



Clinical Microscopy

Medical Technology (Southwestern University PHINMA)



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CLINICAL MICROSCOPY

A collection of transes on Clinical Microscopy

Bachelor of Science
in Medical Technology

BACKGROUND

Safety and Quality Assessment

OUTLINE

- I. Introduction
 - A. Safety
 - B. Safety Hazards
 - C. Chain of Infection
 - D. Personal Protective Equipment
 - E. Handwashing Steps
- II. Biologic Hazards
 - A. Disposal of Biological Waste
- III. Sharp Hazards
- IV. Chemical Hazards
 - A. Material Safety Data Sheets (MSDS)
- V. Radioactive Hazards
- VI. Electrical Hazards
- VII. Fire / Explosive Hazard
- VIII. Physical Hazards
- IX. Hazardous Material Classification
- X. Quality Assessment
 - A. Preexamination Variables
 - B. Examination Variables
 - C. Postexamination Variables

INTRODUCTION

A. Safety

- Mandated by
 - Centers for Disease Control and Prevention (CDC)
 - Occupational Safety and Health Administration (OSHA) Clinical and Laboratory Standards Institute (CLSI) - provides the guidelines for writing procedures and policies

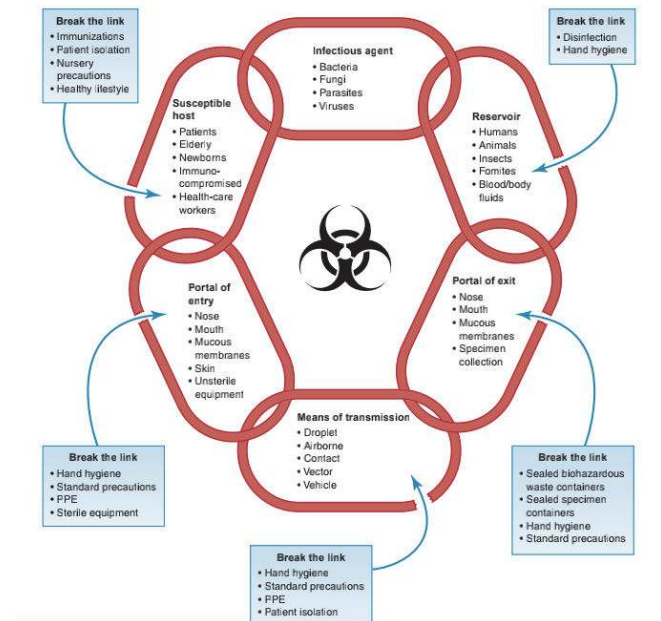
Hazard vs Risk

- Hazard - anything that has a potential to cause harm
- Risk - the likelihood that hazard will occur

B. Safety Hazards

- Biological – infectious agents such as parasitic, viral, fungal and bacterial
- Sharps – sharps
- Chemical – e.g. preservatives and reagents
- Radioactive – radioisotopes
- Electrical – grounded wires
- Fire / explosive
- Physical – even patients can cause hazard

C. Chain of Infection



- Source
- Method of transmission – where the intervention happens
 - Direct contact – a unprotected host touches a patient or any specimen or any contaminated specimen or reservoir
 - Airborne – in relation of dried aerosol particles circulating to air currents attached to air particles
 - Droplet – host inhales material from the reservoir or uncapped centrifuge tubes, or tubes are being allocated; has certain distance
 - Vehicle – ingestion of contaminated substance through food or water
 - Vector – from an animal or insect bite
- Susceptible host
 - important for completing the chain of infection
 - essential for the spread of infection



Biohazard symbol

- ∴ Universal Precaution – treat all biological samples as potentially infectious

D. Personal Protective Equipment

How to put on PPE



NOTE:

Personal protective equipments are comprised of gloves, gowns, eye protection and medical masks. Items that are not PPEs are the caps for the hair and closed footwear, which are used only for the comfort of HCWs and to avoid accidents with sharp objects.

SOURCE: Infection prevention and control of epidemic- and pandemic-prone acute respiratory diseases in health care. Interim Guidelines. Geneva, World Health Organization, 2007 (WHO/CDS/EPB/2007.0).

E. Handwashing Steps

1. Wet hands with warm water
2. Apply antimicrobial soap
3. Rub to form a lather, create friction, and loosen debris
4. Thoroughly clean between fingers for at least 15 seconds
5. Rinse hand in a downward position
6. Dry with a paper towel
7. Turn off faucets with clean paper towel to prevent recontamination
 - ∴ nowadays, the standard is a liquid soap
 - ∴ hand contact – primary source of transmission

BIOLOGIC HAZARDS

A. Disposal of Biological Waste

- All biological waste must be placed in appropriate containers labelled with the biohazard symbol
- Disinfection: 1:5 or 1:10 dilution of sodium hypochlorite (10%)
 - e.g. 1 L of 1:10 sodium hypochlorite
 - 900 mL of H₂O
 - 100 mL of sodium hypochlorite
 - biological waste and sodium hypochlorite can't be disposed in the sink
 - urine can be disposed in the sink
 - sodium hypochlorite will disintegrate if exposed to sunlight

SHARP HAZARDS

- Be disposed in puncture resistant containers
 - ∴ by standard, we do not recap
 - ∴ by standard, in ETS we also dispose the adapter
 - ∴ by standard, tourniquet is per patient

CHEMICAL HAZARDS

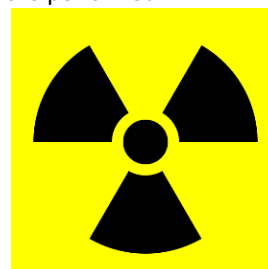
- Skin contact: flush with large amount of water
- Proper chemical handling
- Chemical labelling

A. Material Safety Data Sheets (MSDS)

- Information contained in MSDS
 - Physical and chemical character
 - Fire and explosion potential
 - Reactivity potential
 - Health hazards
 - Methods of safe handling

RADIOACTIVE HAZARDS

- Radioisotopes are performed



ELECTRICAL HAZARDS

- All electrical equipment must be grounded with three-pronged plugs
- Never touch the person or the equipment involved (in electrical accidents)

FIRE / EXPLOSIVE HAZARD

- Rescue
- Alarm
- Contain
- Extinguish
 - or evacuate depends on the situation
- Pull pin
- Aim
- Squeeze
- Sweep

A. Types of Fires

- A - wood, paper, clothing
- B - flammable organic chemicals
- C - electrical
- D - combustible metals

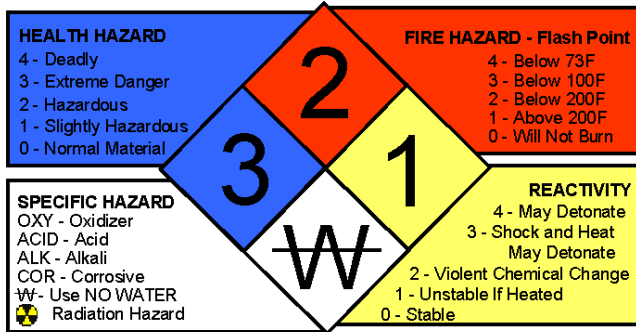
B. Types of fire extinguisher

- A - water
- B - dry chemicals, CO₂, foam or halon
- C - dry chemicals, CO₂ or halon
 - ∴ no D. because to stop the fire from combustible metal, you need to use sand or dry powders

PHYSICAL HAZARDS

- General precautions inside the lab
 - No running!
 - No playing!

HAZARDOUS MATERIAL CLASSIFICATION



QUALITY ASSESSMENT

A. Preexamination Variables

- turnaround time (TAT) - amount of time required from the point at which a test is ordered by the health-care provider until the results are reported to the health-care provider

Specimen Collection and Handling

- the actual date and time of specimen collection
- whether the specimen was refrigerated before transporting
- the time the specimen was received in the laboratory and the time the test was performed
- tests requested,
- an area for specific instructions that might affect the results of the analysis
- patient identification information

B. Examination Variables

Reagents

- Distilled or deionized water or clinical laboratory reagent water (CLRW)
- properly labeled with the date of preparation or opening, purchase and received date, expiration date, and appropriate safety information

Instrumentation and Equipment

- calibrated on each shift against
- deionized water (1.000)
- known control - 5% saline (1.022 ± 0.001) or 9% sucrose (1.034 ± 0.001)
- Calibration of centrifuges - performed every 3 months
- preventive maintenance (PM) - prepared as mandated by the JC or CAP guidelines

Testing Procedure

- Detailed, concise testing instructions are written in a step-by-step manner

Quality Control

- performed to ensure that acceptable standards are met during the process of patient testing
- Control results must be recorded in a log

a. External Quality Control

- accuracy - ability to obtain the expected result

- precision - ability to obtain the same result on the same specimen
- uses a mechanical or electrical device in place of a liquid QC specimen
- control mean - average of all data points
- standard deviation (SD) - measurement statistic that describes the average distance each data point in a normal distribution is from the mean
- coefficient of variation (CV) - SD expressed as a percentage of the mean
- Control ranges - determined by setting confidence limits that are within ±2 SD or ±3 SD of the mean; plotted on Levy Jennings control charts
- trend - gradual changing in the mean in one direction
- shift - abrupt change in the mean

b. Internal Quality Control

- internal monitoring systems built in to the test system
- internal or procedural controls

c. Proficiency Testing (External Quality Assessment)

- mandated by Clinical Laboratory Improvement Amendments (CLIA)
- testing of unknown samples received from an outside agency
- provides unbiased validation of the quality of patient test results

Personnel and Facilities

- quality control is only as good as the personnel performing
- and monitoring it

C. Postexamination Variables

Reporting Results

- Electronic transmission - most common method for reporting results
- delta check - QA procedure that compares a patient's test results with the previous results
- telephone - frequently used to transmit results of stat tests and critical values

Interpreting Results

- All known interfering substances should be listed for evaluation of patient test data

Review Questions

- In the urinalysis laboratory the primary source in the chain of infection would be:
 - Patients
 - Needlesticks
 - Specimens
 - Biohazardous waste
- The best way to break the chain of infection is:
 - Hand sanitizing
 - Personal protective equipment
 - Aerosol prevention
 - Decontamination
- The current routine infection control policy developed by CDC and followed in all health-care settings is:
 - Universal Precautions
 - Isolation Precautions
 - Blood and Body Fluid Precautions
 - Standard Precautions

4. An employee who is accidentally exposed to a possible blood-borne pathogen should immediately:
 - D. Report to a supervisor
 - E. Flush the area with water
 - F. Clean the area with disinfectant
 - G. Receive HIV prophylaxis
5. Personnel in the urinalysis laboratory should wear lab coats that:
 - A. Do not have buttons
 - B. Are fluid-resistant
 - C. Have short sleeves
 - D. Have full-length zippers
6. All of the following should be discarded in biohazardous waste containers except:
 - A. Urine specimen containers
 - B. Towels used for decontamination
 - C. Disposable lab coats
 - D. Blood collection tubes
7. An employer who fails to provide sufficient gloves for the employees may be fined by the:
 - A. CDC
 - B. NFPA
 - C. OSHA
 - D. FDA
8. An acceptable disinfectant for blood and body fluid decontamination is:
 - A. Sodium hydroxide
 - B. Antimicrobial soap
 - C. Hydrogen peroxide
 - D. Sodium hypochlorite
9. Proper handwashing includes all of the following except:
 - A. Using warm water
 - B. Rubbing to create a lather
 - C. Rinsing hands in a downward position
 - D. Turning on the water with a paper towel
10. Centrifuging an uncapped specimen may produce a biologic hazard in the form of:
 - A. Vectors
 - B. Sharps contamination
 - C. Aerosols
 - D. Specimen contamination
11. An employee who accidentally spills acid on his arm should immediately:
 - A. Neutralize the acid with a base
 - B. Hold the arm under running water for 15 minutes
 - C. Consult the MSDSs
 - D. Wrap the arm in gauze and go to the emergency department
12. When combining acid and water, ensure that:
 - A. Acid is added to water
 - B. Water is added to acid
 - C. They are added simultaneously
 - D. Water is slowly added to acid
13. An employee can learn the carcinogenic potential of potassium chloride by consulting the:
 - A. Chemical hygiene plan
 - B. Material safety data sheets
 - C. OSHA standards
 - D. Urinalysis procedure manual
14. Employees should not work with radioisotopes if they are:
 - A. Wearing contact lenses
 - B. Allergic to iodine
 - C. Sensitive to latex
 - D. Pregnant
15. All of the following are safe to do when removing the source of an electric shock except:
 - A. Pulling the person away from the instrument
 - B. Turning off the circuit breaker
 - C. Using a glass container to move the instrument
 - D. Unplugging the instrument
16. The acronym PASS refers to:
 - A. Presence of vital chemicals
 - B. Operation of a fire extinguisher
 - C. Labeling of hazardous material
 - D. Presence of radioactive substances
17. The system used by firefighters when a fire occurs in the laboratory is:
 - A. MSDS
 - B. RACE
 - C. NFPA
 - D. PASS
18. A class ABC fire extinguisher contains:
 - A. Sand
 - B. Water
 - C. Dry chemicals
 - D. Acid
19. The first thing to do when a fire is discovered is to:
 - A. Rescue persons in danger
 - B. Activate the alarm system
 - C. Close doors to other areas
 - D. Extinguish the fire if possible
20. If a red rash is observed after removing gloves, the employee:
 - A. May be washing her hands too often
 - B. May have developed a latex allergy
 - C. Should apply cortisone cream
 - D. Should not rub the hands so vigorously
21. Pipetting by mouth is:
 - A. Acceptable for urine but not serum
 - B. Not acceptable without proper training
 - C. Acceptable for reagents but not specimens
 - D. Not acceptable in the laboratory
22. The NFPA classification symbol contains information on all of the following except:
 - A. Fire hazards
 - B. Biohazards
 - C. Reactivity
 - D. Health hazards
23. The classification of a fire that can be extinguished with water is:
 - A. Class A
 - B. Class B
 - C. Class C
 - D. Class D
24. Employers are required to provide free immunization for:
 - A. HIV
 - B. HTLV-1
 - C. HBV
 - D. HCV
25. A possible physical hazard in the hospital is:
 - A. Wearing closed-toed shoes
 - B. Not wearing jewelry
 - C. Having short hair
 - D. Running to answer the telephone
26. Quality assessment refers to:
 - A. Analysis of testing controls
 - B. Increased productivity
 - C. Precise control results
 - D. Quality of specimens and patient care

27. During laboratory accreditation inspections, procedure manuals are examined for the presence of:
- Critical values
 - Procedure references
 - Procedures for specimen preservation
 - All of the above
28. As supervisor of the urinalysis laboratory, you have just adopted a new procedure. You should:
- Put the package insert in the procedure manual
 - Put a complete, referenced procedure in the manual
 - Notify the microbiology department
 - Put a cost analysis study in the procedure manual
29. Indicate whether each of the following would be considered a 1) preexamination, 2) examination, or 3) postexamination factor by placing the appropriate number in the blank:
- _____ Reagent expiration date
- _____ Rejecting a contaminated specimen
- _____ Constructing a Levy-Jennings chart
- _____ Telephoning a positive Clinitest result on a newborn
- _____ Calibrating the centrifuge
- _____ Collecting a timed urine specimen
30. The testing of sample from an outside agency and the comparison of results with participating laboratories is called:
- External QC
 - Electronic QC
 - Internal QC
 - Proficiency testing
31. A color change that indicates that a sufficient amount of patient's specimen or reagent is added correctly to the test system would be an example of:
- External QC
 - Equivalent QC
 - Internal QC
 - Proficiency testing
32. What steps are taken when the results of reagent strip QC are outside of the stated confidence limits?
- Check the expiration date of the reagent strip
 - Run a new control
 - Open a new reagent strips container
 - All of the above
33. When a new bottle of QC material is opened, what information is placed on the label?
- The supervisor's initials
 - The lot number
 - The date and the laboratory worker's initials
 - The time the bottle was opened
34. When a control is run, what information is documented?
- The lot number
 - Expiration date of the control
 - The test results
 - All of the above

Introduction to Urinalysis

OUTLINE

- I. History & Importance
- II. Urine
- III. Specimen Collection
- IV. Specimen Rejection
- V. Specimen Handling
- VI. Types of Specimens

HISTORY & IMPORTANCE

- Edwin Smith Surgical Papyrus
- Hippocrates - 5th century BCE, wrote a book on "uroscopy"
- Frederik Dekkers - chemical testing progressed from "ant testing" and "taste testing" of albuminuria by boiling urine
- Pisse prophets - charlatans without medical credentials began offering their predictions to the public for a healthy fee
- Thomas Addis - methods for quantitating the microscopic sediment
- Richard Bright - concept of urinalysis as part of a doctor's routine patient examination

Two unique characteristics of a urine specimen

1. Urine is a readily available and easily collected specimen.
2. Urine contains information, which can be obtained by inexpensive laboratory tests, about many of the body's major metabolic functions.

URINE

A. Formation

- Kidneys continuously form urine
- Ultrafiltrate of plasma
- Reabsorption of water and filtered substances essential to body function converts approximately 170,000 ml of filtered plasma to the average daily urine output of 1200 ml.

B. Composition

- 95% water and 5% solutes
- Other substances found in urine include hormones, vitamins, and medications
- Urine also may contain formed elements, such as cells, casts, crystals, mucus, and bacteria
- Increased amounts of these formed elements are often indicative of disease

Component	Comment
Urea	Primary organic component (nearly half). Metabolic waste product produced in the liver from the breakdown of protein and amino acid
Creatinine	Product of creatine metabolism by muscles
Uric acid	Product of nucleic acid breakdown in food and cells

Chloride	Primary inorganic component of urine. Found in combination with sodium (table salt) and many other inorganic substance
Sodium	Primarily from salt, varies by intake
Potassium	Combined with chloride and other salts
Phosphate	Combines with sodium to buffer the blood
Ammonium	Regulates blood and tissue fluid acidity
Calcium	Combines with chloride, sulfate, and phosphate

C. Volume

- Depends on the amount of water that the kidneys excrete
- Water - major body constituent
- Normal urine output: 1200 to 1500 mL
- Range of normal urine output: 600 to 2000 mL

MUST-KNOW

Is this fluid urine?

- Specimen can be tested for its urea and creatinine content.
- Both these substances are present in much higher concentrations in urine than in other body fluids

Factors that influence urine volume

1. fluid intake
2. fluid loss from nonrenal sources
3. variations in the secretion of antidiuretic hormone
4. need to excrete increased amounts of dissolved solids, such as glucose or salts

Terms

- Oliguria
 - decrease in urine output
 - commonly seen when the body enters a state of dehydration as a result of excessive water loss from vomiting, diarrhea, perspiration, or severe burns
 - less than 1 mL/kg/hr in infants
 - less than 0.5 mL/kg/hr in children
 - less than 400 mL/day in adults
- Anuria
 - commonly from oliguria
 - cessation of urine flow
 - may result from
 - any serious damage to the kidneys
 - decrease in the flow of blood to the kidneys
- Nocturia
 - increase in the nocturnal excretion of urine
 - normal excretion: kidneys excrete two to three times more urine during the day than during the night

- Polyuria
 - increase in daily urine volume
 - often associated with diabetes mellitus and diabetes insipidus
 - artificially induced by diuretics, caffeine, or alcohol (suppress the secretion of antidiuretic hormone)
 - greater than 2.5 L/day in adults
 - to 3 mL/kg/day in children

NICE-TO-KNOW

Diabetes mellitus

- caused by a defect either in the pancreatic production of insulin or in the function of insulin
- results in an increased body glucose concentration.
- The kidneys do not reabsorb excess glucose, necessitating excretion of increased amounts of water to remove the dissolved glucose from the body
- Specimen: Appearing dilute but has a high specific gravity because of increased glucose

Diabetes insipidus

- results from a decrease in the production or function of antidiuretic hormone (ADH)
- water necessary for adequate body hydration is not reabsorbed from the plasma filtrate
- Specimen: urine is truly dilute and has a low specific gravity

- ∴ compensated by increased ingestion of water (polydipsia) → greater urine volume
- ∴ Polyuria accompanied by increased fluid intake - often the first symptom of either disease

SPECIMEN COLLECTION

- Urine - biohazardous substance that requires the observance of Standard Precautions (gloves is worn when in contact)

A. Containers

- clean, dry, leak-proof containers
- disposable containers - eliminate the chance of contamination owing to improper washing
- adhesive for the collection of pediatric specimens
- large containers for 24-hour specimens
- should have
 - wide mouth
 - wide, flat bottom to prevent overturning
 - clear material to allow for determination of color and clarity
 - tubes with conical bottoms - for sediment analysis
 - round bottoms - for automated reagent strip testing
- recommended capacity: 50 mL
 - allows 12 mL of specimen needed for microscopic analysis
 - additional specimen for repeat analysis
 - enough room for the specimen to be mixed by swirling the container
- Individually packaged sterile containers with secure closures - used for microbiologic urine studies
- Sterile containers - suggested if more than 2 hours elapse between specimen collection and analysis
- transfer straw - transfer device that has a needle and an evacuated tube holder

B. Labels

- patient's name and identification number, the date and time of collection, and additional information
- attached to the container, not in the lid

C. Requisitions

- must accompany specimens delivered to the laboratory
- must match the information on the specimen label
- additional information:
 - method of collection or type of specimen
 - possible interfering medications
 - patient's clinical information
- time the specimen is received in the laboratory should be recorded on the form

SPECIMEN REJECTION

Unacceptable situations include:

1. Specimens in unlabeled containers
2. Nonmatching labels and requisition forms
3. Specimens contaminated with feces or toilet paper
4. Containers with contaminated exteriors
5. Specimens of insufficient quantity
6. Specimens that have been improperly transported

- ∴ Never discard a specimen before checking with a supervisor.

SPECIMEN HANDLING

- Changes in urine composition take place not only in vivo but also in vitro, thus requiring correct handling procedures.

A. Specimen Integrity

- should be delivered to the laboratory promptly and tested within 2 hours
- if can't: should be refrigerated or have an appropriate chemical preservative added

Changes in Unpreserved Urine		
Analyte	Change	Cause
Color	Modified/darkened	Oxidation or reduction of metabolites
Clarity	Decreased	Bacterial growth and precipitation of amorphous material
Odor	Increased	Bacterial multiplication causing breakdown of urea to ammonia
pH	Increased	Breakdown of urea to ammonia by urease-producing bacteria/loss of CO ₂
Glucose	Decreased	Glycolysis and bacterial use
Ketones	Decreased	Volatilization and bacterial metabolism
Bilirubin	Decreased	Exposure to light/photo oxidation to biliverdin
Urobilinogen	Decreased	Oxidation to urobilin
Nitrite	Increased	Multiplication of nitrate-reducing bacteria
Red and white blood cells and casts	Decreased	Disintegration in dilute alkaline urine

Bacteria	Increased	Multiplication
Trichomonas	Decreased	Loss of motility, death

B. Specimen Preservation

- refrigeration at 2°C to 8°C
 - most routinely used method of preservation
 - decreases bacterial growth and metabolism
- specimen to be cultured
 - should be refrigerated during transit
 - kept refrigerated until cultured up to 24 hours
 - must return to room temperature before chemical testing by reagent strips (enzyme reactions on the strips perform best at room temperature)
- specimen to be transported over a long distance with impossible refrigeration
 - chemical preservatives may be added
 - use of commercially prepared transport tubes

Ideal preservative

- ✓ bactericidal
- ✓ inhibit urease
- ✓ preserve formed elements in the sediment
- ✓ should not interfere with chemical tests
- ✓ does not exist
- ✓ best suits the needs of the required analysis should be chosen

Urine Preservatives			
Preservatives	Advantages	Disadvantages	Additional Information
Refrigeration	Does not interfere with chemical tests	Precipitates amorphous phosphates and urates	Prevents bacterial growth for 24 hours
Boric acid	Prevents bacterial growth and metabolism	Interferes with drug and hormone analyses	Keeps pH at about 6.0 Can be used for urine culture transport
Formalin (formaldehyde)	Excellent sediment preservative	Acts as a reducing agent, interfering with chemical tests for glucose, blood, leukocyte esterase, and copper reduction	Rinse specimen container with formalin to preserve cells and casts
Sodium fluoride	Is a good preservative for drug analyses	Inhibits reagent strip tests for glucose, blood, and leukocytes	
Commercial preservative Tablets	Convenient when	Check tablet composition	

	refrigeration not possible Have controlled concentration to minimize interference	to determine possible effects on desired tests	
Urine Collection Kits (Becton, Dickinson, Rutherford, NJ)	Contains collection cup, transfer straw, culture and sensitivity (C&S) preservative tube, or UA tube		
Light gray and gray C&S tube	Sample stable at room temperature (RT) for 48 hours; prevents bacterial growth and metabolism	Do not use if urine is below minimum fill line	Preservative is boric acid, sodium borate and sodium formate. Keeps pH at about 6.0
Yellow UA Plus tube	Use on automated instruments	Must refrigerate within 2 hours	Round or conical bottom, no preservative
Cherry red/yellow Preservative Plus tube	Stable for 72 hours at RT; instrument-compatible	Must be filled to minimum fill line. Bilirubin and urobilinogen may be decreased if specimen is exposed to light and left at RT	Preservative is sodium propionate, ethyl paraben, and chlorhexidine; round or conical bottoms

TYPES OF SPECIMENS

Type of Specimen	Purpose
Random	Routine screening
First morning	Routine screening Pregnancy tests Orthostatic protein
24-hour (or timed)	Quantitative chemical tests
Catheterized	Bacterial culture
Midstream clean-catch	Routine screening Bacterial culture
Suprapubic aspiration	Bladder urine for bacterial culture Cytology
Three-glass collection	Prostatic infection

A. Random Specimen

- most commonly received specimen
- ease of collection and convenience for the patient
- collected at any time
- actual time of voiding should be recorded on the container
- may also show erroneous results resulting from dietary intake or physical activity just before collection
- resolution: requested to collect an additional specimen under more controlled conditions

B. First Morning Specimen

- ideal screening specimen
- concentrated specimen
- assuring detection of chemicals and formed elements that may not be present in a dilute random specimen
- collect the specimen immediately on arising and to deliver it to the laboratory within 2 hours or keep it refrigerated
- essential for
 - preventing false-negative pregnancy tests
 - evaluating orthostatic proteinuria

C. 24-Hour (or Timed) Specimen

- exhibit diurnal variations such as catecholamines, 17-hydroxysteroids, and electrolytes in which the lowest concentration is in the early morning and the highest concentration occurs in the afternoon
- required when the concentration of the substance to be measured changes with diurnal variations and with daily activities such as exercise, meals, and body metabolism
- if the concentration of a particular substance remains constant, the specimen may be collected over a shorter period

Procedure

- patient must begin and end the collection period with an empty bladder
- Day 1: 7 a.m.: patient voids and discards specimen; collects all urine for the next 24 hours.
- Day 2: 7 a.m.: patient voids and adds this urine to previously collected urine.
- 24-hour specimen must be thoroughly mixed and the volume accurately measured and recorded
- If only an aliquot is needed for testing, the amount saved must be adequate to permit repeat or additional testing
- If collection is in two containers, it should be mixed before aliquoting
- All specimens should be refrigerated or kept on ice during the collection period and may also require addition of a chemical preservative

D. Catheterized Specimen

- collected under sterile conditions by passing a hollow tube (catheter) through the urethra into the bladder
- bacterial culture - most commonly requested test on a catheterized specimen

E. Midstream Clean-Catch Specimen

- alternative to catheterized specimen
- safer, less traumatic method for obtaining urine for bacterial culture and routine urinalysis
- less contaminated by epithelial cells and bacteria
- provided with appropriate cleansing materials, a sterile container, and instructions for cleansing and voiding
 - strong bacterial agents - should not be used (such as hexachlorophene or povidone-iodine)

- recommended: mild antiseptic towelettes

Procedure

For females

- Wash hands.
- Remove the lid from the container without touching the inside of the container or lid.
- Separate the skin folds (labia).
- Cleanse from front to back on either side of the urinary opening with an antiseptic towelette, using a clean one for each side.
- Hold the skin folds apart and begin to void into the toilet.
- Bring the urine container into the stream of urine and collect an adequate amount of urine. Do not touch the inside of the container or allow the container to touch the genital area.
- Finish voiding into the toilet.
- Cover the specimen with the lid. Touch only the outside of the lid and container.
- Label the container with the name and time of collection and place in the specified area or follow institutional policy.

For males

- Wash hands.
- Remove the lid from the sterile container without touching the inside of the container or lid.
- Cleanse the tip of the penis with antiseptic towelette and let dry. Retract the foreskin if uncircumcised.
- Void into the toilet. Hold back foreskin if necessary.
- Bring the sterile urine container into the stream of urine and collect an adequate amount of urine. Do not touch the inside of the container or allow the container to touch the genital area.
- Finish voiding into the toilet.
- Cover the specimen with the lid. Touch only the outside of the lid and container.
- Label the container with the name and time of collection and place in the specified area or follow institutional policy.

F. Suprapubic Aspiration

- external introduction of a needle through the abdomen into the bladder
- bladder is sterile under normal conditions
- completely free of extraneous contamination
- also used for cytologic examination

G. Prostatitis Specimen

a. Three-Glass Collection

Procedure

- cleansed using the male midstream clean-catch procedure
- instead of discarding the first urine passed, it is collected in a sterile container
- midstream portion is collected in another sterile container
- prostate is then massaged so that prostate fluid will be passed with the remaining urine into a third sterile container
 - quantitative cultures performed on all specimen
 - first and third glass - microscopically examined
 - third specimen in prostatic infection - will have a white blood cell/high-power field count and a bacterial count 10 times that of the first specimen (macrophages with lipids can be detected)

- second specimen - used as a control for bladder and kidney infection and should be negative (if it is positive, the results from the third specimen are invalid because infected urine has contaminated the specimen)

b. Pre- and Post-Massage Test (PPMT)

- clean-catch mid-stream urine specimen is collected
- second urine sample - collected after the prostate is massaged
- positive result - significant bacteriuria in the post-massage specimen of greater than 10 times the premassage count

c. Stamey-Mears Test for Prostatitis

- four-glass method
- consists of:
 - initial voided urine (VB1) - tested for urethral infection or inflammation
 - midstream urine (VB2) - tests for urinary bladder infection
 - expressed prostatic secretions (EPS)
 - post-prostatic massage urine specimen (VB3) - cultured and examined for white blood cells
 - having more than 10 to 20 white blood cells per high-power field is considered abnormal

H. Pediatric Specimens

- uses a soft, clear plastic bag with hypoallergenic skin adhesive to attach to the genital area of both boys and girls
- may be obtained by catheterization or by suprapubic aspiration

Procedure

1. Attach the bag firmly over the genital area avoiding the anus.
2. When enough specimen has been collected, remove the bag and label it or pour the specimen into container and label the container following institutional policy.

For microbiology specimens

1. Clean the area with soap and water and sterily dry the area, removing any residual soap residue.
2. Firmly apply a sterile bag.
3. Sterily transfer collected specimen into a sterile container and label the container

∴ 15 minutes - time the applied bags should be checked

I. Drug Specimen

- Chain of Custody (COC)
 - process that provides documentation of proper sample identification from the time of collection to the receipt of laboratory results
 - standardized form that must document and accompany every step of drug testing, from collector to courier to laboratory to medical review officer to employer
- Urine specimen collections may be "witnessed" or "unwitnessed."
 - Witnessed - same-gender collector will observe the collection of 30 to 45 mL of urine
- Urine temperature - must be taken within 4 minutes from the time of collection to confirm the specimen has not been adulterated (32.5°C to 37.7°C)

Procedure

- a. The collector washes hands and wears gloves.
- b. The collector adds bluing agent (dye) to the toilet water reservoir to prevent an adulterated specimen.
- c. The collector eliminates any source of water other than toilet by taping the toilet lid and faucet handles.
- d. The donor provides photo identification or positive identification from employer representative.
- e. The collector completes step 1 of the chain-of-custody (COC) form and has the donor sign the form.
- f. The donor leaves his or her coat, briefcase, and/or purse outside the collection area to avoid the possibility of concealed substances contaminating the urine.
- g. The donor washes his or her hands and receives a specimen cup.
- h. The collector remains in the restroom but outside the stall, listening for unauthorized water use, unless a witnessed collection is requested.
- i. The donor hands specimen cup to the collector. Transfer is documented.
- j. The collector checks the urine for abnormal color and for the required amount (30 to 45 mL).
- k. The collector checks that the temperature strip on the specimen cup reads 32.5°C to 37.7°C. The collector records the in-range temperature on the COC form (COC step 2). If the specimen temperature is out of range or the specimen is suspected of having been diluted or adulterated, a new specimen must be collected and a supervisor notified.
- l. The specimen must remain in the sight of the donor and collector at all times.
- m. With the donor watching, the collector peels off the specimen identification strips from the COC form (COC step 3) and puts them on the capped bottle, covering both sides of the cap.
- n. The donor initials the specimen bottle seals.
- o. The date and time are written on the seals.
- p. The donor completes step 4 on the COC form.
- q. The collector completes step 5 on the COC form.
- r. Each time the specimen is handled, transferred, or placed in storage, every individual must be identified and the date and purpose of the change recorded.
- s. The collector follows laboratory-specific instructions for packaging the specimen bottles and laboratory copies of the COC form.
- t. The collector distributes the COC copies to appropriate personnel.

Review Questions

1. The average daily output of urine is:
 - A. 200 mL
 - B. 500 mL
 - C. 1200 mL
 - D. 2500 mL
2. An unidentified fluid is received in the laboratory with a request to determine whether the fluid is urine or another body fluid. Using routine laboratory tests, what tests would determine that the fluid is most probably urine?
 - A. Glucose and ketones
 - B. Urea and creatinine
 - C. Uric acid and amino acids
 - D. Protein and amino acids

3. The primary inorganic substance found in urine is:
 - A. Sodium
 - B. Phosphate
 - C. Chloride
 - D. Calcium
4. A patient presenting with polyuria, nocturia, polydipsia, and a low urine specific gravity is exhibiting symptoms of:
 - A. Diabetes insipidus
 - B. Diabetes mellitus
 - C. Urinary tract infection
 - D. Uremia
5. A patient with oliguria might progress to having:
 - A. Nocturia
 - B. Polyuria
 - C. Polydipsia
 - D. Anuria
6. All of the following are characteristics of recommended urine containers except:
 - A. A flat bottom
 - B. A capacity of 50 mL
 - C. A snap-on lid
 - D. Are disposable
7. Labels for urine containers are:
 - A. Attached to the container
 - B. Attached to the lid
 - C. Placed on the container prior to collection
 - D. Not detachable
8. A urine specimen may be rejected by the laboratory for all of the following reasons except the fact that the:
 - A. Requisition states the specimen is catheterized
 - B. Specimen contains toilet paper
 - C. Label and requisition do not match
 - D. Outside of the container has fecal material contamination
9. A cloudy specimen received in the laboratory may have been preserved using:
 - A. Boric acid
 - B. Chloroform
 - C. Refrigeration
 - D. Formalin
10. For general screening the most frequently collected specimen is a:
 - A. Random one
 - B. First morning
 - C. Midstream clean-catch
 - D. Timed
11. The primary advantage of a first morning specimen over a random specimen is that it:
 - A. Is less contaminated
 - B. Is more concentrated
 - C. Is less concentrated
 - D. Has a higher volume
12. If a routine urinalysis and a culture are requested on a catheterized specimen, then:
 - A. Two separate containers must be collected
 - B. The routine urinalysis is performed first
 - C. The patient must be recatheterized
 - D. The culture is performed first
13. If a patient fails to discard the first specimen when collecting a timed specimen the:
 - A. Specimen must be recollected
 - B. Results will be falsely elevated
 - C. Results will be falsely decreased
 - D. Both A and B
14. The primary cause of unsatisfactory results in an unpreserved routine specimen not tested for 8 hours is:
 - A. Bacterial growth
 - B. Glycolysis
 - C. Decreased pH
 - D. Chemical oxidation
15. Prolonged exposure of a preserved urine specimen to light will cause:
 - A. Decreased glucose
 - B. Increased cells and casts
 - C. Decreased bilirubin
 - D. Increased bacteria
16. Which of the following would be least affected in a specimen that has remained unpreserved at room temperature for more than 2 hours?
 - A. Urobilinogen
 - B. Ketones
 - C. Protein
 - D. Nitrite
17. Bacterial growth in an unpreserved specimen will:
 - A. Decrease clarity
 - B. Increase bilirubin
 - C. Decrease pH
 - D. Increase glucose
18. The most sterile specimen collected is a:
 - A. Catheterized
 - B. Midstream clean-catch
 - C. Three-glass
 - D. Suprapubic aspiration
19. Which of the following would not be given to a patient prior to the collection of a midstream clean-catch specimen?
 - A. Sterile container
 - B. Iodine cleanser
 - C. Antiseptic towelette
 - D. Instructions
20. Urine specimen collection for drug testing requires the collector to do all of the following except:
 - A. Inspect the specimen color
 - B. Perform reagent strip testing
 - C. Read the specimen temperature
 - D. Fill out a chain-of-custody form

Renal Function

OUTLINE

- I. Renal Physiology
 - A. Nephrons
 - B. Renal Blood Flow
 - C. Glomerular Filtration
 - D. Tubular Reabsorption
 - E. Tubular Secretion
- II. Renal Function Tests
 - A. Glomerular Filtration Tests
 - B. Tubular Reabsorption Tests
 - C. Tubular Secretion and Renal Blood Flow Tests

RENAL PHYSIOLOGY

A. Nephrons

- functional units of kidney
- each kidney contains approximately **1 to 1.5 million nephrons**
- controls the ability of the kidneys to clear waste products selectively from the blood and simultaneously to maintain the body's essential water and electrolyte balances

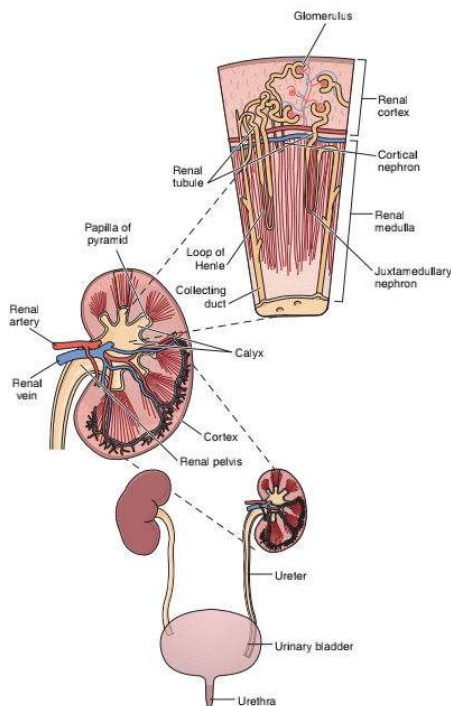
Types of Nephrons

a. Cortical nephrons

- approximately **85%** of nephrons
- situated primarily in the cortex of the kidney
- responsible primarily for **removal of waste products and reabsorption of nutrients**

b. Juxtamedullary nephrons

- have longer loops of Henle that extend deep into the medulla of the kidney
- primary function: primary function is **concentration of the urine**

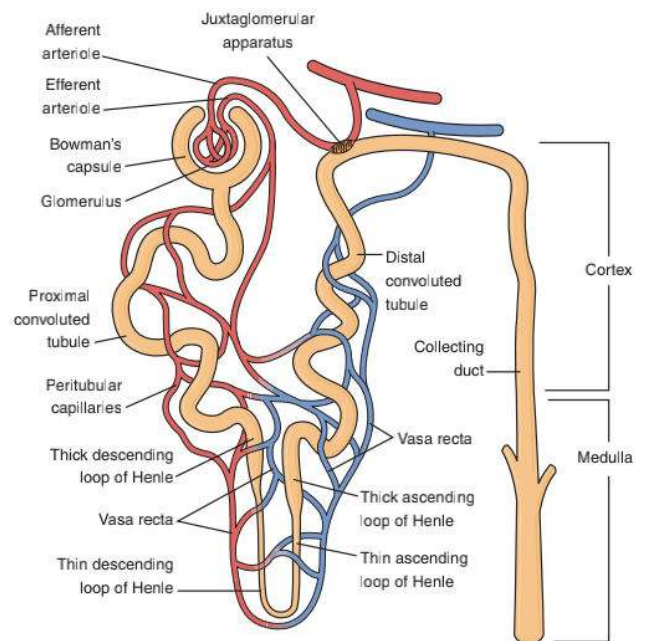


Renal Functions

- glomerular filtration
- tubular reabsorption
- tubular secretion

B. Renal Blood Flow

- renal artery** - **supplies blood to the kidney**
- kidneys receive approximately **25%** of the blood pumped through the heart at all times
- afferent arteriole** - capillaries of the nephron where blood enters that flows to the glomerulus then into the efferent arteriole
- efferent arteriole** - **smaller**, increases the glomerular capillary pressure
 - varying size of arterioles - help to create the hydrostatic pressure differential important for glomerular filtration and to maintain consistency of glomerular capillary pressure and renal blood flow within the glomerulus



- Total renal blood flow: **1200 mL/min**
- Total renal plasma flow: **600 to 700 mL/min**
 - based on an average body size of **1.73 m²** of surface

Path of Urine

Blood to kidney → Afferent arteriole → glomerulus → Efferent arteriole → Peritubular capillaries and vasa recta → flows to the cortex and medulla of the kidney close to the tubules → Bowman's space → Proximal Convoluted tubule (PCT) → Loop of Henle → Distal Convoluted tubule (DCT) → Collecting Tubule → Ureter → Bladder → Urethra

- Distal convoluted tubule - **final adjustment of the urinary composition**
- vasa recta - located adjacent to the ascending and descending loops of Henle in juxtamedullary nephron; in

this area, major exchanges of water and salts take place between the blood and the medullary interstitium

- this exchange maintains the osmotic gradient (salt concentration) in the medulla

C. Glomerular Filtration

Glomerulus

- consists of a coil of approximately **eight** capillary lobes
 - located **within Bowman's capsule** (forms the beginning of the renal tubule)
 - serves as a **nonselective filter** of plasma substances with **molecular weights less than 70,000**
 - **glomerular filtration barrier**
 - walls of glomerulus
 - **contains a shield of negativity** - repels molecules with a positive charge even though they are small enough to pass through the three layers of the barrier
 - **albumin**
 - the **primary protein associated with renal disease**
 - has a positive charge and would easily pass through the barrier
- ∴ If it were not for the shield of negativity, all routine urines would have positive reagent strip readings for protein/albumin

Factors that Influence the Filtration Process

1. cellular structure of the capillary walls and Bowman's capsule
2. hydrostatic pressure and oncotic pressure
3. feedback mechanisms of the renin-angiotensin-aldosterone system (RAAS)

Three Glomerular Filtration Barrier Cellular Layers

1. **fenestrated capillary wall membrane** - pores increase capillary permeability but do not allow the passage of large molecules and blood cells
2. **basement membrane (basal lamina)** - further restriction of large molecules
3. **visceral epithelium of Bowman's capsule** - further restriction of large molecules in the thin membranes covering the filtration slits formed by the intertwining foot processes of the podocytes of the inner layer of Bowman's capsule

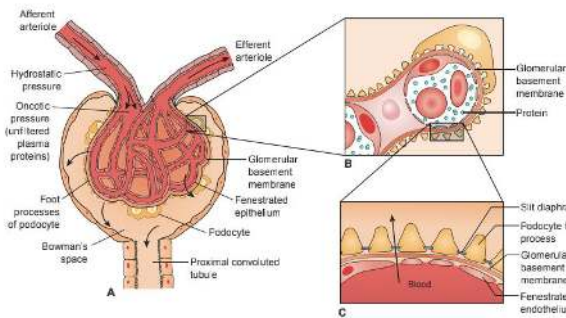


Figure 3-3 Factors affecting glomerular filtration in the renal corpuscle. (A) Inset B, glomerular filtration barrier. Inset C, the shield of negativity.

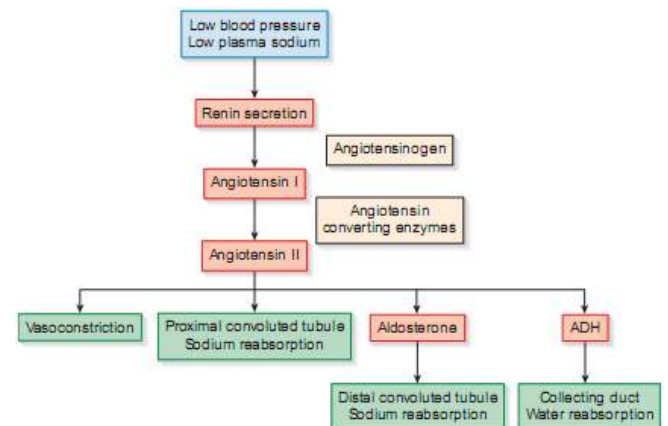
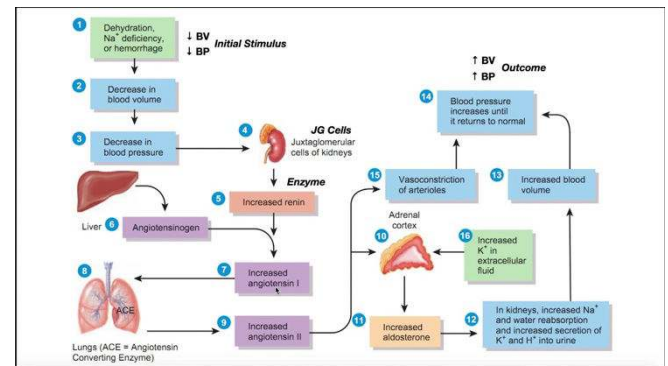
Glomerular Pressure

- Autoregulatory mechanism within the juxtaglomerular apparatus - maintains the glomerular blood pressure at a relatively constant rate regardless of fluctuations in systemic blood pressure

- Dilation of the afferent arterioles and constriction of the efferent arterioles when blood pressure drops prevents a marked decrease in blood flowing through the kidney → preventing an increase in the blood level of toxic waste products
- Increase in blood pressure → constriction of the afferent arterioles to prevent overfiltration or damage to the glomerulus.

Renin-Angiotensin-Aldosterone System

- regulates the flow of blood to and within the glomerulus
- responds to changes in blood pressure and plasma sodium content
- **monitored by the juxtaglomerular apparatus**
- consists of
 - juxtaglomerular cells in the afferent arteriole
 - macula densa of the distal convoluted tubule



- Renin - an enzyme produced by the juxtaglomerular cells
- Aldosterone - sodium-retaining hormone
- Every minute approximately two to three million glomeruli filter approximately 120 mL of water-containing low-molecular-weight substances
- Difference between compositions of the filtrate and the plasma: absence of plasma protein, any protein-bound substances, and cells
- Filtrate - specific gravity of 1.010

Actions of the RAAS

1. Dilates the afferent arteriole and constricts the efferent arteriole
2. Stimulates sodium reabsorption in the proximal convoluted tubule
3. Triggers the adrenal cortex to release the sodium-retaining hormone aldosterone to cause sodium

reabsorption and potassium excretion in the distal convoluted tubule and collecting duct

4. Triggers antidiuretic hormone release by the hypothalamus to stimulate water reabsorption in the collecting duct

D. Tubular Reabsorption

- Body cannot lose 120 mL of water-containing essential substances every minute → when the plasma ultrafiltrate enters the proximal convoluted tubule, the nephrons, through cellular transport mechanisms, begin reabsorbing these essential substances and water

Reabsorption Mechanisms

- a. active transport
 - substance to be reabsorbed must combine with a carrier protein contained in the membranes of the renal tubular epithelial cells → electrochemical energy → transfers the substance across the cell membranes and back into the bloodstream
 - responsible for
 - reabsorption of glucose, amino acids, and salts in the proximal convoluted tubule
 - chloride in the ascending loop of Henle
 - sodium in the distal convoluted tubule
- b. passive transport
 - movement of molecules across a membrane as a result of differences in their concentration or electrical potential on opposite sides of the membrane
 - gradients - physical differences
 - all parts of the nephron - where passive reabsorption
 - of water takes place
 - ascending loop of Henle - impermeable to water

maximal reabsorptive capacity (T_m)

- normal plasma filtrate concentration reabsorbed
- exceeds maximal reabsorptive capacity (T_m) → appears in the urine

renal threshold

- the plasma concentration at
- which active transport stops
- glucose plasma renal threshold: 160 to 180 mg/dL

Tubular Concentration

- Countercurrent mechanism
 - serves to maintain the osmotic gradient of the medulla
 - excessive reabsorption of water as the filtrate passes through the highly concentrated medulla is prevented by the water-impermeable walls of the ascending loop
 - selective reabsorption process
 - sodium and chloride leaving the filtrate in the ascending loop → prevent dilution of the medullary interstitium by the water reabsorbed from the descending loop
 - aldosterone - controls the reabsorption of sodium continues in the distal convoluted tubule

Collecting Duct Concentration

- A high level of ADH increases permeability → increased reabsorption of water, and a low-volume concentrated urine

- ADH renders the walls impermeable to water → resulting in a large volume of dilute urine
- production of ADH - determined by the state of body hydration

ADH Control:

$\uparrow \text{Body Hydration} = \downarrow \text{ADH} = \uparrow \text{Urine Volume}$
 $\downarrow \text{Body Hydration} = \uparrow \text{ADH} = \downarrow \text{Urine Volume}$

E. Tubular Secretion

- Involves the passage of substances from the blood in the peritubular capillaries to the tubular filtrate
- proximal convoluted tubule - major site for removal of nonfiltered substance (e.g. foreign medications that cannot be filtered by the glomerulus because they are bound to plasmaproteins. When these protein-bound substances enter the peritubular capillaries → develop a stronger affinity for the tubular cells and dissociate from their carrier proteins → transport into the filtrate by the tubular cells.
- Two major functions
 - 1) eliminating waste products not filtered by the glomerulus
 - 2) regulating the acid-base balance in the body through the secretion of hydrogen ions

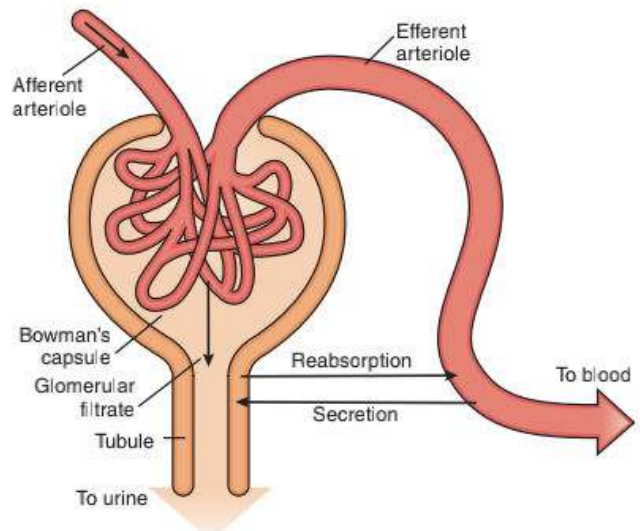


Figure 3-7 Summary of movement of substances in the nephron.

Acid-Base Balance

- Buffer and elimination of excess acid (from dietary intake and body metabolism) - to maintain normal pH of the blood of 7.4
- bicarbonate (HCO_3^-) ions - where buffering capacity is dependent
- secretion of hydrogen ions (H^+)
 - prevents the filtered bicarbonate from being excreted in the urine and causes the return of a bicarbonate ion to the plasma
 - small molecular size
 - readily filtered and reabsorbed

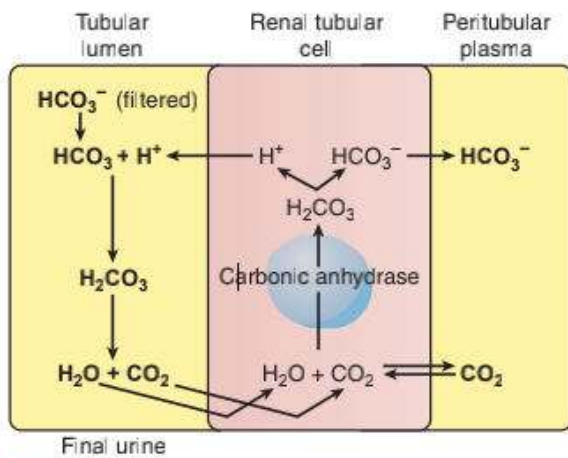


Figure 3-8 Reabsorption of filtered bicarbonate.

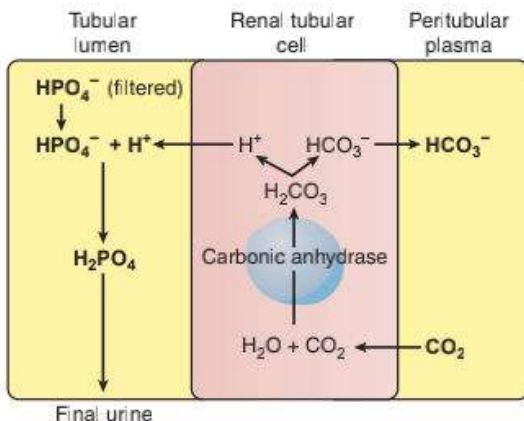


Figure 3-9 Excretion of secreted hydrogen ions combined with phosphate.

- The secreted hydrogen ion combines with a filtered phosphate ion instead of a bicarbonate ion and is excreted rather than reabsorbed.

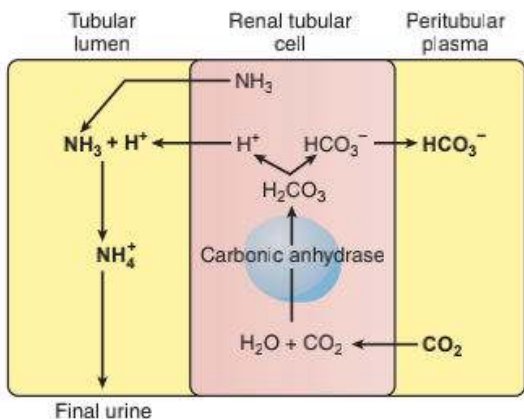


Figure 3-10 Excretion of secreted hydrogen ions combined with ammonia produced by the tubules.

- In the proximal convoluted tubule, ammonia is produced from the breakdown of the amino acid glutamine. The ammonia reacts with the H^+ to form the ammonium ion (NH_4^+).
- Metabolic acidosis or renal tubular acidosis - caused by a disruption in secretory functions can result such the inability to produce an acid urine.

RENAL FUNCTION TESTS

A. Glomerular Filtration Tests

- Clearance tests
 - standard tests used to measure the filtering capacity of the glomeruli
 - measures the rate at which the kidneys are able to remove (to clear) a filterable substance from the blood
 - primary substances: creatinine, beta₂-microglobulin, cystatin C

Factors to Consider in Selecting a Clearance Tests

- stability of the substance in urine during a possible 24-hour collection period
- plasma level consistency
- substance's availability to the body
- availability of tests to analyze the substance

1. Urea Clearance

- earliest glomerular filtration test
- 40% of the filtered urea is reabsorbed
- patients were hydrated to produce a urine flow of 2 mL/min to ensure that no more than 40% of the urea was reabsorbed

2. Inulin Clearance

- polymer of fructose
- an extremely stable substance that is not reabsorbed or secreted by the tubules
- must be infused by IV at a constant rate throughout the testing period
- original reference method for clearance tests

3. Creatinine Clearance

- waste product of muscle metabolism
- produced enzymatically by creatine phosphokinase from creatine
- normally found at a relatively constant level in the blood

Disadvantages

- Some creatinine is secreted by the tubules, and secretion increases as blood levels rise.
- Chromogens present in human plasma react in the chemical analysis. Their presence, however, may help counteract the falsely elevated rates caused by tubular secretion.
- Medications, including gentamicin, cephalosporins, and cimetidine (Tagamet), inhibit tubular secretion of creatinine, thus causing falsely low serum levels.¹
- Bacteria will break down urinary creatinine if specimens are kept at room temperature for extended periods.²
- A diet heavy in meat consumed during collection of a 24-hour urine specimen will influence the results if the plasma specimen is drawn before the collection period because the increased intake of meat can raise the urine and plasma levels of creatinine during the 24-hour collection period.
- Measurement of creatinine clearance is not a reliable indicator in patients suffering from muscle-wasting diseases or persons involved in heavy exercise or athletes supplementing with creatine.
- Accurate results depend on the accurate completeness of a 24-hour collection.

- 8) It must be corrected for body surface area, unless normal is assumed, and must always be corrected for children.

Traditional Creatinine Clearance Procedure

Example

Calculate the urine volume (V) for a 2-hour specimen measuring 240 mL:

$$\begin{aligned} 2 \text{ hours} \times 60 \text{ minutes} &= 120 \text{ minutes} \\ 240 \text{ mL} / 120 \text{ minutes} &= 2 \text{ mL/min} \\ V &= 2 \text{ mL/min} \end{aligned}$$

The plasma and urine concentrations are determined by chemical testing. The standard formula used to calculate the milliliters of plasma cleared per minute (C) is:

$$C = \frac{UV}{P}$$

This formula is derived as follows. The milliliters of plasma cleared per minute (C) times the mg/dL of plasma creatinine (P) must equal the mg/dL of urine creatinine (U) times the urine volume in mL/min (V), because all of the filtered creatinine will appear in the urine. Therefore:

$$CP = UV \text{ and } C = \frac{UV}{P}$$

estimated glomerular filtration rate (eGFR)

- newer methods that do not require the collection of timed (24-hour) urine specimens
- formulas can be programmed into automated instruments
 - Modification of Diet in Renal Disease (MDRD) study - most frequently used formula
 - MDRD-IDMS-traceable - formula recommended by the National Kidney Disease Education Program (NKDEP)
- not accurate for pediatric patients
- most accurate when results are lower than 60 mL/min

$$\text{GFR} = 175 \times \text{serum creatinine} - 1154 \times \text{age} - 0.203 \times 0.742 \text{ (if patient is female)} \times 1.202 \text{ (if patient is black)}$$

Original MDRD Calculation

$$\text{GFR} = 173 \times \text{serum creatinine} - 1.154 \times \text{age} - 0.203 \times 0.742 \text{ (if patient is female)} \times 1.212 \text{ (if patient is black)}$$

4. Cystatin C

- a small protein (molecular weight 13,359)
- produced at a constant rate by all nucleated cells
- readily filtered by the glomerulus and reabsorbed and broken down by the renal tubular cells → no cystatin C is secreted by the tubules
- serum concentration can be directly related to the GFR
- Recommended for: pediatric patients, persons with diabetes, the elderly, and critically ill patients

- Advantage: independent of muscle mass
- both serum or plasma cystatin C and creatinine can provide even more accurate information on a patient's GFR

5. Beta₂-Microglobulin

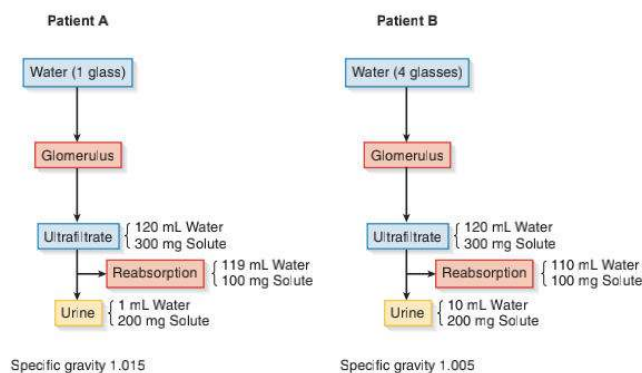
- molecular weight 11,800
- dissociates from human leukocyte antigens at a constant rate
- rapidly removed from the plasma by glomerular filtration
- rise in the plasma level of beta₂-microglobulin has been shown to be a more sensitive indicator of a decrease in GFR than creatinine clearance
- not reliable in patients with history of immunologic disorders or malignancy

6. Radionucleotides

- such as iothalamate
- exogenous procedures
- provides a method for determining glomerular filtration through the plasma disappearance of the radioactive material and enables visualization of the filtration in one or both kidneys
- valuable to measure the viability of a transplanted kidney

B. Tubular Reabsorption Tests

- loss of tubular reabsorption capability - often the first function affected in renal disease
- specimens no higher than 1.010 specific gravity yet no renal disease present → because urine concentration is largely determined by the body's state of hydration, and the normal kidney will reabsorb only the amount of water necessary to preserve an adequate supply of body water.



