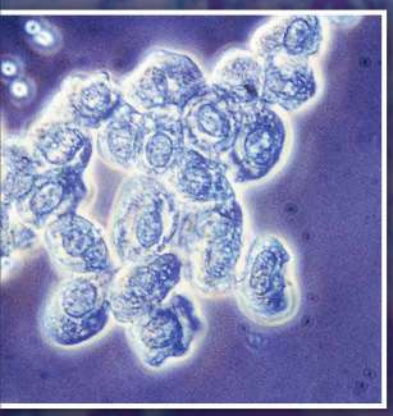
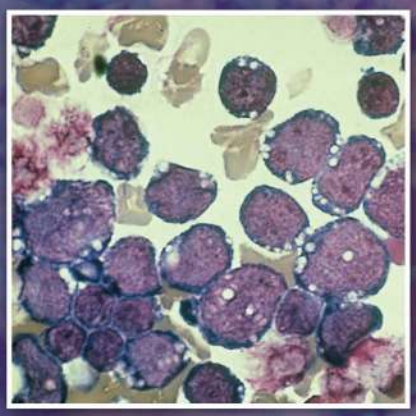
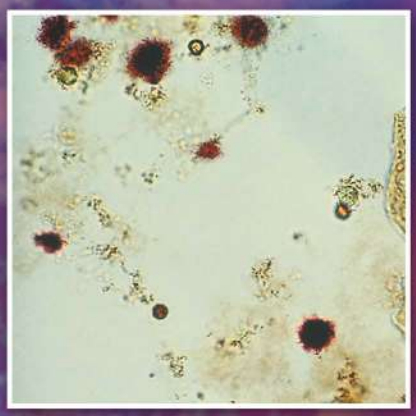
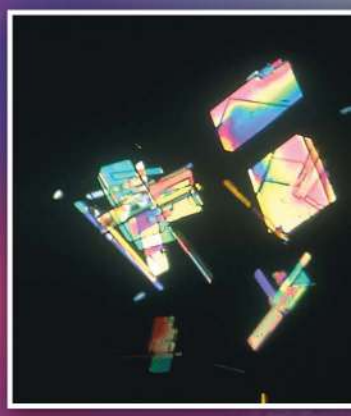
Susan King Strasinger
Marjorie Schaub Di Lorenzo

Urinalysis and Body Fluids

SEVENTH EDITION

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Contents

Atlas of Images 1

PART ONE: **Basic Principles** 48

CHAPTER 1

Safety and Quality Management 49
- Safety 50
- Biological Hazards 50
  - Infectious Agent (Pathogen) 50
  - Reservoir 50
  - Portal of Exit 51
  - Means of Transmission 51
  - Portal of Entry 51
  - Susceptible Host 51
  - Standard Precautions 51
- Sharp Hazards 56
- Chemical Hazards 56
  - Chemical Spills and Exposure 56
  - Chemical Handling 57
  - Chemical Hygiene Plan 57
  - Chemical Labeling 57
  - Safety Data Sheets 57
  - The Globally Harmonized System of Classification and Labeling of Chemicals 58
  - Chemical Waste Disposal 58
- Radioactive Hazards 58
- Electrical Hazards 59
- Fire/Explosive Hazards 60
- Physical Hazards 61
- Quality Management 61
- Urinalysis Procedure Manual 62
  - Preexamination (Preanalytical) Variables 62
  - Examination (Analytical) Variables 64
  - Postexamination Variables 68

CHAPTER 2

Urine and Body Fluid Analysis Automation 75
- Urinalysis Automation 76
  - Reflectance Photometry 76
  - Analyzers 76
  - Fully Automated Urine Chemistry Analyzers 78
  - Automated Microscopy Analyzers 79
  - Automated Urinalysis Systems 86
  - Body Fluid Analysis Automation 87

CHAPTER 3

Introduction to Urinalysis 91
- History and Importance 92
- Urine Formation 93
- Urine Composition 93
- Urine Volume 93
- Specimen Collection 94
  - Containers 94
  - Labels 95
  - Requisition Form 95
- Specimen Rejection 95
- Specimen Handling 95
  - Specimen Integrity 95
  - Specimen Preservation 96
- Types of Specimens 96
  - Random Specimen 96
  - First Morning Specimen 96
  - 24-Hour (or Timed) Specimen 97
  - Catheterized Specimen 98
  - Midstream Clean-Catch Specimen 98
  - Suprapubic Aspiration 98
  - Prostatitis Specimen 98
  - Pediatric Specimens 100
  - Drug Specimen Collection 100

CHAPTER 4

Renal Function 105
- Introduction 106
- Renal Physiology 106
  - Renal Blood Flow 106
  - Glomerular Filtration 106
  - Tubular Reabsorption 110
  - Tubular Secretion 111
- Renal Function Tests 113
  - Glomerular Filtration Tests 113
  - Tubular Reabsorption Tests 116
  - Tubular Secretion and Renal Blood Flow Tests 119

PART TWO: **Urinalysis** 124

CHAPTER 5

Physical Examination of Urine 125
- Introduction 126
- Color 126
  - Normal Urine Color 126
  - Abnormal Urine Color 127
- Clarity 129
  - Normal Clarity 129
  - Nonpathological Turbidity 129
  - Pathological Turbidity 130
- Specific Gravity 131
  - Refractometer 131
  - Osmolality 132
  - Reagent Strip Specific Gravity 133
- Odor 134

CHAPTER 6

Chemical Examination of Urine 139
- Introduction 140
- Reagent Strips 140
  - Reagent Strip Technique 140
  - Handling and Storing Reagent Strips 141
  - Quality Control of Reagent Strips 142
  - Confirmatory Testing 142
- pH 142
  - Clinical Significance 143
  - Reagent Strip Reactions 143
- Protein 143
  - Clinical Significance 144
  - Prerenal Proteinuria 144
  - Renal Proteinuria 144
  - Postrenal Proteinuria 145
  - Reagent Strip Reactions 146
  - Reaction Interference 146
- Glucose 148
  - Clinical Significance 148
  - Reagent Strip (Glucose Oxidase) Reaction 149
  - Reaction Interference 150
  - Copper Reduction Test (Clinitest) 150
  - Clinical Significance of Clinitest 151
- Ketones 151
  - Clinical Significance 151
  - Reagent Strip Reactions 152
  - Reaction Interference 152
- Blood 152
  - Clinical Significance 153
  - Reagent Strip Reactions 153
  - Reaction Interference 154
- Bilirubin 154
  - Bilirubin Production 154
  - Clinical Significance 155
  - Reagent Strip (Diazo) Reactions 155
  - Reaction Interference 156
- Urobilinogen 156
  - Clinical Significance 157
  - Reagent Strip Reactions and Interference 157
  - Reaction Interference 157
- Nitrite 157
  - Clinical Significance 157
  - Reagent Strip Reactions 158
  - Reaction Interference 158
- Leukocyte Esterase 159
  - Clinical Significance 159
  - Reagent Strip Reaction 160
  - Reaction Interference 160
- Specific Gravity 160
  - Reagent Strip Reaction 160
  - Reaction Interference 160

CHAPTER 7

Microscopic Examination of Urine 167
- Introduction 168
- Macroscopic Screening 168
  - Specimen Preparation 168
  - Specimen Volume 168
  - Centrifugation 168
  - Sediment Preparation 169
  - Volume of Sediment Examined 169
  - Commercial Systems 169
  - Examining the Sediment 169
  - Reporting the Microscopic Examination 170
  - Correlating Results 170
- Sediment Examination Techniques 170
  - Sediment Stains 171
  - Cytodiagnostic Urine Testing 173
  - Microscopy 173
  - Types of Microscopy 176
- Urine Sediment Constituents 178
  - Red Blood Cells 179
  - White Blood Cells 181
  - Epithelial Cells 182
  - Bacteria 187
  - Yeast 189
  - Parasites 189
  - Spermatozoa 189
  - Mucus 190
  - Casts 191

Urinary Crystals 199
Urinary Sediment Artifacts 209

CHAPTER 8
Renal Disease 219
Introduction 220
Glomerular Disorders 220
Glomerulonephritis 220
Nephrotic Syndrome 222
Tubular Disorders 222
Acute Tubular Necrosis 222
Hereditary and Metabolic Tubular Disorders 225
Interstitial Disorders 226
Urinary Tract Infection 226
Acute Pyelonephritis 226
Chronic Pyelonephritis 227
Acute Interstitial Nephritis 228
Renal Failure 228
Renal Lithiasis 229

CHAPTER 9
Urine Screening for Metabolic Disorders 235
Introduction 236
Overflow Versus Renal Disorders 236
Newborn Screening Tests 236
Amino Acid Disorders 236
Phenylalanine-Tyrosine Disorders 236
Branched-Chain Amino Acid Disorders 239
Tryptophan Disorders 241
Cystine Disorders 242
Porphyrin Disorders 243
Mucopolysaccharide Disorders 246
Purine Disorders 247
Carbohydrate Disorders 247

PART THREE: Other Body Fluids 252

CHAPTER 10
Cerebrospinal Fluid 253
Introduction 254
Formation and Physiology 254
Specimen Collection and Handling 254
Appearance 255
Traumatic Collection (Tap) 256
Uneven Blood Distribution 256
Clot Formation 257
Xanthochromic Supernatant 257
Cell Count 257
Methodology 257
Total Cell Count 258
WBC Count 258
Quality Control of CSF and Other Body Fluid Cell Counts 258
Differential Count on a CSF Specimen 258
Cytocentrifugation 259
CSF Cellular Constituents 259
Chemistry Tests 266
Cerebrospinal Protein 266
CSF Glucose 268
CSF Lactate 268
CSF Glutamine 268
Microbiology Tests 268
Gram Stain 269
Immunologic Assays 270
PCR Molecular Diagnostic Testing 270
Serological Testing 271

CHAPTER 11
Semen 277
Introduction 278
Physiology 278
Specimen Collection 278
Specimen Handling 280
Semen Analysis 280
Appearance 280
Liquefaction 280
Volume 280
Viscosity 281
pH 281
Sperm Concentration and Sperm Count 281
Sperm Motility 283
Automated Semen Analysis Systems 283
Sperm Morphology 283
Additional Testing 285
Sperm Vitality 286
Seminal Fluid Fructose 286
Antisperm Antibodies 286
Microbial and Chemical Testing 287
Postvasectomy Semen Analysis 287
Sperm Function Tests 288
Semen Analysis Quality Control 288

CHAPTER 12
Synovial Fluid 293
Physiology 294
Specimen Collection and Handling 294
Color and Clarity 295
Viscosity 296
Cell Counts 296
Differential Count 296

Crystal Identification 297
Types of Crystals 297
Slide Preparation 297
Crystal Polarization 298
Chemistry Tests 300
Glucose 300
Total Protein 300
Uric Acid 301
Lactate 301
Enzymes 301
Microbiological Tests 301
Serological Tests 301

CHAPTER 13
Serous Fluid 305
Introduction 306
Formation 306
Specimen Collection and Handling 306
Transudates and Exudates 307
General Laboratory Procedures 307
Pleural Fluid 308
Appearance 308
Hematology Tests 309
Chemistry Tests 310
Microbiological and Serological Tests 312
Pericardial Fluid 312
Appearance 312
Laboratory Tests 312
Peritoneal Fluid 314
Transudates Versus Exudates 314
Appearance 314
Laboratory Tests 314

CHAPTER 14
Bronchoalveolar Lavage Fluid 321
Clinical Significance 322
Specimen Collection 322
Specimen Handling and Transport 322
Color and Clarity 322
Cell Counts 322
Leukocytes 323
Macrophages 323
Lymphocytes 323
Neutrophils 323
Eosinophils 323
Mast Cells 324
Erythrocytes 324
Epithelial Cells 324
Microbiological Tests 324
Cytology 324

CHAPTER 15
Amniotic Fluid 327
Introduction 328
Physiology 328
Function 328
Volume 328
Chemical Composition 329
Differentiating Maternal Urine From Amniotic Fluid 329
Specimen Collection 329
Indications for Amniocentesis 329
Collection 330
Specimen Handling and Processing 330
Color and Appearance 331
Tests for Fetal Distress 331
Hemolytic Disease of the Fetus and Newborn 331
Neural Tube Defects 332
Tests for Fetal Maturity 333
Fetal Lung Maturity 333
Lecithin-Sphingomyelin Ratio 333
Phosphatidyl Glycerol 334
Foam Stability Index 334
Lamellar Bodies 334

CHAPTER 16
Fecal Analysis 339
Introduction 340
Physiology 340
Diarrhea and Steatorrhea 341
Diarrhea 341
Steatorrhea 342
Specimen Collection 342
Macroscopic Screening 342
Color 343
Appearance 343
Microscopic Examination of Feces 343
Fecal Leukocytes 343
Muscle Fibers 344
Qualitative Fecal Fats 344
Chemical Testing of Feces 345
Occult Blood 345
Quantitative Fecal Fat Testing 347
APT Test (Fetal Hemoglobin) 348
Fecal Enzymes 349
Carbohydrates 349

CHAPTER 17

Vaginal Secretions 355
- Introduction 356
- Specimen Collection and Handling 357
- Color and Appearance 357
- Diagnostic Tests 357
  - pH 357
  - Microscopic Procedures 358
  - Other Diagnostic Tests 363
- Vaginal Disorders 364
  - Bacterial Vaginosis 364
  - Trichomoniasis 364
  - Candidiasis 364
  - Desquamative Inflammatory Vaginitis 365
  - Atrophic Vaginitis 365
- Additional Procedures for Vaginal Secretion Associated With Pregnancy 365
  - Fetal Fibronectin Test 365
  - AmniSure Test 366
  - Actim PROM 366
  - ROM Plus 366

Answers to Study Questions, Case Studies, and Clinical Situations 371

Abbreviations 381

Glossary 385

Index 393

Atlas of Images

Kathleen Finnegan

URINALYSIS 2

Cells 2
- **Red Blood Cells** 2
- **White Blood Cells** 3
- **Epithelial Cells** 5
- **Transitional Epithelial (Urothelial) Cell** 7
- **Renal Tubular Epithelial Cells** 8
- **Oval Fat Bodies** 10

Microorganisms 11
- **Bacteria** 11
- **Yeast** 12

Parasites 14
- **Trichomonads** 14
- **Pinworm** 14
- ***Schistosoma Haematobium*** 15

Miscellaneous Structures 16
- **Sperm** 16
- **Mucus** 16

Casts 17
- **Hyaline Casts** 17
- **Red Blood Cell Casts** 18
- **White Blood Cell Casts** 19
- **Bacterial Casts** 20
- **Epithelial Cell Casts** 20
- **Granular Casts** 21
- **Mixed Cell Casts** 23
- **Fatty Casts** 23
- **Waxy Casts** 24
- **Broad Casts** 25

Crystals/Acid 26
- **Uric Acid Crystals** 26
- **Amorphous Urate Crystals** 27
- **Calcium Oxalate Crystals** 28
- **Sodium Urate Crystals** 29

Crystals/Alkaline 30
- **Amorphous Phosphate Crystals** 30
- **Triple Phosphate Crystals** 30
- **Calcium Phosphate Crystals** 32
- **Calcium Carbonate Crystals** 32
- **Ammonium Biurate Crystals** 33

Abnormal Crystals 34
- **Cystine Crystals** 34
- **Leucine Crystals** 34
- **Tyrosine Crystals** 35
- **Cholesterol Crystals** 35
- **Bilirubin Crystals** 36
- **Sulfonamide Crystals** 37
- **Ampicillin Crystals** 37

Artifacts 38
- **Radiographic Dye Crystals** 38
- **Starch** 38
- **Fibers** 39
- **Oil Droplets or Air Bubbles** 40

BODY FLUIDS 41

Cerebrospinal Fluid 41

Peritoneal Fluid 43

Pleural Fluid 44

Synovial Fluid 46

Red Blood Cells

Description

- Normal: Pale nonnucleated biconcave discs, membrane regular, 6 to 8 µm in size
- Hypotonic urine: Red blood cells (RBCs) appear colorless with membrane intact and regular; often lose their hemoglobin in a dilute urine with low specific gravity
- Hypertonic urine: RBCs have lost their biconcave shape, the membrane becomes irregular, and the cells appear crenated; they appear like small spheres with spicules in a urine with high specific gravity
- Dysmorphic: RBCs vary in size and shape, are often fragmented, or have cellular protrusions (burr cells or acanthrocytes)

Clinical Significance

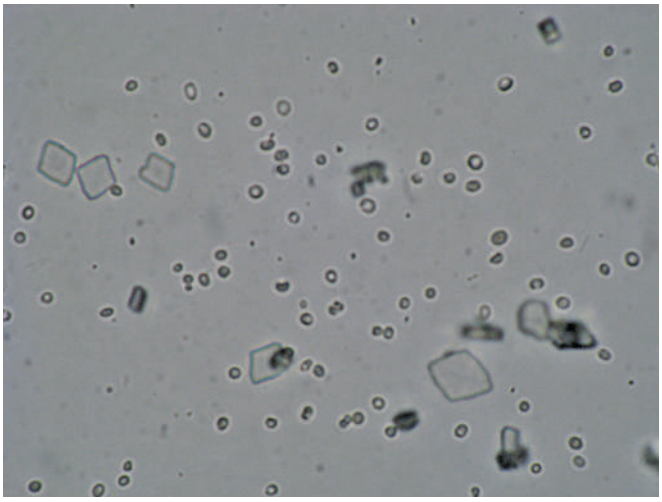
- Increased RBCs are seen with urinary tract infections (UTIs), trauma to the kidneys, or menstrual contamination
- Dysmorphic RBCs are associated with glomerular membrane damage

UA Correlations

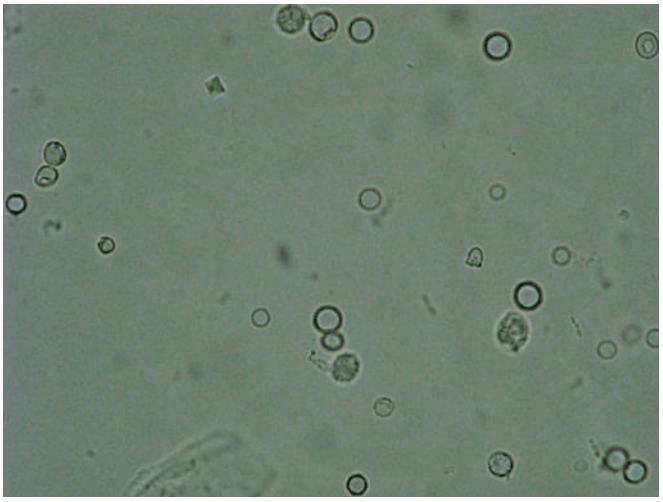
- Color: Pale pink to bright red
- Specific gravity
- Blood

Tips

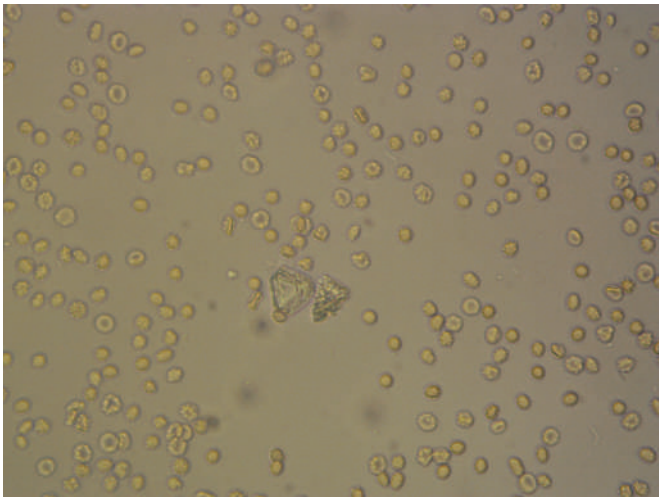
- RBCs can be confused with yeast cells (round to oval and smaller in shape and tend to bud) or oil droplets and air bubbles (tend to be refractile)



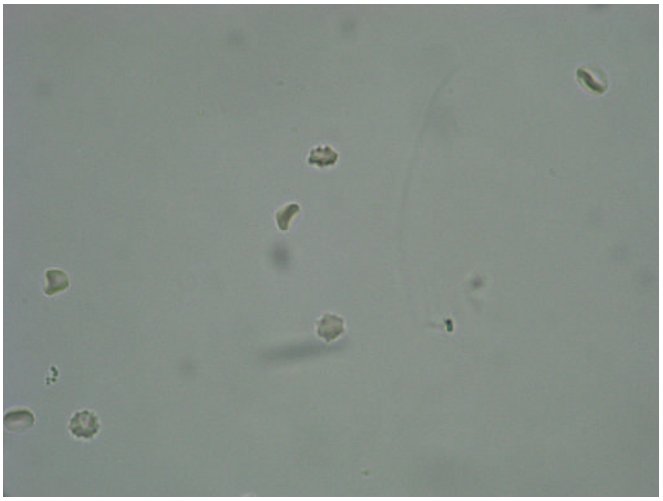
A-01. Normal RBCs. Note uric acid crystals.



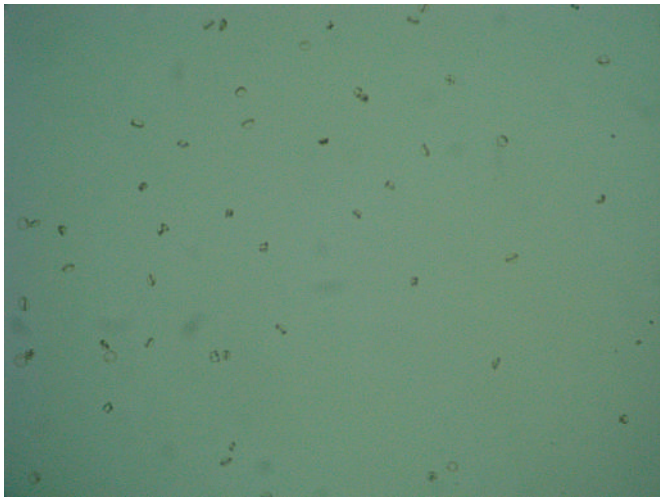
A-03. Ghost RBCs.



A-02. Normal and crenated RBCs.



A-04. Crenated RBCs.



A-05. Dysmorphic RBCs.

White Blood Cells

Description

- Colorless, 12 µm in size
- Neutrophils are granular and multinucleated; the majority of white blood cells (WBCs) in the peripheral blood are neutrophils
- Lymphocytes and monocytes are agranular
- Hypotonic urine: Sparkling cytoplasm in the neutrophils associated with Brownian movement are called glitter cells; they absorb water and swell

Clinical Significance

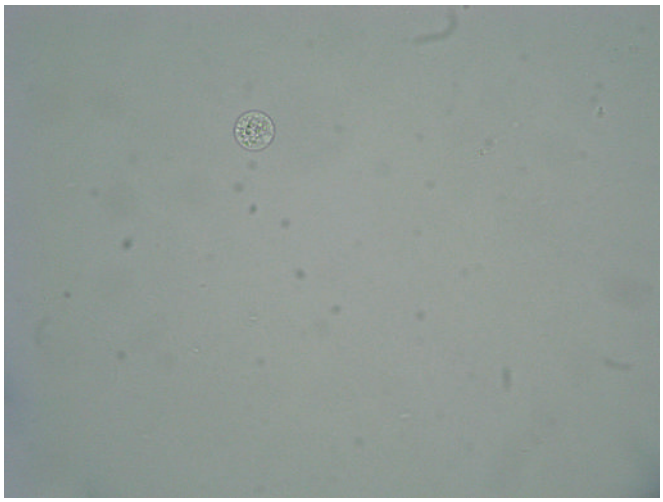
- Neutrophils are associated with bacterial infection, cystitis, and inflammation
- Eosinophils are associated with drug-induced interstitial nephritis
- Lymphocytes are associated with renal transplant
- Monocytes are associated with chronic inflammation

UA Correlations

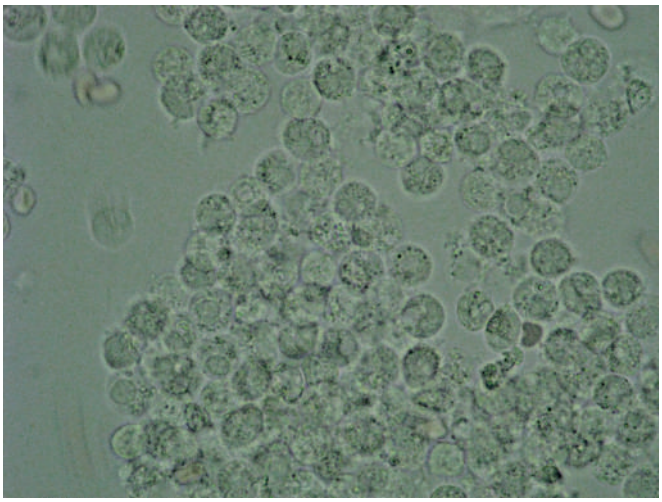
- Clarity
- Leukocyte esterase
- Nitrite with bacterial infection

Tips

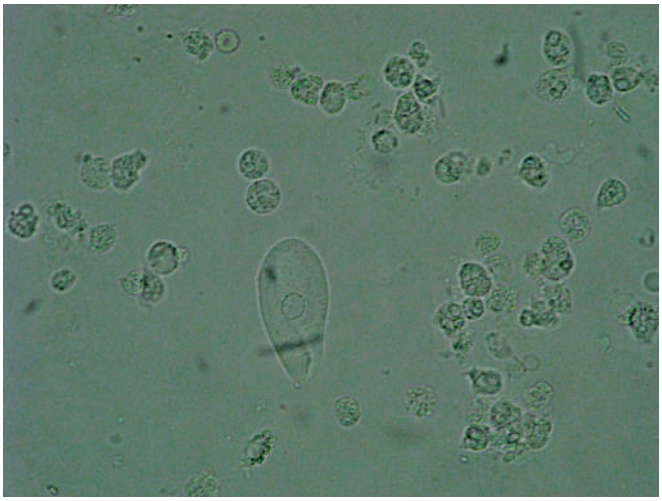
- Neutrophils lose their nuclear detail in dilute urine and can be confused with renal tubular epithelial (RTE) cells, which tend to be larger with an eccentric nucleus
- During active inflammation of the urinary tract, the WBCs may have extended pseudopodia



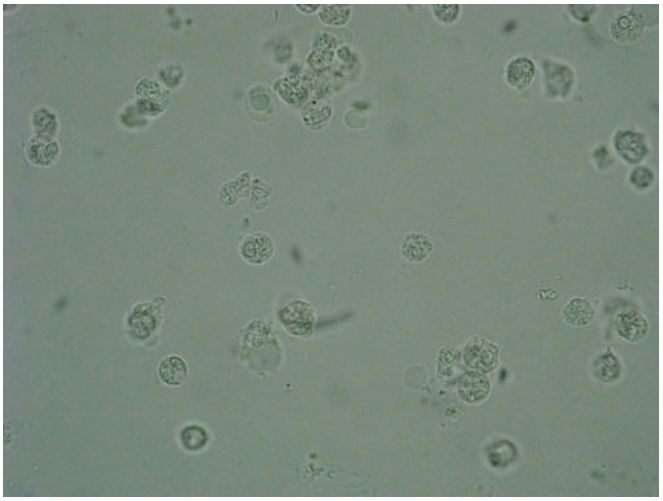
A-06. One-segmented WBC. Note the multilobed nucleus.



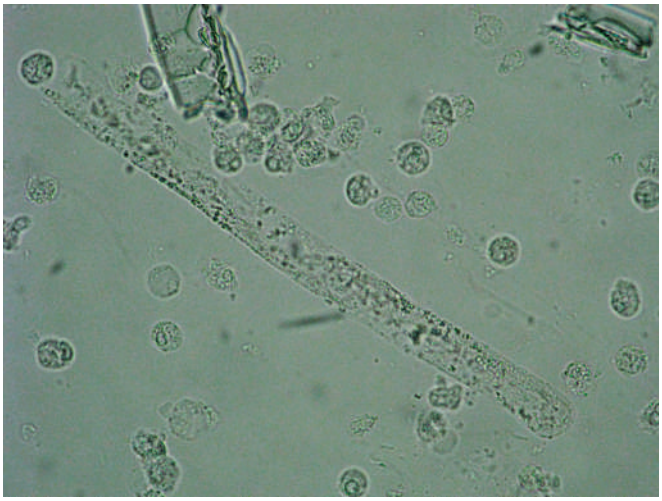
A-07. Clump of WBCs.



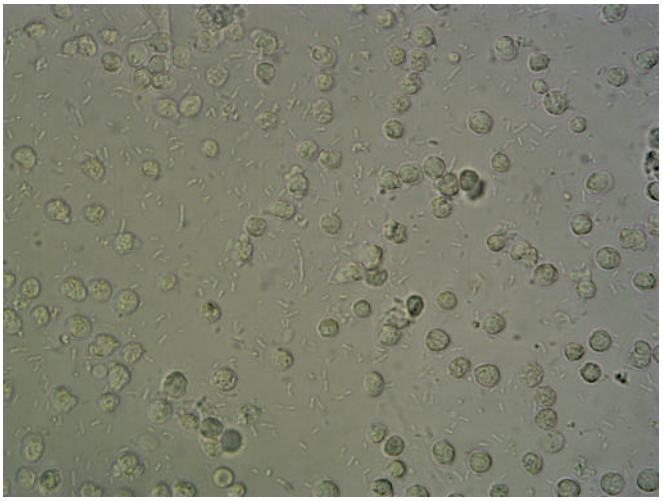
A-08. WBCs and a single transitional epithelial cell.



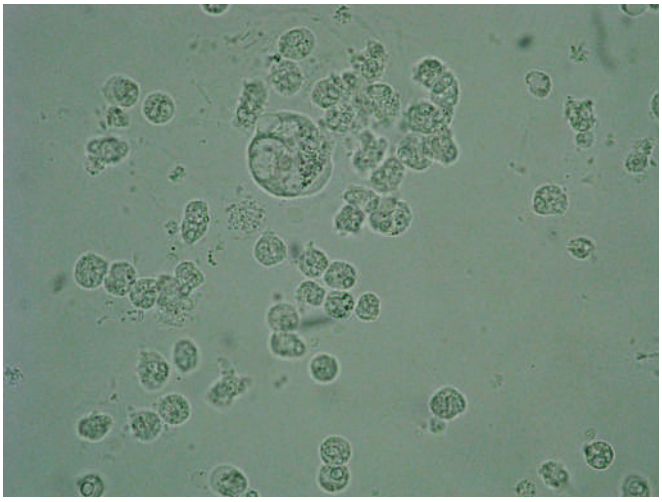
A-11. WBCs with three RBCs.



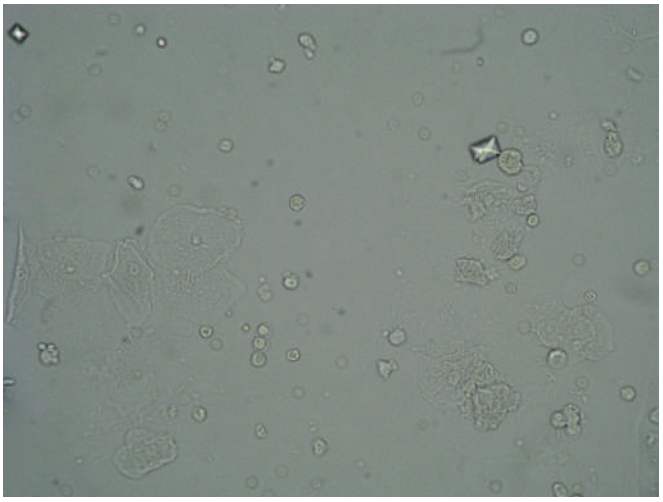
A-09. WBCs with a fine granular cast.



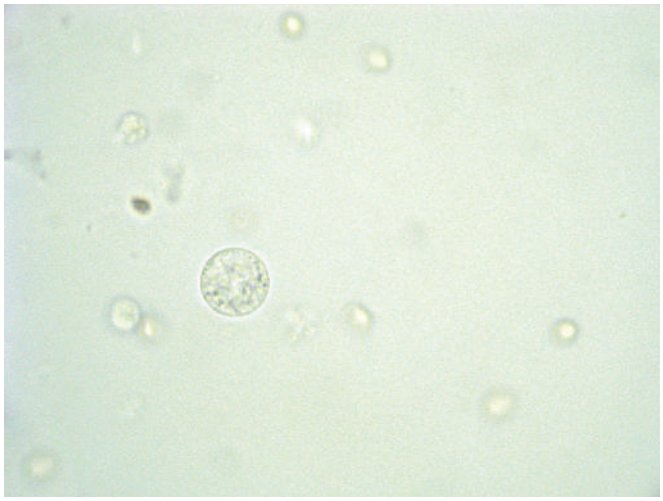
A-12. Numerous WBCs, bacteria, and two RBCs.



A-10. Segmented WBCs.



A-13. WBCs, RBCs, squamous epithelial cells, and two calcium oxalate crystals.



A-14. Glitter cell. Observe the granules. RBCs are also present.

Epithelial Cells

Description

- Large cells, 60 to 100 µm in size, with abundant irregular cytoplasm and wrinkled borders
- Nucleus is in the center and is the size of an RBC; can be binucleated, tend to have multiridge borders that tend to fold or curl
- Clue cell: Variation of squamous epithelial cells; covered with *Gardnerella coccobacillus,* which gives the cell a granular appearance

Clinical Significance

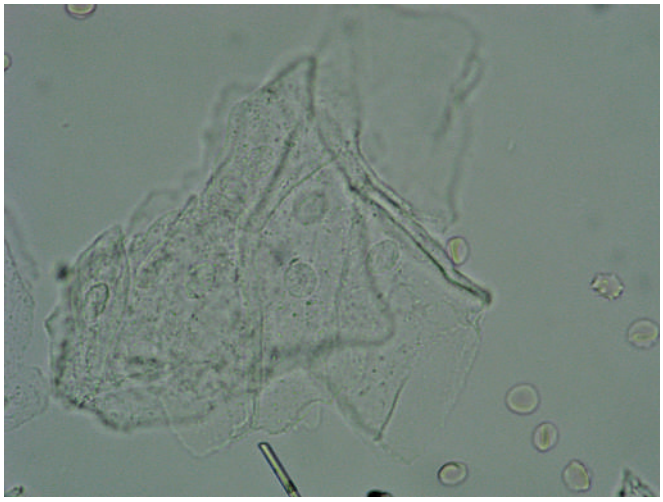
- Originate from the mucous membrane around the urethra
- Squamous epithelial cells can be associated with wear and tear from urethra or vaginal mucosa; seen more frequently in females
- Clue cells are pathological and indicative of vaginal infection caused by *Gardnerella vaginalis*

UA Correlations

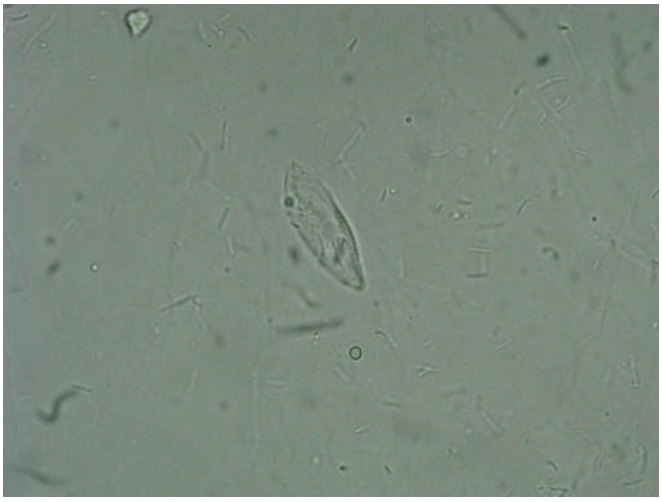
- Clarity

Tips

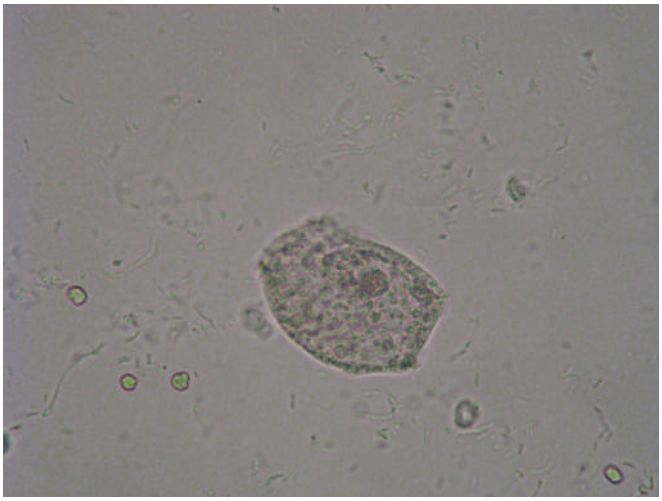
- Squamous epithelial cells tend to resemble a fried egg
- When rolled or curled, they tend to look like casts
- Casts do not have a nucleus
- When seen in high numbers, they can indicate contamination from vaginal secretions or poor collection



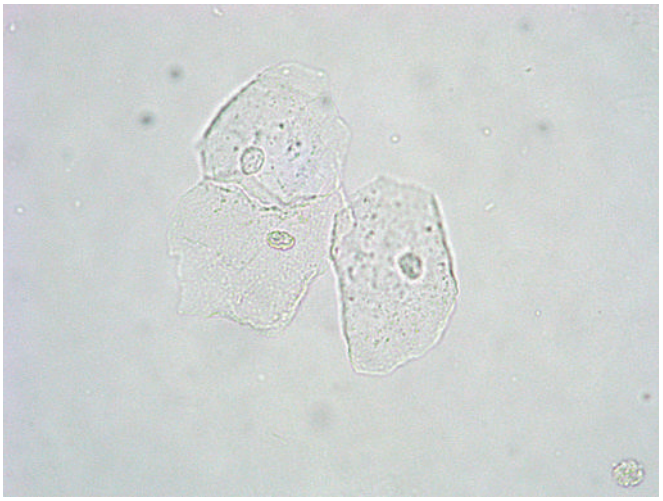
A-15. Clump of squamous epithelial cells. Note normal RBCs and one crenated RBC.



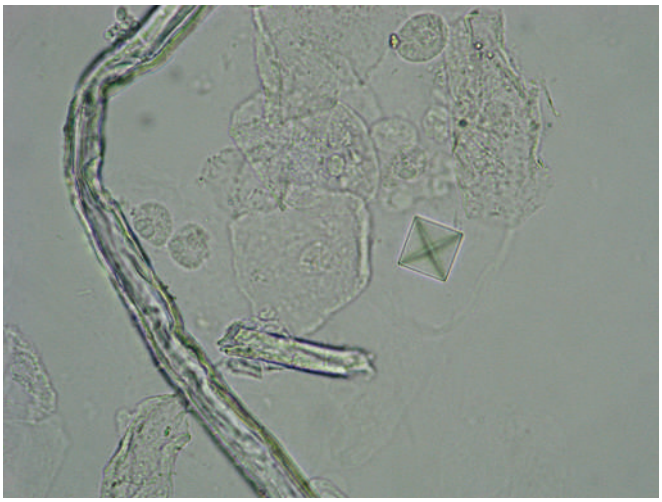
A-16. Rolled squamous epithelial cell. Note bacteria.



