sample.

Answers to Questions to Consider

1. The important analytical factors to consider when preparing dilutions include:
   - Linearity of the analytical reaction: The aim is to dilute the analyte to a level within the established linear range, also called the reportable range, of the assay. Remember, all assays have lower limits of linearity; one never reports out '0' for a result, but "less than" (<). This lower limit is sometimes referred to as the lower limit of sensitivity of the assay.
   - Analytical sensitivity: One does not want to over-dilute the analyte to a level below the limit of sensitivity; the final result is often bizarre if this caution is not observed.

- Precision: One usually gets better precision with a larger signal (e.g., absorbance, Chapter 23; or counts per minute for radioactivity, p 198-Chapter 9). Therefore, one would not want to dilute the analyte down into the very lowest region of the reportable range. In addition, each pipetting step for dilution has some inherent imprecision which is now added onto the assay's degree of imprecision. Larger error assocaited with the new analysis can affect the final result, sometimes producing erroneous results. Thus the dilution should be one that is easily attainable with the resources at hand to produce an accurate dilution.
- Sample volume: One needs sufficient volume of sample to produce the optimal dilution. If insufficient sample is available, a larger, less optimal dilution may be necessary.
- The "hook" effect: Many immunoassays have an anomolous "hook" effect which occurs at very high levels of analyte and results in lower results than the true value. A "hook" effect is another limit of linearity. One wants to dilute an analyte measured by an immunoassay below the region of a "hook" effect.

2. Important clinical factors to be considered when preparing dilutions include:
   - The levels of analyte that are usually associated with specific disease states.
   - The "usual" results obtained on a specific population or an individual.
   - The current level of that analyte in the specific patient in question.

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4.29: Potassium

The head nurse of a cardiac care unit (CCU) calls the central chemistry laboratory about a low potassium result on a recently drawn plasma sample. The potassium level was measured in a small laboratory near the CCU. The CCU's head nurse complains to the central chemistry laboratory's supervisor that not only is this potassium result too low, but that a problem of "low" potassium results from the CCU laboratory has been noticed for the past week. The CCU's results are "low" when compared to the results obtained from the central laboratory.

Potassium is measured in the CCU laboratory by an analyzer that uses a nondilutional ion selective electrode and whole blood, plasma, or serum as a sample. The central laboratory uses a dilutional ion selective electrode system for measuring potassium in serum or plasma.

QUESTION

What steps can the laboratory supervisor take immediately to validate the CCU's measurement of potassium on the samples from the CCU?

The following potassium results are reported back to the supervisor:

Specimen	CCU	Central Lab	Origin of Sample
1	3.8*	3.7	plasma from CCU sample
2	3.6*	3.7	plasma from CCU sample
3	4.1	4.1*	serum from central lab
4	3.7*	3.6	plasma from CCU sample
5	4.0	4.0*	serum from central lab

The starred (*) results are essentially equal to those obtained upon initial analysis of the whole blood or serum samples.

The laboratory supervisor takes these results and meets with the CCU nurse. The supervisor explains that there does not appear to be an analytical bias between the two laboratories; each instrument measures essentially the same result for each sample. Instead there appears to be a sampling problem - the potassium concentrations in the plasma (whole blood) samples is approximately 10% to 11% less than the potassium concentrations the serum samples.

QUESTION

What kind of phlebotomy error can lead to this discrepancy between the plasma and serum results?

Questions to Consider

1. Are there any sources of interference that might affect one potassium method but not another?
2. Are there any sample requirements that might affect the potassium results?
3. Is the magnitude of the difference between the plasma and serum potassium results to be expected?
4. What assumptions are usually made when phlebotomy tubes for plasma or whole blood are used?

Answer

1. The laboratory supervisor asks a medical technologist to take the following steps:
   - retrieve the most recent samples from the CCU for this patient including samples sent to the central laboratory
   - verify the patient's name and identification code on the samples
   - note if there is anything unusual about the samples (lipemia, hemolysis, icteria, etc.)
   - perform a potassium analysis on all samples in both the CCU and central laboratories

   The supervisor should ask a technologist to verify that the patient's name and hospital identification number are on each tube to ensure that there was no sample mix-up. The plasma fractions from the whole blood samples and recent serum samples from this patient should be re-analyzed for potassium in both the CCU and central laboratories. This will help determine if an analytical bias exists between the two laboratories.

2. The most likely explanation for the significantly lower plasma levels for potassium is an underfilling of the phlebotomy tubes (p.71). If a 3.5 mL green-top tube containing 50 μL of heparin solution (see Table 3-2, p.71) is used, and the tube is only filled with 1.5 mL of blood, the dilution error would be 3.2% instead of the 1.4% that would exist if the tube were filled to capacity. The error for a blood potassium level of 4.0 mmol/L would be -0.130 mmol/L vs. -0.056 mmol/L, respectively. When this dilutional error is added onto the normal bias between plasma and serum potassium values, the total error becomes clinically obvious. As soon as the CCU's phlebotomy tubes were properly filled, this bias disappeared. Obviously, if the phlebotmy tubes did not contain anticoagulant in a liquid form (that is, in a dried form instead), this problem would not have arisen.

Answers to Questions to Consider

1. One interference that would affect one method but not another is the effect of extreme hypertriglyceridemia (See Sodium and Potassium Method on CD-ROM), which would *not* affect the non-dilution method but might severely affect the dilutional method, depending upon how severe was the hypertriglyceridemia.
2. Serum, plasma, and whole blood samples can be used for the measurement of potassium (See Sodium and Potassium Method on CD-ROM). Sodium heparin is usually the anticoagulant of choice for whole blood and plasma samples. Ammonium heparin is unacceptable because the ammonium ions cause a positive interference with potassium ISEs (See Sodium and Potassium Method on CD-ROM).
3. No. There usually is a slight positive bias of 0.2 to 0.3 mmol/L between serum vs plasma because of the release of potassium from blood cells during the clotting process (pp.70, 73, and see Sodium and Potassium on CD-ROM). That is, plasma samples tend to run about 5% lower than a corresponding serum sample. The differences observed in this case are much greater than might normally be expected.
4. The assumption made when using phlebotomy tubes containing an anticoagulant are: 1) the tubes are filled to stated capacity, and 2) the filled tubes will be sufficiently mixed to ensure even dispersion of the anticoagulant in the sample.

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4.30: Ammonia Analysis

The laboratory receives a specimen from the Neonatal Intensive Care Unit (NICU) for an ammonia measurement. The analysis is performed on a centrifugal analyzer using an enzymatic method (glutamate dehydrogenase, GLDH). The result is reported as 1170 mmol/L (upper limit of the reference range is 72 mmol/L).

A second sample from the same infant is received later that evening on third shift. Because ammonia levels cannot be performed on third shift, the sample is sent to a nearby hospital that does have stat. ammonia analysis capabilities. The other hospital also employs an enzymatic method for measuring ammonia levels, but uses a different analyzer. The result for this sample is 48 mmol/L.

The NICU physician calls the laboratory wanting an explanation for the difference between results.

QUESTIONS

1. What can the laboratory do to confirm these results?
2. The laboratory supervisor asks a technologist to retrieve both specimens and re-analyze them for ammonia. After verifying the infant's identification on both specimens, the technologist measures the ammonia levels, reporting results of 1245 and 67 mmol/L for the first and second results, respectively.

   The laboratory supervisor talks with the NICU physician and determines that the ammonia levels were requested because of the infant's failure to thrive; the physician wants to rule out an inborn error of metabolism as the cause of the infant's problems. The physician does not know of any clinical state that would cause such a rapid change in ammonia levels. A third sample is obtained from the infant, and the ammonia level is found to be normal.

   The laboratory's clinical chemist asks the supervisor to find out what kind of tubes were available in the NICU for obtaining the samples for ammonia analysis. The supervisor reports back that heparinized capillary tubes were available for the initial analysis but that they contained neither sodium nor potassium heparin. The second and third specimens were drawn into small vacutainer tubes containing sodium heparin. What might be the cause of the apparently falsely elevated ammonia level in the first specimen?

Questions to Consider

1. What is the principle of analysis of the enzymatic method for ammonia that employs the same GLDH reaction employed in the enzymatic measurement of urea?
2. What other methods are available for ammonia measurements?
3. For what clinical problems would an ammonia measurement be requested?
4. What are the specimen requirements for an ammonia measurement?
5. What kind of phlebotomy tube would be used for an Infant?

Answer

1. The laboratory should verify these results by repeating the ammonia analyses on both specimens. If possible, the first specimen should be analyzed by the other hospital's GLDH procedure. It would probably be useful to request a third sample. The repeat analyses might be significantly higher than the initial results because the samples are unstable with time, generating ammonia-like substances upon sitting.
2. The most likely explanation for the elevated ammonia levels in the first sample was the use of ammonium heparin as the anticoagulant in the capillary tube.

Answers to Questions to Consider

1. The GLDH reaction converts alpha-ketoglutarate to glutamic acid in the presence of, NADH, and NH_4^+ ((Link to Methods CD Ammonia). As the reaction proceeds, NADH is oxidized to NAD^+, and the decrease in absorbance at 340 nm is directly related to the ammonia concentration.
2. Another method available for the measurement of ammonia is a potentiometric one, using an ion-selective electrode (p. 277).
3. Ammonia levels can be highly elevated in severe liver disease, such as seen in Reye's syndrome, liver cirrhosis, and hepatic failure (Chapter 27). Hyperalimentation can also cause a hyperammonemia (Chapter 27). A rare inborn error of metabolism

that affects the urea cycle can cause a marked elevation in blood ammonia levels. These infants are usually labeled “unable to thrive” as a result of the genetic abnormality (Chapter 47).

4. Ammonia is usually measured in plasma or whole blood, using heparin as the preferred anticoagulant. The specimen must be protected from air and be refrigerated. If analysis can not be performed with a few hours, the sample should be frozen.
5. In order to minimize the volume of blood taken from the infant, a capillary tube would probably be used for phlebotomy (p. 70).

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4.31: Toxicology (CO Poisoning)

The laboratory received three venous blood samples for carboxyhemoglobin (HbCO) analysis. The samples, drawn from three individuals at a fire, had the following results:

Patient	% HbCO
DJ (>30 years, pregnant female)	41.4%
AJ (approx. 7-8 years, female)	32.4%
EW (>40 years, female)	33.9%

Because the results were highly elevated, the medical technologist repeated the entire analysis and obtained similar results. The QC (Hi and Lo) in both sets of analyses were within normal range.

Upon receiving these results, the Emergency Unit physician called and asked to speak to a supervisor. The physician stated that he believed the results were inaccurate since the patients appeared normal. He mentioned that a blood gas specimen drawn on DJ while she was breathing room air had been recently sent, and asked if the lab would repeat the HbCO analysis on it.

The specimen was retrieved and the repeat HbCO on this sample was 42.8%. The blood gas values were: pH = 7.40, PCO_2 = 32, PO_2 = 110; TCO_2 = 21.

QUESTIONS

1. Are the HbCO results at all compatible with the physical condition of the patients?
2. Approximately 120 minutes later, a second set of specimens was received in the lab for HbCO analysis and the following results were obtained: AJ = 0%; DJ = 5.6%; EW = 5.4%. Can you explain the differences between the initial and final CO levels and the results of the blood gas analysis?

Questions to Consider

1. What is the biochemical mechanism for CO toxicity?
2. What is the physiological mechanism for CO toxicity?
3. What factors affect the degree of CO toxicity?
4. What is the treatment for CO poisoning?
5. Is there anything additional the lab could do to confirm the elevated CO levels?

Answer

1. On the face of it, these HbCO values are highly elevated. HbCO levels this high are usually associated with severe symptoms of CO poisoning: severe headache, dizziness, vomiting and even coma. So these very elevated levels of HbCO are not consistent with the observed condition of the patients. Moreover, an even severer reaction would be expected in the pregnant female and the child.
2. The physician was concerned about the validity of the results because, although the three patients had been exposed to CO in a fire, they did not demonstrate the typical signs of CO toxicity associated with the reported values (headache, dizziness, vomiting). The reason for the discrepancy was obvious once the clinical chemist and medical technologist pieced together the order of events. The three patients were treated with 100% oxygen while they were being brought to the hospital. However, the blood samples were drawn at the time that the oxygen therapy was initiated. Thus, the analysis accurately reflected the degree of CO exposure and poisoning of the three individuals at that point in time. However, by the time the results were returned to the emergency unit, enough time had elapsed since the exposure for the O_2 to replace most of the CO from the blood and reduce the symptoms.

   The bright cherry-red color of the retrieved blood gas sample did confirm the initial HbCO analysis and convince the laboratory that the results were valid. The apparent discrepancy between the high % HbCO and the high PO2 is explained by the fact that the oxygen electrode does not measure total O_2 being carried by blood, but only that portion actually dissolved in the plasma portion of blood. Usually there is a direct releationship between the Hb binding of O_2, total O_2 carried in blood and the PO_2 of blood. However, any impairment of the ability of Hb to bind O2 will cause a dissociation between O_2

capacity and the PO_2 of blood. This dissociation can occur in CO poisoning and certain hemoglobinopathies which involve defective hemoglobin molecules.