1. Fishberg concentration tests

- patients were deprived of fluids for 24 hours before measuring specific gravity
- no longer used

2. Mosenthal concentration tests

- compared the volume and specific gravity of day and night urine samples to evaluate concentrating ability
- no longer used

3. Osmolality

- A urine to serum ratio (U:S ratio) of >3:1
- A osmolality of >800 mOsm
 - indicates normal tubular reabsorption
- Serum osmolality: 275 to 300 mOsm
- Urine osmolality: 50 to 1400 mOsm

- a. Freezing Point Osmometers
 - first principle incorporated into clinical osmometers
 - determine the freezing point of a solution by supercooling a measured amount of sample to approximately 27°C
 - NaCl concentration - reference standard
- b. Vapor Pressure Osmometers
 - measures the dew point (temperature at which water vapor condenses to a liquid)

4. Free Water Clearance

- determined by first calculating the osmolar clearance using the standard clearance formula

$$C_{\text{osm}} = \frac{U_{\text{osm}} \times V}{P_{\text{osm}}}$$

- and then subtracting the osmolar clearance value from the urine volume in mL/min.

Example

$$C_{\text{osm}} = \frac{600 (U) \times 2 (V)}{300 (P)} = 4.0 \text{ mL/min}$$

$$C_{\text{H}_2\text{O}} = 2 (V) - 4.0 (C_{\text{osm}}) = -2.0 \text{ (free water clearance)}$$

- Calculating osmolar clearance indicates how much water must be cleared each minute to produce a urine with the same osmolality as the plasma.
- The ultrafiltrate contains the same osmolality as the plasma; therefore, the osmotic differences in the urine are the result of renal concentrating and diluting mechanisms.

C. Tubular Secretion and Renal Blood Flow Tests

1. p-aminohippuric acid (PAH) test

- nontoxic substance
- loosely bound to plasma proteins, which permits its complete removal as the blood passes through the peritubular capillaries
- all the plasma PAH is secreted by the proximal convoluted tubule (except for a small amount of PAH contained in plasma that does not come in contact with functional renal tissue)
- effective renal plasma flow: 600 to 700 mL/min (8% of the renal blood flow does not come into contact with the functional renal tissue)
- average renal blood flow: 1200 mL/min

$$C_{\text{PAH}} (\text{mL/min}) = \frac{U (\text{mg/dL PAH}) \times V (\text{mL/min urine})}{P (\text{mg/dL PAH})}$$

2. Titratable Acidity and Urinary Ammonia

- ability of the kidney to produce an acid urine depends on the tubular secretion of hydrogen ions and production and secretion of ammonia by the cells of the distal convoluted tubule
- normal secretion: 70 mEq/day of acid in the form of titratable acid (H^+), hydrogen phosphate ions (H_2PO_4^-), or ammonium ions (NH_4^+)
- night - lowest pH

- specimen: fresh or toluene-preserved urine specimens collected at 2-hour intervals from patients who have been primed with an acid load consisting of oral ammonium chloride

renal tubular acidosis

- inability to produce an acid urine in the presence of metabolic acidosis
- result from impaired tubular secretion of hydrogen ions associated with the proximal convoluted tubule or defects in ammonia secretion associated with the distal convoluted tubule

3. Phenolsulfonphthalein (PSP) Test

- Difficult because of interference by medications, elevated waste products in patients' serum, the necessity to obtain several very accurately timed urine specimens, and the possibility of producing anaphylactic shock

Review Questions

- The type of nephron responsible for renal concentration is the:
 - Cortical
 - Juxtaglomerular
- The function of the peritubular capillaries is:
 - Reabsorption
 - Filtration
 - Secretion
 - Both A and C
- Blood flows through the nephron in the following order:
 - Efferent arteriole, peritubular capillaries, vasa recta, afferent arteriole
 - Peritubular capillaries, afferent arteriole, vasa recta, efferent arteriole
 - Afferent arteriole, peritubular capillaries, vasa recta, efferent arteriole
 - Efferent arteriole, vasa recta, peritubular capillaries, afferent arteriole
- Filtration of protein is prevented in the glomerulus by:
 - Hydrostatic pressure
 - Oncotic pressure
 - Renin
 - The glomerular filtration barrier
- The renin-angiotensin-aldosterone system is responsible for all of the following except:
 - Vasoconstriction of the afferent arteriole
 - Vasoconstriction of the efferent arteriole
 - Reabsorbing sodium
 - Releasing aldosterone
- The primary chemical affected by the renin-angiotensin-aldosterone system is:
 - Chloride
 - Sodium
 - Potassium
 - Hydrogen
- Secretion of renin is stimulated by:
 - Juxtaglomerular cells
 - Angiotensin I and II
 - Macula densa cells
 - Circulating angiotensin-converting enzyme
- The hormone aldosterone is responsible for:
 - Hydrogen ion secretion
 - Potassium secretion
 - Chloride retention
 - Sodium retention

9. The fluid leaving the glomerulus has a specific gravity of:
- 1.005
 - 1.010
 - 1.015
 - 1.020
10. For active transport to occur, a chemical:
- Must combine with a carrier protein to create electrochemical energy
 - Must be filtered through the proximal convoluted tubule
 - Must be in higher concentration in the filtrate than in the blood
 - Must be in higher concentration in the blood than in the filtrate
11. Which of the tubules is impermeable to water?
- Proximal convoluted tubule
 - Descending loop of Henle
 - Ascending loop of Henle
 - Distal convoluted tubule
12. Glucose will appear in the urine when the:
- Blood level of glucose is 200 mg/dL
 - T_m for glucose is reached
 - Renal threshold for glucose is exceeded
 - All of the above
13. Concentration of the tubular filtrate by the countercurrent mechanism depends on all of the following except:
- High salt concentration in the medulla
 - Water-impermeable walls of the ascending loop of Henle
 - Reabsorption of sodium and chloride from the ascending loop of Henle
 - Reabsorption of water in the descending loop of Henle
14. ADH regulates the final urine concentration by controlling:
- Active reabsorption of sodium
 - Tubular permeability
 - Passive reabsorption of urea
 - Passive reabsorption of chloride
15. Decreased production of ADH:
- Produces a low urine volume
 - Produces a high urine volume
 - Increases ammonia excretion
 - Affects active transport of sodium
16. Bicarbonate ions filtered by the glomerulus are returned to the blood:
- In the proximal convoluted tubule
 - Combined with hydrogen ions
 - By tubular secretion
 - All of the above
17. If ammonia is not produced by the distal convoluted tubule, the urine pH will be:
- Acidic
 - Basic
18. Place the appropriate letter in front of the following clearance substances:
- Exogenous
 - Endogenous
- ____ beta2-microglobulin
 ____ creatinine
 ____ cystatin C
 ____ 125I-iothalamate
19. The largest source of error in creatinine clearance tests is:
- Secretion of creatinine
 - Improperly timed urine specimens
 - Refrigeration of the urine
 - Time of collecting blood sample
20. Given the following information, calculate the creatinine clearance:
 24-hour urine volume: 1000 mL; serum creatinine: 2.0 mg/dL; urine creatinine: 200 mg/dL
21. Clearance tests used to determine the glomerular filtration rate must measure substances that are:
- Not filtered by the glomerulus
 - Completely reabsorbed by the proximal convoluted tubule
 - Secreted in the distal convoluted tubule
 - Neither reabsorbed or secreted by the tubules
22. Performing a clearance test using radionucleotides:
- Eliminates the need to collect urine
 - Does not require an infusion
 - Provides visualization of the filtration
 - Both A and C
23. Variables that are included in the MDRD-IDSM estimated creatinine clearance calculations include all of the following except:
- Serum creatinine
 - Weight
 - Age
 - Gender
24. An advantage to using cystatin C to monitor GFR is that:
- It does not require urine collection
 - It is not secreted by the tubules
 - It can be measured by immunoassay
 - All of the above
25. Solute dissolved in solvent will:
- Raise the vapor pressure
 - Lower the boiling point
 - Decrease the osmotic pressure
 - Lower the freezing point
26. Substances that may interfere with freezing point measurement of urine and serum osmolarity include all of the following except:
- Ethanol
 - Lactic acid
 - Sodium
 - Lipids
27. Clinical osmometers use NaCl as a reference solution because:
- 1 g molecular weight of NaCl will lower the freezing point 1.86°C
 - NaCl is readily frozen
 - NaCl is partially ionized similar to the composition of urine
 - 1 g equivalent weight of NaCl will raise the freezing point 1.86°C
28. The normal serum osmolarity is:
- 50 to 100 mOsm
 - 275 to 300 mOsm
 - 400 to 500 mOsm
 - 3 times the urine osmolarity
29. After controlled fluid intake, the urine-to-serum osmolarity ratio should be at least:
- 1:1
 - 2:1
 - 3:1
 - 4:1
30. Calculate the free water clearance from the following results:
 urine volume in 6 hours: 720 mL; urine osmolarity: 225 mOsm; plasma osmolarity: 300 mOsm

31. To provide an accurate measure of renal blood flow, a test substance should be completely:
- A. Filtered by the glomerulus
 - B. Reabsorbed by the tubules
 - C. Secreted when it reaches the distal convoluted tubule
 - D. Cleared on each contact with functional renal tissue
32. Given the following data, calculate the effective renal plasma flow:
urine volume in 2 hours: 240 mL; urine PAH: 150 mg/dL;
plasma PAH: 0.5 mg/dL
33. Renal tubular acidosis can be caused by the:
- A. Production of excessively acidic urine due to increased filtration of hydrogen ions
 - B. Production of excessively acidic urine due to increased secretion of hydrogen ions
 - C. Inability to produce an acidic urine due to impaired production of ammonia
 - D. Inability to produce an acidic urine due to increased production of ammonia
34. Tests performed to detect renal tubular acidosis after administering an ammonium chloride load include all of the following except:
- A. Urine ammonia
 - B. Arterial pH
 - C. Urine pH
 - D. Titratable acidity

URINALYSIS

Physical Examination of Urine

OUTLINE

- I. Color
- II. Clarity
- III. Specific Gravity
- IV. Odor

COLOR

A. Laboratory Correlation of Urine Color

Color	Cause	Clinical/Laboratory Correlations
Colorless	Recent fluid consumption	Commonly observed with random specimens
Pale yellow	Polyuria or diabetes insipidus	Increased 24-hour volume and low specific gravity
	Diabetes mellitus	Elevated specific gravity and positive glucose test result
	Dilute random specimen	Recent fluid consumption
Dark yellow	Concentrated specimen	May be normal after strenuous exercise or in first morning specimen
	B complex vitamins	
	Dehydration	Fever or burns
	Bilirubin	Yellow foam when shaken and positive chemical test results for bilirubin
	Acriflavine	Negative bile test results and possible green fluorescence
	Nitrofurantoin	Antibiotic administered for urinary tract infections
Orange-yellow	Phenazopyridine (Pyridium)	Drug commonly administered for urinary tract infections
	Phenindione	Anticoagulant, orange in alkaline urine, colorless in acid urine
Yellow-green	Bilirubin oxidized to biliverdin	Colored foam in acidic urine and false-negative chemical test results for bilirubin
Green	<i>Pseudomonas</i> infection	Positive urine culture
Blue-green	Amitriptyline	Antidepressant
	Methocarbamol (Robaxin)	Muscle relaxant, may be green-brown
	Clorets	None
	Indican	Bacterial infections, intestinal disorders
	Methylene Blue	Fistulas
	Phenol	When oxidized
Pink	RBCs	Cloudy urine with positive chemical test results for blood and RBCs visible microscopically

Red	Hemoglobin	Clear urine with positive chemical test results for blood; intravascular hemolysis
	Myoglobin	Clear urine with positive chemical test results for blood; muscle damage
	Beets	Alkaline urine of genetically susceptible persons
	Rifampin	Tuberculosis medication
	Menstrual contamination	Cloudy specimen with RBCs, mucus, and clots
Port wine	Porphyryns	Negative test for blood, may require additional testing
Red-brown	RBCs oxidized to methemoglobin	Seen in acidic urine after standing; positive chemical test result for blood
	Myoglobin	
Brown	Homogentisic acid (alkaptonuria)	Seen in alkaline urine after standing; specific tests are available
Black	Malignant melanoma	Urine darkens on standing and reacts with nitroprusside and ferric chloride
	Melanin or melanogen	
	Phenol derivatives	Interfere with copper reduction tests
	Argyrol (antiseptic)	Color disappears with ferric chloride
	Methyldopa or levodopa	Antihypertensive
	Metronidazole (Flagyl)	Darkens on standing, intestinal and vaginal infections

B. Normal Urine Color

Yellow color of urine

- caused by the presence of a pigment which Thudichum named urochrome

Pigments of Urine

a. urochrome

- a product of endogenous metabolism, and under normal conditions the body produces it at a constant rate
- increases in urine that stands at room temperature

b. uroerythrin

- a pink pigment
- most evident in specimens that have been refrigerated → resulting in the precipitation of amorphous urates
- attaches to the urates, producing a pink color to the sediment

- c. urobilin
 - oxidation product of the normal urinary constituent urobilinogen
 - imparts an orange-brown color to urine that is not fresh.

C. Abnormal Urine Color

1. Dark Yellow/Amber/Orange

- can be caused by the presence of the abnormal pigment bilirubin
- presence is suspected if yellow foam appears when the specimen is shaken
- normal urine - produces only a small amount of rapidly disappearing foam when shake
- large amount of white foam = indicates an increased concentration of protein
- yellow-orange
 - photo-oxidation of large amounts of excreted urobilinogen to urobilin (no yellow foam when the specimen is shaken)
 - caused by the administration of phenazopyridine (brand name Pyridium) or azo-gantrisin compounds to people who have urinary tract infections (with yellow foam when shaken)
 - interferes with chemical tests that are based on color reactions
- yellow-green
- photo-oxidation of bilirubin caused by the presence of biliverdin

2. Red/Pink/Brown

- usual color that blood produces in urine
- range is pink to brown
- depending on the amount of blood, the pH of the urine, and the length of contact
- brown
 - red blood cells in acidic urine for several hours
 - due to the oxidation of hemoglobin to methemoglobin
- fresh brown
 - may also indicate glomerular bleeding resulting from the conversion of hemoglobin to methemoglobin
- hemoglobin and myoglobin
 - produce a red urine and result in a positive chemical test result for blood
 - examination of plasma - differentiation between hemoglobinuria and myoglobinuria
 - myoglobin - breakdown of skeletal muscles; more rapidly cleared in the plasma (does not affect the color of plasma)
 - hemoglobin - in vivo breakdown of RBCs is accompanied by red plasma
- red and cloudy
 - RBCs are present
- port wine
 - caused by porphyrins resulting from the oxidation of porphobilinogen to porphyrins
- nonpathogenic causes: menstrual contamination, ingestion of highly pigmented foods, and medications, eating fresh beets, black berries, medications (rifampin, phenolphthalein, phenindione, and phenothiazines)

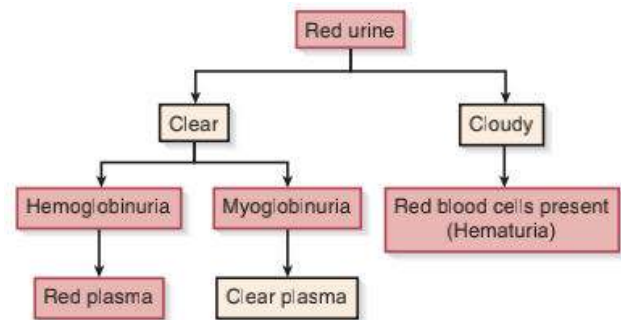


Figure 4-1 Differentiation of red urine testing chemically positive for blood.

3. Brown/Black

- urine may contain melanin or homogentisic acid
 - melanin - an oxidation product of the colorless pigment, melanogen, produced in excess when a malignant melanoma is present
 - homogentisic acid - a metabolite of phenylalanine, imparts a black color to alkaline urine from persons with the inborn-error of metabolism (alcaptonuria)
- can also be caused by levodopa, methyl dopa, phenol derivatives, and metronidazole (Flagyl)

4. Blue/Green

- limited to bacterial infections
- urinary tract infection by *Pseudomonas* species
- intestinal tract infections resulting in increased urinary indican
- green
 - ingestion of deodorizers (Clorets)
 - phenol derivatives found in certain intravenous medications
- blue
 - medications: methocarbamol (Robaxin), methylene blue, and amitriptyline (Elavil)
- purple
 - catheter bags and is caused by indican in the urine or a bacterial infection
 - frequently caused by *Klebsiella* or *Providencia* species

CLARITY

- general term that refers to the transparency or turbidity of a urine specimen
- visually examining the mixed specimen while holding it in front of a light source

Clarity	Term
Clear	No visible particulates, transparent
Hazy	Few particulates, print easily seen through] urine
Cloudy	Many particulates, print blurred through urine
Turbid	Print cannot be seen through urine
Milky	May precipitate or be clotted

A. Normal Clarity

- clear - freshly voided normal urine
- white cloudiness - precipitation of amorphous phosphates and carbonates

B. Nonpathologic Turbidity

- hazy urine in women (normal)
 - presence of squamous epithelial cells and mucus
 - specimens that are allowed to stand or are refrigerated
- improper preservation of a specimen → results in bacterial growth
 - not representative of the actual specimen
 - caused by the precipitation of amorphous phosphates, carbonates, and urates
- amorphous phosphates and carbonates
 - alkaline pH: white precipitate
 - acidic pH: pink brick dust due to the presence of uroerythrin
- Others: semen, fecal contamination, radiographic contrast media, talcum powder, and vaginal creams

C. Pathologic Turbidity

Most common

- RBCs
- white blood cells (WBCs)
- bacteria caused by infection or a systemic organ disorder

Less frequent

- abnormal amounts of nonsquamous epithelial cells (RBCs)
- yeast
- abnormal crystal
- lymph fluid
- lipids

SPECIFIC GRAVITY

- the density of a solution compared with the density of a similar volume of distilled water (SG 1.000) at a similar temperature
- influenced not only by the number of particles present but also by their size
- 1.010 - specific gravity of the plasma filtrate entering the glomerulus
 - isotherm - urine with a specific gravity of 1.010
 - hyposthenuric - below 1.010
 - hypersthenuric - above 1.010
- Normal random specimens: 1.002 to 1.035 (depending on the hydration)
- Specimens with <1.002 - probably not urine
- Abnormal amount: above 1.040
 - in an intravenous pyelogram
 - caused by the excretion of the injected radiographic contrast media
 - receiving dextran or other receiving dextran or other]
 - can be measured using the reagent strip chemical test or osmometry because they are not affected by these high-molecular-weight substances

Method	Principle
Refractometry	Refractive index
Osmolality	Changes in colligative properties by particle number
Reagent strip	pK _a changes of a polyelectrolyte by ions present

A. Refractometer

- Refractive index - comparison of the velocity of light in air with the velocity of light in a solution
- The concentration of dissolved particles present in the solution determines the velocity and angle at which light passes through a solution.
- Specific gravity scale is calibrated in terms of the angles (in prism) at which light passes through the specimen.
- Advantage: using a small volume of specimen (one or two drops)
- Temperature is compensated between 15°C and 38°C (corrections not needed)
- Corrections for glucose and protein must be calculated by subtracting
 - 0.003 for each gram of protein present
 - 0.004 for each gram of glucose present
- Calibration: distilled water that should read 1.000, further checked using 5% NaCl, which as shown in the refractometer conversion tables should read 1.022 ± 0.001, or 9% sucrose that should read 1.034 ± 0.001

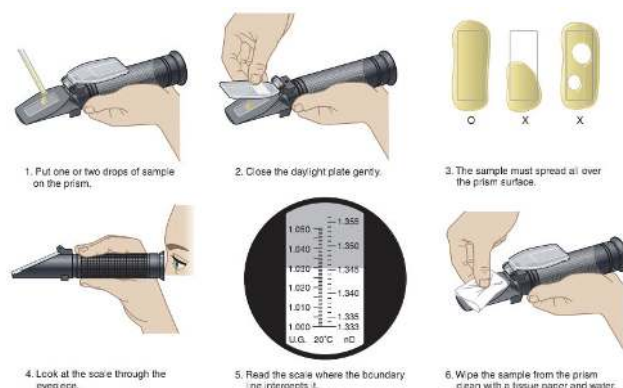
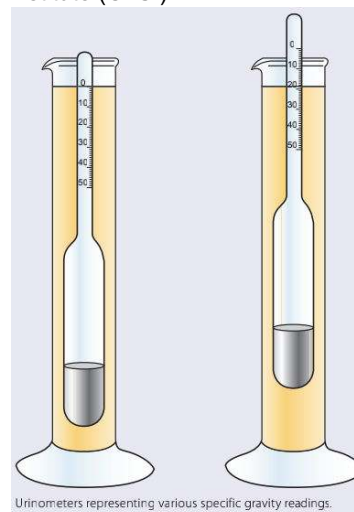


Figure 4-2 Steps in the use of the urine specific gravity refractometer. (Courtesy of HSG Precision Cells, Inc., 195G Central Ave., Farmingdale, NY, 11735.)

B. Urinometry

- Urinometer - consists of a weighted float attached to a scale that has been calibrated in terms of urine specific gravity
- The weighted float displaces a volume of liquid equal to its weight and has been designed to sink to a level of 1.000 in distilled water
- less accurate than the other methods currently available
- not recommended by the Clinical and Laboratory Standards Institute (CLSI)



Urinometers representing various specific gravity readings.

Example

A specimen containing 1 g/dL protein and 1 g/dL glucose has a specific gravity reading of 1.030. Calculate the corrected reading.

$$1.030 - 0.003 (\text{protein}) = 1.027 - 0.004 (\text{glucose}) = 1.023 \text{ corrected specific gravity}$$

C. Osmolality

- specific gravity depends on the number of particles present in a solution and the density of these particles
- osmolality is affected only by the number of particles present
- evaluating concentration ability
 - sodium (molecular weight 23)
 - chloride (molecular weight 35.5)
 - urea (molecular weight 60) - will contribute more to the specific gravity than will the sodium and chloride molecules
- unit of measure: milliosmole (mOsm)

Osmole

$\frac{1 \text{ g molecular weight of a substance}}{\text{number of particles into which it dissociates}}$

- Nonionizing substance such as glucose (molecular weight, 180) contains 180 g per osmole, whereas sodium chloride (NaCl) (molecular weight 58.5), if completely dissociated, contains 29.25 g per osmole

Osmolality vs Osmolarity

- An osmolal solution of glucose has 180 g of glucose dissolved in 1 kg of solvent.
- An osmolar solution of glucose has 180 g of glucose dissolved in 1 L of solvent.
 - can be determined by measuring colligative property and comparing this value with the value obtained from the pure solvent
 - colligative property - a property that is mathematically related to the number of particles in the solution

Changes in Colligative Properties

- ✓ lower freezing point
- ✓ higher boiling point
- ✓ increased osmotic pressure
- ✓ lower vapor pressure

Particle Changes to Colligative Properties		
Property	Normal Pure Water Point	Effect of 1 Mole of Solute
Freezing Point	0°C	Lowered 1.86°C
Boiling Point	100°C	Raised 0.52°C
Vapor Pressure	2.38 mm/ Hg at 25°C	Lowered 0.3 mm/ Hg at 25°C
Osmotic Pressure	0 mm/Hg	Increased 1.7×10^9 mm/Hg

A₂O Advanced Automated Osmometer

- uses freezing point depression to measure osmolality
- providing a more automated method for measuring both urine and serum osmolality

D. Reagent Strip Specific Gravity

- convenient way to perform the routine urinalysis by eliminating the need for an additional procedure
- based on the change in pK_a (dissociation constant) of a polyelectrolyte in an alkaline medium
- polyelectrolyte ionizes, releasing hydrogen ions in proportion to the number of ions in the solution
- higher the concentration of urine → the more hydrogen ions are released → lowering the pH
- Indicator: bromthymol blue
- Higher specific gravity: from blue (1.000 [alkaline]) to shades of green, to yellow (1.030 [acid])
- Intervals: 0.005

ODOR

- not a part of the routine urinalysis
 - Freshly voided urine - has a faint aromatic odor
 - ammonia odor - breakdown of urea
 - strong, unpleasant odor - bacterial infections
 - sweet or fruity odor - diabetic ketones
 - strong odor of maple syrup - maple syrup urine disease
 - unusual or pungent urine odor - onions, garlic, and asparagus

Odor	Cause
Aromatic	Normal
Foul, ammonia-like	Bacterial decomposition, urinary tract infection
Fruity, sweet	Ketones (diabetes mellitus, starvation, vomiting)
Maple syrup	Maple syrup urine disease
Mousy	Phenylketonuria
Rancid	Tyrosinemia
Sweaty feet	Isovaleric acidemia
Cabbage	Methionine malabsorption
Bleach	Contamination

Review Questions

- The concentration of a normal urine specimen can be estimated by which of the following?
 - Color
 - Clarity
 - Foam
 - Odor
- The normal yellow color of urine is produced by:
 - Bilirubin
 - Hemoglobin
 - Urobilinogen
 - Urochrome
- The presence of bilirubin in a urine specimen produces a:
 - Yellow foam when shaken
 - White foam when shaken
 - Cloudy specimen
 - Yellow-red specimen
- A urine specimen containing melanin will appear:
 - Pale pink
 - Dark yellow
 - Blue-green

- D. Black
5. Specimens that contain hemoglobin can be visually distinguished from those that contain RBCs because:
 - A. Hemoglobin produces a clear, yellow specimen
 - B. Hemoglobin produces a cloudy pink specimen
 - C. RBCs produce a cloudy red specimen
 - D. RBCs produce a clear red specimen
 6. A patient with a viscous orange specimen may have been:
 - A. Treated for a urinary tract infection
 - B. Taking vitamin B pills
 - C. Eating fresh carrots
 - D. Taking antidepressants
 7. The presence of a pink precipitate in a refrigerated specimen is caused by:
 - A. Hemoglobin
 - B. Urobilin
 - C. Uroerythrin
 - D. Beets
 8. Microscopic examination of a clear urine that produces a white precipitate after refrigeration will show:
 - A. Amorphous urates
 - B. Porphyrins
 - C. Amorphous phosphates
 - D. Yeast
 9. The color of urine containing porphyrins will be:
 - A. Yellow-brown
 - B. Green
 - C. Orange
 - D. Port wine
 10. Which of the following specific gravities would be most likely to correlate with a pale yellow urine?
 - A. 1.005
 - B. 1.010
 - C. 1.020
 - D. 1.030
 11. A urine specific gravity measured by refractometer is 1.029, and the temperature of the urine is 14°C. The specific gravity should be reported as:
 - A. 1.023
 - B. 1.027
 - C. 1.029
 - D. 1.032
 12. The principle of refractive index is to compare:
 - A. Light velocity in solutions with light velocity in solids
 - B. Light velocity in air with light velocity in solutions
 - C. Light scattering by air with light scattering by solutions
 - D. Light scattering by particles in solution
 13. A correlation exists between a specific gravity by refractometer of 1.050 and a:
 - A. 2+ glucose
 - B. 2+ protein
 - C. First morning specimen
 - D. Radiographic dye infusion
 14. A cloudy urine specimen turns black upon standing and has a specific gravity of 1.012. The major concern about this specimen would be:
 - A. Color
 - B. Turbidity
 - C. Specific gravity
 - D. All of the above
 15. A specimen with a specific gravity of 1.035 would be considered:
 - A. Isosthenuric
 - B. Hyposthenuric
 - C. Hypersthenuric
 - D. Not urine
 16. A specimen with a specific gravity of 1.001 would be considered:
 - A. Hyposthenuric
 - B. Not urine
 - C. Hypersthenuric
 - D. Isosthenuric
 17. A strong odor of ammonia in a urine specimen could indicate:
 - A. Ketones
 - B. Normalcy
 - C. Phenylketonuria
 - D. An old specimen
 18. The microscopic of a clear red urine is reported as many WBCs and epithelial cells. What does this suggest?
 - A. Urinary tract infection
 - B. Dilute random specimen
 - C. Hematuria
 - D. Possible mix-up of specimen and sediment
 19. Which of the following would contribute the most to a urine osmolality?
 - A. One osmole of glucose
 - B. One osmole of urea
 - C. One osmole of sodium chloride
 - D. All contribute equally
 20. Which of the following colligative properties is not stated correctly?
 - A. The boiling pointing is raised by solute
 - B. The freezing point is raised by solute
 - C. The vapor pressure is lowered by solute
 - D. The osmotic pressure is raised by solute
 21. An osmole contains:
 - A. One gram molecular weight of solute dissolved in one liter of solvent
 - B. One gram molecular weight of solute dissolved in one kilogram of solvent
 - C. Two gram molecular weights of solute dissolved in one liter of solvent
 - D. Two gram molecular weights of solute dissolved in one kilogram of solvent
 22. The unit of osmolality measured in the clinical laboratory is the:
 - A. Osmole
 - B. Milliosmole
 - C. Molecular weight
 - D. Ionic charge
 23. In the reagent strip specific gravity reaction the polyelectrolyte:
 - A. Combines with hydrogen ions in response to ion concentration
 - B. Releases hydrogen ions in response to ion concentration
 - C. Releases hydrogen ions in response to pH
 - D. Combines with sodium ions in response to pH
 24. Which of the following will react in the reagent strip specific gravity test?
 - A. Glucose
 - B. Radiographic dye
 - C. Protein
 - D. Chloride

Chemical Examination of Urine

OUTLINE

- I. Reagent Strips
- II. pH
- III. Protein
- IV. Glucose
- V. Ketones
- VI. Blood
- VII. Bilirubin
- VIII. Urobilinogen
- IX. Nitrite
- X. Leukocyte Esterase
- XI. Specific Gravity

REAGENT STRIPS

A. Introduction

- simple, rapid means for performing medically significant chemical analysis of urine
- including
 - ✓ pH
 - ✓ protein
 - ✓ glucose
 - ✓ ketones
 - ✓ blood
 - ✓ bilirubin
 - ✓ urobilinogen
 - ✓ nitrite
 - ✓ leukocytes
 - ✓ specific gravity
- consist of chemical-impregnated absorbent pads attached to a plastic strip
- color-producing chemical reaction takes place when the absorbent pad comes in contact with urine
- interpreted by comparing the color produced on the pad within the required time frame with a chart supplied by the manufacturer
- a semiquantitative value of trace: 1+, 2+, 3+, or 4+
- estimate of the milligrams per deciliter present is available for appropriate testing areas

Two Major Types of Reagent Strips

1. Multistix (Siemens Healthcare Diagnostics, Deerfield, IN)
2. Chemstrip (Roche Diagnostics, Indianapolis, IN)

B. Reagent Strip Technique

Procedure

1. Dip the reagent strip briefly into a well-mixed uncentrifuged urine specimen at room temperature.
2. Remove excess urine by touching the edge of the strip to the container as the strip is withdrawn.
3. Blot the edge of the strip on a disposable absorbent pad.
4. Wait the specified amount of time for the reaction to occur.
5. Compare the color reaction of the strip pads to the manufacturer's color chart in good lighting.

Errors Caused by Improper Technique

1. Formed elements such as red and white blood cells sink to the bottom of the specimen and will be undetected in an unmixed specimen.

2. Allowing the strip to remain in the urine for an extended period may cause leaching of reagents from the pads.
3. Excess urine remaining on the strip after its removal from the specimen can produce a run-over between chemicals on adjacent pads, producing distortion of the colors. To ensure against run-over, blotting the edge of the strip on absorbent paper and holding the strip horizontally while comparing it with the color chart is recommended.
4. The timing for reactions to take place varies between tests and manufacturers, and ranges from an immediate reaction for pH to 120 seconds for leukocyte esterase. For the best semiquantitative results, the manufacturer's stated time should be followed; however, when precise timing cannot be achieved, manufacturers recommend that reactions be read between 60 and 120 seconds, with the leukocyte esterase reaction read at 120 seconds.
5. A good light source is essential for accurate interpretation of color reactions.
6. The strip must be held close to the color chart without actually being placed on the chart. Automated reagent strip instruments standardize the color interpretation and timing of the reaction and are not subject to room lighting deficiencies or inconsistency among laboratory personnel (Appendix A).
7. Reagent strips and color charts from different manufacturers are not interchangeable.
8. Specimens that have been refrigerated must be allowed to return to room temperature prior to reagent strip testing, as the enzymatic reactions on the strips are temperature dependent.

C. Handling and Storing Reagent Strips

- Deterioration caused by moisture, volatile chemicals, heat, and light
- Packaged in opaque containers with a desiccant to protect them from light and moisture
- Stored at room temperature below 30°C (but never refrigerated)
- Care must be taken not to touch the chemical pads when removing the strips.

D. Quality Control of Reagent Strips

- every 24 hours
- checked with both positive and negative controls
- new bottle of reagent strips is opened
- Distilled water - not recommended as a negative control because reagent strip chemical reactions are designed to perform at ionic concentrations similar to urine

Reagent Strip Interference

- masking of color reactions by the orange pigment present in the urine of persons taking phenazopyridine compounds

E. Confirmatory Testing

- Nonreagent strip testing procedures using tablets and liquid chemicals
- use of automated strip readers

PH

A. Introduction

- healthy individual **first morning specimen** - slightly acidic pH of **5.0 to 6.0**
- after meals - more alkaline pH (alkaline tide)
- normal random samples - range from **4.5 to 8.0**
- must be considered in conjunction with other patient
- information: acid-base content of the blood, the patient's renal function, the presence of a urinary tract infection, the patient's dietary intake, and the age of the specimen

B. Clinical Significance

- **determining the existence of systemic acid-base disorders of metabolic or respiratory origin and in the management of urinary conditions that require the urine to be maintained at a specific pH**
 - respiratory or metabolic acidosis not related to renal function disorders - the urine is acidic
 - respiratory or metabolic alkalosis is present - the urine is alkaline
- precipitation of inorganic chemicals dissolved in the urine forms urinary crystals and renal calculi (depends on urinary pH)
- **maintaining urine at an alkaline pH - discourages formation of the calculi**
- **maintaining an acidic urine - valuable in treating urinary tract infections caused by urea-splitting organisms** because they do not multiply as readily in an acidic medium
- persons on **high-protein and high-meat diets - acidic urine**,
- **vegetarians - alkaline**, due to the formation of bicarbonate following digestion of many fruits and vegetables
- **freshly excreted urine - does not reach above 8.5 in normal or abnormal conditions**

Causes of Acid and Alkaline Urine	
Acid Urine	Alkaline Urine
Emphysema	Hyperventilation
Diabetes mellitus	Vomiting
Starvation	Renal tubular acidosis
Dehydration	Presence of urease-producing bacteria
Diarrhea	Vegetarian diet
Presence of acid-producing bacteria (<i>Escherichia coli</i>)	Old specimens
High-protein diet	
Cranberry juice	
Medications (methenamine mandelate [Mandelamine], fosfomycin tromethamine [Monurol])	

C. Reagent Strip Reactions

- use a **double-indicator system of methyl red and bromthymol blue**
 - Methyl red
 - **red to yellow - pH range 4 to 6**
 - Bromthymol blue
 - **yellow to blue - pH range of 6 to 9**
- orange at pH 5 through yellow and green to a final deep blue at pH 9

Methyl red + H⁺ → bromthymol blue – H⁺
(Red-orange → yellow) (green → blue)

- ∴ Care must be taken to prevent run-over between the pH testing area and the adjacent, highly acidic protein testing area on Multistix, as this may produce a falsely acidic reading in an alkaline urine.

pH Reagent Strip	
Reagents	Methyl red, bromthymol blue
Sensitivity	Multistix: 5.0 to 8.5 in 0.5 increments Chemstrip: 5.0 to 9.0 in 1.0 increments
Sources of error/interference	No known interfering substances Run-over from adjacent pads Old specimens
Correlations with other tests	Nitrite Leukocytes Microscopic

PROTEIN

A. Introduction

- **Proteinuria**
 - **most indicative of renal disease**
- associated with early renal disease
- **albumin - major serum protein found in normal urine due to its low molecular weight**
- **Normal protein in urine: less than 10 mg/dL or 100 mg per 24 hours**
- protein consists primarily of low-molecular-weight serum proteins that have been filtered by the glomerulus and proteins produced in the genitourinary tract
- Other proteins:
 - small amounts of serum and tubular microglobulins
 - **Tamm-Horsfall protein (uromodulin) - produced by the renal**
 - tubular epithelial cells, forms the matrix of casts formed in the distal convoluted tubule
 - proteins from prostatic, seminal, and vaginal secretions

B. Clinical Significance

- **Clinical proteinuria - indicated at 30 mg/dL or greater (300 mg/L)**

Causes of Proteinuria

1. Prerenal Proteinuria

- **caused by conditions affecting the plasma prior to its reaching the kidney**
- **not indicative of actual renal disease**
- frequently transient
- caused by increased levels of low-molecular-weight plasma proteins (hemoglobin, myoglobin, and the **acute phase reactants associated with infection and inflammation**)
- increased filtration of these proteins exceeds the normal reabsorptive capacity of the renal tubules → overflow of the proteins into the urine
- **not discovered in a routine urinalysis**

a. Bence Jones Proteins

- **multiple myeloma - proliferative disorder of the immunoglobulin-producing plasma cells**

2. Renal Proteinuria

- **true renal disease**
- either **glomerular or tubular damage**

a. Glomerular Proteinuria

- glomerular membrane is damaged → selective filtration is impaired → increased amounts of serum protein and eventually red and white blood cells pass through the membrane → excreted in the urine
- Benign proteinuria - strenuous exercise, high fever, dehydration, and exposure to cold (transient)

b. Microalbuminuria

- diabetic nephropathy leading to reduced glomerular filtration and eventual renal failure - common in both type 1 and type 2 diabetes mellitus
- associated with an increased risk of cardiovascular disease
- onset of renal complications can first be predicted with the detection of microalbuminuria
- Collected in 24-hour specimen before with albumin/24 hours or as the albumin excretion (AER) in µg/min
 - significant
 - 30 to 300 mg of albumin is excreted in 24 hours
 - AER 20 to 200 µg/min

c. Orthostatic (Postural) Proteinuria

- persistent benign proteinuria occurs frequently in young adults
- occurs following periods spent in a vertical posture and disappears when a horizontal position is assumed
- requested to empty the bladder before going to bed, collect a specimen immediately upon arising in the morning, and collect a second specimen after remaining in a vertical position for several hours
- negative reading - first morning specimen
- positive result - second specimen

d. Tubular Proteinuria

- exposure to toxic substances and heavy metals, severe viral infections, and Fanconi syndrome
- slightly above normal to 4 g/day
- markedly elevated protein levels are seldom
- seen in tubular disorders

3. Postrenal Proteinuria

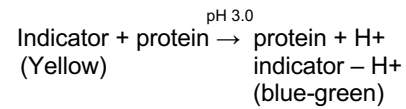
- Protein can be added to a urine specimen as it passes through the structures of the lower urinary tract (ureters, bladder, urethra, prostate, and vagina)
- Bacteria and inflammations → exudates that has protein from interstitial fluid
- presence of blood as the result of injury or menstrual contamination contributes protein
- presence of prostatic fluid and large amounts of spermatozoa

C. Reagent Strip Reactions

- Principle: protein error of indicators to produce a visible colorimetric reaction
- protein (primarily albumin) accepts hydrogen ions from the indicator
- more sensitive to albumin (contains more amino groups to accept the hydrogen ions than other proteins)
- Contains
 - Multistix: tetrabromophenol blue
 - Chemstrip: 3',3'',5',5''-tetrachlorophenol or 3,4,5,6 tetrabromo-sulfonphthalein
 - acid buffer to maintain the pH at a constant level

Reactions:

- yellow - pH level of 3, absence of protein
- green to blue - as pH increases
- Trace values: less than 30 mg/dL



D. Reaction Interference

- Major source of error: highly buffered alkaline urine that overrides the acid buffer system → rise in pH and a color change unrelated to protein concentration
- False-positive readings - obtained when the reaction does not take place under acidic conditions, high specific gravity, presence of contamination in the container

1. Sulfosalicylic Acid Precipitation (SSA) Test

- cold precipitation test that reacts equally with all forms of protein

2. Testing for Microalbuminuria

- Immunochemical assays: the Micral-Test (Roche Diagnostics, Indianapolis, IN) and the ImmunoDip (Sakiusi Diagnostics, Framingham, MA)

3. Albumin:Creatinine Ratio

- simultaneous measurement of albumin/protein and creatinine that permits an estimation of the 24-hour microalbumin excretion
- comparing the albumin excretion to the creatinine excretion, the albumin reading can be corrected for overhydration and dehydration in a random sample
- albumin low-test pad is changed to a dye-binding reaction that is more specific for albumin than the protein error of indicators' reaction on strips measuring protein

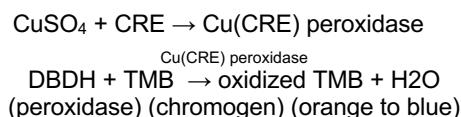
Reagent Strip Reactions

a. Albumin

- dye - bis(3',3''-diiodo-4',4''-dihydroxy-5',5''-dinitrophenyl)-3,4,5,6-tetrabromosulphonphthalein (DIDNTB)
- can measure albumin between 8 and 15 mg/dL (80 to 150 mg/L)
- Reaction interference: highly buffered alkaline urine
- Resolution: using paper treated with bis-(heptapropylene glycol) carbonate
- Colors: pale green to aqua blue

b. Creatinine

- Principle: based on the pseudoperoxidase activity of copper-creatinine complexes
- Reagent strips contain
 - copper sulfate (CuSO₄)
 - 3,3',5,5'-tetramethyl-benzidine (TMB)
 - diisopropyl benzene dihydroperoxide (DBDH)
- Creatinine in the urine combines with the copper sulfate to form copper-creatinine peroxidase.
- This reacts with the peroxide DBDH, releasing oxygen ions that oxidize the chromogen TMB and producing a color change from orange through green to blue



- Falsely elevated results: **visibly bloody urine or presence of the gastric acid-reducing medication cimetidine (Tagamet)**
 - Reaction interference: **abnormally colored urine**
 - Normally present in concentrations of **10 to 300 mg/dL**
- c. Albumin/Protein:Creatinine Ratio
- Automated and manual methods
 - Clinitek Microalbumin reagent strips

Protein Reagent Strip	
Reagents	Multistix: Tetrabromophenol blue Chemstrip: 3',3'',5',5''-tetrachlorophenol 3,4,5,6-tetrabromosulfophthalein
Sensitivity	Multistix: 15 to 30 mg/dL albumin Chemstrip: 6 mg/dL albumin
Sources of error/interference	<u>False-positive</u> Highly buffered interference alkaline urine Pigmented specimens, phenazopyridine Quaternary ammonium compounds (detergents) Antiseptics, chlorhexidine Loss of buffer from prolonged exposure of the strip to the specimen reagent High specific gravity <u>False-negative</u> Proteins other than albumin Microalbuminuria Blood
Correlations with other tests	Nitrite Leukocytes Microscopic

GLUCOSE

A. Introduction

- **most frequently performed chemical analysis on urine**

B. Clinical Significance

- almost all the glucose filtered by the glomerulus is actively reabsorbed in the proximal convoluted tubule
- urine contains only minute amounts of glucose
- hyperglycemia - tubular transport of glucose has reached its renal threshold → appears in the urine
- **160 to 180 mg/dL** - blood level at which tubular reabsorption stops (renal threshold)
- glycosuria - following a meal containing a high glucose content
- most informative glucose results are obtained from specimens collected under controlled conditions (**fasting**)
 - tested 2 hours after meals
 - first morning specimen - does not always represent a fasting specimen because glucose from an evening meal may remain in the bladder overnight

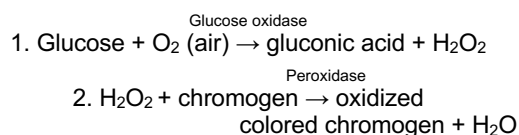
- gestational diabetes - during pregnancy and disappears after delivery (**6th month**)
 - Hormones secreted by the placenta block the action of insulin → insulin resistance and hyperglycemia
 - **glucose crosses the placenta whereas insulin does not**
- renal glycosuria - glycosuria occurs in the absence of hyperglycemia when the reabsorption of glucose by the renal tubules is compromised

Clinical Significance of Urine Glucose	
Hyperglycemia-Associated	Renal-Associated
Diabetes mellitus Pancreatitis Pancreatic cancer Acromegaly Cushing syndrome Hyperthyroidism Pheochromocytoma Central nervous system damage Stress Gestational diabetes	Fanconi syndrome Advanced renal disease Osteomalacia Pregnancy

C. Reagent Strip Reaction

1. Glucose oxidase

- impregnating the testing area with a mixture of glucose oxidase, peroxidase, chromogen, and buffer to produce a **double sequential enzyme reaction**
- In the first step, glucose oxidase catalyzes a reaction between glucose and room air (oxygen) to produce gluconic acid and peroxide.
- In the second step, peroxidase catalyzes the reaction between peroxide and chromogen to form an oxidized colored compound that is produced in direct proportion to the concentration of glucose



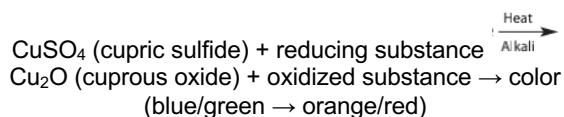
Reagent strips chromogens

- Multistix: **potassium iodide** (green to brown)
- Chemstrip: **tetramethylbenzidine** (yellow to green)

∴ Keep in mind that **table sugar is sucrose**, a nonreducing sugar, and does not react with Clinitest or glucose oxidase strips and therefore cannot be used as a control or in preparation of a laboratory exercise for glucose testing.

2. Copper Reduction Test (Clinitest)

- relies on the ability of glucose and other substances to reduce **copper sulfate to cuprous oxide** in the presence of alkali and heat
- A color change progressing from a negative blue (CuSO_4) through green, yellow, and orange/red (Cu_2O) occurs when the reaction takes place.



- classic Benedict solution - contained copper sulfate, sodium carbonate, and sodium citrate buffer
- Urine was added to the solution, heat was applied, and the resulting precipitate was observed for color.

Clinical Significance

- Clinitest is often performed on pediatric specimens from patients up to at least the age of 2 years.
- The appearance of other reducing sugars is usually of minimal clinical significance, and **lactose is frequently found in the urine of nursing mothers**

D. Reaction Interference

- False-positive reactions are not obtained from other urinary constituents, including reducing sugars that may be present
- Strong reducing agents (such as ascorbic acid) - prevent oxidation of the chromogen may produce false-negative results
- Resolution: incorporating additional chemicals into the test pads (iodate that oxidizes ascorbic acid)
- False-negative - **technical error of allowing specimens to remain unpreserved at room temperature for extended periods, subjecting the glucose to bacterial degradation**

Glucose Reagent Strip	
Reagents	<u>Multistix</u> Glucose oxidase Peroxidase Potassium iodide <u>Chemstrip</u> Glucose oxidase Peroxidase Tetramethylbenzidine
Sensitivity	Multistix: 75 to 125 mg/dL Chemstrip: 40 mg/dL
Sources of error/interference	<u>False-positive</u> Contamination by oxidizing agents and detergents <u>False-negative</u> High levels of ascorbic acid High levels of ketones High specific gravity Low temperatures Improperly preserved specimens
Correlations with other tests	Ketones Protein

KETONES

A. Introduction

Three intermediate products of fat metabolism

1. **acetone (2%)**
 2. **acetoacetic acid (20%)**
 3. **β -hydroxybutyrate (78%)**
- Metabolized fat should be broken down into **carbon dioxide** and **water** (normally).

B. Clinical Significance

Increased fat metabolism

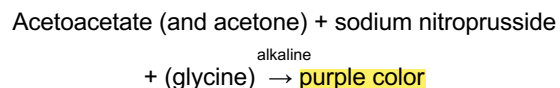
- ✓ ability to metabolize carbohydrate
- ✓ increased loss of carbohydrate from vomiting
- ✓ inadequate intake of carbohydrate associated with starvation and malabsorption
- **most valuable in the management and monitoring of insulin dependent (type 1) diabetes mellitus**
- Ketonuria - **shows a deficiency in insulin**
- Increased accumulation of ketones in the blood $\rightarrow$ electrolyte imbalance, dehydration $\rightarrow$ acidosis and eventual diabetic coma

Clinical Significance of Urine Ketones

Diabetic acidosis
 Insulin dosage monitoring
 Starvation
 Malabsorption/pancreatic disorders
 Strenuous exercise
 Vomiting
 Inborn errors of amino acid metabolism

C. Reagent Strip Reactions

- **sodium nitroprusside (nitroferricyanide)** - used to measure ketones
- **purple color** - acetoacetic acid in an alkaline medium reacts with sodium nitroprusside
- does not measure β -hydroxybutyrate
- only slightly sensitive to acetone when glycine is also present
 - **both are derived from acetoacetic acid**, so presence can be assumed
- Results:
 - small (1+)
 - moderate (2+)
 - large (3+) or semiquantitatively as negative
 - trace (5 mg/dL)
 - small (15 mg/dL)
 - moderate (40 mg/dL)
 - large (80 to 160 mg/dL)



D. Reaction Interference

- Large amounts of **levodopa** and medications containing **sulfhydryl groups**, including mercaptoethane sulfonate sodium (MESNA) and captopril
- **Improperly timed readings**: frequently fade on standing, whereas color development from acetoacetic acid increases, resulting in false-positive

- Falsely decreased values: volatilization of acetone and the breakdown of acetoacetic acid by bacteria (seen in **improperly preserved specimens**)

Acetest tablet

- **confirmatory testing**
- provides sodium nitroprusside, glycine, disodium phosphate, and lactose in tablet form
- lactose - gives better color differentiation
- hygroscopic
- not completely absorbed within 30 seconds, a new tablet should be used

Ketone Reagent Strip	
Reagents	Sodium nitroprusside Glycine (Chemstrip)
Sensitivity	Multistix: 5 to 10 mg/dL acetoacetic acid Chemstrip: 9 mg/dL acetoacetic acid; 70 mg/dL acetone
Sources of error/interference	False-positive: Phthalein dyes Highly pigmented red urine Levodopa Medications containing free sulfhydryl groups False-negative: Improperly preserved specimens
Correlations with other tests	Glucose

BLOOD

A. Introduction

- Hematuria
 - intact red blood cells
 - produces a **cloudy urine**
- Hemoglobinuria
 - product of red blood cell destruction
 - **clear red specimen**
- **>5 cells/uL of urine - considered significant**
- **chemical tests for hemoglobin - provide most accurate means for determining the presence of blood**

B. Clinical Significance

1. Hematuria

- closely related to **disorders of renal or genitourinary origin**
- bleeding is the result of trauma or damage to the organs of these systems
- causes: renal calculi, glomerular diseases, tumors, trauma, pyelonephritis, exposure to toxic chemicals, and anticoagulant therapy
- nonpathologic cause: strenuous exercise and during menstruation

2. Hemoglobinuria

- **lysis of red blood cells** produced in the urinary tract, particularly in dilute, alkaline urine → mixture of hemoglobinuria and hematuria
- **intravascular hemolysis** and the subsequent filtering of hemoglobin through the glomerulus → no red blood cells
- Reabsorption of filtered hemoglobin → results in the appearance of **large yellow-brown granules of denatured ferritin called hemosiderin** in the renal tubular epithelial cells and in the urine sediment

3. Myoglobinuria

- **heme-containing protein found in muscle tissue**
- reacts positively with the reagent strip test for blood
- produces a **clear red-brown urine**
- associated with **muscle destruction (rhabdomyolysis)**
- causes: trauma, crush syndromes, prolonged coma, convulsions, muscle-wasting diseases, alcoholism, heroin abuse, and extensive exertion
- heme portion of myoglobin - toxic to the renal tubules, and high concentrations can cause acute renal failure

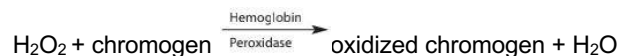
Hemoglobinuria Versus Myoglobinuria

- larger hemoglobin molecules - **precipitated by the ammonium sulfate**
- **myoglobin - remains in the supernatant**

Clinical Significance of a Positive Reaction for Blood		
Hematuria	Hemoglobinuria	Myoglobinuria
Renal calculi Glomerulonephritis Pyelonephritis Tumors Trauma Exposure to toxic chemicals Anticoagulants Strenuous exercise	Transfusion reactions Hemolytic anemias Severe burns Infections/malaria Strenuous exercise/red blood cell trauma Brown recluse spider bites	Muscular trauma/crush syndromes Prolonged coma Convulsions Muscle-wasting diseases Alcoholism/overdose Drug abuse Extensive exertion Cholesterol-lowering statin medications

C. Reagent Strip Reactions

- use the **pseudoperoxidase activity of hemoglobin** to catalyze a reaction between the heme component of both hemoglobin and myoglobin and the chromogen tetramethylbenzidine to produce an oxidized chromogen, which has a **green-blue color**.
- presence of free hemoglobin/myoglobin - negative yellow through green to a strongly positive green-blue appears on the pad
- **liberated hemoglobin - speckled pattern on the pad**



D. Reaction Interference

- False-positive reactions and false-negative reactions may occur

Ketone Reagent Strip	
Reagents	Multistix: Diisopropylbenzene dihydroperoxide and 3,3',5,5'-tetramethylbenzidine Chemstrip: dimethyldihydroperoxy-hexane and tetramethylbenzidine
Sensitivity	Multistix: 5 to 20 RBCs/mL, 0.015 to 0.062 mg/dL hemoglobin Chemstrip: 5 RBCs/mL, hemoglobin corresponding to 10 RBCs/mL

Sources of error/interference	False-positive: Strong oxidizing agents Bacterial peroxidases Menstrual contamination False-negative: High specific gravity/crenated cells Formalin Captopril High concentrations of nitrite Ascorbic acid greater than 25 mg/dL Unmixed specimens
Correlations with other tests	Protein Microscopic

BILIRUBIN

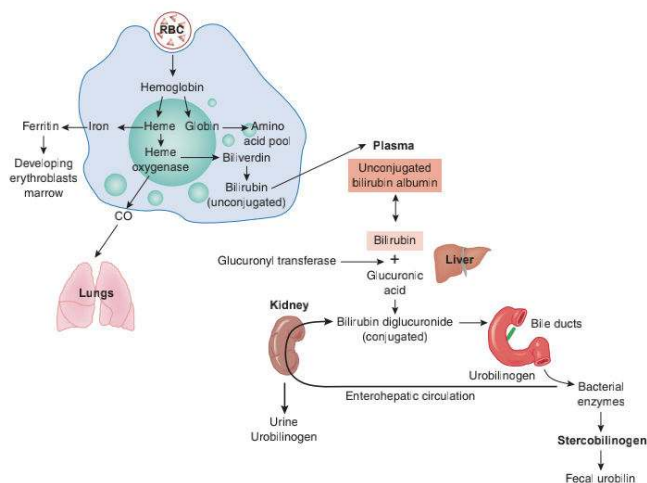
A. Introduction

early indication of liver disease

often detected long before the patient exhibits jaundice

Bilirubin Production

- Bilirubin
 - highly pigmented yellow compound
 - degradation product of hemoglobin
 - liberated hemoglobin components
 - iron - reused
 - protein - reused
 - protoporphyrin - converted to bilirubin and released to circulation → binds to albumin → transported to the liver
- unconjugated bilirubin - bound to albumin and water insoluble that kidneys cannot excrete for the same reasons
- conjugated bilirubin - conjugated with glucuronic acid by the action of glucuronyl transferase to form water-soluble bilirubin diglucuronide in the liver
- does not appear in the urine because it is passed directly from the liver into the bile duct and on to the intestine
- intestinal bacteria reduce bilirubin to urobilinogen → oxidized and excreted in the feces in the form of stercobilinogen and urobilin



B. Clinical Significance

- Only conjugated bilirubin- appear in the urine when the normal degradation cycle is disrupted by bile duct obstruction (post-hepatic jaundice) or when the integrity of the liver is damaged (hepatic jaundice)
- Jaundice due to increased destruction of RBCs - does not produce bilirubinuria (this is because the serum bilirubin is present in the unconjugated form and the kidneys cannot excrete it)

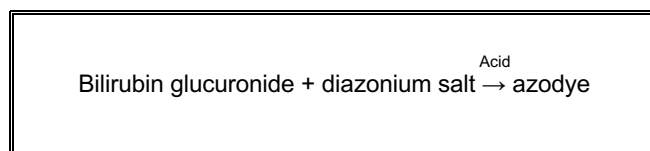
Urine Bilirubin and Urobilinogen in Jaundice		
	Urine Bilirubin	Urine Urobilinogen
Bile duct obstruction	+++	Normal
Liver damage	+ or -	++
Hemolytic disease	Negative	+++

Clinical Significance of Urine Bilirubin	
Hepatitis	Other liver disorders
Cirrhosis	Biliary obstruction (gallstones, carcinoma)

C. Reagent Strip Reactions

Diazo

Bilirubin combines with 2,4-dichloroaniline diazonium salt or 2,6-dichlorobenzene-diazonium-tetrafluoroborate in an acid medium to produce an azodye, with colors ranging from increasing degrees of tan or pink to violet most difficult easily influenced by other pigments present in the urine



D. Reaction Interference

- False-positive reaction - persons taking phenazopyridine compounds, because the thick pigment produced may be mistaken for bilirubin on initial examination
- Bilirubin is an unstable compound that is rapidly photo-oxidized to biliverdin when exposed to light.
- Biliverdin does not react with diazo tests.