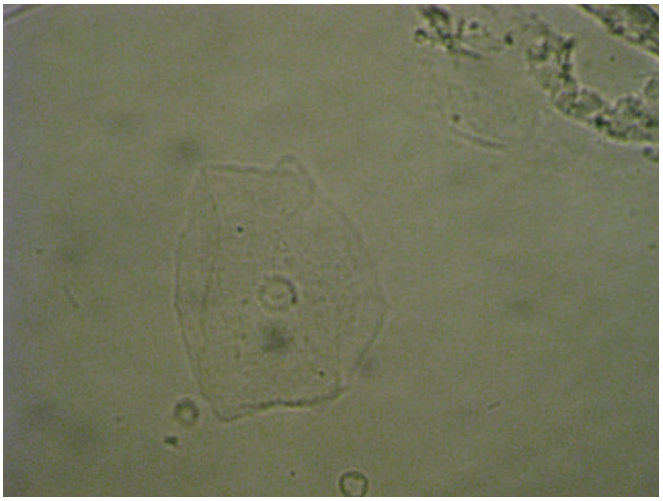
A-17. Clue cell. Note bacteria covering the epithelial cell.



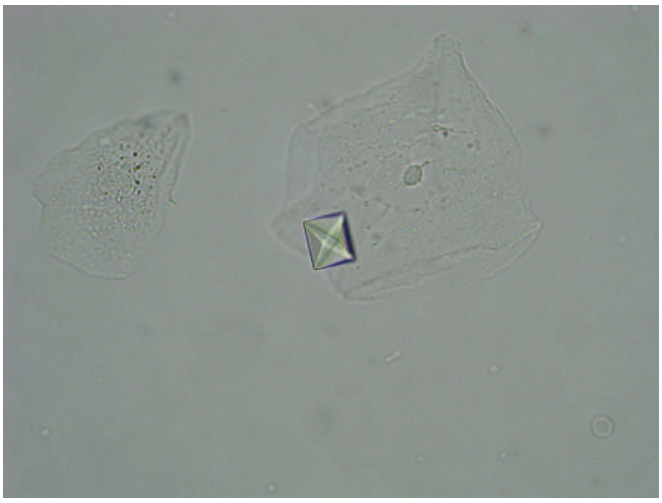
A-20. Three squamous epithelial cells and one WBC.



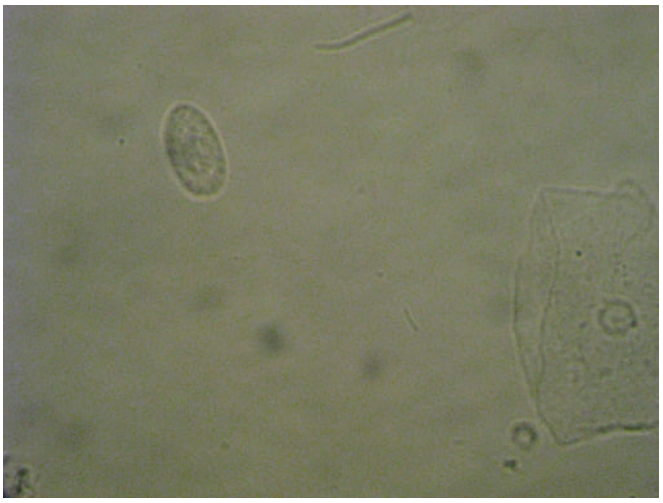
A-18. Squamous epithelial cells with WBCs and one calcium oxalate crystal. Note refractile fiber.



A-21. Squamous epithelial cell. Note the abundant cytoplasm and prominent centrally located nucleus.



A-19. Two squamous epithelial cells, one calcium oxalate crystal, and one RBC.



A-22. Squamous epithelial cell and transitional epithelial cell. The amount of cytoplasm is much larger in the squamous epithelial cell.

Transitional Epithelial (Urothelial) Cell

Description

- Smaller than squamous epithelial cells
- Can be spherical, pear-shaped, polyhedral, or caudate shaped with a centrally located nucleus
- In elongated cells, the nucleus tends to be off-center
- Cells contain a moderate amount of cytoplasm

Clinical Significance

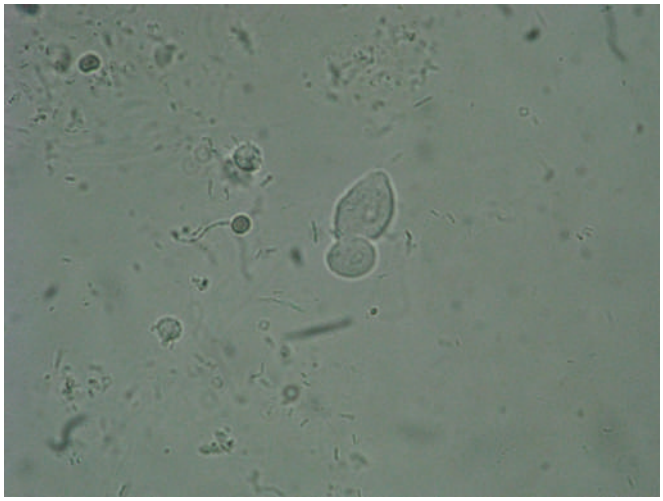
- Originate from mucosa of renal pelvis, ureter, and bladder
- Appear in urine with infection, inflammation, renal stones, bladder cancer, and catheterization

UA Correlations

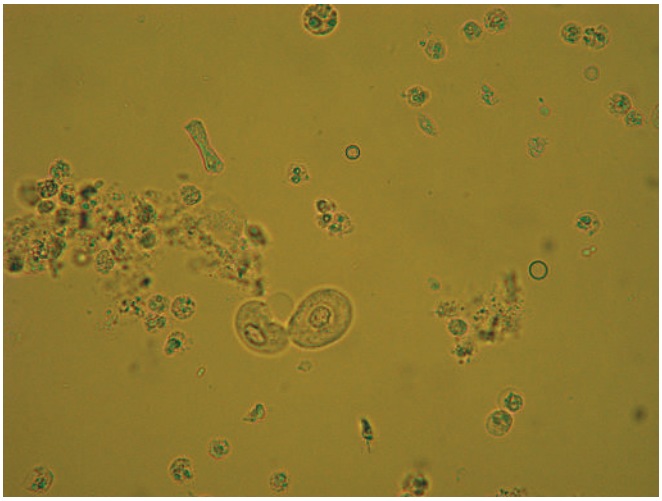
- Clarity
- Blood, if malignancy associated

Tips

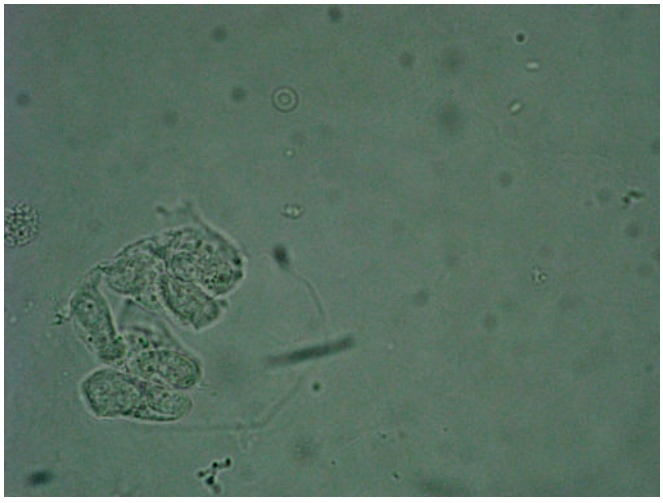
- Spherical forms resemble RTE cells
- When seen in clusters or sheets, they are associated with catheterized specimens
- Transitional epithelial cells tend to take on water and can look like large round balloons



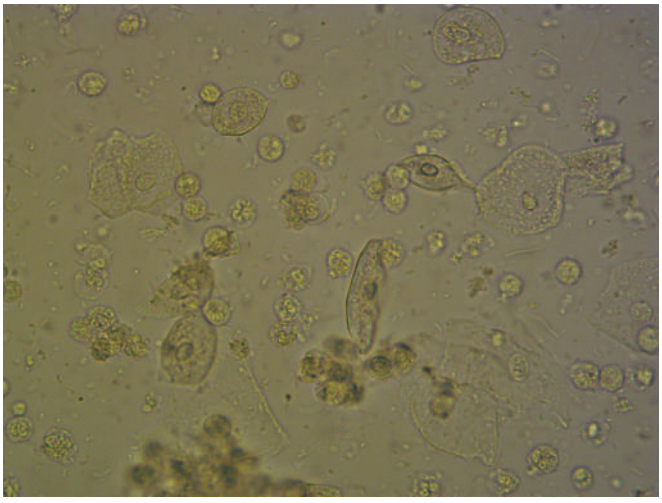
A-23. Pear-shaped transitional epithelial cell. Note bacteria.



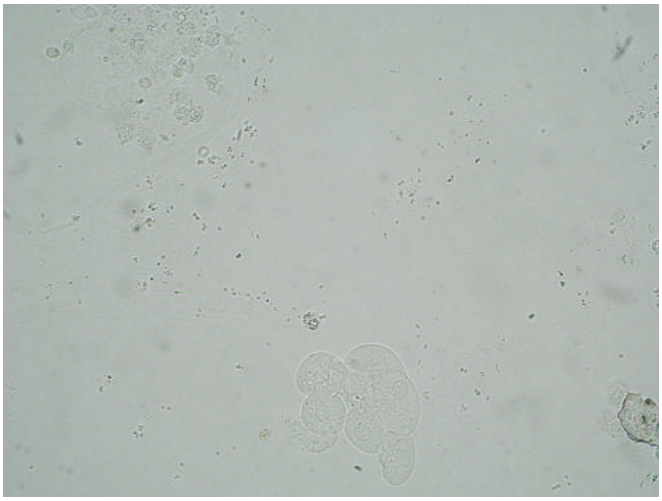
A-25. Two spherical transitional epithelial cells. WBCs, RBCs, and yeast (refractile) are also present.



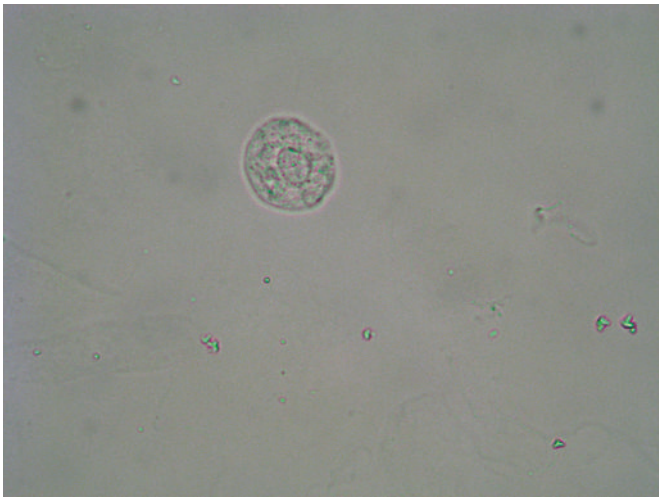
A-24. Clump of transitional epithelial cells. Note RBC.



A-26. Squamous and transitional epithelial cells. Notice the central location of the nucleus of the epithelial cells. The squamous epithelial cell has a greater amount of cytoplasm. Many WBCs and bacteria are also present.



A-27. A clump of transitional epithelial cells.



A-28. Spherical transitional epithelial cell with a hyaline cast in the left lower corner.

Renal Tubular Epithelial Cells (RTE)

Description

- Originate from proximal renal tubules, loop of Henle, distal convoluted renal tubules, and collecting ducts; vary in size and shape due to different origins
- Proximal tubular cells are large in size and oblong in shape with grainy cytoplasm; nucleus is eccentric
- Distal tubular cells are smaller and more round to oval, with small, round eccentric nucleus
- Collecting duct cells are polygonal or cuboidal with a large nucleus

Clinical Significance

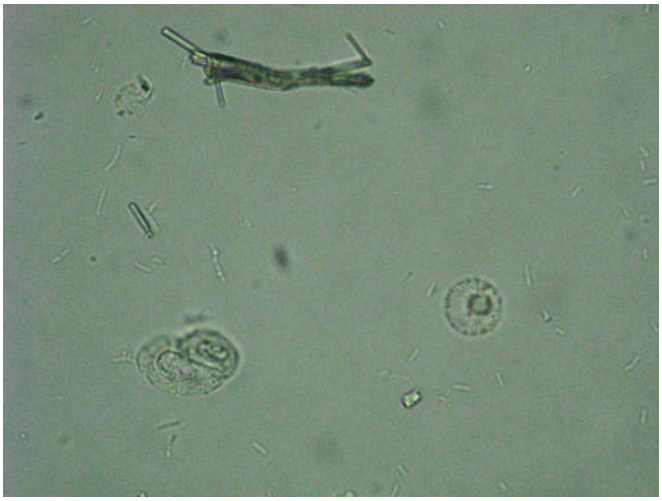
- Increased amounts are indicative of necrosis of the renal tubules, renal tubular damage, exposure to heavy metals, drug-induced toxicity, hemoglobin and myoglobin toxicity, ischemic events, and viral infections

UA Correlations

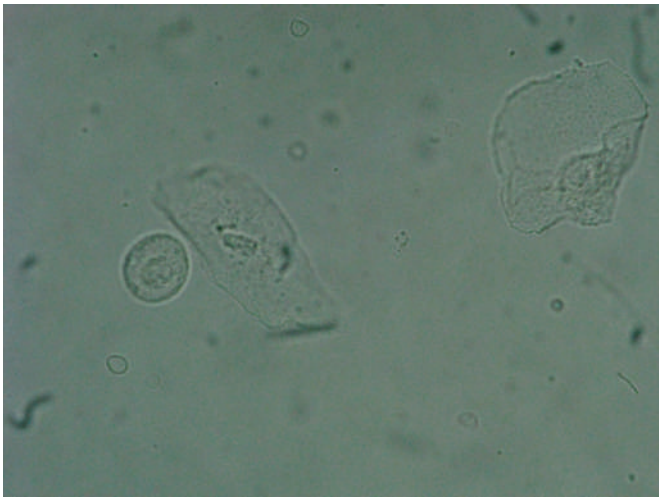
- Clarity
- Color
- Protein
- Blood
- Bilirubin (hepatitis)
- Leukocyte esterase (pyelonephritis)
- Nitrite (pyelonephritis)

Tips

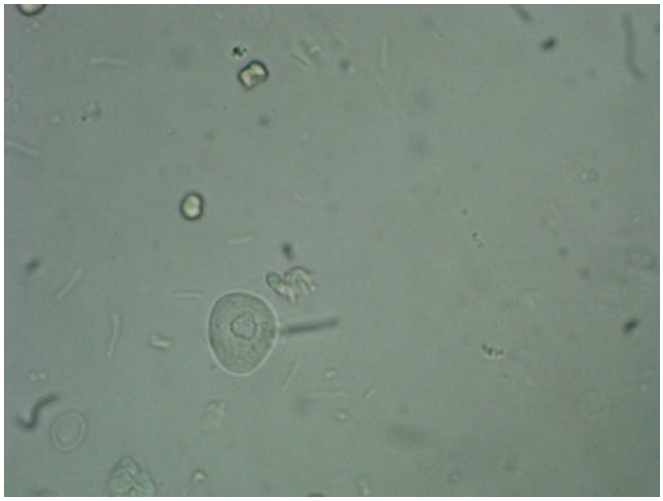
- RTEs from the collecting ducts have a flat edge to one side of the membrane; cells from the proximal convoluted tubules are more rectangular and can be mistaken for casts
- Cells from the distal convoluted tubule are small and can be mistaken for WBCs



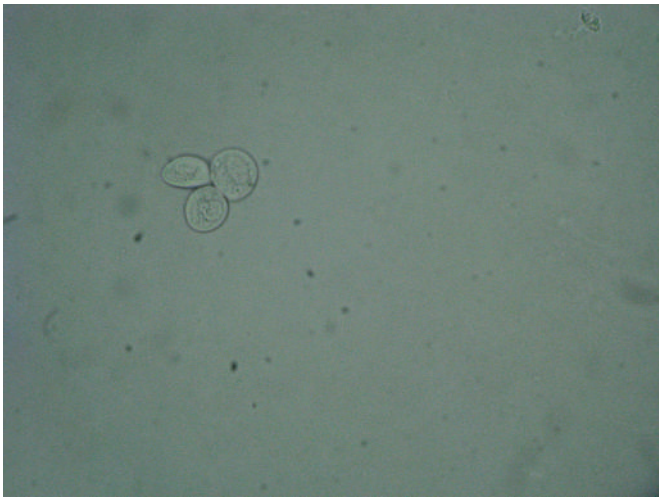
A-29. Single and double RTE cells. Note fiber.



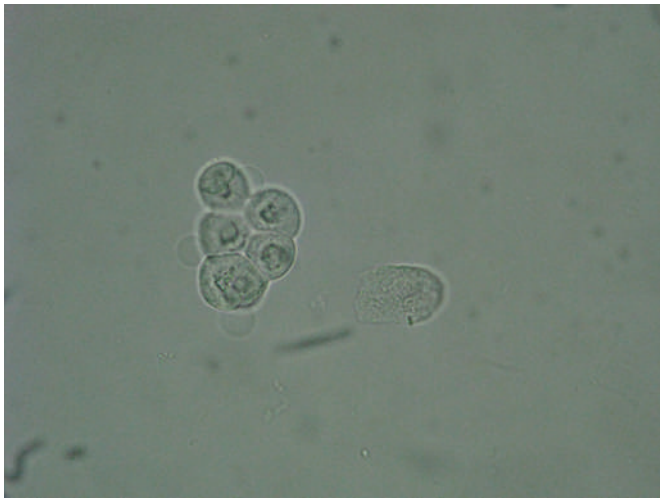
A-30. One RTE cell with two squamous epithelial cells.



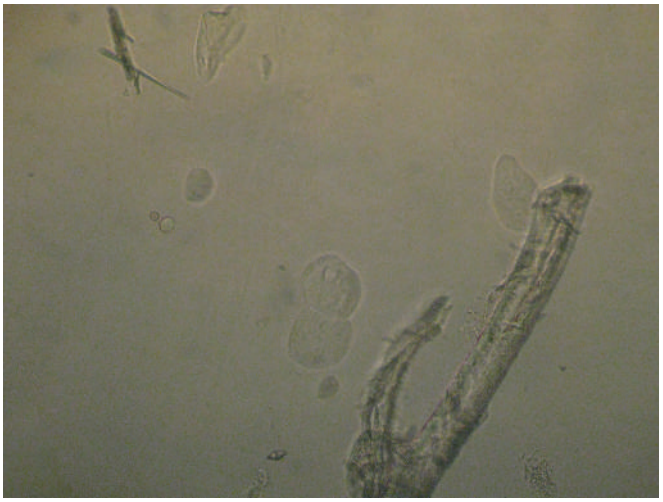
A-31. One RTE cell. Note bacteria.



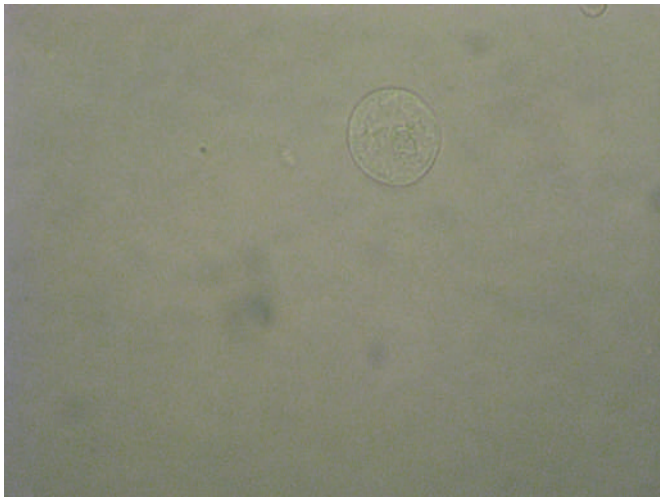
A-34. Three RTE cells. Notice the eccentrically placed nuclei.



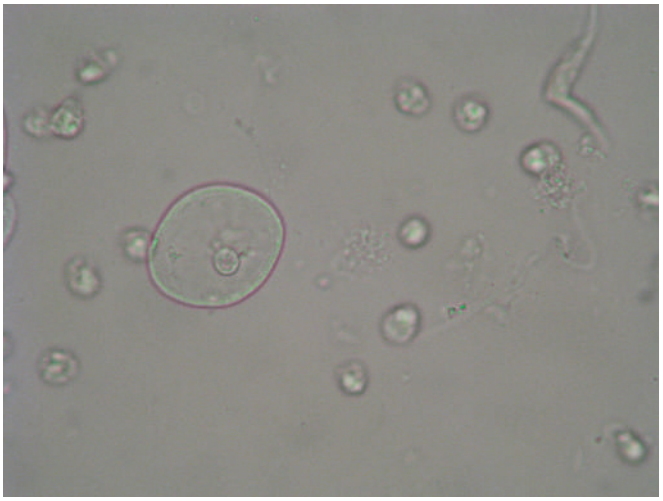
A-32. Clump of RTE cells.



A-35. RTE cells. Fiber artifact present.



A-33. RTE cell.



A-36. RTE cell and WBCs.

Oval Fat Bodies

Description

- Oval fat bodies are renal tubular cells laden with fat
- They are highly refractile and globular in shape, with a nucleus that is difficult to see
- Polarizing microscopy demonstrates a Maltese cross formation

Clinical Significance

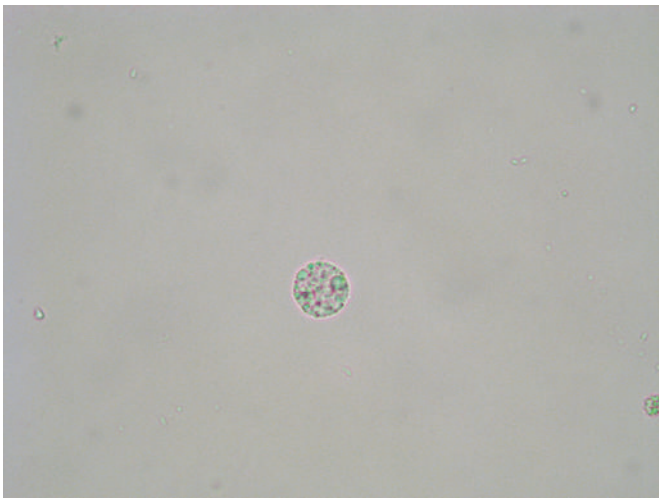
- Oval fat bodies can be associated with nephrotic syndrome, glomerular dysfunction, and renal tubular cell death

UA Correlations

- Clarity
- Protein
- Blood
- Free fat droplets
- Fatty casts

Tips

- Oval fat bodies can be seen in conjunction with free floating fat droplets
- Lubricants used in specimen collection can be confused with fat droplets



A-37. Oval fat body.

Microorganisms

Bacteria

Description

- Small, spherical cocci and rod-shaped (bacilli) structures
- Vary in size from short to long
- Can appear single or in pairs or chains

Clinical Significance

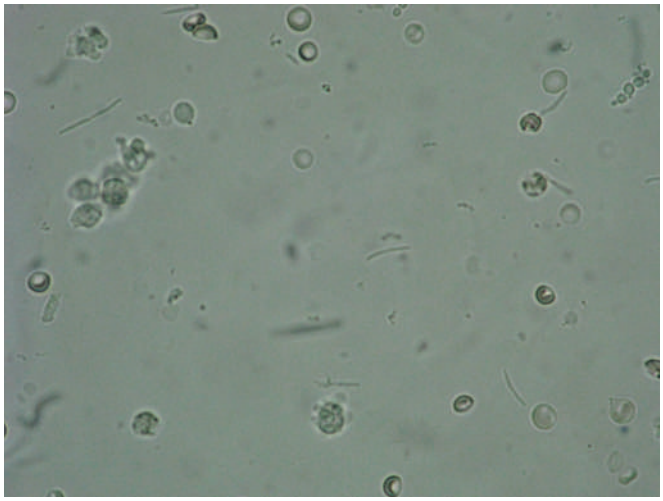
- Increased bacteria indicate a UTI
- Bacteria should be seen with increased WBCs, positive leukocyte esterase, an alkaline pH, and a +/– nitrite

UA Correlations

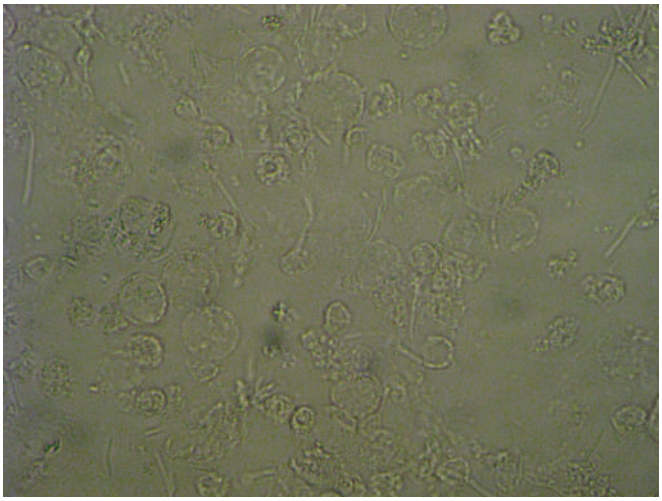
- Alkaline pH
- Leukocyte esterase
- Nitrite
- WBCs
- WBCs in clumps

Tips

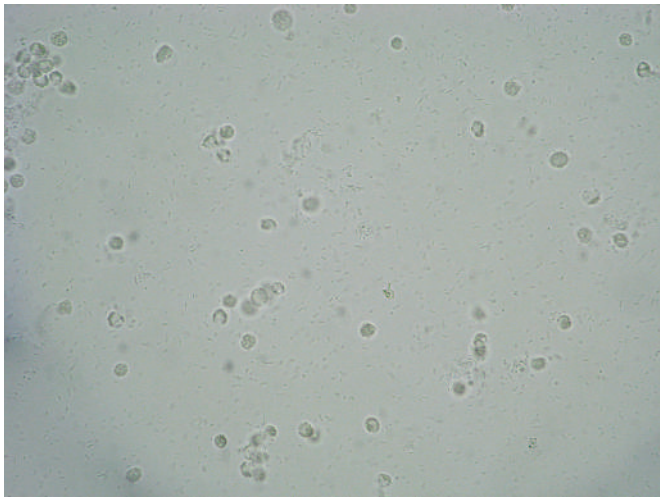
- Motility will help distinguish bacteria from amorphous urates (pink-colored sediment) or amorphous phosphates (white-colored sediment) or calcium carbonate crystals, which can look like bacteria
- Bacteria multiply in urine if left at room temperature for several hours



A-38. Bacteria in chains with WBCs and RBCs.



A-40. Many bacteria and WBCs.



A-39. Rod-shaped bacteria with WBCs.

Yeast

Description

- Appear as colorless, small, refractile, round to oval structures that may be single, budding, or with pseudohyphae and branching

Clinical Significance

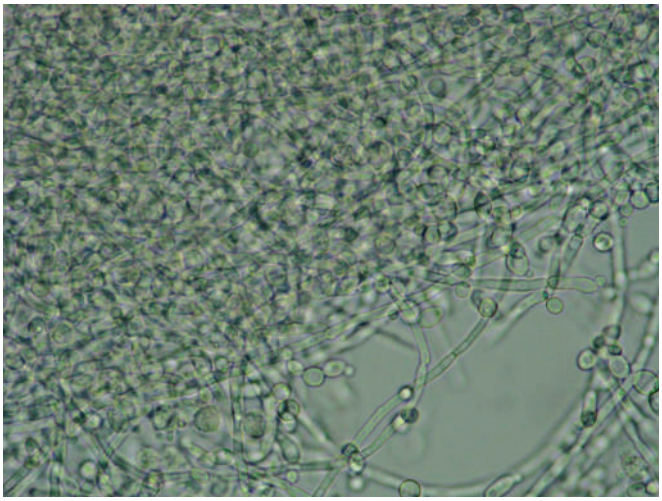
- *Candida albicans* is yeast found commonly in urine of women, it most often indicates vaginal secretion contamination or vaginal infection
- It can also be seen in patients who are diabetic or immunocompromised

UA Correlations

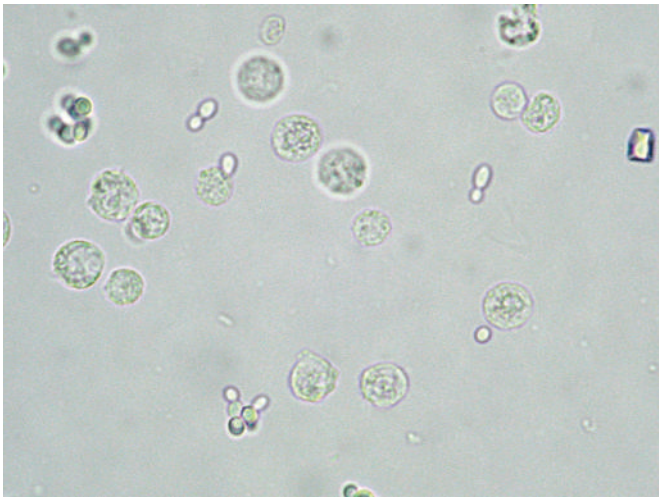
- Glucose
- Increased WBCs
- Leukocyte esterase

Tips

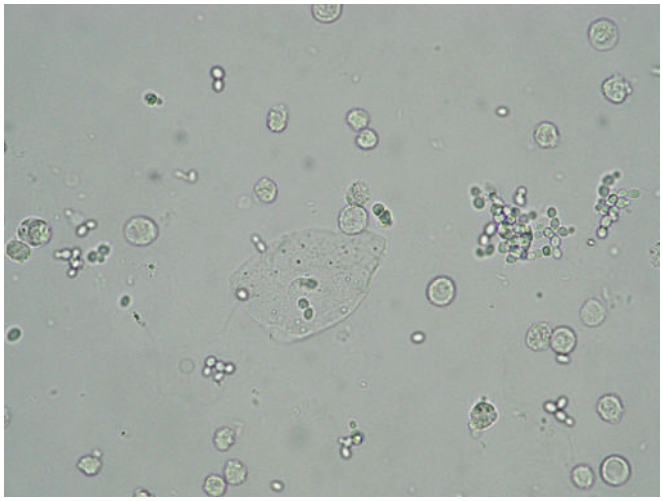
- Yeast can be confused with RBCs
- Budding yeast can help differentiate
- Yeast does not dissolve when acetic acid is added
- As with bacteria, yeast will multiply in urine if left at room temperature for several hours



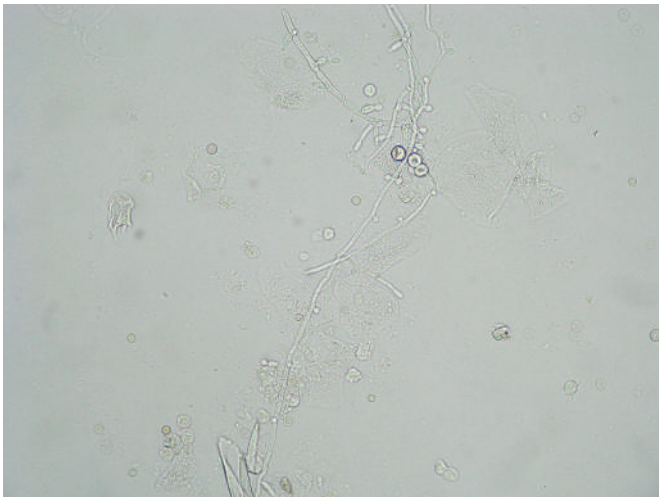
A-41. Packed field of budding yeast and pseudohyphae.



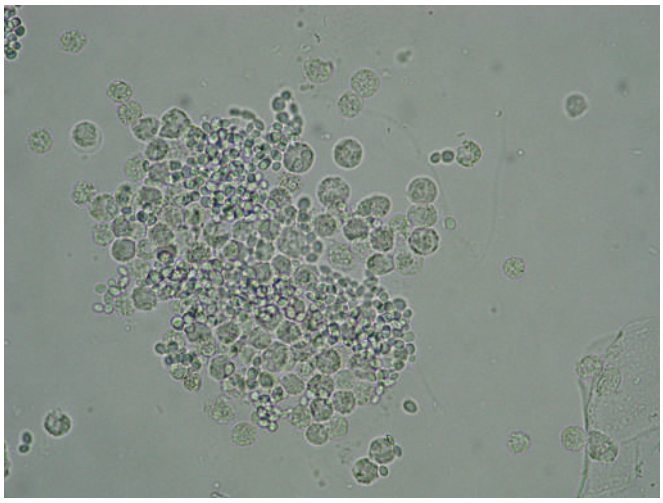
A-42. Budding yeast with WBCs and one RBC.



A-43. Budding yeast with RBCs, WBCs, and one squamous epithelial cell. Note the halo around RBCs.



A-45. Budding yeast with pseudohyphae and RBCs.



A-44. Clump of budding yeast and WBCs.

Parasites

Trichomonads

Description

- A trophozoite that is a pear-shaped flagellate with an underlying membrane that causes a rapid flitting motion; resembles WBC with tail-like flagella

Clinical Significance

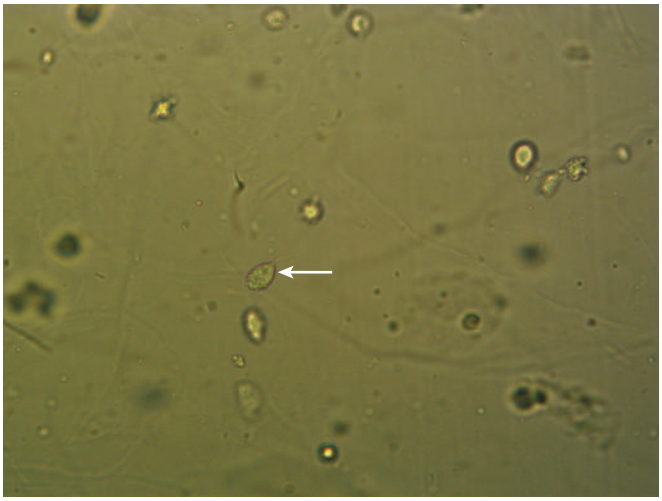
- *Trichomonas vaginalis* is a sexually transmitted pathogen associated with vaginal or urethral inflammation
- In males, it is asymptomatic

UA Correlations

- Increased WBCs
- Increased epithelial cells
- WBC clumps may be present
- Leukocyte esterase

Tips

- *T. vaginalis* must be identified in fresh urine
- They begin to die, and when not moving, they resemble WBCs
- Organisms must be moving or have motility not to be confused with WBCs and RTE cells



A-46. A trichomonad. Notice the flagella and undulating membrane.

Pinworm

Description

- Eggs or ova are football shaped with one flattened side and one round side
- Eggs are transparent, and developing larva can be seen sometimes

Clinical Significance

- Can be found as a result of fecal contamination
- Usually seen with school-age children

UA Correlations

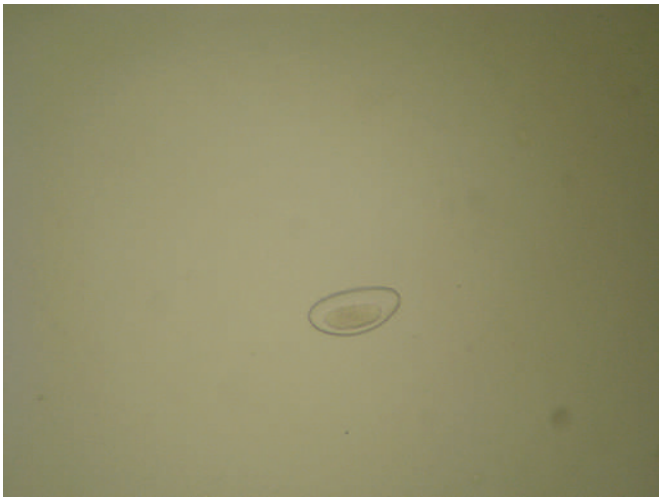
- N/A

Tips

- Eggs or ova tend to be quite large compared with WBCs and epithelial cells



A-47. *Enterobius vermicularis* worm (pinworm).



A-48. *Enterobius vermicularis* ova.

Schistosoma Haematobium

Description

- Very large in size and football shaped with a terminal spine
- Cell wall looks thick

Clinical Significance

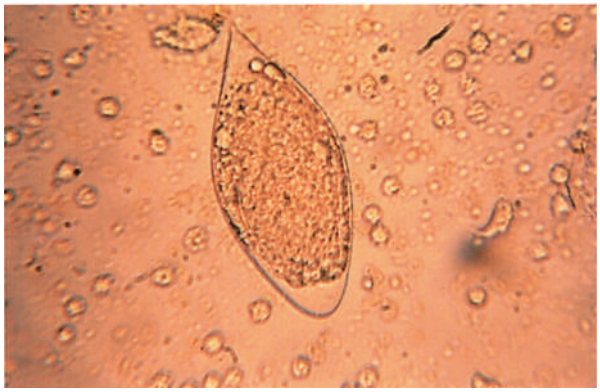
- Eggs can be found in urine and are endemic to Africa and the Middle East

UA Correlation

- Increased RBCs
- Increased WBCs

Tips

- Sometimes the terminal spine can be small and hard to see
- Examine several ova for confirmation



A-49. *Schistosoma haematobium* ova.

Miscellaneous Structures

Sperm

Description

- Spermatozoa have tapered oval heads with long, delicate, flagella-like tails

Clinical Significance

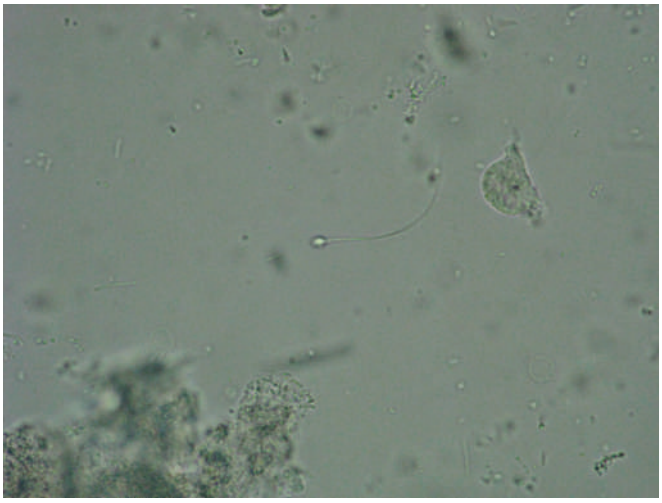
- Sperm can be found in the urine of both males and females after sexual intercourse, masturbation, and nocturnal emission; rarely have any clinical significance

UA Correlations

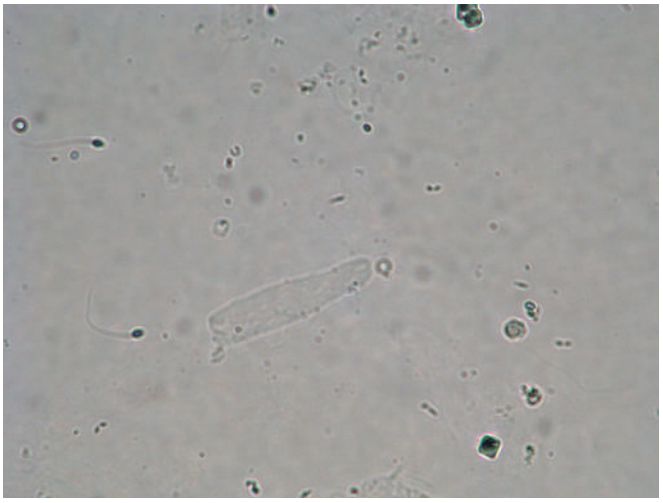
- Protein

Tips

- Urine is very toxic to sperm, so it rarely shows motility in urine



A-50. Sperm and bacteria in urine sediment.



A-51. Two sperm and a hyaline cast.