Answers to Questions to Consider

3. The most important factors are those that would increase the body's oxygen requirements. Thus, preexisting cardiac failure, fever, pregnancy, or low hematocrit in an individual each would exacerbate the toxicity shown at any given level of CO in the blood. In addition, young children have higher rates of metabolism than adults and thus show a greater degree of toxicity at a given level of CO. Some Web sites on the toxicity of CO include: ash.xanthia.com/co.html, www.health.state.mn.us/divs/eh/indoorair/co/
4. The primary treatment for CO poisoning is the displacement of the CO from the hemoglobin molecule by increasing the PO_2 of the blood (see p 1011). This patients with CO poisoning are treated with 100% oxygen. In very severe cases, patients can be treated with hypebaric oxygen, that is, oxygen at 2-4 atmospheres of pressure. Hyperbaric oxygen treatment not only reduces the HbCO concentration faster, but it raises the PO_2 of plasma so that more can be carried in the dissolved state. The half-life of HbCO with O_2 treatment is approximately 30-40 minutes, greatly reduced from the 180-240 minute half-life when only room air is inspired. For additional information on hyperbaric treatment, see these Web sites: http://www.pulmonaryreviews.com/feb0...yperbaric.html www.hyperchamber.com/co_poisoning/
5. To confirm the presence of CO the medical technologists could inspect the color of the blood gas specimen that was retrieved. HbCO has a characteristic bright cherry-red color. In fact, at high levels of CO poisoning, the tips of fingers can be noticeably brighter red and CO poisoning is readily diagnosed by pathologists at autopsy by the bright red color of tissues.

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4.32: Spectrophotometry- ELISA Reader

A technologist is performing a colorimetric assay using 96-well ELISA microtiter plates. The ELISA plate reader measures the absorbance of the solution in each well (cuvette) by recording the light passing through the solution from the top to the bottom of each well. The assay's color, when tested in a standard spectrophotometer, is linear to an absorbance of 2.3. The ELISA reader has a range of linearity which extends to an absorbance of 1.2. When a microtiter plate run is placed in the ELISA reader, the technologist notes that the solution in several wells has an approximate absorbance of 1.5. The assay material is very expensive and each assay takes one day to perform. Considerable effort is made to get as much data as possible from each run. Therefore, in order to measure the absorbance of the solution in these wells, the technologist dilutes the samples by adding a volume of buffer equal to the volume in the well. To her surprise, the absorbance values remain the same.

QUESTIONS

1. Why didn't the absorbance value change?
2. The technologist consults with the clinical chemist who suggests that she remove half of the solution from each well and recalculate. Why should this step now bring the absorbances within the linear range of the ELISA reader?

Questions to Consider

1. What is the equation, which relates absorbance to concentration?
2. What happens to each of the parameters in the equation in question 1 when buffer is added to the microtiter well to double the sample volume?

Answer

1. In this case, diluting the solution increased the path length through which the ELISA reader measures by a factor of two. This exactly counters the halving of the concentration by doubling the volume in the well, so in the above equation as c decreased by two-fold, the path length increased by a factor of 2, and the net absorbancy remained the same. This is in contrast to the usual spectrophotometry reading which is made through a cell of fixed path length and is independent of the volume in the cuvette.
2. Removing half the volume of the diluted reaction mixture from the well reduces the path length by one half. Since the solution has been diluted, the measured absorbance will be reduced by a factor of 2, the pathlength is restored to the initial value, and the absorbance of the solution will now be in the instrument's range. The technologist must simple multiply the calculated concentration by a the dilution factor of 2.

Answers to Questions to Consider

1. The equation which relates concentration to absorbance is Beer's law:

$$A = abc \tag{4.32.1}$$

where A is absorbance; a, absorptivity; b, light path of the solution in centimeters; and c, concentration of the substance of interest (p. 88).

2. When buffer is added to a microtiter plate well, both b and c in the Beer's law equation change in inverse proportion to each other. The concentration decreases to one-half its initial value because the solution is being diluted. However, the path length (b) is increased because the volume in the well has doubled and the height of the solution in the well is approximately doubled. The configuration of the reader is such that the path length is proportional to the amount of solution in each well.

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4.33: Enzyme Analysis

A laboratory sets up an assay for total alkaline phosphatase (ALP) by a modified AACC method. This kinetic method uses 0.30 mL of reagent, 0.05 mL of sample, and 0.15 mL of diluent in the final reaction mixture. A month after beginning to use this assay, the clinical chemist notes that a large percentage (15%) of specimens require dilution before analysis because the results exceed the assay's upper limit of linearity. The clinical chemist asks a senior medical technologist to reformat the ALP assay to give it a wider linear range and reduce the number of required dilutions.

QUESTIONS

1. How can the technologist modify the assay to increase its linear range?
2. The technologist decides to reduce the sample volume to 0.04 mL and then determines that the linearity of the assay is now satisfactory. However, when comparing results for the ALP assay with 0.05 mL and 0.04 mL sample volumes, the technologist finds that the assay with the smaller sample volume gives consistently lower results than the original assay. What can the technologist do to have the modified assay yield the same results as the original assay?

Questions to Consider

1. What components of an enzymatic assay determine its linear range?
2. How would decreasing only the temperature of the assay affect the assay's linearity?
3. How would decreasing only the reagent volume affect the assay's linearity?
4. How would decreasing only the sample volume affect the assay's linearity?
5. How would decreasing only the sample volume affect the assay's precision?
6. How are the results of an enzyme assay calculated?
7. How does an automated instrument calculate the final answer for an enzyme measurement?

Answer

1. The technologist can increase the linear range af the ALP assay by monitoring the reaction over a shorter time period, increasing the reagent volume, incubating the reaction at a lower temperature, or decreasing the sample. volume. The ease in which these changes can be made and the impact of any change on analytical performance would have to be monitored.
2. The technologist merely has to recalculate the instrument's multiplication "factor," substituting the new sample volume for the old value. The "factor" is

$$\frac{\text{Total Volume} \times \text{Sample Volume}}{\text{extinction coefficient } (\epsilon) \times \text{path length}}. \tag{4.33.1}$$

   For example, the calculation for ALP (Link to Methods CD Alkaline Phosphatase) has the "factor" as 3245.

Answers to Questions to Consider

1. The components of an enzyme assay that determine linear range are the timing of measurements of the assay and the final concentration of substrates in the reaction mixture (Chapter 54). Both are related to the need to monitor the enzyme reaction under conditions of zero order kinetics (Chapter 54). If the reaction is monitored for too long a time, substrate may become exhausted. If the reaction is monitored for too short a time, the change in absorbance (ΔA) may be so small that precision of analysis is decreased. The greater the amount of substrate per amount of added enzyme analyte, the greater the linear range of the assay.
2. Decreasing the temperature of the reaction will decrease the apparent enzyme activity (Chapter 54). This means that, for a given time interval for measurement, more substrate will be available for the enzyme. Thus, the linear range of the assay should increase. However, it is often not desirable, or even possible, to change the incubation temperature of a multi-test chemistry analyzer, especially for a single assay.
3. Decreasing the reagent volume will decrease the substrate in the final reaction mixture and thus decrease the linearity of the assay.
4. Decreasing the sample volume will decrease the analyte (enzyme) in the final reaction mixture and thus allow more substrate to be available for reaction. This would increase the linearity.
5. Decreasing the sample volume while keeping the monitoring parameters constant might decrease the assay's precision, especially at lower levels of enzyme activity. The smaller the sample volume, the lower the final activity in, the reaction

mixture, the smaller the ΔA/min. The smaller the ΔA, the greater the error of analysis. The laboratory would have to evaluate the effect of changing the sample volume on the assay's precision and decide whether the decrease, if any, in precision was acceptable.

6. $$\text{Enzyme activity}, \frac{U}{L} = \frac{\Delta A/minute \times \text{total reaction volume (mL)} \times \text{sample volume}}{\text{Micromolar extinction coefficient } (\epsilon) \times \text{optical pathlength}} \tag{4.33.2}$$

   See Chapter 54.

7. An automated instrument calculates the final result for an enzyme measurement by multiplying the measured change of absorbance (ΔA /min) by a "factor." This factor consists of all the constants of the equation in answer #6, that is

   $$\frac{\text{Total Volume} \times \text{Sample Volume}}{\text{extinction coefficient } (\epsilon) \times \text{path length}}. \tag{4.33.3}$$

   See calculation example for ALP (See Alkaline Phosphatase method in CD-ROM).

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4.34: Renal Function - Creatinine Clearance Calculation

A technologist is asked to calculate a patient's 24 hour creatinine clearance. The urine sample is an aliquot from the total sample reported on the laboratory slip to be 800 mL. The plasma creatinine level is observed to be 14 mg/L. The urine creatinine concentration is found to be 900 mg/L.

QUESTIONS

1. How is a creatinine clearance calculated?
2. The technologist calculates the creatinine clearance and reports the value in the LIS as 36 mL/min. That afternoon, a nephrologist calls the laboratory, questioning the accuracy of the result and asks for a reanalysis of the specimens.

   The technologist repeats both the creatinine analyses and the calculation and comes up with essentially the same result. The technologist brings the problem to the laboratory's clinical chemist. Together they review the patient's demographics in the laboratory computer and find out that the patient is a 23 year old, 225 lb., 6' 3", white male. Does this information help resolve the question of the validity of the results?

Questions to Consider

1. What are the units for creatinine clearance?
2. What is the formula for the calculation of creatinine clearance, and what additional data are needed?
3. What is the usual volume of urine output for a healthy male? What is the usual amount of creatinine excreted in urine of such individuals?
4. Will the correction of the creatinine clearance for body surface area give a more valid result?
5. What is the formula for correcting creatinine clearance for body surface area?
6. What additional data are needed to calculate a corrected creatinine clearance?
7. What is the corrected value for creatinine clearance for this patient?
8. What is the reference range for serum creatinine and creatinine clearance?

Answer

1. The creatinine clearance is calculated from the formula given in Answer #2. It is important to remember that only the urine and plasma creatinine values were directly measured by the technologist. The 24-hour creatinine clearance is calculated as follows:

$$\begin{aligned} C_{cr} &= \frac{UV}{P} \\ &= \frac{(900\ mg/L)(\frac{800\ mL}{24\ hrs})}{(14\ mg/L)(\frac{1440\ min}{L \cdot 24\ hours})} \\ &= 36\ mL/min \end{aligned} \tag{4.34.1}$$

2. No! The ca1culations are all correct, but the final result is obviously not appropriate for an individual whose serum creatinine is 14 mg/L. Since the creatinine measurements were probably accurate one must assume the urine volume of 800 mL did not reflect the actual 24-hour urine excretion. This supposition is supported by the very low urine output (approximately one-half to one-third that of a normal male) and by the total creatinine output.

   A proper 24-hour urine sample is one of the most difficult specimens to obtain. The laboratory should request a new 24-hour urine sample after consulting with the patient's nephrologist.

Answers to Questions to Consider

1. The units for creatinine clearance are mL/min. This is the theoretical equivalent amount of plasma from which creatinine is cleared by the kidneys per minute. See p 487, Chapter 26.
2. The equation for creatinine clearance described on Chapter 26 p 487 is:

$$\text{Creatinine clearance (mL/min)} = \frac{UV}{P} \tag{4.34.2}$$

   which requires the term "urine volume per minute" (V) as well as the urine (U) and plasma (P) creatinine concentrations. The additional information which is needed to perform the calculation is the time over which the sample was collected. This

allows the calculation of the rate of plasma clearance in mL/min.

The creatinine clearance is a way of estimating a patient's glomerular filtration rate (GFR). Many now argue (See Web site: www.kidney.org/professionals/...elineindex.cfm) that, given the difficulties and errors associated with a timed (i.e., a 24-hour urine collection) urine sample, that it is actually *better* to estimate the GFR directly from the measured serum creatinine value. One of the popular equations is the Cockcroft & Gault equation also listed on p 487).

3. The usual urine volume output for an adult is approximately 1.5 L per day (Chapter 26). The usual urinary output of creatinine per day is 1 g to 2 g for a healthy male (See Method Creatinine on CD-ROM).
4. The creatinine output is dependent upon a body's muscle mass and, of course, renal function. Thus normalizing the calculated creatinine clearance to an "average" body size is usually performed to correct for differences in muscle mass.
5. The formula for correction for body surface area is the calculated clearance (Cl) times the ratio of the ideal body surface (1.73 m^2)/patient body surface.

$$Corrected\ C_{cr} = Cl \times \frac{1.73\ m^2}{\text{Patient body surface}} \tag{4.34.3}$$

6. The additional data needed are the patient's height and weight. These will allow calculation of the body surface from the nomogram of Appendix I.
7. The calculation of the normalized value is as follows:

$$37\ mL/min \times \frac{1.73\ m^2}{2.30\ m^2} = 28\ mL/min \tag{4.34.4}$$

8. The reference range for uncorrected creatinine clearance is 97-137 mL/min. While the plasma creatinine level reference range is 6.4-10.4 mg/L. The creatinine level is only a little outside the reference range. See CREATININE metehod on CD-ROM.

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4.35: Amniotic Fluid Analysis - Fetal Maturity Studies

A medical technologist receives an amniotic fluid sample from the Labor and Delivery Unit requesting fetal maturity studies. These include a lecithin/sphingomyelin (L/S) ratio and phosphatidyl glycerol (PG) measurement by one and two-dimensional, thin-layer chromatography (TLC), respectively.

The technologist performs the TLC analysis and obtains an L/S ratio of 1:2 and a borderline positive result for PG (that is, the presence of a PG spot). The technologist is not satisfied with these results and reviews them with the laboratory's clinical chemist.

QUESTIONS

1. Why is the technologist doubting the accuracy of these results?
2. The technologist and the clinical chemist review the TLC plates and decide that there was excessive streaking of the analyte spots. The actual amniotic fluid sample is clear and uncolored. There was no visible pellet of cells following the centrifugation step. Taht is, there seems to be no blood contamination.

   The clinical chemist calls the patient's physician, who explains that the sample was obtained from the fluid draining from the patient's vagina. The clinical chemist asks the technologist to perform a "renal profile" (electrolytes, creatinine, urea, and glucose) on the sample. Why is the "renal profile" being performed on the sample?
3. The results of the "renal profile" are: Na 50, mmol/L; K, 10 mmol/L; glucose, 100 mg/L; urea, 10,000 mg/L; and creatinine, 1,320 mg/L. What do these results indicate about the nature of the sample?