Ictotest Tablets

- confirmatory test
- testing mats and tablets containing p-nitrobenzene-diazonium-p-toluenesulfonate, SSA, sodium carbonate, and boric acid

Procedure

- Place 10 drops of urine onto one square of the absorbent test mat.
- Using forceps, remove one Ictotest reagent tablet, recap the bottle promptly, and place the tablet in the center of the moistened area.
- Place 1 drop of water onto the tablet and wait 5 seconds.
- Place a second drop of water onto the tablet so that the water runs off the tablet onto the mat.
- Observe the color of the mat around the tablet at the end of 60 seconds. The presence of a blue-to-purple

color on the mat indicates that bilirubin is present. A slight pink or red color should be ignored. Report as positive or negative.

UROBILINOGEN

A. Introduction

- When conjugated bilirubin is excreted through the bile duct into the intestine, the intestinal bacteria convert the bilirubin to a combination of urobilinogen and stercobilinogen
- Urobilinogen - reabsorbed from the intestine into the blood, recirculates to the liver, and is excreted back into the intestine through the bile duct.
 - Urobilinogen in the urine - it circulates in the blood back to the liver, it passes through the kidney and is filtered by the glomerulus
 - less than 1 mg/dL or Ehrlich uni
- Stercobilinogen - cannot be reabsorbed and remains in the intestine where it is oxidized to stercobilin.
- Both are excreted in the feces
- Pigments responsible for the characteristic brown color of feces

B. Clinical Significance

- >1 mg/dL - indicative of liver disease and hemolytic disorders
- Impairment of liver function decreases the ability of the liver to process the urobilinogen recirculated from the intestine.
- The excess urobilinogen remaining in the blood is filtered by the kidneys and appears in the urine.

Clinical Significance of Urine Urobilinogen

Early detection of liver disease
Liver disorders, hepatitis, cirrhosis, carcinoma
Hemolytic disorders

C. Reagent Strip Reactions and Interference

1. Multistix

- uses Ehrlich's aldehyde reaction
- reacts with p-dimethylaminobenzaldehyde (Ehrlich reagent)
- light to dark pink
- Ehrlich's unit (EU) - unit of measure, equal to mg/dL
- normal readings: 0.2 and 1
- abnormal readings: 2, 4, and 8

Urobilinogen + p-dimethylaminobenzaldehyde $\xrightarrow{\text{acid}}$ red color
(Ehrlich's reagent)
reactive substances)

2. Chemstrip

- azo-coupling (diazo) reaction using 4-methoxybenzene-diazonium-tetrafluoroborate to react with urobilinogen,
- white to pink
- more specific for urobilinogen
- mg/dL - unit of measure

Urobilinogen + diazonium salt $\xrightarrow{\text{acid}}$ red azodye
(4-methoxybenzene-diazonium-tetrafluoroborate)

D. Reaction Interference

- Ehrlich-reactive compounds - produce false-positive reactions
- Testing should be performed at room temperature (sensitivity of the Ehrlich reaction increases with temperature)
- Highly pigmented urines - cause atypical readings with both brands of reagent strips
- increased excretion of bile salts $\rightarrow$ normally highest following a meal

Urobilinogen Reagent Strip	
Reagents	Multistix: p-dimethylaminobenzaldehyde Chemstrip: 4-methoxybenzene-diazonium-tetrafluoroborate
Sensitivity	Multistix: 0.2 mg/dL urobilinogen Chemstrip: 0.4 mg/dL urobilinogen
Sources of error/interference	<u>Multistix</u> <u>False-positive:</u> Porphobilinogen Indican p-aminosalicylic acid Sulfonamides Methyldopa Procaine Chlorpromazine Highly pigmented urine <u>False-negative:</u> Old specimens Preservation in formalin <u>Chemstrip</u> <u>False-positive:</u> Highly pigmented urine <u>False-negative:</u> Old specimens Preservation in formalin High concentrations of nitrite
Correlations with other tests	Bilirubin

NITRITE

A. Introduction

- rapid screening test for the presence of urinary tract infection (UTI)
- not intended to replace the urine culture
- valuable for detecting initial bladder infection (cystitis)
- evaluate the success of antibiotic therapy and to periodically screen persons with recurrent infections, patients with diabetes, and pregnant women

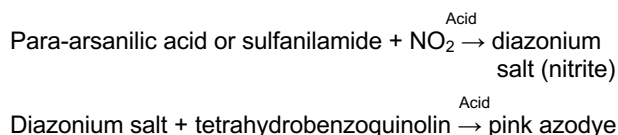
B. Clinical Significance

Clinical Significance of Urine Nitrite

Cystitis
Pyelonephritis
Evaluation of antibiotic therapy
Monitoring of patients at high risk for urinary tract infection
Screening of urine culture specimens

C. Reagent Strip Reactions

- ability of certain bacteria to reduce nitrate to nitrite
 - nitrate - a normal constituent of urine
 - nitrite - does not normally appear in the urine
- detected by the **Greiss reaction**, in which nitrite at an acidic pH reacts with an aromatic amine (para-arsanilic acid or sulfanilamide) to form a diazonium compound that then reacts with tetrahydrobenzoquinolin compounds to produce a **pink-colored azodye**
- bacterial culture criterion**: 100,000 organisms per milliliter



D. Reaction Interference

- Bacteria that lack the **enzyme reductase** do not possess the ability to reduce nitrate to nitrite. **Reductase is found in the gram-negative bacteria (Enterobacteriaceae)** that most frequently cause UTIs. Non-nitrate-reducing gram-positive bacteria and yeasts, however, cause a significant number of infections, and the nitrite test does not detect the presence of these organisms.
- Bacteria capable of reducing nitrate must remain in contact with the urinary nitrate long enough to produce nitrite.** Therefore, nitrite tests should be performed on **first morning specimens** or specimens collected after urine has remained in the bladder for at least 4 hours. The correlation between positive cultures and positive nitrite test results is significantly lower when testing is performed on random samples.
- The reliability of the test depends on the presence of adequate amounts of nitrate in the urine. This is seldom a problem in patients on a normal diet that contains green vegetables; however, because diet usually is not controlled prior to testing, the possibility of a false-negative result owing to lack of dietary nitrate does exist.
- Further reduction of nitrite to nitrogen may occur when large numbers of bacteria are present, and this causes a false-negative reaction.
- Other causes of false-negative results include inhibition of bacterial metabolism by the presence of antibiotics, large quantities of ascorbic acid interfering with the diazo reaction, and decreased sensitivity in specimens with a high specific gravity. Large amounts of ascorbic acid compete with nitrite to combine with the diazonium salt, therefore preventing a true nitrite measurement.

Nitrite Reagent Strip	
Reagents	Multistix: p-arsanilic acid Tetrahydrobenzo(h)-quinolin-3-ol Chemstrip: Sulfanilamide, hydroxytetrahydro benzoquinoline
Sensitivity	Multistix: 0.06 to 0.1 mg/dL nitrite ion Chemstrip: 0.05 mg/dL nitrite ion
Sources of error/interference	<u>False-positive:</u> Nonreductase-containing bacteria Insufficient contact time between bacteria and urinary nitrate Lack of urinary nitrate

	Large quantities of bacteria converting nitrite to nitrogen Presence of antibiotics High concentrations of ascorbic acid High specific gravity <u>False-negative:</u> Improperly preserved specimens Highly pigmented urine
Correlations with other tests	Protein Leukocytes Microscopic

LEUKOCYTE ESTERASE

A. Introduction

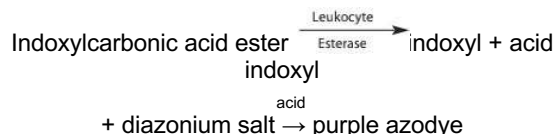
- Advantage: **detects the presence of leukocytes that have been lysed, particularly in dilute alkaline urine**, and would not appear in the microscopic examination.

B. Clinical Significance

- Normal values: 0 to 2 to 0 to 5 per high-power field
- Women tend to have higher numbers than men as a result of vaginal contamination**
- Increased amount - indicators of UTI
- detects the **presence of esterase in the granulocytic white blood cells (neutrophils, eosinophils, and basophils) and monocytes, but not lymphocytes**
- Neutrophils - leukocytes most frequently associated with bacterial infections
- Infections caused by *Trichomonas*, *Chlamydia*, yeast, and inflammation of renal tissues (i.e., interstitial nephritis) produce leukocyturia without bacteriuria.
- Bacterial and nonbacterial urinary tract infection
- Inflammation of the urinary tract
- Screening of urine culture specimens

C. Reagent Strip Reaction

- uses the action of LE** to catalyze the hydrolysis of an acid ester embedded on the reagent pad to produce an aromatic compound and acid
- The aromatic compound then combines with a diazonium salt present on the pad to produce a **purple azodye**.



D. Reaction Interference

- Highly pigmented urines and the presence of the antibiotic nitrofurantoin** may obscure the color reaction.
- Crenation of leukocytes preventing release of esterases may
- occur in urines with a high specific gravity.
- The presence of the antibiotics gentamicin, cephalixin, cephalothin, and tetracycline decreases the sensitivity of the reaction.

Leukocyte Esterase Reagent Strip	
Reagents	Multistix: Derivatized pyrrole amino acid ester Diazonium salt Chemstrip: Indoxylcarbonic acid ester Diazonium salt
Sensitivity	Multistix: 5 to 15 WBC/hpf Chemstrip: 10 to 25 WBC/hpf
Sources of error/interference	False-positive: Strong oxidizing agents Formalin Highly pigmented urine, nitrofurantoin False-negative: High concentrations of protein (greater than 500 mg/dL), glucose (greater than 3 g/dL), oxalic acid, ascorbic acid, gentamicin, cephalosporins, tetracyclines; inaccurate timing
Correlations with other tests	Protein Nitrite Microscopic

SPECIFIC GRAVITY

A. Clinical Significance

- ✓ Monitoring patient hydration and dehydration
- ✓ Loss of renal tubular concentrating ability
- ✓ Diabetes insipidus
- ✓ Determination of unsatisfactory specimens due to low concentration

B. Reagent Strip Reaction

- based on the change in pK_a (dissociation constant) of a polyelectrolyte in an alkaline medium

C. Reaction Interference

- Specimens with a pH of 6.5 or higher have decreased readings caused by interference with the bromthymol blue indicator (the blue-green readings associated with an alkaline pH correspond to a low specific gravity reading).
- Therefore, manufacturers recommend adding 0.005 to specific gravity readings when the pH is 6.5 or higher.
- The correction is performed by automated strip readers.

Urine Specific Gravity Reagent Strip	
Reagents	Multistix: Poly (methyl vinyl ether/maleic anhydride) bromthymol blue Chemstrip: Ethylene glycol diaminoethyl ether tetraacetic acid, bromthymol blue
Sensitivity	1.000 to 1.030
Sources of error/interference	False-positive: High concentrations of protein False-negative: Highly alkaline urines (greater than 6.5)

Review Questions

- Leaving excess urine on the reagent strip after removing it from the specimen will:
 - Cause run-over between reagent pads
 - Alter the color of the specimen
 - Cause reagents to leach from the pads
 - Not affect the chemical reactions
- Failure to mix a specimen before inserting the reagent strip will primarily affect the:
 - Glucose reading
 - Blood reading
 - Leukocyte reading
 - Both B and C
- Testing a refrigerated specimen that has not warmed to room temperature will adversely affect:
 - Enzymatic reactions
 - Dye-binding reactions
 - The sodium nitroprusside reaction
 - Diazo reactions
- The reagent strip reaction that requires the longest reaction time is the:
 - Bilirubin
 - pH
 - Leukocyte esterase
 - Glucose
- Quality control of reagent strips is performed:
 - Using positive and negative controls
 - When results are questionable
 - At least once every 24 hours
 - All of the above
- All of the following are important to protect the integrity of reagent strips except:
 - Removing the desiccant from the bottle
 - Storing in an opaque bottle
 - Storing at room temperature
 - Resealing the bottle after removing a strip
- The principle of the reagent strip test for pH is the:
 - Protein error of indicators
 - Greiss reaction
 - Dissociation of a polyelectrolyte
 - Double indicator reaction
- A urine specimen with a pH of 9.0:
 - Indicates metabolic acidosis
 - Should be recollected
 - May contain calcium oxalate crystals
 - Is seen after drinking cranberry juice
- In the laboratory, a primary consideration associated with pH is:
 - Identifying urinary crystals
 - Monitoring vegetarian diets
 - Determining specimen acceptability
 - Both A and C
- Indicate the source of the following proteinurias by placing a 1 for prerenal, 2 for renal, or 3 for postrenal in front of the condition.
 - 2 Microalbuminuria
 - 1 Acute phase reactants
 - 1 Pre-eclampsia
 - 2 Vaginal inflammation
 - 2 Multiple myeloma
 - 2 Orthostatic proteinuria
 - 3 Prostatitis
- The principle of the protein error of indicators reaction is that:
 - Protein keeps the pH of the urine constant
 - Albumin accepts hydrogen ions from the indicator

- C. The indicator accepts hydrogen ions from albumin
D. Albumin changes the pH of the urine
12. All of the following will cause false-positive protein reagent strip values except:
A. Microalbuminuria
B. Highly buffered alkaline urines
C. Delay in removing the reagent strip from the specimen
D. Contamination by quaternary ammonium compounds
13. A patient with a 2+ protein reading in the afternoon is asked to submit a first morning specimen. The second specimen has a negative protein reading. This patient is:
A. Positive for orthostatic proteinuria
B. Negative for orthostatic proteinuria
C. Positive for Bence Jones protein
D. Negative for clinical proteinuria
14. Testing for microalbuminuria is valuable for early detection of kidney disease and monitoring patients with:
A. Hypertension
B. Diabetes mellitus
C. Cardiovascular disease risk
D. All of the above
15. The primary chemical on the reagent strip in the Micral-Test for microalbumin binds to:
A. Protein
B. Antihuman albumin antibody
C. Conjugated enzyme
D. Galactoside
16. All of the following are true for the ImmunoDip test for microalbumin except:
A. Unbound antibody migrates farther than bound antibody
B. Blue latex particles are coated with antihuman albumin antibody
C. Bound antibody migrates further than unbound antibody
D. It utilizes an immunochromographic principle
17. The principle of the protein-high pad on the Multistix Pro reagent strip is the:
A. Diazo reaction
B. Enzymatic dye-binding reaction
C. Protein error of indicators
D. Microalbumin-Micral-Test
18. Which of the following is not tested on the Multistix Pro reagent strip?
A. Urobilinogen
B. Specific gravity
C. Creatinine
D. Protein-high
19. The principle of the protein-low reagent pad on the Multistix Pro is the:
A. Binding of albumin to sulphonphthalein dye
B. Immunologic binding of albumin to antibody
C. Reverse protein error of indicators reaction
D. Enzymatic reaction between albumin and dye
20. The principle of the creatinine reagent pad on microalbumin reagent strips is the:
A. Double indicator reaction
B. Diazo reaction
C. Pseudoperoxidase reaction
D. Reduction of a chromogen
21. The purpose of performing an albumin:creatinine ratio is to:
A. Estimate the glomerular filtration rate
B. Correct for hydration in random specimens
C. Avoid interference for alkaline urines
D. Correct for abnormally colored urines
22. A patient with a normal blood glucose and a positive urine glucose should be further checked for:
A. Diabetes mellitus
B. Renal disease
C. Gestational diabetes
D. Pancreatitis
23. The principle of the reagent strip tests for glucose is the:
A. Peroxidase activity of glucose
B. Glucose oxidase reaction
C. Double sequential enzyme reaction
D. Dye-binding of glucose and chromogen
24. All of the following may produce false-negative glucose reactions except:
A. Detergent contamination
B. Ascorbic acid
C. Unpreserved specimens
D. Low urine temperature
25. The primary reason for performing a Clinitest is to:
A. Check for high ascorbic acid levels
B. Confirm a positive reagent strip glucose
C. Check for newborn galactosuria
D. Confirm a negative glucose reading
26. The three intermediate products of fat metabolism include all of the following except:
A. Acetoacetic acid
B. Ketoacetic acid
C. β -hydroxybutyric acid
D. Acetone
27. The most significant reagent strip test that is associated with a positive ketone result is:
A. Glucose
B. Protein
C. pH
D. Specific gravity
28. The primary reagent in the reagent strip test for ketones is:
A. Glycine
B. Lactose
C. Sodium hydroxide
D. Sodium nitroprusside
29. Ketonuria may be caused by all of the following except:
A. Bacterial infections
B. Diabetic acidosis
C. Starvation
D. Vomiting
30. Urinalysis on a patient with severe back and abdominal pain is frequently performed to check for:
A. Glucosuria
B. Proteinuria
C. Hematuria
D. Hemoglobinuria
31. Place the appropriate number or numbers in front of each of the following statements. Use both numbers for an answer if needed.
1. Hemoglobinuria
 2. Myoglobinuria
 - A. ____ Associated with transfusion reactions
 - B. ____ Clear red urine and pale yellow plasma
 - C. ____ Clear red urine and red plasma
 - D. ____ Associated with rhabdomyolysis
 - E. ____ Produces hemosiderin granules in urinary sediments
 - F. ____ Associated with acute renal failure

32. The principle of the reagent strip test for blood is based on the:
- Binding of heme and a chromogenic dye
 - Peroxidase activity of heme
 - Reaction of peroxide and chromogen
 - Diazo activity of heme
33. A speckled pattern on the blood pad of the reagent strip indicates:
- Hematuria
 - Hemoglobinuria
 - Myoglobinuria
 - All of the above
34. List the following products of hemoglobin degradation in the correct order by placing numbers 1 to 4 in the blank.
- ____ Conjugated bilirubin
 - ____ Urobilinogen and stercobilinogen
 - ____ Urobilin
 - ____ Unconjugated bilirubin
35. The principle of the reagent strip test for bilirubin is the:
- Diazo reaction
 - Ehrlich reaction
 - Greiss reaction
 - Peroxidase reaction
36. An elevated urine bilirubin with a normal urobilinogen is indicative of:
- Cirrhosis of the liver
 - Hemolytic disease
 - Hepatitis
 - Biliary obstruction
37. The primary cause of a false-negative bilirubin reaction is:
- Highly pigmented urine
 - Specimen contamination
 - Specimen exposure to light
 - Excess conjugated bilirubin
38. The purpose of the special mat supplied with the Ictotest tablets is that:
- Bilirubin remains on the surface of the mat.
 - It contains the dye needed to produce color.
 - It removes interfering substances.
 - Bilirubin is absorbed into the mat.
39. The reagent in the Multistix reaction for urobilinogen is:
- A diazonium salt
 - Tetramethylbenzidine
 - p-Dimethylaminobenzaldehyde
 - Hoesch reagent
40. The primary problem with urobilinogen tests using Ehrlich reagent is:
- Positive reactions with porphobilinogen
 - Lack of specificity
 - Positive reactions with Ehrlich's reactive substances
 - All of the above
41. The reagent strip test for nitrite uses the:
- Greiss reaction
 - Ehrlich reaction
 - Peroxidase reaction
 - Pseudoperoxidase reaction
42. All of the following can cause a negative nitrite reading except:
- Gram-positive bacteria
 - Gram-negative bacteria
 - Random urine specimens
 - Heavy bacterial infections
43. A positive nitrite test and a negative leukocyte esterase test is an indication of a:
- Dilute random specimen
 - Specimen with lysed leukocytes
 - Vaginal yeast infection
 - Specimen older than 2 hours
44. All of the following can be detected by the leukocyte esterase reaction except:
- Neutrophils
 - Eosinophils
 - Lymphocytes
 - Basophils
45. Screening tests for urinary infection combine the leukocyte esterase test with the test for:
- pH
 - Nitrite
 - Protein
 - Blood
46. The principle of the leukocyte esterase reagent strip test uses a:
- Peroxidase reaction
 - Double indicator reaction
 - Diazo reaction
 - Dye-binding technique
47. The principle of the reagent strip test for specific gravity uses the dissociation constant of a(n):
- Diazonium salt
 - Indicator dye
 - Polyelectrolyte
 - Enzyme substrate
48. A specific gravity of 1.005 would produce the reagent strip color:
- Blue
 - Green
 - Yellow
 - Red
49. Reagent strip-specific gravity readings are affected by:
- Glucose
 - Radiographic dye
 - Alkaline urine
 - All of the above

Microscopic Examination of Urine

OUTLINE

- I. Introduction
- II. Macroscopic Screening
- III. Sediment Examination Techniques
- IV. Urine Sediment Constituents

INTRODUCTION

- These include
 - ✓ red blood cells (RBCs)
 - ✓ white blood cells (WBCs)
 - ✓ epithelial cells
 - ✓ casts
 - ✓ bacteria
 - ✓ yeast,
 - ✓ parasites
 - ✓ mucus
 - ✓ spermatozoa
 - ✓ crystals
 - ✓ artifacts
- considered normal unless they are present in increased amounts

MACROSCOPIC SCREENING

- microscopic examination of the urine sediment is performed only on specimens meeting specified criteria

Macroscopic Screening and Microscopic Correlations Screening Test Significance	
Screening Test	Significance
Color	Blood
Clarity	Hematuria versus hemoglobinuria/ myoglobinuria Confirm pathologic or nonpathologic cause of turbidity
Blood	RBCs, RBC casts
Protein	Casts, cells
Nitrite	Bacteria, WBCs
Leukocyte esterase	WBCs, WBC casts, bacteria
Glucose	Yeast

A. Specimen Preparation

- Formed elements (primarily RBCs, WBCs, and hyaline casts) - disintegrate rapidly, particularly in dilute alkaline urine
- Refrigeration - cause precipitation of amorphous urates and phosphates and other nonpathologic crystals that can obscure other elements in the urine sediment
- Warming the specimen to 37°C prior to centrifuging - may dissolve some of these crystals
- Midstream clean-catch specimen - minimizes external contamination of the sediment
- Dilute random specimens - may cause false-negative readings

B. Specimen Volume

- Standard amount: 10 and 15 mL
- Most common: 12 mL volume
- multiparameter reagent strips are easily immersed in this volume

- capped centrifuge tubes are often calibrated to this volume
- Pediatric patients - get 6 mL and multiplied by 2

C. Centrifugation

- 5 minutes at a relative centrifugal force (RCF) of 400 - optimum amount of sediment with the least chance of damaging the elements
- RCF - correct for differences in the diameter of centrifuge heads (rather than RPM)

Conversion of RPM to RCF

$$RCF = 1.118 \times 10^{-5} \times \text{radius in centimeters} \times \text{RPM}^2$$

D. Sediment Preparation

- 0.5 and 1.0 mL - uniform amount of urine and sediment should remain in the tube after decantation
- volume of urine centrifuged divided by the sediment volume equals the concentration factor

sediment concentration factor

- probability of detecting elements present in low quantities and is used when quantitating the number of elements present per milliliter
- should be aspirated off rather than poured off

sediment

- thoroughly resuspended by gentle agitation
- performed using a commercial-system pipette or by repeatedly tapping the tip of the tube with the finger
- vigorous agitation should be avoided

E. Volume of Sediment Examined

1. Conventional Glass-Slide Method

- 20 µL (0.02 mL)
- covered by a 22 × 22 mm glasscover slip
- allowing the specimen to flow outside of the cover slip → may result in the loss of heavier elements such as casts

2. Commercial Systems

- providing slides with chambers capable of containing a specified volume

F. Examining the Sediment

- minimum of 10 fields under both low (10×) and high (40×) power
 - low power - to detect casts and to ascertain the general composition of the sediment
 - high power - casts that require identification
- conventional glass-slide method
 - casts have a tendency to locate near the edges of the cover slip
 - under reduced light when using bright-field microscopy

G. Reporting the Microscopic Examination

- Reported as
 - number per low-power field (lpf)
 - average number per 10 high-power fields (hpf)

H. Correlating Results

- Should be correlated with the physical and chemical findings to ensure the accuracy of the report

Routine Urinalysis Correlations			
Microscopic Elements	Physical	Chemical	Exceptions
RBCs	Turbidity Red color	+ Blood + Protein	Number Hemolysis
WBCs	Turbidity	+ Protein + Nitrite Lysis + LE	Number
Epithelial cells	Turbidity		Number
Casts		+ Protein	Number
Bacteria	Turbidity	↑ pH + Nitrite + Leukocytes	Number and type
Crystals	Turbidity Color	pH + Bilirubin	Number and type

SEDIMENT EXAMINATION TECHNIQUES

A. Sediment Stains

- changing their refractive index
- Sternheimer-Malbin stain
 - aka KOVA stain or Sedi-Stain
 - most frequently used stain in urinalysis
 - consists of crystal violet and safranin O
 - dye is absorbed well by WBCs, epithelial cells, and casts, providing clearer delineation of structure and contrasting colors of the nucleus and cytoplasm
- 0.5% solution of toluidine blue
 - metachromatic stain
 - provides enhancement of nuclear detail
 - useful in the differentiation between WBCs and renal tubular epithelial cells and is also used in the examination of cells from other body fluids
- addition of 2% acetic acid
 - nuclear detail is enhanced

Lipid Stains

- passage of lipids (triglycerides, neutral fats, and cholesterol) across the glomerular membrane → results in the appearance of free fat droplets and lipid-containing cells and casts in the urinary sediment
- contains Oil Red O and Sudan III
- polarizing microscopy - used for confirmation
- orange-red - triglycerides and neutral fats
- cholesterol - does not stain but capable of polarization

Gram Stain

- blue - gram-positive
- red - gram-negative
- limited to the identification of bacterial casts

Hansel Stain

- Polynuclear WBCs - neutrophils associated with microbial infection
- Eosinophils - drug-induced allergic reaction producing inflammation of the renal interstitium
- consists of methylene blue and eosin Y

Prussian Blue Stain

- after episodes of hemoglobinuria, yellow-brown granules may be seen in renal tubular epithelial cells and casts or free-floating in the urine sediment
- to confirm the granules are hemosiderin
- blue color

Urine Sediment Stain Characteristics		
Stain	Action	Function
Sternheimer-Malbin	Delineates structure and contrasting colors of the nucleus and cytoplasm	Identifies WBCs, epithelial cells, and casts
Toluidine blue	Enhances nuclear detail	Differentiates WBCs and renal tubular epithelial (RTE) cells
2% acetic acid	Lyses RBCs and enhances nuclei of WBCs	Distinguishes RBCs from WBCs, yeast, oil droplets, and crystals
Lipid stains: Oil Red O and Sudan III	Stain triglycerides and neutral fats orange-red Do not stain cholesterol	Identify free fat droplets and lipid-containing cells and casts
Gram stain	Differentiates gram-positive and gram-negative bacteria	Identifies bacterial casts
Hansel stain	Methylene blue and eosin Y stains eosinophilic granules	Identifies urinary eosinophils
Prussian blue stain	Stains structures containing iron	Identifies yellow-brown granules of hemosiderin in cells and casts

Expected Staining Reactions of Urine Sediment Constituents			
Elements in Urinary Sediment	Usual Distinguishing Color		Comment
RBCs	Neutral - pink to purple Acid- pink (unstained) Alkaline - purple		
	Nuclei	Cytoplasm	
WBCs (dark staining cells)	Purple	Purple granules	
Glitter cells (Sternheimer-Malbin positive cells)	Colorless or light blue	Pale blue or gray	Some glitter cells exhibit brownian movement

Renal tubular epithelial cells	Dark shade of blue-purple	Light shade of blue-purple	
Bladder tubular epithelial cells	Blue-purple	Light purple	
Squamous epithelial cells	Dark shade of orange-purple	Light purple or blue	
Inclusions and Matrix		Comments	
Hyaline casts	Pale pink or pale purple	Very uniform color; slightly darker than mucous threads	
Coarse granular inclusion casts	Dark purple granules in purple Matrix		
Finely granular inclusion casts	Fine dark purple granules in pale pink or pale purple matrix		
Waxy casts	Pale pink or pale purple	Darker than hyaline casts, but of a pale even color; distinct broken ends	
Fat inclusion casts	Fat globules unstained in a pink matrix	Rare; presence is confirmed if examination under polarized light indicates double refraction	
Red cell inclusion casts	Pink to orange-red	Intact cells can be seen in matrix	
Blood (hemoglobin) casts	Orange-red	No intact cells	
Bacteria	Motile: do not stain Nonmotile: stain purple	Motile organisms are not Impaired	
<i>Trichomonas vaginalis</i>	Light blue-green	Motility is unimpaired in fresh specimens when recommended volumes of stain are used; immobile organisms	

		also identifiable
Mucus	Pale pink or pale blue	
Background	Pale pink or pale purple	

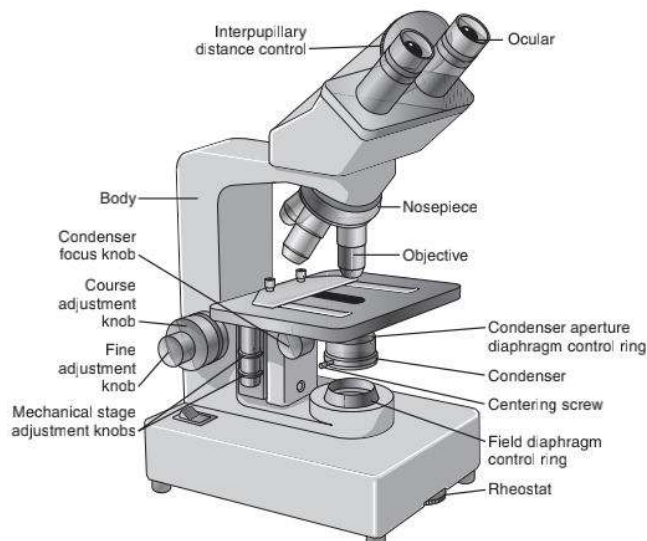
B. Cytodiagnostic Urine Testing

- Additional method for detection renal disease
- preparation of permanent slides using cytocentrifugation followed by staining with Papanicolaou stain
- voided first morning specimen - recommended
- definitive information about renal tubular changes associated with transplant rejection; viral, fungal, and parasitic infections; cellular inclusions; pathologic casts; and inflammatory conditions

C. Microscopy

- Bright-field microscopy - most common type of microscopy performed in the urinalysis laboratory
- depends on the
 - specimen type
 - refractive index of the object
 - ability to image unstained living cells

Microscope

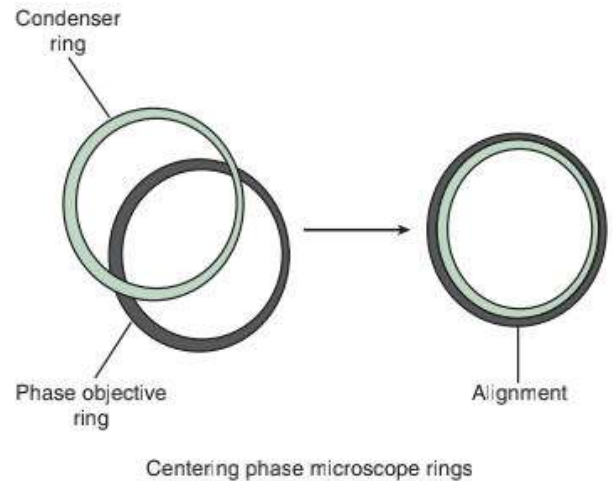
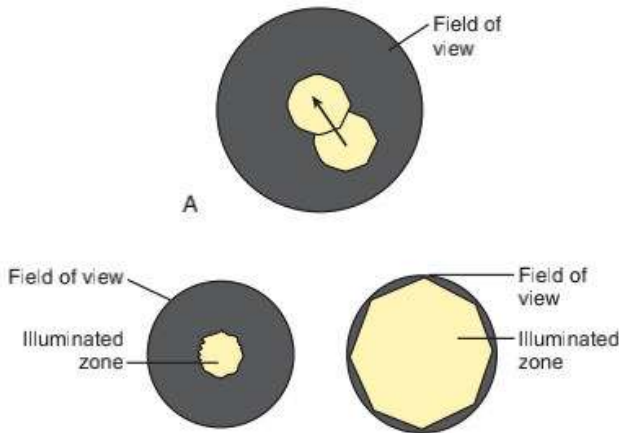


- contains
 - lens system (oculars, the objectives, and the coarse- and fine-adjustment knobs)
 - 10× (low power, dry)
 - 40× (high power, dry)
 - 100× (oil immersion)
 - final magnification - product of the objective magnification times the ocular magnification
 - illumination system (light source, condenser, and field and iris diaphragms)
 - body
 - base
 - body tube
 - nosepiece
 - mechanical stage

Köhler Illumination

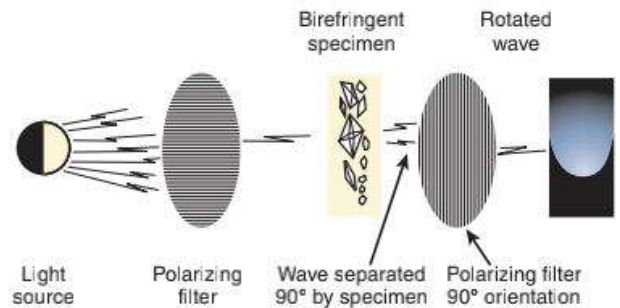
1. Two adjustments to the condenser: centering and Köhler illumination
2. Place a slide on the stage and focus the object using the low-power objective with the condenser raised.

3. Close the field diaphragm.
4. Lower the condenser until the edges of the field diaphragm are sharply focused.
5. Center the image of the field diaphragm with the condenser centering screws.
6. Open the field diaphragm until its image is at the edge of the field.
7. Remove an eyepiece and look down through the eyepiece tube.
8. Adjust the aperture diaphragm until approximately 75% of the field is visible.
9. Replace the eyepiece.



3. Polarizing Microscopy

- identification of crystals and lipids
- have the ability to rotate the path of the unidirectional polarized light beam to produce characteristic colors in crystals and Maltese cross formation in lipids
- birefringent - property indicating that the element can refract light in two dimensions at 90 degrees to each other
- crossed configuration filters
- confirm the identification of fat droplets, oval fat bodies, and fatty casts that produce a characteristic Maltese cross pattern



Types of Microscopy

1. Bright-Field Microscopy

- objects appear dark against a light background
- can present problems when the amount of light reaching the specimen is not properly controlled
- sediments must be examined using decreased light controlled by adjusting the rheostat on the light source, not by lowering the condenser

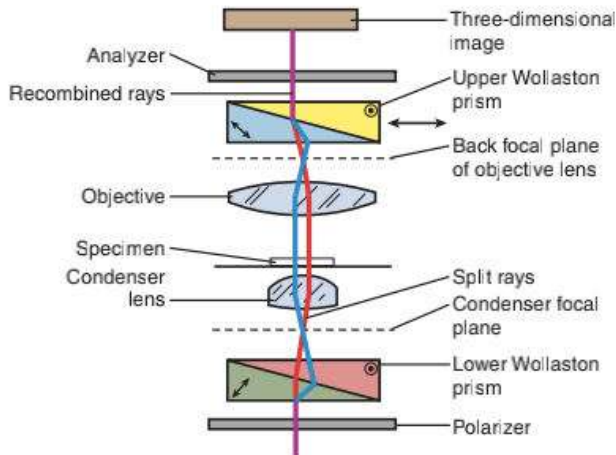
2. Phase-Contrast Microscopy

- As light rays pass through an object, they are slowed in comparison to the rays passing through air (media), thereby decreasing the intensity of the light and producing contrast.
- best contrast - s obtained when the light that does not pass through the specimen is shifted one quarter of a wavelength and compared with the phase difference of the specimen
- Advantage: identifying low refractive hyaline casts or mixed cellular casts and mucous threads

4. Interference-Contrast Microscopy

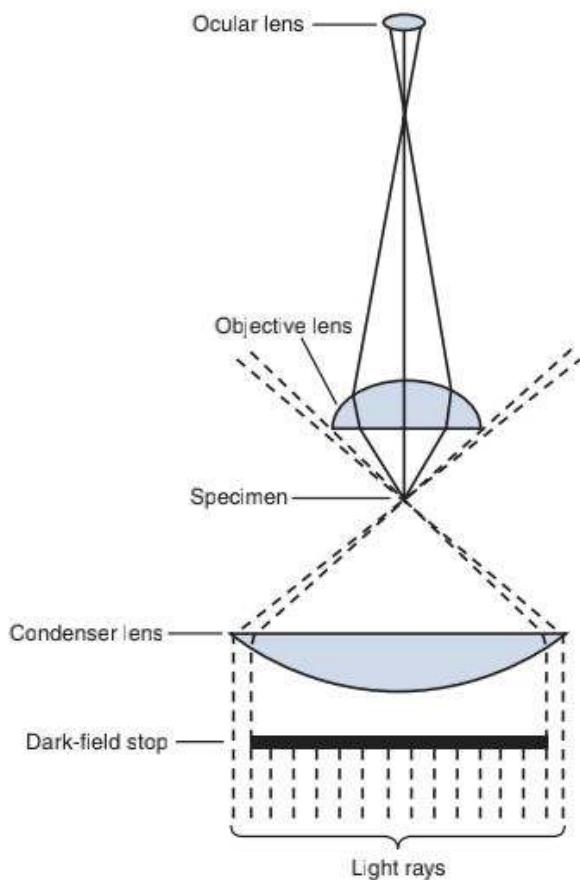
- a three-dimensional image showing very fine structural detail by splitting the light ray so that the beams pass through different areas of the specimen
- Advantage: object appears bright against a dark background but without the diffraction halo associated with phase contrast microscopy
- a. modulation contrast (Hoffman)
 - split aperture is placed below the condenser, a polarizer is placed below the split aperture, and an amplitude filter is placed in back of each objective
 - three zones of light transmission
 - dark zone - transmits 1% of light
 - gray zone - transmits 15% of light
 - clear zone - transmits 100% of light

- b. differential-interference contrast (Nomarski)
- uses prisms



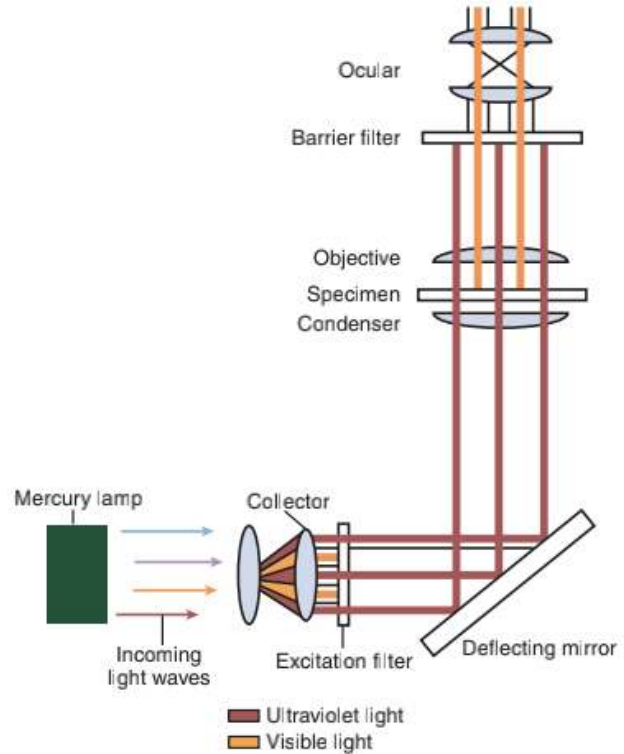
5. Dark-Field Microscopy

- technique used in the clinical laboratory to enhance visualization of specimens that cannot be seen easily viewed with a bright-field microscope
- unstained specimens (spirochete *Treponema pallidum*)
- specimen appears light against the black background or dark-field



6. Fluorescence Microscopy

- used to detect bacteria and viruses within cells and tissues through a technique called immunofluorescence
- fluorochrome or fluorophore (fluorescent dyes) - stains to produce an image
- excitation filter and the emission filter - special filters
 - excitation filter - selects the excitation wavelength of light from a light source
 - emission filter - selects a specific wavelength of emitted light from the specimen to become visible



URINE SEDIMENT CONSTITUENTS

A. Red Blood Cells (RBCs)

- appear as smooth, non-nucleated, biconcave disks approximately 7 mm in diameter
- identified using high-power (40×) objective (×400 magnification)
- reported as the average number seen in 10 hpfs
- concentrated (hypersthenuric urine): crenated or irregularly shaped, cells shrink due to loss of water
- dilute (hyposthenuria) urine: cells absorb water, swell, and lyse rapidly, releasing their hemoglobin and leaving only the cell membrane → called ghost cells
- when in doubt
 - add acetic acid to a portion of the sediment
 - lyse - RBCs
 - intact - yeast, oil droplets, and WBCs
- dysmorphic
 - vary in size, have cellular protrusions, or are fragmented
 - acanthocyte with multiple protrusions
 - associated primarily with glomerular bleeding

Clinical Significance

- associated with
 - damage to the glomerular membrane
 - vascular injury within the genitourinary tract

- number of cells present - indicative of the extent of the damage or injury
- macroscopic hematuria: cloudy with a red to brown color

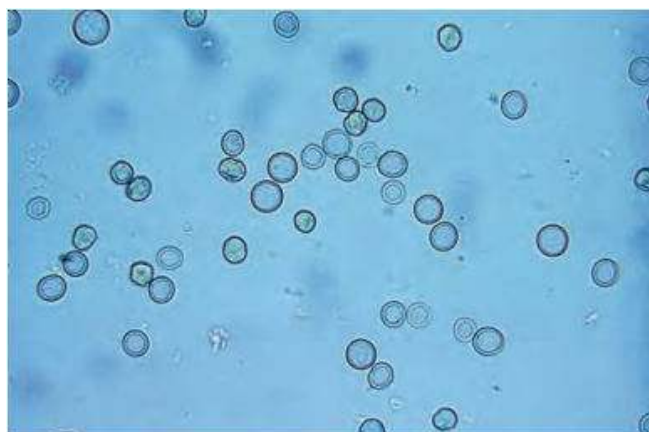


Figure 6-8 Normal RBCs (x400).



Figure 6-9 Microcytic and crenated RBCs (x100).

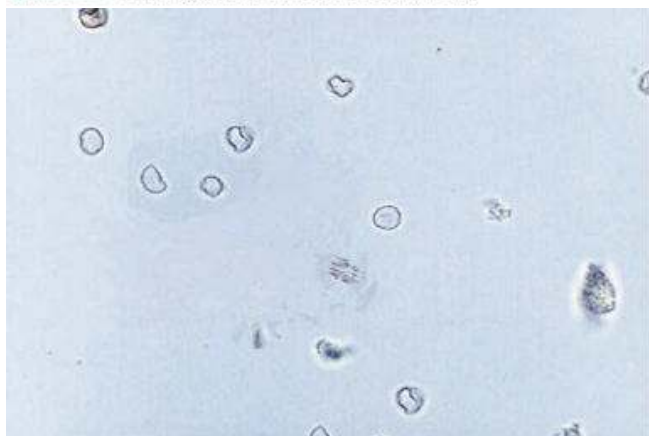


Figure 6-13 Dysmorphic RBCs (x400). Notice the smaller size and fragmentation.



Figure 6-12 Air bubble. Notice no formed elements are in focus (<100).

Microscopic RBCs	
Appearance	Non-nucleated biconcave disks Crenated in hypertonic urine Ghost cells in hypotonic urine Dysmorphic with glomerular membrane damage
Sources of identification error	Yeast cells Oil droplets Air bubbles
Reporting	Average number per 10 hpfs
Complete urinalysis correlations	Color Reagent strip blood reaction

B. White Blood Cells

- larger than RBCs
- 12 mm in diameter
- pyuria - increases urinary WBC indicates the presence of an infection or inflammation in the genitourinary system

1. Neutrophils

- predominant WBC found in the urine sediment
- contain granules and multilobed nuclei
- lyse rapidly in dilute alkaline urine and begin to lose nuclear detail
- exposed to hypotonic urine absorb water and swell
- glitter cells - sparkling appearance due to brownian movement of the granules, no pathologic significance
- blue in Sternheimer-Malbin stain

2. Eosinophils

- not normally seen in the urine
- >1% - significant
- associated with drug-induced interstitial nephritis
- small numbers in urinary tract infection (UTI) and renal transplant rejection
- Hansel - preferred stain

3. Mononuclear Cells

- not identified in the wet preparation urine microscopic analysis
- lymphocyte - smallest WBCs (may resemble RBCs)
- may appear vacuolated or contain inclusions
- primary concern: differentiation of mononuclear cells and disintegrating neutrophils from round renal tubular epithelial (RTE) cells

- RTE cells
 - larger than WBCs with an eccentrically located nucleus
 - Supravital staining or the addition of acetic acid - enhance nuclear detail
- <5 leukocytes per hpf - found in urine (high number in females)

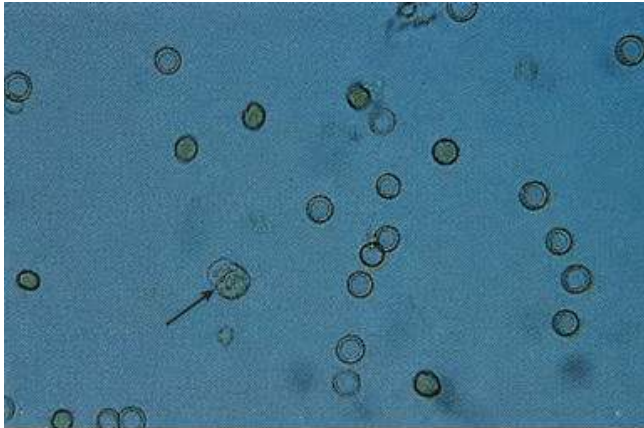


Figure 6-14 RBCs and one WBC (x400). Notice the larger size and granules in the WBC.

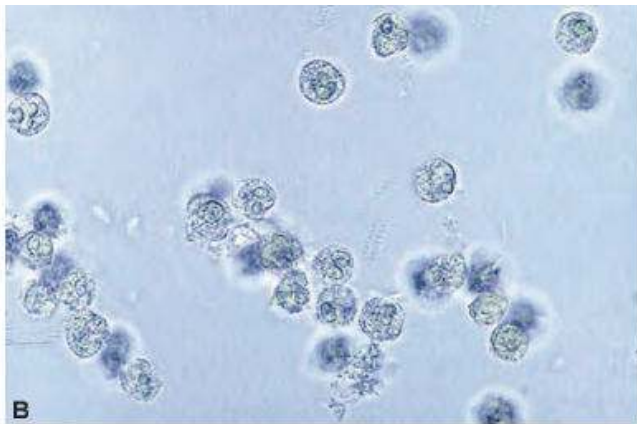


Figure 6-15 WBCs. **A.** One segmented and one nonsegmented WBC (x400). **B.** Notice the multilobed nucleoli (x400).



Figure 6-16 Glitter cells (x400). Observe the very noticeable granules.

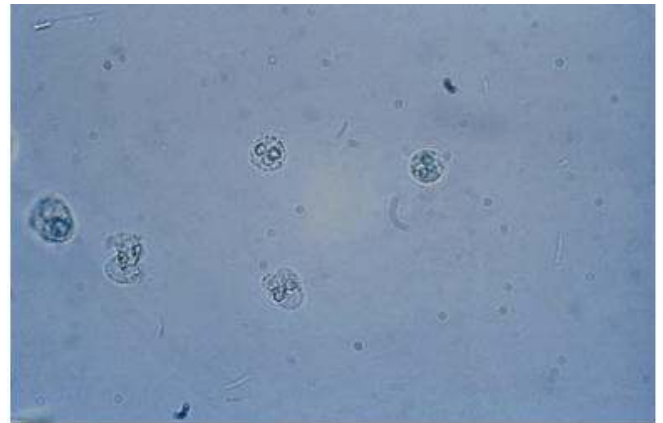


Figure 6-18 WBCs with acetic acid nuclear enhancement. Notice the amoeboid shape in some of the WBCs.

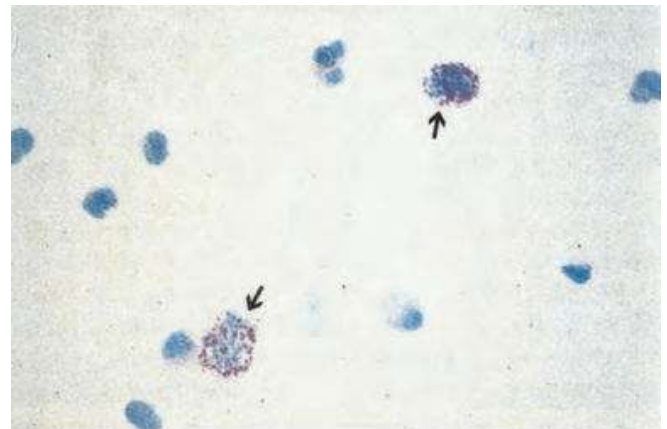


Figure 6-17 Hansel-stained eosinophils (x400).

Microscopic WBCs	
Appearance	Larger than RBCs Granulated, multilobed neutrophils Glitter cells in hypotonic urine Mononuclear cells wi
Sources of identification error	Yeast cells Oil droplets Renal tubular epithelial cells
Reporting	Average number per 10 hpfs
Complete urinalysis correlations	Leukocyte esterase Nitrite Specific gravity pH

C. Epithelial Cells

- not unusual
- derived from the linings of the genitourinary system
- represent normal sloughing of old cells

Types

1. squamous epithelial cells
 - largest cells found in the urine sediment
 - abundant, irregular cytoplasm and a prominent nucleus
 - first structures observed when the urine sediment is examined under low-power magnification
 - linings of the vagina and female urethra and the lower portion of the male urethra

- clue cell - indicative of vaginal infection by the bacterium *Gardnerella vaginalis*, covered with the *Gardnerella* coccobacillus
- transitional (urothelial) epithelial cells
 - smaller than squamous cells
 - spherical, polyhedral, and caudate - caused by the ability of transitional epithelial cells to absorb large amounts of water
 - centrally located nuclei
 - lining of the renal pelvis, calyces, ureters, and bladder, and from the upper portion of the male urethra
 - syncytia - increased numbers of transitional cells seen singly, in pairs, or in clumps following invasive urologic procedures such as catheterization and are of no clinical significance
 - malignancy or viral infection - abnormal morphology such as vacuoles and irregular nuclei
 - renal tubular epithelial cells
 - proximal convoluted tubule (PCT) RTE - larger than other RTE cells
 - distal convoluted tubule (DCT) - oval or round
 - Collecting duct RTE - cuboidal
 - rectangular shape
 - columnar or convoluted cells
 - difference with cast: with nucleus
 - often present as a result of tissue destruction (necrosis)

Clinical Significance

- RTE cells
 - most clinically significant of the epithelial cells
 - may contain the characteristic yellow-brown hemosiderin granules
 - one of the functions of RTE cells is reabsorption of the glomerular filtrate, it is not unusual for them to contain substances from the filtrate
- Oval Fat Bodies
 - RTE cells absorb lipids that are present in the glomerular filtrate
 - confirmed by staining with Sudan III or Oil Red O fat stains
 - examined by polarized microscopy
 - orange-red droplets
- Lipiduria - most frequently associated with damage to the glomerulus caused by the nephrotic syndrome
 - differentiated from oval fat bodies by their large size
- bubble cells - nonlipid-filled vacuoles in cases of acute tubular necrosis, injured cells

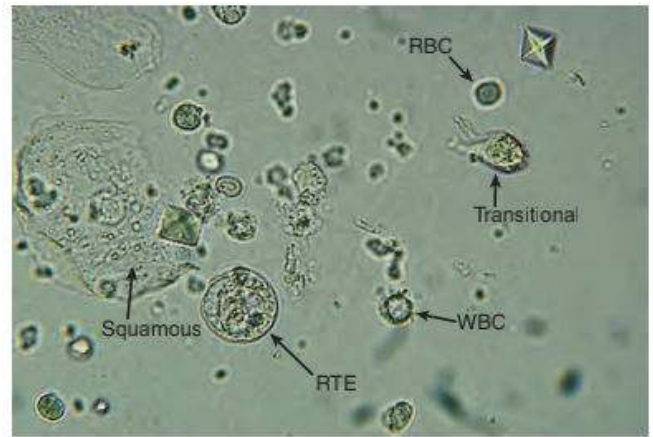


Figure 6-19 Sediment-containing squamous, caudate transitional, and RTE cells (x400).

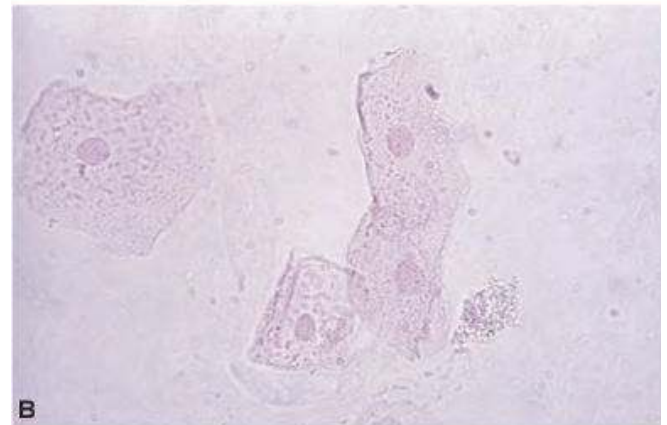
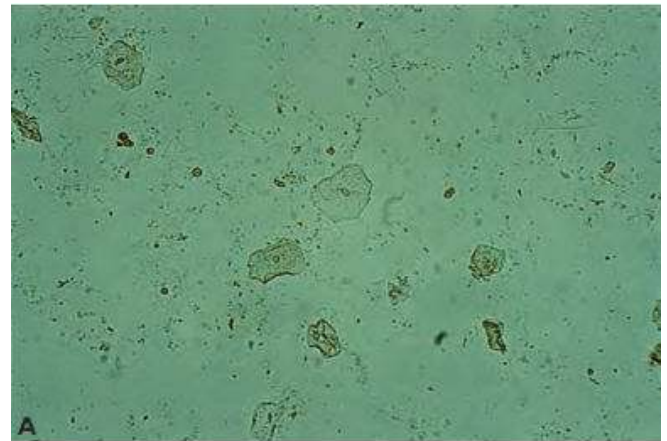


Figure 6-20 **A.** Squamous epithelial cells identifiable under low power (x100). **B.** KOVA-stained squamous epithelial cells (x400). Compare the size of the nucleus with the RBCs in Figure 6-8.



Figure 6-21 Phenazopyridine-stained sediment showing squamous epithelial cells and phenazopyridine crystals formed following refrigeration (x400).



Figure 6-24 Transitional epithelial cells.



Figure 6-22 Clump of squamous epithelial cells (x400).



Figure 6-25 KOVA-stained spherical transitional epithelial cell (x400).



Figure 6-23 Clump of squamous epithelial cells with folded forms (x400).



Figure 6-26 Caudate transitional epithelial cells (x400).

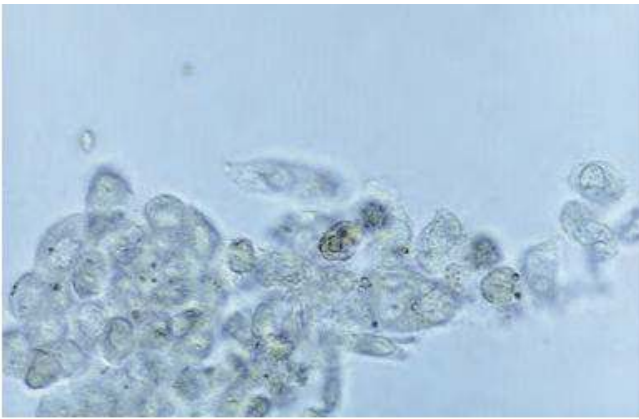


Figure 6-27 Syncytia of transitional epithelial cells from catheterized specimen (x400).



Figure 6-29 RTE cells. Oval distal convoluted tubule cells. Notice the eccentrically placed nuclei (x400).



Figure 6-28 RTE cell. Columnar proximal convoluted tubule cell with granules and attached fat globules (x400). N, nucleus.

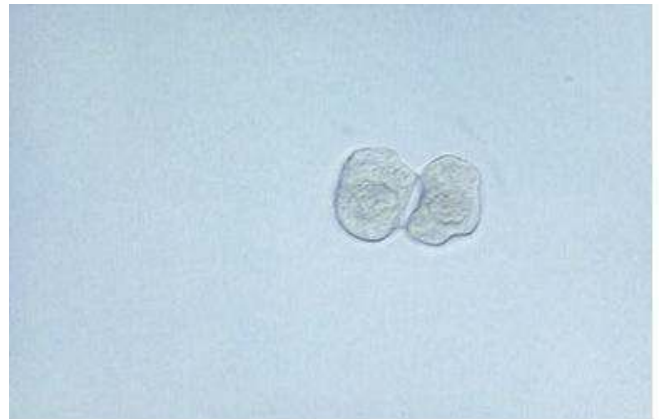


Figure 6-30 RTE cells, cuboidal from the collecting duct (x400).

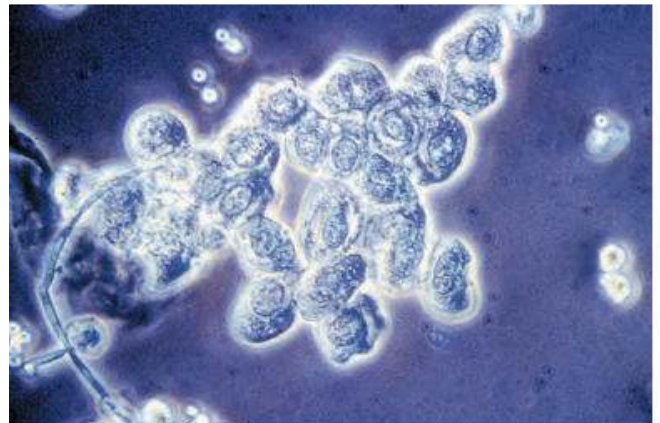


Figure 6-31 Fragment of RTE cells from the collecting duct under phase microscopy (x400).

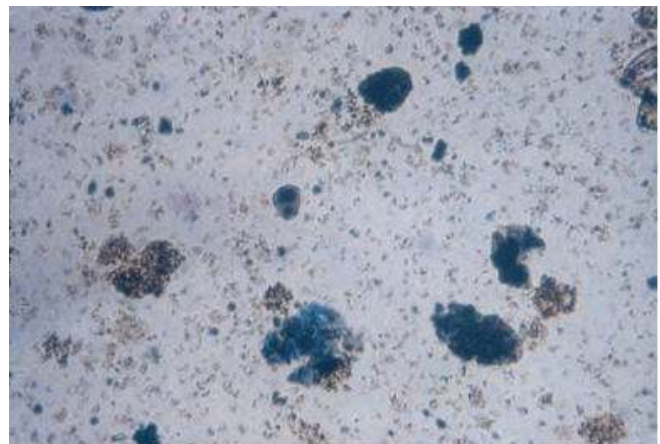


Figure 6-32 Prussian blue-stained hemosiderin granules.



Figure 6-33 Oval fat body (x400).