Mucus

Description

- Single or clumped, ribbon-like or thread-like structures that have irregular or serrated ends with low refractive index
- Subdued light is required when using bright-field microscopy

Clinical Significance

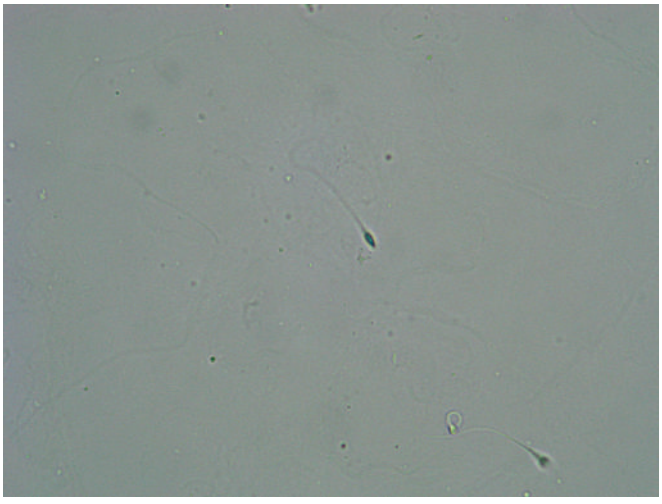
- No clinical significance
- Mucus can be abundant in the presence of inflammation or irritation to the urinary tract

UA Correlations

- Clarity

Tips

- Mucus is hard to see with increased light due to its low refractive index
- Heavy mucus threads have WBCs among them
- These structures can be misidentified as hyaline casts
- Casts have rounded edges and are more cylindrical



A-52. Mucus with sperm present.

Casts

Hyaline Casts

Description

- Composed of uromodulin protein and appear colorless and transparent under low power (100×)
- Low refractive index similar to urine, making them difficult to see using bright-field microscopy
- Consist of parallel sides with rounded ends, with a variety of sizes and shapes
- May contain incisions or have adhering cells or granules under them

Clinical Significance

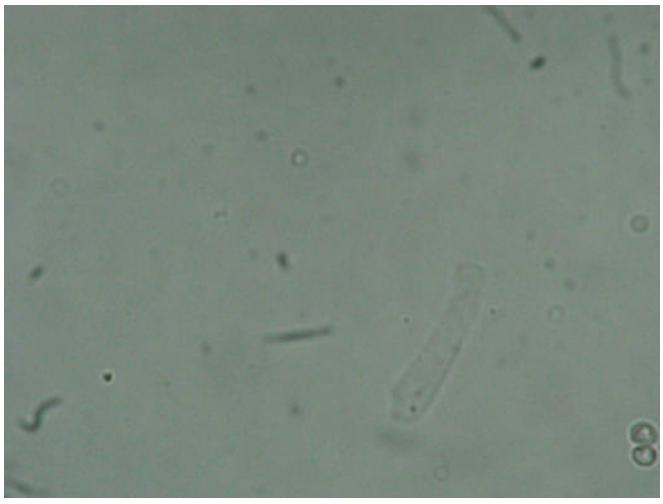
- Can be found in normal individuals; increased numbers with strenuous exercise, stress, fever, and dehydration
- Can be seen in patients with chronic renal disease and congestive heart failure

UA Correlations

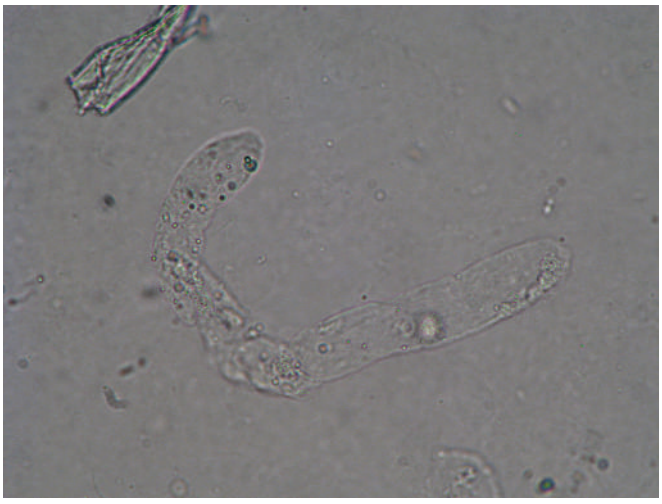
- Protein
- Blood

Tips

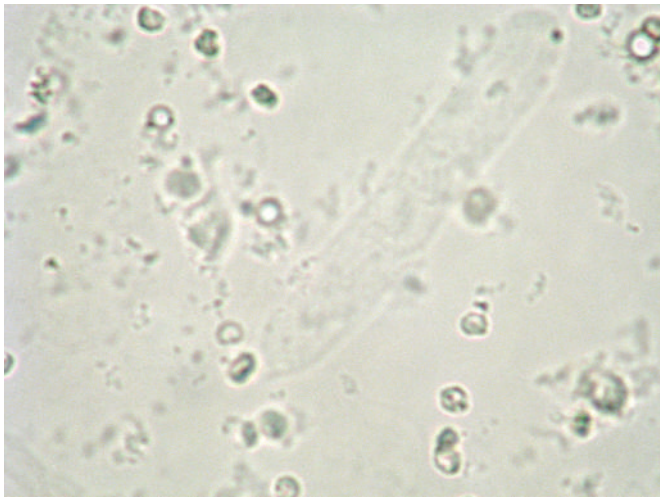
- Hyaline casts have a low refractive index and should be viewed under low light
- Under bright-light microscopy, they can be missed
- Hyaline casts can be confused with mucus and fibers
- Mucus is thin and ribbon-like, whereas fibers do not have parallel sides or rounded edges and tend to have dark edges



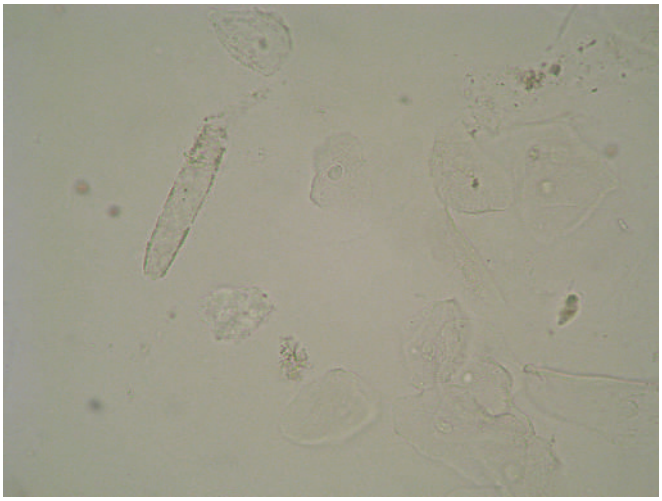
A-53. Hyaline cast.



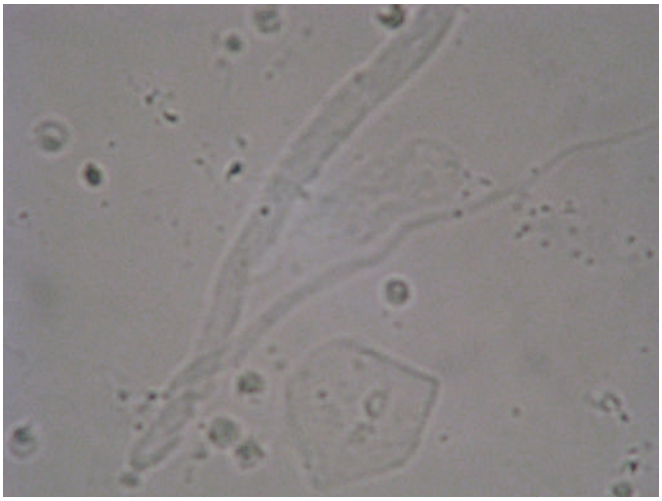
A-55. Hyaline cast. Note refractile fiber in the top left corner.



A-54. Hyaline cast. Several RBCs present.



A-56. Hyaline cast and numerous squamous epithelial cells.



A-57. Cylindroid.

Red Blood Cell Casts

Description

- There are few to many RBCs dispersed in the protein matrix
- They appear red to brown but vary in size and shape

Clinical Significance

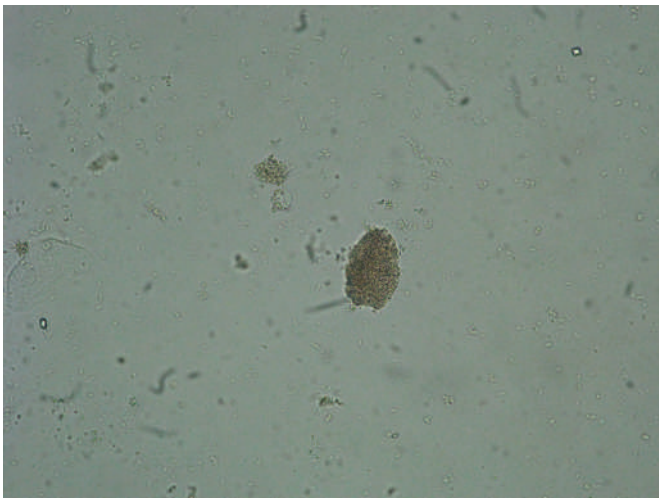
- RBC casts are associated with damage to the glomerulus or glomerulonephritis, bleeding into the nephron, and lupus

UA Correlations

- Increased RBCs
- Protein
- Blood

Tips

- RBC clumps can be confused with RBC casts
- Casts have parallel sides with rounded edges
- The RBCs should be distinct and inside the protein matrix to be called a cast
- If the RBCs have degenerated to a yellow-brown homogenous structure, it is called a hemoglobin cast



A-58. Hemoglobin cast.

White Blood Cell Casts

Description

- WBCs with prominent nuclei are found in a cylindrical matrix with parallel sides and rounded edges that vary in size and length

Clinical Significance

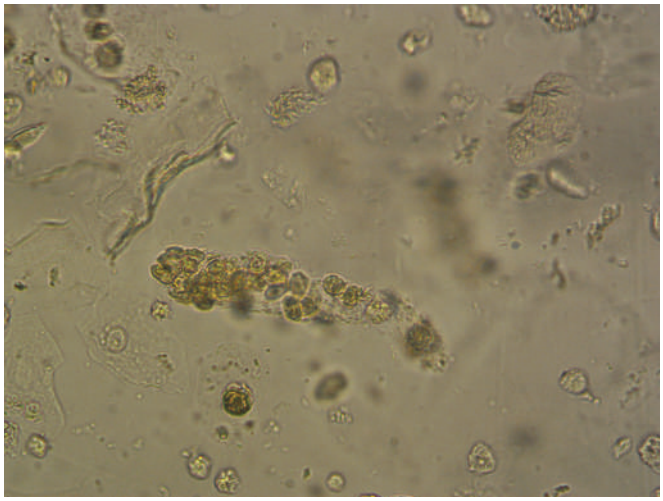
- The presence of WBC casts can indicate renal inflammation, infection, acute pyelonephritis, or interstitial nephritis
- The presence of WBC casts can distinguish pyelonephritis or upper UTI from cystitis or lower UTI

UA Correlations

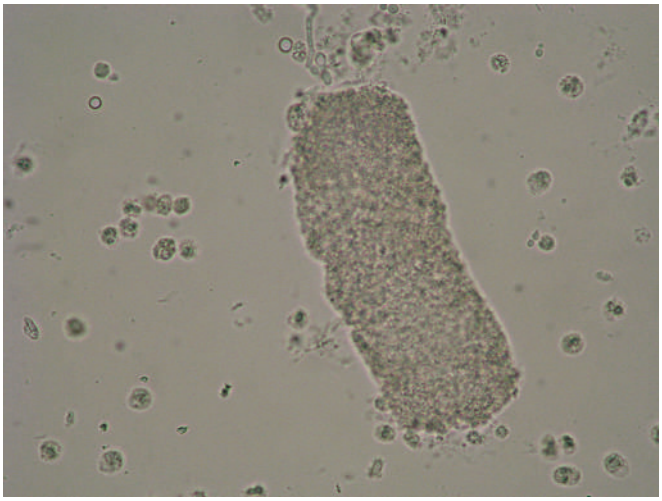
- Protein
- Leukocyte esterase
- Increased WBCs

Tips

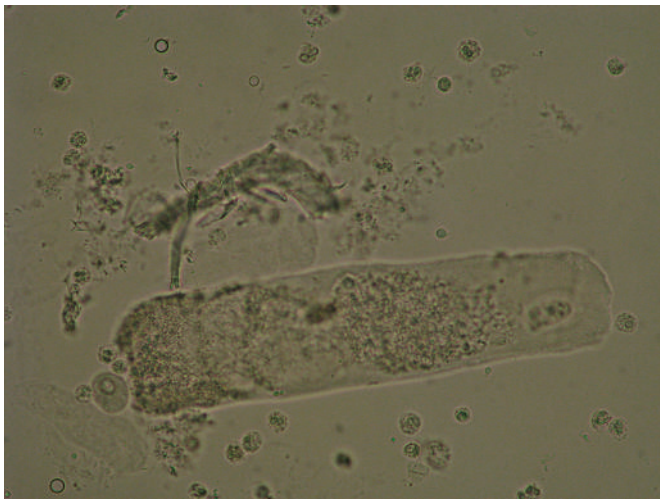
- WBC clumps can be confused with WBC casts
- To identify the cast, the WBCs are intact with visible nuclei within the protein matrix



A-59. WBC cast. Notice the WBCs within the matrix of the cast and free-floating in the urine sediment.



A-61. Broad WBC cast with many free-floating WBCs, two RBCs, and budding yeast present.



A-60. Degenerating WBC cast. WBCs, RBCs, a hyaline cast, a RTE, and an artifact fiber are also present.

Bacterial Casts

Description

- Casts contain bacilli within the protein matrix

Clinical Significance

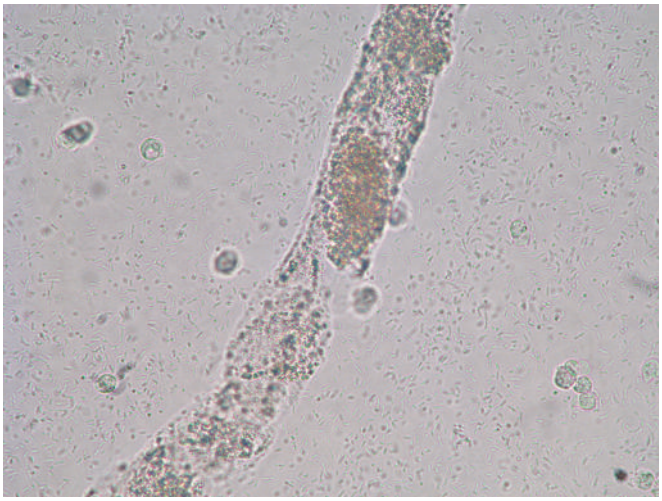
- Diagnostic for pyelonephritis or upper UTI

UA Correlations

- WBCs
- Bacteria
- Protein
- Leukocyte esterase
- Nitrite

Tips

- These casts can also contain WBCs and are called mixed casts
- These casts can be confused with granular casts; confirmation is through a Gram stain



A-62. Mixed granular cast with bacteria embedded in the matrix. Note bacteria surrounding in cast. Bacterial casts are not common.

Epithelial Cell Casts

Description

- RTE cells with large nuclei are seen within protein matrix; they have a high refractive index
- As the RTE cells undergo degenerating changes, they become smaller and round resembling WBCs

Clinical Significance

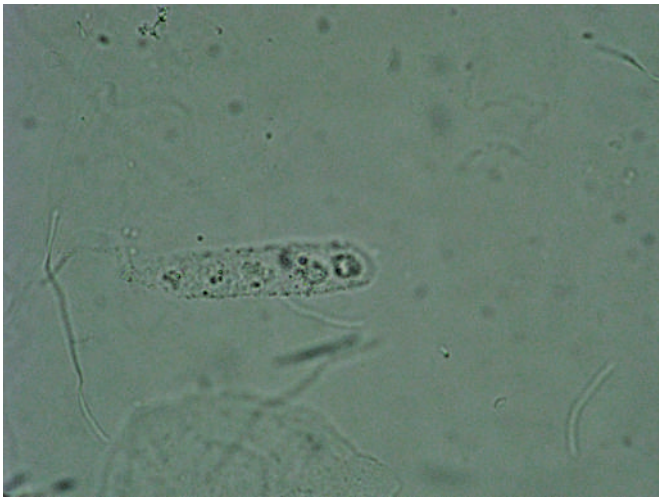
- Most often associated with acute tubular necrosis, advanced tubular destruction, and heavy metal or drug toxicity

UA Correlations

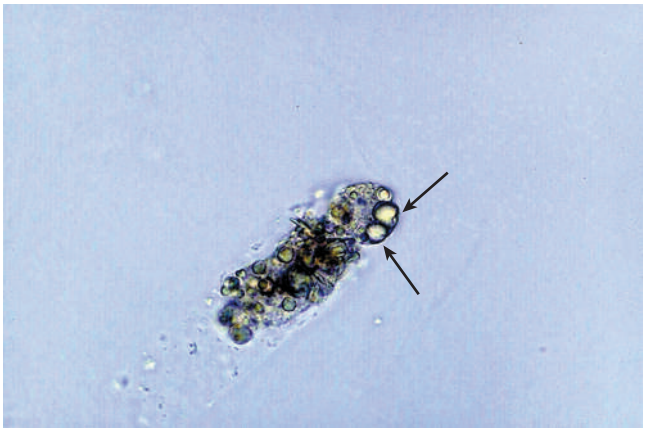
- Protein
- RTE cells

Tips

- Epithelial cell casts can be confused with WBC casts
- A negative leukocyte esterase can help distinguish the epithelial cell cast from a WBC cast



A-63. Renal epithelial cell cast.



A-64. Cast with oval fat bodies.

Granular Casts

Description

- Casts are a degeneration of cellular casts; granules begin large and are coarse and granular
- As stasis continues, granules break down further and are called fine granular casts
- Coarse granules are dark in color, and fine granules tend to be pale
- Casts can be of various sizes and shapes

Clinical Significance

- These casts can indicate urinary stasis, tubular disease, glomerulonephritis, pyelonephritis, or may be seen in stress or after exercise

UA Correlations

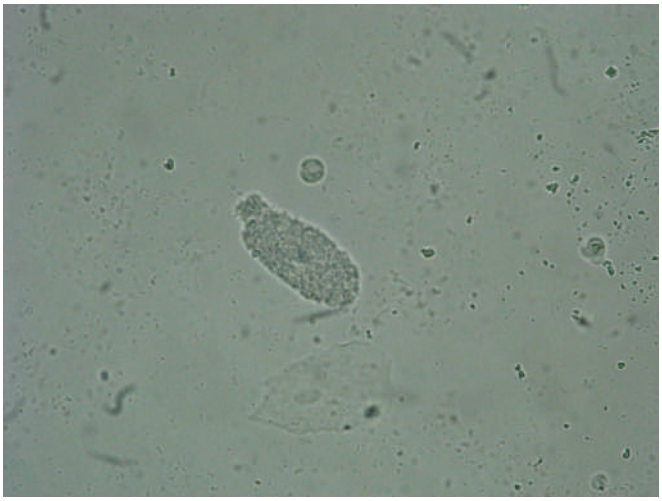
- Protein
- RBCs
- WBCs
- Cellular casts

Tips

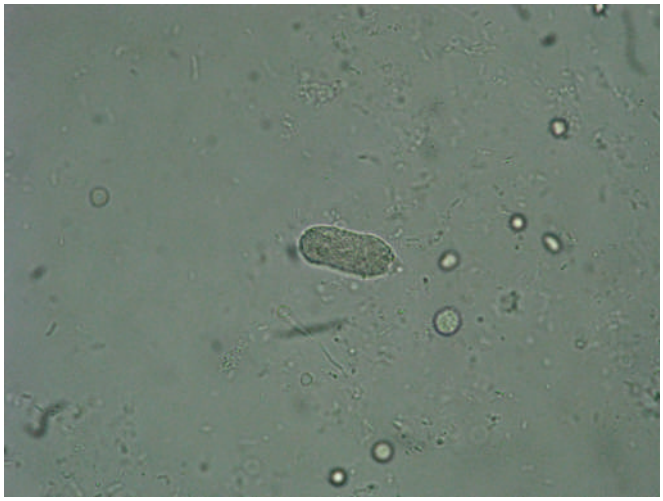
- Examine under low power, but casts are identified under high power
- These casts can be reported as coarse, fine, or just granular
- They can be confused with fibers or clumps of sediment
- For it to be a cast, granules must be in a surrounding protein matrix



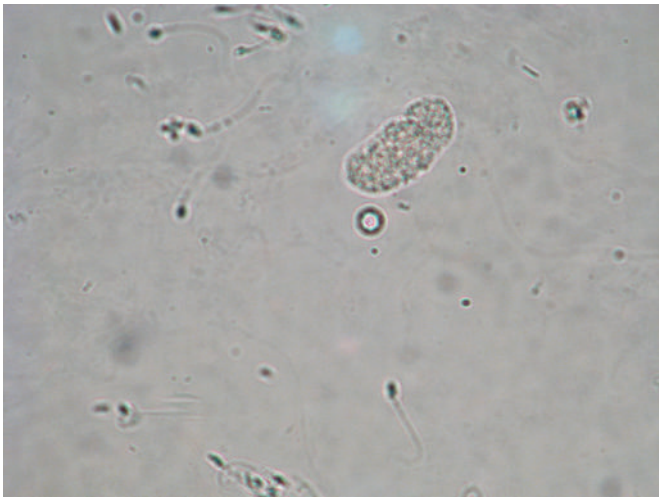
A-65. Granular cast.



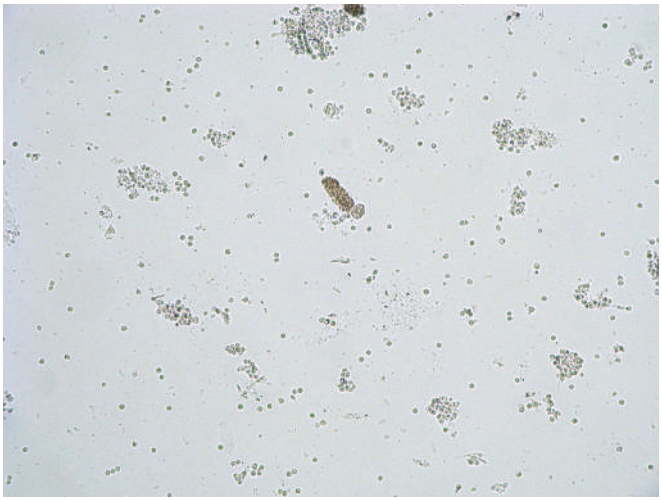
A-67. Granular cast with a squamous epithelial cell and RBC.



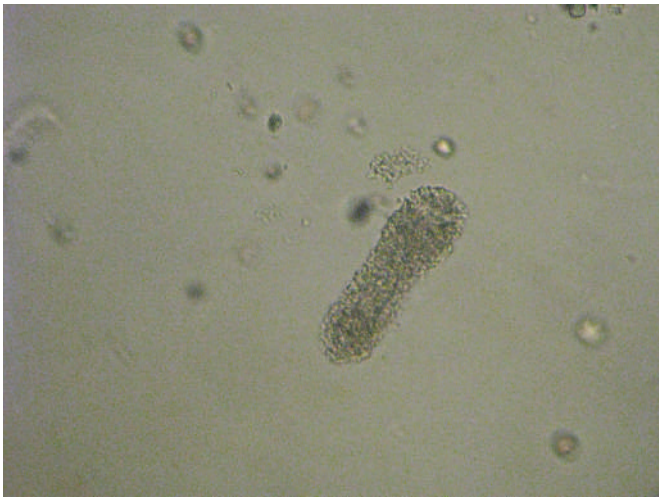
A-66. Granular cast. Note RBCs, WBCs, and bacteria.



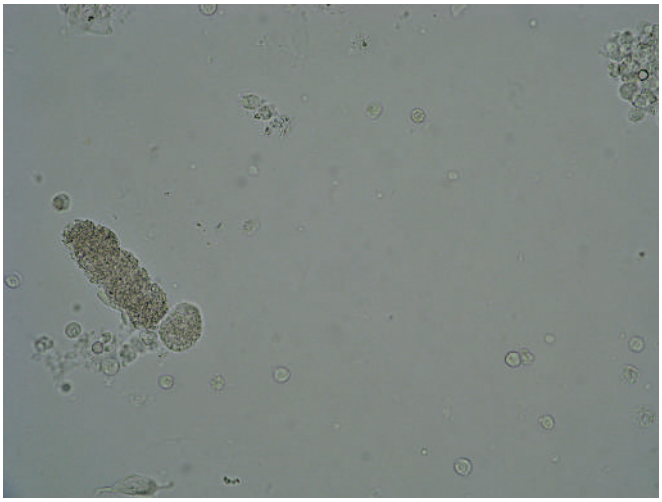
A-68. One granular cast. Numerous sperm present.



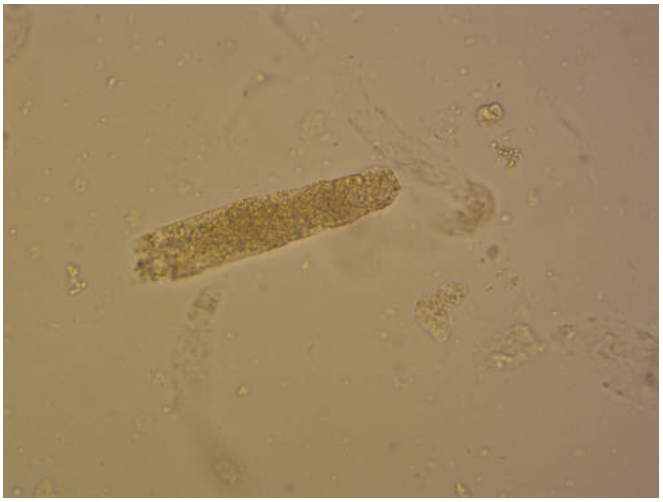
A-69. Granular cast.



A-72. Granular cast.



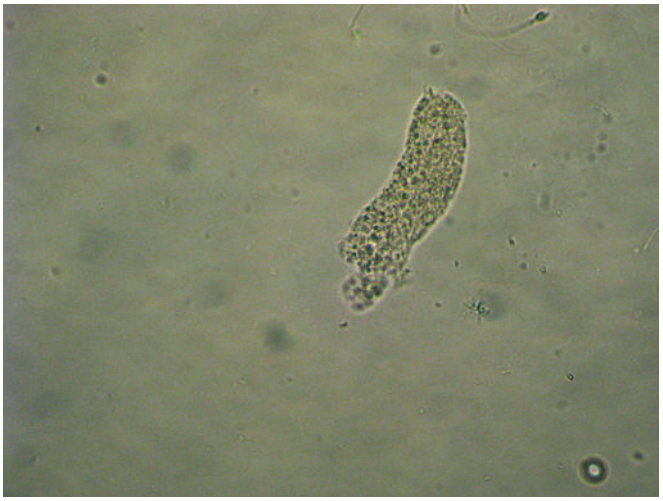
A-70. Granular cast. RBCs are also present.



A-73. One granular cast and two hyaline casts.



A-71. One granular cast with four squamous epithelial cells. Note the highly refractile artifact.



A-74. Granular cast. Sperm present at the top of image. Amorphous is also present.

Mixed Cell Casts

Description

- Contain multiple cellular constituents, which can include RBCs, WBCs, RTE cells, and bacteria

Clinical Significance

- Can indicate renal disease and are usually seen with other cellular casts

UA Correlations

- Protein
- Leukocyte esterase
- RBCs
- WBCs
- RTE cells

Tips

- Mixed casts should have separate free-floating cellular elements present



A-75. Mixed-cell cast. Note free-floating RBCs and WBCs.

Fatty Casts

Description

- Highly refractile
- Casts may contain fat droplets and intact oval fat bodies of varying sizes within the protein matrix

Clinical Significance

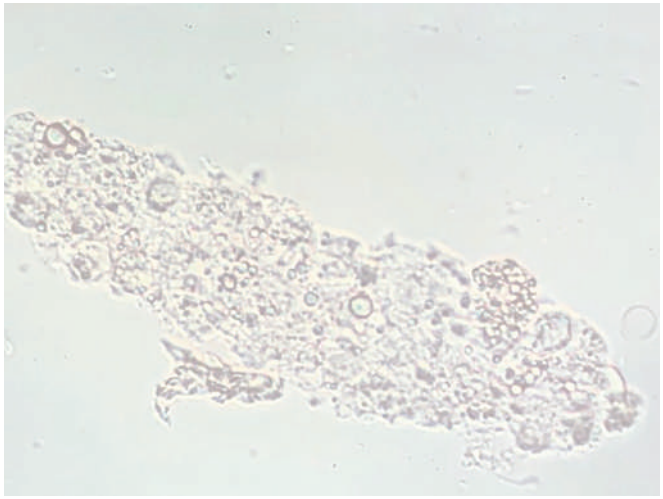
- Associated with nephrotic syndrome, acute tubular necrosis, diabetes mellitus, toxic tubular necrosis, and crushing injuries

UA Correlations

- Protein
- Free fat droplets
- Oval fat bodies

Tips

- Maltese cross formations can be seen with cholesterol under polarization



A-76. Fatty cast. Note intact oval fat bodies within the matrix.

Waxy Casts

Description

- Highly refractile with broken, jagged, or blunt ends with cracks on the margin of the matrix
- May be colorless to gray

Clinical Significance

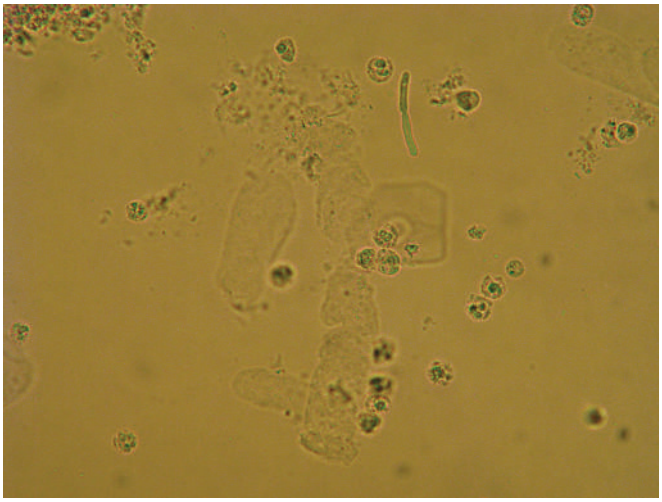
- Casts indicate urinary stasis, chronic renal failure, or end-stage renal disease

UA Correlations

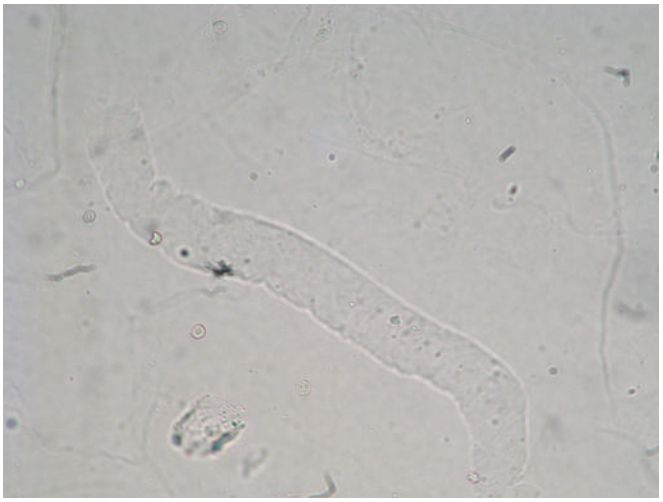
- Protein
- Increased RBCs
- Increased WBCs
- Cellular casts
- Granular casts

Tips

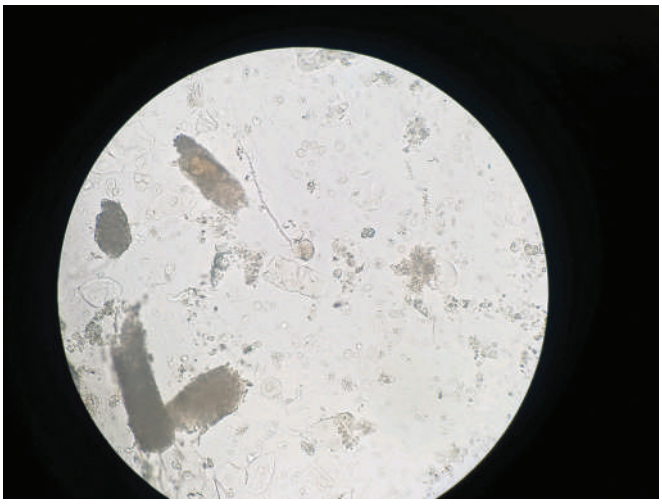
- These casts tend to look like paraffin shavings sitting on top of the urine
- They are often broad in size due to renal stasis



A-77. Waxy cast with a fragmented end and notches on its side. WBCs are also present.



A-79. Waxy cast.



A-78. A waxy cast is located in the center with four granular casts to the side. Also present are RTE cells, RBCs, and squamous epithelial cells.

Broad Casts

Description

- Named for their wide size
- Formed due to distention of the collecting tubules where they are formed

Clinical Significance

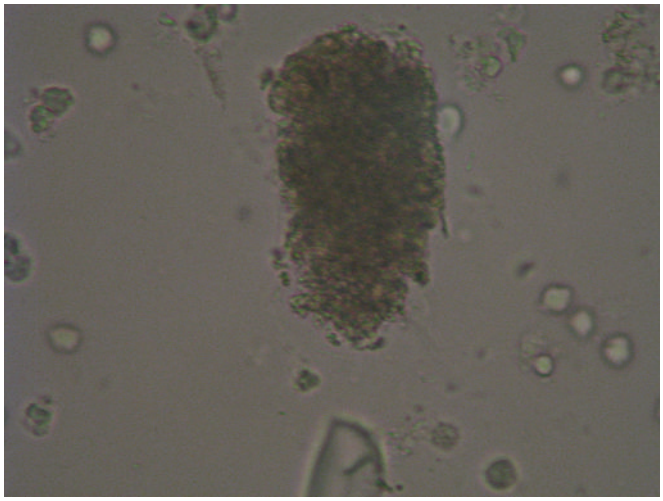
- Represent extreme urinary stasis and renal failure

UA Correlations

- Protein
- Increased RBCs
- Increased WBCs
- Granular casts
- Waxy casts

Tips

- Granular or waxy casts may be classified as broad



A-80. Broad granular cast.

Crystals/Acid

Uric Acid Crystals

Description

- Yellow to brown in color; can occur in many shapes, including rhombic, four-sided flat plates, rosettes, barrels, and wedges

Clinical Significance

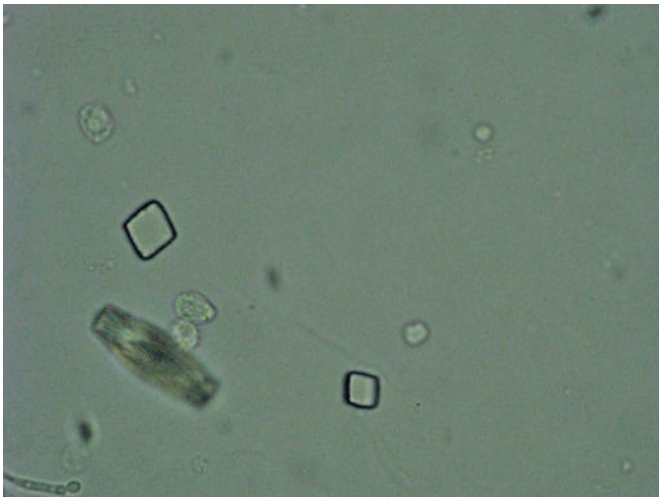
- Can be found in normal individuals
- Can be associated with pathological conditions, such as urinary stones, gout, high purine metabolism, acute febrile conditions, newborns, and patients with leukemia receiving chemotherapy

UA Correlations

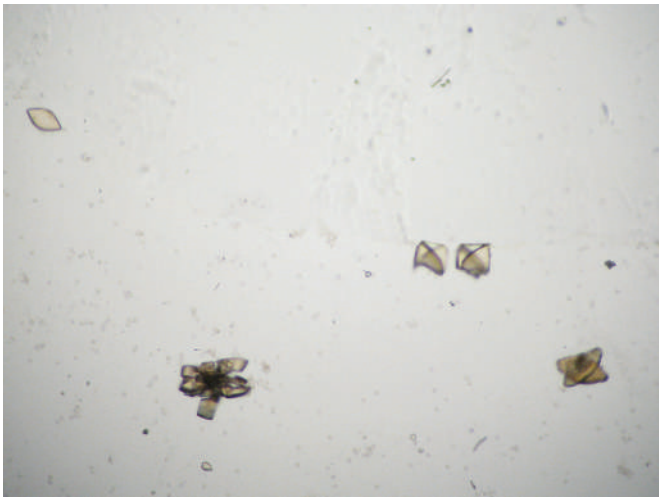
- Acid pH

Tips

- These crystals can be confused with cystine crystals
- Uric acid crystals are birefringent under polarized light
- Crystals have four sides and look more like a football or lemon wedge
- Cystine have six sides or look like a stop sign



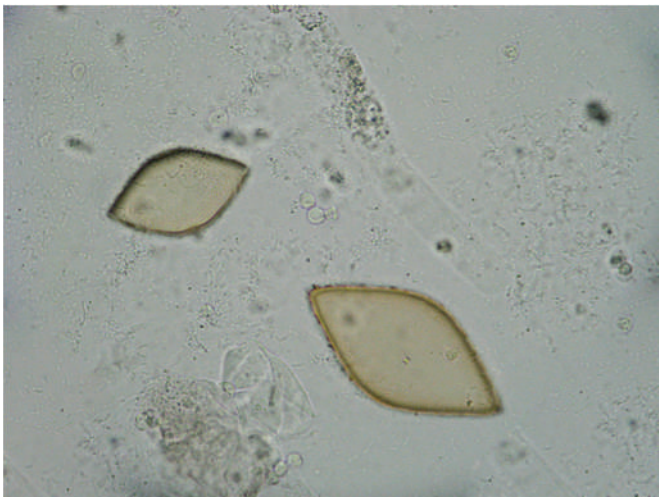
A-81. Uric acid crystals, with barrel shape.



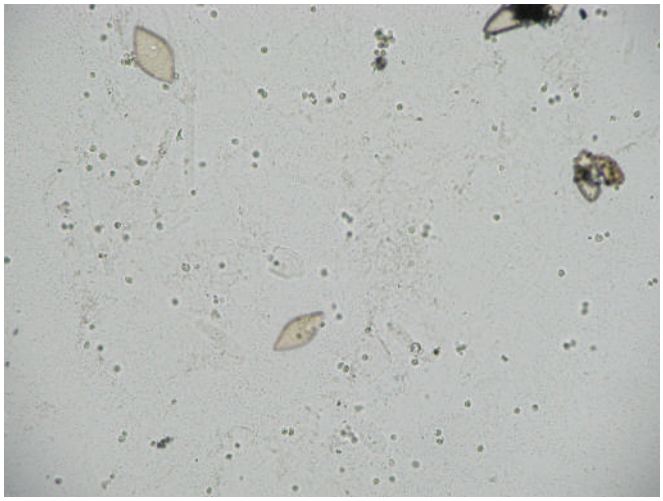
A-83. Uric acid crystals. Note the rosette and wedge shapes.



A-82. Uric acid crystal clump.



A-84. Two uric acid crystals observed using high-power magnification. One hyaline cast, WBCs, and bacteria are also present.



A-85. Uric acid crystals observed using low-power magnification. Note the numerous hyaline casts.