Questions to Consider

1. What values for the L/S ratio are associated with fetal lung maturity and immaturity?
2. How is the detection of PG used to assess fetal lung maturity?
3. What are the major interferents in the TLC assay for the L/S ratio and PG?
4. What other fluids can contaminate an amniotic fluid sample?
5. Which fluid do you think the clinical chemist is most concerned about?
6. What effect would the contamination named in #5 have on the TLC pattern for the L/S ratio?

Answer

1. The technologist doubts the accuracy of these results because they are discordant: a very low L/S ratio but a borderline positive PG result.
2. The "renal profile" was ordered to determine whether its urea and creatinine composition was more like urine or amniotic fluid (more like serum levels, Chapter 26). This will indicate the degree of contamination with maternal urine.
3. The results of the renal profile indicate that the sample is heavily contaminated with maternal urine. The sample should be rejected as unacceptable, and a proper amniotic fluid sample requested.

Answers to Questions to Consider

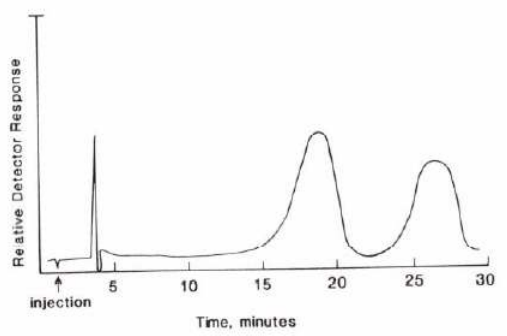
1. The TLC analysis for the L/S ratio is very imprecise and is subject to methodological and technologist-induced biases. However, L/S ratios greater than 2.0 are generally associated with mature fetal lungs, while L/S values less than 1.5 tend to be associated with fetal lung immaturity (Chapter 40, and L/S and PG Methods in CD-ROM).
2. The *presence* of PG is usually associated with mature fetal lungs, while the absence of PG is generally associated with fetal lung irrmaturity (Chapter 40 and L/S and PG Methods in CD-ROM). However, the test has a low sensitivity and a negative PG result does not necessarily indicate the absence of mature fetal lungs. The most clinically important results are concordant ones, that is, L/S > 2, positive PG; and L/S < 1.5, negative PG. These results indicate a high probability of mature and immature fetal lungs, respectively.
3. The primary interferents in the TLC assays are blood or meconium (fetal stool). The L/S ratio of blood is approximately 1.0 while that of meconium is greater than 2. So the presence of blood tends to lower the L/S ratio, while meconium tends to falsely increase the L/S ratio (Chapter 40and L/S and PG Methods in CD-ROM).
4. The fluids that can contaminate an amniotic fluid sample obtained by amniocentesis (Chapter 40) are blood (maternal of fetal) or, rarely, fetal urine from the fetal bladder. If the amniotic fluid sample is obtained from fluid leaking from the placental membranes and draining from the vagina, maternal urine is a possible contaminating fluid.
5. The clinical chemist is concerned about contamination from maternal urine.

6. If maternal urine contaminates the amniotic fluid to a great extent, streaky TLC chromatograms can be produced with poorly defined spots for lecithin, sphingomyelin, and PG.

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4.36: Chromatography II

A laboratory is attempting to establish an HPLC assay for a drug (compound X). The assay employs an internal standard (compound I) for calculating the concentration of the drug. The initial HPLC conditions are as follows: Column type, C18; column temperature, ambient; flow rate, 2 mL/min (at 2000 psi); mobile phase, 30% methanol in water; and column effluent, monitored at 260 nm. The initial chromatogram for the standards, in which the initial peak is the internal standard and the second peak is compound X, is as follows:



The elution times for I and X are considered unacceptably long that is, the k of the compounds are too large.

QUESTIONS

1. How can the elution time be reduced?
2. The clinical chemist suggests that the medical technologist decrease the polarity index of the mobile phase to 70% of the current value in order to decrease the retention times and decrease the capacity factor (k). How should the technologist approach this problem?
3. In order to obtain a mobile phase with a lower polarity index, the suggestion is made to prepare a mobile phase containing acetonitrile and chloroform. How can the technologist prepare the proper mobile phase with these new solvents?
4. The new chromatogram produced using the mobile phase with the reduced polarity index calculated in question gives a ks that are now acceptable, but the resolution is now unacceptable. The clinical chemist now suggests replacing the chloroform by a solvent with the same polarity index but with a different chemical reactivity. Why was this suggestion made?
5. The clinical chemist suggests preparing a new mobile phase consisting of a mixture of isopropanol and acetonitrile. What proportions of these two solvents should be used to maintain the same degree of polarity?

Questions to Consider

1. How would changes in the temperature of the column, polarity of the mobile phase, and flow rate affect the elution times from this column? How would changing these parameters affect the k' of the compounds?
2. If the chromatographic conditions remain the same but the length of the bonded phase chain is decreased from 18 carbons (C18) to 8 carbons (C8), how would the elution time of I and X change?
3. What is the polarity index of the currently constituted mobile phase?
4. What is the minimum polarity index that can be obtained with methanol and water?
5. Which combinations of chloroform and acetonitrile will give a polarity index approximately 70% that of the current mobile phase?
6. What is the relationship of the solvent group to the chemical reactivity of the solvents? To the polarity index? What factor in chromatography theory affects separations on the basis of differences in chemical reactivity?
7. Can solvents of the same polarity belong to different solvent groups?

Answer

1. In most cases, the most practical approach for reducing the elution time is to decrease, in the case of reversed-phase columns, the polarity of the mobile phase. Columns with different bonded phases may not be suitable for this analysis and may radically change the elution profile if used, and proper equipment for temperature equilibration of the column is rarely available in routine laboratories.

2. Since the lowest polarity index possible with this reagent pair is greater than that desired (that is 5.8, or 70% of 8.28), a different set of reagents will need to be examined by the technologist.
3. The final mobile phase will be 21.1% chloroform and 78.9% acetonitrite and will have a polarity index of 5.80. The initial approach the technologist will take is to change the polarity of the mobile phase using these new solvents according to the calculations described in questions 3 and 4. Decreasing the polarity by 70% is only an initial approximation. In actuality, a series of mobile phases with varying polarities, i.e. different proportions of strong and weak solvents, would be used to optimize retention time without totally decreasing resolution.
4. The suggestion to maintain solvent polarity but change the chemical nature of the mobile phase is based on the usual chromatography practice of first optimizing the elution times and then changing the selectivity factor of the mobile phase in order to optimize resolution of solute peaks. Now that the retention time is acceptable, the technologist needs to improve resolution by changing the chemistry of the mobile phase.
5. Since the polarity index of isopropanol and of chloroform are the same, and only their solvent group or chemical selectivity is different, the proportions of each in the mobile phase will remain the same, and the values calculated in question 4 can still be used:

$$\begin{aligned} \text{isopropanol (or chlorofom)} &= 21.1\% \\ \text{acetonitrile} &= 78.9\% \end{aligned} \tag{4.36.1}$$

Answers to Questions to Consider

1. Increasing the column temperature will decrease the retention time; decreasing the polarity of the mobile phase will decrease the retention time in this reversed-phase system, and increasing the flow rate will decrease the retention time (pp. 115-117, 133). Only changes in temperature and mobile phase polarity can change the k. Increasing column temperature and increasing the polarity of the mobile phase will decrease the k.
2. In reversed-phase HPLC, the chromatographic interaction is the partitioning of the salutes between the mobile phase and the bonded, apolar stationary phase. Shortening the length of the bonded hydrocarbon will increase the stationary phase's polarity, reduce the interactions between solute and stationary phase, and decrease retention time (p. 117-118).
3. Using the equation on p. 119, the polarity index can be calculated by summing the products of the volume fraction (ϕ) and polarity index (P') of each of the constituents of the mobile phase (from Table 5-3, p. 120) (see also internet sites such as: http://home.planet.nl/~skok/techniques/hplc/eluotropic_series_extended.html#3, http://www.lc-ms.com/lcsolvent.htm , http://www.jtbaker.com/conversion/solventphydata.htm?#polar):

$$\begin{aligned} P'\ combined &= \phi_{methanol} P'_{methanol} + \phi_{water} P'_{water} \\ &= 0.3(6.6) + 0.7(9.0) \\ &= 8.28 \end{aligned} \tag{4.36.2}$$

4. The least polar (strongest) mobile phase that can be obtained with methanol and water would employ only methanol. Therefore:

$$P = 1.0(6.6) = 6.6 \tag{4.36.3}$$

   is the lowest polarity index possible.
5. The final desired polarity index is 70% (8.28) or 5.80. Let x be the volume fraction of chloroform (polarity index, 4.3) and y be the volume fraction of acetonitrile (polarity index, 6.2). Thus:

$$5.80 = x(4.3) + y(6.2) \tag{4.36.4}$$

   Since x + y = 1 and y = 1- x,

$$\begin{aligned} 5.80 &= x(4.3) + (1-x)(6.2) \\ 5.80 &= 4.3x + 6.2 - 6.2x \\ -0.40 &= -1.9x \\ 0.211 &= x = \text{volume fraction of chloroform} \\ \textit{and } y &= 0.789 = \text{volume fraction of acetonitrile} \end{aligned} \tag{4.36.5}$$

   That is, in 100 mL of mobile phase, 21.1 will be chloroform and 78.9 mL will be acetonitrile.
6. The polarity index of a solvent describes the overall interactive forces of that chemical. Basically, as the polarity of a chemical increases, its polarity index increases. The type of attractive force or chemical reactivity of each solvent is described by its solvent group (see pp 119-120). Thus all the solvents of group 9(IX) exhibit very strong hydrogen bonding

and dipole properties while the solvents of group 0 have only temporary dipoles as their basis of interaction. There is no relationship between the polarity index of a solvent and its solvent group. The chromatographic factor that incorporates the concept of the solvent group is the alpha (α) or selectivity factor (p.115).

7. As mentioned in the previous answer, there is no relationship between polarity index and solvent group. Thus, both chloroform (polarity index, 4.3) and water (polarity index, 9.0) belong to the same solvent group [9(IX)].

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4.37: Chromatography III

An HPLC assay is being developed to measure the serum concentration of an active drug metabolite. The following information is known about this metabolite: Its molecular weight is 531 daltons; it, like the parent compound, has an aromatic hydroxyl group; and it has a hydroxyl group that the parent does not have. The compound strongly absorbs light at 254 nm. Since the hydroxyl group is found at a single position on the aromatic ring, there are no known structural isomers. Its therapeutic range is approximately 25-100 mg/L.

QUESTION

Which type of chromatography (adsorption, partition, ion-exchange, or molecular exclusion) should be used?

Questions to Consider

1. What are the principles governing each of the major classes of chromatography?
2. How do the low molecular weight of the compound and the lack of structural isomers affect the choice of a chromatographic mode?
3. Would ion exchange chromatography be useful?
4. What is the physicochemical basis of polarity?
5. What is the relationship between polarity and solvent strength in a normal phase chromatography system? In a reversed-phase chromatography system?

Answer

Therefore, the most appropriate chromatographic system would be reversed phase.

Answers to Questions to Consider

1. Adsorption chromatography in HPLC makes use of the interaction between polar functional groups on a compound and the polar groups on the solid surfaces of silica or alumina (p. 120).

   Partition chromatography makes use of differences in the solubility of a compound in two liquid phases: the mobile phase and the stationary phase (p. 122). The liquid in the stationary phase can be coated or chemically bonded to the stationary phase. It can be either apolar or polar in nature.

   Ion exchange (p 123) can only be used when the analyte contains an ionizable group. Separation depends upon the ionic attractions that exist between the counterions on the stationary phase and the analyte, and between the analyte and the mobile phase.
2. Using Fig. 6.5 (p. 136), one can make the following decisions. Since the molecular weight of the analyte is <1000 daltons, steric exclusion chromatography can be eliminated as a practical approach. The molecule cannot forn hydrogen bonds (phenol group) and is not known to have structural isomers. This allows one to rule out adsorption chromatography.
3. There is no ionizable group on this compound and therefore ion exchange chromatography would not be useful.
4. Polarity is a concept used to describe the overall interactions between molecules; in liquid chromatography, between solute and solvent molecules and between solute and stationary phase. The four major forces, described in Fig. 5-11 (p. 118), that are the basis of polar interactions all involve the attraction of charges, either induced or permanent. See internet sites: www.cem.msu.edu/~cem333/Week16.pdf, www.laballiance.com/la_info/support/hplc3.htm
5. Nomal phase chromatography employs a polar stationary phase. In this system, a strong solvent is one that can remove the most retained, i.e. the most polar, compounds. Therefore, solvent strength in normal phase chromatography increases with the degree of polarity of the mobile phase. In reversed-phase chromatography, the opposite is true. With very apolar stationary phases, the most non-polar compounds are retained, and solvent strength increases as the stationary phase becomes more nonpolar. In other words, solvent strength of the mobile phase is directly related to the polarity of the stationary phase (see pp. 120-121, 135-142). See the internet site in the previous

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4.38: Electrophoresis

A laboratory is setting up an electrophoretic method for identification of serum proteins; however, the appearance of the protein bands appears to be too diffuse. The technologist notes that the pH of the freshly prepared buffer is correct but a bit warm; the apparatus appears to be warm to the touch as well. One possibility is that the problem could be due to excess heating (Table 10-5, p. 213). The amount of heat generated by an electric current is related to the power (wattage) dissipated by the system. The relationship between wattage, current (i), and resistance (R) is:

$$w = i^2 R \tag{4.38.1}$$

The technologist has a meter which can read both voltage and amperage. During an electrophoretic run, these values are recorded as 100 volts and 0.01 amps. The power generated by the assay, according to the manufacturer, is not to exceed 0.5 watts.