Figure 6-34 Sudan III-stained oval fat body (x400).

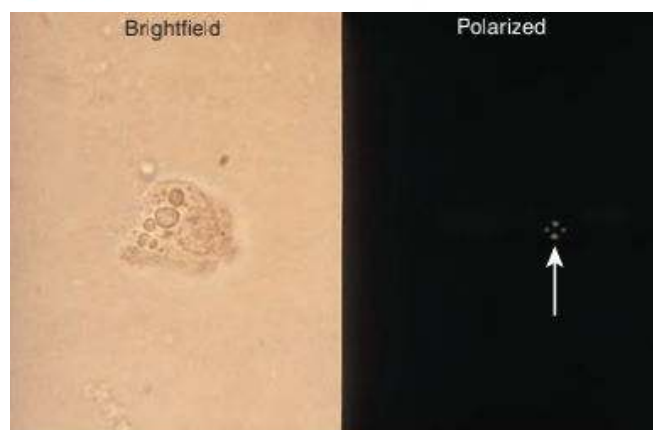


Figure 6-35 Oval fat body under bright-field (left) and polarized (right) microscopy. Notice the Maltese cross formation (arrow x400).

Epithelial Cells	
Squamous Cells	
Appearance	Largest cells in the sediment with abundant, irregular cytoplasm and prominent nuclei
Sources of identification error	Rarely encountered, folded cells may resemble casts
Reporting	Rare, few, moderate, or many per lpf
Complete urinalysis correlations	Clarity
Transitional Cells	

Appearance	Spherical, polyhedral, or caudate with centrally located nucleus
Sources of identification error	Spherical forms resemble RTE cells
Reporting	Rare, few, moderate, or many per hp
Complete urinalysis correlations	Clarity Blood, if malignancy-associated
RTE Cells	
Appearance	Rectangular, columnar, round, oval or, cuboidal with an eccentric nucleus possibly bilirubin stained or hemosiderin-laden
Sources of identification error	Spherical transitional cells Granular casts
Reporting	Average number per 10 hpfs
Complete urinalysis correlations	Leukocyte esterase and nitrite (pyelonephritis) Color Clarity Protein Bilirubin (hepatitis) Blood
Oval Fat Bodies	
Appearance	Highly refractile RTE cells
Sources of identification error	Confirm with fat stains and polarized microscopy
Reporting	Average number per 10 hpfs
Complete urinalysis correlations	Clarity Blood Protein Free fat droplets/fatty casts

D. Bacteria

- not normally present in urine
- few bacteria are usually present as a result of vaginal, urethral, external genitalia, or collection-container contamination
- positive nitrite test result and also frequently result in a pH above 8 → unacceptable specimen
- bacteria should be accompanied by WBCs
 - *Enterobacteriaceae* (gram-negative rods)
 - *Staphylococcus*
 - *Enterococcus*



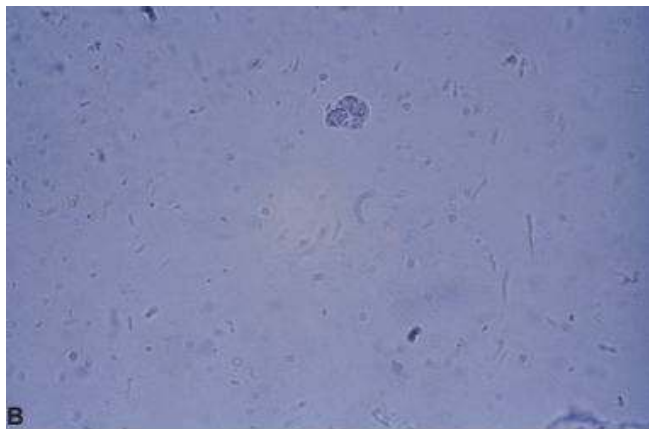


Figure 6-36 A. Rod-shaped bacteria often seen in urinary tract infections. B. KOVA-stained bacteria and WBC (x400).

E. Yeasts

- small, refractile oval structures that may or may not contain a bud
- branched, mycelial forms
- true yeast infection - accompanied by the presence of WBCs
 - *Candida albicans*
 - diabetic patients (glucose-containing urine - ideal medium for yeast growth)
 - immunocompromised patients
 - women with vaginal moniliasis

F. Parasites

- *Trichomonas vaginalis* - most common
- *Schistosoma haematobium* - bladder schistosoma
- ova *Enterobius vermicularis* - most common contaminant is ova



Figure 6-38 *Trichomonas vaginalis*. Notice the flagella and undulating membrane. (From Leventhal and Cheadle, Ed 6, p 87).



Figure 6-39 *Schistosoma haematobium* ova (x300). Eggs are often contained in the last few drops of urine expelled from the bladder.



Figure 6-40 A. *Enterobius vermicularis* ova (x100) B. *Enterobius vermicularis* ova (x400).

G. Spermatozoa

- oval, slightly tapered heads and long, flagella-like tails
- urine is toxic to spermatozoa
- increased amounts of semen are present → positive reagent strip test for protein



Figure 6-41 Spermatozoa (x400).

H. Mucus

- protein material produced by the glands and epithelial cells of the lower genitourinary tract and the RTE cells
- uromodulin - major constituent of mucus that is a glycoprotein
- no clinical significance when present in either female or male urine

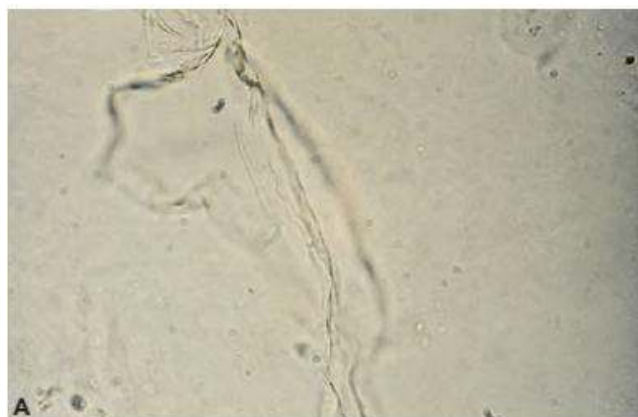


Figure 6-42 A. Mucus threads (×400). B. Mucus clump (×400).

Miscellaneous Structures	
Bacteria	
Appearance	Small spherical and rod-shaped structures
Sources of identification error	Amorphous phosphates and urates
Reporting	Few, moderate, or many per hpf, the presence of WBCs may be required
Complete urinalysis correlations	pH Nitrite LE WBCs
Yeast	
Appearance	Small, oval, refractile structures with buds and/or mycelia
Sources of identification error	RBCs
Reporting	Rare, few, moderate, or many per hpf, the presence of WBCs may be required
Complete urinalysis correlations	Glucose LE WBCs
Trichomonas	
Appearance	Pear-shaped, motile, flagellated
Sources of identification error	WBCs, renal tubular epithelial cells

Reporting	Rare, few, moderate, or many per hpf
Complete urinalysis correlations	LE WBCs
Spermatozoa	
Appearance	Tapered oval head with long, thin tail
Sources of identification error	None
Reporting	Present, based on laboratory protocol
Complete urinalysis correlations	Protein
Mucus	
Appearance	Single or clumped threads with a low refractive index
Sources of identification error	Hyaline casts
Reporting	Rare, few, moderate, or many per lpf
Complete urinalysis correlations	None

I. Casts

- only elements found in the urinary sediment that are unique to the kidney
- performed using lower power magnification
- low refractive index
- observed under subdued light
- dissolves quickly in dilute, alkaline urine
- reported as the
- average number per 10 lpf

Cast Composition and Formation

- uromodulin
- major constituent of casts
- rate of excretion appears to increase under conditions of stress and exercise → transient appearance of hyaline casts
- not detected by reagent strip protein methods
- width - depends on the size of the tubule in which it is formed
- cylindroids - casts formed at the junction of the ascending loop of Henle and the distal convoluted tubule may produce structures with a tapered end
- cylindruria - presence of urinary casts

Step-By-Step Analysis of the Formation of the Uromodulin Protein Matrix

1. Aggregation of uromodulin protein into individual protein fibrils attached to the RTE cells
2. Interweaving of protein fibrils to form a loose fibrillar network (urinary constituents may become enmeshed in the network at this time)
3. Further protein fibril interweaving to form a solid structure
4. Possible attachment of urinary constituents to the solid matrix
5. Detachment of protein fibrils from the epithelial cells
6. Excretion of the cast

Types

1. Hyaline Casts

- most frequent cast
- 0 to 2/lpf - normal
- appear colorless in unstained sediments
- have a refractive index similar to that of urine
- Increased numbers: strenuous exercise, dehydration, heat exposure, and emotional stress
- Pathologic: acute glomerulonephritis, pyelonephritis, chronic renal disease, and congestive heart failure
- pink color in Sternheimer-Malbin
- phase microscopy - increased visualization



Figure 6-43 Hyaline casts under low power (x100).



Figure 6-44 Hyaline cast (A) and amorphous urates (B) attached to mucus pseudocast (x100).

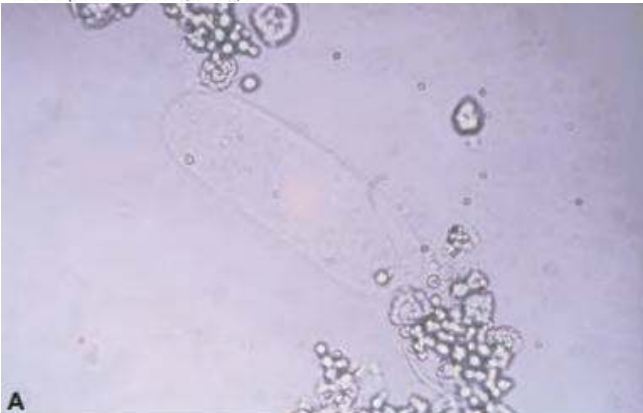


Figure 6-45 A. Hyaline cast (x400). B. Hyaline cast under phase microscopy (x400).

2. RBC Casts

- bleeding within the nephron
- primarily associated with damage to the glomerulus (glomerulonephritis) that allows passage of the cells through the glomerular membrane
- usually associated with proteinuria and dysmorphic erythrocytes
- detected under low power by their orange-red color
- more fragile than other casts
- highly improbable that RBC casts will be present in the absence of free-standing RBCs and a positive reagent strip test for blood
- granular, dirty, brown casts - representing hemoglobin degradation products such as methemoglobin

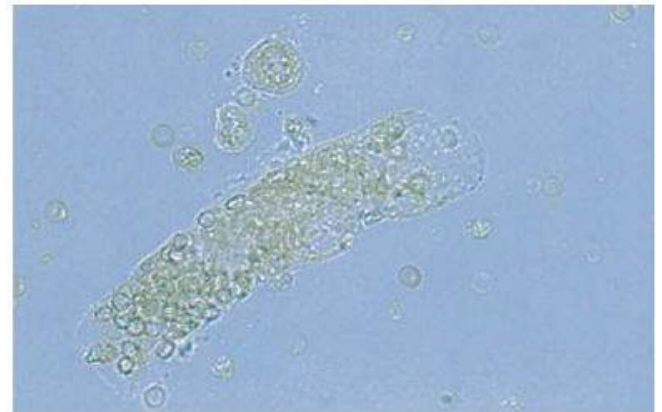


Figure 6-48 RBC cast (x400).



Figure 6-49 KOVA-stained RBC cast under phase microscopy (x400).

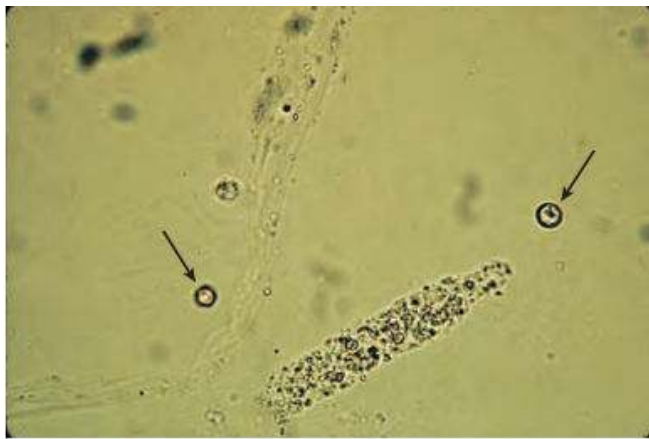


Figure 6-50 Disintegrating RBC cast. Notice the presence of free RBCs (arrows) to confirm identification.



Figure 6-51 Cast containing hemoglobin pigment. A comparison of RBCs (A) and yeast (B) also can be made (x400).



Figure 6-52 Granular, dirty, brown cast (x400).

3. WBC Casts

- signifies infection or inflammation within the nephron
- associated with pyelonephritis
- primary marker for distinguishing pyelonephritis (upper UTI) from cystitis (lower UTI)
- visible under low-power magnification but must be positively identified using high power



Figure 6-53 WBC cast. Notice the free WBCs to aid in identification.

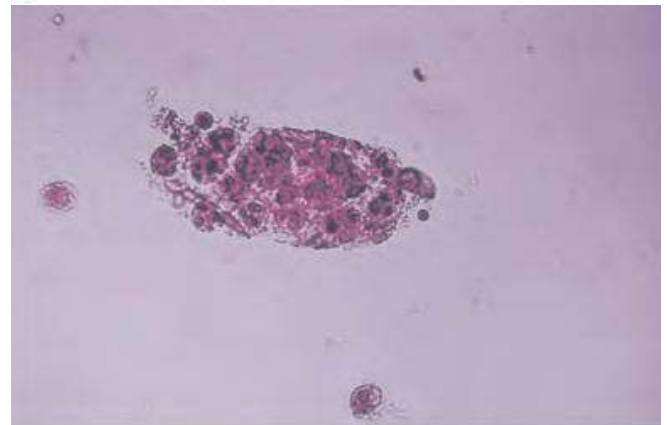


Figure 6-54 KOVA-stained WBC cast (x400).

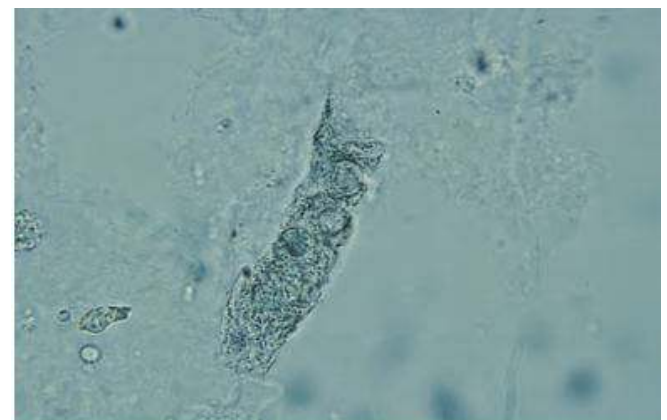


Figure 6-55 Disintegrating WBC cast (x400).

4. Bacterial Casts

- containing bacilli both within and bound to the protein matrix are seen in pyelonephritis
- presence should be considered when WBC casts and many free WBCs and bacteria are seen in the sediment

5. Epithelial Cell Casts

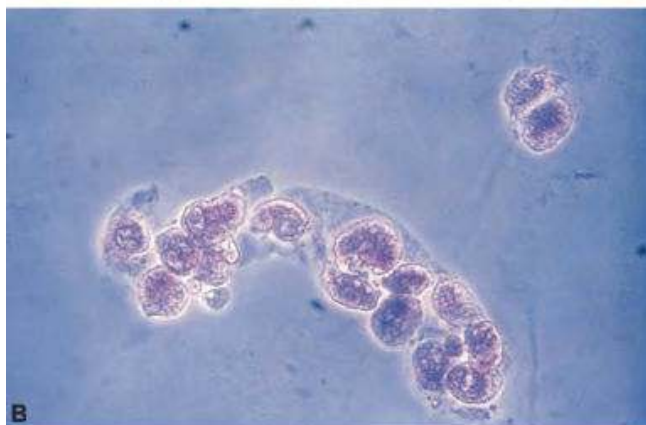
- associated with heavy metal and chemical or drug-induced toxicity, viral infections, and allograft rejection
- Bilirubin-stained RTE cells - cases of hepatitis



Figure 6-57 RTE cell cast (x400).



A



B

Figure 6-58 A. KOVA-stained RTE cell cast (x400). B. KOVA-stained RTE cell cast under phase microscopy (x400).

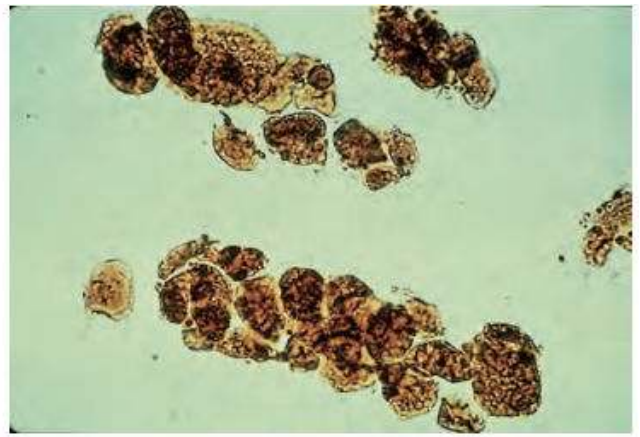


Figure 6-59 RTE cast with bilirubin-stained cells (x400).

6. Fatty Casts

- seen in conjunction with oval fat bodies and free fat droplets in disorders causing lipiduria
- highly refractile under bright-field microscopy
- performed using polarized microscopy and Sudan III or Oil Red O fat stains
- Fats do not stain with Sternheimer-Malbin stains



Figure 6-61 Fatty cast (x400).

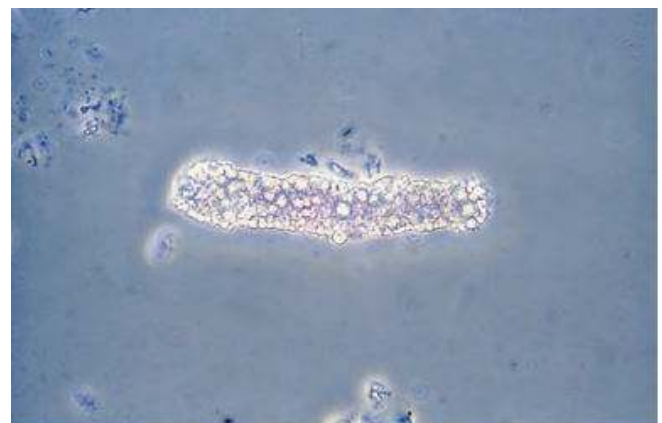


Figure 6-62 Fatty cast under phase microscopy (x400).

7. Mixed Cellular Casts

- include RBC and WBC casts in glomerulonephritis and WBC and RTE cell casts, or WBC and bacterial casts in pyelonephritis

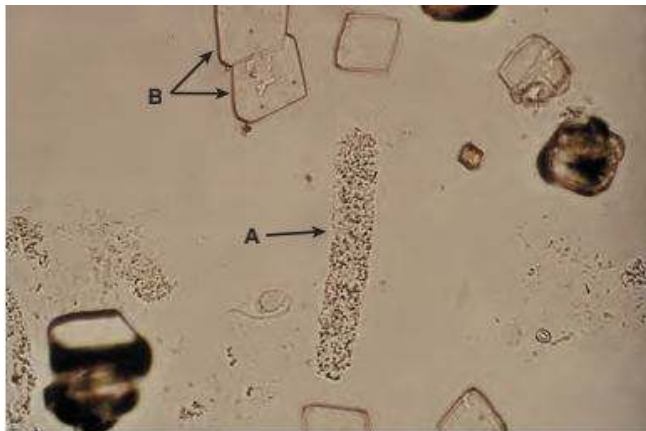


Figure 6-63 Finely granular cast (A) and uric acid crystals (B) (x400)



Figure 6-66 Coarsely granular cast (A), squamous epithelial cell (B) and mucus (C) (x400).

8. Granular Casts

- nonpathologic casts
 - from the lysosomes excreted by RTE cells during normal metabolism
- pathologic casts
 - disintegration of cellular
 - casts and tubule cells or protein aggregates filtered by the glomerulus
- waxy appearance - when granular casts remain in the tubules for extended periods

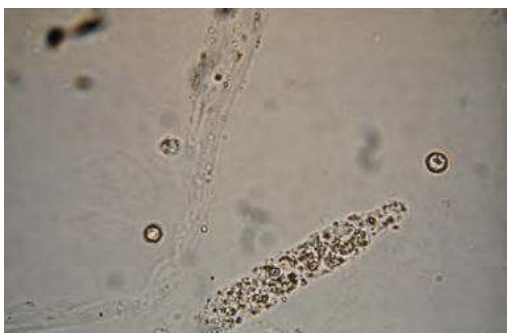


Figure 6-65 Granular disintegrating cellular cast (x400).



Figure 6-66 Coarsely granular cast (A), squamous epithelial cell (B), and mucus (C) (x400).

9. Waxy Casts

- extreme urine stasis
- indicating chronic renal failure
- degeneration of the hyaline cast matrix and any cellular elements or granules contained in the matrix
- higher refractive index
- fragmented with jagged ends and have notches in their sides
- dark pink in supravital stains

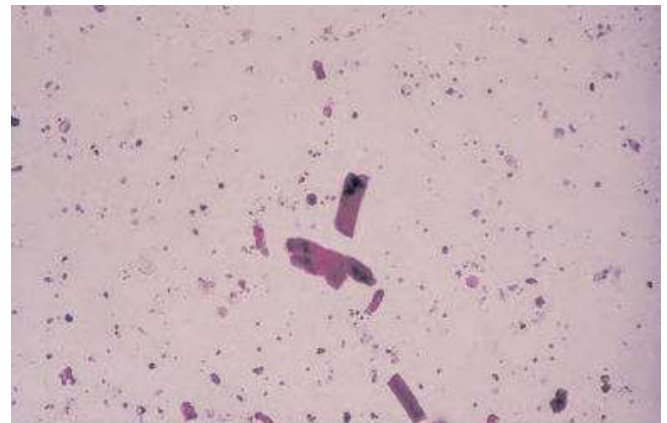


Figure 6-68 KOVA-stained waxy casts (x100).



Figure 6-69 KOVA-stained waxy casts (x200).

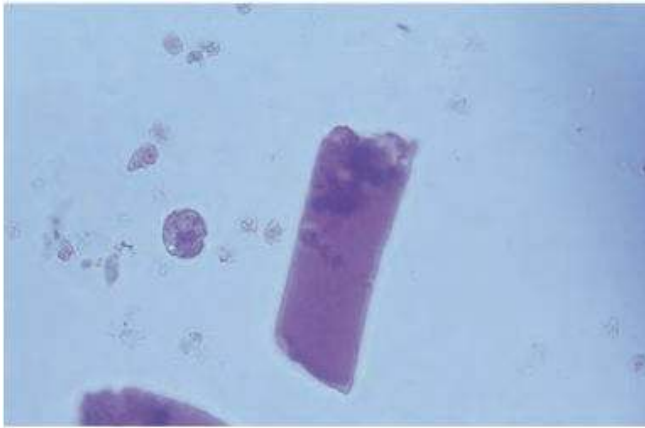


Figure 6-70 KOVA-stained waxy cast (x400).

10. Broad Casts

- renal failure casts
- extreme urine stasis
- indicates destruction (widening) of the tubular walls
- when the flow of urine to the larger collecting ducts becomes severely compromised
- viral hepatitis - bile-stained broad, waxy casts are seen as the result of the tubular necrosis



Figure 6-71 KOVA-stained broad waxy cast (x400).

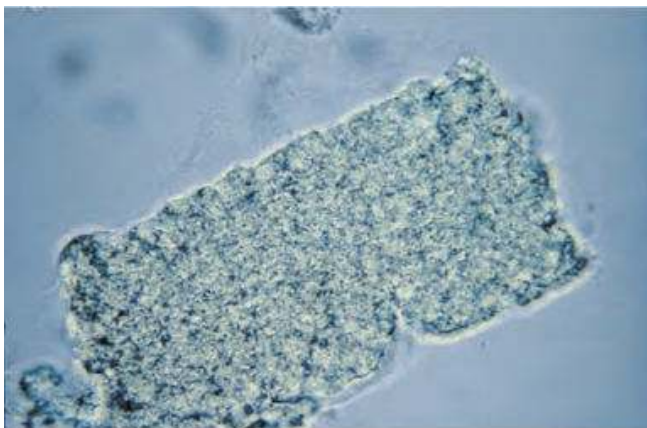


Figure 6-72 Broad granular cast becoming waxy (x400).

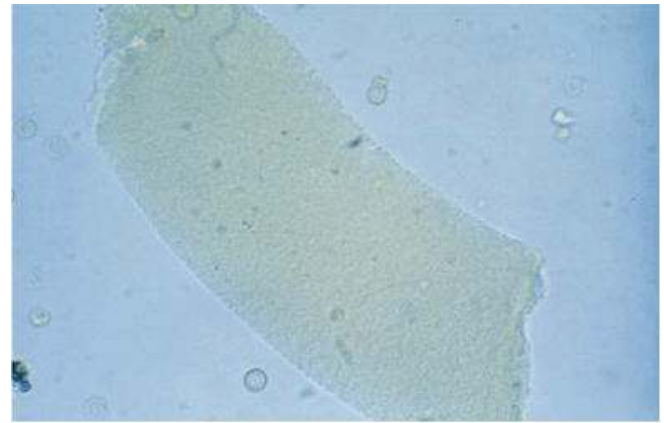


Figure 6-73 Broad bile-stained waxy cast (x400).

J. Urinary Crystals

- rarely of clinical significance
- may appear as
 - true geometrically formed structures
 - amorphous material
- Abnormal types: disorders as liver disease, inborn errors of metabolism, or renal damage caused by crystallization of medications compounds within the tubules

Crystal Formation

- formed by the precipitation of urine solutes
 - inorganic salts
 - organic compounds
 - medications (iatrogenic compounds)
- subject to changes in
 - temperature - lower temperatures is needed
 - solute concentration - concentration of urinary solutes increases, their ability to remain in solution decreases → resulting in crystal formation
 - pH
 - acidic pH - organic and iatrogenic compounds (all abnormal crystals are found in acidic urine)
 - neutral and alkaline pH - inorganic salts
 - exception: calcium oxalate (precipitates in both acidic and neutral urine)
- affect solubility
- reversal of these changes can cause crystals to dissolve

General Identification Techniques

- urine pH - first consideration when identifying crystals
- All abnormal crystals are found in acidic urine

Normal Crystals Seen in Acidic Urine

- Urates - appear yellow to reddish brown

1. amorphous urates
- yellow-brown granules
 - very characteristic pink sediment when refrigerated (caused by uroerythrin)
 - found in >5.5 pH



Figure 6-74 Amorphous urates (x400).

2. uric acid

- found in <5.5 pH
- rhombic, four-sided flat plates (whetstones), wedges, and rosettes
- appear yellow-brown, but may be colorless
- six-sided shape, similar to cystine crystals
- highly birefringent under polarized light
- associated with
 - increased levels of purines and nucleic acids and are seen in patients with leukemia who are receiving chemotherapy
 - in patients with Lesch-Nyhan syndrome
 - patients with gout



Figure 6-77 Clump of uric acid crystals (x400). Notice the whetstone, not hexagonal, shape that differentiates uric acid crystals from cystine crystals.

3. acid urates

- rarely encountered
- seen in less acidic urine
- larger granules and may have spicules similar to the ammonium biurate crystals

4. sodium urates

- rarely encountered
- seen in less acidic urine
- needle-shaped and are seen in synovial fluid during episodes of gout, but may also appear in the urine

5. calcium oxalate crystals

- associated with foods high in oxalic acid and ascorbic acid
- a. dihydrate form
 - most common form of calcium oxalate crystal
 - colorless
 - octahedral envelope
 - two pyramids joined at their bases
 - birefringent under polarized light
- b. monohydrate form
 - oval or dumbbell shaped
 - birefringent under polarized light
 - in cases of ethylene glycol (antifreeze) poisoning



Figure 6-79 Classic dihydrate calcium oxalate crystals (x400).

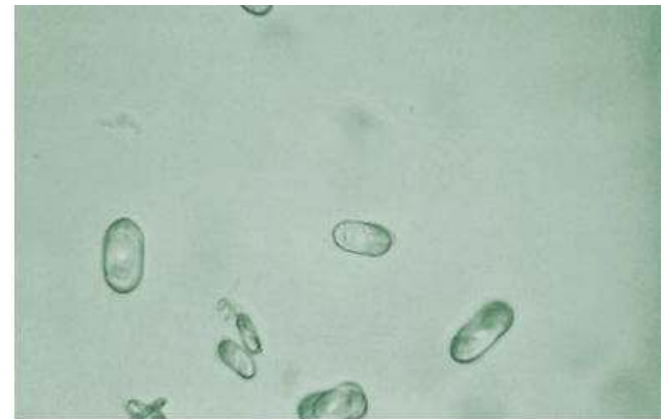


Figure 6-82 Monohydrate calcium oxalate crystals (x400).

Normal Crystals Seen in Alkaline Urine

- Phosphates - majority of the crystals seen in alkaline urine

1. amorphous phosphate

- granular in appearance
- similar to amorphous urates
- differentiated by color of the sediment and the urine pH
- present in large quantities following specimen refrigeration
- cause a white precipitate that does not dissolve on warming

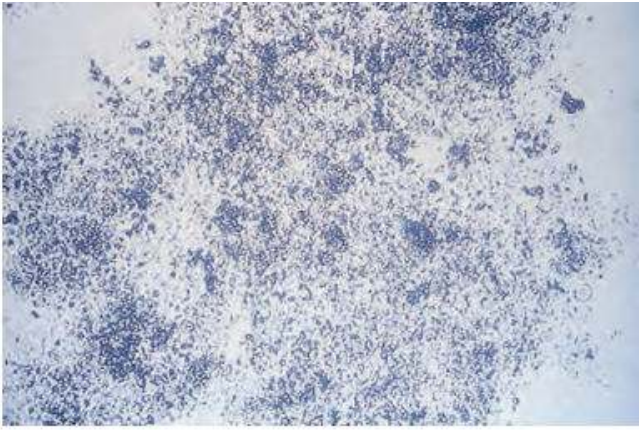


Figure 6-83 Amorphous phosphates (x400) Urine pH 7.0.



Figure 6-84 Amorphous phosphates (x400)

2. triple phosphate (ammonium magnesium phosphate)

- prism shape
- "coffin lid"
- disintegration → feathery appearance
- birefringent under polarized light
- no clinical significance

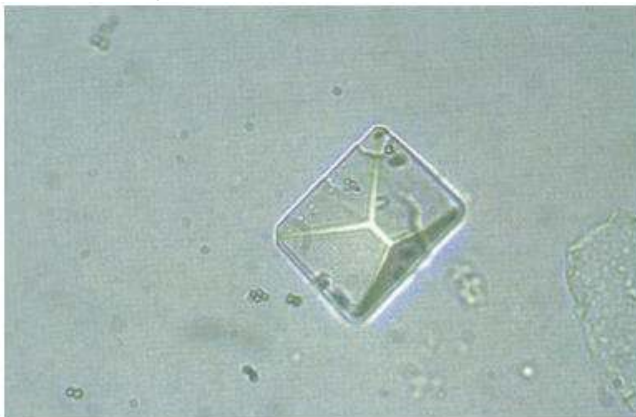


Figure 6-85 Triple phosphate crystal (x400).



Figure 6-86 Triple phosphate crystals (arrow) and amorphous phosphates (x400).

3. calcium phosphate

- colorless, flat rectangular plates or thin prisms often in rosette formations
- confused with sulfonamide crystals when the urine pH is in the neutral range
- calcium phosphates - dissolve in dilute acetic acid
- sulfonamides - does not dissolve in dilute acetic acid
- no clinical significance

4. calcium carbonate

- small and colorless
- with dumbbell or spherical shapes
- can be distinguished by the formation of gas after the addition of acetic acid
- birefringent
- no clinical significance

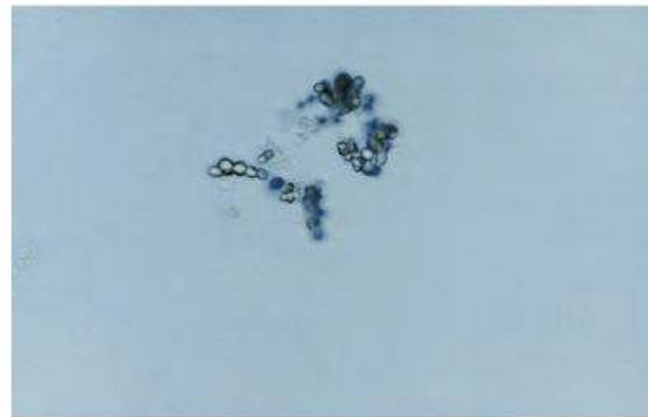


Figure 6-87 Calcium carbonate crystals (x400).

5. ammonium biurate

- yellow-brown color of the urate crystals
- "thorny apples"
- dissolve at 60°C
- convert to uric acid crystals when glacial acetic acid is added
- always encountered in old specimens
- associated with the presence of the ammonia produced by urea-splitting bacteria

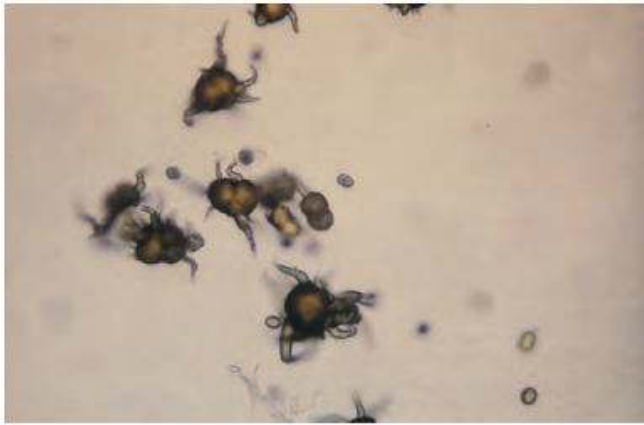


Figure 6–88 Ammonium biurate crystals (x400). Notice the "thorny apple" appearance. (Courtesy of Kenneth L. McCoy, MD.)

Major Characteristics of Normal Urinary Crystals			
Crystal	pH	Color	Appearance
Uric acid	Acid	Yellow-brown (rosettes, wedges)	
Amorphous urates	Acid	Brick dust or yellow brown	
Calcium oxalate	Acid/neutral (alkaline)	Colorless (envelopes, oval, dumbbell)	
Amorphous phosphates	Alkaline/neutral	White-colorless	
Calcium phosphate	Alkaline/neutral	Colorless	
Triple phosphate	Alkaline	Colorless ("coffin lids")	
Ammonium biurate	Alkaline	Yellow-brown ("thorny apples")	
Calcium carbonate	Alkaline	Colorless (dumbbells)	

Abnormal Urine Crystals

found in acidic urine

1. Cystine crystals
 - associated with cystinuria
 - metabolic disorder that prevents reabsorption of cystine by the renal tubules
 - colorless, hexagonal plates and may be thick or thin
 - only thick cystine crystals have polarizing capability
 - confirmation: cyanide-nitroprusside test

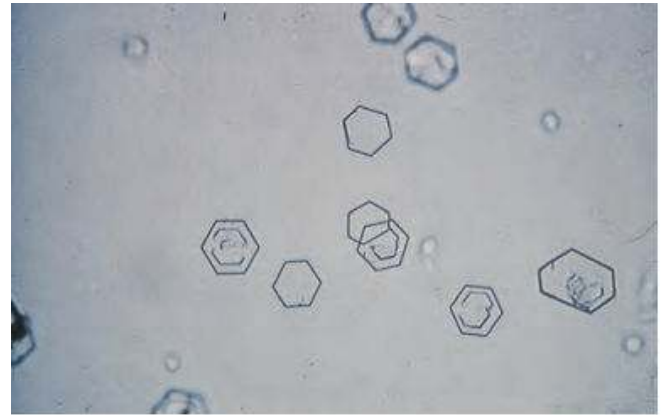


Figure 6–91 Cystine crystals (x400).

2. Cholesterol Crystals
 - rarely seen unless specimens have been refrigerated
 - lipids remain in droplet form
 - rectangular plate with a notch in one or more corners
 - lipiduria (such as the nephrotic syndrome)
 - seen in conjunction with fatty casts and oval fat bodies
 - highly birefringent with polarized light

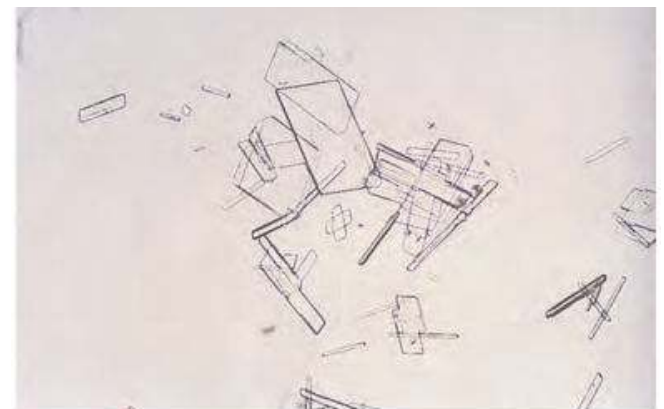


Figure 6–93 Cholesterol crystals. Notice the notched corners (x400).

3. Radiographic Dye Crystals
 - specific gravity of a specimen containing radiographic contrast media is markedly elevated

4. Crystals Associated With Liver Disorders

a. tyrosine

- fine colorless to yellow needles that frequently form clumps or rosettes
- seen in conjunction with leucine crystals in specimens with positive chemical test results for bilirubin
- encountered in inherited disorders of amino acid metabolism



Figure 6-96 Tyrosine crystals in rosette forms (x400).

b. leucine

- yellow-brown spheres that demonstrate concentric circles and radial striations
- less frequently than tyrosine crystals
- when present, should be accompanied by tyrosine crystals

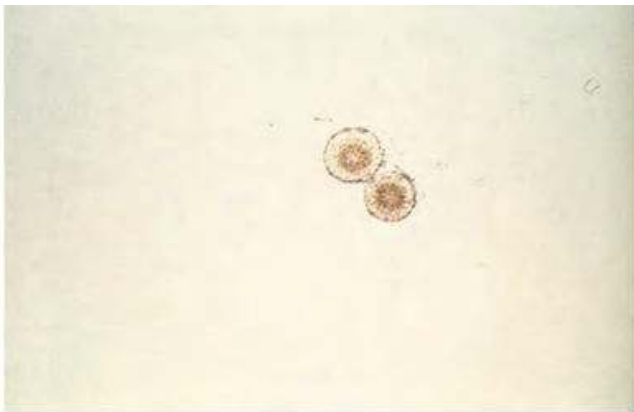


Figure 6-97 Leucine crystals (x400). Notice the concentric circles.

c. bilirubin

- present in hepatic disorders producing large amounts of bilirubin in the urine
- clumped needles or granules
- yellow color of bilirubin

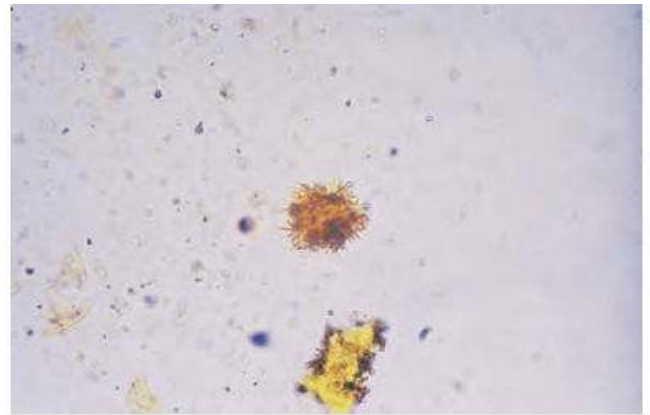


Figure 6-98 Bilirubin crystals. Notice the classic bright yellow color (x400).

5. Sulfonamide Crystals

- patients being treated for UTIs
- inadequate patient hydration - primary cause of sulfonamide crystallization
- needles, rhombics, whetstones, sheaves of wheat, and rosettes
- colors ranging from colorless to yellow-brown

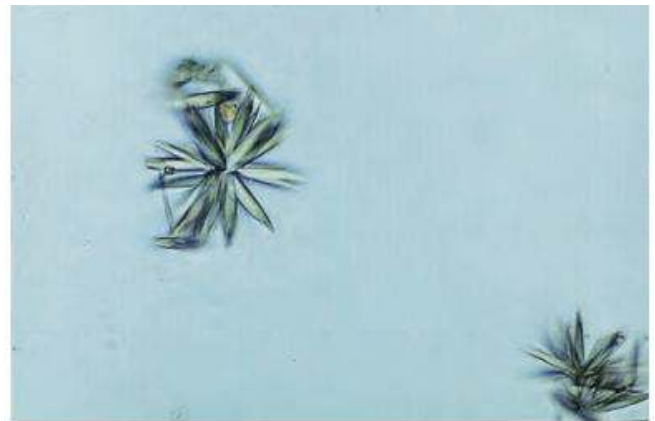


Figure 6-99 Sulfa crystals in rosette form (x400).

6. Ampicillin Crystals

- precipitation of antibiotics
- massive doses of this penicillin compound without adequate hydration
- colorless needles that tend to form bundles following refrigeration



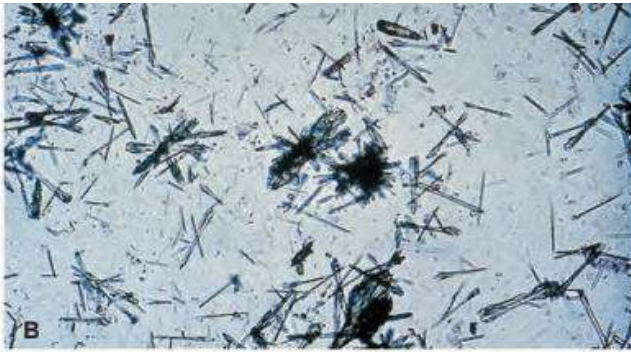


Figure 6-101 Ampicillin crystals **A.** Nonrefrigerated ampicillin crystals. (x400). **B.** Ampicillin crystals after refrigeration (x400).

K. Urinary Sediment Artifacts

- collected under improper conditions or in dirty containers
- such as starch, oil droplets, air bubbles, pollen grains, fibers, and fecal contamination
- occur in a different microscopic plane than the true sediment constituents
- reporting of artifacts is not necessary

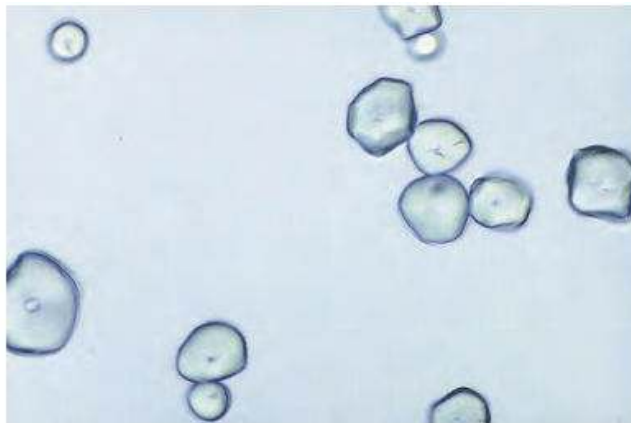


Figure 6-102 Starch granules. Notice the dimpled center (x400).

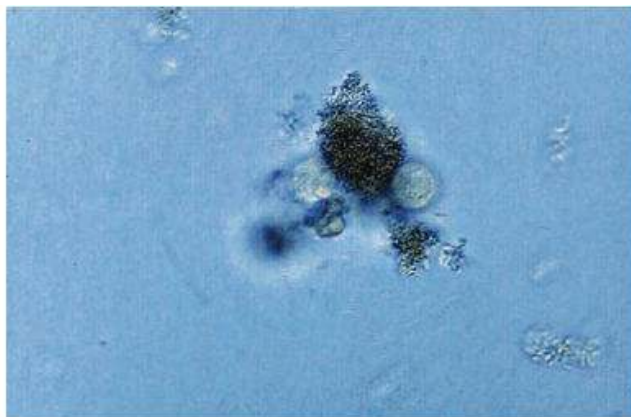


Figure 6-103 Fecal material and oil artifacts (x400).

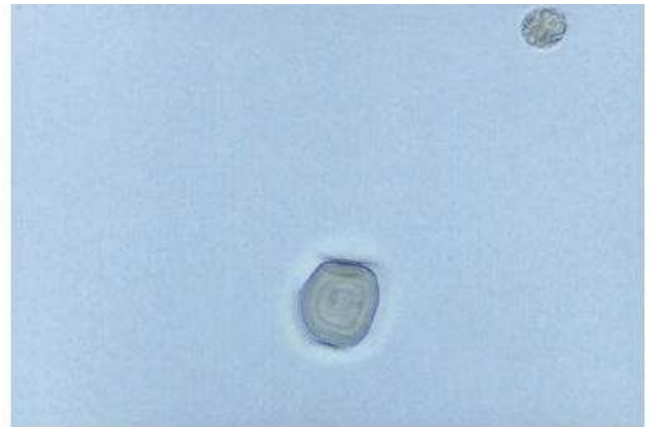


Figure 6-104 Pollen grain. Notice the concentric circles (x400).



Figure 6-105 Fiber and squamous epithelial cell (x400).

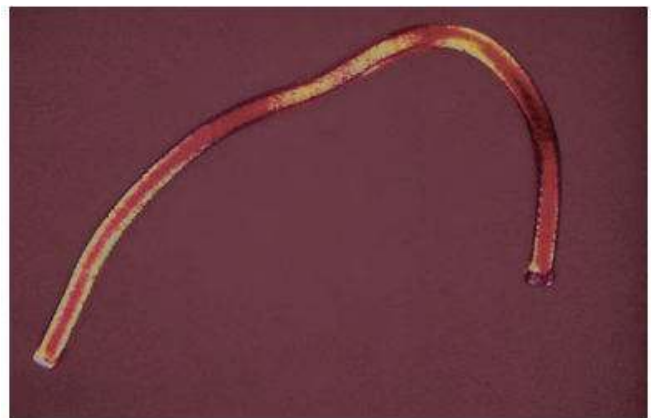


Figure 6-106 Fiber under polarized light (x100).

Review Questions

- Macroscopic screening of urine specimens is used to:
 - Provide results as soon as possible
 - Predict the type of urinary casts present
 - Increase cost-effectiveness of urinalysis
 - Decrease the need for polarized microscopy
- Variations in the microscopic analysis of urine include all of the following except:
 - Preparation of the urine sediment
 - Amount of sediment analyzed
 - Method of reporting
 - Identification of formed elements
- All of the following can cause false-negative microscopic results except:
 - Braking the centrifuge
 - Failing to mix the specimen
 - Dilute alkaline urine
 - Using midstream clean-catch specimens
- The two factors that determine relative centrifugal force are:
 - Radius of rotor head and rpm
 - Radius of rotor head and time of centrifugation
 - Diameter of rotor head and rpm
 - RPM and time of centrifugation
- When using the glass slide and cover-slip method, which of the following might be missed if the cover slip is overflowed?
 - Casts
 - RBCs
 - WBCs
 - Bacteria
- Initial screening of the urine sediment is performed using an objective power of:
 - 4×
 - 10×
 - 40×
 - 100×
- Which of the following should be used to reduce light intensity in bright-field microscopy?
 - Centering screws
 - Aperture diaphragm
 - Rheostat
 - Condenser aperture diaphragm
- Which of the following are reported as number per lpf?
 - RBCs
 - WBCs
 - Crystals
 - Casts
- The Sternheimer-Malbin stain is added to urine sediments to do all of the following except:
 - Increase visibility of sediment constituents
 - Change the constituents' refractive index
 - Decrease precipitation of crystals
 - Delineate constituent structures
- Nuclear detail can be enhanced by:
 - Prussian blue
 - Toluidine blue
 - Acetic acid
 - Both B and C
- Which of the following lipids is/are stained by Sudan III?
 - Cholesterol
 - Neutral fats
 - Triglycerides
 - Both B and C
- Which of the following lipids is/are capable of polarizing light?
 - Cholesterol
 - Neutral fats
 - Triglycerides
 - Both A and B
- The purpose of the Hansel stain is to identify:
 - Neutrophils
 - Renal tubular cells
 - Eosinophils
 - Monocytes
- Crenated RBCs are seen in urine that is:
 - Hyposthenuric
 - Hypersthenuric
 - Highly acidic
 - Highly alkaline
- Differentiation among RBCs, yeast, and oil droplets may be accomplished by all of the following except:
 - Observation of budding in yeast cells
 - Increased refractility of oil droplets
 - Lysis of yeast cells by acetic acid
 - Lysis of RBCs by acetic acid
- A finding of dysmorphic RBCs is indicative of:
 - Glomerular bleeding
 - Renal calculi
 - Traumatic injury
 - Coagulation disorders
- Leukocytes that stain pale blue with Sternheimer-Malbin stain and exhibit brownian movement are:
 - Indicative of pyelonephritis
 - Basophils
 - Mononuclear leukocytes
 - Glitter cells
- Mononuclear leukocytes are sometimes mistaken for:
 - Yeast cells
 - Squamous epithelial cells
 - Pollen grains
 - Renal tubular cells
- When pyuria is detected in a urine sediment, the slide should be carefully checked for the presence of:
 - RBCs
 - Bacteria
 - Hyaline casts
 - Mucus
- Transitional epithelial cells are sloughed from the:
 - Collecting duct
 - Vagina
 - Bladder
 - Proximal convoluted tubule
- The largest cells in the urine sediment are:
 - Squamous epithelial cells
 - Urothelial epithelial cells
 - Cuboidal epithelial cells
 - Columnar epithelial cells
- A clinically significant squamous epithelial cell is the:
 - Cuboidal cell
 - Clue cell
 - Caudate cell
 - Columnar cell
- Forms of transitional epithelial cells include all of the following except:
 - Spherical
 - Caudate
 - Convoluted
 - Polyhedral
- Increased transitional cells are indicative of:
 - Catheterization
 - Malignancy
 - Pyelonephritis
 - Both A and B

27. A primary characteristic used to identify renal tubular epithelial cells is:
 - A. Elongated structure
 - B. Centrally located nucleus
 - C. Spherical appearance
 - D. Eccentrically located nucleus
28. Following an episode of hemoglobinuria, RTE cells may contain:
 - A. Bilirubin
 - B. Hemosiderin granules
 - C. Porphobilinogen
 - D. Myoglobin
29. The predecessor of the oval fat body is the:
 - A. Histiocyte
 - B. Urothelial cell
 - C. Monocyte
 - D. Renal tubular cell
30. A structure believed to be an oval fat body produced a Maltese cross formation under polarized light but does not stain with Sudan III. The structure:
 - A. Contains cholesterol
 - B. Is not an oval fat body
 - C. Contains neutral fats
 - D. Is contaminated with immersion oil
31. The finding of yeast cells in the urine is commonly associated with:
 - A. Cystitis
 - B. Diabetes mellitus
 - C. Pyelonephritis
 - D. Liver disorders
32. The primary component of urinary mucus is:
 - A. Bence Jones protein
 - B. Microalbumin
 - C. Uromodulin
 - D. Orthostatic protein
33. The majority of casts are formed in the:
 - A. Proximal convoluted tubules
 - B. Ascending loop of Henle
 - C. Distal convoluted tubules
 - D. Collecting ducts
34. Cylindruria refers to the presence of:
 - A. Cylindrical renal tubular cells
 - B. Mucus-resembling casts
 - C. Hyaline and waxy casts
 - D. All types of casts
35. A person submitting a urine specimen following a strenuous exercise routine can normally have all of the following in the sediment except:
 - A. Hyaline casts
 - B. Granular casts
 - C. RBC casts
 - D. WBC casts
36. Prior to identifying an RBC cast, all of the following should be observed except:
 - A. Free-floating RBCs
 - B. Intact RBCs in the cast
 - C. Presence of a cast matrix
 - D. A positive reagent strip blood reaction
37. WBC casts are primarily associated with:
 - A. Pyelonephritis
 - B. Cystitis
 - C. Glomerulonephritis
 - D. Viral infections
38. The shape of the RTE cell associated with renal tubular epithelial casts is primarily:
 - A. Elongated
 - B. Cuboidal
 - C. Round
 - D. Columnar
39. When observing RTE casts, the cells are primarily:
 - A. Embedded in a clear matrix
 - B. Embedded in a granular matrix
 - C. Attached to the surface of a matrix
 - D. Stained by components of the urine filtrate
40. The presence of fatty casts is associated with:
 - A. Nephrotic syndrome
 - B. Crush injuries
 - C. Diabetes mellitus
 - D. All of the above
41. Nonpathogenic granular casts contain:
 - A. Cellular lysosomes
 - B. Degenerated cells
 - C. Protein aggregates
 - D. Gram-positive cocci
42. All of the following are true about waxy casts except they:
 - A. Represent extreme urine stasis
 - B. May have a brittle consistency
 - C. Require staining to be visualized
 - D. Contain degenerated granules
43. Observation of broad casts represents:
 - A. Destruction of tubular walls
 - B. Dehydration and high fever
 - C. Formation in the collecting ducts
 - D. Both A and C
44. All of the following contribute to urinary crystals formation except:
 - A. Protein concentration
 - B. pH
 - C. Solute concentration
 - D. Temperature
45. The most valuable initial aid for identifying crystals in a urine specimen is:
 - A. pH
 - B. Solubility
 - C. Staining
 - D. Polarized microscopy
46. Crystals associated with severe liver disease include all of the following except:
 - A. Bilirubin
 - B. Leucine
 - C. Cystine
 - D. Tyrosine
47. All of the following crystals routinely polarize except:
 - A. Uric acid
 - B. Cholesterol
 - C. Radiographic dye
 - D. Cystine
48. Casts and fibers can usually be differentiated using:
 - A. Solubility characteristics
 - B. Patient history
 - C. Polarized light
 - D. Fluorescent light

47. Match the following crystals seen in acidic urine with their description/identifying characteristics:

- | | |
|---------------------------------|----------------------------|
| ___ Amorphous urates | 1. Envelopes |
| ___ Uric acid | 2. Thin needles |
| ___ Calcium oxalate monohydrate | 3. Yellow-brown, whetstone |
| ___ Calcium oxalate dihydrate | 4. Pink sediment |
| | 5. Ovoid |

48. Match the following crystals seen in alkaline urine with their description/identifying characteristics:

- | | |
|-------------------------|----------------------|
| ___ Triple phosphate | 1. Yellow granules |
| ___ Amorphous phosphate | 2. Thin prisms |
| ___ Calcium phosphate | 3. "Coffin lids" |
| ___ Ammonium biurate | 4. Dumbbell shape |
| ___ Calcium carbonate | 5. White precipitate |
| | 6. Thorny apple |

49. Match the following abnormal crystals with their description/identifying characteristics:

- | | |
|----------------------|--|
| ___ Cystine | 1. Bundles following refrigeration |
| ___ Tyrosine | 2. Highly alkaline pH |
| ___ Cholesterol | 3. Bright yellow clumps |
| ___ Leucine | 4. Hexagonal plates |
| ___ Ampicillin | 5. Flat plates, high specific gravity |
| ___ Radiographic dye | 6. Concentric circles, radial striations |
| ___ Bilirubin | 7. Notched corners |
| | 8. Fine needles seen in liver disease |

50. Match the following types of microscopy with their descriptions:

- | | |
|---------------------------|---|
| ___ Bright-field | 1. Indirect light is reflected off the object |
| ___ Phase | 2. Objects split light into two beams |
| ___ Polarized | 3. Low refractive index objects may be overlooked |
| ___ Dark-field | 4. Three-dimensional images |
| ___ Fluorescent | 5. Forms halo of light around object |
| ___ Interference contrast | 6. Detects electrons emitted from objects |
| | 7. Detects specific wavelengths of light emitted from objects |

Renal Disease

OUTLINE

- I. Introduction
- II. Glomerular Disorders
- III. Tubular Disorders
- IV. Interstitial Disorders
- V. Renal Failure
- VI. Renal Lithiasis

INTRODUCTION

- Renal disease - classified as being glomerular, tubular, or interstitial, based on the area of the kidney primarily affected

GLOMERULAR DISORDERS

Immunologic Causes

- Immune complexes formed as a result of immunologic reactions and increased serum immunoglobulins, such as immunoglobulin A (IgA), circulate in the bloodstream and are deposited on the glomerular membranes

Nonimmunologic Causes

- exposure to chemicals and toxins that also affect the tubules
- disruption of the electrical membrane charges as occurs in the nephrotic syndrome
- deposition of amyloid material from systemic disorders that may involve chronic inflammation and acute-phase reactants
- basement membrane thickening associated with diabetic nephropathy

A. Glomerulonephritis

- sterile, inflammatory process that affects the glomerulus and is associated with the finding of blood, protein, and casts in the urine

1. Acute Poststreptococcal Glomerulonephritis (AGN)

- marked by the sudden onset of symptoms consistent with damage to the glomerular membrane
- caused by certain strains of group A streptococcus that contain M protein in the cell wall.
- Symptoms: fever, edema (most noticeably around the eyes), fatigue, hypertension, oliguria, and hematuria
- Secondary complications: hypertension and electrolyte imbalance
- Urinalysis findings: hematuria, proteinuria, and oliguria, accompanied by red blood cell (RBC) casts, dysmorphic RBCs, hyaline and granular casts, and white blood cells (WBCs)
- Confirmation: positive anti-group A streptococcal enzyme

2. Rapidly Progressive (Crescentic) Glomerulonephritis

- more serious form of acute glomerular disease
- poorer prognosis
- terminating in renal failure
- complication of another form of glomerulonephritis or an immune systemic disorder such as systemic lupus erythematosus (SLE)
- Urinalysis findings: markedly elevated protein levels and very

- low glomerular filtration rates

3. Goodpasture Syndrome

- autoimmune disorder
- antiglomerular basement membrane antibody
 - cytotoxic autoantibody can appear against the glomerular and alveolar basement membranes after viral respiratory infections
 - attachment of this autoantibody to the basement membrane, followed by complement activation, produces the capillary destruction
- Initial pulmonary complaints: hemoptysis and dyspnea, followed by the development of hematuria
- Laboratory findings: proteinuria, hematuria, and the presence of RBC casts

4. Wegener Granulomatosis

- causes a granuloma-producing inflammation of the small blood vessels primarily of the kidney and respiratory system
- Key diagnosis: antineutrophilic cytoplasmic antibody (ANCA) in the patient's serum
 - initiate the immune response and the resulting granuloma formation
- Laboratory findings: hematuria, proteinuria, RBC casts, and elevated serum creatinine and BUN
- Testing:
 - incubating the patient's serum with either ethanol or formalin/formaldehyde-fixed neutrophils
 - examining the preparation using indirect immunofixation to detect the serum antibodies attached to the neutrophils
 - ethanol - perinuclear pattern (p-ANCA)
 - formalin/formaldehyde - granular throughout the cytoplasm (c-ANCA)