Amorphous Urate Crystals

Description

- Appear as yellow to reddish granules

Clinical Significance

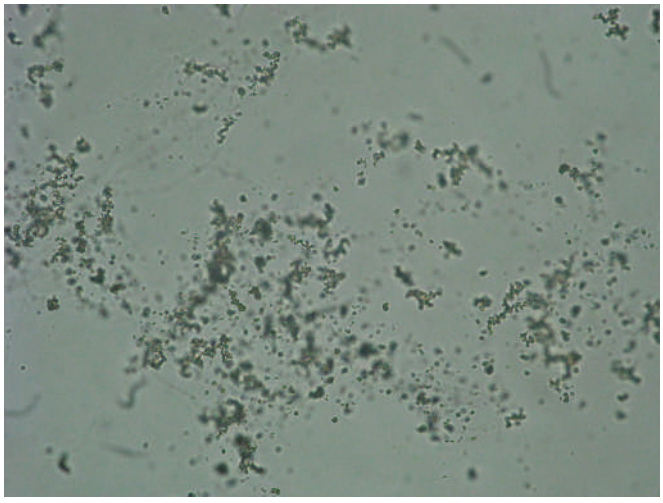
- No clinical significance
- These are salts of sodium, potassium, magnesium, and calcium; usually appear in older urine

UA Correlations

- Acid pH
- Sediment has a pink to brick dust–like color after centrifugation

Tips

- When amorphous urates clump, they tend to look like a granular cast
- Always look for the protein matrix to define a cast
- They also can hide bacteria and RBCs
- Amorphous urates dissolve when heated to 60°C



A-86. Amorphous urates distinguished by acid pH.

Calcium Oxalate Crystals

Description

- Often appear as colorless, envelope-shaped crystals with an X or cross in the middle
- Can appear as dumbbell- or small oval–shaped

Clinical Significance

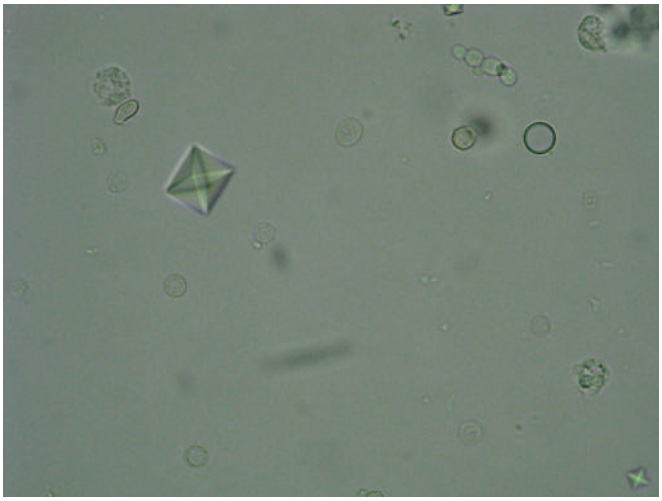
- Frequently can be found in normal urine after ingesting oxalate-rich food, such as tomatoes, spinach, oranges, fruit juice, vitamin C, and chocolate
- Pathological conditions include ethylene glycol poisoning, liver disease, and renal calculi

UA Correlations

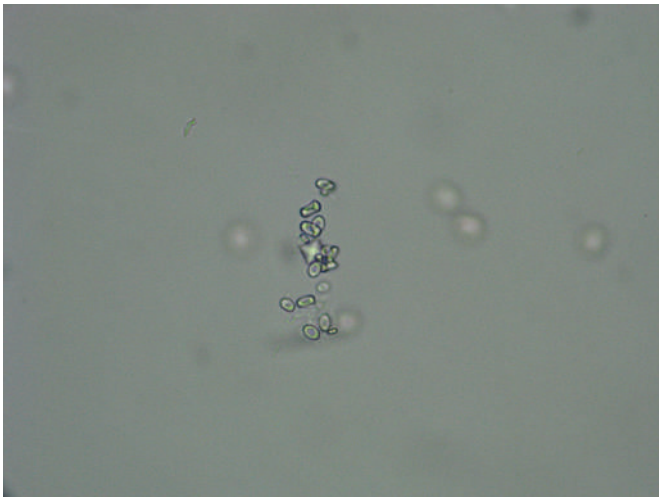
- Acid or neutral pH

Tips

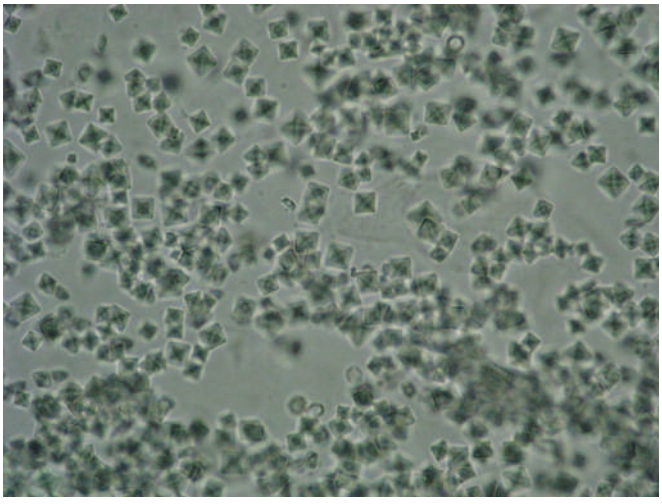
- The more common calcium oxalate crystals have the shape of an emerald-cut diamond
- The oval shapes tend to look like RBCs, but they are more elliptical and tend to have a dark outline along their edge



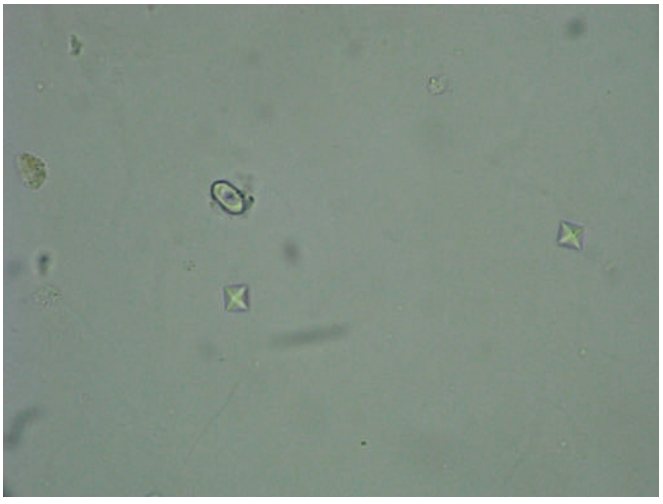
A-87. Calcium oxalate crystals. Note RBCs and budding yeast.



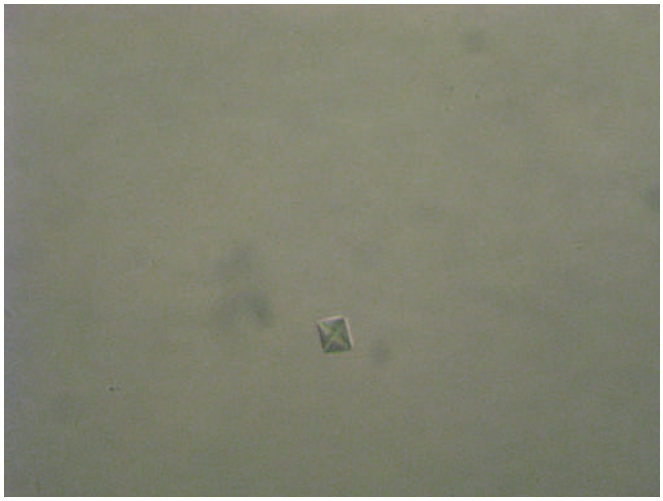
A-89. Monohydrate calcium oxalate crystals.



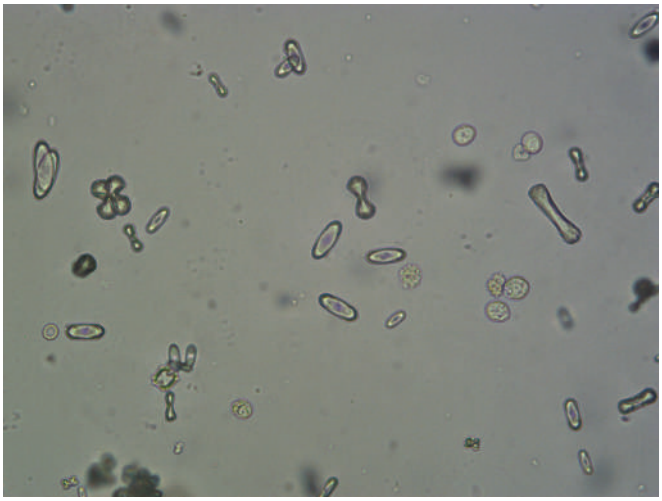
A-88. Clump of calcium oxalate crystals.



A-90. One monohydrate calcium crystal and two classic dihydrate calcium oxalate crystals.



A-91. Calcium oxalate crystal.



A-92. Monohydrate calcium oxalate crystals.

Sodium Urate Crystals

Description

- Colorless or yellowish, slender needles with blunt ends that can be found singly or in clusters

Clinical Significance

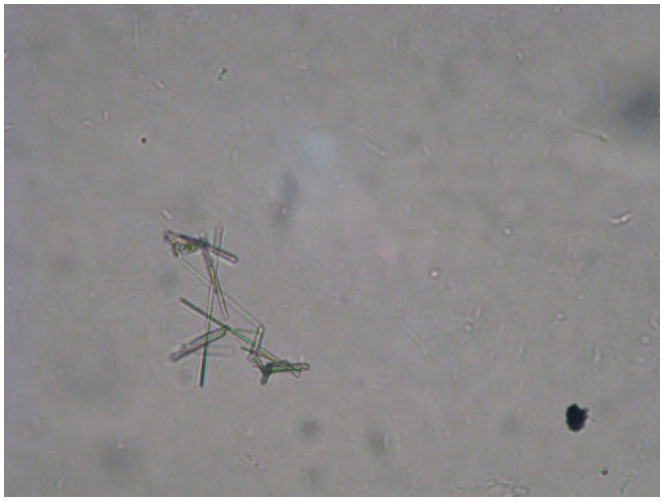
- No clinical significance

UA Correlations

- Acid pH

Tips

- These are usually seen when urine has been standing
- When found in clusters, they look like "pick-up sticks"



A-93. Sodium urate crystals.

Crystals/Alkaline

Amorphous Phosphate Crystals

Description

- Fine colorless granules that can be hard to distinguish from amorphous urates; differentiated by sediment color and urine pH

Clinical Significance

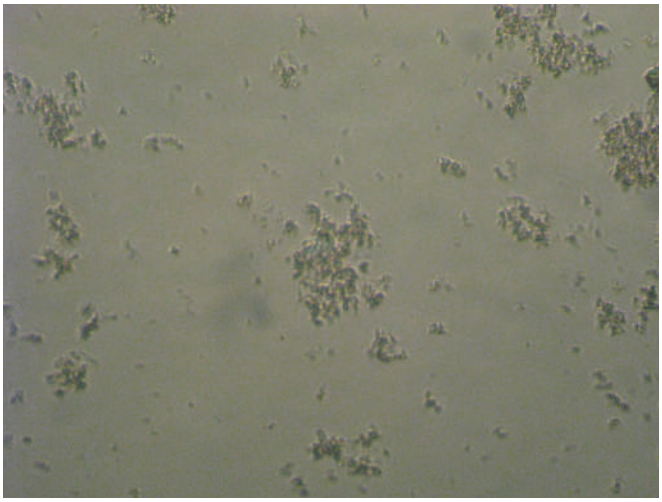
- No clinical significance

UA Correlations

- Urine is cloudy when many crystals are present
- Alkaline or neutral pH
- White sediment after centrifugation

Tips

- Amorphous phosphates can interfere with the microscopic examination
- RBCs and bacteria may not be visualized
- Amorphous phosphates do not dissolve when heated at 60˚C
- Refrigeration increases their presence
- They are distinguished from urates by urine pH



A-94. Amorphous phosphate crystals.

Triple Phosphate Crystals

Description

- Most common form is colorless prisms with three to six sides referred to as coffin covers
- Vary in size
- When they denature, they become feathery or leaf-like

Clinical Significance

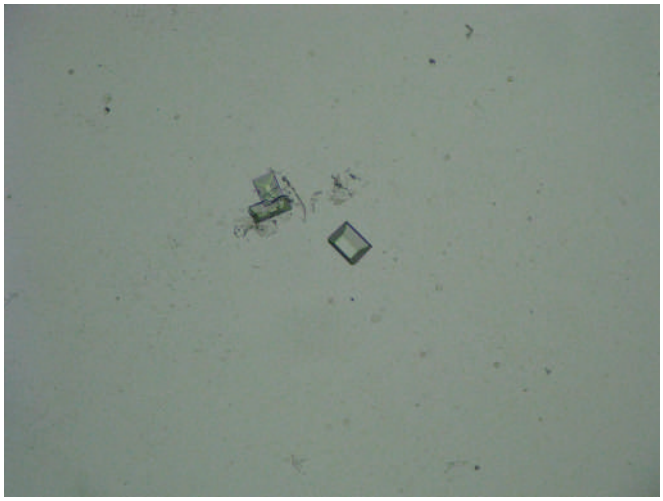
- Can be seen in healthy individuals with no clinical significance
- Can be associated with urinary stones and UTI

UA Correlations

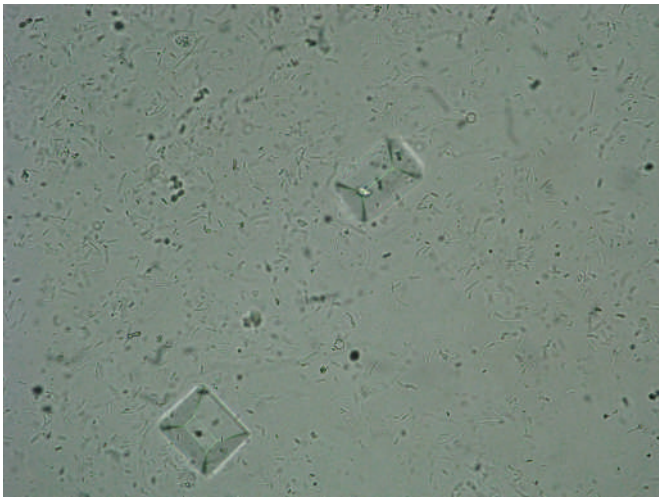
- Alkaline or neutral pH

Tips

- Triple phosphate crystals vary in size and sometimes have the shape and size of a calcium oxalate
- The cross on top of the crystal overlaps or meets in the middle of the triple phosphate crystal
- For calcium oxalate crystals, the X crosses in the middle



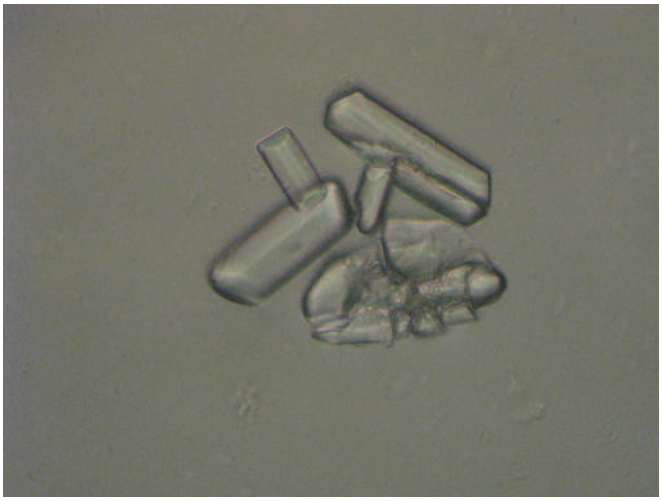
A-95. Triple phosphate crystals.



A-98. Square triple phosphate crystals with bacteria and yeast present in urine sediment.



A-96. Clump of triple phosphate crystals.



A-99. Triple phosphate crystals.



A-97. Triple phosphate crystal with RBCs.

Calcium Phosphate Crystals

Description

- Colorless to grayish-white thin needles
- Arranged in bundles or thin, long prisms and can be arranged in rosettes or can be flat plates
- Tend to float on the top of the urine

Clinical Significance

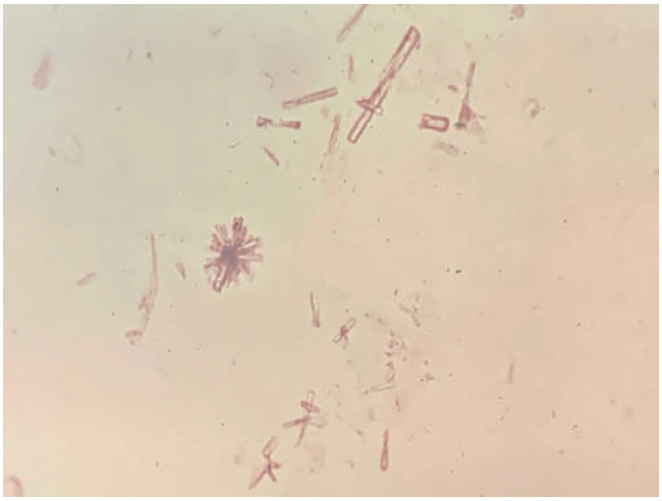
- Can be found in normal urine and are associated with renal stones

UA Correlations

- Alkaline or neutral pH

Tips

- Sometimes the sheets resemble large degenerating squamous cells



A-100. Calcium phosphate crystals.

Calcium Carbonate Crystals

Description

- Small, colorless, dumbbell-shaped; appear in pairs or may have a spherical shape

Clinical Significance

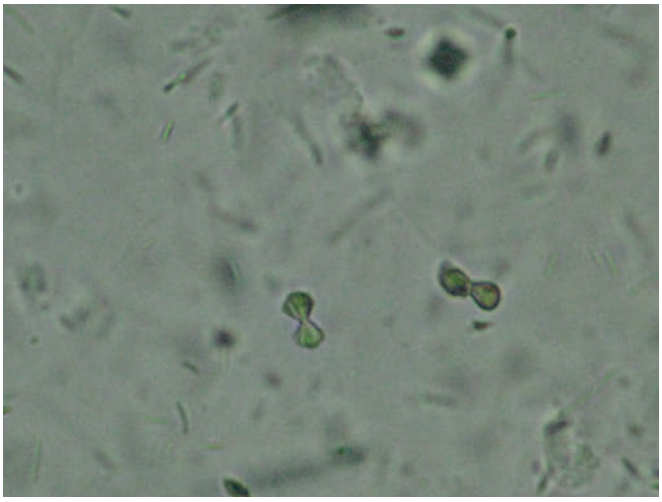
- No clinical significance

UA Correlations

- Alkaline pH

Tips

- Calcium carbonate can appear in clumps and resemble amorphous phosphates or be mistaken for bacteria
- They tend to be a little larger than amorphous phosphates



A-101. Calcium carbonate crystals.

Ammonium Biurate Crystals

Description

- Appear as yellow to brown spheres with long spicules surrounding the crystal; resemble a "thorny apple"

Clinical Significance

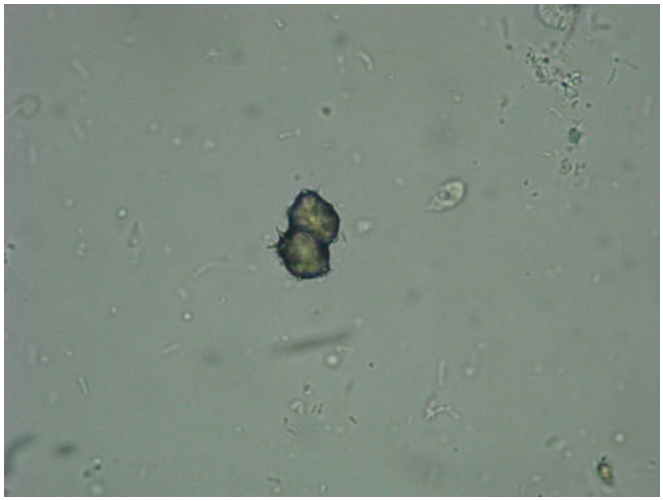
- Usually seen in urines that have not been stored properly
- May be associated with the presence of ammonia produced by urea-splitting bacteria

UA Correlations

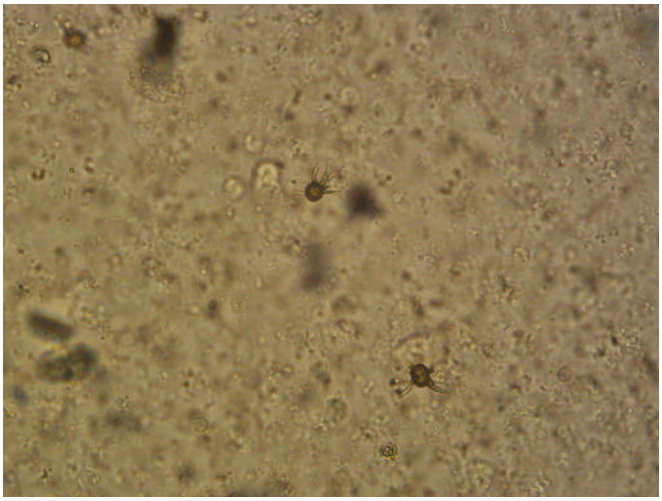
- Alkaline pH

Tips

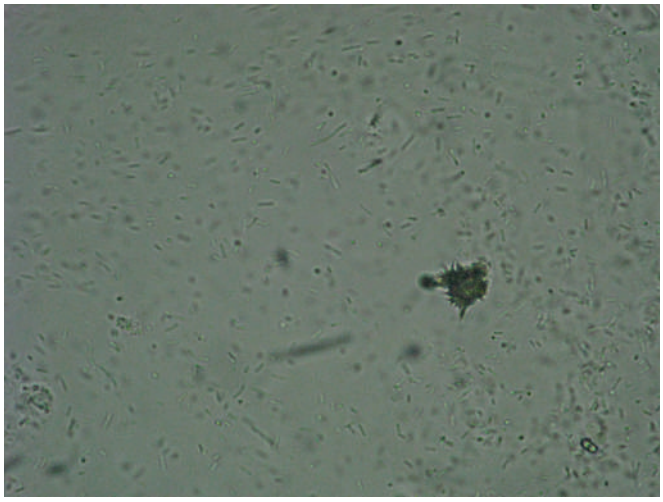
- Usually encountered in old specimens



A-102. Two ammonium biurate crystals.



A-104. Ammonium biurate crystals.



A-103. Ammonium biurate crystal. Note bacteria.

Abnormal Crystals

Cystine Crystals

Description

- Appear as colorless hexagonal plates with sides that are not always even, resembling a stop sign
- Can be found single or overlapping each other to form rosettes

Clinical Significance

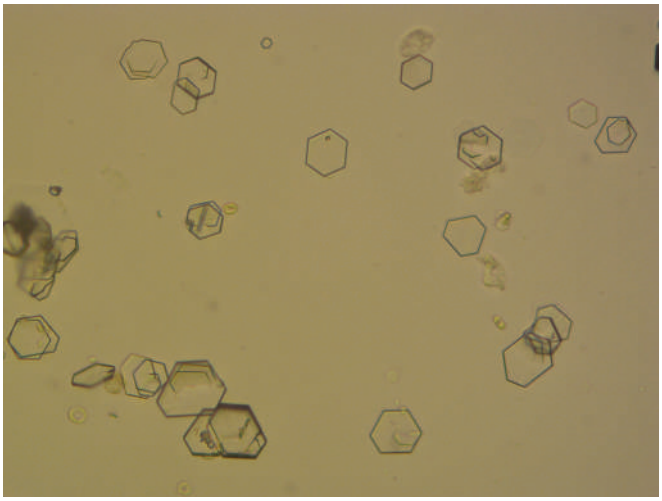
- Clinically significant and indicate a metabolic hereditary disorder called cystinosis or cystinuria

UA Correlations

- Acid pH

Tips

- Cystine crystals can be distinguished from uric acid crystals using polarization
- Uric acid crystals are birefringent under polarization, whereas cystine crystals are not
- Cystine crystals appear as a six-sided stop sign, whereas uric acid crystals appear as a four-sided stop sign



A-105. Cystine crystals.

Leucine Crystals

Description

- Amino acid crystals
- Appear as oily or highly refractile yellow to brown spheres with radial striations

Clinical Significance

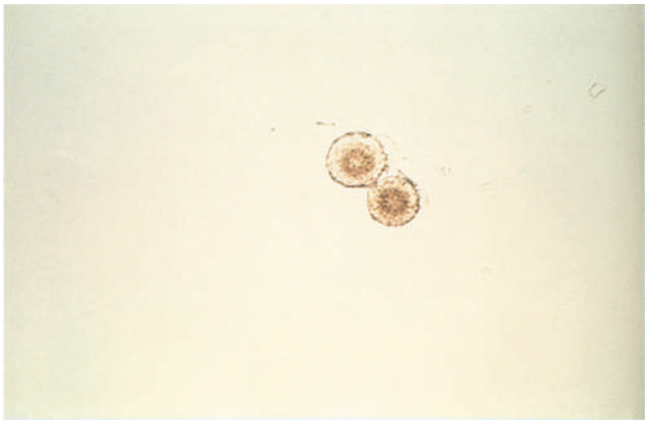
- Can appear in patients with severe liver disease, terminal cirrhosis, viral hepatitis, and maple syrup disease
- Can also be seen in patients with tyrosine crystals

UA Correlations

- Acid or neutral pH
- Bilirubin

Tips

- These crystals can sometimes resemble fat globules
- Under polarization, they produce a pseudo–Maltese cross pattern
- They also can resemble wagon wheels or concentric circles resembling a tree's growth pattern or striations of a tree
- These crystals are rarely seen in urinalysis



A-106. Leucine crystals (×400). Note the concentric circles.

Tyrosine Crystals

Description

- Amino acid crystals
- Appear as fine needles occurring in shelves or clusters that are colorless or yellow

Clinical Significance

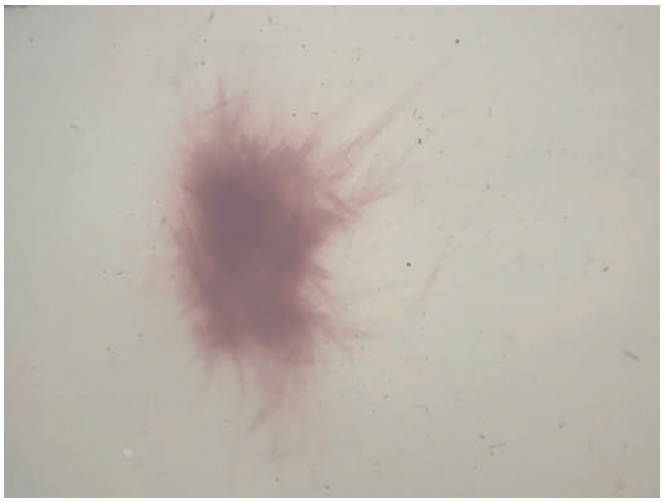
- Associated with liver disease and with inherited disorders of amino acid metabolism

UA Correlations

- Acid or neutral pH
- Bilirubin

Tips

- This is a rare crystal not seen very often in urine sediment
- They are often observed with leucine crystals



A-107. Tyrosine crystal.

Cholesterol Crystals

Description

- Appear as irregular, rectangular, large transparent plates with a notch in one or more corners
- Often appear in stacks

Clinical Significance

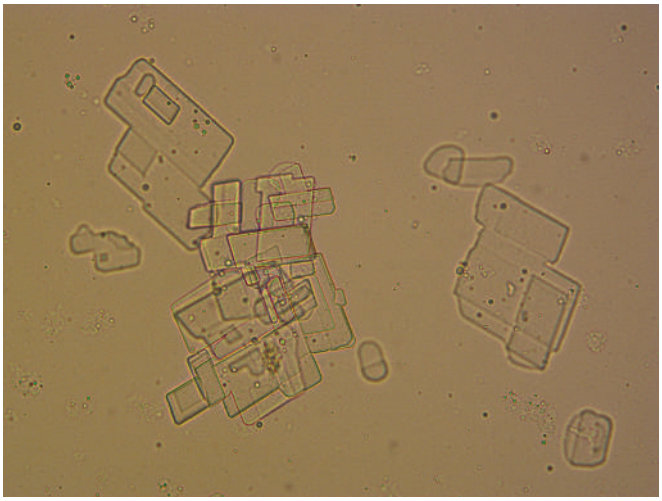
- Can be seen in patients with nephrotic syndrome, chyluria, and disorders producing lipiduria

UA Correlations

- Acid pH
- Protein
- Oval fat bodies
- Fatty casts
- Fat globules

Tips

- These crystals are seen after refrigeration
- They are highly birefringent with polarized light



A-108. Cholesterol crystals.

Bilirubin Crystals

Description

- Appear as small clusters of fine clumped needles or spheres
- Dark yellow in color

Clinical Significance

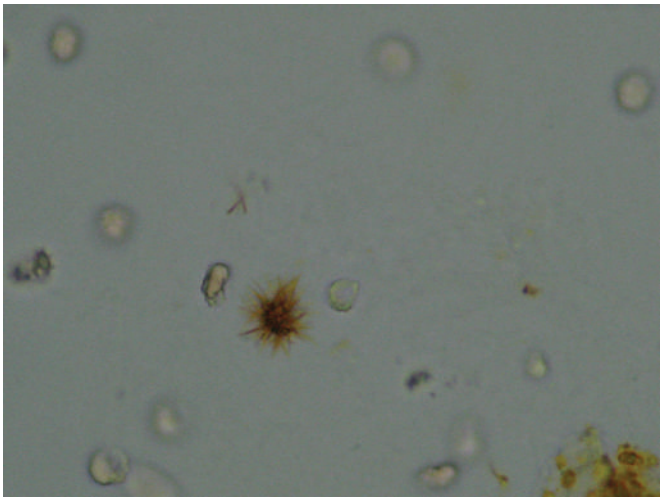
- Associated with hepatic disease, producing large amounts of bilirubin

UA Correlations

- Acid pH
- Bilirubin

Tips

- A positive result for bilirubin must be present for these crystals to be identified



A-109. Bilirubin crystal. Note the classic bright yellow color.

Sulfonamide Crystals

Description

- Can appear in many different forms; most shapes are bundles of needles that resemble stacks of wheat or have a fan shape
- Can be brown in color

Clinical Significance

- With the development of more soluble sulfur drugs, these crystals are not found often
- Patients with UTIs are treated with sulfonamides
- Also can be associated with renal calculi or tubular damage

UA Correlations

- Acid pH

Tips

- These crystals can be confused with ammonium biurate
- Ammonium biurate crystals are found with an alkaline pH



A-110. Sulfa crystal.

Ampicillin Crystals

Description

- Appear as colorless long needles that tend to form bundles after refrigeration

Clinical Significance

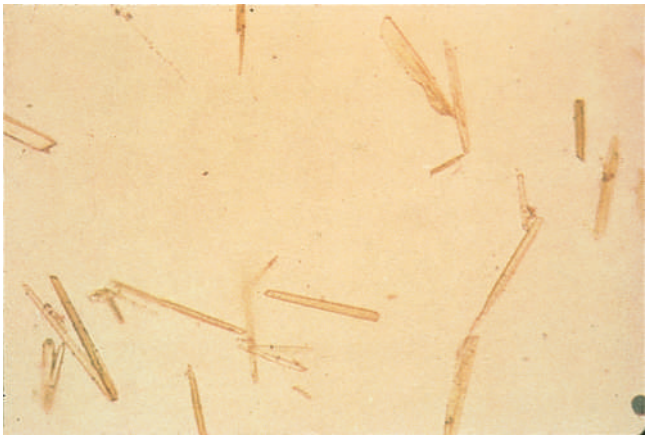
- Indicative of large doses of ampicillin without adequate hydration

UA Correlations

- Acid or neutral pH

Tips

- Knowledge of patient history is helpful to identify these crystals



A-111. Ampicillin crystals.

Artifacts

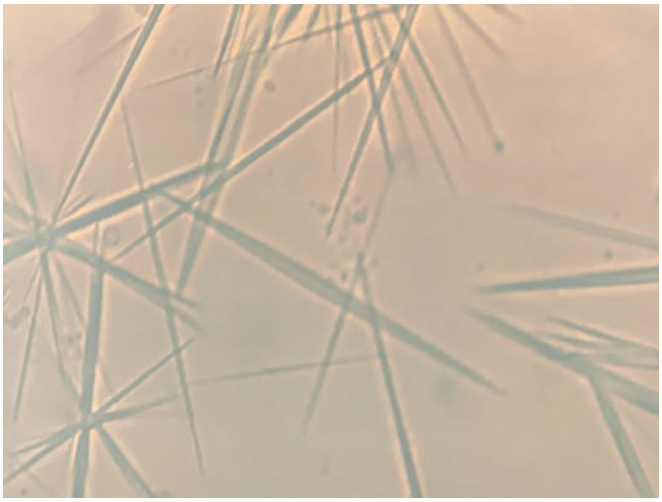
Radiographic Dye Crystals

Description

- When ionic radiographic crystals precipitate out in urine, they appear as flat, elongated rectangles that are strongly birefringent

Clinical Significance

- X-ray procedures, such as a pyelogram, use dye to visualize the urinary tract



A-112. Radiographic dye crystals.

UA Correlations

- High specific gravity when using a refractometer

Tips

- These have a similar appearance to cholesterol crystals
- Cholesterol crystals are accompanied by other lipid elements
- May persist for 3 days after the procedure

Starch

Description

- Vary in size
- Have a Y indentation in the center
- Not perfectly round, but have more scalloped edges

Clinical Significance

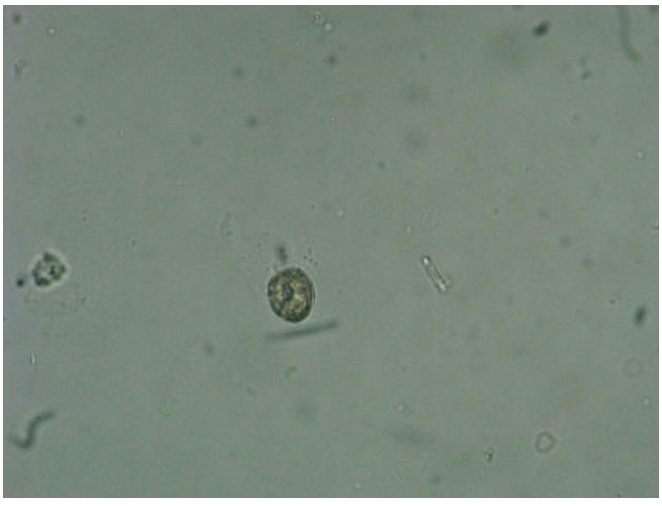
- Caused by contamination from baby powder or powder from health care worker gloves

UA Correlations

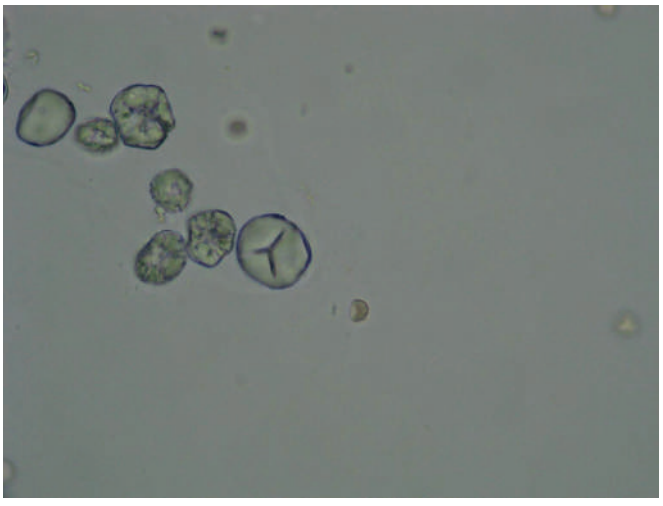
- N/A

Tips

- Starch or pollen granules can be confused with fat droplets
- Under polarized light, starch can appear as the Maltese cross



A-113. Starch granule.



A-114. Starch granules. Notice the dimpled centers.

Fibers

Description

- Large with distinct, flat, dark edges
- Refractile

Clinical Significance

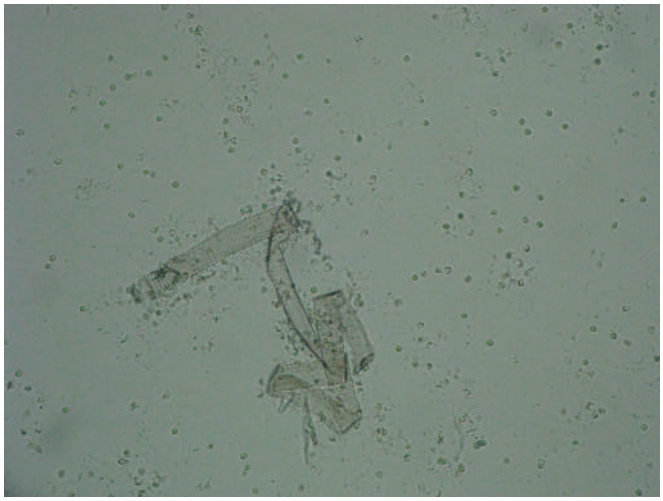
- Considered contaminants

UA Correlations

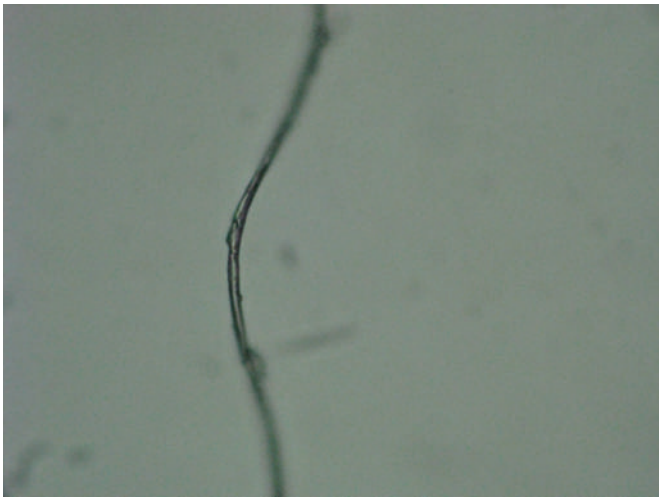
- N/A

Tips

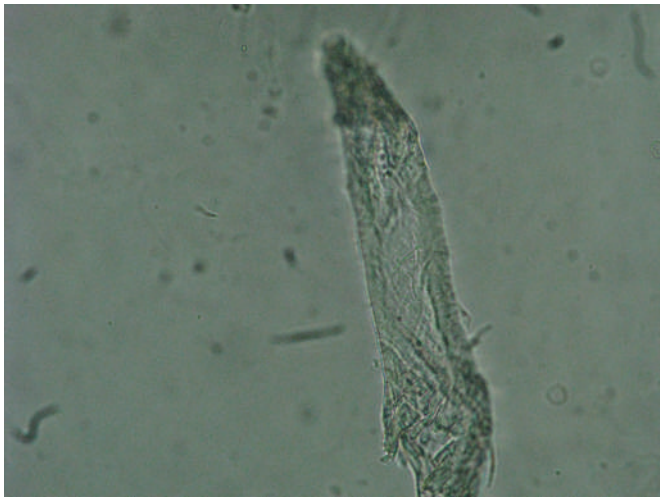
- Fibers can be plant, vegetable, fabric threads, toilet paper, cotton, or diaper
- They can resemble hyaline casts
- Fibers are thicker at their margins and polarize



A-115. Fiber. Note RBCs.



A-117. Fiber. Note refractibility.



A-116. Fiber.



A-118. Fiber.



A-119. Refractile fiber, dysmorphic RBCs, and an RTE cell.