QUESTIONS

1. Does an excess amount of power appear to be the problem?
2. After discussion with the clinical chemist, it is decided to reduce the power passing through the system. What approaches are available to do this?
3. After much discussion, it is decided that the length of time needed for the assay should be as short as possible in order to complete the assay conveniently in a working day. With this additional information, what is the best change to make in the assay system to correct the original problem?
4. The buffer solution in this assay contains 0.1 molar sodium barbital (Na Bar) and 0.01 molar magnesium phosphate ($MgPO_4$). How much would this buffer need to de diluted to reduce the power to 0.5 watts? What will be the ionic strength of the diluted buffer?

Questions to Consider

1. What is the relationship between voltage, amperage, and resistance?
2. What is the power at 100 volts and 0.01 amps?
3. What is the relationship between voltage, current, and power?
4. What is the relationship between ionic strength of an electrophoresis buffer, the current passing through it, and the temperature generated in the buffer?
5. How does changing the voltage affect the rate of migration (velocity) of molecules in an electrophoretic field?
6. What is ionic strength of a solution calculated? What is the ionic strength of the current buffer?

Answer

1. This amount of power, 1.0 watt, exceeds the manufacturer's specifications.
2. In order to decrease the power, either i or R must be decreased. From equation 10.3, if R is decreased at constant voltage, i will increase proportionately. However, power increases as the square of the value of 1 and only proportionately to R. Therefore, if R is decreased, there will be a net increase in power. Thus, to decrease power at constant voltage, R must be increased, causing a decrease in i. Alternatively, if R is held constant, decreasing the voltage will decrease i and thus reduce the power. If voltage is to remain constant, then R must be increased.
3. Simply decreasing voltage to reduce the power is not an acceptable solution in this particular case. The best solution is to increase resistance by decreasing ionic strength.
4. By diluting this buffer two-fold, the ionic strength will decrease by one half as will the conductivity, increasing the resistance by two-fold and decreasing current by one half (at a constant voltage):

$$\begin{aligned} R &= 2 \times 10,000\ ohms = 20,000\ ohms \\ i &= \frac{1}{2} \times 0.01\ amps = 0.005\ amps \end{aligned} \tag{4.38.2}$$

The wattage of this system is now.

$$\begin{aligned} W &= i^2 R \\ &= (0.005)^2 \times 20,000 = 0.5\ watts \end{aligned} \tag{4.38.3}$$

This will decrease the heat produced by the system and increase the sharpness of the bands, but time for analysis will remain the same.

Answers to Questions to Consider

1. The relationship between voltage, amperage, and resistance is:

$$V = R \times i \tag{4.38.4}$$

(Equation 10-3, p. 204).

2. In order to calculate wattage from the information given, the resistance must first be determined. Using Equation 10-3,

$$\begin{aligned} V &= Ri \\ R &= \frac{V}{i} \\ &= \frac{100\ volts}{0.01\ amps} \\ &= 10,000\ ohms \end{aligned} \tag{4.38.5}$$

Now, using the relationship between power, current, and resistance, W can be calculated as follows:

$$\begin{aligned} W &= i^2 R \\ &= (0.01\ amps)^2 (10,000\ ohms) \\ &= 1.0\ watt \end{aligned} \tag{4.38.6}$$

3. By combining the equations in Answer 2, it is seen that W = iV.
4. Ionic strength is proportional to current; the greater the ionic strength, the greater the current the solution is capable of passing at the same voltage. Increased current increases wattage and therefore results in more heat generated by the system. See p 210-211.
5. The rate of migration is directly related to the voltage. The time the electric field is allowed to act on the molecule is directly related to resolution. Thus, decreasing voltage will result in a proportional decrease in rate of migration and increase in the amount of time required to achieve the same degree of resolution, i.e. for all the molecules to travel the same distance.
6. The ionic strength (I) is related to the concentration of the ions and their charge by the following equation:

$$I = \frac{1}{2} C_1 Q_1^2 + \frac{1}{2} C_2 Q_2^2 + \ldots \frac{1}{2} C_N Q_N^2 \tag{4.38.7}$$

In this case

$$\begin{aligned} I &= \frac{1}{2} C_{Na} Q_{Na}^2 + \frac{1}{2} C_{Bar} Q_{Bar}^2 + \frac{1}{2} C_{Mg} Q_{Mg}^2 + \frac{1}{2} C_{PO_4} Q_{PO_4}^2 \\ &= \frac{1}{2}(0.1)(1)^2 + \frac{1}{2}(0.1)(1)^2 + \frac{1}{2}(0.01)(2)^2 + \frac{1}{2}(0.01)(2)^2 \\ &= 0.14\ molar \end{aligned} \tag{4.38.8}$$

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4.39: Immunochemistry

A laboratory is interested in measuring albumin in urine at concentrations > 10 mg/L (μg/mL). One approach being considered is to use an immunochemical assay.

QUESTIONS

1. Which immunoassay would be most suitable for the desired assay?
2. The laboratory currently has no analyzer to which reagent for urine albumin can be adapted. After reviewing the specifications of the available assays, the laboratory decides to concentrate the urine one-hundredfold using an Amicon B-15 concentrating apparatus. Which of the immunoassays is now most suitable for urinary albumin measurements?

Questions to Consider

1. Which immunoassays are sensitive enough to measure as little as 10 mg/L of antigen?
2. Which of these assays are able to quantitate urine albumin?
3. Which of these assay are technically complex or require dedicated, expensive instrumentation?
4. Which additional immunoassays are sensitive enough to quantitatively measure antigens present at a level of 1 mg/mL?

Answer

1. There is not one best assay for this situation. The available equipment and experience of the laboratory must be considered, and can often be limiting factors. The most critical factor may have to do with WHICH instruments are currently available in the laboratory.
2. Since the RID assay is the easier assay to set up and requires no expensive equipment, this is the most suitable technique for the desired assay. In addition, RID plates for measuring albumin are commercially available.

Answers to Questions to Consider

1. As shown in Table 12-1 (p. 242-243), assays sensitive enough to measure 10 mg/L include counterimmunoelectrophoresis, immunonephelometry, complement fixation, fluoroimmunoassay, ELISA, and competitive ELISA.
2. Counterimmunoelectrophoresis and complement fixation are semi-quantitative techniques (Table 12-1), and therefore are not useful for the desired assay. Imunonephelometry, turbidometry, immunometric assays using a wide variety of labels (such as fluoresence, chemiluminescence, enzymes), ELISA, and competitive ELISA are all quantitative assay methods. See also pp 239-240).
3. Automated immunometric or immunonephelometric analyzers are costly, dedicated instruments. The immunometric analyzers usually have fixed menus and may or may not have the ability to measure a new analyte. The nephelometric analyzers are more felixible with respect to adding a new test. Tubidimetric analysis for proteins in the mg/L range can be accomplished on the newer random access, 'chemistry' analyzers (see Chapter 16) and it is relatively easy to add a new test. Most technologists are familiar with these routine instruments.

   ELISA and competitive ELISA are assays that are less frequently used in non-specialty labroatories and the laboratory may not have sufficient experience with these techniques to be able to use them for the assay.
4. Radial immunodiffusion (RID) (p 225 and Figure 11-7) is suitable for the measurement of proteins in the range of 1 mg/mL. RID is relatively easy to do and has a coefficient of variation on the order of 10%, which is suitable for this type of measurement.

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4.40: Competitive Binding

A laboratory has been performing a competitive immunoassay for the quantitative measurement of hCG in urine. The physicians of the hospital have complained that the lower limit of sensitivity is not adequate for their needs. The assay employs 0.5 mL of urine, 0.5 mL of antibody reagent (diluted 1:10 from the stock), and 0.1 mL of labeled β-HCG. Under these conditions, approximately 70% of the label is bound to antibody in the absence of competing HCG (% B0 = 70%, see Table 13-9, p. 255).

QUESTION

How can the laboratory increase the sensitivity of the assay?

Questions to Consider

1. What factors affect the sensitivity of a competitive binding immunoassay?
2. How do proportional changes in the concentration of antibody and labeled ligand affect the sensitivity of the assay? The precision of the assay?
3. If only one reagent is diluted, what will be the effect on the binding and assay sensitivity?
4. If additional sample volume is added, how might this alter the reaction in an unacceptable manner?

Answer

The laboratory can increase the sensitivity of the assay by diluting antibody and labeled, antigen proportionately, or by adding more specimen.

Answers to Questions to Consider

1. The factors that affect sensitivity are the concentrations of labeled antigen, antibody, and antigen from sample, and the affinity of the antibody for binding the antigen (pp. 248, 251-252).
2. Decreasing the concentrations of both labeled antigen (or ligand) and antibody proportionately usually changes the binding equation equilibrium (Eq. 13-3 and p. 248)

$$Ab + Ag^* \leftrightarrow AbAg^* \tag{4.40.1}$$

by shifting the reaction to the left, i.e. dissociation with consequently less total binding.

In this circumstance, the percent labeled antigen bound at zero exogenous ligand (B_0) will become significantly smaller with lower concentrations of Ab and labeled Ag. In this case, less exogenous, unlabeled Ag is required to displace the bound labeled Ag and give 50% binding. This shifts the binding curve to the left, increasing the sensitivity of the assay. The upper limit of linearity will also become less and the lower limit will be extended to a new lower range. Usually, the assay becomes less precise, and there will be an increase in the standard deviation.

3. Usually it is not possible to increase or decrease the concentration of only one of the two reactants, antibody or labeled ligand, without making the reaction less sensitive. The antigen and antibody are usually titered to be at equivalence. Increasing the concentration of labeled antigen in relationship to the amount of antibody present in the reaction results in the need for a greater amount of unlabeled ligand to yield the same amount of displacement, and the percent bound labeled ligand will decrease in proportion to the excess added. Decreasing the amount of labeled antigen or increasing the amount of antibody results in available binding sites remaining on the antibody. Added unlabeled antigen will bind to these available sites rather than displace bound, labeled Ag; that is, no displacement. This will shift the binding curve to the right. In either circumstance there is a decrease in sensitivity.
4. Addition of a larger volume of sample to the reaction mixture would theoretically increase the sensitivity of the assay (see #2 above). However, substances such as urine can adversely affect the matrix of the reaction by changing pH, salt concentrations, etc. Therefore, it is not always possible to increase sensitivity by increasing the amount of test sample.

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4.41: Methods Evaluation

A new procedure is being introduced in your laboratory to replace one in current use. The new assay is considered to be more accurate based upon literature reports as well as survey data (i.e. CAP and AACC surveys). The clinicians who will most likely utilize this assay have suggested that at a concentration of the analyte (Xc) of 1000 mg/L, i.e. a medical decision level, they would like a precision of 75 mg/L (E_A) since, at changes of this magnitude, they will begin to treat the patient. The upper limit of the reference range of this analyte is 0.9 gm/L and you would like a range of linearity up to 5 gm/L. A series of experiments were run to validate the method.

Experiment 1. The following data were obtained on aqueous standards:

Concentration in mg/L	Absorbance Units
400	0.075
800	0.148
1200	0.227
2000	0.370
4000	0.650
6000	0.950

QUESTIONS

1. What is the linearity of this assay? If the linearity is unacceptable to you, how can the assay be modified to increase the linearity?
2. Experiment 2. A quality control pool was analyzed 26 times within a single run. The calculated standard deviation (SD or STM, see p 346, Chapter 19 or p 422, Chapter 22) was 28 mg/L at a mean analyte value of 1000 mg/L. Is the within-run precision acceptable at a 95% confidence limit?
3. Without doing a calculation, do you consider the between-run and within-run data to be consistent? If so, why? If not, why not?
4. Experiment 3. A clear non-hemolyzed serum pool ("ZERO") is spiked with Intralipid and a stock hemolysate to give a series of samples with increasing concentration of interferent. The samples are analyzed as six time replicates with the following results:

Sample	Mean (mg/L)	Standard Deviation (mg/L)	Difference from ZERO (mg/L)
hemolysate, Intralipid	950	30	
Slightly hemolyzed	928	29	-22
Moderately hemolyzed	897	31	-53
Grossly hemolyzed	827	28	-153

Sample	Mean (mg/L)	Standard Deviation (mg/L)	Difference from ZERO (mg/L)
Slightly turbid	24	15	
Moderately turbid	983	37	33
Grossly turbid	1025	45	75
Slightly icteric	937	29	-13
Moderately icteric	968	30	18
Grossly icteric	952	34	2

Do any of these substances cause a significant interference? If so, at what level does significant interference occur?

5. Experiment 4. Fifty patient samples are analyzed by the current glucose method and the data (in mg/L) are given below. All these specimens will then be analyzed by the current method in a correlation experiment.

   720, 1000, 430, 1900, 450, 550, 520, 800, 4100, 880, 630, 405, 500, 460, 420, 304, 400, 599, 563, 338, 445, 1167, 2700, 470, 425, 575, 820, 500, 640, 450, 538, 419, 495, 360, 955, 470, 378, 745, 1355, 401, 525, 705, 355, 480, 359, 437, 900, 446, 410, 525

   Should all these samples be used? If so, why? If not, why not?
6. What further steps should be taken to increase the confidence of the correlation data?

Question to Consider

1. How can one determine the linearity of the assay?
2. How is the total random error of the assay determined and evaluated?
3. The same sample is analyzed on 20 successive days, and the between-run precision is calculated to be 26 mg/L. Do the between-run precision data meet your criteria for error at a 95% confidence limit?
4. How can one determine the extent of an interference?