5. Henoch-Schönlein Purpura

- primarily in children after upper respiratory infection
- Initial symptoms: appearance of raised, red patches on the skin
- Respiratory and gastrointestinal symptoms: blood in the sputum and stools
- Renal involvement - most serious complication

6. Membranous Glomerulonephritis

- pronounced thickening of the glomerular basement membrane resulting from the deposition of immunoglobulin G immune complexes
- Associated with: systemic lupus erythematosus, Sjögren syndrome, secondary syphilis, hepatitis B, gold and mercury treatments, and malignancy
- Laboratory findings: microscopic hematuria and elevated urine protein excretion

7. Membranoproliferative Glomerulonephritis (MPGN)

- Type 1 - increased cellularity in the subendothelial cells of the mesangium (interstitial area of Bowman's capsule), causing thickening of the capillary walls
- Type 2 - extremely dense deposits in the glomerular basement membrane

- Laboratory findings: hematuria, proteinuria, and decreased serum complement levels

8. Chronic Glomerulonephritis

- Symptoms: fatigue, anemia, hypertension, edema, and oliguria
- Laboratory findings: hematuria, proteinuria, glucosuria as a result of tubular dysfunction, and many varieties of casts, including broad casts
- markedly decreased glomerular filtration rate is present in conjunction with increased BUN and creatinine levels and electrolyte imbalance

9. Immunoglobulin A Nephropathy

- Berger disease
- most common cause of glomerulonephritis
- immune complexes containing IgA are deposited on the glomerular membrane
- asymptomatic for 20 years or more
- Laboratory findings: macroscopic hematuria, asymptomatic microhematuria and elevated serum levels of IgA

B. Nephrotic Syndrome

- marked by
 - massive proteinuria (greater than 3.5 g/day)
 - low levels of serum albumin → increased lipid production by the liver
 - high levels of serum lipids
 - pronounced edema
- Acute onset of the disorder
 - circulatory disruption producing systemic shock that decrease the pressure and flow of blood to the kidney
- Increased permeability of the glomerular membrane - damage to the shield of negativity and the podocytes that produces a less tightly connected barrier
- Laboratory findings: proteinuria; urinary fat droplets; oval fat bodies; renal tubular epithelial (RTE) cells; epithelial, fatty, and waxy casts; and microscopic hematuria

1. Minimal Change Disease

- lipid nephrosis
- associated with human leukocyte antigen-B12 (HLA-B12) antigen
- produces little cellular change in the glomerulus, except for some damage to the podocytes and the shield of negativity, allowing for increased protein filtration
- Laboratory findings: heavy proteinuria, transient hematuria, and normal BUN and creatinine
- responds to corticosteroids

2. Focal Segmental Glomerulosclerosis (FSGS)

- affects only numbers and areas of glomeruli
- Immune deposits: immunoglobulin M and C3
- association with abuse of heroin and analgesics and with AIDS
- Laboratory findings: Moderate to heavy proteinuria and microscopic hematuria

Laboratory Testing in Glomerular Disorders		
Disorder	Primary Urinalysis Result	Other Significant Tests
Acute glomerulonephritis	Macroscopic hematuria Proteinuria RBC casts Granular casts	Anti-group A streptococcal enzyme tests
Rapidly progressive glomerulonephritis	Macroscopic hematuria Proteinuria RBC casts	BUN Creatinine eGFR
Goodpasture syndrome	Macroscopic hematuria Proteinuria RBC casts	Antiglomerular basement membrane antibody
Wegener granulomatosis	Macroscopic hematuria Proteinuria RBC casts	Antineutrophilic peripheral or cytoplasmic antibody
Henoch-Schönlein purpura	Macroscopic hematuria Proteinuria RBC casts	Stool occult blood
Membranous glomerulonephritis	Microscopic hematuria Proteinuria	Antinuclear antibody Hepatitis B surface antigen Fluorescent treponemal antibody-absorption test (FTA-ABS)
Membranoproliferative glomerulonephritis	Hematuria Proteinuria	Serum complement levels
Chronic glomerulonephritis	Hematuria Proteinuria Glucosuria Cellular and granular casts Waxy and broad casts	BUN Serum creatinine eGFR Electrolytes
IgA nephropathy (early stages)	Macroscopic or microscopic hematuria	Serum IgA
IgA nephropathy (late stages)	See Chronic-glomerulonephritis	
Nephrotic syndrome	Heavy proteinuria Microscopic hematuria Renal tubular cells Oval fat bodies Fat droplets Fatty and waxy casts	Serum albumin Cholesterol Triglycerides
Minimal change disease	Heavy proteinuria Transient hematuria Fat droplets	Serum albumin Cholesterol Triglycerides

Focal segmental glomerulosclerosis	Proteinuria Microscopic hematuria Macroscopic or microscopic hematuria	Drugs of abuse HIV tests
Alport syndrome (early stages) (late stages)	See Nephrotic syndrome Microalbuminuria	Genetic testing
Diabetic nephropathy (late stages)	See Chronic glomerulonephritis	Blood glucose

Clinical Information Associated With Glomerular Disorders		
Disorder	Etiology	Clinical Course
Acute glomerulonephritis	Deposition of immune complexes, formed in conjunction with group A <i>Streptococcus</i> infection, on the glomerular membranes	Rapid onset of hematuria and edema, permanent renal damage seldom occurs
Rapidly progressive Glomerulonephritis	Deposition of immune complexes from systemic immune disorders on the glomerular membrane	Rapid onset with glomerular damage and possible progression to end-stage renal failure
Goodpasture syndrome	Attachment of a cytotoxic antibody formed during viral respiratory infections to glomerular and alveolar basement membranes	Hemoptysis and dyspnea followed by hematuria Possible progression to end-stage renal failure
Wegener granulomatosis	Antineutrophilic cytoplasmic autoantibody binds to neutrophils in vascular walls producing damage to small vessels in the lungs and glomerulus	Pulmonary symptoms including hemoptysis develop first followed by renal involvement and possible progression to end-stage renal failure
Henoch-Schönlein Purpura	Occurs primarily in children following viral	Initial appearance of purpura followed by blood in sputum

	respiratory infections; a decrease in platelets disrupts vascular integrity	and stools and eventual renal involvement Complete recovery is common, but may progress to renal failure
Membranous glomerulonephritis	Thickening of the glomerular membrane following IgG immune complex deposition associated with systemic disorders	Slow progression to the nephrotic syndrome or possible remission
Membranoproliferative glomerulonephritis	Cellular proliferation affecting the capillary walls or the glomerular basement membrane, possibly immune-mediated	Slow progression to chronic glomerulonephritis or nephrotic syndrome
Chronic glomerulonephritis	Marked decrease in renal function resulting from glomerular damage precipitated by other renal disorders	Noticeable decrease in renal function progressing to renal failure
IgA nephropathy	Deposition of IgA on the glomerular membrane resulting from increased levels of serum IgA	Recurrent macroscopic hematuria following exercise with slow progression to chronic glomerulonephritis
Nephrotic syndrome	Disruption of the shield of negativity and damage to the tightly fitting podocyte barrier resulting in massive loss of protein and lipids	Acute onset following systemic shock Gradual progression from other glomerular disorders and then to renal failure
Minimal change disease	Disruption of the podocytes occurring	Frequent complete remission

	primarily in children following allergic reactions and immunizations	following corticosteroid treatment
Focal segmental glomerulosclerosis	Disruption of podocytes in certain areas of glomeruli associated with heroin and analgesic abuse and AIDS	May resemble nephrotic syndrome or minimal change disease
Alport syndrome	Genetic disorder showing lamellated and thinning glomerular basement membrane	Slow progression to nephrotic syndrome and end-stage renal disease

TUBULAR DISORDERS

A. Acute Tubular Necrosis (ATN)

- primary disorder associated with damage to the renal tubules or RTEs
- produced by decreased blood flow that causes a lack of oxygen presentation to the tubules (ischemia) or the presence of toxic substances in the urinary filtrate
- Ischemic ATN: shock, trauma (such as crushing injuries), and surgical procedures
- Nephrotoxic agents: aminoglycoside antibiotics, the antifungal agent amphotericin B, cyclosporine, radiographic dye, organic solvents such as ethylene glycol, heavy metals, and toxic mushrooms
- Laboratory findings: mild proteinuria, microscopic hematuria, and, most noticeably, the presence of RTE cells and RTE cell casts containing tubular fragments consisting of three or more cells

B. Hereditary and Metabolic Tubular Disorders

- caused by systemic conditions that affect or override the tubular reabsorptive maximum (T_m)

1. Fanconi Syndrome

- generalized failure of tubular reabsorption in the proximal convoluted tubule
- affected are glucose, amino acids, phosphorous, sodium, potassium, bicarbonate, and water
- inherited in association with cystinosis and Hartnup disease
- acquired through exposure to toxic agents, including heavy metals and outdated tetracycline, or seen as a complication of multiple myeloma and renal transplant
- Laboratory findings: glycosuria with a normal blood glucose and possible mild proteinuria, low pH

2. Alport Syndrome

- inherited disorder of collagen production affecting the glomerular basement membrane
- sex-linked or autosomal genetic disorder
- glomerular basement membrane has a lamellated appearance with areas of thinning

- Laboratory diagnosis: macroscopic hematuria, microscopic hematuria

3. Uromodulin-Associated Kidney Disease

- glycoprotein and is the only protein produced by the kidney
- produced by the proximal and distal convoluted tubules
- inherited disorder caused by an autosomal mutation in the gene that produces uromodulin
- causes a decrease in the production of normal uromodulin that is replaced by the abnormal form
- Laboratory findings: serum uric acid → resulting in persons developing gout as early as the teenage years before the onset of detectable renal disease

4. Diabetic Nephropathy

- most common cause of end-stage renal disease
- result of increased proliferation of mesangial cells and increased deposition of cellular and noncellular material within the glomerular matrix, resulting in accumulation of solid substances around the capillary tufts
- associated with deposition of glycosylated proteins resulting from poorly controlled blood glucose levels

5. Nephrogenic Diabetes Insipidus

- inherited as a sex-linked recessive gene or acquired from medications, including lithium and amphotericin B
- seen as a complication of polycystic kidney disease and sickle cell anemia
- Urinalysis findings: low specific gravity, pale yellow color, and possible false-negative results for chemical tests

6. Renal Glycosuria

- affects only the reabsorption of glucose (as opposed to Fanconi syndrome)
- inherited as an autosomal recessive trait
- glucose is not present in the urine unless the blood glucose level reaches the maximal tubular reabsorption capacity for glucose (TM_G)
- 160 to 180 mg/dL - glucose TM_G
- Laboratory findings: urine glucose concentrations with
- normal blood glucose concentrations

Laboratory Testing in Metabolic and Hereditary Tubular Disorders		
Disorder	Primary Urinalysis Result	Other Significant Tests
Acute tubular necrosis	Microscopic hematuria Proteinuria Renal tubular epithelial cells Renal tubular epithelial cell casts Hyaline, granular, waxy, broad casts	Hemoglobin Hematocrit Cardiac enzymes
Fanconi syndrome	Glucosuria Possible cystine crystals	Serum and urine electrolytes Amino acid chromatography Serum uric acid

Uromodulin-associated kidney disease (early stages)	Renal tubular epithelial cells	
Late stages	See chronic glomerulonephritis	
Nephrogenic diabetes insipidus	Low specific gravity, polyuria	ADH testing
Renal glucosuria	Glucosuria	Blood glucose

Clinical Information Associated With Metabolic and Tubular Disorders		
Disorder	Etiology	Clinical Course
Acute tubular necrosis	Damage to renal tubular cells caused by ischemia or toxic agents	Acute onset of renal dysfunction usually resolved when underlying cause is corrected
Fanconi syndrome	Inherited in association with cystinosis and Hartnup disease or acquired through exposure to toxic agents	Generalized defect in renal tubular reabsorption requiring supportive therapy
Uromodulin-associated kidney disease	Inherited defect in the production of normal uromodulin by the renal tubules and increased uric acid causing gout	Continual monitoring of renal function for progression to renal failure and possible kidney transplantation
Nephrogenic diabetes insipidus	Inherited defect of tubular response to ADH or acquired from medications	Requires supportive therapy to prevent Dehydration
Renal glucosuria	Inherited autosomal recessive trait	Benign disorder

INTERSTITIAL DISORDERS

- tubulointerstitial disease - close proximity between the renal tubules and the renal interstitium, disorders affecting the interstitium also affect the tubules
- urinary tract infection (UTI) - most common renal disease
 - upper - renal pelvis, tubules, and interstitium
 - lower - urethra and bladder (cystitis)
 - presence of numerous WBCs and bacteria, often accompanied by mild proteinuria and hematuria and an increased pH

A. Acute Pyelonephritis

- pyelonephritis - infection of the upper urinary tract
- most frequently occurs as a result of ascending movement of bacteria from a lower UTI
 - renal calculi, pregnancy, and reflux of urine from the bladder back into the ureters (vesicoureteral reflux)
- Symptoms: urinary frequency, burning on urination, and lower back pain
- Laboratory findings: numerous leukocytes and bacteria with mild proteinuria and hematuria, WBC casts

B. Chronic Pyelonephritis

- serious disorder that can result in permanent damage to the renal tubules and possible progression to chronic renal failure
- Congenital urinary structural defects producing reflux nephropathy - most frequent cause

MUST-KNOW

Cystitis vs Pyelonephritis

- presence of WBC casts

C. Acute Interstitial Nephritis (AIN)

- marked by inflammation of the renal interstitium followed by inflammation of the renal tubules
- Symptoms: oliguria, edema, decreased renal concentrating ability, and a possible decrease in the glomerular filtration rate
- develop approximately 2 weeks following administration of medication
- penicillin, methicillin, ampicillin, cephalosporins, sulfonamides, NSAIDs, and thiazide diuretics
- Initial symptoms: fever and the presence of a skin rash
- primarily associated with an allergic reaction to medications that occurs within the renal interstitium, possibly caused by the medication binding to the interstitial protein
- Laboratory findings: hematuria, possibly macroscopic, mild to moderate proteinuria, numerous WBCs, and WBC casts without bacteria
- Confirmation: Differential leukocyte staining for the presence of increased eosinophils

Laboratory Testing in Metabolic and Hereditary Tubular Disorders		
Disorder	Primary Urinalysis Result	Other Significant Tests
Cystitis	Leukocyturia Bacteriuria Microscopic hematuria Mild proteinuria Increased pH	Urine culture
Acute pyelonephritis	Leukocyturia Bacteriuria WBC casts Bacterial casts Microscopic hematuria Proteinuria	Urine culture
Chronic pyelonephritis	Leukocyturia Bacteriuria WBC casts Bacterial casts Granular, waxy, broad casts Hematuria Proteinuria	Urine culture BUN Creatinine eGFR
Acute interstitial nephritis	Hematuria Proteinuria Leukocyturia WBC casts	Urine eosinophils BUN Creatinine eGFR

Clinical Information Associated With Interstitial Disorders		
Disorder	Etiology	Clinical Course
Cystitis	Ascending bacterial infection of the bladder	Acute onset of urinary frequency and burning resolved with antibiotics
Acute pyelonephritis	Infection of the renal tubules and interstitium related to interference of urine flow to the bladder, reflux of urine from the bladder, and untreated cystitis	Acute onset of urinary frequency, burning, and lower back pain resolved with antibiotics
Chronic pyelonephritis	Recurrent infection of the renal tubules and interstitium caused by structural abnormalities affecting the flow of urine	Frequently diagnosed in children; requires correction of the underlying structural defect

		Possible progression to renal failure
Acute interstitial nephritis	Allergic inflammation of the renal interstitium in response to certain medications	Acute onset of renal dysfunction often accompanied by a skin rash Resolves following discontinuation of medication and treatment with corticosteroids

RENAL FAILURE

- gradual progression from the original disorder to chronic renal failure or end-stage renal disease
- Marked by decrease in the glomerular filtration rate (less than 25 mL/min); steadily rising serum BUN and creatinine values (azotemia); electrolyte imbalance; lack of renal concentrating ability producing an isosthenuric urine; proteinuria; renal glycosuria; and an abundance of granular, waxy, and broad casts

A. Acute Renal Failure (ARF)

- exhibits a sudden loss of renal function and is frequently reversible
- General: decreased glomerular filtration rate, oliguria, edema, and azotemia

Causes and Symptoms

- prerenal
 - sudden decrease in blood flow to the kidney
 - presence of RTE cells and casts
 - Decreased blood pressure/cardiac output
 - Hemorrhage
 - Burns
 - Surgery
 - Septicemia
- renal
 - acute glomerular and tubular disease
 - RBCs indicate glomerular injury; WBC casts with or without bacteria
 - Acute glomerulonephritis
 - Acute tubular necrosis
 - Acute pyelonephritis
 - Acute interstitial nephritis
- postrenal
 - renal calculi or tumor obstructions
 - normal- and abnormal-appearing urothelial cells possibly associated with malignancy
 - Renal calculi
 - Tumors

RENAL LITHIASIS

- Renal calculi (kidney stones) - may form in the calyces and pelvis of the kidney, ureters, and bladder
- Renal lithiasis - vary in size from barely visible to large, staghorn calculi resembling the shape of the renal pelvis and smooth, round bladder stones with diameters of 2 or more inches
- Small stones - may be passed in the urine severe pain radiating from the lower back to the legs
- Larger stones - cannot be passed and may not be detected until patients develop symptoms of urinary obstruction

Lithotripsy

- procedure using high-energy shock waves, can be used to break stones located in the upper urinary tract into pieces that can then be passed in the urine
- stones removed surgically
- Calculus formation: finding of clumps of crystals in freshly voided urine

Primary calculi constituents

- Magnesium ammonium phosphate (struvite) - frequently accompanied by urinary infections involving urea-splitting bacteria
- uric acid - associated with increased intake of foods with high purine content and with uromodulin-associated kidney disease
- cystine - seen in conjunction with hereditary disorders of cystine metabolism

∴ Calcium calculi - metabolic calcium and phosphate disorders and occasionally diet

Review Questions

1. Most glomerular disorders are caused by:
 - A. Sudden drops in blood pressure
 - B. Immunologic disorders
 - C. Exposure to toxic substances
 - D. Bacterial infections
2. Dysmorphic RBC casts would be a significant finding with all of the following except:
 - A. Goodpasture syndrome
 - B. Acute glomerulonephritis
 - C. Chronic pyelonephritis
 - D. Henoch-Schönlein purpura
3. Occasional episodes of macroscopic hematuria over periods of 20 or more years are seen with:
 - A. Crescentic glomerulonephritis
 - B. IgA nephropathy
 - C. Nephrotic syndrome
 - D. Wegener granulomatosis
4. Antiglomerular basement membrane antibody is seen with:
 - A. Wegener granulomatosis
 - B. IgA nephropathy
 - C. Goodpasture syndrome
 - D. Diabetic nephropathy
5. Antineutrophilic cytoplasmic antibody is diagnostic for:
 - A. IgA nephropathy
 - B. Wegener granulomatosis
 - C. Henoch-Schönlein purpura
 - D. Goodpasture syndrome
6. Respiratory and renal symptoms are associated with all of the following except:
 - A. IgA nephropathy
 - B. Wegener granulomatosis
 - C. Henoch-Schönlein purpura
 - D. Goodpasture syndrome
7. The presence of fatty casts is associated with all of the following except:
 - A. Nephrotic syndrome
 - B. Focal segmental glomerulosclerosis
 - C. Nephrogenic diabetes insipidus
 - D. Minimal change disease
8. The highest levels of proteinuria are seen with:
 - A. Alport syndrome
 - B. Diabetic nephropathy
 - C. IgA nephropathy
 - D. Nephrotic syndrome
9. Ischemia frequently produces:
 - A. Acute renal tubular necrosis
 - B. Minimal change disorder
 - C. Renal glycosuria
 - D. Goodpasture's syndrome
10. A disorder associated with polyuria and low specific gravity is:
 - A. Renal glucosuria
 - B. Minimal change disease
 - C. Nephrogenic diabetes insipidus
 - D. Focal segmental glomerulosclerosis
11. An inherited disorder producing a generalized defect in tubular reabsorption is:
 - A. Alport syndrome
 - B. Acute interstitial nephritis
 - C. Fanconi syndrome
 - D. Renal glycosuria
12. A teenage boy who develops gout in his big toe and has a high serum uric acid should be monitored for:
 - A. Fanconi syndrome
 - B. Renal calculi
 - C. Uromodulin-associated kidney disease
 - D. Chronic interstitial nephritis
13. The only protein produced by the kidney is:
 - A. Albumin
 - B. Uromodulin
 - C. Uroprotein
 - D. Globulin
14. The presence of renal tubular epithelial cells and casts is an indication of:
 - A. Acute interstitial nephritis
 - B. Chronic glomerulonephritis
 - C. Minimal change disease
 - D. Acute tubular necrosis
15. Differentiation between cystitis and pyelonephritis is aided by the presence of:
 - A. WBC casts
 - B. RBC casts
 - C. Bacteria
 - D. Granular casts
16. The presence of WBCs and WBC casts with no bacteria is indicative of:
 - A. Chronic pyelonephritis
 - B. Acute tubular necrosis
 - C. Acute interstitial nephritis
 - D. Both B and C

17. End-stage renal disease is characterized by all of the following except:
- A. Hypersthenuria
 - B. Isosthenuria
 - C. Azotemia
 - D. Electrolyte imbalance
18. Prerenal acute renal failure could be caused by:
- A. Massive hemorrhage
 - B. Acute tubular necrosis
 - C. Acute interstitial nephritis
 - D. Malignant tumors
19. The most common composition of renal calculi is:
- A. Calcium oxalate
 - B. Magnesium ammonium phosphate
 - C. Cystine
 - D. Uric acid
20. Urinalysis on a patient with severe back pain being evaluated for renal calculi would be most beneficial if it showed:
- A. Heavy proteinuria
 - B. Low specific gravity
 - C. Uric acid crystals
 - D. Microscopic hematuria

Urine Screening for Metabolic Disorders

OUTLINE

- I. Overflow Versus Renal Disorders
- II. Newborn Screening Tests
- III. Amino Acid Disorders
- IV. Porphyrin Disorders
- V. Mucopolysaccharide Disorders
- VI. Purine Disorders
- VII. Carbohydrate Disorders

OVERFLOW VERSUS RENAL DISORDERS

Overflow Disorders

- result from disruption of a normal metabolic pathway that causes increased plasma concentrations of the nonmetabolized substances
- these chemicals either override the reabsorption ability of the renal tubules or are not normally reabsorbed from the filtrate because they are present in only minute amounts
- inborn error of metabolism (IEM) - gene failed to inherit that can cause disruption of enzyme function

Abnormal Metabolic Constituents or Conditions Detected in the Routine Urinalysis

Color	Odor	Crystals
Homogentisic acid	Phenylketonuria	Cystine
Melanin	Maple syrup urine disease	Leucine
Indican	Isovaleric acidemia	Tyrosine
Porphyrins	Cystinuria	Lesch-Nyhan Disease
	Cystinosis	
	Homocystinuria	

Major Disorders of Protein and Carbohydrate Metabolism Associated With Abnormal Urinary Constituents, Classified by Functional Defect

Overflow Inherited	Metabolic	Renal
Phenylketonuria	Infantile tyrosinemia	Hartnup disease
Tyrosinemia	Melanuria	Cystinuria
Alkaptonuria	Indicanuria	
Maple syrup urine disease	5-Hydroxyindoleacetic acid	
Organic acidemias	Porphyrin	
Cystinosis		
Porphyrin		
Mucopolysaccharidoses		
Galactosemia		
Lesch-Nyhan disease		

NEWBORN SCREENING TESTS

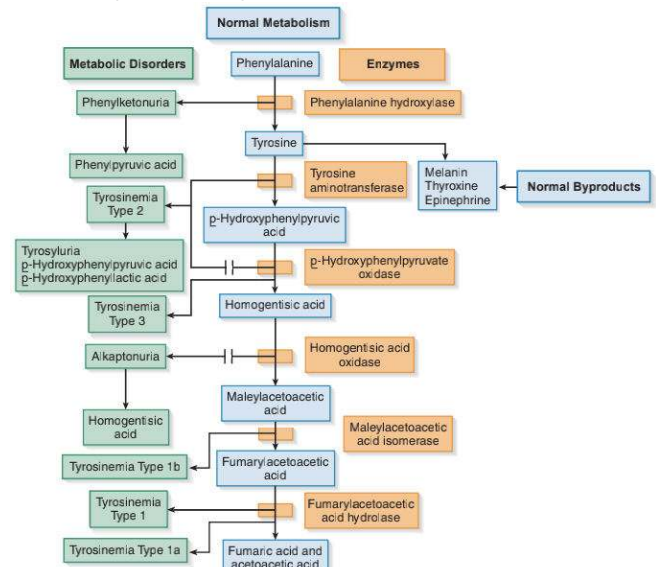
- detect and monitor newborns for IEMs
- as many as 30 or more metabolic disorders
- buildup of unmetabolized toxic food ingredients
- blood collected by infant heel puncture
- performed using tandem mass spectrophotometry (MS/MS)



Figure 8-1 Specimen collection form for MS/MS newborn screening test.

AMINO ACID DISORDERS

A. Phenylalanine-Tyrosine Disorders



Phenylalanine and tyrosine metabolic pathway including the normal pathway (blue), enzymes (yellow), and disorders caused by failure to inherit particular enzymes (green).

1. Phenylketonuria (PKU)

- most well known of the aminoacidurias
- results in severe mental retardation
- peculiar mousy odor urine
- amounts of the keto acids, including phenylpyruvate
- occurs when the normal conversion of phenylalanine to tyrosine is disrupted
- failure to inherit the gene to produce the enzyme phenylalanine hydroxylase
- excessive buildup of serum phenylalanine
- Urine tests for phenylpyruvic acid are based on the ferric chloride reaction performed by tube test.

Procedure: Ferric Chloride Tube Test

- 1) Place 1 mL of urine in a tube.
- 2) Slowly add five drops of 10% ferric chloride.
- 3) Observe color for a permanent blue-green color.

2. Tyrosyluria

- accumulation of excess tyrosine in the plasma (tyrosinemia)
- inherited or metabolic defects
- can be found as its degradation products: p-hydroxyphenylpyruvic acid and p-hydroxyphenyllactic acid
- transitory tyrosinemia in premature infants - underdevelopment of the liver function required to produce the enzymes necessary to complete the tyrosine metabolism

a. Type 1

- caused by the deficiency of the enzyme fumarylacetoacetate hydrolase (FAH)
- produces a generalized renal tubular disorder and progressive liver failure in infants soon after birth

b. Type 2

- caused by lack of the enzyme tyrosine aminotransferase
- develop corneal erosion and lesions on the palms, fingers, and soles of the feet believed to be caused by crystallization of tyrosine in the cells

c. Type 3

- caused by lack of the enzyme p-hydroxyphenylpyruvic acid dioxygenase

Procedure: Nitroso-Naphthol Test for Tyrosine

- 1) Place five drops of urine in a tube.
- 2) Add 1 mL of 2.63N nitric acid.
- 3) Add one drop of 21.5% sodium nitrite.
- 4) Add 0.1 mL 1-nitroso-2-naphthol.
- 5) Mix.
- 6) Wait 5 minutes.
- 7) Observe for an orange-red color, indicating tyrosine metabolites.

3. Melanuria

- melanin - pigment responsible for the dark color of hair, skin, and eyes
- albinism - deficient production of melanin
- increased urinary melanin darkens the urine
- appears after the urine is exposed to air
- indicates proliferation of the normal melanin-producing cells (melanocytes) → malignant melanoma
- 5,6-dihydroxyindole - melanin precursor
- a sodium nitro-ferricyanide (acetone reagent strip) - red color

4. Alkaptonuria

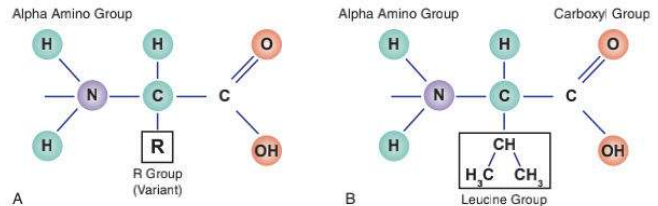
- name derived from the observation that urine from patients with this condition darkened after becoming alkaline from standing at room temperature
- "alkali lover"
- third major defect in the phenylalanine-tyrosine pathway
- failure to inherit the gene to produce the enzyme homogentisic acid oxidase
- brown-stained or black-stained cloth diapers and reddish-stained disposable diapers
- brown pigment (particularly noticeable in the ears)
 - ferric chloride test - transient deep blue color
 - Clinitest - yellow precipitate
 - add alkali to freshly voided urine - darkening of the color

Procedure: Homogentisic Acid Test

- 1) Place 4 mL of 3% silver nitrate in a tube.
- 2) Add 0.5 mL of urine.
- 3) Mix.
- 4) Add 10% NH₄OH by drops.
- 5) Observe for black color.

B. Branched-Chain Amino Acid Disorders

- differ from other amino acids by having a methyl group that branches from the main aliphatic carbon chain
- Laboratory finding: presence of ketonuria in a newborn



α -Alpha amino acid and branched chain amino acid structures. A. Structure of an α -amino acid. B. Structure of the branched chain amino acid leucine.

1. Maple Syrup Urine Disease (MSUD)

- inherited as an autosomal recessive trait
- amino acids: leucine, isoleucine, and valine
- transamination of the three amino acids in the liver to the keto acids
 - α -ketoisovaleric
 - α -ketoisocaproic
 - α -keto-β -methylvaleric
- Symptoms: failure to thrive after approximately 1 week
- strong odor resembling maple syrup that is caused by the rapid accumulation of keto acids in the urine

Procedure: 2,4-Dinitrophenylhydrazine (DNPH) Test for MSUD

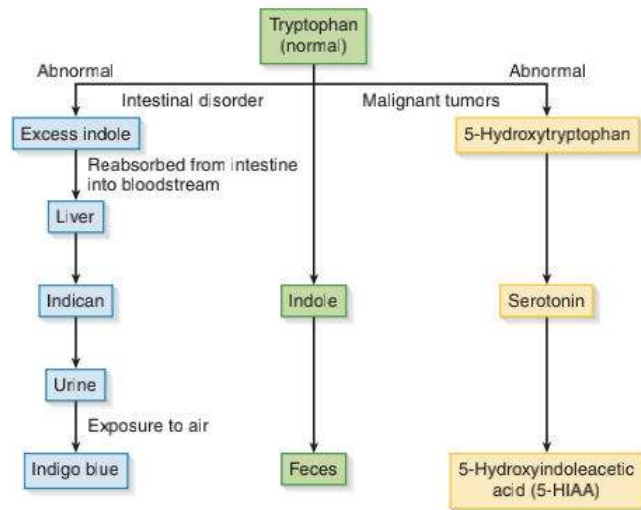
- 1) Place 1 mL of urine in a tube.
- 2) Add 10 drops of 0.2% 2,4-DNPH in 2N HCl.
- 3) Wait 10 minutes.
- 4) Observe for yellow turbidity or precipitate.

2. Organic Acidemias

- Generalized symptoms: early severe illness, often with vomiting accompanied by metabolic acidosis; hypoglycemia; ketonuria; and increased serum ammonia
- a. Isovaleric acidemia
 - odor of "sweaty feet"
 - accumulation of isovaleryl glycine due to a deficiency of isovaleryl coenzyme A in the leucine pathway
- b. Propionic and methylmalonic acidemias
 - result from errors in the metabolic pathway converting isoleucine, valine, threonine, and methionine to succinyl coenzyme A
 - propionic - immediate precursor to methylmalonic acid in this pathway

C. Tryptophan Disorders

- increased urinary excretion of the metabolites indican and 5-hydroxyindoleacetic acid (5-HIAA)



1. Indicanuria

- increased amounts of tryptophan are converted to indole
- excess indole is then reabsorbed from the intestine → bloodstream and circulated to the liver → converted to indican and then excreted in the urine
- colorless until oxidized to the dye indigo blue by exposure to air
- Hartnup disease - "blue diaper syndrome"

2. 5-Hydroxyindoleacetic Acid

- production of serotonin - second metabolic pathway of tryptophan
- Serotonin - produced from tryptophan by the argentaffin cells in the intestine and is carried through the body primarily by the platelets
- argentaffin (enterochromaffin) cells → elevation of urinary 5-HIAA levels
- 2 to 8 mg - normal daily excretion of 5-HIAA
- >25 mg/24 h - indication of argentaffin cell tumors
 - preserved with hydrochloric or boric acid
- nitrous acid and 1-nitroso-2-naphthol - purple to black

Procedure: Silver Nitroprusside Test

- Place 1 mL of urine in a tube.
- Add two drops concentrated NH_4OH .
- Add 0.5 mL 5% silver nitrate.
- Wait 10 minutes.
- Add five drops sodium nitroprusside.
- Observe for purple-black color.

D. Cystine Disorders

- odor of sulfur

1. Cystinuria

- marked by elevated amounts of the amino acid cystine in the urine
- inability of the renal tubules to reabsorb cystine filtered by the glomerulus
- two modes of inheritance:
 - reabsorption of all four amino acids- cystine, lysine, arginine, and ornithine- is affected
 - cystine and lysine are not reabsorbed

- produce calculi
- cystine - also the only amino acid found during the analysis of calculi from these patients

Procedure: Cyanide-Nitroprusside Test for Cystine

- Place 3 mL of urine in a tube.
- Add 2 mL sodium cyanide.
- Wait 10 minutes.
- Add five drops 5% sodium nitroprusside.
- Observe for red-purple color.

2. Cystinosis

- nephropathic
 - infantile - rapid progression to renal failure
 - late-onset cystinosis - gradual progression to total renal failure
- nonnephropathic - relatively benign but may cause some ocular disorders
- defect in the lysosomal membranes prevents the release of cystine into the cellular cytoplasm for metabolism
- incomplete metabolism of cystine → in crystalline deposits of cystine
- Laboratory findings: polyuria, generalized aminoaciduria, positive Clinistest results for reducing substances, and lack of urinary concentration

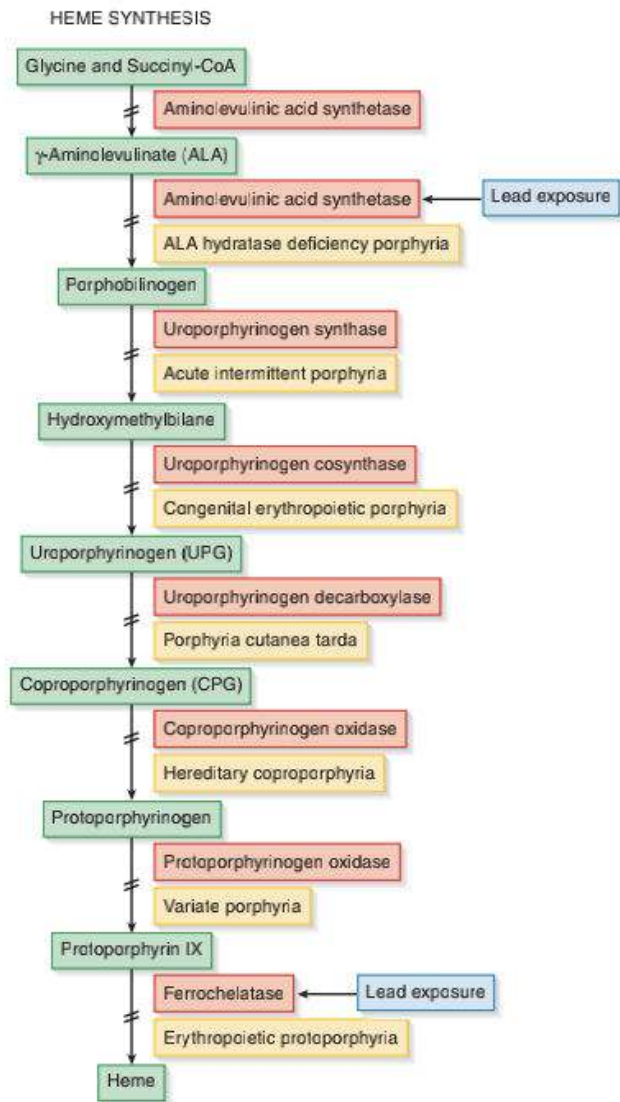
3. Homocystinuria

- defects in the metabolism of the amino acid methionine → increase in homocysteine
- failure to thrive, cataracts, mental retardation, thromboembolic problems, and death
- use of silver nitrate in place of sodium cyanide reduces homocystine to its nitroprusside-reactive form but does not reduce cystine
- Confirmation: silver-nitroprusside test
- Specimen: fresh urine

Procedure: Silver Nitroprusside Test for Homocystine

- Place 1 mL of urine in a tube.
- Add two drops concentrated NH_4OH .
- Add 0.5 mL 5% silver nitrate.
- Wait 10 minutes.
- Add five drops sodium nitroprusside.
- Observe for red-purple color.

PORPHYRIN DISORDERS



- Porphyrins - intermediate compounds in the production of heme
- porphyrias - disorders of porphyrin metabolism
- red or port wine color to the urine after exposure to air
- red discoloration of an infant's diapers
- blockage of a pathway reaction results in the accumulation of the product formed

A. Three Primary Porphyrins

- uroporphyrin - most soluble and readily appear in the urine
- coproporphyrin - less soluble but is found in the urine
- protoporphyrin - not seen in the urine

B. Porphyrin Precursors

- α-aminolevulinic acid [ALA] - most soluble and readily appear in the urine
- porphobilinogen - most soluble and readily appear in the urine

C. Screening Tests

- Screening test for lead poisoning: analysis of whole blood for the presence of free erythrocyte protoporphyrin (FEP)
- Screening test:

- Ehrlich reaction - ALA and porphobilinogen
- fluorescence under ultraviolet light in the 550 to 600nm - other porphyrins
 - positive: violet, pink, or red
 - negative: faint blue fluorescence
- + acetyl acetone - ALA → porphobilinogen

Common Porphyrins			
Porphyrin	Elevated Compound (s)	Clinical Symptoms	Laboratory Testing
Acute intermittent porphyria	ALA Porphobilinogen	Neurologic/psychiatric	Urine/Ehrlich reaction
Porphyria cutanea tarda	Uroporphyrin	Photosensitivity	Urine fluorescence
Congenital erythropoietic porphyria	Uroporphyrin Coproporphyrin	Photosensitivity	Urine or feces fluorescence
Variegate porphyria	Coproporphyrin	Photosensitivity/neurologic	Bile or feces fluorescence
Erythropoietic protoporphyria	Protoporphyrin	Photosensitivity	Blood FEP Bile or feces fluorescence
Lead poisoning	ALA Protoporphyrin	Neurologic	Acetoacetic acid + urine/ Ehrlich reaction Blood FEP

MUST-KNOW

Vampires in Old Europe

- Did you ever wonder how the legend of vampires got started? Think about the previous discussion on the symptoms and inheritance of porphyrias.
 - Photosensitivity→ Avoidance of sunlight
 - Pale coloring→ Anemia caused by heme disorder
 - Port wine- colored urine, red-stained teeth→ Drinking blood
 - Psychiatric symptoms→ Abnormal behavior
 - Inherited disorder→ Familial association, small gene pool
- Dracula is associated with Transylvania, now Romania. Porphyria was a common disease of early royalty in Europe as a result of intermarriage among the royals of different countries. King George III reportedly died blind, deaf, and mad from porphyria.

MUCOPOLYSACCHARIDE DISORDERS

- aka glycosaminoglycans
- group of large compounds located primarily in the connective tissue
- prevent complete breakdown of the polysaccharide portion of the compounds, resulting in accumulation of the incompletely metabolized polysaccharide portions in the lysosomes of the connective tissue cells and their increased excretion in the urine
- dermatan sulfate, keratan sulfate, and heparan sulfate

A. Disorders

1. Hurler syndrome

- skeletal structure is abnormal and there is severe mental
- mucopolysaccharides accumulate in the cornea of the eye

2. Hunter syndrome

- skeletal structure is abnormal and there is severe mental
- retardation
- inherited as sex-linked recessive and is rarely seen in females

3. Sanfilippo syndrome

- only abnormality is mental retardation

B. Screening tests

1. acid-albumin turbidity test

- 30 minutes
- a thick, white turbidity forms

2. cetyltrimethylammonium bromide (CTAB) turbidity test

- 5 minutes
- a thick, white turbidity forms

3. metachromatic staining spot tests

PURINE DISORDERS

A. Lesch-Nyhan Disease

- sex-linked recessive
- massive excretion of urinary uric acid crystals
- failure to inherit the gene to produce the enzyme hypoxanthine guanine phosphoribosyltransferase
- motor defects, mental retardation, a tendency toward self-destruction, gout, and renal calculi
- orange sand in diapers

CARBOHYDRATE DISORDERS

- Suggestive of a carbohydrate metabolism disorder: positive copper reduction + negative reagent strip glucose oxidase test

A. Melituria

- presence of increased urinary sugar
- cause no disturbance to body metabolism

B. Galactosuria

- inability to properly metabolize galactose to glucose
- failure to thrive, combined with liver disorders, cataracts, and severe mental retardation
- prevention: removal of lactose (a disaccharide containing galactose and glucose) from the diet

Enzyme Deficiency

1. galactose-1-phosphate uridyl transferase (GALT) - severe, possibly fatal symptoms
2. galactokinase - cataracts
3. UDP-galactose-4-epimerase - asymptomatic or produce mild symptoms

C. Lactosuria

- during pregnancy and lactation

D. Fructosuria

- parenteral feeding

E. Pentosuria

- ingestion of large amounts of fruit

Review Questions

1. All states require newborn screening for PKU for early:
 - A. Modifications of diet
 - B. Administration of antibiotics
 - C. Detection of diabetes
 - D. Initiation of gene therapy
2. All of the following disorders can be detected by newborn screening except:
 - A. Tyrosyluria
 - B. MSUD
 - C. Melanuria
 - D. Galactosemia
3. The best specimen for early newborn screening is a:
 - A. Timed urine specimen
 - B. Blood specimen
 - C. First morning urine specimen
 - D. Fecal specimen
4. Abnormal urine screening tests categorized as an overflow disorder include all of the following except:
 - A. Alkaptonuria
 - B. Galactosemia
 - C. Melanuria
 - D. Cystinuria
5. Which of the following disorders is not associated with the phenylalanine-tyrosine pathway?
 - A. MSUD
 - B. Alkaptonuria
 - C. Albinism
 - D. Tyrosinemia
6. The least serious form of tyrosylemia is:
 - A. Immature liver function
 - B. Type 1
 - C. Type 2
 - D. Type 3
7. An overflow disorder of the phenylalanine-tyrosine pathway that would produce a positive reaction with the reagent strip test for ketones is:
 - A. Alkaptonuria
 - B. Melanuria
 - C. MSUD
 - D. Tyrosyluria
8. An overflow reaction that could produce a false-positive reaction with Clinitest procedure is:
 - A. Cystinuria
 - B. Alkaptonuria
 - C. Indicanuria
 - D. Porphyrinuria

9. A urine that turns black after sitting by the sink for several hours could be indicative of:
 - A. Alkaptonuria
 - B. MSUD
 - C. Melanuria
 - D. Both A and C
10. Ketonuria in a newborn is an indication of:
 - A. MSUD
 - B. Isovaleric acidemia
 - C. Methylmalonic acidemia
 - D. All of the above
11. Urine from a newborn with MSUD will have a significant:
 - A. Pale color
 - B. Yellow precipitate
 - C. Milky appearance
 - D. Sweet odor
12. Hartnup disease is a disorder associated with the metabolism of:
 - A. Organic acids
 - B. Tryptophan
 - C. Cystine
 - D. Phenylalanine
13. 5-HIAA is a degradation product of:
 - A. Heme
 - B. Indole
 - C. Serotonin
 - D. Melanin
14. Elevated urinary levels of 5-HIAA are associated with:
 - A. Carcinoid tumors
 - B. Hartnup disease
 - C. Cystinuria
 - D. Platelet disorders
15. False-positive levels of 5-HIAA can be caused by a diet high in:
 - A. Meat
 - B. Carbohydrates
 - C. Starch
 - D. Bananas
16. Place the appropriate letter in front of the following statements.
 - A. Cystinuria
 - B. Cystinosis
 - ___ IEM
 - ___ Inherited disorder of tubular reabsorption
 - ___ Fanconi syndrome
 - ___ Cystine deposits in the cornea
 - ___ Early renal calculi formation
17. Blue diaper syndrome is associated with:
 - A. Lesch-Nyhan syndrome
 - B. Phenylketonuria
 - C. Cystinuria
 - D. Hartnup disease
18. Homocystinuria is caused by failure to metabolize:
 - A. Lysine
 - B. Methionine
 - C. Arginine
 - D. Cystine
19. The Ehrlich reaction will only detect the presence of:
 - A. Uroporphyrin
 - B. Porphobilinogen
 - C. Coproporphyrin
 - D. Protoporphyrin
20. Acetyl acetone is added to the urine before performing the Ehrlich test when checking for:
 - A. Aminolevulinic acid
 - B. Porphobilinogen
 - C. Uroporphyrin
 - D. Coproporphyrin
21. The classic urine color associated with porphyria is:
 - A. Dark yellow
 - B. Indigo blue
 - C. Pink
 - D. Port wine

OTHER BODY FLUIDS

Cerebrospinal Fluid

OUTLINE

- I. Introduction
- II. Formation and Physiology
- III. Specimen Collection and Handling
- IV. Appearance
- V. Traumatic Collection (Tap)
- VI. Cell Count
- VII. Differential Count on CSF Specimen
- VIII. Chemistry Tests
- IX. Microbiology Tests
- X. Serologic Testing

INTRODUCTION

A. Cerebrospinal Fluid

- major fluid in the body
- Functions
 - supply nutrients to the nervous tissue
 - remove metabolic wastes
 - produce a mechanical barrier to cushion the brain and spinal cord against trauma.

FORMATION AND PHYSIOLOGY

A. Meninges

- Lining of the brain and spinal cord

Three Layers

- dura mater
 - Latin for "hard mother"
 - lines the skull and vertebral canal
- arachnoid
 - "spiderweb-like"
 - filamentous (spider-like) inner membrane
- pia mater Latin for "gentle mother"
 - membrane lining the surfaces of the brain and spinal cord

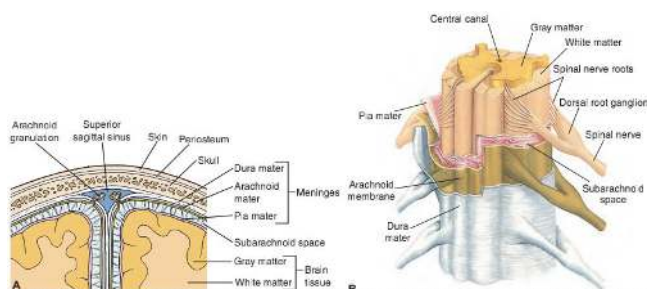


Figure 9-1 The layers of the meninges. A, the layers of the meninges in the brain. B, the layers of the meninges in the spinal cord. (A, from Scanlon & Sanders, *Essentials of Anatomy and Physiology*, 6th ed., F. A. Davis Company, Philadelphia, 2011, with permission.)

B. Cerebrospinal Fluid (CSF)

- produced in the choroid plexuses of the two lumbar ventricles and the third and fourth ventricles
- fluid flows through the subarachnoid space (between the arachnoid and pia mater)
- reabsorbed back into the blood capillaries in the arachnoid granulations/villae at a rate equal to its production
- Adults: 20mL every hour is produced
- Volume
 - Adults: 90 to 150 mL
 - Neonates: 10 to 60 mL

C. Blood-brain barrier

- In the choroid plexuses, the endothelial cells have very tight fitting junctures that prevent the passage of many molecules
- essential to protect the brain from chemicals and other substances circulating in the blood that could harm the brain tissue

SPECIMEN COLLECTION AND HANDLING

A. Collection

- collected by lumbar puncture
- between the third, fourth, or fifth lumbar vertebra

Precautions

- measurement of intracranial pressure
- careful technique to prevent infection or neural tissue damage

Volume to be collected

- based on the
 - volume available in the patient (adult vs. neonate)
 - opening pressure of the CSF
 - measured when the needle first enters the subarachnoid space

Collected in Three Sterile Tubes

- Tube 1
 - used for chemical and serologic tests
 - least affected by blood or bacteria introduced as a result of the tap procedure
- Tube 2
 - designated for the microbiology laboratory
- Tube 3
 - used for the cell count
 - least likely to contain cells introduced by the spinal tap procedure
- Tube 4
 - may be drawn
 - exclude skin contamination
 - additional serologic tests
 - ∴ Excess fluid - frozen

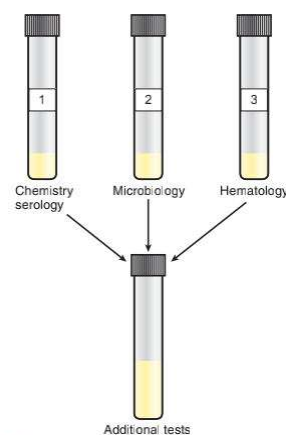


Figure 9-3 CSF specimen collection tubes.

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B. Handling

- performed on a STAT basis

Maintenance

- Hematology tubes are refrigerated.
- Microbiology tubes remain at room temperature.
- Chemistry and serology tubes are frozen.

MUST-KNOW

- If only one tube can be collected, it must be tested first by microbiology.

APPEARANCE

A. Cloudy, turbid, or milky specimen

- increased protein or lipid concentration
- may be indicative of an infection (WBCs for cloudiness)

B. Xanthochromia

- CSF supernatant that is
 - pink - very slight amount of oxyhemoglobin
 - orange - heavy hemolysis
 - yellow - conversion of oxyhemoglobin to unconjugated bilirubin
- Caused by presence of RBC degradation products

C. Other Clinical Significance of CSF Appearance

Clinical Significance of CSF Appearance		
Appearance	Cause	Major Significance
Crystal clear		Normal
Hazy, turbid, milky, cloudy	WBCs	Meningitis
	Microorganisms	Meningitis
	Protein	Disorders affecting blood-brain barrier Production of IgG within the CNS
Oily	Radiographic contrast media	
Bloody	RBCs	Hemorrhage Traumatic tap
Xanthochromic	Hemoglobin	Old hemorrhage Lysed cells from traumatic tap
	Bilirubin	RBC degradation Elevated serum bilirubin level
	Carotene	Increased serum levels
	Protein	Disorders affecting blood-brain barrier
Clotted	Melanin	Meningeal melanoma
	Protein	Disorders affecting blood-brain barrier
	Clotting factors	Introduced by

		traumatic tap
Pellicle	Protein	Disorders affecting blood-brain barrier
	Clotting factors	Tubercular meningitis

TRAUMATIC COLLECTION (TAP)

A. Grossly bloody CSF

- intracranial hemorrhage
- may also be due to the puncture of a blood vessel during the spinal tap procedure

B. Visual Examinations

1. Uneven Blood Distribution

- Cerebral hemorrhage - evenly distributed throughout the three CSF specimen tubes
- Traumatic tap - heaviest concentration of blood in tube 1, and gradually diminishing amounts in Tubes 2 and 3

2. Clot Formation

- Cerebral hemorrhage - does not contain enough fibrinogen to clot
- Traumatic tap - form clots owing to the introduction of plasma fibrinogen into the specimen

3. Xanthochromic Supernatant

- RBCs remain in the CSF for approximately 2 hours before noticeable hemolysis begins
- Result of blood that has been present longer than that introduced by the traumatic tap
- Centrifuged in a microhematocrit tube and the supernatant examined against a white background
- Additional tests: microscopic examination and the D-dimer test
- Cerebral hemorrhage - macrophages containing ingested RBCs (erythrophagocytosis) or hemosiderin granules
- Traumatic tap - fibrin degradation product D-dimer

CELL COUNT

A. Leukocyte (white blood cell [WBC]) count

- should be performed immediately
- WBCs (particularly granulocytes) and RBCs
 - begin to lyse within 1 hour
 - 40% of the leukocytes disintegrate after 2 hours

B. Methodology

- Normal adult CSF: 0 to 5 WBCs/ μ L
- Newborns: 30 mononuclear cells/ μ L
- Clear: 200 WBCs or 400 RBCs/ μ L
- uses Neubauer counting chamber

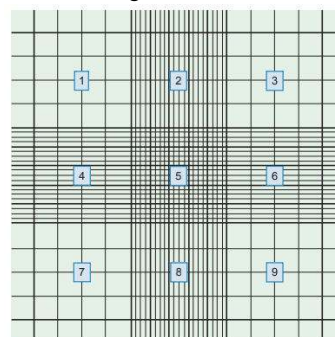


Figure 9-5 Neubauer counting chamber depicting the nine large square counting areas.

Calculating CSF Cell Counts

$$\frac{\text{Number of cells counted} \times \text{dilution}}{\text{Number of cells counted} \times \text{volume of 1 square}} = \text{cells}/\mu\text{L}$$

- used both diluted and undiluted specimens
- convert the number of cells counted in a specific amount of fluid to the number of cells that would be present in 1 μL of fluid
- eliminates the need to correct for the volume counted by counting the four large corner squares (0.4 μL) and the large center square (0.1 μL) on each side of the counting chamber

C. Total Cell Count

- counted
- in the four corner squares and the center square on both sides of the hemocytometer

D. WBC Count

- Lysis of RBCs - obtained before performing the WBC count
- diluted with 3% glacial acetic acid (to lyse the RBCs)
- methylene blue - stains the WBCs
- counted in the four corner squares, and the center square on both sides of the hemocytometer
- number is multiplied by the dilution factor to obtain the number of WBCs per microliter

DIFFERENTIAL COUNT ON CSF SPECIMEN

A. Differential Count on CSF Specimens

- performed on a stained smear
- not from the cells in the counting chamber
- concentrated before preparing the smear
- 100 cells should be counted
- reported in percentage

B. Methods

1. Sedimentation

- not routinely used in the clinical laboratory
- produce less cellular distortion

2. Filtration

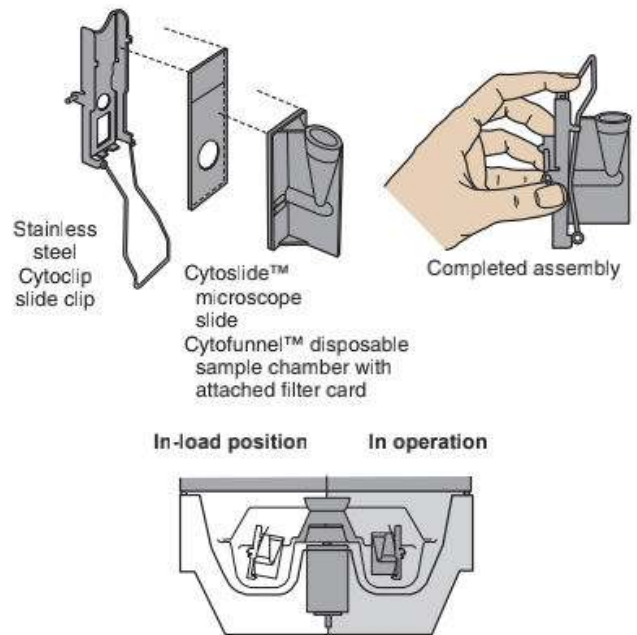
- not routinely used in the clinical laboratory
- produce less cellular distortion

3. Centrifugation

- 5 to 10 minutes
- supernatant fluid is removed and saved for additional tests
- slides made from the suspended sediment are allowed to air dry and are stained with Wright's stain

4. Cytocentrifugation

- 0.1 mL of CSF + 1 drop of 30% albumin



This cutaway drawing illustrates, at left, the in-load position which shows the sample chamber assembly in a tilted-back position, so that the sample is not absorbed by the filter card. During spinning, centrifugal force tilts the assembly upright and forces the sample to flow toward the microscope slide.

Figure 9-6 Cytospin 3 cytocentrifuge specimen processing assembly (Courtesy of Shandon, Inc., Pittsburgh, PA).

C. CSF Cellular Constituents

- primarily lymphocytes and monocytes
- 70:30
 - ratio is essentially reversed in children
- pleocytosis - increased numbers of neutrophils
- high CSF WBC count - indicative of bacterial meningitis
- moderately elevated CSF WBC count with a high percentage of lymphocytes and monocytes - meningitis of viral, tubercular, fungal, or parasitic origin

Predominant Cells Seen in CSF		
Type of Cell	Major Clinical Significance	Microscopic Findings
Lymphocytes	Normal Viral, tubercular, and fungal meningitis Multiple sclerosis	All stages of development may be found
Neutrophils	Bacterial meningitis Early cases of viral, tubercular, and fungal meningitis Cerebral hemorrhage	Granules may be less prominent than in blood Cells disintegrate rapidly
Monocytes	Normal Viral, tubercular, and fungal meningitis Multiple sclerosis	Found mixed with lymphocytes
Macrophages	RBCs in spinal fluid	May contain phagocytized RBCs appearing as empty

		vacuoles or ghost cells, hemosiderin granules, and hematoidin crystals
Blast forms	Acute leukemia	Lymphoblasts, myeloblasts, or monoblasts
Lymphoma cells	Disseminated lymphomas	Resemble lymphocytes with cleft nuclei
Plasma cells	Multiple sclerosis Lymphocyte reactions	Traditional and classic forms seen Reactive lymphs
Ependymal, choroidal, and spindle-shaped cells	Diagnostic procedures	Seen in clusters with distinct nuclei and distinct cell walls
Malignant cells	Metastatic carcinomas Primary central nervous system carcinoma	Seen in clusters with fusing of cell borders and nuclei

1. Neutrophils

- early stages (1 to 2 days) of viral, fungal, tubercular, and parasitic meningitis
- pyknotic nuclei - indicate degenerating cells
- nucleated red blood cells (NRBCs) - seen as a result of bone marrow contamination during the spinal tap

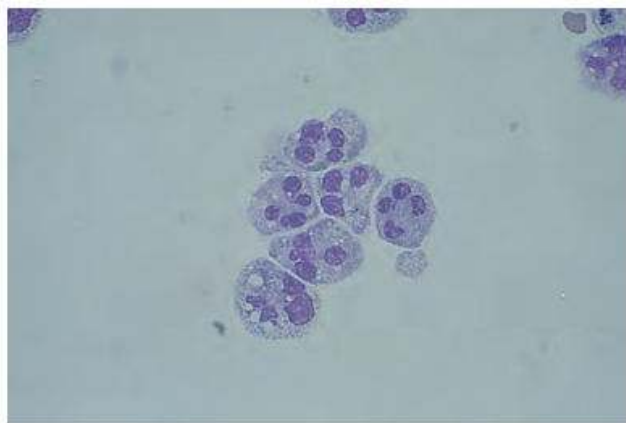


Figure 9-9 Neutrophils with cytoplasmic vacuoles resulting from cytocentrifugation (x500).