Oil Droplets or Air Bubbles

Description

- Highly refractile; may resemble RBCs

Clinical Significance

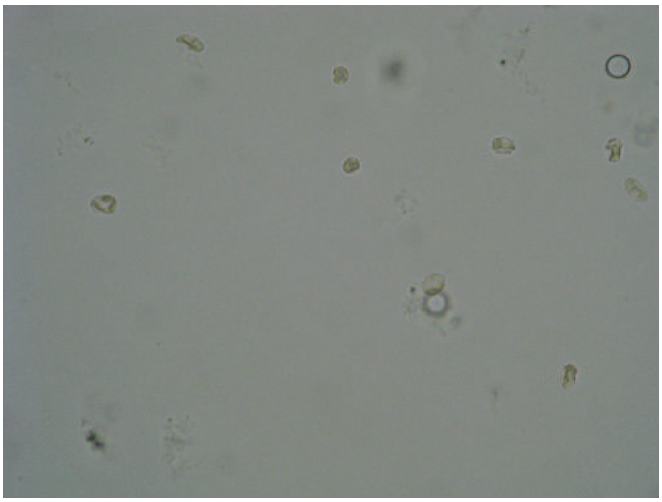
- Contamination; oil droplets from immersion oil and air bubbles formed when the specimen is placed under a cover slip are highly refractile using low power

UA Correlations

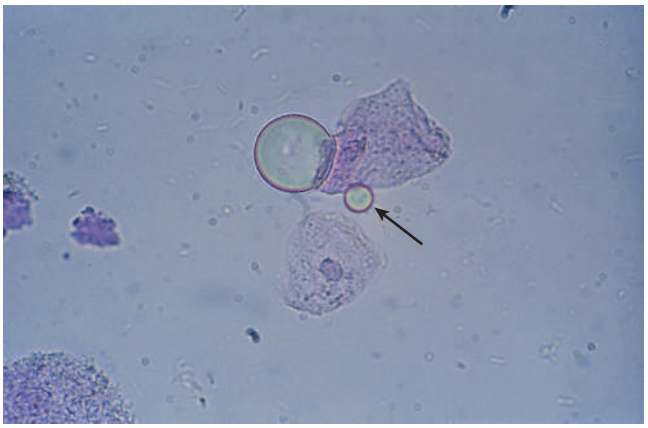
- N/A

Tips

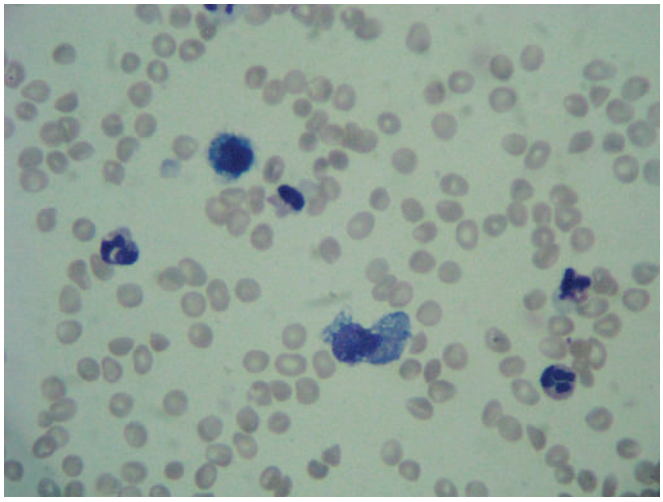
- Oil droplets and air bubbles can look like RBCs; however, their high refractibility distinguishes them



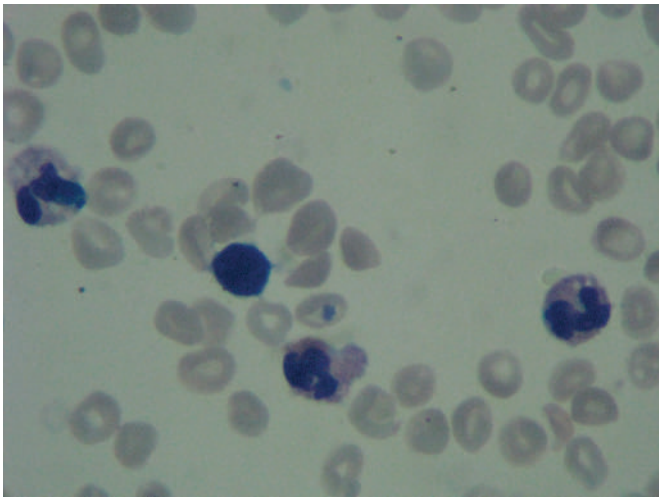
A-120. Air bubble. Note high refractibility.



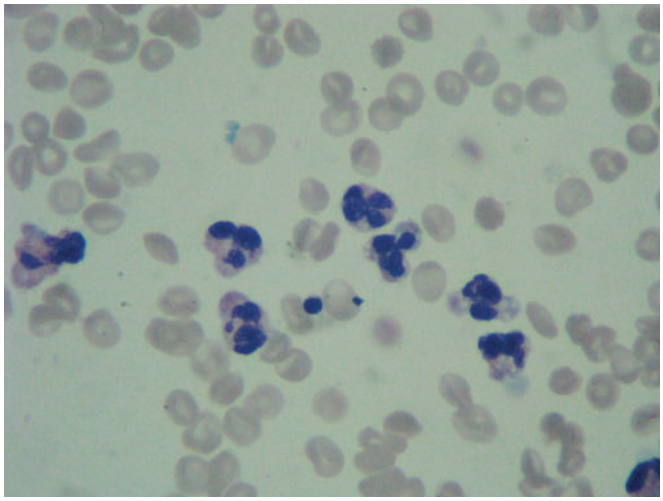
A-121. Note the oil droplet indicated by the arrow.



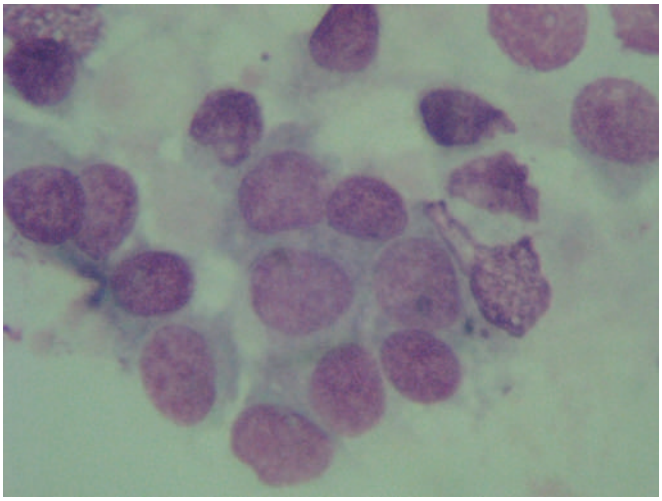
A-122. Bloody CSF with neutrophils, lymphocytes, and one monocyte.



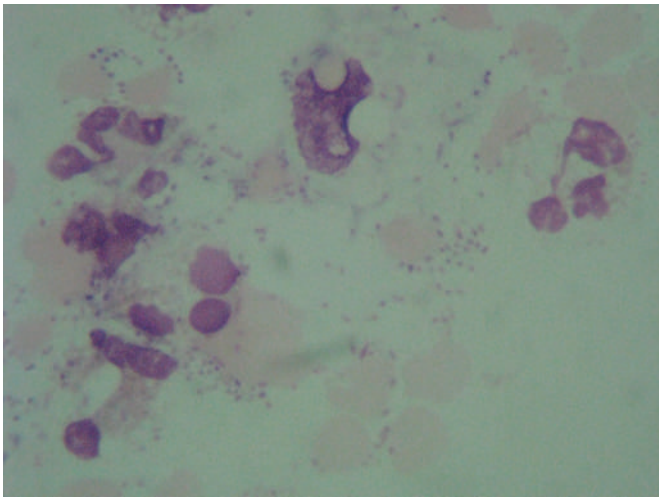
A-124. Bloody CSF with neutrophils, with cytoplasmic vacuoles resulting from cytoplasmic centrifugation.



A-123. Bloody CSF with neutrophils.

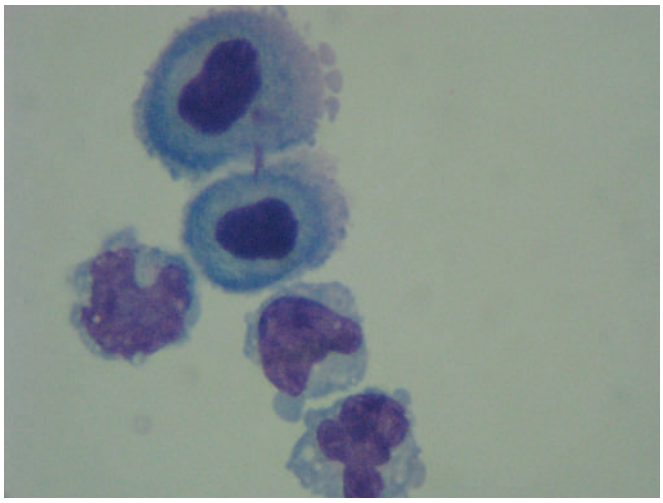


A-125. Ependymal cells in CSF. Notice the nucleoli and less distinct borders.

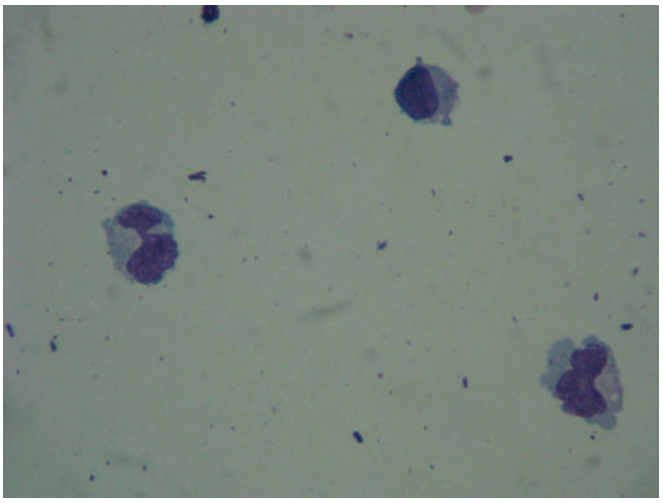


A-126. Neutrophils with intracellular and extracellular bacteria in CSF.

Peritoneal Fluid

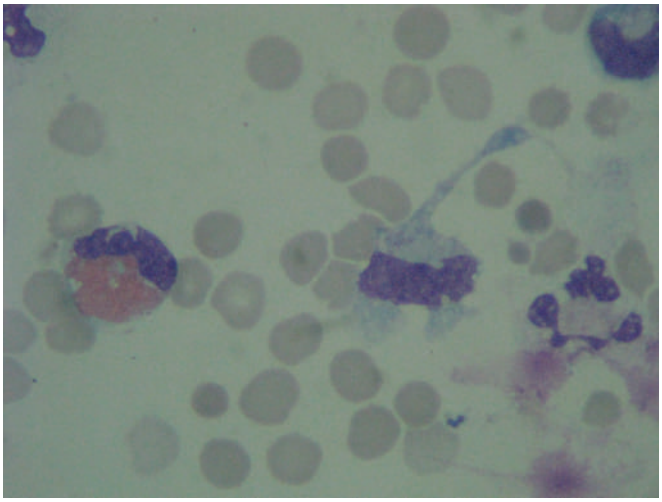


A-127. Two large mesothelial cells and three monocytes in peritoneal fluid.

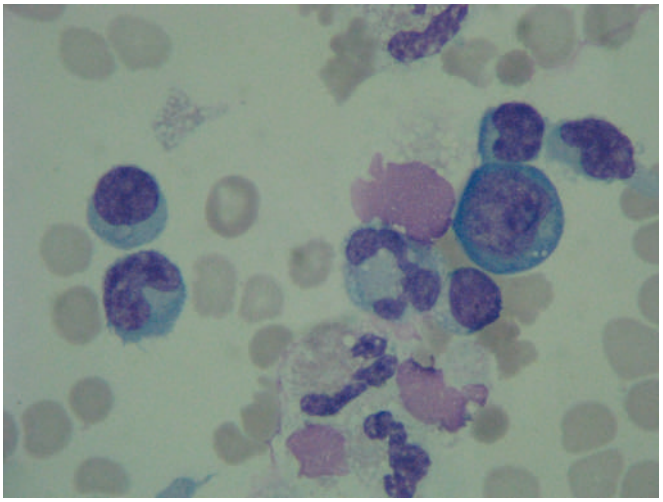


A-128. Two monocytes and one lymphocyte in peritoneal fluid.

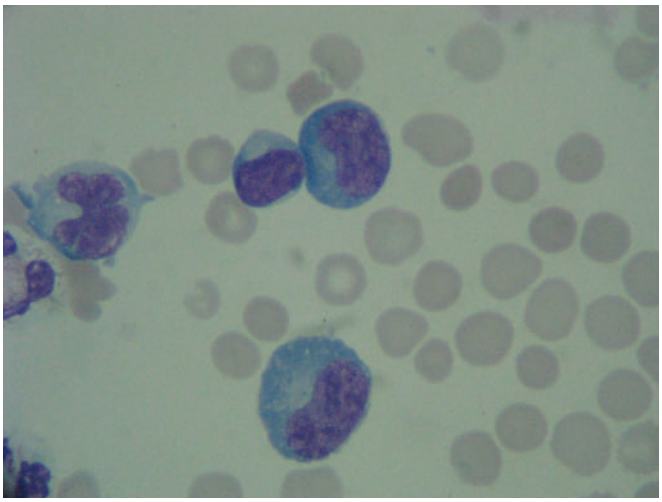
Pleural Fluid



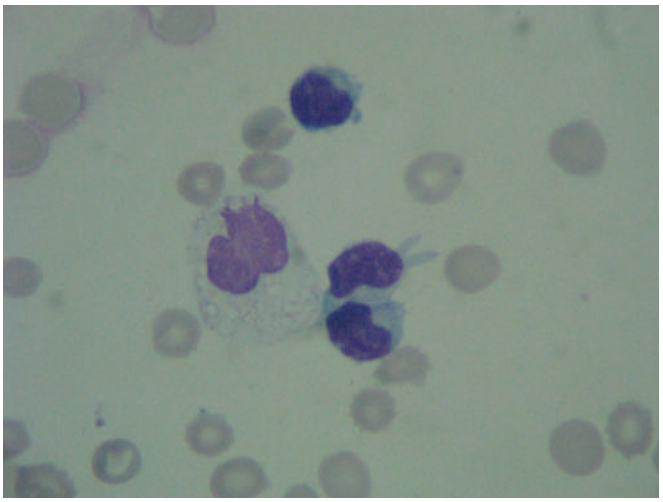
A-129. Two monocytes, one eosinophil, and one neutrophil in pleural fluid.



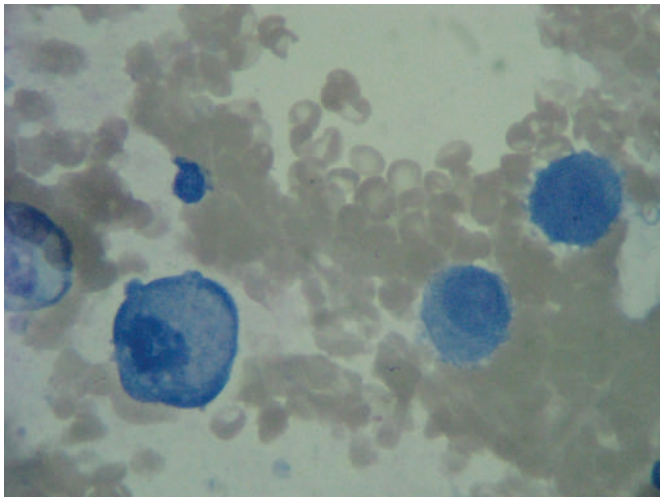
A-131. Lymphocytes, neutrophils, and monocytes in pleural fluid. Note the large reactive lymphocyte.



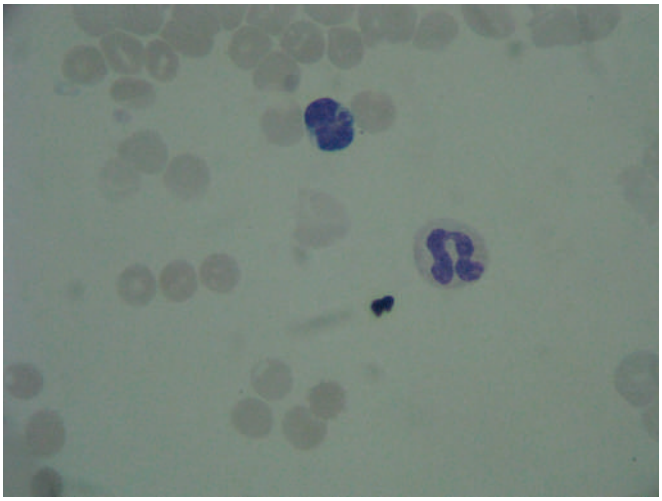
A-130. Reactive lymphocytes in pleural fluid.



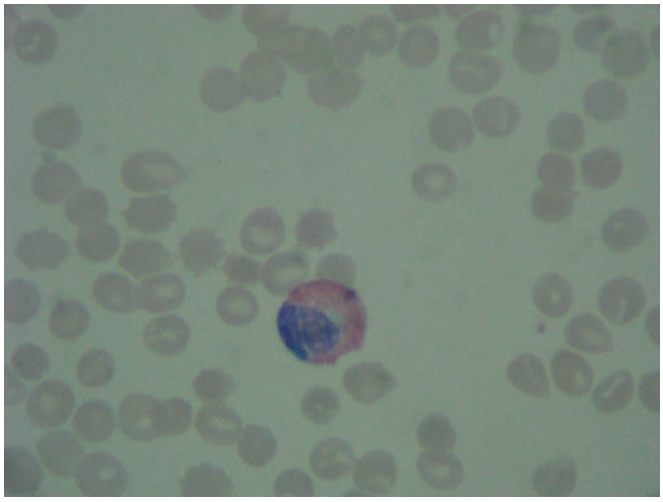
A-132. Reactive lymphocytes in pleural fluid.



A-133. Mesothelial cells in pleural fluid.

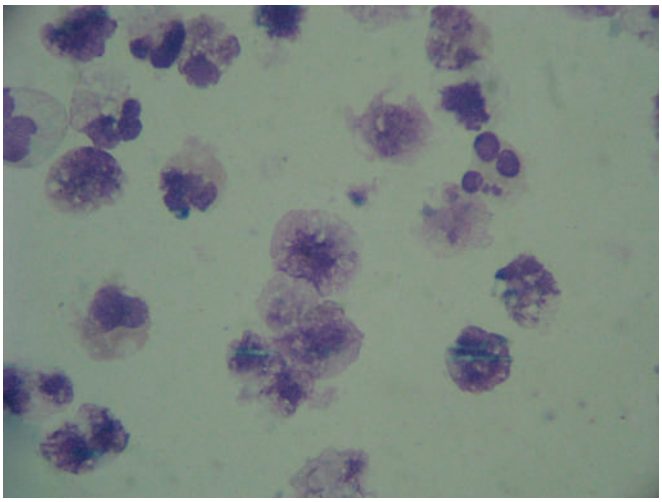


A-135. RBCs, lymphocyte, and neutrophil in pleural fluid.

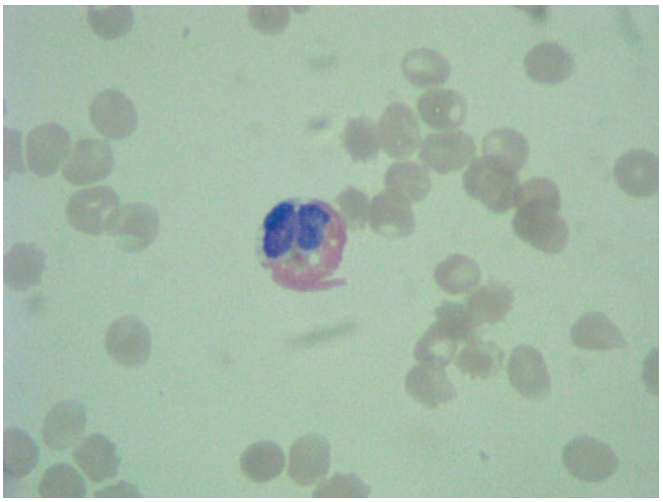


A-134. Eosinophil and RBCs in pleural fluid.

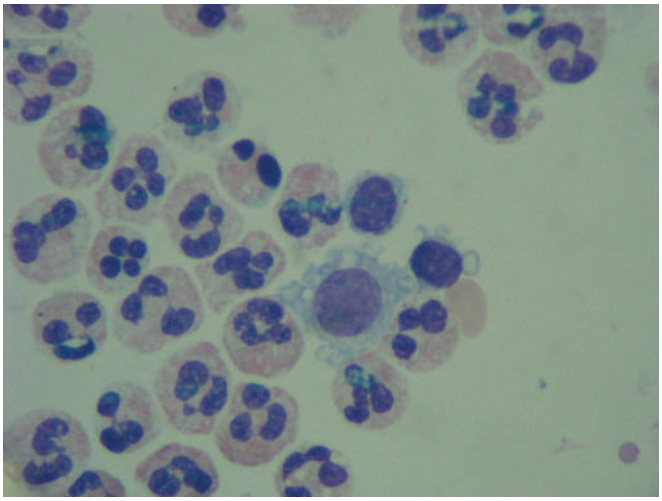
Synovial Fluid



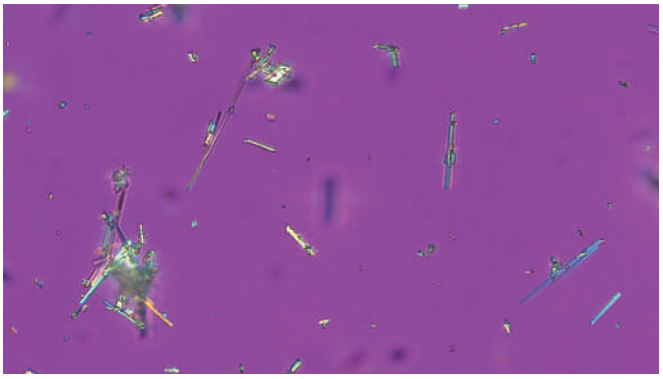
A-136. Wright-stained neutrophils containing CPPD crystals in synovial fluid.



A-138. Wright-stained eosinophil in synovial fluid.



A-137. Numerous Wright-stained neutrophils with vacuoles in synovial fluid, indicative of inflammation.



A-139. Polarized urate crystals in synovial fluid.

PART ONE

Basic Principles

Chapter 1: Safety and Quality Management

Chapter 2: Urine and Body Fluid Analysis Automation

Chapter 3: Introduction to Urinalysis

Chapter 4: Renal Function

CHAPTER 1

Safety and Quality Management

LEARNING OUTCOMES

Upon completing this chapter, the reader will be able to:

1-1 Define and give an example of the types of safety hazards encountered in the laboratory.

1-2 List the six components of the chain of infection and the laboratory safety precautions that break the chain.

1-3 State the purpose of the Standard Precautions policy, and describe its guidelines.

1-4 State the requirements mandated by the Occupational Exposure to Bloodborne Pathogens Compliance Directive.

1-5 Describe the types of personal protective equipment (PPE) that laboratory personnel wear, including when, how, and why each article is used.

1-6 Correctly perform hand-hygiene procedures following guidelines provided by the Centers for Disease Control and Prevention (CDC).

1-7 Describe the acceptable methods for handling and disposing of biological waste and sharp objects in the urinalysis laboratory.

1-8 Discuss the components and purpose of a chemical hygiene plan and a Safety Data Sheet (SDS).

1-9 Describe the use of the Globally Harmonized System (GHS).

1-10 Recognize standard hazard warning symbols.

1-11 State and interpret the components of the National Fire Protection Association (NFPA) hazardous material labeling system.

1-12 Describe precautions that laboratory personnel should take with regard to radioactive, electrical, fire, and physical hazards.

1-13 Explain the RACE and PASS actions to be taken when a fire is discovered.

1-14 Explain the role of quality management (QM) in the urinalysis laboratory.

1-15 Define the preexamination (preanalytical), examination (analytical), and postexamination (postanalytical) components of QM.

1-16 Distinguish among internal, external, and electronic quality control, as well as external quality assessment (proficiency testing) in a QM program.

KEY TERMS

Accreditation
Accuracy
Autoverification
Biohazardous
Chain of infection
Chemical hygiene plan (CHP)
Clinical Laboratory Improvement Amendments (CLIA)
Clinical and Laboratory Standards Institute (CLSI)
Delta check
Electronic quality control
Examination variable
External quality assessment (EQA)
External quality control
Fomite
Globally Harmonized System (GHS)
Infection control
Internal quality control
Occupational Safety and Health Administration (OSHA)
Personal protective equipment (PPE)
Postexamination variable

Continued

KEY TERMS—cont'd

Postexposure prophylaxis (PEP)
Precision
Preexamination variable
Preventive maintenance (PM)
Proficiency testing (PT)
Quality assessment (QA)
Quality control (QC)
Quality management (QM)
Quality management system (QMS)
Radioisotope
Reliability
Root cause analysis (RCA)
Safety Data Sheet (SDS)
Sensitivity
Specificity
Standard Precautions (SP)
Test utilization
Turnaround time (TAT)
Universal Precautions (UP)

Safety

The clinical laboratory contains a variety of safety hazards, many capable of producing serious injury or life-threatening disease. To work safely in this environment, laboratory personnel must learn what hazards exist, the basic safety precautions associated with them, and how to apply the basic rules of common sense required for everyday safety for patients, coworkers, and themselves.

As seen in Table 1-1, some hazards are unique to the health-care environment, whereas others are encountered routinely throughout life. Safety procedure manuals that describe the safety policies mandated by the Centers for Disease Control and Prevention (**CDC**) and the **Occupational Safety and Health Administration** (**OSHA**) must be readily available in the laboratory, and it is essential for laboratory personnel to adhere closely to these guidelines. The laboratory director must update and review the manual annually. The **Clinical and Laboratory Standards Institute** (**CLSI**) provides the guidelines for writing these procedures and policies.[1-3]

Table 1–1 Types of Safety Hazards

Type	Source	Possible Injury
Biological	Infectious agents	Bacterial, fungal, viral, or parasitic infections
Sharps	Needles, lancets, broken glass	Cuts, punctures, or exposure to bloodborne pathogens
Chemical	Preservatives and reagents	Exposure to toxic, carcinogenic, or caustic agents
Radioactive	Equipment and radioisotopes	Exposure to radiation
Electrical	Ungrounded or wet equipment; frayed cords	Burns or shock
Fire/explosive	Open flames, organic chemicals	Burns or dismemberment
Physical	Wet floors, heavy boxes, patients	Falls, sprains, or strains

From Strasinger, SK, and DiLorenzo, MA: The Phlebotomy Textbook, Fourth Edition, FA Davis, Philadelphia, 2019, p. 44, with permission.

Biological Hazards



The health-care setting provides abundant sources of potentially harmful microorganisms. These microorganisms are frequently present in the specimens received in the clinical laboratory. Understanding how microorganisms are transmitted (**chain of infection**) is essential to preventing infection. All health-care facilities have developed procedures to control and monitor infections occurring within their facilities, which is referred to as **infection control**. The chain of infection requires a continuous link among six elements: an infectious agent, a reservoir, a portal of exit, a means of transmission, a portal of entry, and a susceptible host.[4]

Infectious Agent (Pathogen)

Infectious agents can include bacteria, fungi, parasites, and viruses. The chain of infection is broken by early detection of one of these agents. The treatment of infectious agents can reduce their opportunity for growth.

Reservoir

The reservoir is the location of potentially harmful microorganisms, such as a contaminated clinical specimen or an infected patient. It is the place where the infectious agent can live and possibly multiply. Humans and animals make excellent reservoirs. Equipment and other soiled inanimate objects, called **fomites**, will serve as reservoirs, particularly if they contain blood, urine, or other body fluids. Some microorganisms form spores or become inactive when conditions are not ideal and wait until a suitable reservoir is available. Disinfecting the work area kills the infectious agent and eliminates the reservoir, thereby breaking the chain of infection.

Portal of Exit

The infectious agent must have a way to exit the reservoir to continue the chain of infection. This can be through the mucous membranes of the reservoir's nose, mouth, and eyes, as well as in blood or other body fluids. The chain of infection is broken when contaminated materials are placed in biohazard containers and/or when tubes and specimen containers are sealed. When contaminated materials are in the appropriate containers that remain sealed, the infectious agent still has a reservoir but no means of exit.

Means of Transmission

Once the infectious agent has left the reservoir, it must have a way to reach a susceptible host. Means of transmission include:

1. Direct contact: the unprotected host touches the patient, specimen, or a contaminated object (reservoir)
2. Airborne: the host inhales dried aerosol particles circulating on air currents or attached to dust particles
3. Droplet: the host inhales infected aerosol droplets from the reservoir (e.g., aerosol droplets from a patient or an uncapped centrifuge tube, or when specimens are aliquoted or spilled)
4. Vehicle: the host ingests a contaminated substance (e.g., food, water, specimen)
5. Vector: from an animal or insect bite

Hand sanitizing and adhering to **Standard Precautions** (**SP**) are methods to break the chain of infection.

Portal of Entry

After the infectious agent has been transmitted to a new reservoir, it must have a means to enter the reservoir. The portal of entry can be the same as the portal of exit, which includes the mucous membranes of the reservoir's nose, mouth, and eyes, as well as breaks in the skin and open wounds. Disinfection and sterilization and strict adherence to SPs block the portal of entry, thereby breaking the chain of infection.

Susceptible Host

The susceptible host can be another patient during invasive procedures, visitors, and health-care personnel when exposed to infectious specimens or needlestick injuries. Immunocompromised patients, newborns and infants, and the elderly are often more susceptible hosts. Stress, fatigue, and lack of proper nutrition depress the immune system and contribute to the susceptibility of patients and health-care providers. Once the chain of infection is complete, the infected host becomes another source able to transmit the microorganisms to others.[1] To break the chain of infection, health-care personnel must sanitize hands often, wear gloves, stay current with the required immunizations and tests, and maintain a healthy lifestyle.

In the clinical laboratory, the most direct contact with a source of infection is through contact with patient specimens, although contact with patients and infected objects also occurs. Preventing completion of the chain of infection is a primary objective of biological safety. Figure 1-1 illustrates the universal symbol for **biohazardous** material and demonstrates how following prescribed safety practices can break the chain of infection. Figure 1-1 places particular emphasis on laboratory practices.

Standard Precautions

Proper hand hygiene, correct disposal of contaminated materials, and wearing **personal protective equipment** (**PPE**) are of major importance in the laboratory. Concern over exposure to bloodborne pathogens, such as hepatitis B virus (**HBV**), hepatitis C virus (**HCV**), and human immunodeficiency virus (**HIV**), caused the CDC and OSHA to draft guidelines and regulations to prevent exposure. In 1987, the CDC instituted **Universal Precautions** (**UP**), in which all patients are considered to be possible carriers of bloodborne pathogens. The guideline recommends wearing gloves when collecting or handling blood and body fluids contaminated with blood and wearing face shields when there is danger of blood splashing on mucous membranes and when disposing of all needles and sharp objects in puncture-resistant containers. The CDC excluded urine and body fluids not visibly contaminated by blood from UP, although many specimens can contain a considerable amount of blood before it becomes visible. UP was modified for **body substance isolation** (**BSI**) to help alleviate this concern. BSI guidelines are not limited to bloodborne pathogens; they consider all body fluids and moist body substances to be potentially infectious. According to BSI guidelines, personnel must wear gloves at all times when encountering moist body substances. A major disadvantage of BSI guidelines is that they do not recommend hand sanitizing after removing gloves unless visual contamination is present.

In 1996, the CDC and the Healthcare Infection Control Practices Advisory Committee (**HICPAC**) combined the major features of UP and BSI guidelines and called the new guidelines Standard Precautions. Although SP, as described next, stress patient contact, the principles also can be applied to handling patient specimens in the laboratory.[5]

SP are as follows:

1. **Hand hygiene**: Hand hygiene includes both hand washing and the use of alcohol-based antiseptic cleansers. Sanitize hands after touching blood, body fluids, secretions, excretions, and contaminated items, whether or not gloves are worn. Sanitize hands immediately after removing gloves, between patient contacts, and when otherwise indicated to avoid transferring microorganisms to other patients or environments. Sanitizing hands may be necessary between tasks and procedures on the same patient to prevent cross-contamination of different body sites.
2. **Gloves**: Wear gloves (clean, nonsterile, latex-free gloves are adequate) when touching blood, body fluids, secretions, excretions, and contaminated items. Put on gloves just before touching mucous membranes and nonintact skin. Change gloves between tasks and procedures on

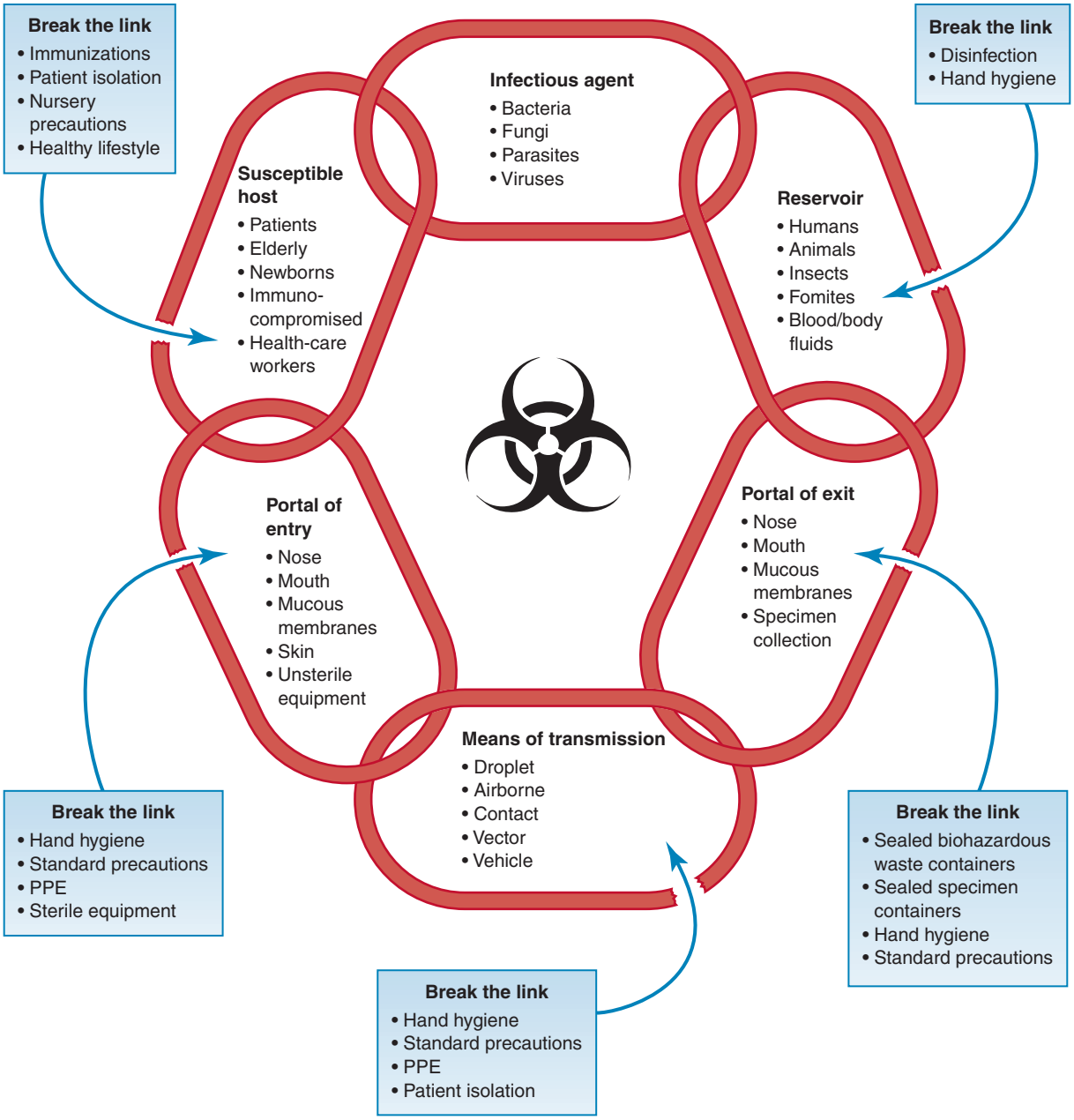


Figure 1–1 Chain of infection and safety practices related to the biohazard symbol. (From Strasinger, SK, and DiLorenzo, MA: The Phlebotomy Textbook, Fourth Edition, FA Davis, Philadelphia, 2011, with permission.)

the same patient after contact with material that may contain a high concentration of microorganisms. Remove gloves promptly after use, before touching noncontaminated items and environmental surfaces, and between patients. Always sanitize your hands immediately after glove removal to avoid transferring microorganisms to other patients or environments.

3. **Mouth, nose, and eye protection**: Wear a mask and eye protection or a face shield to protect mucous membranes of the eyes, nose, and mouth during procedures and patient care activities that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions. A specially fitted respirator (N95) must be used during patient care activities related to suspected *Mycobacterium* exposure.
4. **Gown**: Wear a gown (a clean, nonsterile gown is adequate) to protect skin and to prevent soiling of clothing during procedures and patient care activities that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions. Select a gown that is appropriate for the activity and the amount of fluid likely to be encountered (e.g., fluid-resistant laboratory coat in the laboratory). Remove a soiled gown as promptly as possible, and sanitize hands to avoid transferring microorganisms to other patients or environments.

5. **Patient care equipment**: Handle used patient care equipment soiled with blood, body fluids, secretions, and excretions in a manner that prevents skin and mucous membrane exposure, clothing contamination, and transfer of microorganisms to other patients or environments. Ensure that reusable equipment is not used for the care of another patient until it has been cleaned and reprocessed appropriately. Ensure that single-use items are discarded properly.
6. **Environmental control**: Ensure that the hospital has adequate procedures for the routine care, cleaning, and disinfection of environmental surfaces, beds, bed rails, bedside equipment, and other surfaces that are touched frequently. Ensure that these procedures are being followed.
7. **Linen**: Handle, transport, and process linen soiled with blood, body fluids, secretions, and excretions in a manner that prevents skin and mucous membrane exposures and clothing contamination and that avoids the transfer of microorganisms to other patients and environments.
8. **Occupational health and bloodborne pathogens**: Take care to prevent injuries when using needles, scalpels, and other sharp instruments or devices; when handling sharp instruments after procedures; when cleaning used instruments; and when disposing of used needles. Never recap used needles or otherwise manipulate them using both hands or use any other technique that involves directing the point of a needle toward any part of the body; rather, use self-sheathing needles or a mechanical device to conceal the needle. Do not remove used unsheathed needles from disposable syringes by hand, and do not bend, break, or otherwise manipulate used needles by hand. Place used disposable syringes and needles, scalpel blades, and other sharp items in appropriate puncture-resistant containers, which are located as close as practical to the area in which the items were used, and place reusable syringes and needles in a puncture-resistant container for transport to the reprocessing area. Use mouthpieces, resuscitation bags, or other ventilation devices as an alternative to mouth-to-mouth resuscitation methods in areas where the need for resuscitation is predictable.
9. **Patient placement**: Place a patient in a private room if he or she contaminates the environment or does not (or cannot be expected to) assist in maintaining appropriate hygiene or environment control. If a private room is not available, consult with infection control professionals regarding patient placement or other alternatives.
10. **Respiratory hygiene/cough etiquette**: Educate health-care personnel, patients, and visitors to contain respiratory secretions to prevent droplet and fomite transmission of respiratory pathogens. Offer masks to coughing patients, distance symptomatic patients from others, and practice good hand hygiene to prevent the transmission of respiratory pathogens.