Answer

1. The linearity of this assay does NOT meet the expected value of 5 g/L and therefore is unacceptable. To increase the linearity, decrease the sample size or increase the total volume of the reaction mixture.
2. $$\begin{aligned} RE &= 4\ S_{TM} \\ &= 4(28\ mg/L) \\ &= 112\ mg/L \end{aligned} \tag{4.41.1}$$

   This calculated point estimate random error is greater than the allowable error of 75 mg/L and therefore the point estimate of the random error is not acceptable. The 95% confidence limit of the acceptability of this error is derived by calculating the 5% and 95% interval around the S_{TM} from a one-sided confidence limit table and again taking two standard deviations around these values (see example, p 422, Chapter 22).

   $$\begin{aligned} S_{TM1} &= S_{TM}(A\ 0.05) \\ &= 28.0\ mg/L(0.8149) \\ &= 22.8\ mg/L \end{aligned} \tag{4.41.2}$$

   $$\begin{aligned} S_{TMu} &= S_{TM}(A\ 0.95) \\ &= 28.0\ mg/L(1.308) \\ &= 36.6\ mg/L \end{aligned} \tag{4.41.3}$$

   $$\begin{aligned} RE_1 &= 4\ S_{TM1} \\ &= 4(22.8\ mg/L) \\ &= 91.2\ mg/L \end{aligned} \tag{4.41.4}$$

   $$\begin{aligned} RE_u &= 4\ S_{TMu} \\ &= 4(36.6\ mg/L) \\ &= 146.4\ mg/L \end{aligned} \tag{4.41.5}$$

   Both the upper and lower limits of the RE values are greater than the desired E_A and therefore one is 95% confident that the estimated error is greater than the allowable limits.
3. No! The within-run component of the random error should be smaller than the between-day error component since there are less variables to introduce error within a single run than over a many-day period. Remember, the between-day error includes the within-run component. Therefore, one should look at this data with a critical eye and assume that one of the data sets is incorrect and should be repeated. Since the calcualted estimates of random error exceed teh desired limits, the laboratory either need to speak with the clinicians about revising the limit or else speak with the manufacturer of the assay to discuss ways to improve the assay.

4. Hemolysis constant error due to interference by hemolysis exceeds the acceptable limit of 75 mg/L with the gross hemolysis sample, and therefore grossly hemolyzed samples would be unacceptable for analysis. The remaining question requires determination of a confidence limit for acceptance of the slightly hemolyzed and moderately hemolyzed samples. These can be calculated using a confidence limits (p. 422-425):

$$\begin{aligned} CE_U &= CE + \frac{ts}{\sqrt{N}} \\ &= 53\ mg/L + \frac{2.089(31\ mg/L)}{\sqrt{6}} \\ &= 79\ mg/L \end{aligned} \tag{4.41.6}$$

$$\begin{aligned} CE_1 &= CE - \frac{ts}{\sqrt{N}} \\ &= 53\ mg/L - \frac{2.089(31\ mg/L)}{\sqrt{6}} \\ &= 27\ mg/L \end{aligned} \tag{4.41.7}$$

Moderate hemolysis conclusion: Since the CE_u exceeds the acceptable limits but the CE_1 does not, one is not 95% confident that the moderately hemolyzed samples do not cause significant interference. The question of sample acceptance cannot be statistically answered at this point. The question may be resolved by repeating the experiment with a larger number of analyses or on clinical grounds.

Slight hemolysis conclusion: One can accept slightly hemolyzed samples with a 95% confidence limit that the error is within the allowable limits (75 mg/L).

Turbidity: Grossly turbid samples are obviously unacceptable as the point error introduced is at the limit of acceptable error (75 mg/L). For moderate turbidity, the calculation is as follows:

$$\begin{aligned} CE_u &= CE + ts \\ &= 33\ mg/L + \frac{2.089(37\ mg/L)}{\sqrt{6}} \\ &= 64.6\ mg/L \end{aligned} \tag{4.41.8}$$

Since the upper limit of error is within the acceptable error limit, one can be 95% confident that the error introduced by moderate turbidity is acceptable.

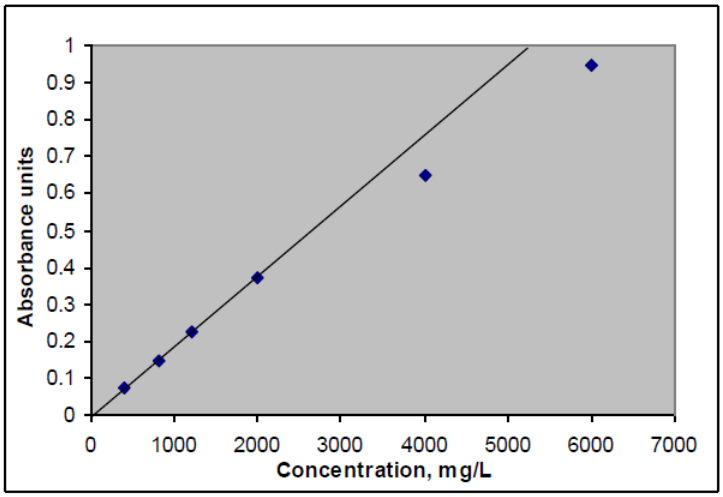
Slight turbidity. Same conclusion as above for slight hemolysis.

5. No! When collecting sample for this type of experiment it is important to include samples from many types of patients (see pp 414-415) and with analyte levels over as wide a range as possible (see p 416). That is, the samples should not be choosen at random, but purposely picked with these criteria in mind. The bulk of the data presented for this experiment falls below the upper limit of the reference range (900 mg/L) and does not include enough patient samples above this point. Insufficient data points within the entire dynamic working range can result in incorrect regression analysis including a falsely low correlation coefficient (r, see p 416).
6. Go back and pick out a number of specimens whose values fall between 1000 and 5000 mg/L. One approach may be to divide the desired range of sample over the linear range of the assays, that is into perhaps 8 to 10 groups. In this case, if the linear range were up to 5000 mg/L, one might divide it into groups of 500 mg/L. Thus, one would choose samples in the range of 0-500, 501-1000, 1001-1500, etc. for analysis by the new method. Use of 10 samples in each of these groups would give an ideal range of data for analysis. If fewer samples are readily available in one or more groups, this must be taken into consideration when analyzing the data.

Answers to Questions to Consider

1. There are two ways of determining the linearity of an assay. One can perform a linear regression analysis (Chapters 19, pp 357-361, and 22, pp 415-418). Usually a regression coefficient ("r") of > 0.999 indicates a linear relationship. However, it is easy to be mislead by the linear regression analysis (see p 416), and one should always plot and visually inspect the data, looking for a deviation from a straight line.

The "r" value for the data by regression analysis was 0.9987, a fairly high value that would suggest good linearity. However, a plot the actual results obtained from the analysis vs. the expected results of the standards suggests a possible problem.



The region of the curve that deviates from the straight line of the initial data points is the concentration at which the method loses its linear response; that is, between 2000 to 4000 mg/L. One might want to redo the experiment with some points between 2 and 4 mg/L to get a more specific estimate of the upper limit of linearity.

2. The point estimate of the random error (RE) is calculated by taking two standard deviations around the mean SD (Chapter 22): RE = 1.96 (STM). It is suggested (Table 22-4, p 421) that acceptable RE be: $4S_{TM} < E_A$. To determine the 95% confidence limit about this point estimate of random error, use the equations found on p 419, Chapter 22):

$$S_{TMl} = S_{TM}(A\ 0.05) S_{TMu} = S_{TM}(A\ 0.95). \tag{4.41.9}$$

3. The point estimate of a constant error ('bias') is simply the DIFFERENCE between the spiked and unspiked ('ZERO') samples. The confidence limits of the point estimate is calculated as shown for the example on p 422-423, Chapter 22.
4. The point estimate of error exceeds the allowable limit of error (75 mg/L) and one would have to assume that the new method has an unacceptable bias compared to the older method. However, the currently used method is not a reference method and therefore one cannot assume that the bias between the methods is an accurate criteria for rejection of this method. One would need additional information concerning the two methods in which both are compared to an acceptable reference method. At this point, one can accept the method on other grounds.

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4.42: “Discrepant Results”

An Emergency Room (ER) physician complains about a series of discrepant hCG results on an ER patient. To determine if a young (17 years old) patient is pregnant, a urine sample had been sent to the Chemistry laboratory for a Stat. qualitative hCG analysis. The results were reported as *negative*.

Convinced that this patient really is pregnant, the physician sends a serum sample to the laboratory almost five hours later for this patient for a Rule/out Ectopic panel (qualitative hCG, followed by, if necessary, quantitative hCG and progesterone analyses. The results of these analyses are: Qualatative hCG, positive; Quantitative hCG, 7051 mIU/mL. Really confused, the ER physician orders another urine sample to be sent for a repeat qualitative hCG test; the result for this sample is positive.

The ER physician calls the laboratory director and asks for some clarification of the discrepant results.

QUESTIONS

1. What steps do you think that the supervisor should take to resolve the problem?
2. What additional analyses can be quickly performed to determine the origin of the two urine samples?

Questions to consider

1. What methods are used for qualitative hCG assays?
2. Do the urine and serum qualitative hCG assays employ different techniques?
3. What are causes of false positive and false negative hCG results?

Answer

1. The laboratory supervisor uses the following process to investigate the samples: 1) Retrieves all three samples and checks both the bar-coded and Addressograph labels to ensure that a positive identification can be made. All the labels correctly have the patient’s name. 2) Has all three analyses repeated. The repeat results agree with the original results.

   The supervisor and laboratory director believe that the basis for the discrepant results is a mislabeled sample. Since results from the serum sample and one of the urine samples agree, they believe that one of the urine samples is not from the patient and has been mislabeled. They take the sample to another laboratory to perform additional analyses.
2. One easy test to perform is a dipstick urinalysis (See Urinalysis methods in CD-ROM) on both samples and see if there are any obvious differences. In addition, one could analyze the samples for creatinine, protein, or any other common urine constituent.

The supervisor choses to perform a dipstick analysis. The significant results of the dipstick analyses are as follows:

Initial (negative for hCG) urine sample

Ketones: very large

Specific gravity: 1.030

pH: 5.0

Appearance: clear, yellow

Second (positive for hCG) urine sample

Ketones: negative

Specific gravity: 1.015

pH: 7.5

Appearance: moderately turbid, yellow

CONCLUSION: The two urine samples are chemically and visually quite different samples, most likely coming from two patients with very different problems. One must assume that the first urine sample is the incorrect, mislabeled one because (1) the second urine sample and the serum results agree and (2) it is unusual for the laboratory to mislabel several specimens from

the same patient taken at different times. These results are communicated to the ER physician, who fully accepts the explanation.

Answers to Questions to Consider

1. Solid-phase ‘sandwich assays are universally employed for qualitative hCG assays, usually using an enzyme to develop the visual results. See methods for hCG in CD-ROM.
2. Modern qualitative hCG assays do not differ at all; usually the same kit, using the same technology, is used for both serum and urine sample types.See methods for hCG in CD-ROM.
3. Serum: False positive results may be seen in patients with compromised renal function; the hCG molecule and its degraded core fragments are filtered at the glomerulus and found in urine. Renal failure may result in these molecules being retained in blood.

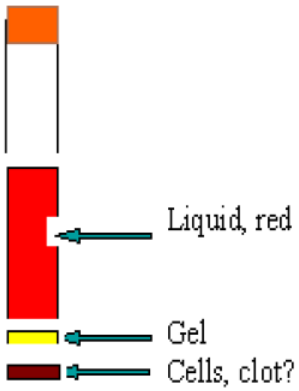
   Urine: A concentrated, morning sample will have approximately the same level of hCG as will serum. However, false negative results may be seen in dilute urine samples, and such samples are the most frequent cause of discrepant results between urine and serum samples taken at approximately the same time of the day. In fact, a negative urine result does not exclude a pregnancy with the same confidence as does a serum hCG result. In addition, postmenopausal women can excrete low quantities of hCG in urine.

   Human anti-mouse antibodies (HAMAs) can cause false positive or false negative resutls in immuoassays, including the ones used for qualitative hCG analysis. See p 262 in textbook. See hCG methods in CD-ROM.

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4.43: Possible Sample Contamination

A technologist working on a chemistry analyzer is reviewing the results of the analysis for a basic metabolic profile; one sample has a very low total calcium of 5.4 mg/dL. The technologist performs a delta check and determines that there has been no previous sample on this patient. The technologist looks at the sample and sees the following.



According to the label on the tube, the tube for this sample was an evacuated phlebotomy tube containing a gel. After a further review of the patient's laboratory data in the laboratory information system (LIS), the technologist reports the result as a 'critical value", but adds the following comment to the result: "Possible contaminating material; send a repeat sample."

QUESTION

Why did the technologist make this comment?

Questions to Consider

1. Is there anything unusual about how the centrifuged sample looked?
2. What other types of samples might give this appearance?
3. What other information do you think the technologist found in the LIS?

Answer

The technologist was properly concerned that the very low result for total serum calcium was not correct because the sample was either not a serum sample or was grossly contaminated with another fluid. The result was reported, but with a warning to the physician that something was amiss.