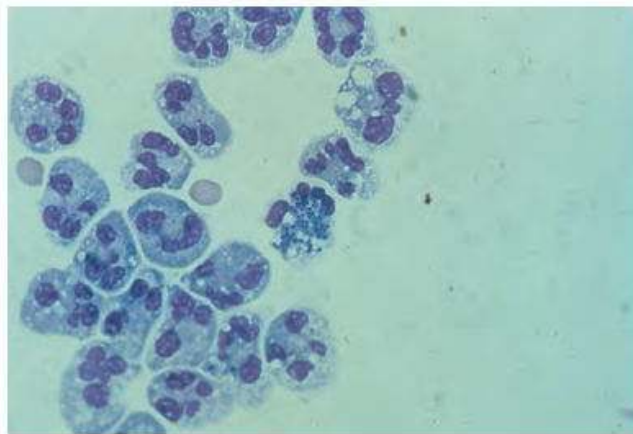


Figure 9-10 Neutrophils with intracellular bacteria (x1000).

2. Lymphocytes and Monocytes

- common in cases of viral, tubercular, and fungal meningitis
- reactive lymphocytes - increased dark blue cytoplasm and clumped chromatin are frequently present during viral infections
- increased lymphocytes - both asymptomatic HIV infection and AIDS
- moderately elevated WBC count (less than 50 WBCs/ μ L) with increased normal and reactive lymphocytes and plasma cells - indicate multiple sclerosis or other degenerative neurologic disorders

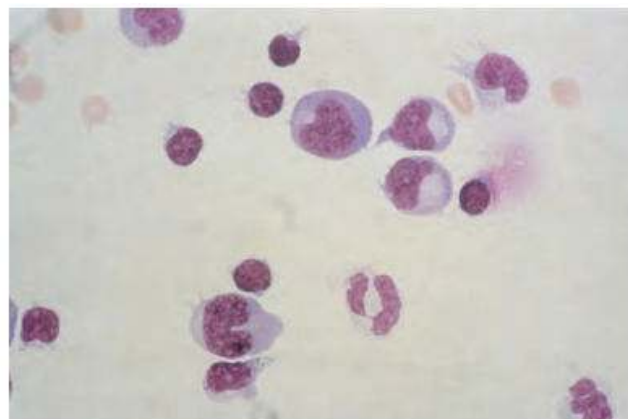


Figure 9-16 Broad spectrum of lymphocytes and monocytes in viral meningitis (x1000).

3. Eosinophils

- Increased eosinophils - association with parasitic infections, fungal infections (primarily *Coccidioides immitis*), introduction of foreign material, including medications and shunts, into the CNS

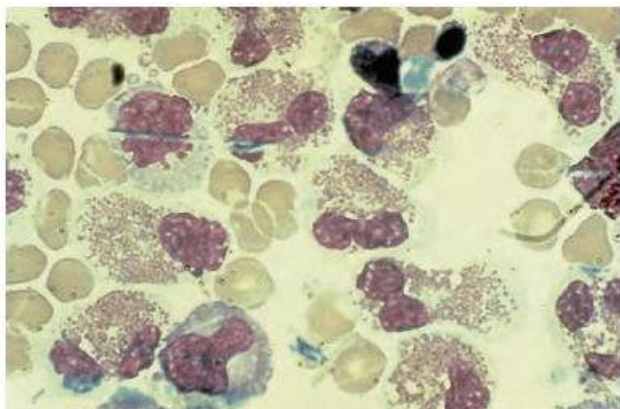


Figure 9-17 Eosinophils (x1000). Notice cytocentrifuge distortion.

4. Macrophages

- remove cellular debris and foreign objects such as RBCs
- appear within 2 to 4 hours after RBCs enter the CSF
- are frequently seen following repeated taps
- increased macrophages - previous hemorrhage
- degradation of the phagocytized RBCs - results in the appearance of dark blue or black iron-containing hemosiderin granules
- further degradation - yellow hematoidin crystals, iron-free, consisting of hemoglobin and unconjugated bilirubin

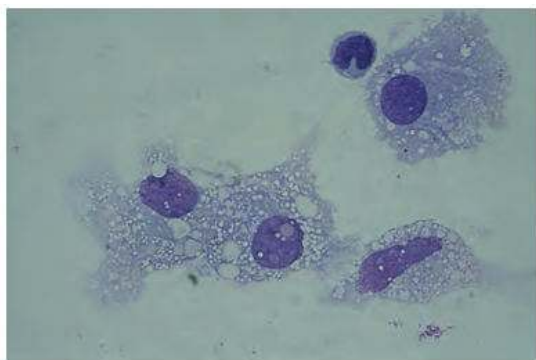


Figure 9-18 Macrophages. Notice the large amount of cytoplasm and vacuoles (x500).

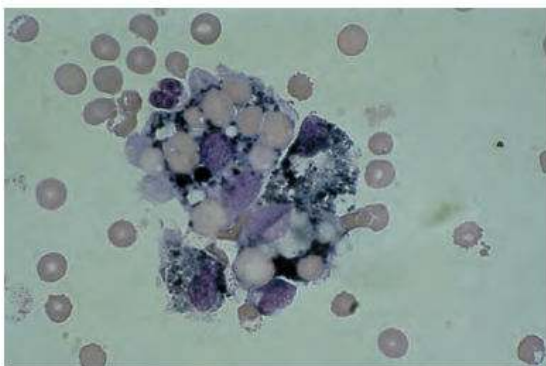


Figure 9-19 Macrophages showing erythrophagocytosis (x500).

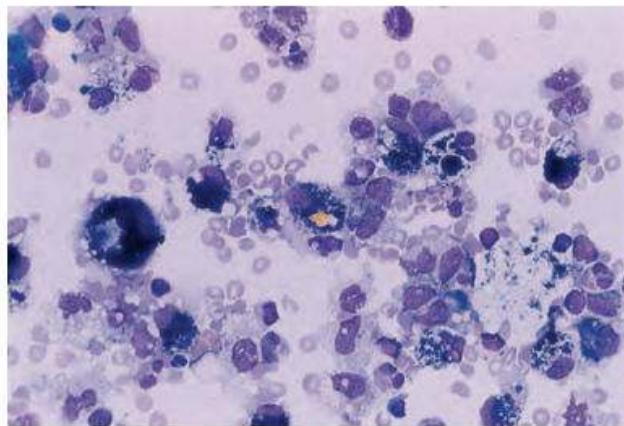


Figure 9-25 Macrophages with hemosiderin and hematoidin (x250). Notice the bright yellow color.

5. Nonpathologically Significant Cells

- Choroidal cells - from the epithelial lining of the choroid plexus
- Ependymal cells - from the lining of the ventricles and neural canal, less defined cell membranes
- Spindle-shaped cells - represent lining cells from the arachnoid, may be seen with systemic malignancies

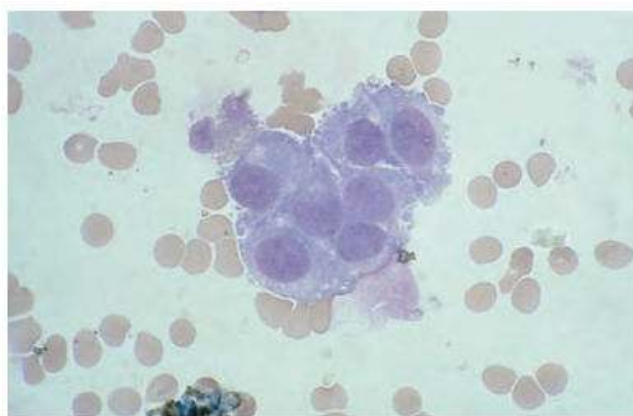


Figure 9-26 Choroidal cells showing distinct cell borders and nuclear uniformity (x500).

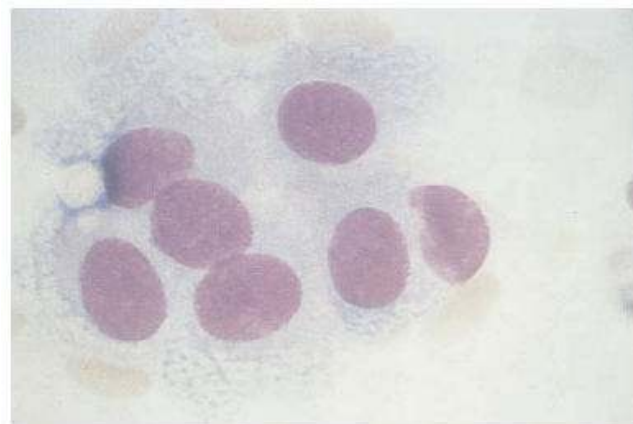


Figure 9-27 Ependymal cells. Notice the nucleoli and less distinct cell borders (x1000).

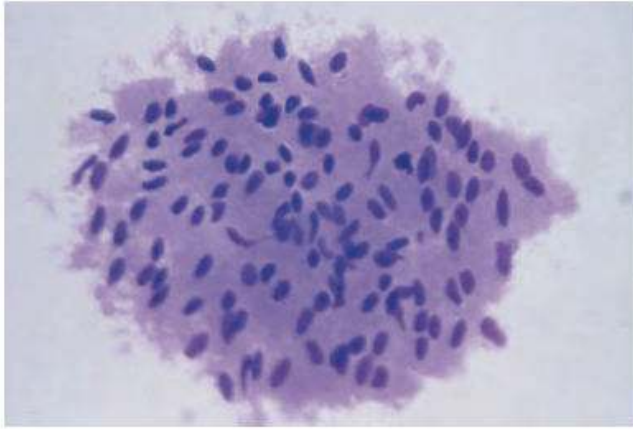


Figure 9-28 Cluster of spindle-shaped cells (x500).

6. Malignant Cells of Hematologic Origin

- Lymphoblasts, myeloblasts, and monoblasts - serious complication of acute leukemias
- Lymphoma cells - indicate dissemination from the lymphoid tissue, resemble large and small lymphocytes and usually appear in clusters of large, small, or mixed cells based on the classification of the lymphoma, nuclei may appear cleaved, and prominent nucleoli are present

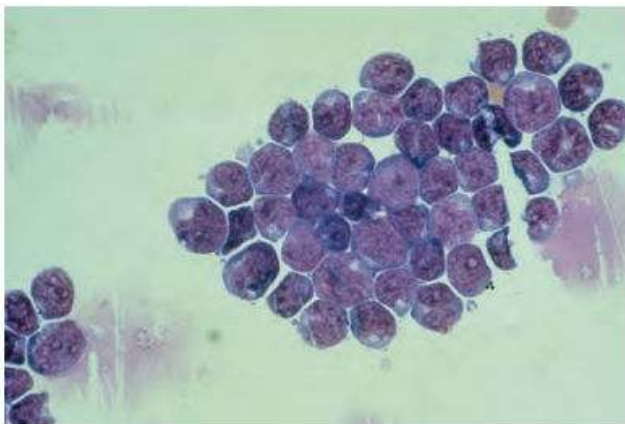


Figure 9-29 Lymphoblasts from acute lymphocytic leukemia (x500).

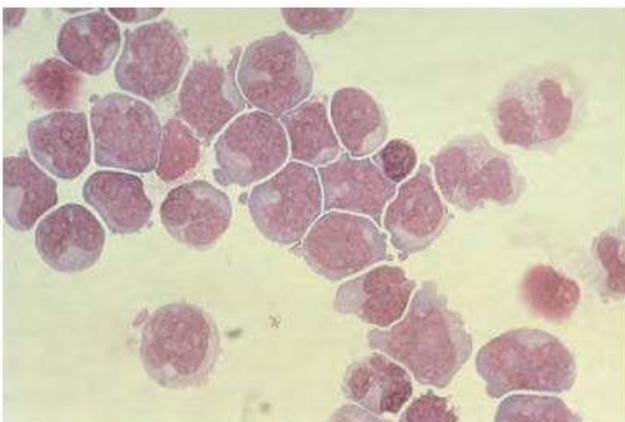


Figure 9-30 Myeloblasts from acute myelocytic leukemia (x500).

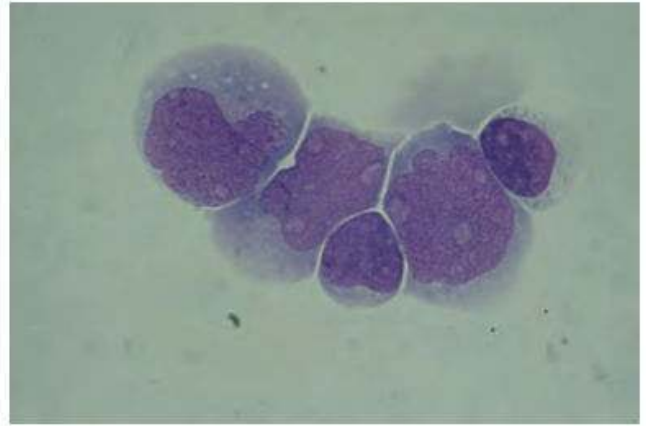


Figure 9-31 Monoblasts and two lymphocytes (x1000). Notice the prominent nucleoli.

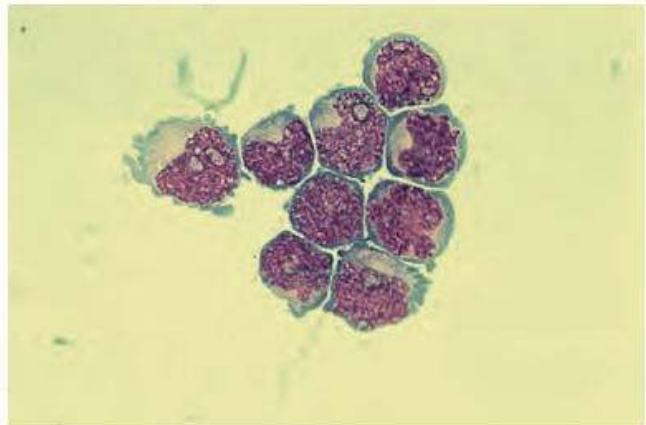


Figure 9-33 Lymphoma cells with nucleoli (x500).

Malignant Cells of Nonhematologic Origin

- from lung, breast, renal, and gastrointestinal malignancies
- astrocytomas, retinoblastomas, and medulloblastomas - cells from primary CNS tumors

CHEMISTRY TESTS

A. CSF Filtration

- process is selective and the chemical composition is controlled by the blood-brain barrier
- reference values for CSF chemicals are not the same as the plasma values
- abnormal values - alterations in the permeability of the blood-brain barrier or increased production or metabolism by the neural cells in response to a pathologic condition

B. Cerebrospinal Protein

- protein determination - most frequently performed chemical test on CSF
- Normal CSF - contains a very small amount of protein (15 to 45 mg/dL)

Proteins

- albumin - makes up most of CSF protein
- prealbumin - second most prevalent fraction in CSF
- alpha globulins - include primarily haptoglobin and ceruloplasmin
- transferrin - major beta globulin present
- tau - separate carbohydrate-deficient transferrin fraction, seen in CSF not in serum

- gamma globulin - primarily immunoglobulin G (IgG), with only a small amount of immunoglobulin A (IgA)
- Not found in normal CSF: Immunoglobulin M (IgM), fibrinogen, and beta lipoprotein
- Myelin Basic protein (MBP) - indicates recent destruction of the myelin sheath that protects the axons of the neurons (demyelination)

Clinical Significance of Elevated Protein Values

- abnormally low values - when fluid is leaking from the CNS
- elevated CSF protein - damage to the blood-brain barrier, immunoglobulin production within the CNS, decreased normal protein clearance from the fluid, and neural tissue degeneration (meningitis and hemorrhage)

Clinical Causes of Abnormal CSF Protein Values	
Elevated Results	Decreased Results
• Meningitis • Hemorrhage • Primary CNS tumors • Multiple sclerosis • Guillain-Barré syndrome • Neurosyphilis • Polyneuritis • Myxedema • Cushing disease • Connective tissue disease • Polyneuritis • Diabetes • Uremia	• CSF leakage/trauma • Recent puncture • Rapid CSF production • Water intoxication
Reference values for protein are usually 15 to 45 mg/dL, but are method dependent, and higher values are found in infants and people older than 40 years.	

Methodology

- Principles: turbidity production or dye-binding ability
- Nephelometry - turbidity production

Protein Fraction

CSF/serum albumin index

$$\text{CSF/serum albumin index} = \frac{\text{CSF albumin (mg/dL)}}{\text{Serum albumin (g/dL)}}$$

- <9 - intact blood-brain barrier

CSF/serum IgG index

$$\text{IgG index} = \frac{\text{CSF IgG (mg/dL) / serum IgG (g/dL)}}{\text{CSF albumin (mg/dL) / serum albumin (g/dL)}}$$

Electrophoresis and Immunophoretic Techniques

Primary purpose for performing CSF protein electrophoresis: detection of oligoclonal bands (represent inflammation within the CNS)

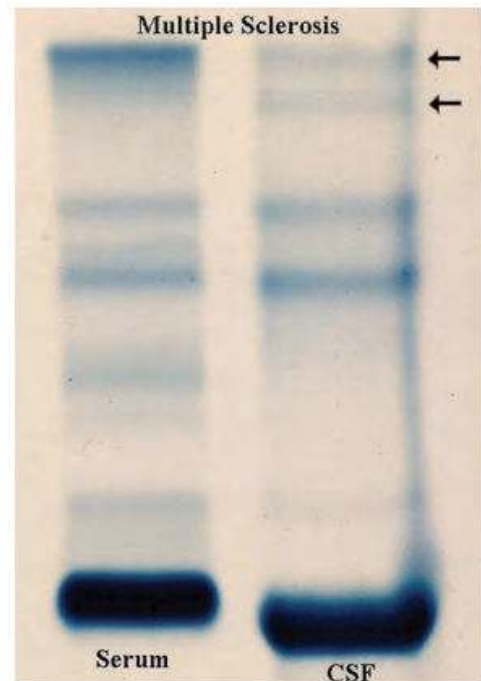


Figure 9-36 Normal and abnormal oligoclonal banding. (Photograph courtesy of the University of Pittsburgh Department of Pathology, Case Index files, Case 059; <http://path.upmc.edu/cases/case59.html>, with permission.)

C. CSF Glucose

- 60% to 70% that of the plasma glucose
- drawn about 2 hours before the spinal tap - allow time for equilibration between the blood and fluid

Significance

- Elevated CSF glucose values - always a result of plasma elevations
- Low CSF glucose values - causative agents in meningitis.
- Markedly decreased CSF glucose level, increased WBC count and a large percentage of neutrophils - indicates bacterial meningitis
- Markedly decreased CSF glucose level, increased lymphocytes count and a large percentage of neutrophils - tubercular meningitis
- normal CSF glucose value with an increased number of lymphocytes - viral meningitis
- Decreased CSF glucose values - caused primarily by alterations in the mechanisms of glucose transport across the blood-brain barrier and by increased use of glucose by the brain cells

D. CSF Lactate

- >25 mg/dL - bacterial, tubercular, and fungal meningitis
- >35 mg/dL - bacterial meningitis

E. CSF Glutamine

- Glutamine - produced from ammonia and α -ketoglutarate by the brain cells
- Normal CSF glutamine: 8 to 18 mg/dL

- >35 mg/dL - disturbance of consciousness
- presence of excess ammonia - indirect test as excess ammonia in the CNS increases glutamine synthesis
- Concentration of ammonia in the CSF increases → the supply of α -ketoglutarate becomes depleted → glutamine can no longer be produced to remove the toxic ammonia → coma ensues
- requested for patients with coma of unknown origin
- elevated levels found in Reye syndrome

CSF Chemistry Tests			
Chemical Substance	Reference Concentration Value Normal CSF	Significance of Increased Concentration	Significance of Decreased Concentration
Protein	15 to 45 mg/dL	Meningitis Hemorrhage Multiple sclerosis	CSF leakage
Glucose	60% to 70% of plasma concentration	None	Bacterial, tubercular, and fungal meningitis
Lactate	10 to 24 mg/dL	>35 mg/dL: Bacterial meningitis	None
Glutamine	8 to 18 mg/dL	>35 mg/dL: Some disturbance of consciousness	None

MICROBIOLOGY TESTS

A. CSF in Microbiology

- 24 hours - bacterial meningitis
- 6 weeks - tubercular meningitis
- Confirmation: CSF culture

B. Major Laboratory Results for Differential Diagnosis of Meningitis

1. Bacterial

- Elevated WBC count
- Neutrophils present
- Marked protein elevation
- Markedly decreased glucose level
- Lactate level >35 mg/dL
- Positive Gram stain and
- bacterial antigen tests

2. Viral

- Elevated WBC count
- Lymphocytes present
- Moderate protein elevation
- Normal glucose level
- Normal lactate level

3. Tubercular

- Elevated WBC count
- Lymphocytes and monocytes present
- Moderate to marked protein elevation
- Decreased glucose level

- Lactate level >25 mg/dL
- Pellicle formation

4. Fungal

- Elevated WBC count
- Lymphocytes and monocytes present
- Moderate to marked protein elevation
- Normal to decreased glucose level
- Lactate level >25 mg/dL
- Positive India ink with *Cryptococcus neoformans*
- Positive immunologic test for *C. neoformans*

C. Gram Stain

- performed on concentrated specimens
- centrifuged at 1500 g for 15 minutes
- slides and cultures should be prepared from the sediment
- classic starburst pattern produced by *Cryptococcus*

D. Other Tests

1. Acid-fast or fluorescent antibody stains - tubercular meningitis is suspected
2. India ink preparation - fungal meningitis
3. Latex agglutination tests
 - presence of *C. neoformans* antigen in serum and CSF
 - false-positive interference: rheumatoid factor
4. Lateral flow assay (LAF) - provide a rapid method for detecting *C. neoformans*
5. Enzyme-linked immunosorbent assay (ELISA) - rapid means for detecting and identifying microorganisms in CSF
6. Bacterial antigen test (BAT) - does not appear to be as sensitive to *N. meningitidis*

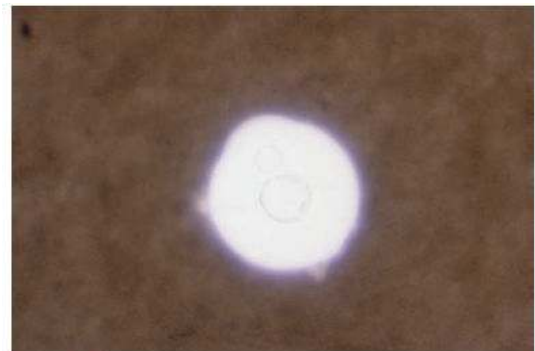


Figure 9-37 India ink preparation of *C. neoformans* (x400). Notice budding yeast form. (Courtesy of Ann K. Fulenwider, MD.)

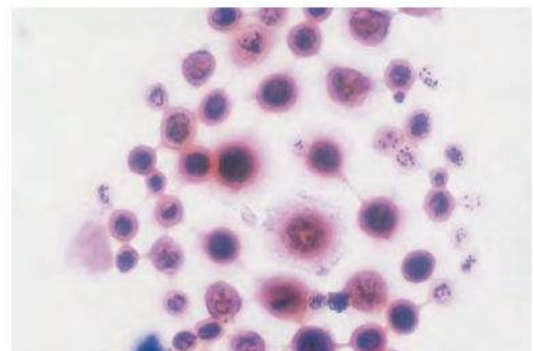


Figure 9-38 Gram stain of *C. neoformans* showing starburst pattern (x1000). (Courtesy of Ann K. Fulenwider, MD.)

SEROLOGIC TESTING

A. Purpose

- detect the presence of neurosyphilis

B. Tests

1. Venereal Disease Research Laboratories (VDRL) - recommended
2. Fluorescent treponemal antibody-absorption (FTA-ABS) - more sensitive, remains positive in the serum of treated cases of syphilis
3. Rapid plasma regain (RPR) - not recommended, less sensitive than the VDRL

Review Questions

1. The functions of the CSF include all of the following except:
 - A. Removing metabolic wastes
 - B. Producing an ultrafiltrate of plasma
 - C. Supplying nutrients to the CNS
 - D. Protecting the brain and spinal cord
2. The CSF flows through the:
 - A. Choroid plexus
 - B. Pia mater
 - C. Arachnoid space
 - D. Dura mater
3. Substances present in the CSF are controlled by the:
 - A. Arachnoid granulations
 - B. Blood-brain barrier
 - C. Presence of one-way valves
 - D. Blood-CSF barrier
4. What department is the CSF tube labeled 3 routinely sent to?
 - A. Hematology
 - B. Chemistry
 - C. Microbiology
 - D. Serology
5. The CSF tube that should be kept at room temperature is:
 - A. Tube 1
 - B. Tube 2
 - C. Tube 3
 - D. Tube 4
6. Place the appropriate letter in front of the statement that best describes CSF specimens in these two conditions:
 - A. Traumatic tap
 - B. Intracranial hemorrhage

____ Even distribution of blood in all tubes

____ Xanthochromic supernatant

____ Concentration of blood in tube 1 is greater than in tube 3

____ Specimen contains clots
7. The presence of xanthochromia can be caused by all of the following except:
 - A. Immature liver function
 - B. RBC degradation
 - C. A recent hemorrhage
 - D. Elevated CSF protein
8. A web-like pellicle in a refrigerated CSF specimen indicates:
 - A. Tubercular meningitis
 - B. Multiple sclerosis
 - C. Primary CNS malignancy
 - D. Viral meningitis
9. Given the following information, calculate the CSF WBC count: cells counted, 80; dilution, 1:10; large Neubauer squares counted, 10.
 - A. 8
 - B. 80
 - C. 800
 - D. 8000
10. A CSF WBC count is diluted with:
 - A. Distilled water
 - B. Normal saline
 - C. Acetic acid
 - D. Hypotonic saline
11. A total CSF cell count on a clear fluid should be:
 - A. Reported as normal
 - B. Not reported
 - C. Diluted with normal saline
 - D. Counted undiluted
12. The purpose of adding albumin to CSF before cytocentrifugation is to:
 - A. Increase the cell yield
 - B. Decrease the cellular distortion
 - C. Improve the cellular staining
 - D. Both A and B
13. The primary concern when pleocytosis of neutrophils and lymphocytes is found in the CSF is:
 - A. Meningitis
 - B. CNS malignancy
 - C. Multiple sclerosis
 - D. Hemorrhage
14. Neutrophils with pyknotic nuclei may be mistaken for:
 - A. Lymphocytes
 - B. Nucleated RBCs
 - C. Malignant cells
 - D. Spindle-shaped cells
15. The presence of which of the following cells is increased in a parasitic infection?
 - A. Neutrophils
 - B. Macrophages
 - C. Eosinophils
 - D. Lymphocytes
16. Macrophages appear in the CSF after:
 - A. Hemorrhage
 - B. Repeated spinal taps
 - C. Diagnostic procedures
 - D. All of the above
17. Nucleated RBCs are seen in the CSF as a result of:
 - A. Elevated blood RBCs
 - B. Treatment of anemia
 - C. Severe hemorrhage
 - D. Bone marrow contamination
18. After a CNS diagnostic procedure, which of the following might be seen in the CSF?
 - A. Choroidal cells
 - B. Ependymal cells
 - C. Spindle-shaped cells
 - D. All of the above
19. Hemosiderin granules and hematoidin crystals are seen in:
 - A. Lymphocytes
 - B. Macrophages
 - C. Ependymal cells
 - D. Neutrophils
20. Myeloblasts are seen in the CSF:
 - A. In bacterial infections
 - B. In conjunction with CNS malignancy
 - C. After cerebral hemorrhage
 - D. As a complication of acute leukemia

21. Cells resembling large and small lymphocytes with cleaved nuclei represent:
 - A. Lymphoma cells
 - B. Choroid cells
 - C. Melanoma cells
 - D. Medulloblastoma cells
22. The reference range for CSF protein is:
 - A. 6 to 8 g/dL
 - B. 15 to 45 g/dL
 - C. 6 to 8 mg/dL
 - D. 15 to 45 mg/dL
23. CSF can be differentiated from serum by the presence of:
 - A. Albumin
 - B. Globulin
 - C. Prealbumin
 - D. Tau transferrin
24. In serum, the second most prevalent protein is IgG; in CSF, the second most prevalent protein is:
 - A. Transferrin
 - B. Prealbumin
 - C. IgA
 - D. Ceruloplasmin
25. Elevated CSF protein values can be caused by all of the following except:
 - A. Meningitis
 - B. Multiple sclerosis
 - C. Fluid leakage
 - D. CNS malignancy
26. The integrity of the blood–brain barrier is measured using the:
 - A. CSF/serum albumin index
 - B. CSF/serum globulin ratio
 - C. CSF albumin index
 - D. CSF IgG index
27. Given the following results, calculate the IgG index: CSF IgG, 50 mg/dL; serum IgG, 2 g/dL; CSF albumin, 70 mg/dL; serum albumin, 5 g/dL.
 - A. 0.6
 - B. 6.0
 - C. 1.8
 - D. 2.8
28. The CSF IgG index calculated in Study Question 27 indicates:
 - A. Synthesis of IgG in the CNS
 - B. Damage to the blood–brain barrier
 - C. Cerebral hemorrhage
 - D. Lymphoma infiltration
29. The finding of oligoclonal bands in the CSF and not in the serum is seen with:
 - A. Multiple myeloma
 - B. CNS malignancy
 - C. Multiple sclerosis
 - D. Viral infections
30. A CSF glucose of 15 mg/dL, WBC count of 5000, 90% neutrophils, and protein of 80 mg/dL suggests:
 - A. Fungal meningitis
 - B. Viral meningitis
 - C. Tubercular meningitis
 - D. Bacterial meningitis
31. A patient with a blood glucose of 120 mg/dL would have a normal CSF glucose of:
 - A. 20 mg/dL
 - B. 60 mg/dL
 - C. 80 mg/dL
 - D. 120 mg/dL
32. CSF lactate will be more consistently decreased in:
 - A. Bacterial meningitis
 - B. Viral meningitis
 - C. Fungal meningitis
 - D. Tubercular meningitis
33. Measurement of which of the following can be replaced by CSF glutamine analysis in children with Reye syndrome?
 - A. Ammonia
 - B. Lactate
 - C. Glucose
 - D. α -Ketoglutarate
34. Before performing a Gram stain on CSF, the specimen must be:
 - A. Filtered
 - B. Warmed to 37°C
 - C. Centrifuged
 - D. Mixed
35. All of the following statements are true about cryptococcal meningitis except:
 - A. An India ink preparation is positive
 - B. A starburst pattern is seen on Gram stain
 - C. The WBC count is over 2000
 - D. A confirmatory immunology test is available
36. The test of choice to detect neurosyphilis is the:
 - A. RPR
 - B. VDRL
 - C. FAB
 - D. FTA-ABS

Semen

OUTLINE

- I. Physiology
- II. Specimen Collection
- III. Semen Analysis
- IV. Additional Testing

PHYSIOLOGY

A. Semen

Four fractions

1. epididymis - sperm maturation
2. seminal vesicles - provide nutrients for sperm and fluid
3. prostate gland - provide enzymes and proteins for coagulation and liquefaction
4. bulbourethral glands - add alkaline mucus to neutralize prostatic acid and vaginal acidity

- Ductus deferens - propel sperm to ejaculatory ducts
- Seminiferous tubules of testes - spermatogenesis
- Sertoli cells - support and nutrients for the germ cells as they undergo mitosis and meiosis (spermatogenesis)

Composition

- Spermatozoa - 5%
- Seminal fluid - 60% to 70%
- Prostate fluid - 20% to 30%
- Bulbourethral glands - 5%

SPECIMEN COLLECTION

A. Collection

- concentrated in the first portion of the ejaculate
- accurate testing of both fertility and postvasectomy specimens
- first portion of the ejaculate is missing → the sperm count will be decreased → the pH falsely increased → and the specimen will not liquefy
- last portion of ejaculate is missing → the semen volume is decreased → the sperm count is falsely increased → the pH is falsely decreased → and the specimen will not clot
- collected following a period of sexual abstinence of at least 2 days to not more than 7 days
- prolonged abstinence - tend to have higher volumes and decreased motility
- Recommended: two or three samples be collected not less than 7 days or more than 3 weeks apart, with two abnormal samples considered significant
- warm sterile glass or plastic containers
- kept at room temperature and delivered to the laboratory within 1 hour of collection
- should be collected by masturbation
- only nonlubricant-containing rubber or polyurethane condoms should be used

B. Handling

- discarded as biohazardous waste
- semen culture - processed for bioassay, intra-uterine insemination (IUI), or in vitro fertilization (IVF)

SEMEN ANALYSIS

Reference Values for Semen Analysis

Volume	2 to 5 mL
Viscosity	Pours in droplets
pH	7.2 to 8.0
Sperm concentration	>20 million/mL
Sperm count	>40 million/ejaculate
Motility	>50% within 1 h
Quality	>2.0 or a, b, c in Table 10-3
Morphology	>14% normal forms (strict criteria) >30% normal forms (routine criteria)
Round cells	<1.0 million/mL

A. Appearance

Normal Characteristics

- gray-white color
- appears translucent
- musty odor

Abnormal Characteristics

- white turbidity - indicates the presence of white blood cells (WBCs) and infection within the reproductive tract, leukocyte esterase reagent strip test
- red - presence of red blood cells (RBCs)
- yellow - urine contamination, abstinence
- urine is toxic to sperm
- culture - done before semen analysis

B. Liquefaction

- Fresh semen specimen - clotted and should liquefy within 30 to 60 minutes after collection
- Recording the time of collection - essential for evaluating semen liquefaction
- Failure of liquefaction within 60 minutes - caused by a deficiency in prostatic enzymes and should be reported
- Sample should be liquified to be analysed

Inducers

- 2 hours the specimen has not liquified - add inducer
 - an equal volume of physiologic Dulbecco's phosphate-buffered saline
 - proteolytic enzymes (alpha-chymotrypsin or bromelain)
 - may affect biochemical tests, sperm motility, and sperm morphology, so their use must be documented
- Jelly-like granules (gelatinous bodies) - no clinical significance

Procedure: Semen Dilution with Physiologic Saline

Prepare an equal volume of diluent and semen (1 part diluent and 1 part semen) using Dulbecco's phosphate-buffered saline. Repeated pipetting of the prepared dilution will induce liquefaction.

Preparation of Dulbecco's Phosphate-Buffered Saline

1. Using a 1-L volumetric flask, add the following:
 - a. 750 ml of purified water
 - b. 0.20 g of potassium chloride (KCL)

- 0.20 g of potassium dihydrogen phosphate (KH_2PO_4)
 - 0.10 g of magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$)
 - 8.0 g of sodium chloride (NaCl)
 - 2.16 g of disodium hydrogen phosphate heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$)
 - 1.00 g of D-glucose
- In a 10-mL volumetric flask, dissolve 0.132 g of calcium chloride dehydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) in 10 mL of purified water.
 - To prevent precipitation, add calcium chloride dehydrated solution to the 1-L flask slowly, stirring continuously.
 - Adjust the pH to 7.4 with 1 mol/L sodium hydroxide (NaOH).
 - Make up to 1000 mL with purified water.

Procedure: Digestion with Bromelain

- Prepare 10 IU/mL bromelain in Dulbecco's phosphate-buffered saline.
 - Into a 100-mL volumetric flask, add 1000 IU of bromelain.
 - Add 60 mL Dulbecco's phosphate-buffered saline.
 - Mix to dissolve. It takes about 15 to 20 minutes. Bring volume to calibration mark using buffered saline.
- Dilute one part semen 1:2 with the 10 IU/mL bromelain (1 part semen + 1 part bromelain solution).
- Stir with a pipette tip.
- Incubate at 37°C for 10 minutes.
- Mix the sample well before analysis.

∴ mixing the sample container during liquefaction → produce a homogeneous sample

C. Volume

- Normal: 2 and 5 mL
- clean graduated cylinder calibrated in 0.1-mL increments
- Increased - abstinence
- Decreased - infertility, improper functioning of one of the semen-producing organs (seminal vesicles)

D. Viscosity

- consistency of the fluid and may be related to specimen liquefaction
- 0 (watery) to 4 (gel-like)
- Normal - easily drawn into a pipette and form small discrete droplets that do not appear clumped or stringy when falling by gravity from the pipette
- Incomplete liquefaction - clumped and highly viscous
 - form threads longer than 2 cm
 - impede testing for sperm motility, sperm concentration, antisperm antibody detection, and measurement of biochemical markers

E. pH

- balance between the pH values from the acidic prostatic secretion and the alkaline seminal vesicles secretion
- measured within 1 hour of ejaculation due to the loss of CO_2 that occurs
- Normal: alkaline (7.2 to 8.0)
- Increased pH - infection within the reproductive tract
- Decreased pH - increased prostatic fluid, ejaculatory duct obstruction, or poorly developed seminal vesicles

F. Sperm Concentration and Sperm Count

Factors that Affect Sperm Concentration

- ✓ days of sexual abstinence before the collection
- ✓ infection
- ✓ stress
- Normal: >20 to 250 million sperm per milliliter
- Borderline - 10 and 20 million per milliliter
- performed using the Neubauer counting chamber
- 1:20 - common dilution
 - immobilizes the sperm before counting
- counted in the four corner and center squares of the large center square
- Fully developed sperm - only counted
- Immature sperm and WBCs - "round" cells, not included
 - >1 million leukocytes per milliliter - associated with inflammation or infection of the reproductive organs that can lead to infertility
 - >1 million spermatids per milliliter - indicates disruption of spermatogenesis (viral infections, exposure to toxic chemicals, and genetic disorders)

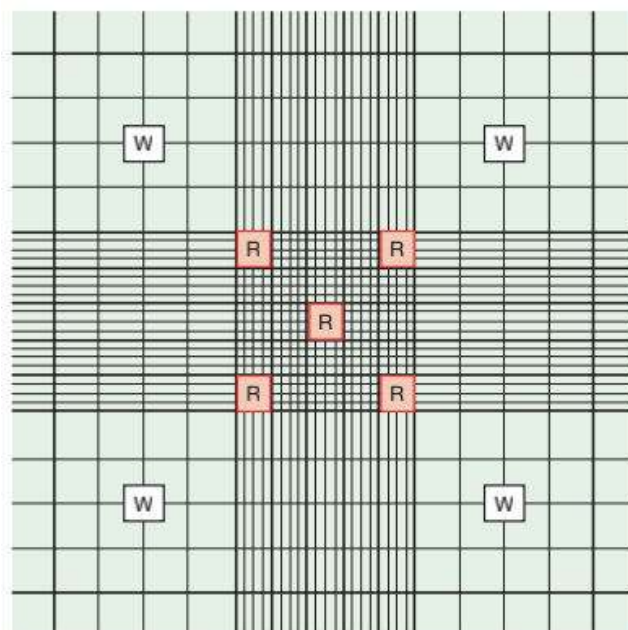


Figure 10-2 Areas of the Neubauer counting chamber used for red and white blood cell counts. W, typical WBC counting area; R, typical RBC counting area.

Calculating Sperm Concentration and Sperm Count

1:20 dilution

number of sperm can be multiplied by 1,000,000 (add 6 zeros) to equal the sperm concentration per milliliter

Examples

- Using a 1:20 dilution, an average of 60 sperm are counted in the five RBC counting squares on both sides of the hemocytometer. Calculate the sperm concentration per milliliter and the total sperm count in a specimen with a volume of 4 mL.

$$60 \text{ sperm counted} \times 1,000,000 = 60,000,000 \text{ sperm/mL}$$

$$60,000,000 \text{ sperm/mL} \times 4 \text{ mL} = 240,000,000 \text{ sperm/ ejaculate}$$

2. In a 1:20 dilution, 600 sperm are counted in two WBC counting squares. Calculate the sperm concentration per milliliter and the total sperm count in a specimen with a volume of 2 μ L.

$$\frac{600 \text{ sperm counted} \times 20 \text{ (dilution)}}{2 \text{ sq mm} \times \text{squares counted} \times 0.1 \text{ mm (depth)}} = 60,000 \text{ sperm}/\mu\text{L}$$

$$60,000 \text{ sperm}/\mu\text{L} \times 1000 = 60,000,000 \text{ sperm/mL}$$

$$60,000,000/\text{mL} \times 2 \text{ mL} = 120,000,000 \text{ sperm/ejaculate}$$

G. Sperm Motility

- forward, progressive movement
- well-mixed, liquefied semen specimen within 1 hour of specimen collection

Procedure

- 10 μ L under a 22 $\times$ 22 mm cover slip
- calibrated positive-displacement pipette
- allow it to settle for 1 minute
- 20 high-power fields
- done in duplicate for accuracy

$\therefore$ computer-assisted semen analysis (CASA) - specialize in andrology and perform a high volume of semen analysis

Sperm Motility Grading		
Grade	WHO Criteria	Sperm Motility Action
4.0	a	Rapid, straight-line motility
3.0	b	Slower speed, some lateral movement
2.0	b	Slow forward progression, noticeable lateral movement
1.0	c	No forward progression
0	d	No movement

H. Sperm Morphology

- head (penetration), neckpiece, midpiece, and tail (motility)
- head - oval-shaped head, 5 μ m long and 3 μ m wide
- tail - 45 μ m long
- midpiece - thickest part of the tail, mitochondrial sheath that produces the energy
- acrosomal cap - tip of the head, critical to ovum penetration

Procedure

- evaluated at room temperature or 37°C
- incubated at this temperature
- preparation made with prewarmed slides and cover slips
- thinly smeared, stained slide under oil immersion
- 10 μ L of semen near the frosted end of a clean microscope slide
- Wright's, Giemsa, Shorr, or Papanicolaou stain
- Air-dried slides are stable for 24 hours
- At least 200 sperm should be evaluated

Kruger's strict criteria

- measuring acrosome size
- evaluating for the presence of vacuoles

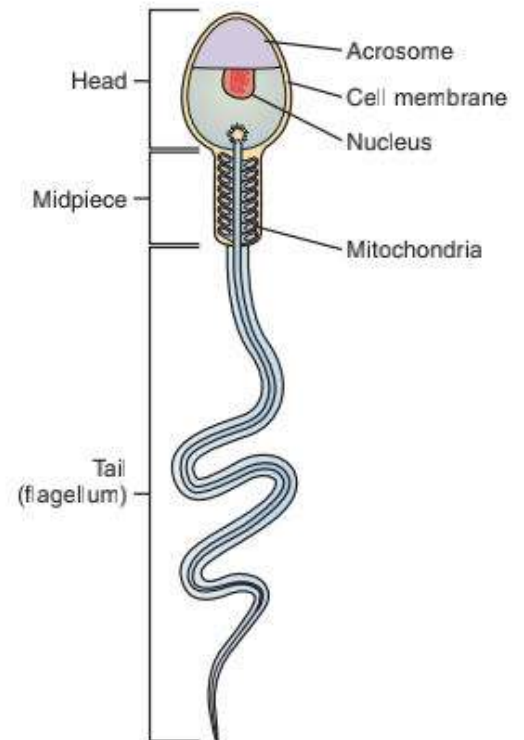


Figure 10-3 Normal spermatozoon structure.



Figure 10-4 Spermatozoon with double head, hematoxylin-eosin ($\times 1000$).



Figure 10-5 Spermatozoon with amorphous head, hematoxylin-eosin ($\times 1000$).

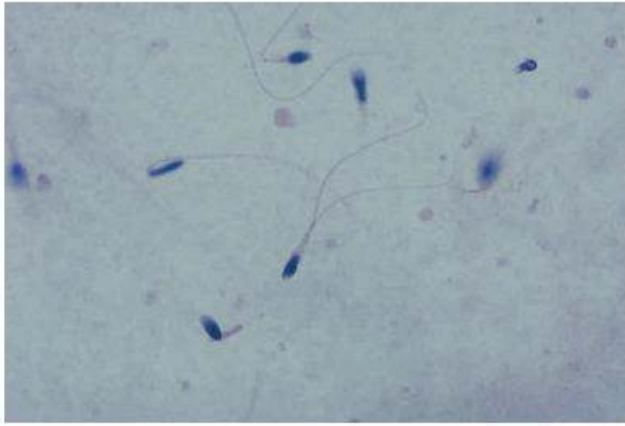


Figure 10-6 Spermatozoon with double tail, hematoxylin-eosin (x1000).



Calculating Round Cells

- made during the morphology examination
- Peroxidase-positive granulocytes - predominant form of leukocyte in semen

$$C = \frac{N \times S}{100}$$

- N - number of spermatids or neutrophils counted per 100 mature sperm
- S - sperm concentration in millions per milliliter
- >1 million WBCs per milliliter per ejaculate - indicates an inflammatory condition associated with infection and poor sperm quality and may impair sperm motility and DNA integrity

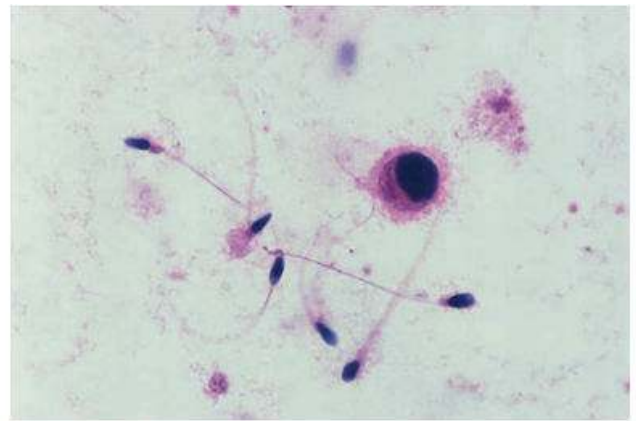


Figure 10-8 Spermatozoon with bent neck and spermatid, hematoxylin-eosin (x1000).

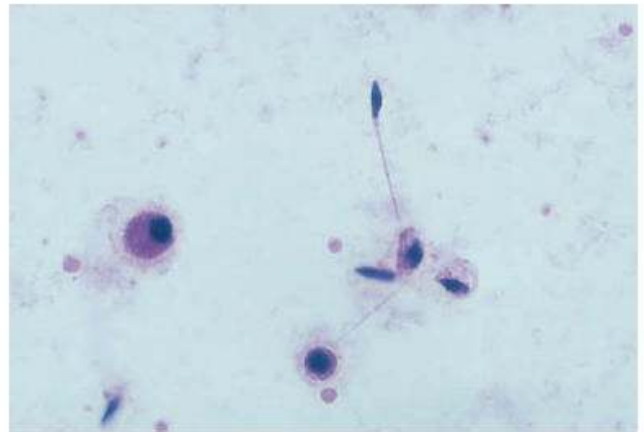


Figure 10-9 Immature spermatozoa, hematoxylin-eosin (x1000).

ADDITIONAL TESTING

A. Sperm Vitality

- assessed within 1 hour of ejaculation
- Normal vitality: 50%
- Decreased sperm vitality - normal sperm concentration with markedly decreased motility
- specimen + eosin-nigrosin stain
- preparing a smear, and counting the number of dead cells in 100 sperm using a bright-field or phase-contrast microscope
- Living cells - remain bluish white
- Dead cells - red against the purple background

B. Seminal Fluid Fructose

- Normal: $\geq 13 \mu\text{mol}$ per ejaculate
- low to absent fructose level - lack support medium $\rightarrow$ low sperm concentration
- resorcinol test
 - orange - present
- tested within 2 hours of collection or frozen to prevent fructolysis.

Procedure

Seminal Fructose Screening Test

1. Prepare reagent (50 mg resorcinol in 33 mL concentrated HCl diluted to 100 mL with water).
2. Mix 1 mL of semen with 9 mL of reagent.
3. Boil.
4. Observe for orange-red color.

C. Antisperm Antibodies

- can be present in both men and women
- may be detected in semen, cervical mucosa, or serum
- considered a possible cause of infertility
- male antisperm antibodies - more frequently encountered

Male

- sperm-agglutinating antibodies - cause sperm to stick to each other in a head-to-head, head-to-tail, or tail-to-tail pattern

Female

- normal semen analysis, continued infertility

Tests

1. Mixed agglutination reaction (MAR) test
 - screening procedure used primarily to detect the presence of immunoglobulin G (IgG) antibodies
 - semen sample containing motile sperm is incubated with IgG antihuman globulin (AHG) and a suspension of latex particles or treated RBCs coated with IgG
 - bivalent AHG binds simultaneously to both the antibody on the sperm and the antibody on the latex particles or RBCs, forming microscopically visible clumps of sperm and particles or cells
 - <10% of the motile sperm attached to the particles - normal
2. Immunobead test
 - more specific procedure
 - demonstrates what area of the sperm (head, neckpiece, midpiece, or tail) the autoantibodies are affecting
 - sperm are mixed with polyacrylamide beads known to be coated with either anti-IgG, anti-IgM, or anti-IgA
 - presence of beads on <50% of the sperm - considered normal

D. Microbial and Chemical Testing

Microbial

- >1 million leukocytes per millimeter - indicates infection within the reproductive system (prostate)
- Cultures commonly for
 - *Chlamydia trachomatis*
 - *Mycoplasma hominis*
 - *Ureaplasma urealyticum*

Chemical

- determining the levels of neutral α -glucosidase, free L-carnitine, glycerophosphocholine, zinc, citric acid, glutamyl transpeptidase, and prostatic acid phosphatase
- decreased neutral α -glucosidase, glycerophosphocholine, and L-carnitine - disorder of the epididymis
- decreased zinc, citric acid, glutamyl transpeptidase, and acid phosphatase - indicate a lack of prostatic fluid

Reference Semen Chemical Values	
Neutral α -glucosidase	≥ 20 mU/ejaculate
Zinc	≥ 2.4 μ mol/ejaculate
Citric acid	≥ 52 μ mol/ejaculate
Acid phosphatase	≥ 200 Units/ejaculate

Alleged rape

- xylene and examining under phase microscopy
 - motile - up to 24 hours after intercourse

- nonmotile sperm - persist for 3 days
- head as the sperm dies - 7 days after intercourse
- high concentration of prostatic acid phosphatase from seminal fluid - aid the detection
- seminal glycoprotein p30 (prostatic specific antigen [PSA]) - test even sperm is absent

E. Postvasectomy Semen Analysis

- only concern: presence or absence of spermatozoa
- Specimens are routinely tested at monthly intervals, beginning at 2 months postvasectomy and continuing until two consecutive monthly specimens show no spermatozoa.
- negative wet preparation $\rightarrow$ specimen centrifugation for 10 minutes and examination of the sediment

F. Sperm Function Tests

1. Hamster egg penetration - sperm are incubated with species-nonspecific hamster eggs and penetration is observed microscopically
2. Cervical mucus penetration - observation of sperm's ability to penetrate partner's midcycle cervical mucus
3. Hypo-osmotic swelling - sperm exposed to low-sodium concentrations are evaluated for membrane integrity and sperm viability
4. In vitro acrosome reaction - evaluation of the acrosome to produce enzymes essential for ovum penetration

Additional Testing for Abnormal Semen Analysis		
Abnormal Result	Possible Abnormality	Test
Decreased motility with normal count	Vitality	Eosin-nigrosin stain
Decreased count	Lack of seminal vesicle support medium	Fructose level
Decreased motility with clumping	Male antisperm antibodies	Mixed agglutination reaction and immunobead tests Sperm agglutination with male serum
Normal analysis with continued infertility	Female antisperm antibodies	Sperm agglutination with female serum

Review Questions

1. Maturation of spermatozoa takes place in the:
 - A. Sertoli cells
 - B. Seminiferous tubules
 - C. Epididymis
 - D. Seminal vesicles
2. Enzymes for the coagulation and liquefaction of semen are produced by the:
 - A. Seminal vesicles
 - B. Bulbourethral glands
 - C. Ductus deferens
 - D. Prostate gland
3. The major component of seminal fluid is:
 - A. Glucose
 - B. Fructose
 - C. Acid phosphatase

- D. Citric acid
4. If the first portion of a semen specimen is not collected, the semen analysis will have which of the following?
 - A. Decreased pH
 - B. Increased viscosity
 - C. Decreased sperm count
 - D. Decreased sperm motility
 5. Failure of laboratory personnel to document the time a semen sample is collected primarily affects the interpretation of semen:
 - A. Appearance
 - B. Volume
 - C. pH
 - D. Viscosity
 6. Liquefaction of a semen specimen should take place within:
 - A. 1 hour
 - B. 2 hours
 - C. 3 hours
 - D. 4 hours
 7. A semen specimen delivered to the laboratory in a condom has a normal sperm count and markedly decreased sperm motility. This indicates:
 - A. Decreased fructose
 - B. Antispermicide in the condom
 - C. Increased semen viscosity
 - D. Increased semen alkalinity
 8. An increased semen pH may be caused by:
 - A. Prostatic infection
 - B. Decreased prostatic secretions
 - C. Decreased bulbourethral gland secretions
 - D. All of the above
 9. Proteolytic enzymes may be added to semen specimens to:
 - A. Increase the viscosity
 - B. Dilute the specimen
 - C. Decrease the viscosity
 - D. Neutralize the specimen
 10. The normal sperm concentration is:
 - A. Less than 20 million/ μ L
 - B. More than 20 million/mL
 - C. Less than 20 million/mL
 - D. More than 20 million/ μ L
 11. Given the following information, calculate the sperm concentration: dilution, 1:20; sperm counted in five RBC squares on each side of the hemocytometer, 80 and 86; volume, 3 mL.
 - A. 80 million/mL
 - B. 83 million/mL
 - C. 86 million/mL
 - D. 169 million/ μ L
 12. Using the above information, calculate the sperm concentration when 80 sperm are counted in 1 WBC square and 86 sperm are counted in another WBC square.
 - A. 83 million/mL
 - B. 166 million per ejaculate
 - C. 16.6 million/mL
 - D. 50 million per ejaculate
 13. The primary reason to dilute a semen specimen before performing a sperm concentration is to:
 - A. Immobilize the sperm
 - B. Facilitate the chamber count
 - C. Decrease the viscosity
 - D. Stain the sperm
 14. When performing a sperm concentration, 60 sperm are counted in the RBC squares on one side of the hemocytometer and 90 sperm are counted in the RBC squares on the other side. The specimen is diluted 1:20. The:
 - A. Specimen should be rediluted and counted
 - B. Sperm count is 75 million/mL
 - C. Sperm count is greater than 5 million/mL
 - D. Sperm concentration is abnormal
 15. Sperm motility evaluations are performed:
 - A. Immediately after the specimen is collected
 - B. Within 1 hour of collection
 - C. After 3 hours of incubation
 - D. At 6-hour intervals for 1 day
 16. The percentage of sperm showing average motility that is considered normal is:
 - A. 25%
 - B. 50%
 - C. 60%
 - D. 75%
 17. The purpose of the acrosomal cap is to:
 - A. Penetrate the ovum
 - B. Protect the the nucleus
 - C. Create energy for tail movement
 - D. Protect the neckpiece
 18. The sperm part containing a mitochondrial sheath is the:
 - A. Head
 - B. Neckpiece
 - C. Midpiece
 - D. Tail
 19. All of the following are associated with sperm motility except the:
 - A. Head
 - B. Neckpiece
 - C. Midpiece
 - D. Tail
 20. The morphologic shape of a normal sperm head is:
 - A. Round
 - B. Tapered
 - C. Oval
 - D. Amorphous
 21. Normal sperm morphology when using the WHO criteria is:
 - A. >30% normal forms
 - B. <30% normal forms
 - C. >15% abnormal forms
 - D. <15% normal forms
 22. Additional parameters measured by Kruger's strict morphology include all of the following except:
 - A. Vitality
 - B. Presence of vacuoles
 - C. Acrosome size
 - D. Tail length
 23. Round cells that are of concern and may be included in sperm counts and morphology analysis are:
 - A. Leukocytes
 - B. Spermatids
 - C. RBCs
 - D. Both A and B
 24. If 5 round cells per 100 sperm are counted in a sperm morphology smear and the sperm concentration is 30 million, the concentration of round cells is:
 - A. 150,000
 - B. 1.5 million
 - C. 300,000
 - D. 15 million
 25. Following an abnormal sperm motility test with a normal sperm count, what additional test might be ordered?
 - A. Fructose level

- B. Zinc level
 - C. MAR test
 - D. Eosin-nigrosin stain
26. Follow-up testing for a low sperm concentration would include testing for:
- A. Antisperm antibodies
 - B. Seminal fluid fructose
 - C. Sperm vitality
 - D. Prostatic acid phosphatase
27. The immunobead test for antisperm antibodies:
- A. Detects the presence of male antibodies
 - B. Determines the presence of IgG, IgM, and IgA
- G. antibodies
- C. Determines the location of antisperm antibodies
 - D. All of the above
28. Measurement of α -glucosidase is performed to detect a disorder of the:
- A. Seminiferous tubules
 - B. Epididymis
 - C. Prostate gland
 - D. Bulbourethral glands
29. A specimen delivered to the laboratory with a request for prostatic acid phosphatase and glycoprotein p30 was collected to determine:
- A. Prostatic infection
 - B. Presence of antisperm antibodies
 - C. A possible rape
 - D. Successful vasectomy
30. Following a negative postvasectomy wet preparation, the specimen should be:
- A. Centrifuged and reexamined
 - B. Stained and reexamined
 - C. Reported as no sperm seen
 - D. Both A and B
31. Standardization of procedures and reference values for semen analysis is primarily provided by the:
- A. Manufacturers of instrumentation
 - B. WHO
 - C. Manufacturers of control samples
 - D. Clinical Laboratory Improvement Amendments

Synovial Fluid

OUTLINE

- I. Physiology
- II. Specimen Collection & Handling
- III. Color & Clarity
- IV. Viscosity
- V. Cell Counts
- VI. Differential Count
- VII. Crystal Identification
- VIII. Chemistry Tests
- IX. Microbiologic Tests
- X. Serologic Tests

PHYSIOLOGY

A. Synovial Fluid

- joint fluid
- viscous liquid found in the cavities of the movable joints (diarthroses) or synovial joints
- formed as an ultrafiltrate of plasma across the synovial membrane
- have concentrations similar to plasma values
- arthritis - damage to the articular membranes produces pain and stiffness in the joints

Beneficial tests

- white blood cell (WBC) count
- differential
- Gram stain
- culture
- crystal examination

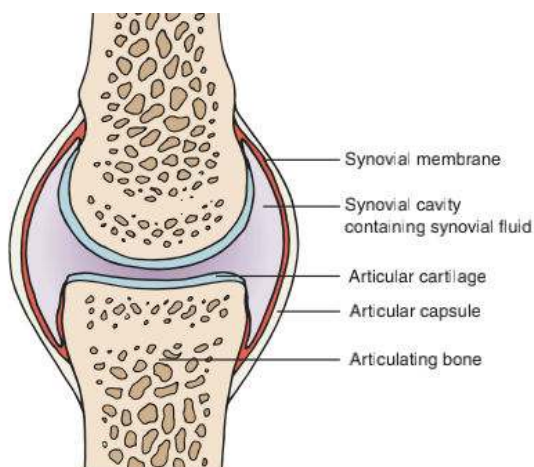


Figure 11-1 A synovial joint.