The Occupational Exposure to Bloodborne Pathogens Standard is a law monitored and enforced by OSHA.[6,7] OSHA requires these controls to be provided by or mandated by the employer for all employees. Specific requirements of this OSHA standard include the following:

Engineering Controls

1. Providing sharps disposal containers and needles with safety devices
2. Requiring discarding of needles with the safety device activated and the holder attached
3. Labeling all biohazardous materials and containers

Work Practice Controls

4. Requiring all employees to practice SP and documenting training on an annual basis
5. Prohibiting eating, drinking, smoking, and applying cosmetics in the work area
6. Establishing a daily work surface disinfection protocol

Personal Protective Equipment

7. Providing laboratory coats, gowns, face shields, and gloves to employees and laundry facilities for nondisposable protective clothing

Medical

8. Providing immunization for HBV free of charge
9. Providing medical follow-up to employees who have been exposed accidentally to bloodborne pathogens

Documentation

10. Documenting annual training of employees in safety standards
11. Documenting evaluations and implementation of safer needle devices
12. Involving employees in the selection and evaluation of new devices and maintaining a list of those employees and the evaluations
13. Maintaining a sharps injury log, including the type and brand of safety device, location and description of the incident, and confidential employee follow-up

Any accidental exposure to a possible bloodborne pathogen through needlestick, mucous membranes, or nonintact skin must be immediately reported to a supervisor. A confidential evaluation of the incident must begin right away to ensure appropriate **postexposure prophylaxis** (**PEP**) is initiated within 24 hours. If it is determined that a significant exposure occurred, an incident report will be completed; blood testing for HIV, HBV, and HCV on both the employee and the source patient is performed; and employee treatment and counseling are initiated. The CDC provides periodically updated guidelines for the management of exposures and recommended PEP.[8,9]

Personal Protective Equipment

PPE used in the laboratory includes gloves, fluid-resistant gowns, eye and face shields, and Plexiglas countertop shields. Shoes must be closed-toed and cover the entire foot.

Gloves should be worn when in contact with patients, specimens (urine, blood, body fluids, secretions, excretions), contaminated items, and laboratory equipment or fixtures. When specimens are collected, gloves must be changed between every patient. In the laboratory, they are changed whenever they become noticeably contaminated or damaged and are always removed when leaving the work area. Wearing gloves is not a substitute for hand hygiene, and hands must be sanitized after gloves are removed.

A variety of gloves is available, including sterile and nonsterile, powdered and unpowdered, and latex and nonlatex. Allergy to latex is increasing among health-care workers, and laboratory personnel should be alert for symptoms of reactions associated with latex. Reactions to latex include irritant contact dermatitis, which produces patches of dry, itchy irritation on the hands; delayed hypersensitivity reactions resembling poison ivy that appear 24 to 48 hours after exposure; and true, immediate hypersensitivity reactions often characterized by facial flushing and breathing difficulties. Hand sanitizing immediately after removing gloves and avoiding powdered gloves may aid in preventing the development of latex allergies. Replacing latex gloves with nitrile or vinyl gloves provides an alternative. Any symptoms of latex allergy should be reported to a supervisor because true latex allergy can be life-threatening.[10]

Laboratory Coats

Full-length, fluid-resistant laboratory coats with wrist cuffs are worn to protect clothing and skin from exposure to patients' body substances. These coats should always be completely buttoned, and gloves should be pulled over the cuffs. They are worn at all times when working with patient specimens and are removed before leaving the work area. They are changed when they become visibly soiled. Disposable coats are placed in containers for biohazardous waste, and nondisposable coats are placed in designated laundry receptacles.

Masks, Goggles, and Face Shields

The mucous membranes of the eyes, nose, and mouth must be protected from specimen splashes and aerosols. A variety of protective equipment is available, including masks and goggles, full-face plastic shields that cover the front and sides of the face, masks with attached shields, and Plexiglas countertop shields. Particular care should be taken to avoid splashes and aerosols when removing container tops, pouring specimens, and centrifuging specimens. Transfer pipettes should be used when aliquoting samples from a specimen container. Specimens must never be centrifuged in uncapped tubes or in uncovered centrifuges. When specimens are received in containers with contaminated exteriors, the exterior of the container must be disinfected or, if necessary, a new specimen may be requested.

Hand Hygiene

Hand hygiene is emphasized in Figure 1-1 and in the SP guidelines. Hand contact is the primary method of infection transmission. Laboratory personnel must always sanitize hands in the following situations:

- Before patient contact
- After gloves are removed
- Before leaving the work area
- At any time when hands have been knowingly contaminated
- Before going to designated break areas
- Before and after using bathroom facilities

Hand hygiene includes both hand washing and using alcohol-based antiseptic cleansers. Alcohol-based cleansers can be used when hands are not visibly contaminated, but those cleansers are not recommended after contact with spore-forming bacteria, including *Clostridium difficile* and *Bacillus* sp. because the spores formed by these bacteria are not killed by alcohol-based cleanser. Hand washing with soap and water and scrubbing to create a friction can remove these spores.

When using alcohol-based cleansers, apply the cleanser to the palm of one hand. Rub your hands together and over the entire cleansing area, including the area between the fingers and thumbs. Continue rubbing until the alcohol dries.

The CDC has developed guidelines to be followed for correct hand washing.[1,11] Procedure 1-1 demonstrates CDC routine hand washing guidelines.[4] More stringent procedures are used in surgery and in areas with patients who are highly susceptible, such as patients who are immunocompromised and/or burn patients.

Biological Waste Disposal

All biological waste, except urine, must be placed in appropriate leakproof containers labeled with the biohazard symbol (Fig. 1-2) or with red or yellow color coding. This includes both specimens and the materials with which the specimens come in contact. Then waste is decontaminated following facility policy: incineration, autoclaving, or pickup by a certified hazardous waste company.

Urine may be discarded by pouring it into a laboratory sink under a Plexiglas countertop shield. Care must be taken to avoid splashing, and the sink should be flushed with water after specimens are discarded. Disinfection of the sink using a 1:5 or 1:10 dilution of sodium hypochlorite (bleach) should be performed daily. Sodium hypochlorite dilutions stored in plastic bottles are effective for 1 month if protected from light after preparation.[12] Empty urine containers can be discarded as nonbiologically hazardous waste (Fig. 1-3).

Decontamination

Regular disinfection is needed for contaminated nondisposable equipment, blood spills, and laboratory areas that process blood and body fluids. Laboratory countertops should be disinfected after every work shift and after any spill occurs. The

CDC recommends the use of a 1:10 dilution of sodium hypochlorite for routinely disinfecting countertops and accidental spills. The solution should be allowed to air-dry on the contaminated area. Absorbent materials used for cleaning countertops and removing spills must be discarded in biohazard containers. Other products are commercially available both in prefilled spray bottles and single-use wipes. However, it must be confirmed that the product is effective enough to eliminate most bacteria, including *Mycobacterium tuberculosis,* fungi, and viruses. It is also important to know the contact time needed for **disinfectant** chemical products to work effectively on laboratory surfaces as prescribed by the manufacturer.

When spills occur, do not mop or wipe the fluid; instead, use an absorbent powder (e.g., Zorbitrol) or paper towels to

PROCEDURE 1-1

Hand Washing Procedure

Visit www.fadavis.com for Phlebotomy Video 3-1 and Video 3-2 (Hand Washing).

Equipment

Antimicrobial soap

Paper towels

Running water

Waste container

Procedure

1. Wet your hands with warm water. Do not allow parts of your body to touch the sink.



2. Apply soap, preferably antimicrobial.



3. Rub to form a lather, create friction, and loosen debris. Thoroughly clean between your fingers and under your fingernails for at least 20 seconds; include your thumbs and wrists in the cleaning.



4. Rinse your hands in a downward position to prevent recontamination of your hands and wrists.



5. Obtain a paper towel from the dispenser.



Continued

PROCEDURE 1-1—cont'd

6. Dry your hands with a paper towel.



7. Turn off the faucets with a clean paper towel to prevent recontamination.



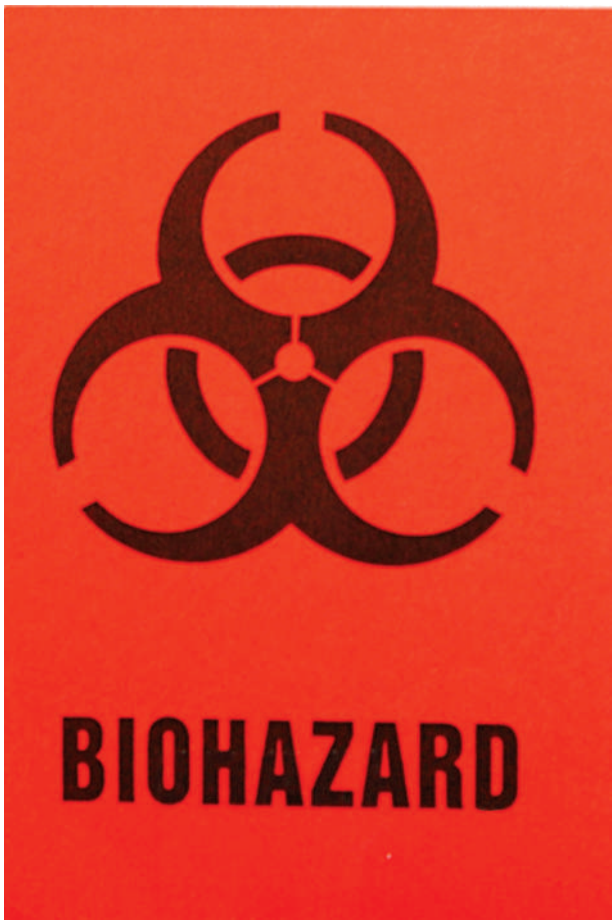


Figure 1–2 Biohazard symbol. (From Strasinger, SK, and DiLorenzo, MA: The Phlebotomy Textbook, Fourth Edition, FA Davis, Philadelphia, 2019, p. 60, with permission.)

remove as much as possible before disinfecting. When using an absorbent powder, the liquid will solidify and then can be scooped up. If paper towels are used to absorb the liquid, pour bleach over the towels. Dispose of absorbent material and paper towels used for disinfection in the appropriate biohazard container. Disinfect the spill area with bleach or a phenol solution.

Sharp Hazards



Sharp objects in the laboratory, including needles, lancets, and broken glassware, present a serious biological hazard, particularly for the transmission of bloodborne pathogens. All sharp objects must be disposed in puncture-resistant, leakproof container with the biohazard symbol. Puncture-resistant containers should be conveniently located within the work area. The biohazard sharp containers should not exceed three-fourths full and must always be replaced when the level of waste inside reaches the safe capacity mark.

Chemical Hazards



The same general rules for handling biohazardous materials apply to chemically hazardous materials; that is, to avoid getting these materials in or on bodies, clothes, or the work area. Every chemical in the workplace should be presumed to be hazardous.

Chemical Spills and Exposure

When skin or eye contact occurs, the best first aid is to flush the area with large amounts of water for at least 15 minutes and then seek medical attention. For this reason, all laboratory personnel should know the location and proper use of emergency showers and eye wash stations. Contaminated clothing should be removed as soon as possible. No attempt should be made to neutralize chemicals that come in contact with the skin.



Figure 1–3 Technologist disposing of urine **(A)** sample and **(B)** container.

Chemical spill kits containing protective apparel, nonreactive absorbent material, and disposable bags and waste tags for disposing of contaminated materials should be available for cleaning up spills. Liquids are absorbed using a neutralizer, such as sodium bicarbonate, and a sand mixture or ground clay. After the liquid is absorbed, it is scooped up and placed into a waste bag and labeled appropriately for disposal. Then the area is cleaned thoroughly.

A chemical spill or exposure must be reported to the employee health department and an incident (variance) report submitted that documents the exposure according to the facility policy. Examples of chemical exposure include any time a chemical was breathed in or touched the skin, two or more chemicals that came in contact with each other and created an odor or fume, or an identified substance spill.

Chemical Handling

Chemicals should never be mixed together unless specific instructions are followed, and they must be added in the order specified. This is particularly important when combining acid and water. Acid should always be added to water to avoid the possibility of sudden splashing caused by the rapid generation of heat in some chemical reactions. Wearing goggles and preparing reagents under a fume hood are recommended safety precautions. Chemicals should be used from containers that are of an easily manageable size. Pipetting by mouth is unacceptable in the laboratory.

Chemical Hygiene Plan

OSHA also requires all facilities that use hazardous chemicals to have a written **chemical hygiene plan** (**CHP**) available to employees.[13] The purpose of the plan is to detail the following:

1. Appropriate work practices
2. Standard operating procedures
3. PPE
4. Engineering controls, such as fume hoods and flammables safety cabinets
5. Employee training requirements
6. Medical consultation guidelines

Each facility must appoint a chemical hygiene officer, who is responsible for implementing and documenting compliance with the plan. Examples of required safety equipment and information are shown in Figure 1-4.

Chemical Labeling

Hazardous chemicals should be labeled with a description of their particular hazard, such as poisonous, corrosive, flammable, explosive, teratogenic, or carcinogenic (Fig. 1-5). The National Fire Protection Association (**NFPA**) has developed the Standard System for the Identification of the Fire Hazards of Materials, NFPA 704.[14] This symbol system is used to inform firefighters of the hazards that they may encounter with fires in a particular area. The diamond-shaped, color-coded symbol contains information relating to health (blue), flammability (red), reactivity (yellow), and specific hazards (white). Each category is graded on a scale of 0 to 4, based on the extent of concern. These symbols are placed on doors, cabinets, and containers. The federal Department of Transportation (**DOT**) regulations state that all chemicals are required to have the NFPA 704-M Hazard Identification System warnings on their shipping containers. An example of this system is shown in Figure 1-6.

Safety Data Sheets

The OSHA Federal Hazard Communication Standard requires that all employees have a right to know about all chemical hazards present in their workplace. All chemicals and reagents containing hazardous ingredients in a concentration greater than 1% are required by OSHA to have a **Safety Data Sheet** (**SDS**) on file in the workplace. By law, vendors are required



Figure 1–4 Chemical safety aids. **A.** Emergency shower. **B.** Eye wash station. (From Strasinger, SK, and DiLorenzo, MA: The Phlebotomy Textbook, Fourth Edition, FA Davis, Philadelphia, 2019, p. 64, with permission.)

to provide these sheets to purchasers; however, the facility itself is responsible for obtaining and making SDSs available to employees. Information contained in an SDS includes the following:

1. Physical and chemical characteristics
2. Fire and explosion potential
3. Reactivity potential
4. Health hazards and emergency first aid procedures
5. Methods for safe handling and disposal
6. Primary routes of entry
7. Exposure limits and carcinogenic potential

The Globally Harmonized System of Classification and Labeling of Chemicals

The **Globally Harmonized System** (**GHS**) of Classification and Labeling of Chemicals is an international effort to standardize both the classification of hazardous chemicals and the symbols used to communicate these hazards on labels and in SDS documentation (Fig. 1-7). It includes criteria for the classification of health, physical, and environmental hazards. The standard label elements include GHS hazard pictograms, signal words, and a GHS hazard statement. The SDS under GHS provides a clear description of the data used to identify hazards and has 16 sections in a specified order (Box 1-1).

OSHA aligned its Hazard Communication Standard with the GHS and requires that all employees be trained on the new label elements and SDS format. The adoption of the GHS in the United States has increased awareness and understanding of hazards in the workplace.

Chemical Waste Disposal

State and federal regulations are in place for the disposal of chemicals and should be consulted. Any hazardous chemical waste should be disposed of per current Environmental Protection Agency (**EPA**) regulations. Most reagents used in the laboratory come with an SDS, which provides specific information for the disposal of particular chemicals. All chemicals should be disposed of by following the SDS directions. Sinks and drains should be flushed with large amounts of water after disposal of chemical waste.

Radioactive Hazards



Radioactivity may be encountered in the clinical laboratory when procedures using **radioisotopes** are performed. The amount of radioactivity present in the clinical laboratory is very small and represents little danger; however, the effects of radiation are cumulative related to the amount of exposure. The amount of radiation exposure is related to a combination of time, distance, and shielding. People working in a radioactive environment are required to wear measuring devices to determine the amount of radiation that they are accumulating.



Figure 1–5 Chemical hazard symbols. (From Strasinger, SK, and DiLorenzo, MA: The Phlebotomy Textbook, Fourth Edition, FA Davis, Philadelphia, 2019, p. 65, with permission.)

Laboratory personnel should be familiar with the radioactive hazard symbol shown here. This symbol must be displayed on the doors of all areas where radioactive material is present. Exposure to radiation during pregnancy presents a danger to the fetus; personnel who are pregnant or think they may be should avoid areas with this symbol.

Electrical Hazards



The laboratory setting contains electrical equipment with which workers have frequent contact. The same general rules of electrical safety observed outside the workplace apply. The danger of water or fluid coming in contact with equipment is greater in the laboratory setting. Equipment should not be operated with wet hands. Designated hospital personnel monitor electrical equipment closely; however, laboratory personnel should continually observe for any dangerous conditions, such as frayed cords and overloaded circuits, and report them to the supervisor. Equipment that has become wet should be unplugged and allowed to dry completely before reusing. Equipment also should be unplugged before cleaning. All electrical equipment must be grounded with three-pronged plugs.

When an accident involving electrical shock occurs, the electrical source must be removed immediately. This must be done without touching the person or the equipment involved to avoid transferring the current. Turning off the circuit breaker, unplugging the equipment, or moving the equipment using a nonconductive glass or wood object are safe procedures

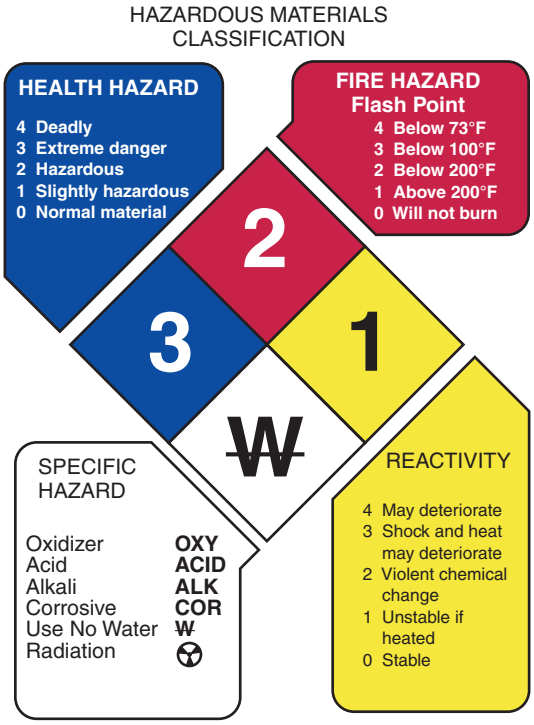


Figure 1–6 NFPA hazardous material symbols.

to follow. The victim should receive immediate medical assistance after discontinuation of the electricity. Cardiopulmonary resuscitation (CPR) may be necessary.

Fire/Explosive Hazards



The Joint Commission (TJC) requires that all healthcare facilities post evacuation routes and detailed plans to follow in the event of a fire. Laboratory personnel should be familiar with these procedures. When a fire is discovered, all employees are expected to take the actions to protect themselves and others, following the acronym RACE:

Rescue—rescue anyone in immediate danger

Alarm—activate the institutional fire alarm system

Contain—close all doors to potentially affected areas

Extinguish/Evacuate—attempt to extinguish the fire, if possible, or evacuate, closing the door

As discussed previously, laboratory workers often use potentially volatile or explosive chemicals that require special procedures for handling and storage. Flammable chemicals should be stored in safety cabinets and explosion-proof refrigerators,

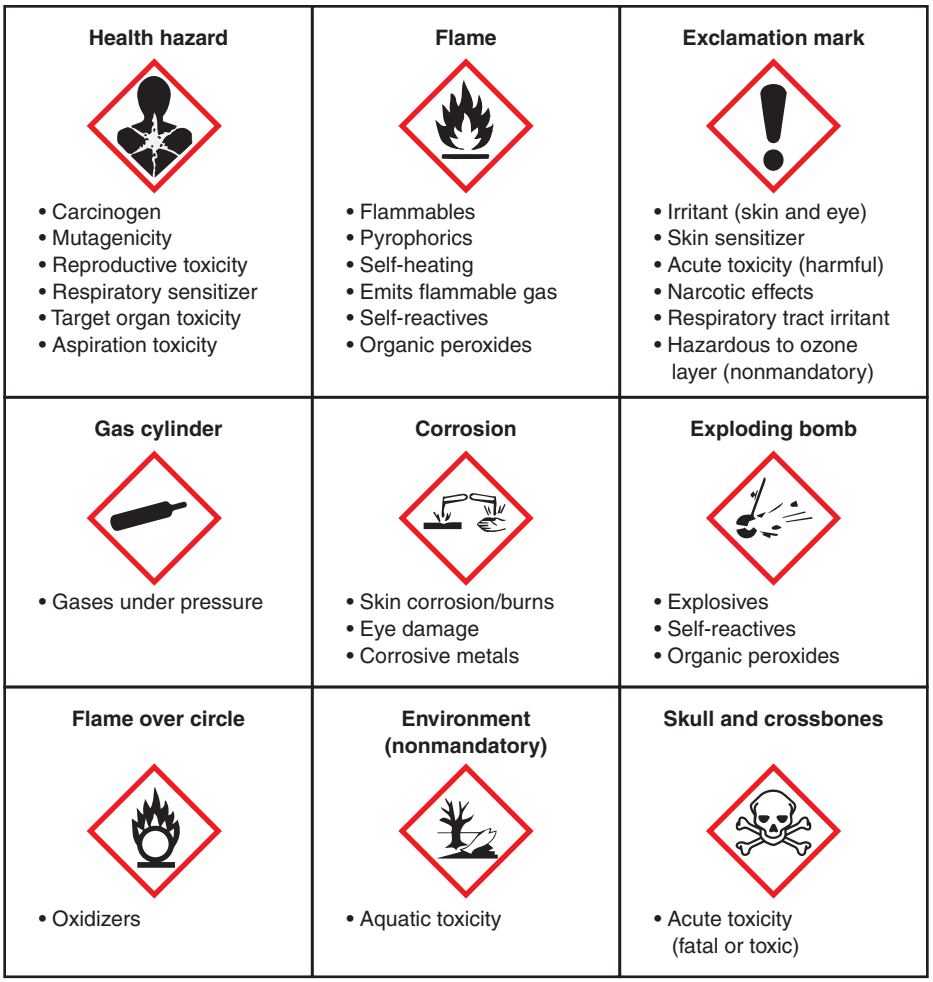


Figure 1–7 GHS pictograms and hazards chart. (From Strasinger, SK, and DiLorenzo, MA: The Phlebotomy Textbook, Fourth Edition, FA Davis, Philadelphia, 2019, Figure 3-16, p. 66, with permission.)

Box 1–1 Globally Harmonized System (GHS) and Safety Data Sheet (SDS) Format

Sixteen-section SDS that includes the following headings in the order specified:

1. Identification
2. Hazard(s) identification
3. Composition/information on ingredients
4. First-aid measures
5. Firefighting measures
6. Accidental release measures
7. Handling and storage
8. Exposure control/personal protection
9. Physical and chemical properties
10. Chemical stability and reactivity
11. Toxicological information
12. Ecological information
13. Disposal considerations
14. Transport information
15. Regulatory information
16. Other information

From Strasinger, SK, and DiLorenzo, MA: The Phlebotomy Textbook, Fourth Edition, FA Davis, Philadelphia, 2019, p. 67, with permission.

and cylinders of compressed gas should be located away from heat and securely fastened to a stationary device to prevent accidental capsizing. Fire blankets may be present in the laboratory. Persons with burning clothes should be wrapped in the blanket to smother the flames.

The NFPA classifies fires with regard to the type of burning material. It also classifies the type of fire extinguisher that is used to control them. This information is summarized in Table 1-2. The multipurpose ABC fire extinguishers are the most common, but the label should always be checked before using. It is important to be able to operate the fire extinguishers. The acronym PASS is about handling a fire extinguisher and can be used to remember the steps in the operation:

1. **P**ull pin
2. **A**im at the base of the fire
3. **S**queeze handles
4. **S**weep nozzle side to side

Physical Hazards



Physical hazards are not unique to the laboratory, and routine precautions observed outside the workplace apply. General precautions to consider are to avoid running in rooms and hallways; watch for wet floors; bend the knees when lifting heavy objects; keep long hair pulled back; avoid dangling jewelry; and maintain a clean, organized work area. Closed-toed shoes that provide maximum support are essential for safety and comfort.

Quality Management

The term **quality management** (QM) refers to the overall process of guaranteeing quality patient care, and it is regulated throughout the total testing system. A **quality management system** (QMS) refers to all of the laboratory's policies, processes, procedures, and resources needed to achieve quality testing.[15] In a clinical laboratory, a QMS includes not only testing controls, referred to as **quality control** (QC), but also encompasses **preexamination** (preanalytical) **variables** (e.g., specimen collection, handling, and storage), **examination** (analytical) **variables** (e.g., reagent and test performance, instrument calibration and maintenance, personnel requirements, and technical competence), **postexamination** (postanalytical) **variables** (e.g., reporting of results and interpretation), and documentation that the program is being followed meticulously. The original terms preanalytical, analytical, and postanalytical have been replaced with the standard terms of preexamination, examination, and postexamination from the International Organization for Standardization (ISO).

Table 1–2 Types of Fires and Fire Extinguishers

Fire Type	Extinguishing Material	Type/Composition of Fire	Extinguisher
Class A	Wood, paper, clothing	Class A	Water
Class B	Flammable organic chemicals	Class B	Dry chemicals, carbon dioxide, foam, or halon
Class C	Electrical	Class C	Dry chemicals, carbon dioxide, or halon
Class D	Combustible metals	None	Sand or dry powder
		Class ABC	Dry chemicals
Class K	Grease, oils, fats	Class K	Liquid designed to prevent splashing and cool the fire

From Strasinger, SK, and DiLorenzo, MA: The Phlebotomy Textbook, Fourth Edition, FA Davis, Philadelphia, 2019, with permission.

All of the following are included in a QMS:

- Procedure manuals
- **Internal quality control**
- **External quality control**
- **Electronic quality control**
- Calibration or calibration verification
- Standardization
- **Proficiency testing** (**PT**), more formally known as **external quality assessment** (**EQA**)[16]
- Record-keeping
- Equipment maintenance
- Safety programs
- Training
- Education and competency assessment of personnel
- A review process that is scheduled and documented

Essentially, QM is the continual monitoring of the entire test process, from test ordering and specimen collection through reporting and interpreting results. Written policies and documented actions as they relate to the patient, the laboratory, ancillary personnel, and the health-care provider are required. Having written remedial actions mandating the steps to take when any part of the system fails is essential to a QM program.

QM in the urinalysis laboratory—or any other laboratory department—is an integration of many factors used to monitor, evaluate, and improve the quality of laboratory services through all three phases of patient testing. This section of the text will provide a collection of the procedures essential for providing quality urinalysis. In the following chapters, the methods of ensuring accurate results will be covered on an individual basis for each of the tests.

Documentation of QM procedures is required by all laboratory **accreditation** agencies, including TJC, College of American Pathologists (**CAP**), American Association of Blood Banks (**AABB**), American Osteopathic Association (**AOA**), American Society of Histocompatibility and Immunogenetics (**ASHI**), American Association for Laboratory Accreditation (**A2LA**), and the Commission on Laboratory Assessment (**COLA**); it is also required for Medicare and Medicaid reimbursement. Guidelines published by CAP and the CLSI provide very complete instructions for documentation and are used as a reference for the ensuing discussion of the specific areas of urinalysis QC and QM.[16-18]

Documentation in the form of a procedure manual is required in all laboratories, and this format is used as the basis for the following discussion.

Urinalysis Procedure Manual

A procedure manual containing all the procedures performed in the urinalysis section must be available for reference in the working area and must comply with the CLSI guidelines. The following information is included for each procedure:

- Principle or purpose of the test
- Clinical significance
- Patient preparation
- Specimen type and method of collection
- Specimen acceptability and criteria for rejection
- Reagents
- Standards and controls
- Instrument calibration and maintenance protocols and schedules
- Step-by-step procedure
- Calculations
- Frequency and tolerance limits for controls and corrective actions
- Reference values and critical values
- Interpretation of results
- Specific procedure notes
- Limitations of the method
- Method validation
- Confirmatory testing
- Recording of results
- References
- Effective date
- Author
- Review schedule

Current package inserts should be reviewed and available at the workplace. Electronic manuals are acceptable and must be readily available to all personnel. As with written procedural manuals, electronic versions must be subjected to proper document control (i.e., only authorized persons may make changes, changes are dated/signed [manually or electronically], and there is documentation of periodic review).[17,18]

Evaluating procedures and adopting new methodologies is an ongoing process in the clinical laboratory. Whenever changes are made (Fig. 1-8), a person with designated authority, such as the laboratory director or section supervisor, should review and reference the procedure and then sign off on the change, and personnel should be notified of the changes. There must be documentation of an annual review of all procedures by the designated authority.

Preexamination (Preanalytical) Variables

Preexamination (preanalytical) variables occur before the actual testing of the specimen and include test requests, patient preparation, timing, specimen collection, handling, and storage. Health-care personnel outside the clinical laboratory control many of these factors, such as ordering tests and specimen collection. Communication between departments and adequate training on the correct procedures for ordering a test (**test utilization**), collecting, and transporting the specimen improves the **turnaround time** (**TAT**) of results, avoids duplication of test orders, and ensures a high-quality specimen. TAT is defined as the 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. Laboratories determine the TAT for tests, including both stat and routine

URINALYSIS SECTION

SPECIMEN ACCEPTABILITY/LABELING

Prepared by:

Initial approval:

Procedure placed in use:

Revised:

Reason for revision:

Effective Date	Supervisor Approval	Medical Director Approval
Reviewed		
Reviewed		
Reviewed		
Reviewed		
Reviewed		

Figure 1–8 Example of procedure review documentation. (Adapted from the Department of Pathology, St. Joseph Hospital, Omaha, NE.)

tests, as appropriate. Then the laboratory can monitor the TATs to determine areas in the process that need improvement. This can be determined by creating a cause-and-effect diagram, as shown in Figure 1-9.

Specimen Collection and Handling

Specific information on specimen collection and handling should be stated at the beginning of each procedure listed in the manual. Requisition forms and computerized entry forms should designate the type of urine specimen to be collected and the date and time of collection. The form should include space for recording

1. The actual date and time of specimen collection
2. Whether the specimen was refrigerated before transporting
3. The time the specimen was received in the laboratory and the time the test was performed
4. Tests requested
5. An area for specific instructions that might affect the results of the analysis
6. Patient identification information[18]

The patient's sex, age or date of birth, and, when appropriate, the source of the specimen and the time it was collected must be documented.[15]

Along with the specific procedure, the requisition form must detail patient preparation (e.g., fasting or elimination of interfering medications), type and volume of specimen required, and the need for sterile or opaque containers. All

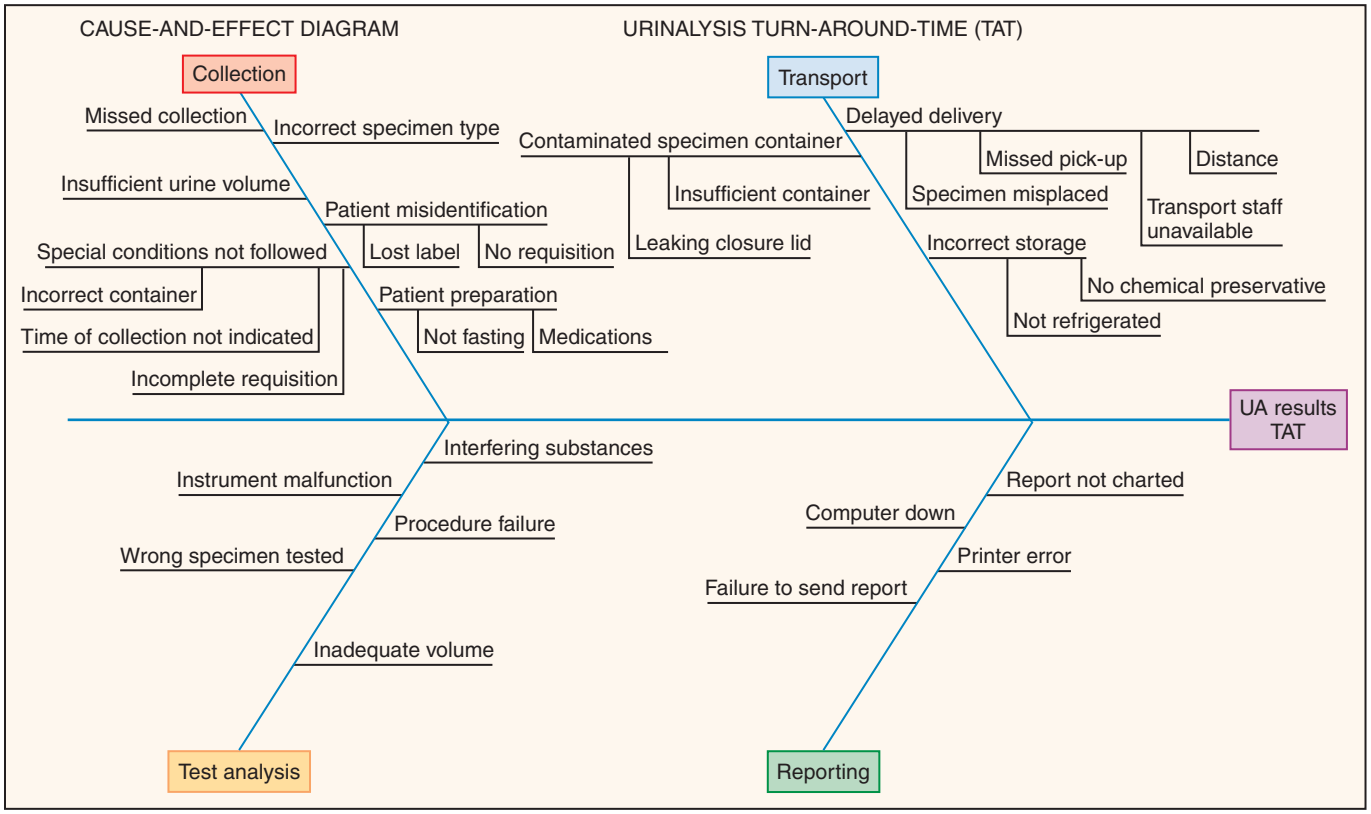


Figure 1–9 Cause-and-effect diagram for analyzing urinalysis TAT.

urine specimens should be examined within 2 hours. If this is not possible, written instructions for preserving the specimen must be available.[18]

The manual should also include instructions of a general nature, such as procedures for collecting clean-catch midstream and timed specimens, as well as information on specimen processing and printed instructions that are given to patients.

Criteria for specimen rejection for both physical characteristics and labeling errors must be present. Box 1-2 gives an example of a policy for handling mislabeled specimens. Written criteria for rejecting specimens must be documented and available to the health-care provider and nursing staff.[18] Box 1-3 lists the criteria for urine specimen rejection.

Laboratory personnel must determine the suitability of a specimen and document any problems and corrective actions taken. An example of an internal laboratory quality improvement form is shown in Figure 1-10. It is used as a tool to document a problem at the point of discovery, describing what happened and the immediate corrective action taken. This enables the laboratory director to capture the information to determine the **root cause analysis** (**RCA**) and develop a preventive or corrective action plan. Laboratory information systems have the capability to electronically generate these forms for review. An acceptable specimen requires verification of the patient's identification information on the requisition form and the container label, timely transport to the laboratory, the presence of refrigeration or recommended preservative if transport was delayed, and collection of an adequate amount of the correct urine specimen type in a noncontaminated, tightly closed container.[18]

Box 1–2 Policy for Handling Mislabeled Specimens

Do NOT assume any information about the specimen or patient.

Do NOT relabel an incorrectly labeled specimen.

Do NOT discard the specimen until investigation is complete.

Leave specimen EXACTLY as you receive it; put in the refrigerator for preservation until errors can be resolved.

Notify nursing station, doctor's office, etc. of problem and why it must be corrected for analysis to continue.

Identify problem on specimen requisition form with date, time, and your initials.

Make person responsible for specimen collection participate in solution of problem(s). Any action taken should be documented on the requisition slip.

Report all mislabeled specimens to the appropriate supervisor.

From Schweitzer, SC, Schumann, JL, and Schumann, GB: Quality assurance guidelines for the urinalysis laboratory. Journal of Medical Technology 3(11): 568, 1986, with permission.

Box 1–3 Criteria for Urine Specimen Rejection

Unlabeled containers

Nonmatching labels and requisition forms

Specimens contaminated with feces or toilet paper

Containers with contaminated exteriors

Insufficient volume of urine

Specimens that were transported or preserved improperly

Delay between time of collection and receipt in the laboratory

Examination (Analytical) Variables

The examination (analytical) variables are the processes that directly affect the testing of specimens. They include reagents, instrumentation and equipment, testing procedure, QC, **preventive maintenance** (**PM**), access to procedure manuals, and competency of personnel performing the tests.

Reagents

The manual should state the name and chemical formula of each reagent used; instructions for preparation, when necessary, or company source of prepared materials; storage requirements; and procedures for reagent QC. The type of water used for preparing reagents and controls must be specified. Distilled or deionized water or clinical laboratory reagent water (**CLRW**) must be available. CLRW quality must be monitored regularly for microbial contamination as well as ionic and organic impurities.[19] A written policy must be available with the procedures to follow when the CLRW quality is not within the tolerance limits. A bold-type statement of any safety or health precautions associated with reagents should be present. An example of this would be the heat produced in the Clinitest reaction.

All reagents and reagent strips must be properly labeled with the date of preparation or opening, purchase and received date, expiration date, and appropriate safety information. Reagent strips should be checked against known negative and positive control solutions on each shift or, at a minimum, once a day and whenever a new bottle is opened. Reagents are checked daily or when tests requiring their use are requested. After checking is performed, laboratory personnel should properly record the results of all reagent checks. Reagent strips must never be refrigerated, must be protected from light, and must be recapped immediately after removing each strip.

Instrumentation and Equipment

The procedure manual should clearly state the instructions regarding the operation, performance, and frequency of calibration; limitations; and procedures to follow when limitations or linearity are exceeded, such as dilution procedures. Instructions detailing the appropriate recording procedures must be included.

The instruments encountered in the urinalysis laboratory most frequently are refractometers, osmometers, automated reagent strip readers, and automated microscopy instruments. Refractometers are calibrated daily or when used against deionized water (1.000) and a known control,

Quality Improvement Follow-up Report

CONFIDENTIAL

Instructions: Section I should be completed by the individual identifying the event.

Date of report: _____ Reported by: _____
Date of incident: _____ Date/time of discovery: _____
Patient MR# _____ Patient accession# _____

Section I.
Summary of incident — *describe what happened* _____

_____

_____

What immediate corrective action was taken? _____

_____

_____

Provide the ORIGINAL to team leader/technical specialist within 24 hours of incident discovery
Date: _____
To: _____

Forwarded for follow-up:
Date: _____
To: _____

Section II. Management investigation: *Tracking #* _____

Instructions: Section II should be completed by laboratory management within 72 hours

Check appropriate problem category

- ☐ Unacceptable patient samples (Due to hemolysis, QNS, or contaminated)
- ☐ Equipment-related event
- ☐ Standard operating procedure deviation
- ☐ Communication problem/complaint
- ☐ Accident
- ☐ Wrong tube type
- ☐ Misidentified sample
- ☐ Wrong location
- ☐ Other (explain)

Explain answers:

_____

_____

Preventive/corrective action recommendations:

_____

_____

Technical specialist/team leader: _____ Date: _____

Medical director review: _____ Date: _____

Quality assurance review: _____ Date: _____

FDA reportable: Yes or no _____ Date reported: _____

Figure 1–10 Sample of quality improvement follow-up report form. (From Danville Regional Medical Center Laboratory, Danville, VA, with permission.)

such as 5% saline (1.022 ± 0.001) or 9% sucrose (1.034 ± 0.001). Two levels of commercial controls are available for the osmometer, urine reagent strip tests, and human chorionic gonadotropin (**hCG**) kit tests. All control values must be recorded. Automated urinalysis systems and reagent strip readers are calibrated using manufacturer-supplied calibration materials, following the protocol specified by the manufacturer. Both positive and negative control values must be run and recorded (Fig. 1-11). There must be documentation showing evidence of corrective action for any failed QC tests. No patient's testing may be performed until QC is acceptable.