Answers to Questions to Consider

1. The usual proportion of blood cells to serum, that is, the hematocrit, is approximately 28-40% (see Chapter 3). This sample obviously has a much lower proportion of blood cells, in this case the clot, to serum. There is obviously something different about this sample.
2. There are other types of body fluids that might not have the usual proportion of blood cells that might also be drawn in a gel-barrier type of tube. For instance, the laboratory often receives an extravascular body fluid that is being investigated to determine if it is a transudate or exudate (see Chapter 41). Another unusual body fluid could be a synovial fluid (also see Chapter 41). Body fluids usually will contain some blood cells, but usually much fewer than what is associated with a serum sample. While synovial fluid is usually clear but viscous, it may be contaminated with blood. In all these cases, because the sample may contain blood and need to be properly processed to yield a clean sample for analysis, the sample is drawn into the usual serum-separator tube (see chapter 3).
3. The technologist first looked up other laboratory data obtained on the day this sample was obtained (see chapter 18). Upon noting that the hematocrit on that day was 35%, the technologist knew that this was probably not a normal blood sample, or if it was, it was grossly contaminated with some other fluid (such as, IV fluid; see chapter 3). The technologist also looked up information on the phlebotomy of this sample, but no explanation could be found.

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4.44: “Quantity Not Sufficient - QNS”

A technologist is working in the sample intake/processing area of the clinical chemistry laboratory. The technologist notices that one sample tube is labeled, but has no blood in it. The doctor is requesting a TSH analysis. One technologist suggests performing the TSH analysis on another sample. Another technologist suggests simply adding the comment, “QNS”.

QUESTION

1. What steps should the technologist take to resolve the problem?
2. The technologist looks in the LIS at the records for that patient and sees that a sample for glycosylated hemoglobin analysis was drawn on the same clinic visit, but several hours after the first sample. The technologist also speaks with the ordering physician who indicates that the sample was not time-dependent. Can the glycosylated hemoglobin sample be used to replace the missing sample?

Questions to Consider

1. Should you attempt to find another sample?
2. How could you determine if another sample were available?
3. What type of tube is a glycosylated hemoglobin drawn in?

Answer

1. The technologist should not QNS the test until it is determined if another sample can be used. By checking in the LIS the technologist can determine if another is available. The technologist must also determine if the original sample was a time-dependent sample.
2. The technologist must then determine if the method in use for measuring TSH can use a sample treated either with heparin or with EDTA. If either type of sample can be used, then the glycosylated hemoglobin sample can be used.

Answers to Questions to Consider

1. The short answer is yes, but the longer answer is to take care when doing so. Very often a sample from a patient cannot be easily redrawn. If the patient is being seen as an outpatient (the usual situation) then the patient must make an additional clinic visit, which could be costly in terms of time (perhaps a loss of a work day) and money (loss of work, babysitter, etc.). So trying to find another sample to replace the missing one can have a very large impact on the patient’s life. On the other hand, if the sample is a challenge test (e.g., glucose, TRH, dexamethasone) or if the result is time-dependent because it is subject to diurnal variation (e.g., early morning cortisol, see page 69), or is affected by specific lifestyle factors (e.g., meals, see page 69); then a sample drawn at another time might not be useful.
2. With a laboratory information system (LIS) now available in every laboratory, it is usually very simple to determine if another sample might be available, somewhere. See Chapter 16, p 323.
3. All glycosylated hemoglobin samples are anticoagulated, either with heparin or with EDTA. The technologist has to determine what type of tube the glycosylated hemoglobin was drawn in.

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4.45: Cocaine Abuse

A 36 y/o Caucasian women gives birth to a premature female child on October 21st. The mother has been enrolled in a methadone clinic. The day before the birth the women had a urine DAU screen with the following results.

October 20th, mother's urine sample

- Barbiturates: negative (below detection limits)
- Benzodiazepines: negative (below detection limits)
- Cocaine: positive
- Methadone: positive
- Opiates: positive

Two days and then again at 4 days after her birth, the baby is given urine toxicology screens with the following results.

October 23rd,newborn's first urine sample

- Barbiturates: negative (below detection limits)
- Benzodiazepines: negative (below detection limits)
- Cocaine: negative (below detection limits)
- Methadone: negative (below detection limits)
- Opiates: negative (below detection limits)

October 25th,newborn's second urine sample

- Barbiturates: negative (below detection limits)
- Benzodiazepines: negative (below detection limits)
- Cocaine: positive
- Methadone: negative (below detection limits)
- Opiates: negative (below detection limits)

QUESTION

What explanation can be given for these results?

Questions to Consider

1. What is the clearance rate for cocaine?
2. Does this explanation (to question #1) the initial negative cocaine result for the newborn?
3. What could cause the baby's second urine to be positive for cocaine? Should the laboratory do any additional work on this sample?

Answer

As an enrollee in a Methadone Clinic program the mother was obviously at risk for taking drugs during the pregnancy. Premature birth often accompanies such behavior. Most likely the mother was frightened about her upcoming delivery and took a dose of cocaine 1 to 2 days prior to delivery. The fact that the breast-fed infant had a positive urine for cocaine metabolite 4 days after being born indicates that the mother was still taking cocaine. The baby was separated from its mother for the approximately the first 12 hours of life. Breast feeding was initiated at ~12 hours after birth, and ~36 hours before the second urine sample. See Chapter 51.

Answers to Questions to Consider

1. The serum half-life for cocaine is ~1 hour; clearance should be complete in ~5 hours. Certainly by 24-36 hours after a single dose of cocaine an individual’s urine should be negative for cocaine metabolite. See Chapter 51 and Toxicology Screen on CD-ROM.
2. Yes because the newborn had cleared it's dose of cocaine from it's system by the time her urine sample was obtained.
3. The most likely explanations for the newborn's second sample to become positive are laboratory error or a repeat dosing of the baby. The laboratory repeated the DAU analysis for the second sample; the results repeated. How could the baby have

received an additional dosing? Oral route, possibly breast feeding.

A second urine sample was obtained from the mother and run for DAU analysis. The results were:

October 27th

- Barbiturates: negative (below detection limits)
- Benzodiazepines: negative (below detection limits)
- Cocaine: positive
- Methadone: positive
- Opiates: negative

The mother was still taking cocaine. The baby was separated form its mother and placed in foster care.

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4.46: Blood Gases

A technologist is working in the blood-gas area of a laboratory in an acute-care hospital. While the technologist is at the instrument, a physician brings a blood-gas sample in a syringe at room temperature (not in ice-water). The laboratory test ordering slip indicates that the sample was drawn about 30 minutes previously. When the physician hands the syringe to the technologist, the technologist notices that the syringe is half-filled with air.

QUESTIONS

1. What should you, the technologist, do with the blood-gas sample?
2. The physician demands that the results of the blood-gas analysis be placed in the LIS. What do you tell the physician?

Questions to Consider

1. How might the 30 minutes delay in bringing the sample, not-iced, to the laboratory change the blood-gas results?
2. How might the large amount of air in the syringe affect the accuracy of the blood-gas results?
3. What is the technologist's responsibility for rejecting a sample that does not appear to have been collected properly?
4. Is there a way of providing the physician with information without compromising the laboratory's policy?
5. Many blood-gas analyzers enable the measurement of electrolytes in addition to traditional blood-gas parameters. If the physician wants only the data on electrolytes placed in the LIS, should you agree to this request? Why?

Answer

1. Further delays in analyzing the sample will only cause further deterioration of the sample; perform the blood-gas analysis immediately, do not immediately report the results, and discuss the value of the results later. See chapter 3 and 25.
2. Tell the physician after analysis that because of an unsatisfactory sample that does not comply with the laboratory's sample policy, the blood-gas results will not be placed in the patient's records. However, the technologist could report Na and Cl results comfortably.

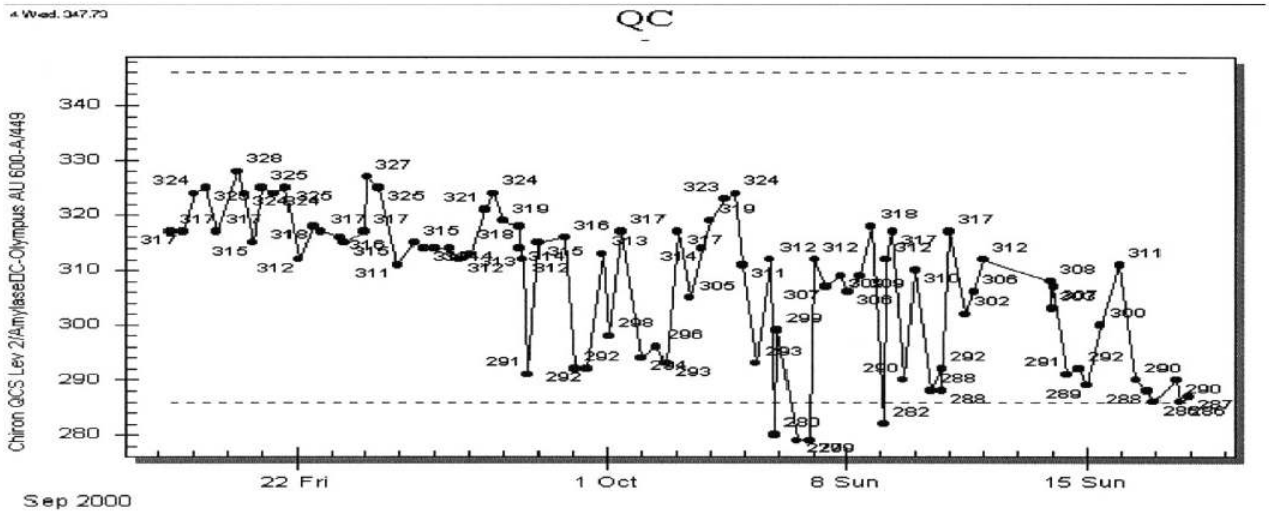
Answers to Questions to Consider

1. Because of the effect of the delay in bringing the non-iced sample to the laboratory the results of a blood-gas analysis will not be reported to the physician because the results will be inaccurate. The blood cells will be able to continue metabolism, producing CO_2 and consuming O_2 and glucose. The result will be falsely decreased PO_2 and glucose levels and falsely increased PCO_2. See chapter 3, 25, and Blood-gas methods on CD-ROM.
2. Air in the syringe will affect the accuracy of blood-gas results by providing oxygen to blood and decreasing the PCO_2 levels and thereby increasing the pH. Overall, the most likely effects on the blood-gas sample will be those listed in Answer #1, with unreliable PCO_2, pH, PO_2, and glucose levels. See chapter 3, 25, and Blood-gas methods (Specimen) on CD-ROM.
3. The laboratory's policy for sample rejection must always be followed. This is a medical-legal responsibility that cannot be ignored. See chapter 2 and 3. If the physician is upset enough, he can speak with the laboratory's supervisor or medical director.
4. It is best that the physician obtain a new sample. However, the technologist can show the results to the physician, but still not place the questionable results in the LIS or patient's chart. The physician will most likely not act on this information because it will not be part of the official records and the physician could be held responsible if any problem occurred with the patient.
5. I would agree to provide the electrolyte data because the effect of changes in PCO_2 and PO levels will most likely not affect the concentration of SODIUM or CHLORIDE. However, potassium levels may increase if the blood pH decreases too much. So the safest response would be to only report Na and Cl, or inform the physician of the possible problems with the remaining results.

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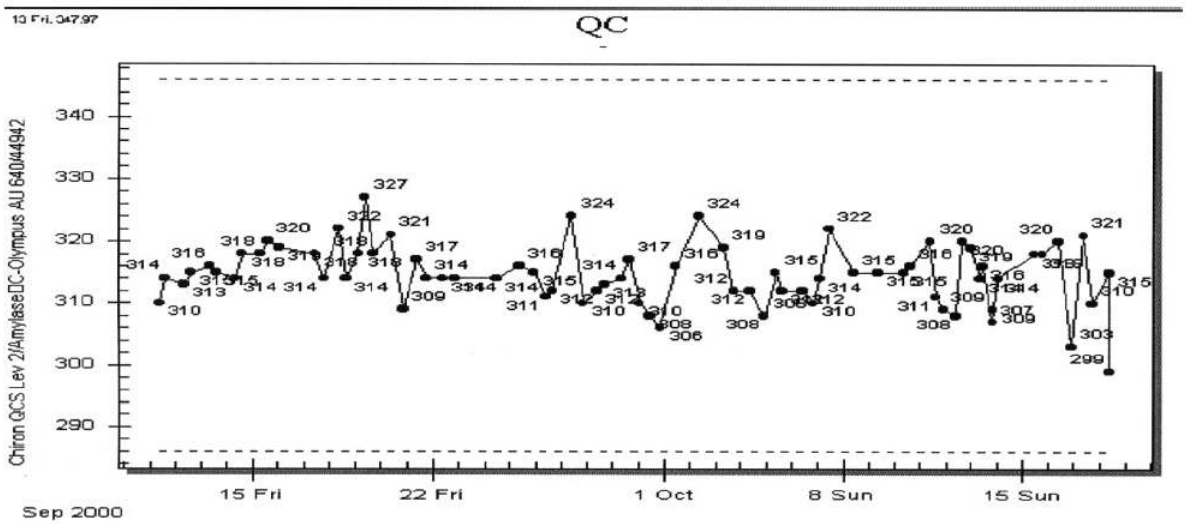
4.47: Quality Control Advanced

A quality control supervisor is reviewing routine quality control (QC) data for the three, random access, multi-channel chemistry analyzer in the laboratory. The supervisor notices the data in the following figures for amylaseon one of the instruments. She becomes very concerned and approached the supervisor responsible for the analyzer.