B. Normal Synovial Values

Normal Synovial Fluid Values	
Volume	<3.5 mL
Color	Colorless to pale yellow
Clarity	Clear
Viscosity	Able to form a string 4 to 6 cm long
Leukocyte count	<200 cells/ uL
Neutrophils	<25% of the differential
Crystals	None present
Glucose: plasma difference	<10 mg/dL lower than the blood glucose level
Total protein	<3 g/dL

C. Joint Disorders

1. Noninflammatory

- Degenerative joint disorders, osteoarthritis

2. Inflammatory

- Immunologic disorders, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, polymyositis, ankylosing spondylitis, rheumatic fever,
- Lyme arthritis
- Crystal-induced gout, pseudogout

3. Septic

- Microbial infection

4. Hemorrhagic

- Traumatic injury, tumors, hemophilia, other coagulation disorders
- Anticoagulant overdose

Laboratory Findings in Joint Disorders	
Group Classification	Laboratory Findings
Noninflammatory	Clear, yellow fluid Good viscosity WBCs <1000 L Neutrophils <30% Similar to blood glucose
Inflammatory Immunologic origin	Cloudy, yellow fluid Poor viscosity WBCs 2,000 to 75,000 uL Neutrophils >50% Decreased glucose level Possible autoantibodies present
Inflammatory Crystal-induced origin	Cloudy or milky fluid Low viscosity WBCs up to 100,000 L Neutrophils <70% Decreased glucose level Crystals present
Septic	Cloudy, yellow-green fluid Variable viscosity WBCs 50,000 to 100,000 L Neutrophils >75% Decreased glucose level Positive culture and Gram stain
Hemorrhagic	Cloudy, red fluid Low viscosity WBCs equal to blood Neutrophils equal to blood Normal glucose level

SPECIMEN COLLECTION & HANDLING

A. Collection

- arthrocentesis - collected by needle aspiration
- Normal adult knee cavity fluid: <3.5 mL
- Normal synovial fluid - does not clot
- Diseased joint - does clot, moistened with heparin
- Powdered anticoagulants - should not be used because they may produce artifacts that interfere with crystal analysis

Required Tube Types for Synovial Fluid Tests	
Gram stain and culture	Sterile heparinized or sodium polyanethol sulfonate
Cell counts	Heparin or liquid ethylenediaminetetraacetic acid (EDTA)
Glucose analysis	Sodium fluoride
All other tests	Nonanticoagulated

COLOR & CLARITY

- "synovial" comes from the Latin word for egg, ovum
 - colorless to pale yellow
 - resembles egg white
- yellow - noninflammatory and inflammatory effusions
- green - bacterial infection
- turbid - WBCs
- milky - crystals

VISCOSITY

- comes from polymerization of the hyaluronic acid
- essential for the proper joints lubrication
- Arthritis - decreasing fluid viscosity

A. Tests

1. String test

- string from the tip of a syringe
- Normal: 4 to 6 cm

2. Ropes (mucin clot) test

- not routinely performed
- identify a questionable fluid as synovial fluid
- measurement of hyaluronate polymerization
- normal synovial fluid + 2% to 5% acetic acid → solid clot surrounded by a clear fluid
- hyaluronate to polymerize decreases → clot becomes less firm → surrounding fluid increases in turbidity
- Reported by
 - good (solid clot)
 - fair (soft clot)
 - low (friable clot)
 - poor (no clot)

CELL COUNTS

A. Synovial Fluid Count

- total leukocyte count - most frequently performed cell count on synovial fluid
- performed as soon as possible or refrigerated
- very viscous fluid - pretreated by adding one drop of 0.05% hyaluronidase in phosphate buffer per milliliter of fluid and incubating at 37°C for 5 minutes
- done using the Neubauer counting chamber
- normal saline - diluent

- hypotonic saline (0.3%) or saline that contains saponin - diluent to lyse RBCs
- Methylene blue - stains the WBC nuclei
- Recommended technique: line a petri dish with moist paper and place the hemocytometer on two small sticks to elevate it above the moist paper
- Normal: < 200 cells/uL WBCs
- Severe infection - 100,000 cells/uL

B. Counting Procedure

- For counts less than 200 WBCs/uL, count all 9 large squares.
- For counts greater than 200 WBCs /uL in the above count, count the 4 corner squares.
- For counts greater than 200 WBCs /uL in the above count, count the 5 small squares used for a RBC count

DIFFERENTIAL COUNT

- performed on cytocentrifuged preparations or on thinly smeared slides
- incubated with hyaluronidase prior to slide preparation

1. Neutrophils

- <25% of differential count
- Increased neutrophils - indicate a septic condition

2. Lymphocytes

- <15% of differential count
- Increased - nonseptic inflammation

Cells and Inclusions Seen in Synovial Fluid		
Cell/Inclusion	Description	Significance
Neutrophil	Polymorphonuclear leukocyte	Bacterial sepsis Crystal-induced inflammation
Lymphocyte	Mononuclear leukocyte	Nonseptic inflammation
Macrophage (monocyte)	Large mononuclear leukocyte, may be vacuolated	Normal Viral infections
Synovial lining cell	Similar to macrophage, but may be multinucleated, resembling a mesothelial cell	Normal Disruption from arthrocentesis
LE cell	Neutrophil containing characteristic ingested "round body"	Lupus erythematosus
Reiter cell	Vacuolated macrophage with ingested neutrophils	Reactive arthritis (infection in another part of the body)
RA cell (ragocyte)	Neutrophil with dark cytoplasmic granules containing immune complexes	Rheumatoid arthritis Immunologic inflammation
Cartilage cells	Large, multinucleated cells	Osteoarthritis

Rice bodies	Macroscopically resemble polished rice Microscopically show collagen and fibrin	Tuberculosis Septic and rheumatoid arthritis
Fat droplets	Refractile intracellular and extracellular globules Stain with Sudan dyes	Traumatic injury Chronic inflammation
Hemosiderin	Inclusions within clusters of synovial cells	Pigmented villonodular synovitis

CRYSTAL IDENTIFICATION

A. Introduction

- Crystal formation
 - in arthritis
 - Caused by metabolic disorders and decreased renal excretion that produce elevated blood levels of crystallizing chemicals, degeneration of cartilage and bone, and injection of medications, such as corticosteroids, into a joint

B. Types of Crystals

Primary Crystals in Synovial Fluid

- monosodium urate (uric acid) (MSU)
 - in cases of gout
- calcium pyrophosphate dihydrate (CPPD)
 - seen with pseudogout
 - associated with degenerative arthritis
 - producing cartilage calcification and endocrine disorders that produce elevated serum calcium levels

Causes of Gout

- ✓ Increased serum uric acid resulting from impaired metabolism of purines
- ✓ Increased consumption of high-purine-content foods, alcohol, and fructose
- ✓ Chemotherapy treatment of leukemias
- ✓ Decreased renal excretion of uric acid

Additional Crystals

- hydroxyapatite (basic calcium phosphate) - associated with calcified cartilage degeneration
- cholesterol crystals - associated with chronic inflammation, corticosteroids after injections
- calcium oxalate crystals - in renal dialysis patient

Artifacts

- ✓ talcum powder and starch from gloves
- ✓ precipitated anticoagulants
- ✓ dust
- ✓ scratches on slides and cover slips

Characteristics of Synovial Fluid Crystals			
Crystals	Shape	Compensated Polarized Light	Significance
Monosodium urate	Needles	Negative birefringence	Gout
Calcium pyrophosphate	Rhomboid square, rods	Positive birefringence	Pseudogout
Cholesterol	Notched, rhomboid plates	Negative birefringence	Extracellular
Corticosteroid	Flat, variable-shaped plates	Positive and negative birefringence	Injections
Calcium oxalate	Envelope shapes	Negative birefringence	Renal dialysis
Apatite (calcium phosphate)	Small particles Require electron microscopy	No birefringence	Osteoarthritis

C. Slide Preparation

- Crystal examination - should be performed soon after fluid collection to ensure that crystals are not affected by changes in temperature and pH
- MSU and CPPD crystals - located extracellularly and intracellularly (within neutrophils), therefore, fluid must be examined before WBC disintegration.
- examined as an unstained wet preparation
- CPPD confirmation: red compensated polarized microscopy

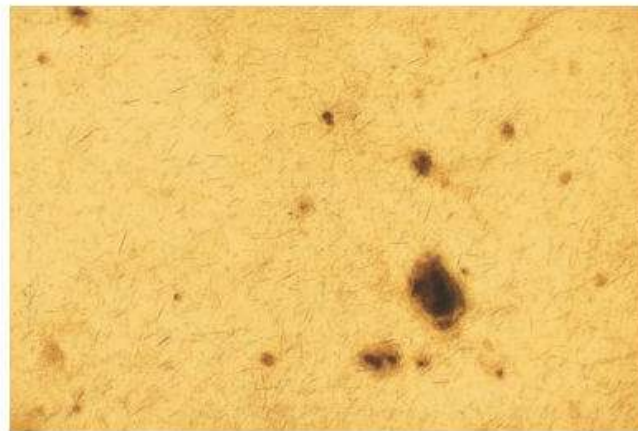


Figure 11-2 Unstained wet prep of MSU crystals (x400). Notice the characteristic yellow-brown of the urate crystals.

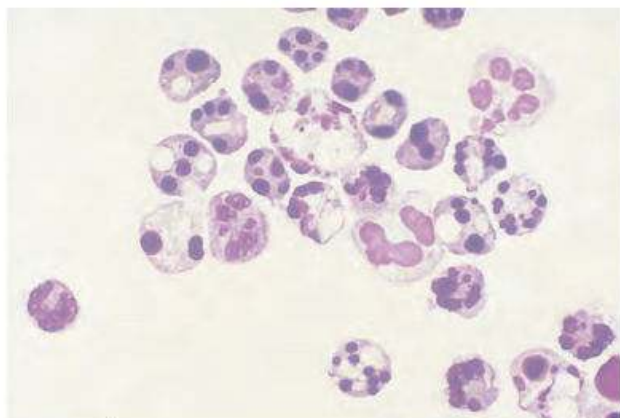


Figure 11-3 Wright's-stained neutrophils containing CPPD crystals (x1000).

D. Crystal Polarization

- Control slide - prepared by betamethasone acetate corticosteroid
- MSU - more highly birefringent and appears brighter against the dark background
- Red compensator - separates the light ray into slow-moving and fast-moving vibrations and produces a red background

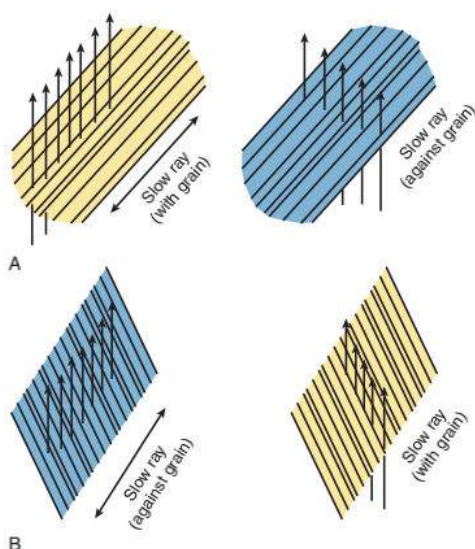


Figure 11-9 Negative and positive birefringence in MSU and CPPD crystals. (A) MSU crystal with grain running parallel to the long axis. The slow ray passes with the grain, producing negative (yellow) birefringence. (B) CPPD crystal with grain running perpendicular to the long axis. The slow ray passes against the grain and is retarded, producing positive (blue) birefringence.

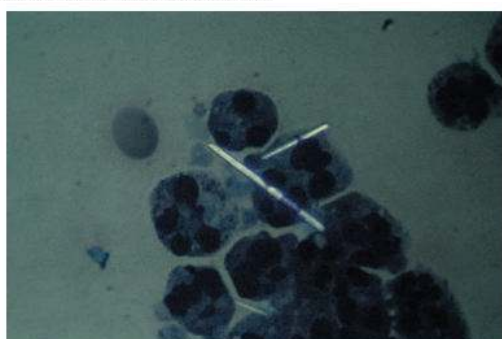


Figure 11-4 Strongly birefringent MSU crystals under polarized light (x500).

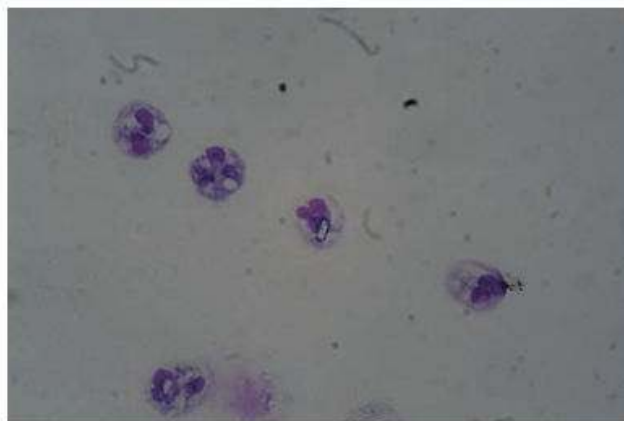


Figure 11-5 Weakly birefringent CPPD crystals under polarized light (x1000).

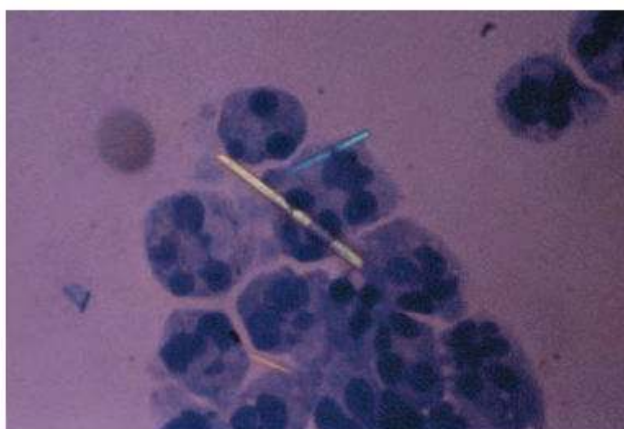


Figure 11-7 MSU crystals under compensated polarized light. The yellow crystal is aligned with the slow vibration (x500).

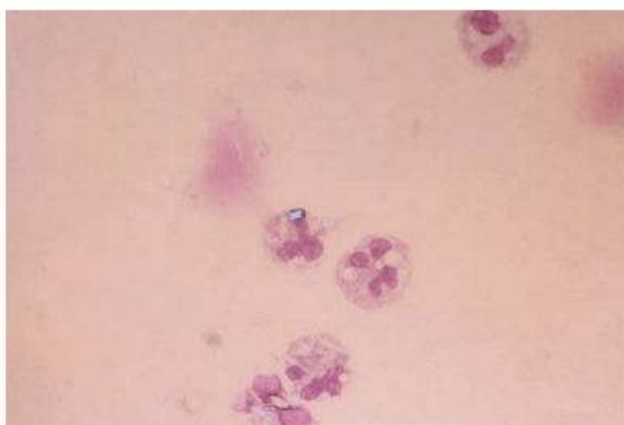


Figure 11-8 CPPD crystals under compensated polarized light. The blue crystal is aligned with the slow vibration (x1000).

CHEMISTRY TESTS

A. Synovial Chemistry Tests

- chemistry test values are approximately the same as serum values
- glucose determination - most frequently requested
- markedly decreased glucose values - indicate inflammatory (group II) or septic (group III) disorders
- fasting for 8 hours - to allow equilibration between serum and synovial fluids
- Synovial fluid - should not be more than 10 mg/dL lower than the blood value

Other Chemistry Tests

- total protein and uric acid determinations
- Synovial fluid - <3 g/dL protein
- Increased levels - inflammatory and hemorrhagic disorders
- Elevation of serum uric acid - in cases of gout
- Fluid lactate or acid phosphatase levels - severity and prognosis of rheumatoid arthritis (RA)

MICROBIOLOGIC TESTS

A. Gram stains and cultures

- must be performed on all specimens
- *Staphylococcus* and *Streptococcus*,
- fastidious *Haemophilus* species and *Neisseria gonorrhoeae* - common organisms that infect synovial fluid
- need an enrichment media

SEROLOGIC TESTS

A. Serology

- synovial fluid analysis - serves as a confirmatory measure in cases that are difficult to diagnose
- autoimmune diseases rheumatoid arthritis and systemic lupus erythematosus - serious joint inflammation, demonstrating the presence of their particular autoantibodies in the patient's serum

Lyme disease

- complication: arthritis
- *Borrelia burgdorferi* in the patient's serum - can confirm the cause of the arthritis

Review Questions

1. The functions of synovial fluid include all of the following except:
 - A. Lubrication for the joints
 - B. Removal of cartilage debris
 - C. Cushioning joints during jogging
 - D. Providing nutrients for cartilage
2. The primary function of synoviocytes is to:
 - A. Provide nutrients for the joints
 - B. Secrete hyaluronic acid
 - C. Regulate glucose filtration
 - D. Prevent crystal formation
3. Which of the following is not a frequently performed test on synovial fluid?
 - A. Uric acid
 - B. WBC count
 - C. Crystal examination
 - D. Gram stain
4. The procedure for collecting synovial fluid is called:
 - A. Synovialcentesis
 - B. Arthrocentesis
 - C. Joint puncture
 - D. Arteriocentesis
5. Match the following disorders with their appropriate group:
 - A. Noninflammatory
 - B. Inflammatory
 - C. Septic
 - D. Hemorrhagic

_____ Gout
_____ Neisseria gonorrhoeae infection
_____ Systemic lupus erythematosus
_____ Osteoarthritis
_____ Hemophilia
_____ Rheumatoid arthritis
_____ Heparin overdose
6. Normal synovial fluid resembles:
 - A. Egg white
 - B. Normal serum
 - C. Dilute urine
 - D. Lipemic serum
7. Before testing, very viscous synovial fluid should be treated with:
 - A. Normal saline
 - B. Hyaluronidase
 - C. Distilled water
 - D. Hypotonic saline
8. Addition of a cloudy, yellow synovial fluid to acetic acid produces a/an:
 - A. Yellow-white precipitate
 - B. Easily dispersed clot
 - C. Solid clot
 - D. Opalescent appearance
9. Which of the following could be the most significantly affected if a synovial fluid is refrigerated before testing?
 - A. Glucose
 - B. Crystal examination
 - C. Mucin clot test
 - D. Differential
10. The highest WBC count can be expected to be seen with:
 - A. Noninflammatory arthritis
 - B. Inflammatory arthritis
 - C. Septic arthritis
 - D. Hemorrhagic arthritis
11. When diluting a synovial fluid WBC count, all of the following are acceptable except:
 - A. Acetic acid
 - B. Isotonic saline
 - C. Hypotonic saline
 - D. Saline with saponin
12. The lowest percentage of neutrophils would be seen in:
 - A. Noninflammatory arthritis
 - B. Inflammatory arthritis
 - C. Septic arthritis
 - D. Hemorrhagic arthritis
13. All of the following are abnormal when seen in synovial fluid except:
 - A. Neutrophages
 - B. Ragocytes
 - C. Synovial lining cells
 - D. Lipid droplets

14. Synovial fluid crystals that occur as a result of purine metabolism or chemotherapy for leukemia are:
 - A. Monosodium urate
 - B. Cholesterol
 - C. Calcium pyrophosphate
 - D. Apatite
15. Synovial fluid crystals associated with inflammation in dialysis patients are:
 - A. Calcium pyrophosphate dihydrate
 - B. Calcium oxalate
 - C. Corticosteroid
 - D. Monosodium urate
16. Crystals associated with pseudogout are:
 - A. Monosodium urate
 - B. Calcium pyrophosphate dihydrate
 - C. Apatite
 - D. Corticosteroid
17. Synovial fluid for crystal examination should be examined as a/an:
 - A. Wet preparation
 - B. Wright's stain
 - C. Gram stain
 - D. Acid-fast stain
18. Crystals that have the ability to polarize light are:
 - A. Corticosteroid
 - B. Monosodium urate
 - C. Calcium oxalate
 - D. All of the above
19. In an examination of synovial fluid under compensated polarized light, rhomboid-shaped crystals are observed. What color would these crystals be when aligned parallel to the slow vibration?
 - A. White
 - B. Yellow
 - C. Blue
 - D. Red
20. If crystals shaped like needles are aligned perpendicular to the slow vibration of compensated polarized light, what color are they?
 - A. White
 - B. Yellow
 - C. Blue
 - D. Red
21. Negative birefringence occurs under red-compensated polarized light when:
 - A. Slow light is impeded more than fast light
 - B. Slow light is less impeded than fast light
 - C. Fast light runs against the molecular grain of the crystal
 - D. Both B and C
22. Synovial fluid cultures are often plated on chocolate agar to detect the presence of:
 - A. *Neisseria gonorrhoeae*
 - B. *Staphylococcus agalactiae*
 - C. *Streptococcus viridans*
 - D. *Enterococcus faecalis*
23. The most frequently performed chemical test on synovial fluid is:
 - A. Total protein
 - B. Uric acid
 - C. Calcium
 - D. Glucose
24. Which of the following chemistry tests can be performed on synovial fluid to determine the severity of RA?
 - A. Glucose
 - B. Protein
 - C. Lactate
 - D. Uric acid
25. Serologic tests on patients' serum may be performed to detect antibodies causing arthritis for all of the following disorders except:
 - A. Pseudogout
 - B. Rheumatoid arthritis
 - C. Systemic lupus erythematosus
 - D. Lyme arthritis

Serous Fluid

OUTLINE

- I. Physiology
- II. Formation
- III. Specimen Collection & Handling
- IV. Transudates & Exudates
- V. General Laboratory Procedures
- VI. Pleural Fluid
- VII. Pericardial Fluid
- VIII. Peritoneal Fluid

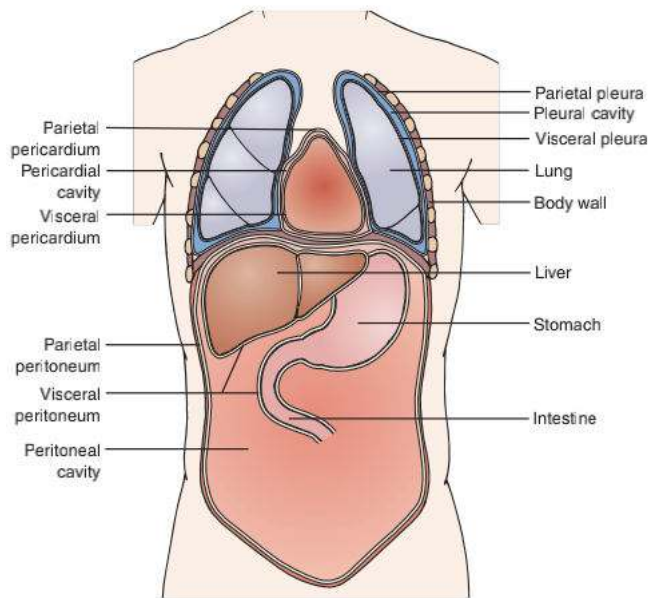
PHYSIOLOGY

A. Serous Fluid

- fluid between the membranes
- provides lubrication between the parietal and visceral membranes
 - parietal membrane - lines the cavity wall
 - visceral membrane - covers the organs within the cavity
- Normal amount: small (because production and reabsorption take place at a constant rate)

Closed Cavities of the Body

1. pleural
2. pericardial
3. peritoneal



FORMATION

A. Serous Fluid Formation

- formed as ultrafiltrates of plasma
- Production and reabsorption are subject to hydrostatic pressure and colloidal pressure (oncotic pressure) from the capillaries that serve the cavities and the capillary permeability
- hydrostatic pressure in the parietal and visceral capillaries - causes fluid to enter between the membranes
- Filtration of the plasma ultrafiltrate → increased oncotic pressure in the capillaries → reabsorption of fluid back into the capillaries.

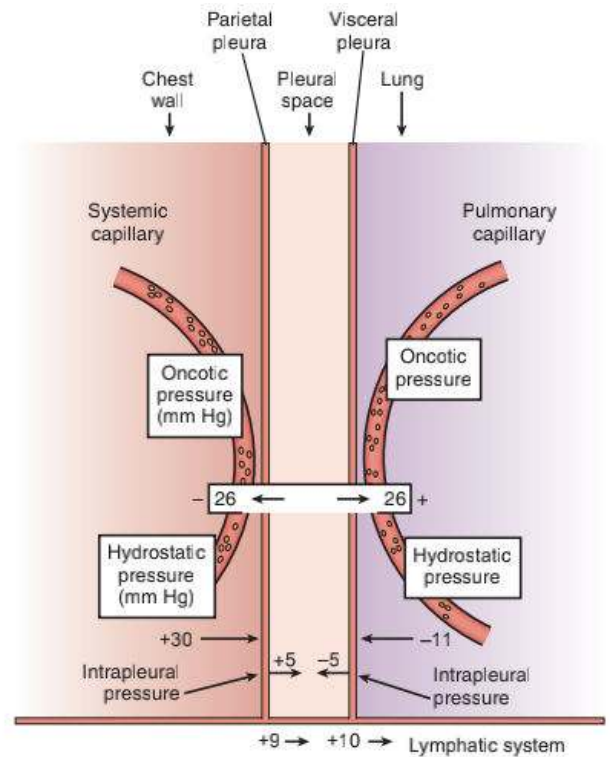


Figure 12-2 The normal formation and absorption of pleural fluid.

Effusion

- increase in fluid between the membranes due to disruption of the mechanisms of serous fluid formation
- and reabsorption

Primary Causes of Effusions

- ✓ increased hydrostatic pressure (congestive heart failure)
- ✓ decreased oncotic pressure (hypoproteinemia)
- ✓ increased capillary permeability (inflammation and infection)
- ✓ lymphatic obstruction (tumors)

Pathologic Causes of Effusions

- Increased capillary hydrostatic pressure
 - Congestive heart failure
 - Salt and fluid retention
- Decreased oncotic pressure
 - Nephrotic syndrome
 - Hepatic cirrhosis
 - Malnutrition
 - Protein-losing enteropathy
- Increased capillary permeability
 - Microbial infections
 - Membrane inflammations
 - Malignancy
- Lymphatic obstruction
 - Malignant tumors, lymphomas
 - Infection and inflammation
 - Thoracic duct injury

SPECIMEN COLLECTION & HANDLING

A. Collection

- Needle aspiration from the cavities
 - thoracentesis (pleural)
 - pericardiocentesis (pericardial)
 - paracentesis (peritoneal)
- >100mL - usually collected

Tubes Used

- EDTA tube - used for cell counts and the differential
- Sterile heparinized or sodium polyanethol sulfonate (SPS) evacuated tubes - used for microbiology and cytology
- plain tubes or in heparin tubes - chemistry tests (compared to plasma sample)

B. Handling

- anaerobically in ice - specimens for pH
- concentration of large amounts of fluid by centrifugation is done for better results

TRANSUDATES & EXUDATES

- both effusion classifications
- differentiation: blood ratios for protein and lactic dehydrogenase

A. Transudates

- Effusions that form because of a systemic disorder that disrupts the balance in the regulation of fluid filtration and reabsorption
- such as the changes in hydrostatic pressure created by congestive heart failure or the hypoproteinemia associated with the nephrotic syndrome
- usually not necessary tested

B. Exudates

- produced by conditions that directly involve the membranes of the particular cavity (infections and malignancies)

Laboratory Differentiation of Transudates and Exudates		
	Transudate	Exudate
Appearance	Clear	Cloudy
Fluid:serum protein ratio	<0.5	>0.5
Fluid:serum LD ratio	<0.6	>0.6
WBC count	<1000/ μ L	>1000/ μ L
Spontaneous clotting	No	Possible
Pleural fluid cholesterol	<45 to 60 mg/dL	>45 to 60 mg/dL
Pleural fluid: serum cholesterol ratio	<0.3	>0.3
Pleural fluid: bilirubin ratio	<0.6	>0.6
Serum-ascites albumin gradient	>1.1	<1.1

GENERAL LABORATORY PROCEDURES

- Included are:
 - evaluation of the appearance
 - differentiation between a transudate and an exudate
 - cell count and differential
 - chemistry
 - microbiology
 - cytology procedures

Additional tests

- Red blood cell (RBC) - 100,000/ μ L
- white blood cell (WBC) counts - > 1000/ μ L

Differential count

- Wright's-stained
- cytocentrifuged specimens or on slides prepared from the sediment of centrifuged specimens

PLEURAL FLUID

A. Introduction

- Tests specific and differentiative for pleural fluid transudate vs exudate
 - pleural fluid cholesterol (>60 mg/dL as exudate) and fluid:serum cholesterol ratio (>0.3 as exudate)
 - pleural fluid:serum total bilirubin ratio (0.6 as exudate)

B. Appearance

Correlation of Pleural Fluid Appearance and Disease	
Appearance	Disorder
Clear, pale yellow	Normal
Turbid, white	Microbial infection (tuberculosis)
Bloody	Hemothorax (traumatic injury) Hemorrhagic effusion, pulmonary embolus, tuberculosis, malignancy
Milky	Chylous material from thoracic duct Leakage (chronic inflammatory conditions, Sudan III) Pseudochylous material from chronic inflammation (higher cholesterol)
Brown	Rupture of amoebic liver abscess
Black	Aspergillus
Viscous	Malignant mesothelioma (increased hyaluronic acid)

*hemothorax - fluid hematocrit is more than 50% of the whole blood hematocrit, because the effusion comes from the inpouring of blood from the injury

*A chronic membrane disease effusion contains both blood and increased pleural fluid, resulting in a much lower hematocrit

Differentiation Between Chylous and Pseudochylous Pleural Effusions		
	Chylous Effusion	Pseudochylous Effusion
Cause	Thoracic duct damage	Chronic inflammation Lymphatic obstruction
Appearance	Milky/white	Milky/green tinge
Leukocytes	Predominantly lymphocytes	Mixed cells
Cholesterol crystals	Absent	Present
Triglycerides	>110 mg/dL	<50 mg/dL
Sudan III staining	Strongly positive	Negative/weakly positive

C. Hematology Tests

- differential cell count - most diagnostically significant hematology test performed on serous fluids

Cells

- Macrophages
 - 64% to 80% of a nucleated cell count
- Neutrophils
 - 1% to 2%
 - indicates a bacterial infection (pneumonia)
 - increased in effusions resulting from pancreatitis and pulmonary infarction
- Lymphocytes
 - 18% to 30%
 - normally noticeably present in both transudates and exudates in a variety of forms
 - tuberculosis, viral infections, malignancy, and autoimmune disorders (rheumatoid arthritis and systemic lupus erythematosus)
- Eosinophils
 - >10% - trauma resulting in the presence of air or blood (pneumothorax and hemothorax)
 - allergic reactions and parasitic infections
- Mesothelial cells
 - membrane lining
 - resemble lymphocytes, plasma cells, and malignant cells
 - normal mesothelial cells - single small or large round cells with abundant blue cytoplasm and round nuclei with uniform dark purple cytoplasm
 - reactive mesothelial cells - may appear in clusters; have varying amounts of cytoplasm, eccentric nuclei, and prominent nucleoli; and be multinucleated, thus more closely resembling malignant cells
 - increased in pneumonia and malignancy

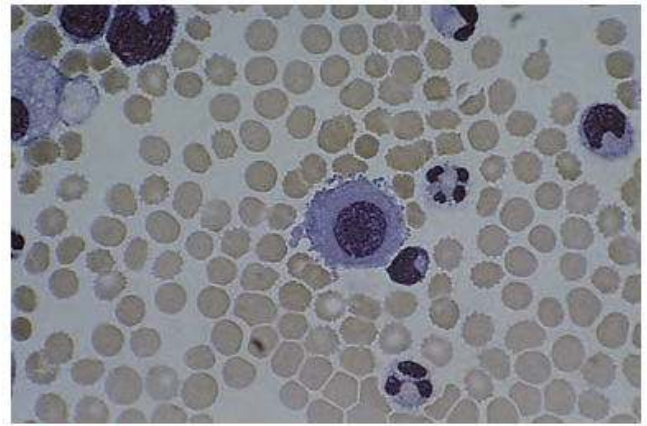


Figure 12-5 Normal mesothelial cell (x500).

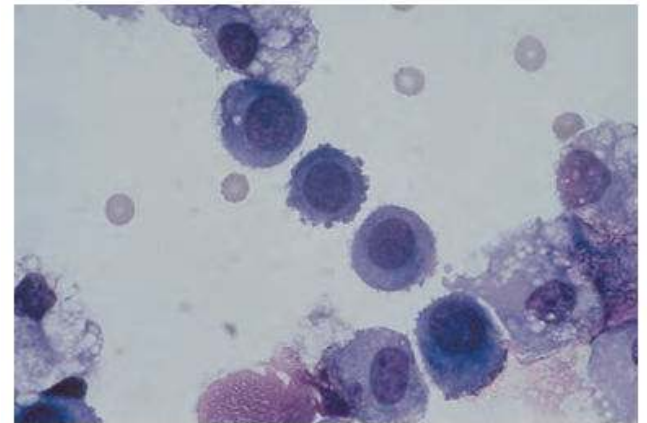


Figure 12-6 Reactive mesothelial cells showing eccentric nuclei and vacuolated cytoplasm (x500).

- Plasma cells
 - increased in tuberculosis
- Malignant cells
 - nuclear and cytoplasmic irregularities, hyperchromatic nucleoli, cellular clumps with cytoplasmic molding (community borders), and abnormal nucleus:cytoplasm ratios
 - contain large, irregular adenocarcinoma cells, small or oat cell carcinoma cells

Characteristics of Malignant Cell

- ✓ Increased nucleus:cytoplasm (N:C) ratio. The higher the ratio, the more poorly differentiated are the cells.
- ✓ Irregularly distributed nuclear chromatin
- ✓ Variation in size and shape of nuclei
- ✓ Increased number and size of nucleoli
- ✓ Hyperchromatic nucleoli
- ✓ Giant cells and multinucleation
- ✓ Nuclear molding
- ✓ Cytoplasmic molding (community borders)
- ✓ Vacuolated cytoplasm, mucin production
- ✓ Cellular crowding, phagocytosis

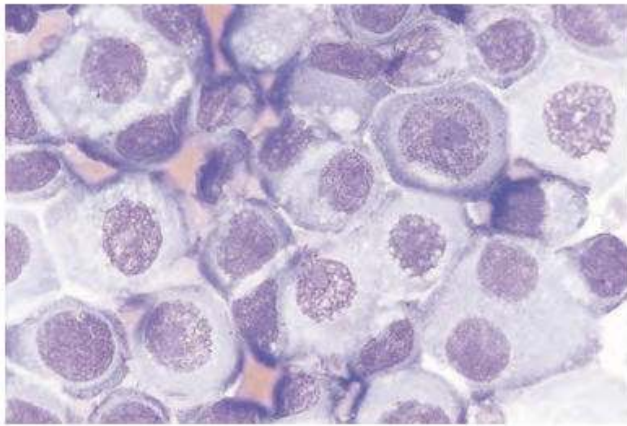


Figure 12-14 Metastatic breast carcinoma cells in pleural fluid. Notice the hyperchromatic nucleoli (x1000).

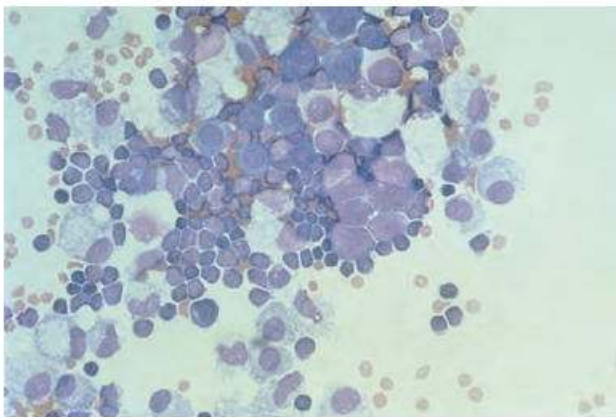


Figure 12-13 Pleural fluid small cell carcinoma showing nuclear molding (x250).

D. Chemistry Tests

- glucose, pH, adenosine deaminase (ADA), and amylase - common
- Triglyceride levels - measured to confirm the presence of a chylous effusion

Significance of Chemical Testing of Pleural Fluid	
Test	Significance
Glucose	Decreased in rheumatoid inflammation Decreased in purulent infection Bacteria (lactate) <60 mg/dL
Lactate	Elevated in bacterial infection
Triglyceride	Elevated in chylous effusions
pH	Decreased in pneumonia not responding to antibiotics Markedly decreased with esophageal rupture 0.30 degrees lower than the blood pH
ADA	Elevated in tuberculosis and malignancy > 40 U/L
Amylase	Elevated in pancreatitis, esophageal rupture, and malignancy

E. Microbiologic and Serologic Tests

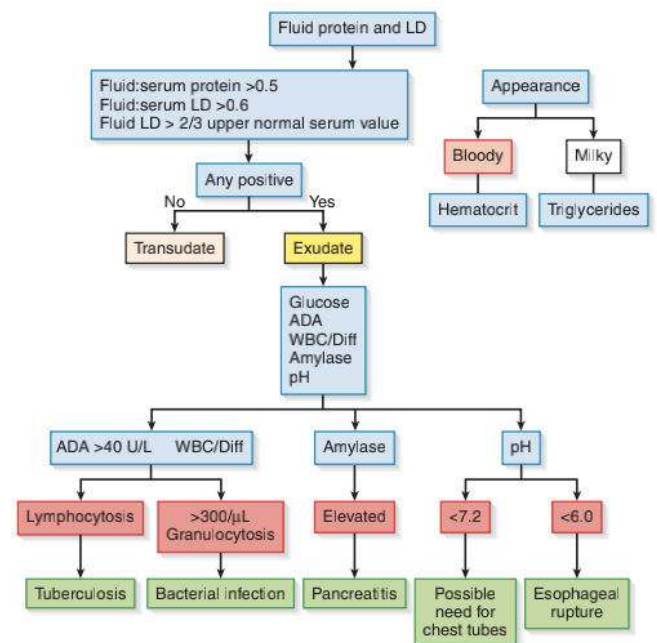
Microbiology

- Common: *Staphylococcus aureus*, Enterobacteriaceae, anaerobes, and *Mycobacterium tuberculosis*
- Gram stains, cultures (both aerobic and anaerobic), acid-fast stains, and mycobacteria cultures

Serologic

- differentiate effusions of immunologic origin from noninflammatory processes
- antinuclear antibody (ANA) and rheumatoid factor (RF) - most frequently performed
- tumor markers - carcinoembryonic antigen (CEA), CA 125 (metastatic uterine cancer), CA 15.3 and CA 549 (breast cancer), and CYFRA 21-1 (lung cancer)

PLEURAL FLUID TESTING ALGORITHM



PERICARDIAL FLUID

A. Introduction

- 10 to 50 mL - found between the pericardial serous membranes
- Effusions - result of changes in the membrane permeability due to infection (pericarditis), malignancy, and trauma-producing exudates, cardiac compression (tamponade) is present
- Primary cause of transudates: uremia, hypothyroidism, and autoimmune disorders are the primary causes of transudates

B. Appearance

Significance of Pericardial Fluid Testing	
Test	Significance
Appearance	
Clear, pale yellow	Normal, transudate
Blood-streaked	Infection, malignancy
Grossly bloody	Cardiac puncture, anticoagulant medications
Milky	Chylous and pseudochylous material

Additional testing	
Increased neutrophils	Bacterial endocarditis
Malignant cells	Metastatic carcinoma
Carcinoembryonic antigen	Metastatic carcinoma
Gram stain and culture	Bacterial endocarditis
Acid-fast stain	Tubercular effusion
Adenosine deaminase	Tubercular effusion

C. Laboratory Tests

Transudate vs exudate

- fluid:serum protein and lactic dehydrogenase (LD) ratios
- >1000 WBCs/ μ L WBCs - bacterial endocarditis, bacterial cultures and Gram stains are performed on concentrated fluids
- Malignant cells - metastatic lung or breast carcinoma, associated with asbestos contact

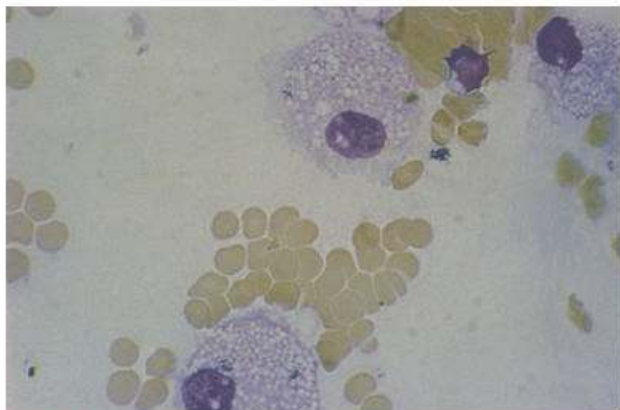


Figure 12-16 Malignant pericardial effusion showing giant mesothelioma cell with cytoplasmic molding and hyperchromatic nucleoli ($\times 1000$).

- Effusions of tubercular origin - AIDS
- acid-fast stains and chemical tests for adenosine deaminase - often requested

PERITONEAL FLUID

A. Introduction

- called ascites
- ascitic fluid rather than peritoneal fluid

Causes of Ascites

- ascitic transudates
 - effusions
 - hepatic disorders (such as cirrhosis)
- ascitic exudates
 - Bacterial infections (peritonitis) and malignancy

B. Specimen Collection

- Normal saline - sometimes introduced into the peritoneal cavity as a lavage to detect abdominal injuries that have not yet resulted in fluid accumulation.
- Peritoneal lavage - sensitive test to detect intra-abdominal bleeding in blunt trauma cases
- >100,000/mL RBCs - indicative of blunt trauma injuries

C. Transudates vs Exudates

- serum-ascites albumin gradient (SAAG) - recommended
 - difference (gradient) ≥ 1.1 or - transudate effusion of hepatic origin
 - <1.1 gradients - exudative effusions

Example

Serum albumin of 3.8 mg/dL – fluid albumin of 1.2 mg/dL = gradient of 2.6 = transudate effusion
 Serum albumin of 3.8 mg/dL – fluid albumin of 3.0 mg/dL = gradient of 0.8 = exudate effusion

D. Appearance

Significance of Peritoneal Fluid Testing	
Test	Significance
Appearance	
Clear, pale yellow	Normal
Turbid	Microbial infection
Green	Bile, gallbladder, pancreatic disorders
Blood-streaked	Lymphatic trauma and blockage
Milky	Trauma, infection, or malignancy (Chylous or pseudochylous material)
Peritoneal lavage	>100,000 RBCs/ μ L indicates blunt trauma injury
WBC count	
<500 cells/ μ L	Normal
>500 cells/ μ L	Bacterial peritonitis, cirrhosis
Differential	Bacterial peritonitis, malignancy
Carcinoembryonic antigen	Malignancy of gastrointestinal origin
CA 125	Malignancy of ovarian origin
Glucose	Decreased in tubercular peritonitis, malignancy
Amylase	Increased in pancreatitis, gastrointestinal perforation
Alkaline phosphatase	Increased in gastrointestinal perforation
Blood urea nitrogen/creatinine	Ruptured or punctured bladder
Gram stain and culture	Bacterial peritonitis
Acid-fast stain	Tubercular peritonitis
Adenosine deaminase	Tubercular peritonitis

D. Laboratory Tests

- neutrophil count
 - >250 cells/ μ L or >50% of the total WBC count - indicates infection

Cellular Examination

- leukocytes, abundant mesothelial cells, and macrophages, including lipophages
- Psammoma bodies - containing concentric striations of collagen-like material can be seen in benign conditions and are also associated with ovarian and thyroid malignancies

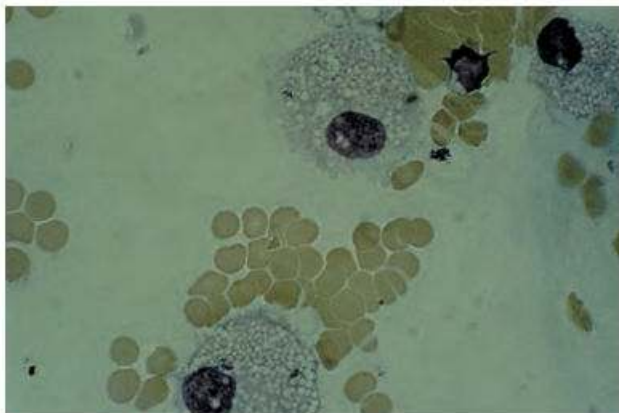


Figure 12-17 Lipophages (macrophages containing fat droplets) in peritoneal fluid (x500).

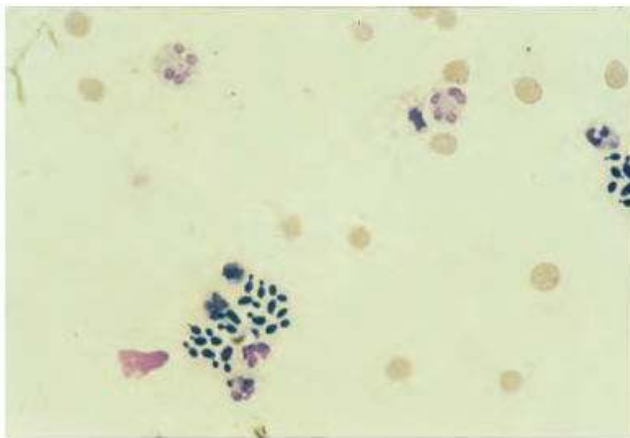


Figure 12-18 Budding yeast in peritoneal fluid (x400).

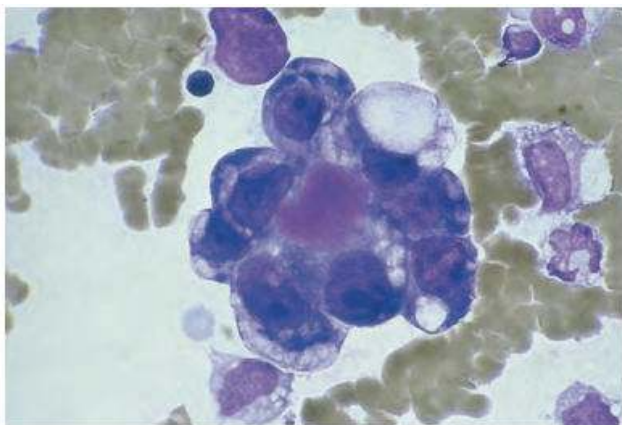


Figure 12-19 Ovarian carcinoma showing community borders, nuclear irregularity, and hyperchromatic nucleoli (x500).

Chemical Testing

- glucose - decreased below serum levels in bacterial and tubercular peritonitis and malignancy
- amylase - pancreatitis, and it may be elevated
- in patients with gastrointestinal perforations
- alkaline phosphatase - highly diagnostic of intestinal perforation
- blood urea nitrogen and creatinine - ruptured bladder or accidental puncture of the bladder during the paracentesis
- bilirubin - leakage of bile into the peritoneum is suspected following trauma or surgery

Microbiology Tests

- Bacterial peritonitis
 - Gram stains and bacterial cultures for both aerobes and anaerobes are performed
- Tuberculosis
 - Acid-fast stains, adenosine deaminase, and cultures

Serologic Tests

- tumor markers CEA and CA 125
- presence of CA 125 antigen with a negative CEA - suggests the source is from the ovaries, fallopian tubes, or endometrium.

Review Questions

- The primary purpose of serous fluid is to:
 - Remove waste products
 - Lower capillary pressure
 - Lubricate serous membranes
 - Nourish serous membranes
- The membrane that lines the wall of a cavity is the:
 - Visceral
 - Peritoneal
 - Pleural
 - Parietal
- During normal production of serous fluid, the slight excess of fluid is:
 - Absorbed by the lymphatic system
 - Absorbed through the visceral capillaries
 - Stored in the mesothelial cells
 - Metabolized by the mesothelial cells
- Production of serous fluid is controlled by:
 - Capillary oncotic pressure
 - Capillary hydrostatic pressure
 - Capillary permeability
 - All of the above
- An increase in the amount of serous fluid is called a/an:
 - Exudate
 - Transudate
 - Effusion
 - Malignancy
- Pleural fluid is collected by:
 - Pleurocentesis
 - Paracentesis
 - Pericentesis
 - Thoracentesis
- Place the appropriate letter in front of the following statements describing transudates and exudates.
 - Transudate
 - Exudate

_____ Caused by increased hydrostatic pressure
 _____ Caused by increased capillary permeability
 _____ Caused by decreased oncotic pressure

- _____ Caused by congestive heart failure
 - _____ Malignancy related
 - _____ Tuberculosis related
 - _____ Endocarditis related
 - _____ Clear appearance
8. Fluid:serum protein and lactic dehydrogenase ratios are performed on serous fluids:
 - A. When malignancy is suspected
 - B. To classify transudates and exudates
 - C. To determine the type of serous fluid
 - D. When a traumatic tap has occurred
 9. Which of the following requires the most additional testing?
 - A. Transudate
 - B. Exudate
 10. An additional test performed on pleural fluid to classify the fluid as a transudate or exudate is the:
 - A. WBC count
 - B. RBC count
 - C. Fluid:cholesterol ratio
 - D. Fluid-to-serum protein gradient
 11. A milky-appearing pleural fluid indicates:
 - A. Thoracic duct leakage
 - B. Chronic inflammation
 - C. Microbial infection
 - D. Both A and B
 12. Which of the following best represents a hemothorax?
 - A. Blood HCT: 42 Fluid HCT: 15
 - B. Blood HCT: 42 Fluid HCT: 10
 - C. Blood HCT: 30 Fluid HCT: 10
 - D. Blood HCT: 30 Fluid HCT: 20
 13. All of the following are normal cells seen in pleural fluid except:
 - A. Mesothelial cells
 - B. Neutrophils
 - C. Lymphocytes
 - D. Mesothelioma cells
 14. A differential observation of pleural fluid associated with tuberculosis is:
 - A. Increased neutrophils
 - B. Decreased lymphocytes
 - C. Decreased mesothelial cells
 - D. Increased mesothelial cells
 15. All of the following are characteristics of malignant cells except:
 - A. Cytoplasmic molding
 - B. Absence of nucleoli
 - C. Mucin-containing vacuoles
 - D. Increased nucleus:cytoplasm ratio
 16. A pleural fluid pH of 6.0 indicates:
 - A. Esophageal rupture
 - B. Mesothelioma
 - C. Malignancy
 - D. Rheumatoid effusion
 17. Plasma cells seen in pleural fluid indicate:
 - A. Bacterial endocarditis
 - B. Primary malignancy
 - C. Metastatic lung malignancy
 - D. Tuberculosis infection
 18. A significant cell found in pericardial or pleural fluid that should be referred to cytology is a:
 - A. Reactive lymphocyte
 - B. Mesothelioma cell
 - C. Monocyte
 - D. Mesothelial cell
 19. Another name for a peritoneal effusion is:
 - A. Peritonitis
 - B. Lavage
 - C. Ascites
 - D. Cirrhosis
 20. A test performed primarily on peritoneal lavage fluid is a/an:
 - A. WBC count
 - B. RBC count
 - C. Absolute neutrophil count
 - D. Amylase
 21. The recommended test for determining whether peritoneal fluid is a transudate or an exudate is the:
 - A. Fluid:serum albumin ratio
 - B. Serum ascites albumin gradient
 - C. Fluid:serum lactic dehydrogenase ratio
 - D. Absolute neutrophil count
 22. Given the following results, classify this peritoneal fluid: serum albumin, 2.2 g/dL; serum protein, 6.0 g/dL; fluid albumin, 1.6 g/dL.
 - A. Transudate
 - B. Exudate
 23. Differentiation between bacterial peritonitis and cirrhosis is done by performing a/an:
 - A. WBC count
 - B. Differential
 - C. Absolute neutrophil count
 - D. Absolute lymphocyte count
 24. Detection of the CA 125 tumor marker in peritoneal fluid indicates:
 - A. Colon cancer
 - B. Ovarian cancer
 - C. Gastric malignancy
 - D. Prostate cancer
 25. Chemical tests primarily performed on peritoneal fluid include all of the following except:
 - A. Lactose dehydrogenase
 - B. Glucose
 - C. Alkaline phosphatase
 - D. Amylase
 26. Cultures of peritoneal fluid are incubated:
 - A. Aerobically
 - B. Anaerobically
 - C. At 37°C and 42°C
 - D. Both A and B

Amniotic Fluid

OUTLINE

- I. Physiology
- II. Specimen Collection
- III. Specimen Handling & Processing
- IV. Color & Appearance
- V. Tests For Fetal Distress
- VI. Tests For Fetal Maturity

PHYSIOLOGY

A. Function

- product of fetal metabolism
- constituents that are present in the fluid provide information about the metabolic processes taking place during—as well as the progress of—fetal maturation
- present in the amnion
- a membranous sac that surrounds the fetus
- provide a protective cushion for the fetus, allow fetal movement, stabilize the temperature to protect the fetus from extreme temperature changes, and permit proper lung development

Tests for Fetal Well-Being and Maturity		
Test	Reference Values at Test Term	Significance
Bilirubin scan	$\Delta A_{450} > .025$	Hemolytic disease of the newborn
Alpha-fetoprotein	$< 2.0 \text{ MoM}$	Neural tube disorders
Lecithin-sphingomyelin ratio	≥ 2.0	Fetal lung maturity
Amniostat-fetal lung maturity	Positive	Fetal lung maturity / phos-phatidyl glycerol
Foam Stability Index	≥ 47	Fetal lung maturity
Optical density 650 nm	≥ 0.150	Fetal lung maturity
Lamellar body count	$\geq 32,000/\text{mL}$	Fetal lung maturity

B. Volume

- regulated by a balance between the production of fetal urine and lung fluid and the absorption from fetal swallowing and intramembranous flow
- increases in quantity throughout pregnancy
- 35 mL - first trimester
- 800 to 1200 mL - during the third trimester
 - $> 1200 \text{ mL}$ - polyhydramnios, failure of the fetus to begin swallowing (associated with fetal structural anomalies, cardiac arrhythmias, congenital infections, or chromosomal abnormalities)
 - $< 800 \text{ mL}$ - oligohydramnios, increased fetal swallowing (associated with congenital malformations, premature rupture of amniotic membranes, and umbilical cord compression, resulting in decelerated heart rate and fetal death)
- gradually decreases prior to delivery

Index of fetal lung maturity

- During each episode of fetal respiratory movement, secreted lung liquid enters the amniotic fluid $\rightarrow$ bathing the lungs and washing pulmonary and alveolar contents (lecithin, sphingomyelin, and phosphatidyl glycerol) $\rightarrow$ into the amniotic fluid surrounding the fetus
- The fetus swallows amniotic fluid $\rightarrow$ absorbed through the gastrointestinal tract $\rightarrow$ reexcreted by the kidneys from the blood into fetal urine and back into amniotic fluid

C. Chemical Composition

- placenta - ultimate source of amniotic fluid water and solutes
- Alpha-fetoprotein and acetylcholinesterase - biomarkers of neural tube defects
- changes when fetal urine production begins
 - creatinine, urea, and uric acid increase
 - glucose and protein concentrations decrease
- creatinine - determine fetal age (1.5 and 2.0 mg/dL prior to 36 weeks' gestation)

D. Differentiating Maternal Urine From Amniotic Fluid

- determine possible premature membrane rupture or accidental puncture of the maternal bladder during specimen collection

1. Chemical Analysis

- a. creatinine
 - much lower in amniotic fluid than in urine
 - amniotic fluid - $< 3.5 \text{ mg/dL}$ in amniotic fluid
 - urine - as high as 10 mg/dL
- b. urea
 - much lower in amniotic fluid than in urine
 - amniotic fluid - $< 30 \text{ mg/dL}$ in amniotic fluid
 - urine - 300 mg/dL
- c. glucose
 - not uncommon urine constituents during pregnancy
- d. protein
 - not uncommon urine constituents during pregnancy

2. Fern Test

- test used to evaluate premature rupture of the membranes
- vaginal fluid specimen is spread on a glass slide and allowed to completely air dry at room temperature
- positive for amniotic fluid: presence of "fern-like" crystals due to the protein and sodium chloride content

SPECIMEN COLLECTION

A. Indications for Amniocentesis

- Amniocentesis
 - recommended for neural tube defects
 - screening blood tests such as the maternal serum alpha-fetoprotein test are abnormal
 - detect genetic disorders or to evaluate the health of the fetus
- 14th week of gestation - safe
- 16 weeks' gestation - chromosome analysis

- near the end of the second trimester - intrauterine growth retardation
- third trimester - fetal distress and maturity

Amniocentesis may be indicated at 15 to 18 weeks' gestation for the following conditions to determine early treatment or intervention:

- Mother's age of 35 or older at delivery
- Family history of chromosome abnormalities, such as trisomy 21 (Down syndrome)
- Parents carry an abnormal chromosome rearrangement
- Earlier pregnancy or child with birth defect
- Parent is a carrier of a metabolic disorder
- Family history of genetic diseases such as sickle cell disease, Tay-Sachs disease, hemophilia, muscular dystrophy, sickle cell anemia, Huntington chorea, and cystic fibrosis
- Elevated maternal serum alpha-fetoprotein
- Abnormal triple marker screening test
- Previous child with a neural tube disorder such as spina bifida, or ventral wall defects (gastroschisis)
- Three or more miscarriages

Amniocentesis is indicated later in the pregnancy (20 to 42 weeks) to evaluate:

- Fetal lung maturity
- Fetal distress
- HDN caused by Rh blood type incompatibility
- Infection

B. Collection

- transabdominal amniocentesis - most commonly performed
- vaginal amniocentesis - greater risk of infection
- maximum of 30 mL
- first 2 or 3 mL collected - contaminated by maternal blood, tissue fluid, and cells and are discarded

SPECIMEN HANDLING & PROCESSING

- Fluid for fetal lung maturity (FLM) tests - placed in ice for delivery to the laboratory and kept refrigerated
- Fluid for bilirubin analysis in cases of hemolytic disease of the newborn (HDN) - must be protected from light
- Specimens for cytogenetic studies or microbial studies - must be processed aseptically and maintained at room temperature or body temperature (37°C incubation) prior to analysis to prolong the life of the cells needed for analysis

COLOR & APPEARANCE

Color	Significance
Colorless	Normal
Blood-streaked	Traumatic tap, abdominal trauma, intra-amniotic hemorrhage
Yellow	Hemolytic disease of the newborn (bilirubin)
Dark green	Meconium
Dark red-brown	Fetal death

*blood-streaked - source of the blood (maternal or fetal) can be determined using the Kleihauer-Betke test

*meconium - newborn's first bowel movement

TESTS FOR FETAL DISTRESS

A. Hemolytic Disease of the Newborn (HDN)

- measured by spectrophotometric analysis using serial dilutions
- optical density (OD) of the fluid - measured in intervals between 365 nm and 550 nm
- normal: OD is highest at 365 nm and decreases linearly to 550 nm (straight line)
- presence of bilirubin: rise in OD is seen at 450 nm (this is the wavelength of maximum bilirubin absorption)
 - with interference (such as hemoglobin) - extraction with chloroform
- plotted on a Liley graph to determine the severity of the hemolytic disease

Liley graph

- Zone I - no more than a mildly affected fetus
- Zone II - moderate hemolysis and require careful monitoring anticipating an early delivery or exchange transfusion upon delivery
- Zone III - severe hemolysis and suggests a severely affected fetus
- Intervention at Zone III: induction of labor or intrauterine exchange transfusion

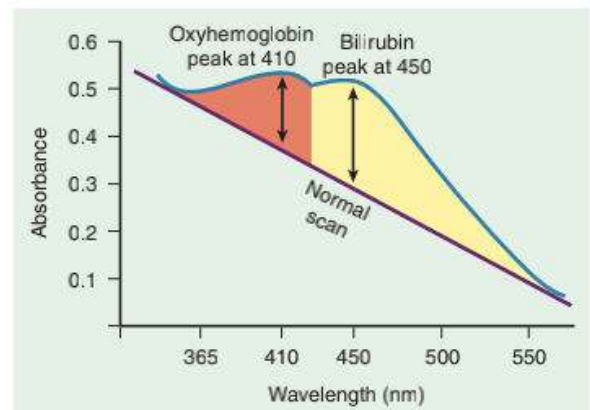
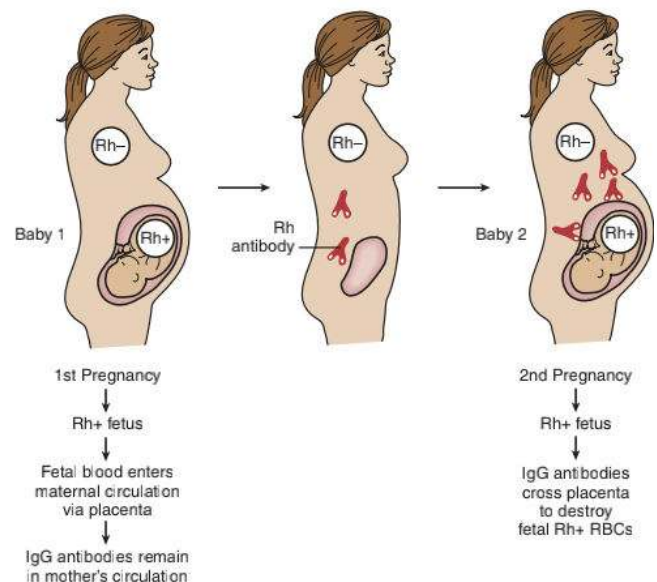
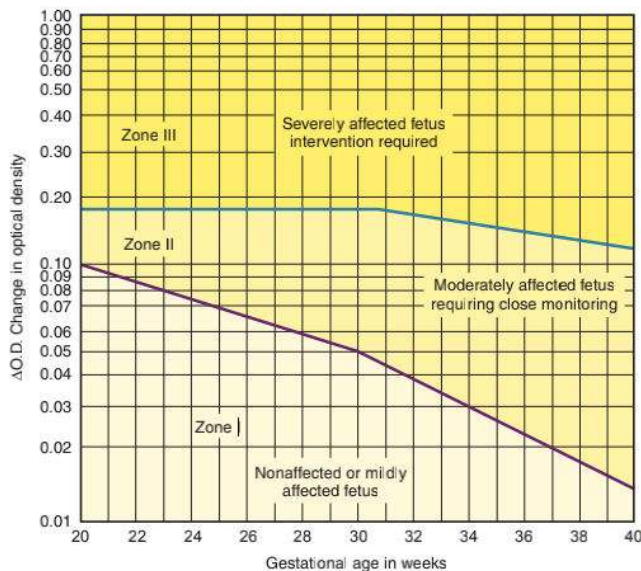


Figure 13-3 Spectrophotometric bilirubin scan showing bilirubin and oxyhemoglobin peaks.