Other common equipment found in the urinalysis laboratory includes refrigerators, freezers, pipettes, centrifuges, microscopes, and water baths. Temperatures of refrigerators and water baths should be taken daily and recorded. Calibration of centrifuges and accuracy of timers are customarily performed every 3 months, and there should be a record of the appropriate relative centrifugal force for each setting. Disinfection of centrifuges should be done routinely on a weekly basis or after spills. Microscopes should be kept clean at all times and have an annual professional cleaning. Automatic pipettes are initially and periodically checked for accuracy and reproducibility. As mandated by the TJC or CAP guidelines, a routine PM schedule for instruments and equipment should be prepared and records kept of all routine and nonroutine maintenance performed.

Deionized water used for reagent preparation is quality controlled by checking pH and purity meter resistance on a weekly basis and the bacterial count on a monthly schedule. All results must be recorded on the appropriate forms.

Testing Procedure

Detailed, concise testing instructions are written in a step-by-step manner. Instructions should begin with specimen preparation, such as time and speed of centrifugation, and include types of glassware needed, time limitations and stability of specimens and reagents, calculation formulas and a sample calculation, health and safety precautions, QC checks, reference ranges, and procedures. Additional procedure information, including reasons for special precautions; sources of error and interfering substances; criteria for the correlation of the physical, chemical, and microscopic urinalysis results; helpful hints; clinical situations that influence the test; alternative procedures; and acceptable TATs for stat tests are listed under the title of Procedure Notes following the step-by-step procedure.

Reference sources should be listed. Manufacturer's package inserts may be included but cannot replace the written procedure. The laboratory director must sign and date new procedures and all modifications of procedures before they are used.[15]

Quality Control

QC refers to the materials, procedures, and techniques that monitor the **accuracy**, **precision**, and **reliability** of a laboratory test. QC procedures are performed to ensure that acceptable standards are met during the process of patient testing. The step-by-step instructions for each test should include specific QC information regarding the type of control specimen preparation and handling, frequency of use, tolerance levels, and methods of recording. QC is performed at scheduled times, such as at the beginning of each shift or before testing patient specimens, and it must always be performed if reagents are changed, an instrument malfunction has occurred, or test results are questioned by the health-care provider. Control results must be recorded in a log, either paper or electronic. Patient test results may not be reported until the QC is verified. Both external quality control monitoring and internal and electronic quality control processes are practiced in the urinalysis laboratory.

External Quality Control

External quality controls are used to verify the accuracy (ability to obtain the expected result) and precision (ability to obtain the same result on the same specimen) of a test, and they are exposed to the same conditions as the patient specimens. Reliability is the ability to maintain both precision and accuracy. Commercial controls are available for the urine chemistry tests, specific gravity, and certain microscopic constituents. Analysis of two levels of control material is required. The concentration

QUALITY CONTROL Month:_____20_____

			Positive control									Negative control								
			GLU	BIL	KET	SP GR	BLD	PH	PROT	NIT	LEU EST	GLU	BIL	KET	SP GR	BLD	PH	PROT	NIT	LEU EST
			mg/dL		mg/dL	1.0			mg/dL			mg/dL		mg/dL	1.0			mg/dL		
Control values																				
Reagent	TECH	DATE																		
Lot #																				
EXP																				

Figure 1–11 Sample instrument QC recording sheet. (Adapted from the Department of Pathology, Methodist Hospital, Omaha, NE, with permission.)

of controls should be at medically significant levels and should be as much like the human specimen as possible. Documentation of QC includes dating and initialing the material when it is first opened and recording the manufacturer's lot number and the expiration date each time a control is tested and the test result is obtained. Food and Drug Administration (**FDA**) standards require that control material test negative for HIV and HBV. The same person who is performing the patient testing should perform the testing and interpretation of external controls in the laboratory.

Control data are evaluated before releasing patient results. Data obtained from repeated measurements have a Gaussian distribution or spread in the values that indicate the ability to repeat the analysis and obtain the same value. The laboratory, after repeated testing, establishes the value for each analyte, and then it should calculate the mean and **standard deviation** (**SD**). The **control mean** is the average of all data points, and the SD is a measurement statistic that describes the average distance each data point in a normal distribution is from the mean. The **coefficient of variation** (**CV**) is the SD expressed as a percentage of the mean. The CV indicates whether the distribution of values about the mean is in a narrow versus broad range and should be less than 5%. Confidence intervals are the limits between which the specified proportion or percentage of results will lie. **Control ranges** are determined by setting confidence limits that are within ±2 SD or ±3 SD of the mean, which indicates that 95.5% to 99.7% of the values are expected to be within that range.

Values are plotted on Levy-Jennings control charts to visually monitor control values. Immediate decisions about patient results are based on the ability of control values to remain within a preestablished limit. Changes in the accuracy of results are indicated by either a **trend**, which is a gradual changing in the mean in one direction, or a **shift**, which is an abrupt change in the mean (Fig. 1-12). Changes in precision are shown by a large amount of scatter about the mean and an uneven distribution above and below the mean that are most often caused by errors in technique.

Corrective action, including the use of new reagents, reagent strips, or controls and the verification of lot numbers and expiration dates, must be taken when control values are outside the tolerance limits. All corrective actions taken are documented. A protocol for corrective action is shown in Figure 1-13. A designated supervisor reviews all QC results.

Laboratories may participate in a commercial QC program. Participating laboratories return results from the same lot of QC material sent by the manufacturer back to the manufacturer for statistical analysis and comparison with other laboratories using the same methodology.

Internal Quality Control

Internal quality control consists of internal monitoring systems built in to the test system and are called internal or procedural controls.[16,20] Internal controls monitor the sufficient addition of a patient sample or reagent, the instruments/reagents interaction, and, for lateral flow test methods, whether the sample migrated through the test strip properly.[16]

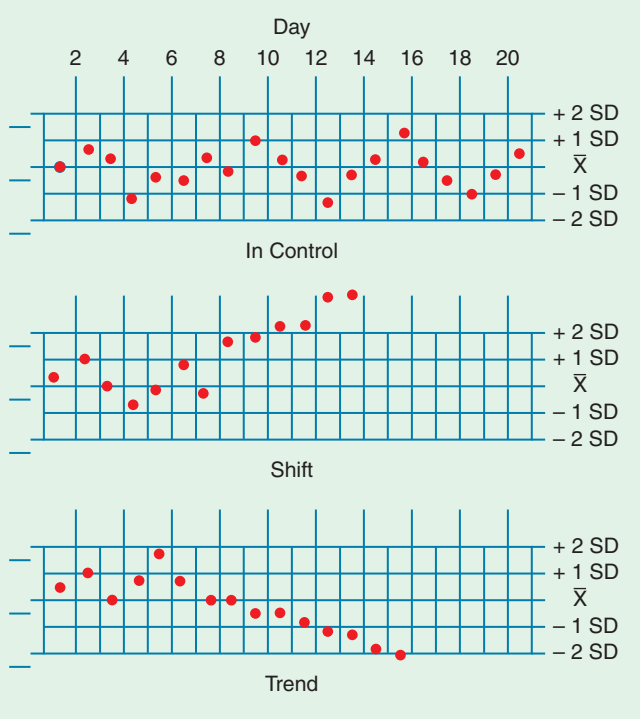


Figure 1–12 Levy-Jennings charts showing in-control, shift, and trend results.

Electronic Controls

Electronic controls use a mechanical or electrical device in place of a liquid QC specimen. This type of QC can be an internal or an external component inserted into a point-of-care (**POC**) instrument. Electronic controls verify the functional ability of a testing device, but they do not verify the integrity of the testing supplies. Many test systems use a combination of external and internal controls to verify that the entire test system is working properly.

Proficiency Testing (External Quality Assessment)

PT, or EQA, is the testing of unknown specimens received from an outside agency and provides unbiased validation of the quality of patient test results. Several commercial vendors, such as CAP, provide PT. Laboratories subscribing to these programs receive lyophilized or ready-to-use specimens for routine urinalysis and digital images or photomicrographs for sediment constituent identification. Personnel in subscribing laboratories test these proficiency survey specimens in the same manner as patient specimens. No communication with other laboratories is permitted. The subscribing laboratory returns the results to the PT vendors, where they are statistically analyzed with those from all participating laboratories. Then the director of the subscribing laboratory receives a report that evaluates laboratory accuracy and compares it with other laboratories using the same method of analysis. Corrective action must be taken for unacceptable results.[16] The **Clinical Laboratory Improvement Amendments** (**CLIA**) mandates comparison testing for laboratory accreditation.[20]

A. Record all actions taken and the resolution of any problems

B. Use the flow diagram below:

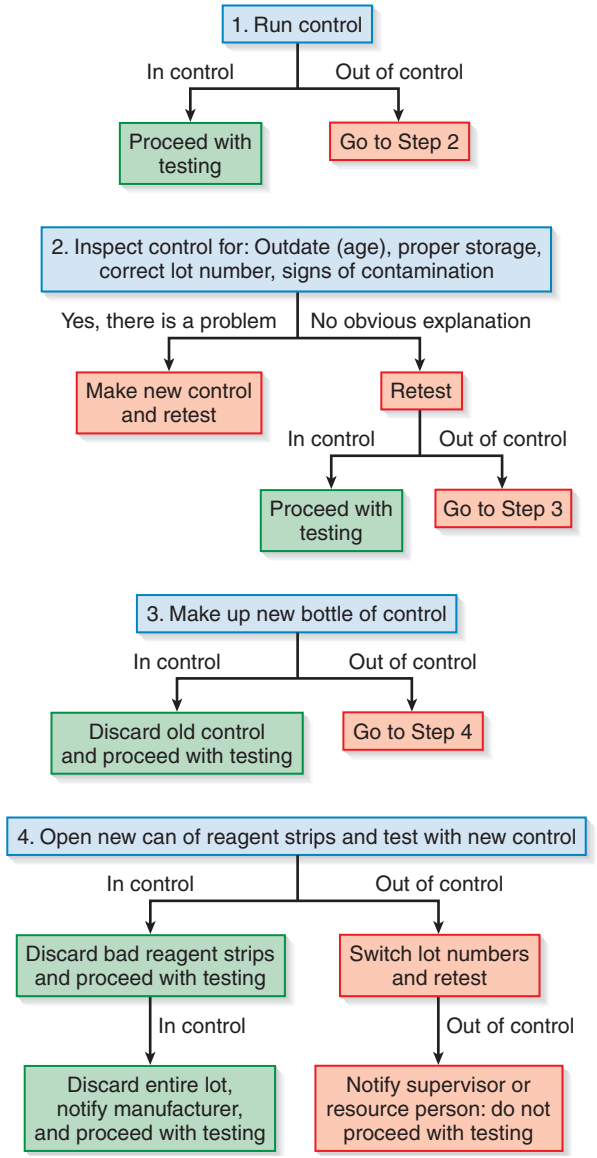


Figure 1–13 "Out-of-control" procedures. (From Schweitzer, SC, Schumann, JL, and Schumann, GB: Quality assurance guidelines for the urinalysis laboratory. Journal of Medical Technology 3(11): 567–572, 1986, with permission.)

Individualized Quality Control Plan

An individualized quality control plan (**IQCP**) is an alternative CLIA QC option that provides equivalent quality testing to meet the CLIA regulations for moderate- and high-complexity tests and provider-performed microscopy procedures (**PPMP**) tests. IQCP considers the entire testing process—preexamination, examination, and postexamination—to ensure quality testing, not just the frequency of QC and number of QC materials. With this option, the laboratory can determine the frequency of QC testing based on information about the test, the risk assessment, and the accreditation agencies' requirements.

An IQCP requires risk assessment (**RA**), a quality control plan (**QCP**), and quality assessment (**QA**).[21] RA identifies and evaluates potential problems that may occur in the entire testing process from preexamination through the examination and postexamination processes. Components that are assessed for potential errors are the specimen, test system, reagents, environment, and testing personnel. QCP establishes control procedures to reduce the possibility of reporting an inaccurate patient test result. It must include the number, type, and frequency of testing, as well as criteria for acceptable results of the QCs. IQCP QA involves selecting quality monitors that include:

- QC records
- PT records
- Review of patient results
- Specimen rejection logs
- TAT reports
- Records of preventive measures, corrective actions, and follow-up
- Personnel competency records

Personnel and Facilities

QC is only as good as the personnel performing and monitoring it. Personnel assessment includes education and training, continuing education, competency assessment, and performance appraisals. Each new employee must have documentation of training during orientation to the laboratory. This is a checklist of procedures and must include the date and initials of the person doing the training and of the employee being trained. Up-to-date reference materials and atlases should be readily available, and documentation of continuing education must be maintained.[16]

An adequate, uncluttered, safe working area is also essential for both quality work and personnel morale. Standard precautions for handling body fluids must be followed at all times.

Postexamination Variables

Postexamination variables are processes that affect the reporting of results and correct interpretation of data. Reporting of test results to the appropriate health-care provider in an efficient and accurate manner is essential to quality patient care. Reports may be handwritten or instrument printouts. Reports may be delivered by telephone or fax or transmitted electronically to the requesting health-care provider.

Laboratory reports must be present in the patient's record. Required information includes:

- Patient's first and last name
- Patient's unique identification number
- Specimen collection date and time
- Specimen source (if pertinent to the test)
- Condition of unsatisfactory specimen

- Tests performed, the results, and the reference ranges of the tests
- Date and time of the final results generated
- Facility where the test was performed

Written Reports

Each procedure covered in the procedure manual should include standardized reporting formats and, when applicable, reference ranges. There must be a written procedure for reporting, reviewing, and correcting errors.

Forms for reporting results should provide adequate space for writing and should present the information in a logical sequence. Standardized reporting methods minimize health-care provider confusion when interpreting results (Fig. 1-14).

Electronic Results

Electronic transmission is now the most common method for reporting results. Many urinalysis instruments have the capability for the operator to transmit results directly from the instrument to the designated health-care provider. It is essential that the operator carefully review results before transmittal. The operator also may enter the results manually into the laboratory computer system and then transmit them to the health-care provider.

MICROSCOPIC QUANTITATIONS

Quantitate an average of 10 representative fields. Do not quantitate budding yeast, mycelial elements, *Trichomonas*, or sperm, but do note their presence with the appropriate LIS code.

Epithelial cells/LPF	
None:	0
Rare:	0–5
Few:	5–20
Moderate:	20–100
Many:	>100
Casts/LPF	
None:	0
Numerical ranges:	0–2, 2–5, 5–10, >10
RBCs/HPF	
None:	0
Numerical ranges:	0–2, 2–5, 5–10, 10–25, 25–50, 50–100, >100
WBCs/HPF	
None:	0
Numerical ranges:	0–2, 2–5, 5–10, 10–25, 25–50, 50–100, >100
Crystals/HPF	
None:	0
Rare:	0–2
Few:	2–5
Moderate:	5–20
Many:	>20
Bacteria/HPF	
None:	0
Rare:	0–10
Few:	10–50
Moderate:	50–200
Many:	>200
Mucous threads	
Rare:	0–1
Few:	1–3
Moderate:	3–10
Many:	>10

Figure 1–14 Sample standardized urine microscopic reporting format. (From University of Nebraska Medical Center, Omaha, NE, with permission.)

Telephone (Verbal) Results

The telephone is frequently used to transmit results of stat tests and critical values. Calls requesting additional results may be received from personnel on hospital units and from health-care providers. When telephoning results, confirm that the results are being reported to the appropriate person. The time of the call and the name of the person receiving the results must be documented according to the facility's policy.

Result Errors

Errors may be discovered in the laboratory through a QM procedure known as the **delta check** that compares a patient's test results with the previous results. Variation outside the established parameters alerts laboratory personnel to the possibility of an error that occurred during the testing procedure or during patient identification. Many laboratory analyzers now have **autoverification**, an automated comparison of patient results with predetermined preapproved criteria, programmed into them.[4]

Erroneous results must be corrected in a timely manner to assure that the patient does not receive treatment based on incorrect results. Errors can occur in patient identification, specimen labeling, or result transcription. The patient's record should be corrected as soon as the error is detected; however, the original result must not be erased in the event that the health-care provider treated the patient based on the erroneous results. An incident (variance) report documenting the problem and corrective action may need to be submitted. Appropriate documentation of erroneous results should follow facility protocol.

Critical Results

There should be written procedures available for the reporting of critical values. Critical values are test results that are significantly lower or higher than the normal reference range. They can be life-threatening and must be relayed to the health-care provider immediately, following facility policy. Figure 1-15 is an example of the procedure instructions for reporting critical values in the urinalysis department analyzing pediatric specimens; this should include the presence of ketones or reducing substances in newborns.

Interpreting Results

The **specificity** and the **sensitivity** for each test should be included in the procedure manual for correct interpretation of results. Sensitivity (the lowest level of an analyte that a test can detect) and specificity (the likelihood of measuring the analyte desired) vary among manufacturers. The manual should list all known interfering substances for evaluation of patient test data. A well-documented QM program ensures quality test results and patient care data.

SUMMARY 1-1 Quality Management Errors

Preexamination

Patient misidentification

Wrong test ordered

Incorrect urine specimen type collected

Insufficient urine volume

Delayed transport of urine to the laboratory

Incorrect storage or preservation of urine

Examination

Sample misidentification

Erroneous instrument calibration

Reagent deterioration

Poor testing technique

Instrument malfunction

Interfering substances present

Misinterpretation of QC data

Postexamination

Patient misidentification

Poor handwriting

Transcription error

Poor quality of instrument printer

Failure to send report

Failure to call critical values

Inability to identify interfering substances

Reporting Critical Results In Urinalysis

POSITIVE KETONES

For children age 2 years and younger:

All results positive for ketones should be telephoned to the appropriate nursing unit.

Document the following information in the computer as a chartable footnote appended to the result:

- ✔ Time of telephone call
- ✔ Initials of the person making the call
- ✔ Name of the person receiving the telephone call

POSITIVE CLINITEST

For children age 2 years and younger:

All Clinitest results should be telephoned to the appropriate nursing unit.

Document the following information in the computer as a chartable footnote appended to the result:

- ✔ Time of telephone call
- ✔ Initials of the person making the call
- ✔ Name of the person receiving the telephone call

Figure 1–15 An example of procedure instructions for reporting critical values in the urinalysis section. A procedure review document similar to that shown in Figure 1–8 would accompany this instruction sheet.



For additional resources please visit
www.fadavis.com

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Study Questions

1. Which of the following organizations publishes guidelines for writing procedures and policies in the urinalysis?

A. CDC
B. OSHA
C. CLSI
D. CLIA

2. Exposure to toxic, carcinogenic, or caustic agents is what type of laboratory safety hazard?

A. Biological
B. Sharps
C. Chemical
D. Fire/explosive

3. In the urinalysis laboratory, the primary source in the chain of infection would be:

A. Patients
B. Needlesticks
C. Specimens
D. Biohazardous waste

4. The best way to break the chain of infection is:

A. Hand sanitizing
B. Personal protective equipment
C. Aerosol prevention
D. Decontamination

5. The current routine infection control policy developed by CDC and followed in all health-care settings is:

A. Universal Precautions
B. Isolation Precautions
C. Blood and Body Fluid Precautions
D. Standard Precautions

6. An employee who is accidentally exposed to a possible bloodborne pathogen should immediately:

A. Report to a supervisor
B. Flush the area with water
C. Clean the area with disinfectant
D. Receive HIV prophylaxis

7. Personnel in the urinalysis laboratory should wear laboratory coats that:
   A. Do not have buttons
   B. Are fluid-resistant
   C. Have short sleeves
   D. Have full-length zippers
8. All of the following should be discarded in biohazardous waste containers *except:*
   A. Urine specimen containers
   B. Towels used for decontamination
   C. Disposable laboratory coats
   D. Blood collection tubes
9. An employer who fails to provide sufficient gloves for the employees may be fined by the:
   A. CDC
   B. NFPA
   C. OSHA
   D. FDA
10. An acceptable disinfectant for decontamination of blood and body fluids is:
   A. Sodium hydroxide
   B. Antimicrobial soap
   C. Hydrogen peroxide
   D. Sodium hypochlorite
11. Correct hand washing 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
12. Centrifuging an uncapped specimen may produce a biological hazard in the form of:
   A. Vectors
   B. Sharps contamination
   C. Aerosols
   D. Specimen contamination
13. 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 SDS
   D. Wrap the arm in gauze and go to the emergency department
14. 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
15. An employee can learn the carcinogenic potential of potassium chloride by consulting the:
   A. Chemical hygiene plan
   B. Safety Data Sheet
   C. OSHA standards
   D. Urinalysis procedure manual
16. Employees should not work with radioisotopes if they are:
   A. Wearing contact lenses
   B. Allergic to iodine
   C. Sensitive to latex
   D. Pregnant
17. 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
18. 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
19. The system used by firefighters to assess the risk potential when a fire occurs in the laboratory is:
   A. SDS
   B. RACE
   C. NFPA
   D. PASS
20. A class ABC fire extinguisher contains:
   A. Sand
   B. Water
   C. Dry chemicals
   D. Acid
21. The first thing to do when a fire is discovered is to:
   A. Rescue people in danger
   B. Activate the alarm system
   C. Close doors to other areas
   D. Extinguish the fire if possible

22. 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 her hands so vigorously

23. 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

24. The NPFA classification symbol contains information on all of the following *except:*

A. Fire hazards
B. Biohazards
C. Reactivity
D. Health hazards

25. The GHS requires the following on a chemical label:

A. Biohazard symbol, warning sign, environmental impact
B. Hazard pictogram, signal words, hazard statement
C. Biological symbol, hazard pictogram, long-term effects
D. Signal words, hazard statement, biological symbol

26. The classification of a fire that can be extinguished with water is:

A. Class A
B. Class B
C. Class C
D. Class D

27. Employers are required to provide free immunization for:

A. HIV
B. HTLV-1
C. HBV
D. HCV

28. 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

29. Quality management refers to:

A. Analysis of testing controls
B. Increased productivity
C. Precise control results
D. Quality of specimens and patient care

30. During laboratory accreditation inspections, procedure manuals are examined for the presence of:

A. Critical values
B. Procedure references
C. Procedures for specimen preservation
D. All of the above

31. As the supervisor of the urinalysis laboratory, you have just adopted a new procedure. You should:

A. Put the package insert in the procedure manual
B. Put a complete, referenced procedure in the manual
C. Notify the microbiology department
D. Put a cost analysis study in the procedure manual

32. 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

33. The testing of a specimen from an outside agency and the comparison of results with participating laboratories is called:

A. External QC
B. Electronic QC
C. Internal QC
D. Proficiency testing

34. A color change indicating that a sufficient amount of a patient's specimen or reagent is added correctly to the test system would be an example of:

A. External QC
B. Equivalent QC
C. Internal QC
D. Proficiency testing

35. What steps are taken when the results of reagent strip QC are outside the stated confidence limits?

A. Check the expiration date of the reagent strip
B. Run a new control
C. Open a new reagent strips container
D. All of the above

36. When a new bottle of QC material is opened, what information is placed on the label?
    A. The supervisor's initials
    B. The lot number
    C. The date and the laboratory worker's initials
    D. The time the bottle was opened

37. When a control is run, what information is documented?
    A. The lot number
    B. Expiration date of the control
    C. The test results
    D. All of the above

Case Studies and Clinical Situations

1. State a possible reason for an accreditation team to report a deficiency in the following situations:
    a. The urine microscopic reporting procedure has been recently revised.
    b. An unusually high number of urine specimens are being rejected because of improper collection.
    c. A key statement is missing from the Clinitest procedure.
    d. Open control bottles in the refrigerator are examined.

2. As the new supervisor of the urinalysis section, you encounter the following situations. Explain whether you would accept them or take corrective action.
    a. You are told that the supervisor always performs the CAP proficiency survey.
    b. QC is not performed daily on the reagent strips.
    c. The urinalysis section is primarily staffed by personnel assigned to other departments for whom you have no personnel data.

3. The medical laboratory scientist was assigned to test 10 urine specimens chemically. She removed 10 strips from the container and proceeded with testing. Several patients' urine indicated a trace positive glucose in the urine. She then opened a new bottle of reagent dipsticks and proceeded to perform the QC. The negative control also tested as a trace positive for glucose. The medical laboratory scientist consulted the supervisor. The supervisor ran the QC, and the results were in the correct range. After observing the medical laboratory scientist's technique, the supervisor realized that the medical laboratory scientist was waiting too long to read the glucose results and therefore reporting erroneous results.
    a. What is wrong with this scenario?
    b. Who should run the QC for each test? Why?
    c. When should controls be run?
    d. What do you do when the QC is out of range?
    e. When can you report patient results?

4. An outpatient urine specimen was delivered to the laboratory at 0800 and placed on the counter in the urinalysis department. The medical laboratory scientist performed urinalysis on the specimen at 1130. The following results were abnormal:

    Clarity: Cloudy

    pH: 9.0

    Nitrite: Positive

    The patient was a known diabetic; however, the glucose result was negative.
    a. What could be a possible cause for the abnormal results?
    b. Where would the information concerning what should have been done with this specimen be found, as well as the criteria for rejection?
    c. What QM procedure may have detected this error?
    d. What form will need to be completed for this scenario?
    e. How might this affect this patient's care?
    f. How will the corrected results be documented?

CHAPTER 2

Urine and Body Fluid Analysis Automation

LEARNING OUTCOMES

Upon completing this chapter, the reader will be able to:

2-1 Explain the principle of reflectance photometry.

2-2 Differentiate between semiautomated urine chemistry analyzers and fully automated urine chemistry analyzers.

2-3 State the advantages of automated microscopy methods over manual microscopy methods for analyzing urine sediment.

2-4 Describe laser-based flow cytometry, digital imaging, and auto particle recognition technologies used in automated urine microscopy analyzers.

2-5 Discuss the advantages of automated body fluid analyzers over the Neubauer hemocytometer for body fluid cell counts.

KEY TERMS

Auto-checks
Auto particle recognition (APR)
Autovalidated
Digital imaging
Flow cytometry
Histograms
Light-emitting diode (LED)
Reflectance photometry
Scattergrams

Urinalysis Automation

Studies have shown that the major variable in urinalysis testing is the conscientiousness of the laboratory personnel in their timing and interpretations of the color reactions. Correct color readings depend on the accuracy of the timing. The ultimate goal of urinalysis automation is to improve reproducibility and color discrimination while increasing productivity and standardization for reporting urinalysis results.

Reflectance Photometry

Subjectivity associated with visual discrimination among colors has been alleviated by the development of automated reagent strip readers that use a spectrophotometric measurement of light reflection, termed "**reflectance photometry**." Reflectance photometry uses the principle that light reflection from the test pads decreases in proportion to the intensity of the color produced by the concentration of the test substance. In reflectance photometry, a monochromatic light source is directed toward the reagent pads by placing a filter between the light source and the reflective surface of the pad or by using a **light-emitting diode** (**LED**) to provide the specific wavelength needed for each test pad color reaction. The light is reflected to a photodetector, as well as a converter that is either analog or digital. The instruments compare the amount of light reflection with that of known concentrations and then display or print concentration units or transmit data to a laboratory information system (**LIS**).

Several automated instruments are available that standardize sample processing, analyze chemistry test strips, perform urine sediment analysis, and report results with consistent quality and reduced hands-on time. The instruments are user-friendly and include visual and audio prompts for operation. The various manufacturers' instruments include different features and principles for testing. See Table 2-1 for a breakdown of the measurement technologies used by the major urine analyzer manufacturers.[1,2]

Additional advantages to automation include online computer capability with an LIS interface; barcoding; manual entry of color, clarity, and microscopic results to be included on the printed report; flagging abnormal results; storing patient and control results; and minimal calibration, cleaning, and maintenance.

Automated instruments in urinalysis include semiautomated and fully automated chemistry analyzers, automated urine cell microscopy analyzers, and systems that are completely automated. Semiautomated instruments still depend on an operator for specimen mixing, test strip dipping, and microscopic results input. In a fully automated chemistry analyzer, the tubes of urine are placed on a rack or a carousel and moved automatically through the instrument. Automated urine cell microscopy analyzers mix, aspirate, dilute, and stain urine to classify urine sediment particles. Automated urine systems perform a complete urinalysis that includes the physical, chemical, and microscopic parts of a routine urinalysis by integrating a fully automated chemistry analyzer with an automated urine cell microscopy analyzer. The automated urinalysis instruments currently available are listed in Table 2-2; however, new instruments are being developed continually.[1,2]

Analyzers

Semiautomated Urine Chemistry Analyzers

Semiautomated urine analyzers test for the chemical components of urine. The instruments read and interpret the reagent strip results consistently, thereby standardizing the interpretation of reagent strip results and eliminating personnel color bias and timing discrepancies. Depending on the instrument and the reagent strip used, the following tests can be performed: leukocyte, nitrite, protein, blood, glucose, ketone, bilirubin, urobilinogen, pH, specific gravity, color, creatinine, and protein-to-creatinine ratio. Semiautomated analyzers are well suited for small- and medium-volume laboratories and physicians' offices and meet the Clinical Laboratory Improvement Amendments (**CLIA**)–waived standards.

Semiautomated analyzers are self-calibrating, and some instruments perform automatic checks (**auto-checks**) to identify strip type and humidity exposure. For a semiautomated instrument, the reagent strips are manually dipped into the urine and placed on the strip reader, the reaction pads are read at the correct time, and the strip is moved to the waste container. The results

Table 2–1 Measurement Technology Methods in Automated Urinalysis[1,2]

	Urine Measurement Technology		
Manufacturer	Color	Clarity	Specific Gravity
U.S. ARKRAY, Inc.	Reflectance photometry	Light scatter	Refractive index
Iris Diagnostics- Beckman Coulter, Inc.	Light transmission/light scatter	Light transmission/light scatter	Refractive index
DIRUI	Reflectance photometry	Turbidity	Refractometry
Roche Diagnostics	Reflectance photometry	Turbidity	Refractometry
Siemens Healthcare Diagnostics, Inc.	Reflectance photometry	Light transmission/light scatter	Refractive index
Sysmex Corporation	Reflectance photometry	Reflectance photometry	Refractometry

Table 2–2 Urinalysis Automation

Equipment	Manufacturer
Semiautomated Chemistry Instruments	
Clinitek Advantus	Siemens Healthineers
Clinitek Status	Siemens Healthineers
Urisys 1800 system	Roche Diagnostics
COBAS u411	Roche Diagnostics
AUTION Eleven AE-4022	U. S. ARKRAY Inc.
Fully Automated Chemistry Instruments	
Clinitek Atlas	Siemens Healthineers
Clinitek Novus	Siemens Healthineers
Urisys 2400 system	Roche Diagnostics
AUTION MAX AX-4030	U.S. ARKRAY, Inc.
IChemVELOCITY	Iris Diagnostics-Beckman Coulter, Inc.
Cobas u 601 urine analyzer	Roche Diagnostics
FUS-100 and FUS-200	DIRUI
Automated Microscopy	
UF-1000*i* Urine Cell Analyzer	Sysmex Corporation
UN-2000	Sysmex Corporation
iQ 200 Automated Urine Microscopy (IQ200SELECT, IQ200ELITE, IQ200SPRINT)	Iris Diagnostics-Beckman Coulter, Inc.
Urine Analyzer (iQ 200 Sprint)	Iris Diagnostics-Beckman Coulter, Inc.
Cobus u 701 Microscopy Analyzer	Roche Diagnostics
LabUMat 2	77 Elektronika
Automated Urinalysis Systems	
iRICELL Urinalysis – IQ Series Urinalysis Workcell	Iris Diagnostics-Beckman Coulter, Inc.
(iRICELL 3000*plus,* iRICELL 2000*plus,* iRICELL 3000pro, iRICELL 2000pro, iRICELL 1500)	
CLINITEK AUWi Pro System	Siemens Healthineers
Cobas 6500	Roche Diagnostics
Dirui FUS – 100/200 with H-800	DIRUI
UriSed 2 or UriSed 3 with LabUMat2	77 Elektronika
UX-2000	Sysmex Corporation
AUTION HYBRID AU-4050	U.S. ARKRAY, Inc.
Body Fluid Analyzers	
ADVIA2120i with Body Fluids Software	Siemens Healthcare Diagnostics, Inc.
Sysmex XN-Series using Body Fluids mode	Sysmex Corporation
iQ 200 using Body Fluids Software	Iris Diagnostics-Beckman Coulter, Inc.
DxH 900 Hematology Analyzer	Beckman Coulter, Inc.

are displayed, printed, or transmitted to an LIS. Patient identification and specimen color and clarity may be entered manually, or a barcode reader can be used to identify samples. Positive results are flagged to indicate a patient sample that requires additional confirmation testing or microscopic evaluation.

The semiautomated instrument requires the operator to:

1. Dip the reagent strip into a urine sample that has been mixed well.
2. Blot the strip to remove excess urine.
3. Place the strip onto the reagent strip platform.
4. Press the analyze/enter button.

The results are printed out, and abnormal results are flagged automatically. As the strip moves through the instrument, reflectance readings are taken at the correct time intervals. Some manufacturers' test strips have a color compensation pad that adjusts results for urine color. This feature allows the instrument to subtract the urine color from the color developed on the reaction pad, providing an accurate result for each pad despite the interference. Then the strips are moved to the waste container. Results are stored in the analyzer, printed, or sent to the LIS. Examples of semiautomated instruments are shown in Figures 2-1 through 2-5.

Daily maintenance is minimal and includes cleaning the reagent strip platform and emptying the reagent strip waste container.

Fully Automated Urine Chemistry Analyzers

Fully automated instruments are designed for a high-volume urinalysis laboratory with user walk-away capability. The various instruments can load many labeled tubes of urine on a carousel or rack at one time with the capability to insert a stat sample during the run. The "start" or "analyze" button is pressed to begin testing, and the sample moves through the instrument automatically. The sample is identified, mixed, and aspirated. A sample probe aspirates an exact amount of urine and dispenses it



Figure 2–1 AUTION ELEVEN™ AE-4022. (Image courtesy of U.S. ARKRAY, Inc.)



Figure 2–2 Cobas u 411 urine chemistry analyzer. (Image courtesy of Roche Diagnostics.)



Figure 2–3 Urisys 1100 semiautomated urine chemistry analyzer. (Image courtesy of Roche Diagnostics.)

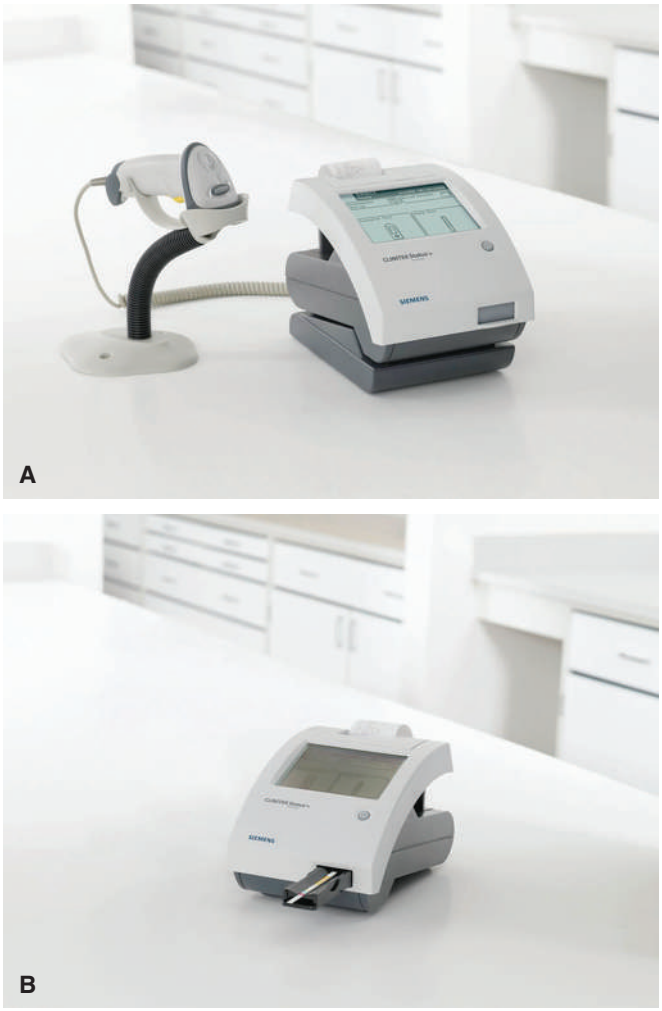

Figure 2–4 Clinitek Status + Analyzer. **A.** Clinitek Status Connect with barcode stand. **B.** Clinitek Status with test strip. (Images courtesy of Siemens Healthcare Diagnostics, Inc.)



Figure 2–5 Clinitek Advantus semiautomated urine chemistry analyzer. (Image courtesy of Siemens Healthcare Diagnostics, Inc.)

directly onto the reagent strip. The reagent strip advances automatically to the reflectance photometer to measure the color change of each reagent pad. Then the strip advances automatically to the disposal area. Tests are measured by the dry chemistry test strip, using reflectance photometry to detect color change and then taking readings at the appropriate time and wavelength for each specific test. Analytes measured vary by the instrument and may include leukocytes, ketones, protein, glucose, nitrite, blood, urobilinogen, pH, bilirubin, color, clarity, creatinine, and protein-low. Color is measured by either reflectance photometry or spectrophotometry at multiple wavelengths. Specific gravity is measured by the refractive index methodology, and clarity is a measurement of transmitted or scattered light. The instruments use integrated barcoded sample identification and allow abnormal ranges to be selected so that samples that require microscopic examination or confirmatory testing can be identified and flagged. Patient results and quality control results and calibrations are stored for visual display, printout, or transmission to an LIS. Standardized controls are run as set by laboratory protocol. Examples of fully automated urine chemistry analyzers are shown in Figures 2-6 through 2-9.

Automated Microscopy Analyzers

In a routine urinalysis, a test strip determines the chemical analytes and the formed elements are determined by microscopy. Manual microscopy is not easily standardized because of the high variation among operators even in the same facility. Routine specimen processing, such as centrifugation, can affect accuracy because rare elements, such as casts or cells, may be lost during handling. It has been demonstrated that recovery of formed elements in the sediment after centrifugation is highly variable. Results are not quantitative because they must be reported in



Figure 2–6 Urisys 2400 automated urine chemistry analyzer. (Image courtesy of Roche Diagnostics.)



Figure 2–7 Clinitek Atlas automated urine chemistry analyzer. (Image courtesy of Siemens Healthcare Diagnostics, Inc.)



Figure 2–8 AUTION MAX AX-4030 fully automated urine chemistry analyzer. (Image courtesy of U.S. ARKRAY, Inc.)



Figure 2–9 iChemVELOCITY automated urine chemistry analyzer. (Image courtesy of Iris Diagnostics-Beckman Coulter, Inc.)

ranges or averages. Overall, manual microscopy is not cost effective because of the labor and time required to process and analyze the specimen, which ultimately increases turnaround times (**TATs**) for results. Automated urine cell microscopy analyzers provide efficient standardized results in less than 1 minute compared with approximately 6 minutes using the manual method, markedly improving TATs. A number of automated urine cell microscopy analyzers are currently available in the United States. Two types of technology are used for urine sediment analysis with these instruments: flow cytometry and digital imaging techniques.

Sysmex UF-1000i *Fully Automated Urine Particle Analyzer* and Sysmex UN-2000 *Automated Urinalysis System*

The Sysmex UF-1000*i* (Sysmex Corporation, Kobe, Japan) uses fluorescent **flow cytometry** to measure the forward-scattered, side-scattered, and fluorescence light characteristics of particles present in urine. The information obtained from these measurements is used to detect and identify stained urine sediment particles (Fig. 2-10).