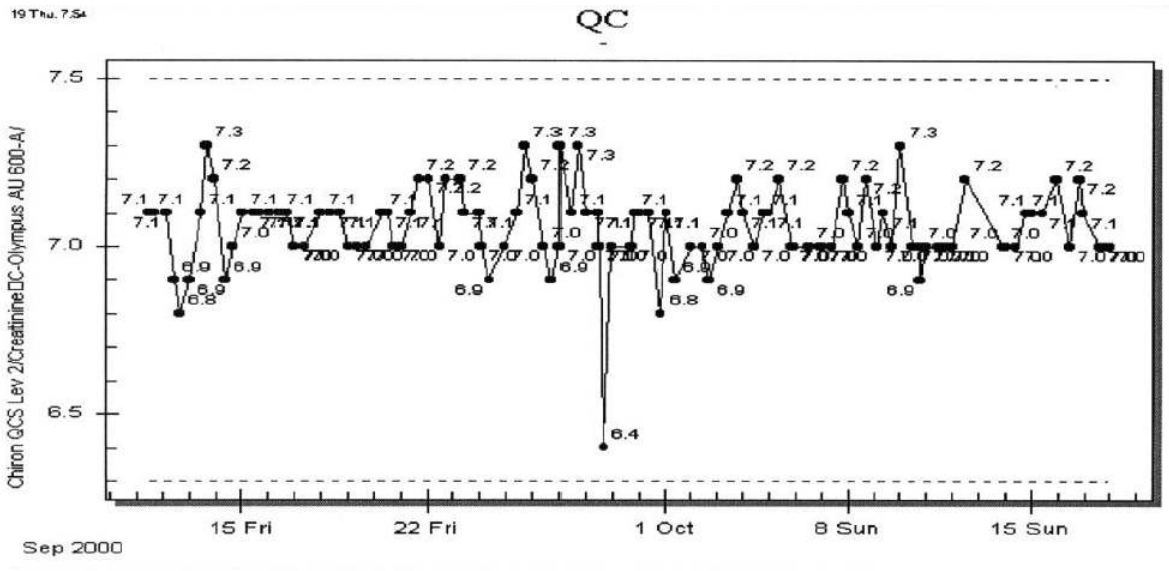
QUESTIONS

1. What do you see in the figure that might have raised the supervisor's concern?
2. The QC supervisor brings the problem to the laboratory director to determine if this is an out-of-control situation requiring possible suspension of this test. The director decides that it is not, but asks that the QC supervisor document the problem (see page 394 of the textbook) and investigate it. The QC supervisor and the instrument supervisor review the QC data and discuss its possible cause. What easy steps could be taken to help determine the possible cause of the significant decrease in precision?
3. The supervisor reviews the QC for amylase on the other two instruments and sees the following data.



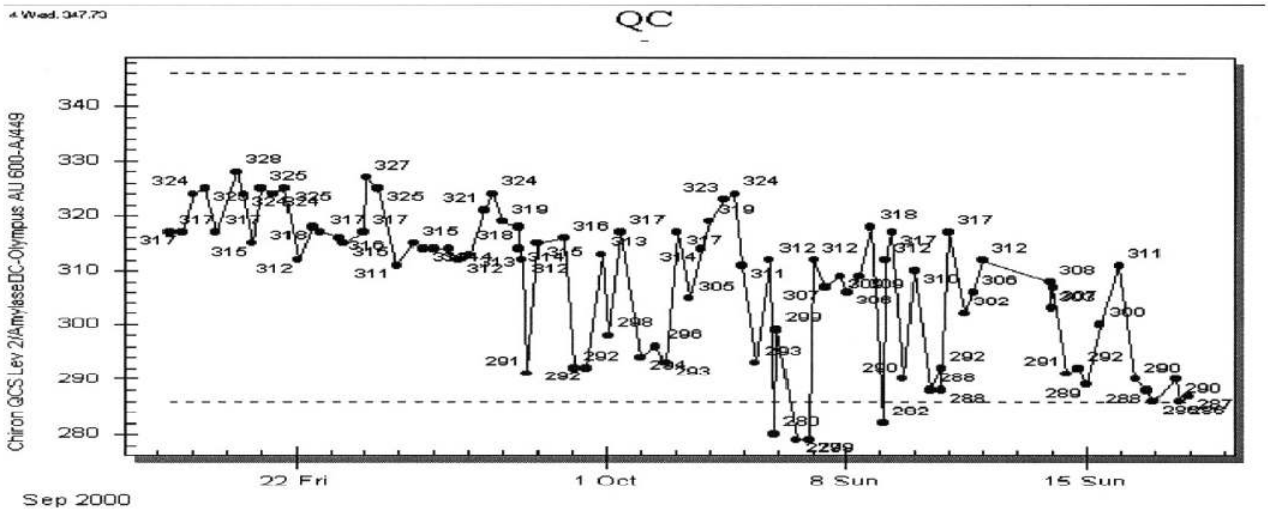
This pattern is pretty much the case for both levels of control material and for both of the other instruments. The supervisor begins to focus on the original instrument by reviewing the QC results for other tests. What test parameters would be of interest to the QC supervisor?

4. The QC supervisor sees a mixed pattern of QC results. As one example, see the following figure for creatinine on the original instrument.



The supervisor is surprised to find that another assay on the same instrument shows excellent, consistent precision (except for one result on September 28th). Reviewing the QC data for the other assays, the pattern of mixed results is that some assays show good precision while others show varying diminishment in precision. One pattern begins to emerge: the degree of decreased precision seems to be related to the sample volume of the assays.

The supervisor believes that the likely cause of the problem is with the sample delivery system. The QC supervisor and the instrument supervisor perform basic maintenance on the instrument, concentrating on the sample delivery system. Changing the syringe and tubing does not seem to improve matters, so the company is called and the instrument is placed out of service. The company's field engineer finds a faulty valve, which is a key component for maintaining precise delivery of sample. After repair, analysis continues. After a week, the QC for amylase is again reviewed; seen below.



The QC results seem to have settled down by the 18th, but now it is obvious that there was a shift of the QC to lower results towards the end of September. The supervisor makes additional investigations. Based upon the previous discussion what do you think these additional investigations included?

Questions to Consider

1. What type of changes might one see in quality control data over time?
2. What factors might cause a changes from a stable pattern of QC data to a shift or trend?
3. What might cause a significant decrease in precision?
4. What different types of assays might be found on an automated chemistry analyzer?
5. What test parameters or factors might result in changes in instrument performance?

There was no new lot of amylase reagent introduced into the laboratory for the past 6 months. However, the data from the PT samples indicate that the instrument in question has consistently lower results than the other two instruments, which are achieving the desired target value. Based on this information, the enzyme factor (see below) on the questionable instrument is adjusted to provide results consistent with the amylase results from the other analyzers. QC results improve and remain stable. All these investigations are documented.

ENZYME FACTOR

The basic calculation of enzyme activity (See chapter 54 and p 39, Chapter 1) is:

$$\frac{U}{L} = \frac{\Delta\text{Absorbance / min.} \times V_{total} \times 10^6\ (\mu\text{moles/mole})}{\epsilon \times V_{sample} \times \text{optical path length}} \tag{4.47.1}$$

For automated instruments the V_{total}, V_{sample}, the optical path length, extinction coefficient (ϵ) are constants for every analysis. These constant can be grouped together and the above equation rearranged to read:

$$\frac{U}{L} = F \times \Delta\text{Absorbance / min.} \tag{4.47.2}$$

where

$$F = \frac{V_{total} \times 10^6\ (\mu\text{moles/mole})}{\epsilon \times V_{sample} \times \text{optical path length}} \tag{4.47.3}$$

Answer

1. The supervisor notices a change from stable QC results beginning about September 29th. The pattern seen above seems to be neither a shift nor a trend, but reflects a likely significant decrease in precision, possible associated with a shift to lower values. The new precision is determined to be significantly worse that the usual standard deviation (USD, see pp 385 and 390).
2. Since there are two additional instruments of the same type, the supervisor could review the QC of the other two instruments. If the same pattern is seen for the other instruments, one could look for the things that are common among all three instruments, that is, reagent and QC. If the QC for the other instruments is acceptable, one needs to focus on the specifics of the first instrument, that is, a mechanical problem.
3. As discussed above, the type of assay, how the assay's signal is generated, and sample/reagent volume might be important factors in the review.
4. The additional investigations included determining if there was a reagent lot change at the end of September and the analysis of frozen proficiency testing (PT) samples to be used as an accuracy check (see p 398). The old PT samples are analyzed on all three instruments.

Answers to Questions to Consider

1. A stable pattern of data, distributed around the mean, target value; shifts; trends; and increases in the dispersion of the data about the target value. A QC shift typically shows the data with approximately the same precision, but changing acutely to a higher or lower value. A QC trend typically shows the data gradually increasing or decreasing over a number of days or weeks. See pp 391-392.
2. A shift might be caused by the use of a new lot of reagent or calibrator, which produce significantly different test results than did the previous lot. A slower change, or trend, of change might be caused by deterioration of some reagent over time . See p 394.
3. Deterioration of QC, deterioration of reagent, or a mechanical problem in the instrument.
4. Types of assays generally found on automated chemistry analyzers include colorimetric and turbidimetric (see chapters 4 and 12), EMIT (see chapter 13), and potentiometric (see chapter 15) assays; see chapter 16 for overview. Each of the different types of assays is affected by instrument deterioration to different extents and for different reasons. Deterioration of the light source might affect to a greater extent those assays that measure the smallest changes of absorbance while potentiometric assays (Na, K, Cl) would not be affected at all.
5. One test factor, as suggested in Question 4, is the technique that is used to generate a measurable signal. Each type of signal-generating procedure may affect peformace in different ways. Test parameters of sample and reagent volumes are very important; tests employing smaller volumes have an increased susceptibility to mechanical deterioration of the volume delivery system.

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4.48: Sample Analysis III, Quantity Not Sufficient (QNS)

A Chemistry technologist is performing sample analysis on a high-throughput analyzer. The printout for one sample indicates that the requested analysis was not performed because of insufficient sample volume. He is about to report the result as QNS when a supervisor intervenes, and asks to see the sample. Once she has seen the sample container, she asks the technologist to view the LIS with her.

QUESTIONS

1. What should a technologist's response be to a possible QNS?
2. The sample in question in the Chemistry laboratory is in a "bullet" tube and the tests requested for the newborn patient included total bilirubin and electrolytes. In addition, a hepatitis B surface antigen test, ordered on a separate tube but drawn at the same time as the Chemistry tests, is performed in the Serology section. Upon investigation ~5 μL of sample can be recovered from the Serology sample. This is taken and added to the ~10 μL of serum obtained after re-centrifuging the sample. What steps can now be taken to produce results?
3. The results of the analysis on the diluted sample are that total bilirubin, AST and alkaline phosphatase all produced values within the reportable range and were verified in the LIS. Analysis of ALT and albumin did not yield results in the reportable range, and had to be reported as QNS. Can different sample types be used to replace the missing sample? For example, if the required test was for a serum cortisol.

Questions to Consider

1. Why would the supervisor want to see the sample container?
2. What information can be gained on this sample by going to the records in the LIS?
3. What is the value of attempting to find another sample?
4. What additional information is needed before proceeding?
5. What type of tube is normally used for a hepatic profile? Does the methodology permit the use of other sample types?
6. Are certain tests time-dependent?

Answer

1. One of the ongoing problems that technologists are faced with is the problem of having insufficient sample (Quantity Not Sufficient, QNS) to perform a requested analysis. There are several approaches to the problem that a technologist should be aware of.
    1. Try to anticipate a QNS before attempting to perform an analysis. You don't want to magnify a small problem of a possible insufficient sample to a real QNS. LOOK at the sample before analysis and if you believe that there may be insufficient sample, deal with the problem now (see points 2 & 3 below). EVERY time you are faced with 'bullets" for babies you must make this decision.
    2. If you believe you might have insufficient sample for the requested analyses before analysis or, if after analysis you are faced with a possible QNS situation, look in the LIS for the previous results on this patient. If previous results are elevated, you can probably perform a dilution that will permit analysis. 'Bullet' samples are examples of samples that are often associated with insufficient sample. Very often they are drawn from newborns for bilirubin analysis, which is almost always highly elevated and can take a 10x dilution, or TSH, which are also usually highly elevated.
    3. If more than one test is being ordered and you are trying to deal with a likely insufficient sample, you must make your best decision as to WHICH test you will try to save and report a value. Again, bilirubin for babies is a good choice because the neonatologists always want a bilirubin. If multiple thyroid tests are ordered, try and run the TSH. Electrolytes usually cannot be run on a dilution, but they require very little sample and probably could be salvaged and run.
    4. When in doubt, check with a supervisor. It is critical that you have a good understanding of both the sample volumes AND the 'dead' volumes required for each instrument and assay. But please remember a QNS report indicates some degree of failure on the laboratory's part to deal with a problem and should be used as infrequently as possible.
2. The dead volume of the analyzer is 25 μL; the sample volume for total bilirubin is 10 μL; the sample volume for AST, alkaline phosphatase, and ALT are 5 μL each; and the sample volume for albumin is 3 μL. Since it is believed that the bilirubin assay is most critical for this newborn, the technologist concentrates on this test. Since the total bilirubin was 15 mg/dL the previous day, the supervisor suggests that the technologist take the available sample and prepare a 7-fold dilution,

that is 10 μL of sample + 60 μL of 0.15 M NaCl. This will allow measurement of the entire hepatic profile with the possible exception of albumin.

3. Very often a sample drawn at another time could replace a sample drawn at a different time. If the nature of the test is that it does NOT change rapidly with time, then replacement is possible, for example, creatinine. If the analyte tends to change rapidly (e.g., glucose) or is time-dependent (such as cortisol), then replacement is not possible.

   In this case, the technologist should speak with the physician to see if the cortisol was specifically a timed sample. If it was, then the alternative sample could NOT be used to replace the missing sample.