B. Neural Tube Defects (NTD)

- such as anencephaly and spina bifida
- can be detected by maternal serum alpha-fetoprotein (MSAFP) blood test, high-resolution ultrasound, and amniocentesis
- increased levels of alpha-fetoprotein (AFP)

alpha-fetoprotein (AFP)

- when the skin fails to close over the neural tissue
- major protein produced by the fetal liver during early gestation (prior to 18 weeks)
- found in the maternal serum due to the combined fetal maternal circulations and in the amniotic fluid from diffusion and excretion of fetal urine
- reported in terms of multiples of the median (MoM)
- value two times the median value is considered abnormal (>2 MoM) for both maternal serum and amniotic fluid

amniotic acetylcholinesterase (AChE)

- followed by measurement of amniotic acetylcholinesterase (AChE)
- more specific for neural tube disorders than AFP
- provided it is not performed on a bloody specimen (because blood contains AChE)

TESTS FOR FETAL MATURITY

A. Fetal Lung Maturity

Respiratory distress syndrome (RDS)

- most frequent complication of early delivery and is the seventh most common cause of morbidity and mortality in the premature infant
- insufficiency of lung surfactant production
- surfactant - keeps the alveoli from collapsing by decreasing surface tension and allows them to inflate with air more easily
- thin-layer chromatography lung profile must include lecithin, sphingomyelin, and PG to provide an accurate measurement of FLM

B. Lecithin-Sphingomyelin Ratio

- reference method
- Prior to 35 weeks' gestation - <1.6 (large amounts of lecithin are not being produced at this time)

- After 35 weeks' gestation - > 2.0 (lecithin concentration increases while the sphingomyelin concentration remains constant)
- Falsely elevated - blood or meconium
- performed using thin-layer chromatography (TLC)
- replaced by quantitative phosphatidyl glycerol immunoassays and lamellar body density procedures

Lecithin

- primary component of the surfactants
- produced at a relatively low and constant rate until the 35th week of gestation, at which time a noticeable increase in its production occurs, resulting in the stabilization of the fetal lung alveoli

Sphingomyelin

- lipid that is produced at a constant rate after about 26 weeks' gestation
- can serve as a control on which to base the rise in lecithin

C. Phosphatidyl Glycerol (PG)

- another lung surface lipid
- essential for adequate lung maturity
- can be detected after 35 weeks' gestation
- production of PG normally parallels that of lecithin, but its production is delayed in cases of maternal diabetes
- Aminostat-FLM - uses antisera containing polyclonal anti-PG antibodies that are specific for PG-containing lamellar bodies in the amniotic fluid

D. Foam Stability Index

- mechanical screening test
- called the "foam" or "shake" test
- presence of bubbles - indicates that a sufficient amount of phospholipid is available to reduce the surface tension of the fluid even in the presence of alcohol, an antifoaming agent
- cannot be used with contaminated amniotic fluid because blood and meconium also reduce surface tension, yielding a falsely mature index result

Procedure: Foam Shake Test

1. Mix equal parts of amniotic fluid with 95% ethanol.
2. Vigorously shake for 15 seconds.
3. Allow to sit undisturbed for 15 minutes.
4. Observe for the presence of a continuous line of bubbles around the outside edge.

Procedure: Foam Stability Index

1. Add 0.5 mL of amniotic fluid to tubes containing increasing amounts of 95% ethanol ranging from 0.42 to 0.55 mL in 0.01-mL increments.
2. Vigorously shake for 15 seconds.
3. Allow to sit undisturbed for 15 minutes.
4. Observe for the presence of a continuous line of bubbles around the outside edge.
5. Values ≥ 47 indicate fetal lung maturity.

E. Lamellar Bodies

- densely packed layers of phospholipids that represent a storage form of pulmonary surfactant
- secreted by the type II pneumocytes of the fetal lung at about 24 weeks of gestation and are absorbed into the alveolar spaces to provide surfactants
- enter the amniotic fluid at about 26 weeks of gestation

- 50,000 to 200,000 per microliter by the end of the third trimester
- directly proportional to fetal lung maturity, phospholipids, the L/S ratio, and OD of amniotic fluid
- centrifuged at 2000 g for 10 minutes
- examined using a wavelength of 650 nm

Lamellar Body Count (LBCs)

- 1.7 to 7.3 fL, or 1 to 5µm
- like small platelets
- obtained using the platelet channel of automated hematology analyzers
- use both impedance and optical methods for counting
- stored at 2°C to 8°C but never frozen

Advantages

- (1) rapid turnaround time
- (2) low reagent cost
- (3) wide availability
- (4) low degree of technical difficulty
- (5) low volume of amniotic fluid required
- (6) excellent clinical performance

Procedure: Lamellar Body Count

1. Mix the amniotic fluid sample by inverting the capped sample container five times.
2. Transfer the fluid to a clear test tube to allow for visual inspection.
3. Visually inspect the specimen. Fluids containing obvious mucus, whole blood, or meconium should not be processed for an LBC.
4. Cap the tube and mix the sample by gentle inversion or by placing the test tube on a tube rocker for 2 minutes.
5. Flush the platelet channel; analyze the instrument's diluents buffer until a background count deemed acceptable by the laboratory is obtained in two consecutive analyses.
6. Process the specimen through the cell counter and record the platelet channel as the LBC.

Results

- noncentrifuged samples
 - >50,000/uL - indication of FLM
 - <15,000/uL - immature

Review Questions

1. Which of the following is not a function of amniotic fluid?
 - A. Allows movement of the fetus
 - B. Allows carbon dioxide and oxygen exchange
 - C. Protects fetus from extreme temperature changes
 - D. Acts as a protective cushion for the fetus
2. What is the primary cause of the normal increase in amniotic fluid as a pregnancy progresses?
 - A. Fetal cell metabolism
 - B. Fetal swallowing
 - C. Fetal urine
 - D. Transfer of water across the placenta
3. Which of the following is not a reason for decreased amounts of amniotic fluid?
 - A. Fetal failure to begin swallowing
 - B. Increased fetal swallowing
 - C. Membrane leakage
 - D. Urinary tract defects
4. Why might a creatinine level be requested on an amniotic fluid?
 - A. Detect oligohydramnios
 - B. Detect polyhydramnios
 - C. Differentiate amniotic fluid from maternal urine
 - D. Evaluate lung maturity
5. Amniotic fluid specimens are placed in amber-colored tubes prior to sending them to the laboratory to prevent the destruction of:
 - A. Alpha-fetoprotein
 - B. Bilirubin
 - C. Cells for cytogenetics
 - D. Lecithin
6. How are specimens for FLM testing delivered to and stored in the laboratory?
 - A. Delivered on ice and refrigerated
 - B. Immediately centrifuged
 - C. Kept at room temperature
 - D. Delivered in a vacuum tube
7. Why are amniotic specimens for cytogenetic analysis incubated at 37°C prior to analysis?
 - A. To detect the presence of meconium
 - B. To differentiate amniotic fluid from urine
 - C. To prevent photo-oxidation of bilirubin to biliverdin
 - D. To prolong fetal cell viability and integrity
8. Match the following colors in amniotic fluid with their significance.

___ A. Colorless	1. Fetal death
___ B. Dark green	2. Normal
___ C. Red-brown	3. Presence of bilirubin
___ D. Yellow	4. Presence of meconium
9. A significant rise in the OD of amniotic fluid at 450 nm indicates the presence of which analyte?
 - A. Bilirubin
 - B. Lecithin
 - C. Oxyhemoglobin
 - D. Sphingomyelin
10. Plotting the amniotic fluid OD on a Liley graph represents the severity of hemolytic disease of the newborn. A value that is plotted in zone II indicates what condition of the fetus?
 - A. No hemolysis
 - B. Mildly affected fetus
 - C. Moderately affected fetus that requires close monitoring
 - D. Severely affected fetus that requires intervention
11. The presence of a fetal neural tube disorder may be detected by:
 - A. Increased amniotic fluid bilirubin
 - B. Increased maternal serum alpha-fetoprotein
 - C. Decreased amniotic fluid phosphatidyl glycerol
 - D. Decreased maternal serum acetylcholinesterase
12. True or False: An AFP MoM value greater than two times the median value is considered an indication of a neural tube disorder.
13. When severe HDN is present, which of the following tests on the amniotic fluid would the physician not order to determine whether the fetal lungs are mature enough to withstand a premature delivery?
 - A. AFP levels
 - B. Foam stability index
 - C. Lecithin/sphingomyelin ratio
 - D. Phosphatidyl glycerol detection

14. True or False: Prior to 35 weeks' gestation, the normal L/S ratio is less than 1.6.
15. When performing an L/S ratio by thin-layer chromatography, a mature fetal lung will show:
 - A. Sphingomyelin twice as concentrated as lecithin
 - B. No sphingomyelin
 - C. Lecithin twice as concentrated as sphingomyelin
 - D. Equal concentrations of lecithin and sphingomyelin
16. True or False: Phosphatidyl glycerol is present with an L/S ratio of 1.1.
17. A rapid immunologic test for FLM that does not require performance of thin-layer chromatography is:
 - A. AFP levels
 - B. Amniotic acetylcholinesterase
 - C. Aminostat-FLM
 - D. Bilirubin scan
18. Does the failure to produce bubbles in the Foam Stability Index indicate increased or decreased lecithin?
 - A. Increased
 - B. Decreased
19. The presence of phosphatidyl glycerol in amniotic fluid fetal lung maturity tests must be confirmed when:
 - A. Hemolytic disease of the newborn is present
 - B. The mother has maternal diabetes
 - C. Amniotic fluid is contaminated by hemoglobin
 - D. Neural tube disorder is suspected
20. A lamellar body count of 50,000 correlates with:
 - A. Absent phosphatidyl glycerol and L/S ratio of 1.0
 - B. L/S ratio of 1.5 and absent phosphatidyl glycerol
 - C. OD at 650 nm of 1.010 and an L/S ratio of 1.1
 - D. OD at 650 nm of 0.150 and an L/S ratio of 2.0

Fecal Analysis

OUTLINE

- I. Physiology
- II. Diarrhea & Steatorrhea
- III. Specimen Collection
- IV. Macroscopic Screening
- V. Microscopic Examination
- VI. Chemical Testing

PHYSIOLOGY

A. Fecal Specimen

- bacteria, cellulose, undigested foodstuffs, GI secretions, bile pigments, cells from the intestinal walls, electrolytes, and water
- 100 to 200 g of feces is excreted in a 24-hour period
- 9000 mL of ingested fluid, saliva, gastric, liver, pancreatic, and intestinal secretions enter the digestive tract each day
- 500 to 1500 mL of this fluid reaches the large intestine
- 150 mL is excreted in the feces
- 3000 mL - amount of water that large intestine could absorb
- >3000 mL - excreted with the solid fecal material, producing diarrhea
- Constipation - provides time for additional water to be reabsorbed from the fecal material, producing small, hard stools
- flatus - intestinal gas
 - excessive gas production occurs in lactose-intolerant people when the intestinal bacteria metabolize the lactose from consumed milk or lactose-containing substances

DIARRHEA & STEATORRHEA

A. Diarrhea

Definition

- an increase in daily stool weight above 200 g
- increased liquidity of stool
- frequency of more than three times per day
 - acute - less than 4 weeks
 - chronic - more than 4 weeks

Classification

- illness duration
- mechanism
- severity
- stool characteristics

Laboratory Tests

- fecal electrolytes
- fecal sodium - 30 mmol/L
- fecal potassium - 75 mmol/L
- fecal osmolality - 290 mOsm/kg
- stool pH - <5.6 indicates a malabsorption of sugars → osmotic diarrhea

$$\text{Osmotic gap} = 290 - [2 (\text{fecal sodium} + \text{fecal potassium})]$$

Major Mechanisms

- a. Secretory Diarrhea
 - increased secretion of water
 - bacterial, viral, and protozoan infections → increased secretion of water and electrolytes → override the reabsorptive ability of the large intestine → secretory diarrhea

Causes

- ✓ Enterotoxin-producing organisms such as Escherichia coli, Clostridium, Vibrio cholerae, Salmonella, Shigella, Staphylococcus, Campylobacter, protozoa, and parasites such as Cryptosporidium
- ✓ drugs
- ✓ stimulant laxatives
- ✓ hormones
- ✓ inflammatory bowel disease (Crohn disease, ulcerative colitis, lymphocytic colitis, diverticulitis)
- ✓ endocrine disorders (hyperthyroidism, Zollinger-Ellison syndrome, VIPoma)
- ✓ neoplasms
- ✓ collagen vascular disease.

b. Osmotic Diarrhea

- caused by poor absorption that exerts osmotic pressure across the intestinal mucosa
- Incomplete breakdown or reabsorption of food → increased fecal material to the large intestine → in water and electrolyte retention in the large intestine (osmotic diarrhea) → excessive watery stool
- contributed by
 - maldigestion - impaired food digestion
 - malabsorption - impaired nutrient absorption by the intestine

Causes

- ✓ disaccharidase deficiency (lactose intolerance)
- ✓ malabsorption (celiac sprue)
- ✓ poorly absorbed sugars (lactose, sorbitol, mannitol)
- ✓ laxatives
- ✓ magnesium-containing
- ✓ antacids
- ✓ amebiasis
- ✓ antibiotic administration

Differential Features for Diarrhea

Laboratory Test	Osmotic Diarrhea	Secretory Diarrhea
Osmotic gap	>50 Osm/kg	<50 Osm/kg
Stool Na	<60 mmol/L	>90 mmol/L
Stool output in 24 hours	<200 g	>200 g
pH	<5.3	>5.6
Reducing substances	Positive	Negative

- c. Increased motility
 - excessive movement of intestinal contents through the GI tract that can cause diarrhea because normal absorption of intestinal contents and nutrients cannot occur
 - used by enteritis, the use of parasympathetic drugs, or with complications of malabsorption
 - Rapid gastric emptying (RGE) dumping syndrome - the hallmark of early dumping syndrome (EDS)
 - normal gastric emptying half-time range: 35 to 100 minutes
 - <35 minutes - considered RGE
 - EDS symptoms - begin 10 to 30 minutes following meal ingestion
 - Late dumping - occurs 2 to 3 hours after a meal

Others

- slow motility (constipation)
- both seen in irritable bowel syndrome (IBS)
 - functional disorder in which the nerves and muscles of the bowel are extra sensitive, causing cramping, bloating, flatus, diarrhea, and constipation

B. Steatorrhea

- fecal fat
- useful in diagnosing pancreatic insufficiency and small-bowel disorders that cause malabsorption
- Absence of bile salts
 - assist pancreatic lipase in the breakdown and subsequent reabsorption of dietary fat (primarily triglycerides)
 - produces an increase in stool fat
- exceeds 6 g per day
- decrease the production of pancreatic enzymes → steatorrhea (cystic fibrosis, chronic pancreatitis, and carcinoma)
- can be distinguished by the D-xylose test (low)
 - a sugar that does not need to be digested but does need to be absorbed to be present in the urine

SPECIMEN COLLECTION

- bedpan or disposable container
- must not be contaminated with urine or toilet water
- random specimens - suitable for qualitative testing for blood and microscopic examination for leukocytes
- timed specimens - quantitative testing, such as for fecal fats
- 3-day collection - most representative sample

MACROSCOPIC SCREENING

A. Color

- brown color - results from intestinal oxidation of stercobilinogen to urobilin
- pale or acholic color - blockage of the bile duct, diagnostic procedures that use barium sulfate
- bright red - blood from the lower GI tract requires less time to appear
- dark red - some foods, including beets
- black - blood that originates from the esophagus, stomach, or duodenum takes approximately 3 days to appear in the stool, degradation of hemoglobin
- green - taking oral antibiotics, because of the oxidation of fecal bilirubin to biliverdin, ingestion of increased amounts of green vegetables

B. Appearance

Macroscopic Stool Characteristics	
Color / Appearance	Possible Cause
Black	Upper GI bleeding Iron therapy Charcoal Bismuth (antacids)
Red	Lower GI bleeding Beets and food coloring Rifampin
Pale yellow, white, gray	Bile-duct obstruction Barium sulfate
Green	Biliverdin/oral antibiotics Green vegetables
Bulky/frothy	Bile-duct obstruction Pancreatic disorders
Ribbon-like	Intestinal constriction
Mucus- or blood-streaked mucus	Colitis Dysentery Malignancy Constipation

MICROSCOPIC EXAMINATION

A. Fecal Leukocytes

- preliminary test to determine whether diarrhea is being caused by invasive bacterial pathogens including *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, and enteroinvasive *E. coli*
- Bacteria that cause diarrhea by toxin production, such as *Staphylococcus aureus* and *Vibrio* spp., viruses, and parasites - usually do not cause the appearance of fecal leukocytes
- methylene blue or as dried smears stained with Wright's or Gram stain
- lactoferrin latex agglutination test - sensitive in refrigerated and frozen specimens
- three neutrophils per high-power field - indicative of an invasive condition

Procedure: Methylene Blue Stain for Fecal Leukocytes

- Place mucus or a drop of liquid stool on a slide.
- Add two drops of Löffler methylene blue.
- Mix with a wooden applicator stick.
- Allow to stand for 2 to 3 minutes.
- Examine for neutrophils under high power.

B. Muscle Fibers

- only undigested striated muscle fibers
- more than 10 is reported as increased
- seen in biliary obstruction and gastrocolic fistulas
- prepared by emulsifying a small amount of stool in 10% alcoholic eosin
- examined for exactly 5 minutes
- undigested fibers - visible striations running both vertically and horizontally
- partially digested fibers - striations in only one direction
- digested fibers - no visible striations
- include red meat in their diet before collecting the specimen

Procedure: Muscle Fibers

- Emulsify a small amount of stool in two drops of 10% eosin in alcohol.
- Apply cover slip and let stand 3 minutes.

- Examine under high power for 5 minutes.
- Count the number of undigested fibers.



Figure 14-2 Meat fibers present in fecal emulsion specimen using brightfield microscopy examination (x400).



Figure 14-3 Note striations on meat fiber present in a fecal emulsion specimen (x1000).

C. Qualitative Fecal Fats

- screened microscopically for the presence of excess fecal fat
- staining with the dyes Sudan III (most common), Sudan IV, or oil red O
- two parts: neutral fat stain and the split fat stain
- large orange-red droplets
- >60 droplets/high-power field - indicate steatorrhea

Procedure: Neutral Fat Stain

- Homogenize one part stool with two parts water.
- Mix emulsified stool with one drop of 95% ethyl alcohol on slide.
- Add two drops of saturated Sudan III in 95% ethanol.
- Mix and apply cover slip.
- Examine under high power.
- Count orange droplets per high-power field.

Procedure: Split Fat Stain

- Mix emulsified stool with one drop of 36% acetic acid.
- Add two drops of saturated Sudan III.
- Mix and apply cover slip.

- Heat gently almost to boiling.
- Examine under high power.
- Count and measure the orange droplets per high-power field.



Figure 14-4 Several orange-red neutral fat globules present in a fecal suspension stained with Sudan III (x400).

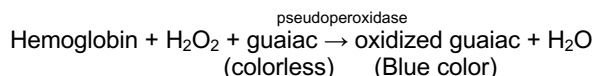
CHEMICAL TESTING

A. Occult Blood

- hidden blood
- fecal occult blood testing (FOBT)
- excess of 2.5 mL/150 g of stool - considered pathologically significant
- no visible signs of bleeding may be present with this amount of blood
- high positive predictive value for detecting colorectal cancer (age 50)

1. Guaiac-Based Fecal Occult Blood Tests (gFOBT)

- most frequently used screening test
- based on detecting the pseudoperoxidase activity of hemoglobin
- more sensitivity - varying the amount and purity of the guaiac reagent used in the test
- testing kits - guaiac-impregnated filter paper enclosed in a cardboard slide, to which the fecal specimen and hydrogen peroxide are added
- patients should be instructed to avoid eating red meats, horseradish, melons, raw broccoli, cauliflower, radishes, and turnips for 3 days before specimen collection



False-Positive

- Aspirin and anti-inflammatory medications
- Red meat
- Horseradish
- Raw broccoli, cauliflower, radishes, turnips
- Melons
- Menstrual and hemorrhoid contamination

False-Negative

- Vitamin C >250 mg/d
- Iron supplements containing vitamin C

- Failure to wait specified time after sample is applied to add the developer reagent

2. Immunochemical Fecal Occult Blood Test (iFOBT)

- specific for the globin portion of human hemoglobin and uses polyclonal anti-human hemoglobin antibodies
- specific for human blood in feces, it does not require dietary or drug restrictions
- more sensitive to lower GI bleeding that could be an indicator of colon cancer or other GI disease
- can be used for patients who are taking aspirin and other anti-inflammatory medications

3. Porphyrin-Based Fecal Occult Blood Test

- HemoQuant
- for hemoglobin based on the conversion of heme to fluorescent porphyrins
- measures both intact hemoglobin and the hemoglobin that has been converted to porphyrins
- As hemoglobin progresses through the intestinal tract, bacterial actions degrade it to porphyrin that the gFOBT cannot detect, thereby making the HemoQuant test more sensitive to upper GI bleeding

B. Quantitative Fecal Fat Testing

- used as a confirmatory test for steatorrhea
- collection of at least a 3-day specimen
- maintain a regulated intake of fat (100 g/d) before and during the collection period
- Van de Kamer titration - method routinely used for fecal fat measurement, gold standard
 - fecal lipids are converted to fatty acids and titrated to a neutral endpoint with sodium hydroxide
- Other: gravimetric, near-infrared reflectance spectroscopy, and nuclear magnetic resonance spectroscopy methods
- hydrogen nuclear magnetic resonance spectroscopy (HNMR) method - safe procedure for analyzing quantitative fecal fat

acid steatocrit

- rapid test to estimate the amount of fat excretion
- reliable tool to monitor a patient's response to therapy and screen for steatorrhea in pediatric populations

fat content

- reported as grams of fat or the coefficient of fat retention per 24 hours
- Reference values based on a 100 g/d intake are 1 to 6 g/d or a coefficient of fat retention of at least 95%.
- The coefficient of fat retention is calculated as follows:

$$\frac{(\text{dietary fat} - \text{fecal fat}) \times 100}{(\text{dietary fat})}$$

Near-infrared reflectance spectroscopy (NIRS)

- rapid procedure for fecal fat that requires less stool handling by laboratory personnel
- requires a 48- to 72-hour stool collection to exclude day-to-day variability
- does not require reagents after homogenization of the sample

- based on the measurement and computed processing of signal data from reflectance of fecal surface
- scanned with infrared light between 1400 nM and 2600 nM wavelength
- quantitates water, fat, and nitrogen in grams per 24 hours

C. APT Test (Fetal Hemoglobin)

- distinguish between the presence of fetal blood or maternal blood in an infant's stool or vomitus
- emulsified in water to release hemoglobin (Hb) and, after centrifugation, 1% sodium hydroxide is added to the pink hemoglobin-containing supernatant

Procedure: APT Test

1. Emulsify specimen in water.
2. Centrifuge.
3. Divide pink supernatant into two tubes.
4. Add 1% sodium hydroxide to one tube.
5. Wait 2 minutes.
6. Compare color with that in the control tube.
7. Prepare controls using cord blood and adult blood.

D. Fecal Enzymes

- focuses primarily on the proteolytic enzymes trypsin, chymotrypsin, and elastase I

Fecal chymotrypsin

- more resistant to intestinal degradation and is a more sensitive indicator of less severe cases of pancreatic insufficiency
- capable of gelatin hydrolysis but is most frequently measured by spectrophotometric methods

Elastase I

- present in high concentrations in pancreatic secretions
- strongly resistant to degradation
- pancreas specific
- concentration is about five times higher than in pancreatic juice
- requires only a single stool sample
- specific in differentiating pancreatic from nonpancreatic causes in patients with steatorrhea

E. Carbohydrates

- increased carbohydrates → produces osmotic diarrhea from the osmotic pressure of the unabsorbed sugar in the intestine drawing in fluid and electrolytes
- increased use of carbohydrates by intestinal bacterial fermentation → increases the lactic acid level and lowers the pH to below 5.5 in cases of carbohydrate disorders
- copper reduction test (Clintest tablet)
 - sucrose - not detected by the Clintest method because it is not a reducing sugar
- 0.5 g/dL - is considered indicative of carbohydrate intolerance

Fecal Screening Tests		
Tests	Methodology / Principle	Interpretation
Examination for neutrophils	Microscopic count of neutrophils in smear stained with methylene blue, Gram stain, or Wright's stain	Three per high-power field indicates condition affecting intestinal wall
Qualitative fecal fats	Microscopic examination of direct smear stained with Sudan III	60 large orange-red droplets indicates Malabsorption
	Microscopic examination of smear heated with acetic acid and Sudan III	100 orange-red droplets measuring 6 to 75 μ m indicates malabsorption
gFOBT	Pseudoperoxidase activity of hemoglobin liberates oxygen from hydrogen peroxide to oxidize guaiac reagent	Blue color indicates gastrointestinal bleeding
iFOBT	Uses polyclonal anti-human antibodies specific for the globin portion of human hemoglobin	Positive test and control lines indicate GI bleeding
APT test	Addition of sodium hydroxide to hemoglobin-containing emulsion determines presence of maternal or fetal blood	Pink color indicates presence of fetal blood
Trypsin	Emulsified specimen placed on x-ray paper determines ability to digest gelatin	Inability to digest gelatin indicates lack of trypsin
Elastase 1	Immunoassay using an ELISA test	Sensitive indicator of exocrine pancreatic insufficiency
Clinitest	Addition of Clinitest tablet to emulsified stool detects presence of reducing substances	Reaction of 0.5 g/dL reducing substances suggests carbohydrate intolerance

Review Questions

- In what part of the digestive tract do pancreatic enzymes and bile salts contribute to digestion?
 - Large intestine
 - Liver
 - Small intestine
 - Stomach
- Where does the reabsorption of water take place in the primary digestive process?
 - Large intestine
 - Pancreas
 - Small intestine
 - Stomach
- Which of the following tests is not performed to detect osmotic diarrhea?
 - Clinitest
 - Fecal fats
 - Fecal neutrophils
 - Muscle fibers
- The normal composition of feces includes all of the following except:
 - Bacteria
 - Blood
 - Electrolytes
 - Water
- What is the fecal test that requires a 3-day specimen?
 - Fecal occult blood
 - APT test
 - Elastase I
 - Quantitative fecal fat testing
- The normal brown color of the feces is produced by:
 - Cellulose
 - Pancreatic enzymes
 - Undigested foodstuffs
 - Urobilin
- Diarrhea can result from all of the following except:
 - Addition of pathogenic organisms to the normal intestinal flora
 - Disruption of the normal intestinal bacterial flora
 - Increased concentration of fecal electrolytes
 - Increased reabsorption of intestinal water and electrolytes
- Stools from persons with steatorrhea will contain excess amounts of:
 - Barium sulfate
 - Blood
 - Fat
 - Mucus
- Which of the following pairings of stool appearance and cause does not match?
 - Black, tarry: blood
 - Pale, frothy: steatorrhea
 - Yellow-gray: bile duct obstruction
 - Yellow-green: barium sulfate
- Stool specimens that appear ribbon-like are indicative of which condition?
 - Bile-duct obstruction
 - Colitis
 - Intestinal constriction
 - Malignancy
- A black tarry stool is indicative of:
 - Upper GI bleeding
 - Lower GI bleeding
 - Excess fat
 - Excess carbohydrates

13. Chemical screening tests performed on feces include all of the following except:
 - A. APT test
 - B. Clinitest
 - C. Pilocarpine iontophoresis
 - D. Quantitative fecal fats
14. Secretory diarrhea is caused by:
 - A. Antibiotic administration
 - B. Lactose intolerance
 - C. Celiac sprue
 - D. *Vibrio cholerae*
15. The fecal osmotic gap is elevated in which disorder?
 - A. Dumping syndrome
 - B. Osmotic diarrhea
 - C. Secretory diarrhea
 - D. Steatorrhea
16. Microscopic examination of stools provides preliminary information as to the cause of diarrhea because:
 - A. Neutrophils are present in conditions caused by toxin-producing bacteria
 - B. Neutrophils are present in conditions that affect the intestinal wall
 - C. Red and white blood cells are present if the cause is bacterial
 - D. Neutrophils are present if the condition is of nonbacterial etiology
17. True or False: The presence of fecal neutrophils would be expected with diarrhea caused by a rotavirus.
18. Large orange-red droplets seen on direct microscopic examination of stools mixed with Sudan III represent:
 - A. Cholesterol
 - B. Fatty acids
 - C. Neutral fats
 - D. Soaps
19. Microscopic examination of stools mixed with Sudan III and glacial acetic acid and then heated will show small orange-red droplets that represent:
 - A. Fatty acids and soaps
 - B. Fatty acids and neutral fats
 - C. Fatty acids, soaps, and neutral fats
 - D. Soaps
20. When performing a microscopic stool examination for muscle fibers, the structures that should be counted:
 - A. Are coiled and stain blue
 - B. Contain no visible striations
 - C. Have two-dimensional striations
 - D. Have vertical striations and stain red
21. A value of 85% fat retention would indicate:
 - A. Dumping syndrome
 - B. Osmotic diarrhea
 - C. Secretory diarrhea
 - D. Steatorrhea
22. Which of the following tests would not be indicative of steatorrhea?
 - A. Fecal elastase-I
 - B. Fecal occult blood
 - C. Sudan III
 - D. Van de Kamer
23. The term "occult" blood describes blood that:
 - A. Is produced in the lower GI tract
 - B. Is produced in the upper GI tract
 - C. Is not visibly apparent in the stool specimen
 - D. Produces a black, tarry stool
24. What is the recommended number of samples that should be tested to confirm a negative occult blood result?
 - A. One random specimen
 - B. Two samples taken from different parts of three stools
 - C. Three samples taken from the outermost portion of the stool
 - D. Three samples taken from different parts of two stools
25. The immunochemical tests for occult blood:
 - A. Test for human globulin
 - B. Give false-positive reactions with meat hemoglobin
 - C. Can give false-positive reactions with aspirin
 - D. Are inhibited by porphyrin
26. Guaiac tests for detecting occult blood rely on the:
 - A. Reaction of hemoglobin with hydrogen peroxide
 - B. Pseudoperoxidase activity of hemoglobin
 - C. Reaction of hemoglobin with ortho-toluidine
 - D. Pseudoperoxidase activity of hydrogen peroxide
27. What is the significance of an APT test that remains pink after addition of sodium hydroxide?
 - A. Fecal fat is present.
 - B. Fetal hemoglobin is present.
 - C. Fecal trypsin is present.
 - D. Vitamin C is present.
28. In the Van de Kamer method for quantitative fecal fat determinations, fecal lipids are:
 - A. Converted to fatty acids prior to titrating with sodium hydroxide
 - B. Homogenized and titrated to a neutral endpoint with sodium hydroxide
 - C. Measured gravimetrically after washing
 - D. Measured by spectrophotometer after addition of Sudan III
29. A patient whose stool exhibits increased fats, undigested muscle fibers, and the inability to digest gelatin may have:
 - A. Bacterial dysentery
 - B. A duodenal ulcer
 - C. Cystic fibrosis
 - D. Lactose intolerance
30. A stool specimen collected from an infant with diarrhea has a pH of 5.0. This result correlates with a:
 - A. Positive APT test
 - B. Negative trypsin test
 - C. Positive Clinitest
 - D. Negative occult blood test
31. Which of the following tests differentiates a malabsorption cause from a maldigestion cause in steatorrhea?
 - A. APT test
 - B. D-xylose test
 - C. Lactose tolerance test
 - D. Occult blood test

Vaginal Secretions

OUTLINE

- I. Introduction
- II. Specimen Collection & Handling
- III. Diagnostic Tests
- IV. Vaginal Disorders
- V. Additional Vaginal Secretion Procedures

INTRODUCTION

A. Vaginal Specimen

- diagnose infections and complications of pregnancy, and for forensic testing
- Vaginitis
 - one of the most common conditions diagnosed by health-care providers for female patients, particularly women of childbearing age
 - characterized by abnormal vaginal discharge or odor, pruritus, vaginal irritation, dysuria, and dyspareunia
 - secondary to bacterial vaginosis (BV), vulvovaginal candidiasis, or trichomoniasis
- detect the placental α 1-microglobulin (PAMG-1) protein
 - to diagnose ruptured fetal membranes, or fetal fibronectin enzyme to assess the risk of preterm delivery

Microscopic methods

- saline wet mount examination
- potassium hydroxide (KOH) examination
- Gram stain - gold standard

Other tests

- litmus pH levels, DNA probe testing, culture, and point of care test kits

Clinical Features and Laboratory Findings in Vaginitis					
Findings	Bacterial Vaginitis	Candidiasis	Trichomoniasis	Desquamative Inflammatory Vaginitis	Atrophic Vaginitis
Appearance	Thin, homogeneous, white-to-gray vaginal discharge	White, curd-like vaginal discharge	Yellow-green frothy adherent vaginal discharge increased in volume	Excessive purulent vaginal discharge, vaginal erythema	Excessive purulent vaginal discharge, vaginal erythema
pH	>4.5	3.8 to 4.5	>4.5	>4.5	>4.5
WBCs	Rare or absent	3+ to 4+	2+ to 4+	3+ to 4+	3+ to 4+
Lactobacilli	Rare or absent	Present	Absent or present	Absent or reduced	Decreased

Clue cells	>20%	Absent	Absent or present		
Other cells		Large clumps of epithelial cells		Occasional parabasal or basal cells >1+ RBCs	Occasional parabasal or basal cells >1+ RBCs
Other organisms	Increase in small curved bacilli, coccobacilli, and pleomorphic bacilli	Budding yeast cells and pseudohyphae	Trichomonas frequently associated with other organisms	2+ gram-positive cocci	Increased gram-positive cocci and gram-negative rods; decreased large rods
Amine (Whiff) test	Positive	Negative	Positive	Negative	Negative
Other tests	Confirmatory tests: DNA probe, proline amino peptidase, OSOM BVBLU E Rapid Test	Confirmatory tests: DNA probe, OSOM BVBLU E Rapid Test	Confirmatory tests: DNA probe or culture, OSOM Trichomonas Rapid Test		

SPECIMEN COLLECTION & HANDLING

A. Collection

- collects vaginal secretions during a pelvic examination
- speculum moistened with warm water - used to visualize the vaginal fornices
- lubricants - can't be used
- collected by swabbing the vaginal walls and vaginal pool to collect epithelial cells along with the vaginal secretions using one or more sterile, polyester-tipped swabs on a plastic shaft or swabs specifically designated by the manufacturer
 - cotton swabs - should not be used
 - cotton - toxic to *Neisseria gonorrhoeae*,
 - wood - toxic to *Chlamydia trachomatis*
 - calcium alginate - can inactivate herpes simplex virus (HSV) for viral cultures

B. Handling

- timely transport to the laboratory is important for optimal detection of the responsible pathogen
- tube: 0.5 to 1.0 mL of sterile physiologic saline
- should be tested with pH paper before being placed in saline
- second sample - placed in 10% KOH solution in the same manner
- Trichomonas vaginalis* and *N. gonorrhoeae* - kept at room temperature to preserve the motility of,
- C. trachomatis* and herpes simplex virus - must be refrigerated to prevent overgrowth of normal flora.
- T. vaginalis* - should be examined within 2 hours of collection

COLOR & APPEARANCE

Normal Findings in Vaginal Secretions	
Appearance	White, flocculent discharge
pH	3.8 to 4.2
Amine (Whiff) test	Negative
WBCs	2+
Lactobacilli	Predominant
Clue cells	Absent
Other cells	Absent (except RBCs during menses)
Other organisms	Other lactobacilli subgroups, occasional yeast

- increased thin, homogeneous white-to-gray discharge - often seen in bacterial vaginosis
- white "cottage cheese"-like discharge - *Candida* infections
- increased yellow-green, frothy, adherent discharge - associated with *T. vaginalis*
- yellow, opaque cervical discharge - *C. trachomatis*

DIAGNOSTIC TESTS

A. pH

- test should be performed before placing the swab into saline or KOH solutions
- placed in the pooled vaginal secretion and the color change is compared to a chart with corresponding pH values
- about 4.5 - vulvovaginal candidiasis
- >4.5 - bacterial vaginosis, trichomoniasis, desquamative inflammatory vaginitis (DIV), and atrophic vaginitis
- predominance of the bacteria lactobacilli - produce the end product lactic acid from glycogen metabolism
 - provides an acidic vaginal environment with a pH value between 3.8 and 4.5.
 - This acidity suppresses the overgrowth of infectious organisms such as *Mobiluncus*, *Prevotella*, and *Gardnerella vaginalis* → maintains the balance of normal vaginal bacteria flora
 - Some subgroups - produce hydrogen peroxide, which is toxic to pathogens, and helps keep the vaginal pH acidic to provide protection from urogenital infections
 - Bacterial vaginosis - associated with the absence of hydrogen peroxide-producing lactobacilli
- Estrogen production - preserve an acidic vaginal environment

Procedure: pH test

- Using a circular motion, gently apply the vaginal secretion over the surface of the pH test paper.
- Immediately observe the color reaction on the paper and compare the color to a color comparison chart to determine the pH of the sample.
- Record the results.

B. Microscopic Procedures

- Saline wet mounts and KOH mounts - initial screening tests
- Gram stain - used as a confirmatory examination for yeast or bacterial vaginosis, three clean glass slides

Wet Mount Examination

- low power (10×) and high dry power (40×) objective with a bright-field microscope
- scanned for an even distribution of cellular components, types and numbers of epithelial cells, clumping of epithelial cells, and the presence of budding yeast or pseudohyphae

Quantitation Scheme for Microscopic Examinations

Rare	Less than 10 organisms or cells/slide
1+	Less than 1 organism or cell/hpf
2+	1 to 5 organisms or cells/hpf
3+	6 to 30 organisms or cells/hpf
4+	>30 organisms or cells/hpf

1. Squamous Epithelial Cells

- measure 25 to 70 µm in diameter
- exhibit a polygonal "flagstone" appearance
- prominent centrally located nucleus about the size of a red blood cell and a large amount of irregular cytoplasm, lacking granularity, with distinct cell margins

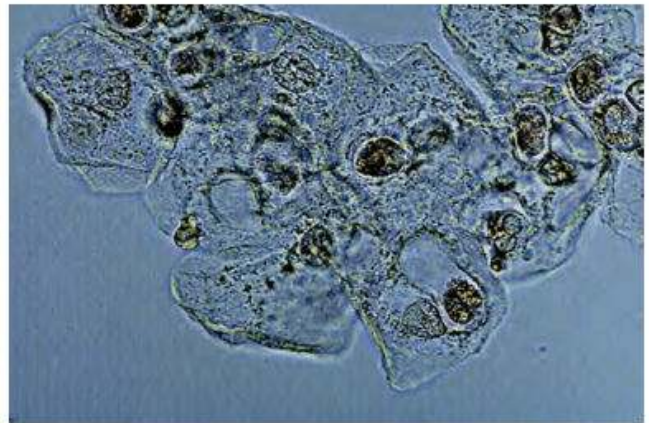


Figure 15-2 Clump of squamous epithelial cells (x400).

2. Clue Cells

- abnormal variation of the squamous epithelial cell
- are distinguished by coccobacillus bacteria attached in clusters on the cell surface
- granular, irregular appearance sometimes described as "shaggy"
- diagnostic of bacterial vaginosis caused by *G. vaginalis*
- also found in found in urine sediment

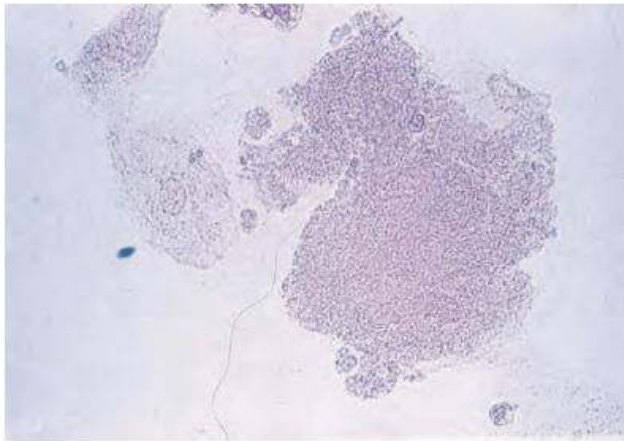


Figure 15-3 Clue cells (x400).

3. White Blood Cells

- 14 to 16 μm in diameter
- granular cytoplasm
- characteristic multi-lobed nucleus
- >3+ WBCs in vaginal secretions - suggest vaginal candidiasis, atrophic vaginitis, or infections with *Trichomonas*, *Chlamydia*, *N. gonorrhoeae*, or Herpes simplex



Figure 15-4 White blood cells. Notice the multi-lobed nucleoli (x400).

4. Red Blood Cells

- smooth, non-nucleated biconcave disks
- approximately 7 to 8 μm in diameter
- usually seen in vaginal secretions
- might be present during menstruation or due to a desquamative inflammatory process
- confused with yeast cells and are distinguished from yeast cells by KOH
 - will lyse the RBCs but allow the yeast cells to remain intact

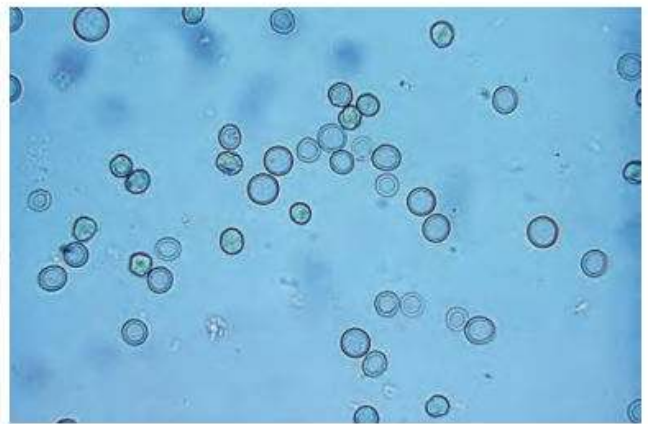


Figure 15-5 Normal red blood cells (x400).

5. Parabasal Cells

- round to oval shaped
- 16 to 40 μm in diameter
- nucleus to cytoplasm ratio is 1:1 to 1:2
- marked basophilic granulation or amorphous basophilic structures ("blue blobs") in the surrounding cytoplasm
- located in the luminal squamous epithelium of the vaginal mucosa
- rare to find parabasal cells in vaginal secretions but less mature cells may be found if the patient is menstruating and in postmenopausal women
- increased + increased WBCs - desquamative inflammatory vaginitis



Figure 15-6 Parabasal cell surrounded by epithelial cells (x400).

6. Basal Cells

- located deep in the basal layer of the vaginal stratified epithelium
- 10 to 16 μm in diameter
- have a nucleus to cytoplasm ratio of 1:2
- distinguished from WBCs that are similar in size by their round rather than lobed nucleus
- found normally
- present + large numbers of WBCs + altered vaginal flora c- an suggest desquamative inflammatory vaginitis

7. Bacteria

a. *Lactobacillus* spp.

- normally comprise the largest portion of vaginal bacteria
- large gram-positive, nonmotile rods on Gram stain and produce lactic acid
- maintains the vaginal pH at 3.8 to 4.5
- Hydrogen peroxide - produced by lactobacilli subgroups, can also help to suppress the overgrowth of other organisms

b. *Trichomonas vaginalis*

- asexual flagellated protozoan
- can cause vaginal inflammation and infection in women
- oval shaped
- measures 5 to 18 μm in diameter
- has four anterior flagella
- has an undulating membrane that extends half the length of the body

8. Yeast Cells

- appear as both budding yeast cells (blastophores) or as hyphae
- differentiation can be made using the KOH test
- stain gram positive

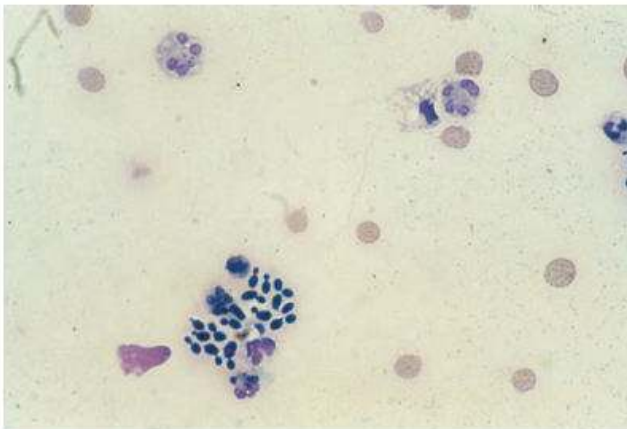


Figure 15-10 Budding yeast cells (x400).



Figure 15-11 Yeast cells showing mycelial forms (x400).

Procedure: Saline Wet Mount

1. Prepare a clean glass slide labeled with the patient's name and unique identifier.
2. Place one drop of vaginal specimen on the slide.
3. Cover the slide with a cover slip, removing any air bubbles.

4. Examine the slide with the 10 $\times$ objective for epithelial cells and any budding yeast cells or pseudohyphae.
5. Examine the slide with the 40 $\times$ objective and quantify organisms and cells per hpv.
6. Record the results.

KOH Preparation and Amine Test

- performed by placing a drop of the saline specimen prepared from the collection swab onto a properly labeled clean slide and adding one drop of 10% KOH solution
- checked for a "fishy" amine odor
- positive: presence of fishy odor
 - suggests bacterial vaginosis caused by increased numbers of *G. vaginalis* in conjunction with *Mobiluncus* spp. and with *T. vaginalis*.
- negative: absence of fishy odor

Procedure: KOH Preparation

1. Prepare a clean glass slide labeled with the patient's name and unique identifier.
2. Place one drop of specimen on the slide.
3. Add one drop of 10% KOH to the slide.
4. Allow the KOH slide preparation to rest for up to 5 minutes to allow cellular tissue and other debris to dissolve. Gentle heating may speed the dissolving process.
5. Cover the specimen with a cover slip, removing any air bubbles.
6. Examine the slide under the 10 $\times$ objective for overall assessment and for yeast pseudohyphae.
7. Switch to the 40 $\times$ objective to examine for budding yeast cells (smaller blastospores).
8. Record the results.

Procedure: Amine (Whiff) Test

1. Apply one drop of the saline vaginal fluid suspension to the surface of a clean glass slide.
2. Add one drop of 10% KOH directly to the vaginal sample.
3. Holding the slide in one hand, gently fan above the surface of the slide with the other hand and assess for the presence of a fishy amine odor.
4. Report as positive or negative.
 - Positive: The presence of a fishy odor after adding KOH.
 - Negative: The absence of a fishy odor after adding KOH.

Other Diagnostic Tests

1. Gram Stain
 - gold standard in identifying the causative organisms for bacterial vaginosis
 - *Lactobacillus acidophilus* (large gram-positive rods)
 - *G. vaginalis* and *Bacteroides* spp. (small gram-variable or gram-negative rods)
 - *Mobiluncus* spp. (curved gram-variable rods)

Nugent's Gram Stain Criteria to Diagnose Bacterial Vaginosis			
Lactobacillus	Gardnerella and Bacteroides spp. Morphophytes	Curved Gram-Variable Rods	Points
4+	0	0	0
3+	1+	1+ or 2+	1
2+	2+	3+ or 4+	2
1+	3+		3
0	4+		4

Note: Points are added according to the morphotypes seen. Add the points for all three columns for a final sum. A score of 7 or higher indicates bacterial vaginosis

2. Culture

- gold standard test for detecting yeast and *Trichomonas*
- more time consuming and requires up to 2 days for a result
- Culture for *G. vaginalis* is not diagnostic for bacterial vaginosis because it is part of the normal flora in 50% of healthy women
- Diamond's medium (special medium) - required for *T. vaginalis*

3. DNA Testing

- easy to perform and results are available in 1 hour with a sensitivity of 95%
- most accurate diagnostic method and it has the advantage of detecting nonviable organisms

4. Point of Care Tests

- provide a higher sensitivity and specificity for the organism sought
- proline aminopeptidase activity in vaginal secretions - can be detected by rapid antigen tests to identify *G. vaginalis*

a. OSOM Trichomonas Rapid Test

- an immunochromatographic strip test that detects *T. vaginalis* antigen from vaginal swabs in 10 minutes visible blue line and a red internal control line indicate a positive result

b. OSOM BVBLUE test

- detects vaginal fluid sialidase
- enzyme produced by the bacterial pathogens associated with bacterial vaginosis, such as *Gardnerella*, *Bacteroides*, *Prevotella*, and *Mobiluncus*
- 1 minute to perform
 - positive: blue or green
 - negative: yellow

VAGINAL DISORDERS

A. Bacterial Vaginosis (BV)

- most common cause of vaginitis
- occurs when there is an imbalance in the ratio of normal vaginal bacterial flora
- associated with new or multiple sex partners, frequent douching, use of intrauterine devices, pregnancy, and a lack of the protective lactobacilli
- increased risk for acquiring some sexually transmitted infections (STIs)

Amsel's Diagnostic Criteria

- three of the following four features must be present for the diagnosis of BV:
 - (1) thin, white, homogeneous discharge
 - (2) vaginal fluid pH greater than 4.5
 - (3) a positive amine (Whiff) test
 - (4) presence of clue cells on microscopic examination

B. Trichomoniasis

- transmitted by sexual intercourse
- causes vaginitis in women and sometimes urethritis in men
- characterized by a green-to-yellow frothy vaginal discharge, malodor, pruritus, irritation, dysuria, dyspareunia, and vaginal mucosa erythema
- "strawberry cervix"

C. Candidiasis

- part of the normal vaginal flora
- infection occurs when there is a change in the vaginal environment that permits the overgrowth of *Candida* and symptoms of the infection to occur
- Estrogen - causes the vagina to mature and produce glycogen, which facilitates the growth and adherence of *C. albican*
- presence of an abnormal thick, white, curd-like vaginal discharge

D. Desquamative Inflammatory Vaginitis (DIV)

- syndrome characterized by profuse purulent vaginal discharge, vaginal erythema, and dyspareunia
- β -hemolytic gram-positive streptococci - cultured from most patients
- occur secondary to atrophic vaginitis in postmenopausal women as a result of decreased estrogen
- pH is greater than 4.5
- amine test is negative
- Treatment
 - 2% clindamycin
 - hormone replacement therapy - effective for patients with DIV secondary to atrophic vaginitis

E. Atrophic Vaginitis

- syndrome found in postmenopausal women
- caused by thinning of the vaginal mucosa because of reduced estrogen production and decreased glycogen production
- Symptoms: vaginal dryness and soreness, dyspareunia, inflamed vaginal mucosa, and purulent discharge
- pH is greater than 4.5
- amine test is negative
- Treatment
 - estrogen replacement
 - topical vaginal ointments
 - oral or transcutaneous (patch) modes - more effective

ADDITIONAL VAGINAL SECRETION PROCEDURES

A. Fetal Fibronectin (fFN) Test

- adhesive glycoprotein in the extracellular matrix at the maternal and fetal interface within the uterus
- presence between 24 and 34 weeks' gestation - associated with preterm delivery
- Collection: swab provided in the specimen collection kit across the posterior fornix of the vagina for 10 seconds to absorb the vaginal secretions
- Methods: solid-phase enzyme-linked immunosorbent assay (ELISA) or lateral flow, solid-phase immunochromatographic assay using the Rapid fFN cassette
- monoclonal antibody FDC-6 - specific for fFN

B. AmniSure Test

- PAMG-1 - reliable marker of fetal membrane rupture
- Normal: 0.05 to 0.22 mg/mL
- Abnormal: increase to 3 mg/mL
- qualitative rapid test that uses an immunochromatographic device
- positive: presence of two lines
- negative: only the control line

Review Questions

1. Which of the following would not be a reason to collect a vaginal fluid for analysis?
 - A. Vaginitis
 - B. Complications of pregnancy resulting in preterm delivery
 - C. Forensic testing in a sexual assault
 - D. Pregnancy testing
2. Which of the following organisms might not be detected if the specimen for vaginal secretion analysis had been refrigerated?
 - A. *Prevotella bivia*
 - B. *Lactobacillus acidophilus*
 - C. *Trichomonas vaginalis*
 - D. *Candida albicans*
3. The appearance of the vaginal discharge in vulvovaginal candidiasis is described as:
 - A. Clear and colorless
 - B. Thin, homogeneous white-to-gray discharge
 - C. White, curd-like
 - D. Yellow-green and frothy
4. A normal range for a vaginal pH is:
 - A. 3.8 to 4.5
 - B. 5.0 to 6.0
 - C. 6.0 to 7.0
 - D. 7.0 to 7.4
5. Which of the following tests differentiates budding yeast cells from RBCs?
 - A. pH
 - B. Saline wet mount
 - C. KOH prep
 - D. Whiff test
6. Which of the following constituents is normal in healthy vaginal fluid secretions?
 - A. Lactobacilli
 - B. Basal cells
 - C. *Trichomonas vaginalis*
 - D. Pseudohyphae
7. Vaginal specimens collected for a saline wet prep should be:
 - A. Refrigerated to preserve motility
 - B. Prepared as soon as possible
 - C. Mailed to a reference laboratory
 - D. Preserved with potassium hydroxide
8. A positive amine (Whiff) test is observed in which of the following syndromes?
 - A. Bacterial vaginosis
 - B. Vulvovaginal candidiasis
 - C. Atrophic vaginitis
 - D. Desquamative inflammatory vaginitis
9. A squamous epithelial cell covered with coccobacilli that extends beyond the cytoplasm margin is a:
 - A. Basal cell
 - B. Parabasal cell
 - C. Clue cell
 - D. Blastospore
10. All of the following are diagnostic of bacterial vaginosis except:
 - A. Vaginal pH of 3.8
 - B. Presence of clue cells
 - C. Positive amine (Whiff) test
 - D. Thin, homogeneous white-to-gray vaginal discharge
11. Which of the following organisms produces lactic acid and hydrogen peroxide to maintain an acid vaginal environment?
 - A. *Gardnerella vaginalis*
 - B. *Mobiluncus* spp.
 - C. *Lactobacilli* spp.
 - D. β -Hemolytic streptococci
12. All of the following are diagnostic of vulvovaginal candidiasis except:
 - A. Large numbers of WBCs
 - B. Presence of clue cells
 - C. Positive KOH test
 - D. Vaginal pH of 4.0
13. All of the following are diagnostic of trichomoniasis except:
 - A. Vaginal pH of 6.0
 - B. Positive amine test
 - C. Positive KOH test
 - D. Motile trichomonads present
14. The bacteria associated with desquamative inflammatory vaginitis is:
 - A. β -Hemolytic streptococci
 - B. *Trichomonas vaginalis*
 - C. *Gardnerella vaginalis*
 - D. *Mycoplasma hominis*
15. The protein present in vaginal secretions that can identify patients who are at risk for preterm delivery is:
 - A. Human chorionic gonadotropin
 - B. Estrogen
 - C. PAMG-1
 - D. Fetal fibronectin