Answers to Questions to Consider

1. By looking at the sample container the supervisor can obtain information regarding the possibility of taking steps that would result in the reporting of a quantitative result and not just reporting "QNS". If the sample container were an aliquot tube ("pour-off") this would indicate that the primary phlebotomy tube should be available elsewhere in the laboratory and might be processed for additional sample. In addition, if the sample container was a "bullet" tube (a Microtainer tube), this would indicate that the sample was most likely drawn from an infant, whose laboratory results might be highly abnormal for the tests requested.
2. The patient's records in the LIS may provide information as to how the sample may be saved and a report of "QNS" avoided. By looking at past test results one can determine if any of the current tests requested were previously highly abnormal. For example, recently highly elevated enzyme or bilirubin results would indicate that a small volume of remaining sample could be diluted sufficiently to provide enough sample to perform one or more of the requested tests (see Case History #21 for questions of proper dilution). If after dilution there is still insufficient sample, then the technologist will need to make a judgment as to which tests can be performed (see Case History #22 for questions of prioritizing test analyses). A supervisor and possibly the ordering physician should help in this process.

   Another reason for looking into the LIS records is to see if another sample drawn for this patient may be present in the Chemistry laboratory or some other Pathology laboratory. For example, the Serology section usually requires serum samples and additional sample may be available.
3. Very often a patient sample cannot be easily re-drawn. If the patient is being seen as an outpatient (the usual situation), then the patient must schedule an additional clinic visit, which could be costly in terms of time (perhaps a loss of a work day) and money (loss of work, babysitter, etc). So trying to find another sample to replace the missing one can have a very large impact on the patient's life.
4. The technologist need to know: (a) the dead volume of the analyzer (b) the tests that can be run on the diluted sample (c) the sample volume for each test to be run (d) the reportable range for each test to be run (upper and lower limits)
5. Routine chemistry tests almost always use a serum sample. But, many tests permit the use of heparin and even EDTA anti-coagulated tubes. IF a sample had been sent to the hematology laboratory for a CBC at about the same time as the Chemistry sample, it is possible that that sample could be centrifuged and the plasma used to measure some portion of the hepatic profile tests.
6. Yes, the value of many tests is time dependent. Examples include twenty-four hour urine collections (many analytes), samples drawn after a fast (e.g., glucose), samples drawn after a challenge (e.g., glucose, cortisol, or TRH stimulation test), samples for analytes that have a specific circadian rhythm (e.g., cortisol). For cortisol, the morning (AM) cortisol is usually higher than the afternoon (PM) sample, and the ratio of the AM cortisol/PM cortisol can be used to determine if a patient has possible adrenal gland dysfunction.

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4.49: Drugs of Abuse in Urine

A 21 y/o Caucasian female has been enrolled in a court-ordered methadone clinic for the past 6 months, two months ago she gave birth to a healthy infant. At the time of delivery, she was drug-free. A routine sample of urine was obtained at her regular methadone clinic visit. The results are:

- OPIATES: POSITIVE
- METHADONE: POSITIVE
- BENZODIAZEPINES: NEGATIVE
- COCAINE: POSITIVE
- BARBITURATES: NEGATIVE
- ALCOHOL: NEGATIVE
- CREATININE: 230 MG/DL

Her physician calls the laboratory director, explaining that the patient is extremely upset, denying taking any drug except methadone. She is concerned that, on the basis of these results the judge assigned to her case will remove her infant from her care.

QUESTION

What steps should you take to investigate the problem and what would you tell the physician?

At her next regular clinic visit she produces another urine.

- OPIATES: NEGATIVE
- METHADONE: POSITIVE
- BENZODIAZEPINES: NEGATIVE
- COCAINE: NEGATIVE
- BARBITURATES: NEGATIVE
- ALCOHOL: NEGATIVE
- CREATININE: <10 MG/DL

The laboratory director and the patient's physician review the results and agree to send the sample for GC/MS confirmation.

QUESTION

Why was the sample sent for GC/MS confirmation?

Questions to Consider

1. What types of techniques are most frequently used to screen for drugs of abuse in urine (DAU)?
2. What type(s) of interferences are most likely to be encountered with DAU assays?
3. Does the patient's physiological state have any impact on your response to the physician?
4. Are there ways that patients undergoing DAU screening might try to confound the laboratory results?

Answer

1. You first review the laboratory data to be sure that there were no obvious problems with the assay at the time of analysis. If the sample is still available in the laboratory, repeat the analysis. If the initial analysis appeared to be without problems and if the repeat analyses yielded similar results to the initial results, let the physician know that likelihood that all the positive results are incorrect is very small. Describe the assay to the physician, explaining the known interferences and cross-reactivities. Suggest the possibility of a mislabeled sample and suggest that the physician order a repeat analysis on a new sample as soon as possible.
2. Because of the low creatinine and the socio-medical nature of the case, the sample was sent for GC/MS analysis. The immeasurable creatinine suggests that the sample was not urine or is a doctored urine sample 'spiked' with methadone. GC/MS analysis finds only the parent drug of methadone present; methadone metabolites are NOT present, which strongly suggests that this was some sort of doctored sample.

Answers to Questions to Consider

1. The most common assays for the detection of drugs in urine are chromatographic (HPLC, GC, and GC/MS; see chapter 6, 7, and 8 respectively) and immunologic (see chapter 13). The greatest percentages of screening tests are performed by competitive binding immunoassays (see chapters 13 and 51).
2. For the all assay types the major problem is one of compounds that closely resemble the drug to be measured interfering in the assay. For chromatographic assays the interfering compounds have physical/solubility properties that closely resemble the drug and so closely- or co-chromatograph with the drug. For immunoassays, the interfering compounds cross-react with the antibody used in the assay. In all cases, the interference causes false positive results. Endogenous antibodies interfering in monoclonal-based assays, HAMAs, can also be a problem.
3. Yes. One of the major problems with people addicted to drugs is that they are in a state of denial, denial of their problem and denial of the steps needed to correct their addiction (see chapter 52). This often leads to addicts who are clearly on drugs to deny that they could possibly have a positive DAU test and complaining of inaccurate drug analysis. Of course there is always the possibility of a mislabeled sample.
4. Yes, the following is just a sampling of such attempts to force the laboratory to produce falsely negative results
    - Adding a compound that might destroy the assay, such as by inhibiting an enzyme
    - Obtaining (often purchasing) urine known to be negative
    - Diluting their urine with toilet water
    - Substituting a liquid that looks like urine but is not.

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4.50: Glucosemeter Recall

A nurse manager is told by one of the clinic nurses that sometimes an icon, which reads “temp”, has appeared on one of the new glucose meters. The nurse manager calls the Chemistry laboratory’s POCT supervisor. The nurse states that the meter is being used, as usual, at room temperature. The nurse did not hold it in her hand long enough for the meter to warm up and, as far as she knows, it was not placed on ice or in a refrigerator. The presence of icons was not discussed in training. The icon has disappeared and has not been seen again in the last day or so. The POCT supervisor’s initial reaction is to tell the nurse manager that if the icon does not appear again, she can ignore the incident.

The POCT supervisor then discusses this with the Lab’s technical supervisor. They agree on another course of action.

QUESTIONS

1. What might the new course of action be?
2. The POCT supervisor calls the instrument manufacturer to determine what are the implications of this icon, that is, does it indicate a problem that will affect the test results? Also have any other complaints about this icon been made to the company? The POCT supervisor is told that the icon indicates temperature problems within the meter and may affect the results. She is informed the problem seems to occur in a small number of cases and that all meters with this icon will be replaced. The company is investigating this and other complaints further. What should the supervisor’s response be now?

Question to Consider

1. What are the recommended responses when irresolvable instrumentation problems are encountered?
2. Should a backup plan exist for instrument downtime?

Answer

1. The lab Director and the supervisor read the detailed device product description to find the meaning of the icon. Unsatisfied with the information provided, they place a high priority call to the manufacturer’s customer service department for assistance. 344A backup plan for instrument downtime must be written for every instrument (see QC chapter, pp 394-395), including POCT instruments. The plan should indicate WHEN an instrument or test is determined to be not usable and WHAT is the alternative testing procedure.

   The immediate response should be to find out how widespread the icon problem is in the institution, that is,how many meters are affected, and to have the affected meters removed from service and replaced if possible by non-affected meters. If non-affected meters are not available, then the central laboratory must be used for the testing. In addition, the laboratory staff must educate nurses and others using instrument about the meaning of the icon.
2. Instruments with the icon problem are removed from service. However, some are kept in critical areas because of the demonstrated need to maintain this testing. Nurses are asked NOT to respond to any result with the icon, but instead send a sample to the central laboratory for analysis.

Answers to Questions to Consider

1. The recommended laboratory response (see p 394) to an unsolvable instrument failure includes the following sequential steps: reading the product information, calling the manufacturer’s ‘hot line’ or technical staff for additional help, and requesting manufacturer service.
2. The nurses and physicians in the critical care areas are told that because of the imprecision associated with the affected meters, ANY result within $\pm$25% of a value that might require patient intervention, especially the introduction of insulin, must be verified in the central laboratory, preferably by glucose analyses performed on the Stat. blood-gas instruments. Another valid option would be to immediately remove the meters and procede with plans to replace them with another brand.

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4.51: Glucosemeter Nonlinearity

Glucose meters are purchased and instrument linearity studies are performed on each instrument using the manufacturer's calibrators (see p 306, Chapter 17). Five calibrators range from about 40 to 460 mg/dL. The initial calibration meets the manufacturer's specifications. After 6 months of use the glucometers are recalibrated and linearity re-validated. The POCT technologist brings to the attention of the laboratory supervisor that the linearity is now only good from 40 to 350 mg/dL. Above 350 mg/dL the values look like they are leveling off.

QUESTIONS

1. Is this a severe problem that requires intervention? Why?
2. The Laboratory Director makes the decision that values above 400 mg/dL are not reliable for proper patient care because of curve nonlinearity and this is an out-of-control situation. The problem is too widespread to use the few replacement instruments on hand. What are the options to improve the quality of results?

Questions to Consider

1. What steps should be taken to determine the severity of the linearity?
2. What factors must be considered when considering the response to an out-of-control situation?

Answer

1. This is an important issue for two reasons. First, patient values often extend to 600 mg/dL or greater and results may be clinically important when diagnosing certain hyperglycemic states, such as hyperosmolar coma. Second, nurses or doctors will adjust insulin dosage using the fingerstick glucose values. Thus meters that will produce falsely low readings when values are above 400 mg/dL, may cause erroneously low doses of insulin for therapy. See p 593-594, chapter 32.
2. Immediately, instruct all users of the meters that all glucose values above 350 mg/dL must be repeated on an automated chemistry instrument in the central laboratory before any treatment is initiated or dosage of medication adjusted. Medium-term; ask the manufacturer for total replacement of all devices. Long-term, consider replacement of the meters.

Answers to Questions to Consider

1. The determination must be made if the reduced linearity is an out-of-control situation that requires either restrictions placed on the use of the glucose meters or cessation of use of the instruments (see p 395, Chapter 21). If this is not an out-of-control situation, the laboratory must determine the immediate steps needed to correct the decreased linearity. If this is an out-of-control situation (see pp 394-396, Chapter 21), the laboratory director needs to determine the extent of response, that is restrictions on use versus total cessation of use. The laboratory should obtain glucose standards (or calibrators with higher values from a different manufacturer) and use these to determine the linear instrument response.
2. One need to determine the extent of the problem, in this case determining how far off the meters are from correct values and how many meters are affected with this poor linear response. What would be the clinical impact if the values >350 mg/dL are too falsely low by the amount determined? If it could adversely impact on patient care, this will increase the likelihood of an-out-of-control decision.

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4.52: Discrepant Urinalysis Values

The technologist is responding to a stat urinalysis request on a patient in the Emergency Unit with abdominal pain. The technologist observes a positive finding for blood. The emergency physician vehemently insists that the result is incorrect. The urinalysis blood test is negative in their hands. The technologist repeats the results and gets the same positive answer.

QUESTIONS

1. What are the possible reasons, or sources of error, for the discrepant results?
2. The technologist checks for mislabeling and finds this has not obviously occurred. The laboratory specimen does not appear grossly contaminated. The technologist opens a new batch of test urine dipsticks and gets the same answer. The technologist calls the physician back and asks how the ER determined that the patient's urine was negative for blood; the answer is that they have urine dipsticks and performed their own test. What possible errors can occur when performing urine dipstick testing?
3. What are some of the ways to resolve this dispute?

The supervisor went down to the Emergency Room to investigate how testing was being performed there. This ER physician was performing tests in an unsanctioned manner. The strips were 3 years out of date and were found in an un-capped storage container, exposed to the air. When the ER physician repeated the urine analysis with fresh strips; the results were the same as those obtained by the technologist. The ER physician was satisfied with the laboratory's resolution of the problem and discarded the outdated strips. The laboratory took steps to place this type of testing under the umbrella of Point-of-Care Testing (POCT) that is controlled by the central laboratory. See Chapter 17, especially pages 308-309.

Answer

1. There are several possible reasons: Specimen mix-up (that is, mislabeling of samples), contamination, or error of analysis by either the ER Physician or the laboratory technologist.
2. The reagents incorporated into the dipstick's reaction pads can deteriorate if the strips are not properly stored in a dry, relatively cool place. The deterioration is time dependent, and so the strips can only be used within the expiration dates indicated on the container (manufacturer's and post-opening of the reagent bottle). Also, each test on the tesat strip must be properly timed for optimal results. Performing QC on a regular basis can validate both the strips and technique. See Chapter 57 and Urinalysis Infobase on the CD-ROM.
3. The laboratory POCT supervisor needs to visit the ER and see how the testing is being performed. The expiration date on the ER team's test strips needs to be verified as well as the manner of storage. In addition, the supervisor can take the patient's specimen and a fresh set of test strips to the ER and ask that the ER test the urine with both the lab's and the ER's strips.

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