To perform an automated urine sediment analysis, 1.2 mL of uncentrifuged urine is aspirated into the instrument and divided into two channels: the sediment channel for urine particle analysis and the bacteria channel for bacteria staining and detection (Fig. 2-11). Each channel has a specific stain that targets surface and/or internal components of the cells. The stained urine sample passes through the flow cell, where it is hydrodynamically focused and presented to a red semiconductor laser (635 nm) (Fig. 2-12). Particles are identified by measuring the height and width of the fluorescent and light scatter signals, which are presented in **scattergrams** and **histograms**. In the bacteria channel, the diluent stabilizes the pH and lyses the nonbacterial particles, reducing interference from amorphous crystals. The stain is specific to the ribonucleic acid (**RNA**) in a bacterial cell, eliminating any nonspecific staining of debris. The width of the fluorescent signal measures cellular inclusions, and the width of forward light scatter measures the length of cells (Fig. 2-13).



Figure 2–10 Sysmex UF 1000*i* urine chemistry analyzer. (Image courtesy of Sysmex Corporation, Kobe, Japan.)

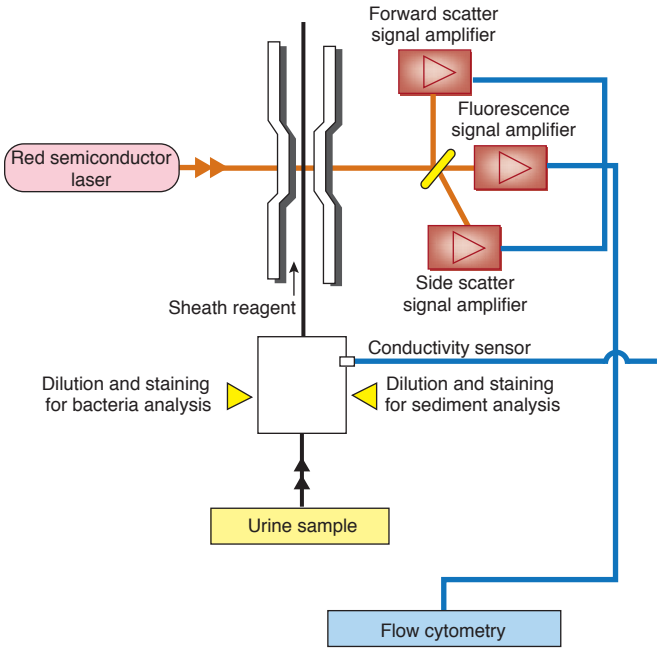

Figure 2–11 Diagram of urine particle analysis in the Sysmex UF1000*i*. (Image courtesy of Sysmex Corporation, Kobe, Japan.)

Resulting values are presented in quantitative cells per microliter and cells per high- or low-powered field. Thresholds to be flagged for primary elements can be established, and abnormal results are flagged for confirmatory review. The main particles enumerated are red blood cells (**RBCs**), white blood cells (**WBCs**), squamous epithelial cells, hyaline casts, and bacteria. The results are displayed as scattergrams (Fig. 2-14). These parameters are reportable directly without technologist intervention and may be **autovalidated**. Flagged particles include pathological casts, crystals, small round cells (renal tubular epithelial cells or transitional epithelial cells), sperm, mucus, and yeastlike cells, and these particles must be confirmed by manual microscopy. A latex particle quality control system monitors performance, and quality control records can be viewed on the instrument screen in a Levy-Jennings graph. The analyzers can store up to 10,000 patient results, including scattergrams, histograms, and specimen characteristics, which can be saved and viewed by the user. A bidirectional interface is provided to download and report results. The UF-1000*i* can be used independently or integrated with an automated urine chemistry strip reader to create a complete urinalysis system.

The newest Sysmex model, the UN-2000 (Fig. 2-15), is an automated, modular, and scalable urinalysis system that combines urine flow cytometry with digital image analysis by integrating the UF-5000 Fully Automated Urine Particle Analyzer (Fig. 2-16) and the UD-10 Fully Automated Urine Particle Digital Imaging Device (Fig. 2-17). The UF-5000 utilizes fluorescent flow cytometry coupled with a blue semiconductor laser to measure particle length, particle volume, particle internal complexity, and total amount of nucleic acid contained within the particle (Figs. 2-18 and 2-19). The shorter wavelength blue (488 nm) semiconductor laser offers the benefit of enhanced detection and differentiation of small particles. The sediment and bacteria channels used in the previous-generation UF-1000*i* have been replaced with two new analysis channels to further enhance the sensitivity and specificity of particle detection in urine sediment.

The core channel (**CR ch**) stains elements with nuclear material, such as WBCs, epithelial cells, and bacteria (Fig. 2-20). In regard to the bacteria analysis in the CR ch, the degree of staining, and thus intensity, of the side fluorescence signal is dependent on the bacterial cell wall structure. The surface channel (**SF ch**) stains and measures anucleate elements, such as RBCs, crystals, and casts (Fig. 2-21).[3]

In addition to the two new measuring channels, the UF-5000 has a new depolarized side scatter (**DSS**) detector that provides information on the particles' ability to depolarize

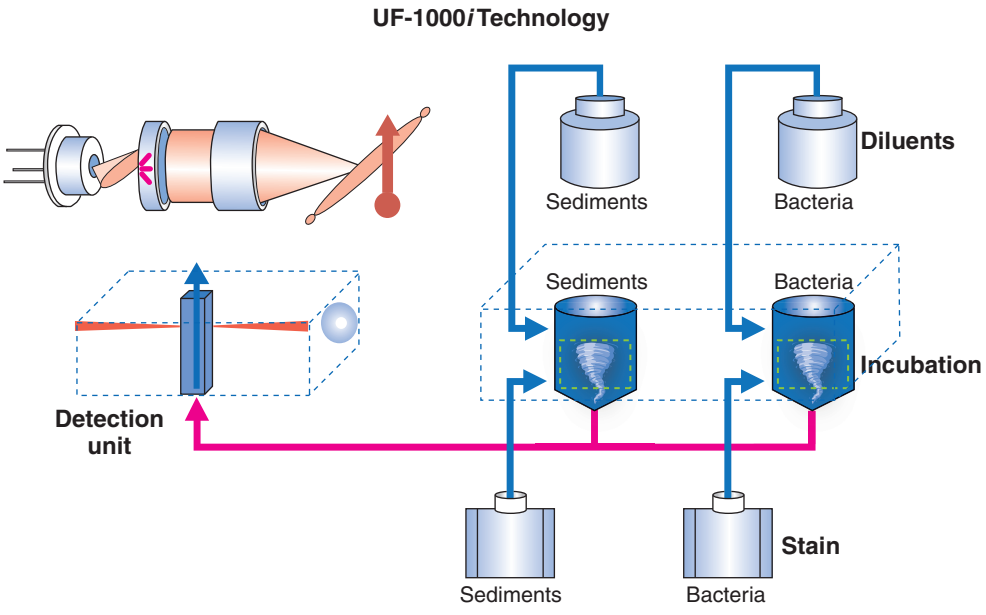

Figure 2–12 Staining elements for the Sysmex UF1000*i*. (Image courtesy of Sysmex Corporation, Kobe, Japan.)

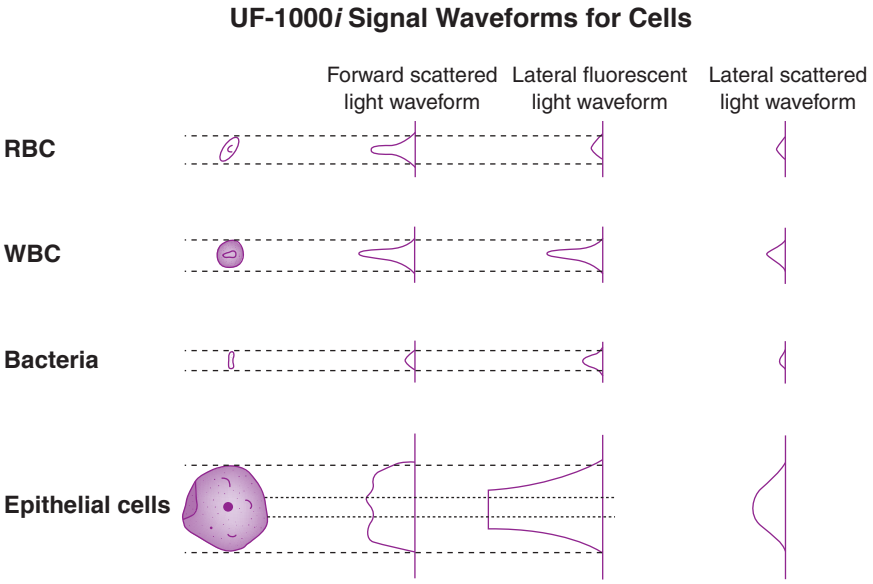

Figure 2–13 UF1000*i* signal waveform for cells. (Image courtesy of Sysmex Corporation, Kobe, Japan.)

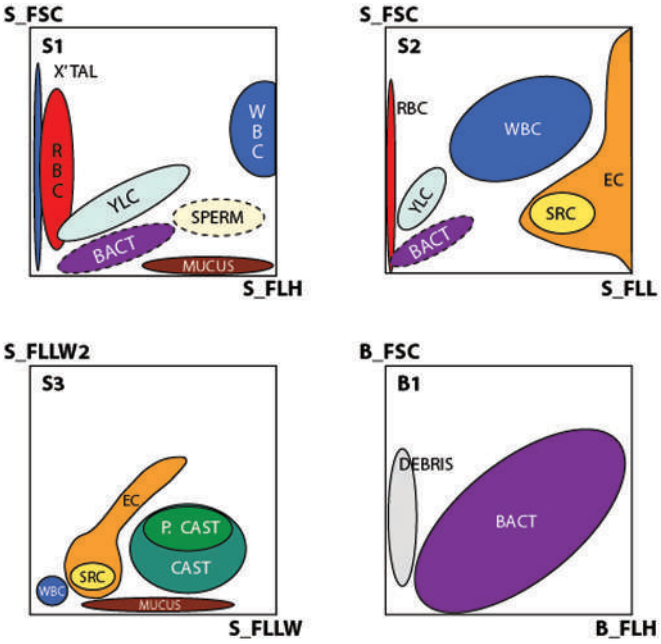

Figure 2–14 Scattergram showing Sysmex UF1000*i* microscopy results. (Image courtesy of Sysmex Corporation, Kobe, Japan.)



Figure 2–15 UN-2000-110™ Sysmex Urinalysis System. (Image courtesy of Sysmex Corporation, Kobe, Japan.)



Figure 2–16 UF-5000™ Fully Automated Urine Particle Analyzer. (Image courtesy of Sysmex Corporation, Kobe, Japan.)

light (Fig. 2-22). Through the detection of depolarized light, the DSS detector works to differentiate urine particles that are birefringent, such as crystals. An advantage of the Sysmex UF-5000 is that it requires a minimal volume of urine for analysis.

The Sysmex UD-10 is a complementary **digital imaging** system designed to capture detailed images of urine particles in urine samples that require further review. All captured urine particle images are classified, based on size, into eight different classes. Then the technologist has the opportunity to review the captured images and identify the type(s) of urine particles present.[3]

The UN-2000 uses a single-screen data management system for monitoring analyzer status, result reporting, and reflex rule setting, as well as providing the connection point for interface with the LIS or middleware system. In the United States and Canada, this data management system is called the Urinalysis Data Manager (**UDM**). In regions outside the United States and Canada, U-WAM is the name of the data management system for UN-Series analyzers.



Figure 2–17 UD-10™ Fully Automated Urine Particle Digital Imaging Device. (Image courtesy of Sysmex Corporation, Kobe, Japan.)

Beckman Coulter's iQ200 Microscopy Analyzer

The iQ200 Automated Urine Microscopy Analyzer (Iris Diagnostics-Beckman Coulter, Brea, CA) uses digital flow morphology (imaging) and **auto particle recognition** (**APR**) to categorize and count urine particles automatically in uncentrifuged urine based on size, shape, texture, and contrast. The instrument also can be used for counts of body fluid cells by adding the optional body fluids software module (Fig. 2-23). The microscopy unit can be integrated with an automated urine chemistry analyzer to provide a complete urinalysis system.

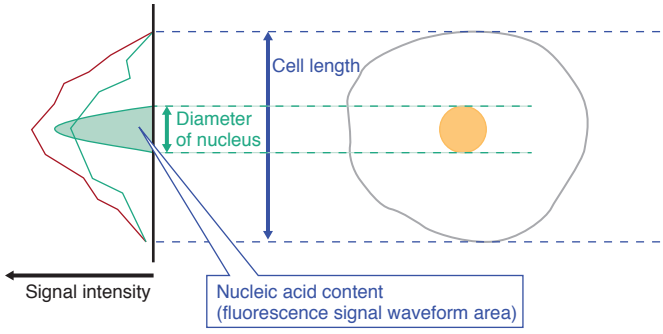

Figure 2–19 UF-5000™ measurement of the cell length, diameter, and nucleic acid content. (Image courtesy of Sysmex Corporation, Kobe, Japan.)

The analyzer mixes the sample and aspirates 1.3 mL of urine. The sample is hydrodynamically focused in a planar flow cell and presented to a digital microscope in a thin moving sheet. Then a digital camera takes 500 images as the sample advances through the flow cell (Fig. 2-24). Individual urine particles are extracted from the raw images and automatically classified using a proprietary neural network algorithm into 12 major classification categories: RBCs, WBCs, WBC clumps, squamous epithelial cells, nonsquamous epithelial cells, unclassified casts, hyaline casts, unclassified crystals, bacteria, yeast, mucus, and sperm (Figs. 2-25 and 2-26).[4]

Results are either reviewed by a trained operator or auto-released to the LIS based on user-defined parameters. Because the images are archived digitally, results can be reviewed easily and reclassified by the operator without the need for manual microscopy.

In addition to the 12 major categories, the software allows the user to subclassify particles into 27 additional categories,

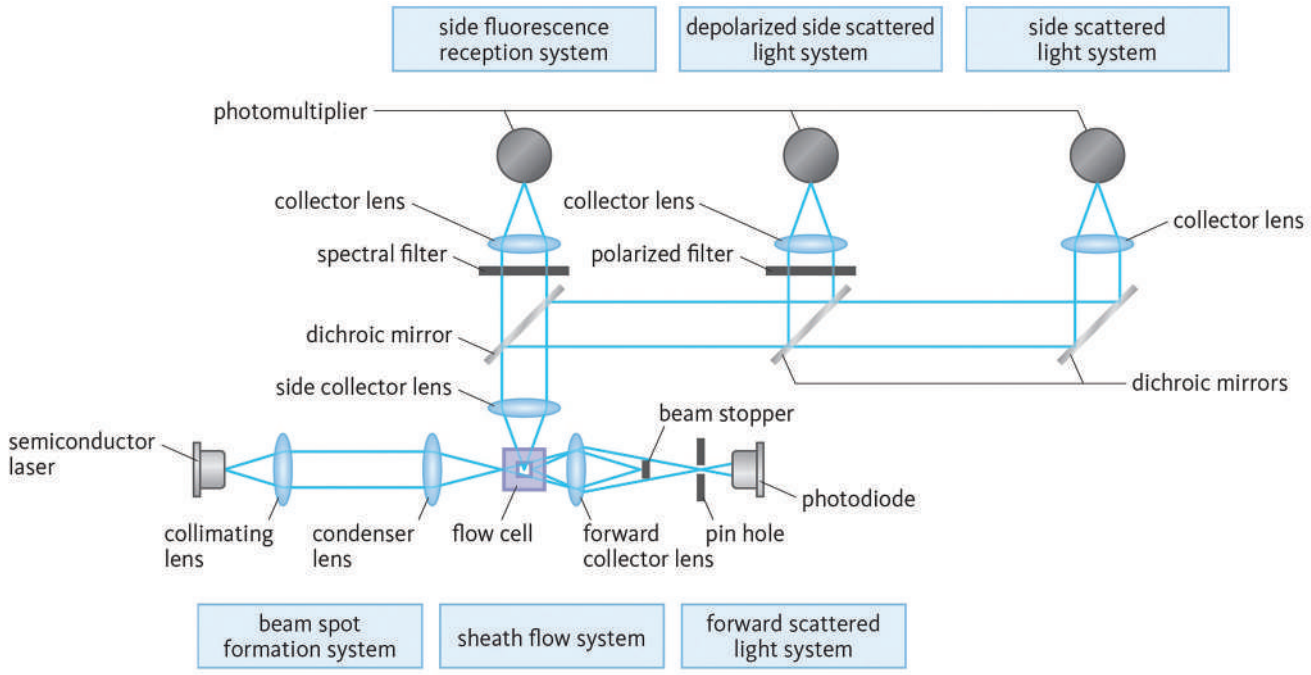

Figure 2–18 Diagram of the urine particle analysis in the Sysmex UF-5000™. (Image courtesy of Sysmex Corporation, Kobe, Japan.)

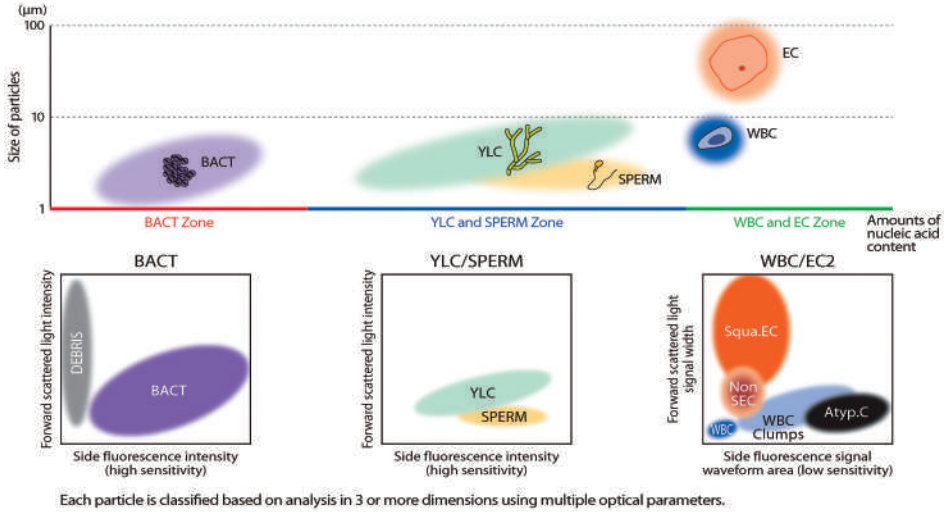

Figure 2–20 UF-5000 core channel. The CR channel analyzes nucleic acid–containing particles such as white blood cells, bacteria, and epithelial cells. Nucleic acid–containing cells are detected and classified through the use of a newly developed nucleic acid staining reagent. The nucleic acid–containing particles are further classified based on the area under the fluorescence signal waveform, which reflects the amounts of nucleic acid content and the known difference in nucleic acid content of different types of particles. A short-wavelength blue semiconductor laser is used to detect small particles, such as bacteria, with greater accuracy. In this channel, red blood cells are lysed and removed by a diluent, and crystals are removed by a chelating agent, contained in the diluent. (Image courtesy of Sysmex Corporation, Kobe, Japan.)

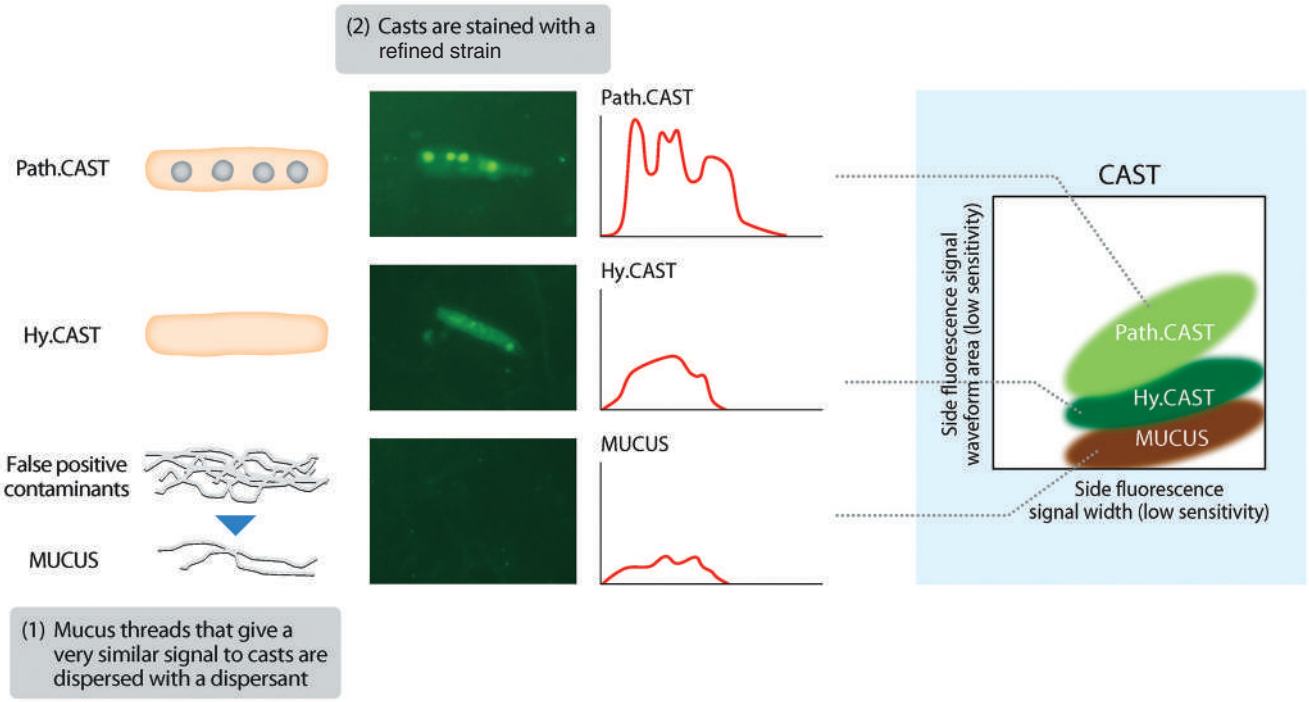

Figure 2–21 UF-5000 surface channel. The SF channel analyzes urine particles that do not contain nucleic acid, such as casts, red blood cells, and crystals. For differentiating casts from castlike elements (mucus threads, salt clumps, bacterial clumps, etc.), those particles are dispersed by reagents, and then the analyzer uses waveform information about particles stained with a newly developed staining reagent for more precise analysis. Besides this, amorphous salts are moved by the chelating action of a reagent and heating process. (Image courtesy of Sysmex Corporation, Kobe, Japan.)

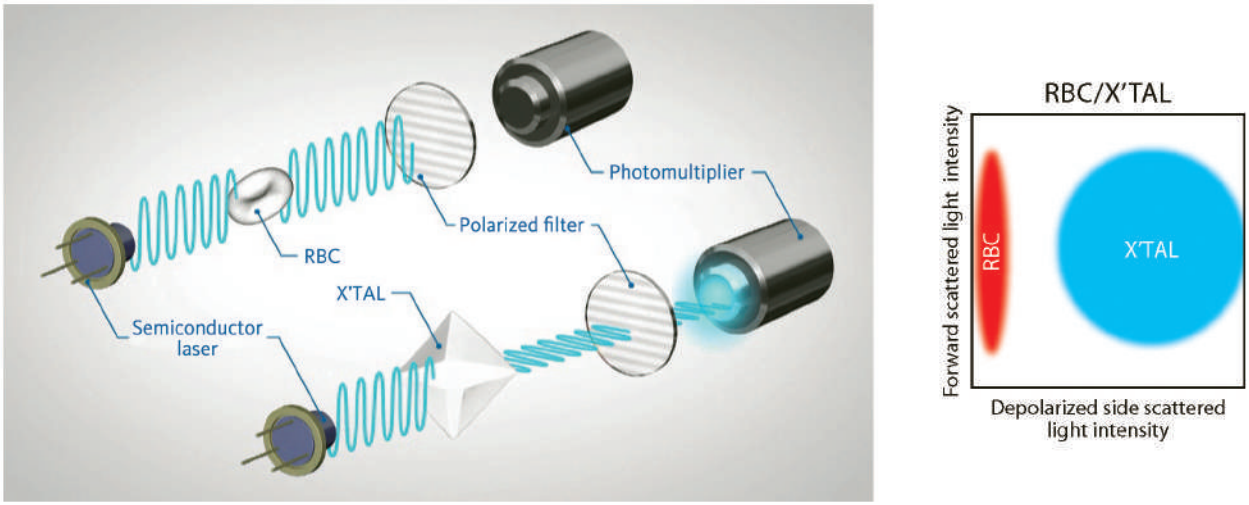

Figure 2–22 UF-5000 depolarized side-scattered light (DSS) detector. (Image courtesy of Sysmex Corporation, Kobe, Japan.)



Figure 2–23 iQ 200 microscopy analyzer. (Image courtesy of Iris Diagnostics-Beckman Coulter, Inc.)

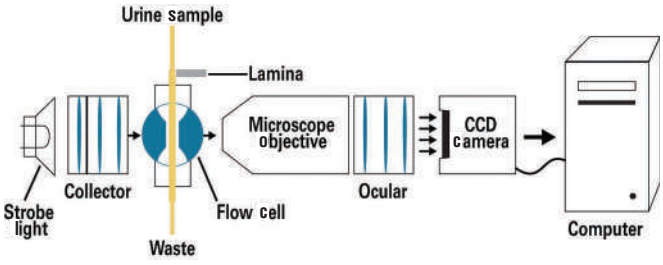

Figure 2–24 Diagram of the iQ 200 digital flow capture process. (Image courtesy of Iris Diagnostics-Beckman Coulter, Inc.)

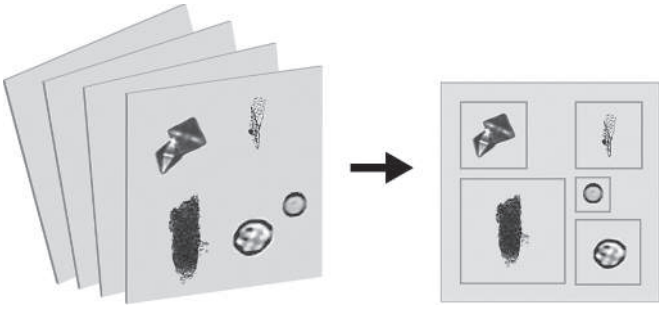

Figure 2–25 Auto particle recognition (APR) process. (Image courtesy of Iris Diagnostics-Beckman Coulter, Inc.)

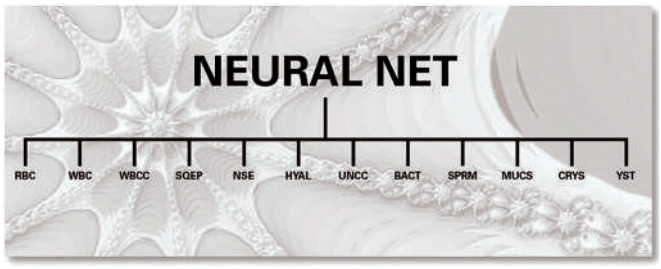

Figure 2–26 iQ 200 urinalysis results display, showing particle categories available for analysis or counting. (Image courtesy of Iris Diagnostics-Beckman Coulter, Inc.)

such as specific types of crystals, casts, nonsquamous epithelial cells, yeast with pseudohyphae, trichomonas, and oval fat bodies. Additional user observations can be added to the report in the Edit Comment section.

77 Electronika UriSed 2 and 3Pro Automated Urine Sediment Analyzer

The UriSed 2 and UriSed 3 Pro (77 Electronika, Budapest, Hungry) perform automated microscopy with digital imaging using auto image evaluation module (AIEM) software. The UriSed 2 requires a minimum of 2 mL of urine that is centrifuged in a special cuvette to produce a monolayer of urine sediment. The sediment is analyzed by a bright-field microscope and digital camera to capture and categorize 15 particle images based upon size and shape using AIEM software.[1,5] An advantage to this instrument is the zoom capability to view images, and interpretation of the images is similar to that of manual microscopic smears. The UriSed 3 Pro incorporates phase-contrast microscopy in addition to bright-field microscopy to improve differentiation of elements, such as hyaline casts, RBC membranes, crystals, and yeast.[1] Both the UriSed 2 and

UriSed 3 can be linked with the chemistry analyzer LabUMat 2 to make a complete automated urinalysis laboratory system.[1]

FUS-100 and FUS-200 Analyzers

The FUS-100 and FUS-200 urine sediment analyzers (DIRUI Industrial Co., Ltd., Changchun City, Jilin Province, China) identify cells using flat-flow cell digital imaging technology and a trained neural network. Artificial imaging identification (**AII**) software is used to classify and quantify the cells and formed particles in the uncentrifuged urine.[6] A digital camera captures up to 820 photos, and the AII identifies and classifies 12 visible components in urine based on shape, contrast, texture, and frequency domain features.[1,6] The FUS instruments can be integrated with the H-800 chemistry analyzer for a total automated urinalysis system.[6]

Automated Urinalysis Systems

Combining automated urine chemistry analyzers and automated urine cell analyzers to create completely automated urinalysis systems has improved TATs for urinalysis significantly. Technologists' hands-on time has been reduced significantly as well. Using similar sample racks and moving on a conveyor system, samples are easily transferred from one instrument to the next, providing complete walkaway capability with minimal sample handling from sampling through results. By interfacing with the LIS, barcoded samples are identified automatically and processed according to the requested tests. The systems can independently perform both physical and chemical testing, microscopy analysis, and a combination of both. A complete urinalysis report can be sent directly to the LIS or printed out, thereby reducing clerical error. Autoverification of results and reflex testing can be validated according to laboratory protocol. Abnormal results are flagged for manual examination by laboratory personnel.

Clinitek AUWi System and AUWi Pro System

The Clinitek Atlas System (Siemens Healthcare Diagnostics, Tarrytown, NY), an automated urine chemistry analyzer, and the Sysmex UF-1000*i* (Sysmex Corporation, Mundelein, IL), an automated urine cell analyzer, have been integrated to develop the Clinitek AUWi System (Siemens), which performs a completely automated urinalysis (Fig. 2-27). A minimum of 5 mL of urine is required in the automated mode. The barcoded tubes are racked and placed onto the system. The rack advances to the Atlas analyzer, where the sample is identified, mixed, aspirated, and tested for physical and chemical components. Then the sample travels across a connecting bridge to the UF-1000*i* for microscopic analysis. The instrument automatically reflexes samples requiring sediment analysis, reducing the time associated with manual microscopic analysis. Results are verified automatically and integrated into a complete urinalysis report to be sent to the LIS or printed.

The Clinitek AUWi PRO Automated Urinalysis System integrates the Clinitek Novus Automated Urine Chemistry Analyzer and the Sysmex UF-1000i Urine Particle Analyzer. This automated system can load up to 200 sample tubes and complete up to 80 tests per hour. As with the AUWi system, the sample volume requirement is 5 mL in the automated mode and 2 mL in the manual mode. Samples can be placed on the instrument with fewer manual steps, and no centrifugation is required. Autoverification of results to be reported to the LIS is based on laboratory protocol. In addition, the system is capable of automatically reflexing samples requiring sediment analysis based on rules defined by each laboratory.[7]



Figure 2–27 AUWi, a fully automated urinalysis system that combines the Siemens Clinitek Atlas Chemistry analyzer and the Sysmex UF-1000*i* particle analyzer. (Image courtesy of Siemens Healthcare Diagnostics, Inc.)

iRICELL Urinalysis Systems

The iRICELL Automated Urinalysis System (Iris Diagnostics-Beckman Coulter, Brea, CA) consists of the iChemVELOCITY urine chemistry analyzer and the iQ200 urine microscopy analyzer (Fig. 2-28). A minimum of 4 mL of urine is required. Barcoded tubes are placed into the 10-position rack and are moved to the iChemVELOCITY. Upon completion of the physical and chemical analysis, the rack moves across the connecting bridge to the iQ200 for microscopy testing, where a reflex rule may be applied based on settings. Combined chemistry and microscopy urinalysis results are transmitted to the LIS or printed.

The IQ series of urinalysis workcells (iQ2000 and iQ3000) pairs the Iris IRICELL series of urine microscopy instrumentation and the ARKRAY AUTION MAX AX4030 fully automated urine chemistry analyzer to provide a complete walk-away urinalysis system (refer back to Table 2-2).



Figure 2–28 iRICELL3000, a fully automated urinalysis system that combines the iChemVELOCITY urine chemistry analyzer and the iQ 200 microscopy analyzer. (Image courtesy of Iris Diagnostics-Beckman Coulter, Inc.)

LabUMat 2 with UriSed 2 or UriSed 3 System

The chemistry analyzer LabUMat 2 (77 Electronika) can be integrated with the UriSed 2 or UriSed 3 (77 Electronika) microscopic urine analyzers to make a complete automated urinalysis laboratory system. The minimum volume is 3 mL of uncentrifuged urine for the combined instruments, and the system can analyze up to 240 tests per hour. The samples are loaded into the 10-position sample racks where the physical (color, clarity, and specific gravity) and chemical tests (10 parameters) are performed by the LabUMat2. Then the sample racks are transferred across a connecting bridge to the UriSed 2 (bright-field microscopy) or the UriSed 3 (both bright-field and phase microscopy) for the urine microscopic analysis.

cobas 6500 Urine Analyzer

The cobas 6500 Urine Analyzer (Roche Diagnostics, Indianapolis, IN) is another fully automated urine system. Its modular design integrates the cobas u 701 microscopy analyzer and the cobas u 601 fully automated urine chemistry analyzer into one platform. Cassettes with urine testing strips and sediment cuvettes are loaded onto the instrument, and 2.8 mL of urine is required. Urine is pipetted on the chemical strip, where 12 physical and chemical urine tests are performed using the cobas u 601 urine analyzer. After chemical testing, the sample is resuspended before pipetting and automatically centrifuged at 2000 rpm for 10 seconds to a monolayer of sediment. The cobas u 701 uses digital imaging to take 15 microscopic images of the sediment, and the images are displayed on the result screen. Particle recognition software determines the identification of RBCs, WBCs, bacteria, epithelial cells, casts, crystals, yeast, sperm, and mucus. Automated result validation and automated reflex testing are available.

UX-2000 Automated Urinalysis Analyzer

The UX-2000 (Sysmex Corporation, Kobe, Japan) is a fully automated integrated urine analyzer. It consists of a chemical component for analyzing the physical and chemical part of urine as well as a flow cytometry component for microscopic examination of sediments contained in a single instrument.[8] The system requires 5 mL of urine. For the physical examination, refractometry, reflectivity measurement, and light scattering are used to measure specific gravity, turbidity, and color. The chemical examination uses a test strip that is measured by the dual-wavelength reflectance methods.[9] The microscopic examination uses fluorescent-flow cytometry to measure RBCs, WBCs, hyaline casts, bacteria, and epithelial cells. Crystals, yeast, small round cells, spermatozoa, and casts are detected and flagged for laboratory personnel to review because this analyzer cannot differentiate between those types of particles in urine sediment.[8]

Visit www.fadavis.com for Video 2-1 (Automated urine chemical strip test).

Body Fluid Analysis Automation

Traditionally body fluid counts for RBCs and WBCs, as well as WBC differentials, are performed manually using a hemocytometer and optical microscopy. Three types of hemocytometers are available—Fuchs-Rosenthal, Nageotte, and Neubauer—and each differs in the design of the calibrated counting area etched on the slide. An exact amount of fluid fills the chamber that provides a defined volume for cell enumeration. These procedures are labor-intensive and time-consuming, and often they are subject to technologist variability.

Visit www.fadavis.com for Video 2-2 (Hemacytometer).

Automation brings quality control, precision, faster TATs, and standardization of results[10] to a method that previously was uncontrolled. However, automated instruments have not eliminated completely the use of a manual hemacytometer count. Body fluids with low cell counts or malignant cells still require a manual differential using a stained cytospin smear. The laboratory must define the limits for the automated instrument and establish the lower limits for cell counting to determine when a manual procedure must be performed.[5] For example, the iQ 200 is linear down to zero (0 to 10,000 cells/L). Linearity can be extended during method comparison. In addition, the laboratory must follow manufacturers' recommended procedures for special treatment required for the specific body fluid analyzed, intended use, and reportable ranges.[11]

Visit www.fadavis.com for Video 2-3 (Making a cytospin smear).

Hematology analyzers that are used to perform body fluids cell counts include the ADVIA 2120i (Siemens), the Sysmex XN-Series analyzers (Sysmex America, Inc.), and the DxH 900 (Beckman Coulter, Inc.).

ADVIA 2120i

The ADVIA 2120i uses flow cytometry, light scattering, and absorbance to count RBCs and WBCs, as well as to perform a WBC differential that includes percentages and absolute numbers of mononuclear cells and polymorphonuclear cells on specimens with more than 20 WBC/µL. The WBC differential includes the numbers of neutrophils, lymphocytes, monocytes, and eosinophils. A specimen of cerebrospinal fluid (CSF) is pretreated with CSF reagent to fix and spherize the cells. The prepared specimen remains stable for 4 minutes to 4 hours when stored at 18°C to 30°C. The specimen is aspirated into the instrument, and cells are differentiated and enumerated by three optical measurements. The signals are digitized and used to construct the CSF cytogram. With this system, more cells are counted, achieving increased accuracy and precision. The automated results for RBC, WBC, polymorphonuclear, mononuclear, and differential are available within 1 minute of sample aspiration. In addition, the ADVIA 2120i can provide a rapid automated diagnostic test for fetal lung maturity by counting lamellar bodies in amniotic fluid. Lamellar bodies are counted in the platelet channel using high and low laser light scattering. The analyzer is approved for counting cells in pleural fluids, peritoneal fluids, and peritoneal dialysates.

Sysmex XN-Series Analyzers

The Sysmex XN-Series analyzer is the newest-generation hematology analyzer that includes a dedicated body fluid mode that

is cleared for analysis of synovial fluid, pleural fluid, and peritoneal fluid, and the XN-10 analyzer is also cleared for CSF specimens in the body fluid mode.[10] Bronchoalveolar lavage and amniotic fluid are not cleared for analysis on the XN-Series analyzers. Table 2-3 lists the fluid types cleared for analysis on the XN-Series analyzers. The body fluid mode has extended cell counting to increase precision in specimens with small numbers of cells. Body fluids can be analyzed without specimen preparation or pretreatment.

The body fluid mode on the XN-Series reports both a body fluid white blood cell count (**WBC-BF**) and a total nucleated body fluid cell count (**TC-BF**) as well as a reportable two-part automated differential that differentiates mononuclear and polymorphonuclear cells using flow cytometry technology.[10] The XN analyzer also identifies high-fluorescing body fluid cells (**HF-BF**), such as mesothelial cells, synovial cells, and malignant/tumor cells, or, rarely, large cell clusters. Digital imaging technology also may be used for body fluid differentials. The CellaVision DI-60 software classifies five different nucleated cell types found in body fluid. This software allows users to add reference cells to a digital library, enables tagging and sharing of cell images electronically, and offers a program to improve staff competency.[10]

The XN analyzer uses impedance counting principles for RBC-BF enumerations and flow cytometry for performing the WBC-BF counts and the two-part differential. The XN series uses Lysercell WDF as an RBC lysing reagent and Fluorocell WDF to stain RNA and DNA in the nucleated cells. In addition, the differential scatterplots should be inspected visually to detect noncellular particulate matter, such as bacteria, *Cryptococcus,* and interference from large cells (macrophages and mesothelial cells).

GloCyte Automated Cell Counter for CSF

The GloCyte analyzer (Sysmex Corporation, Kobe, Japan) combines the principles of both technologies to accurately enumerate cells present in CSF, even at low numbers.[10] A fluorochrome-labeled antibody stains the RBCs, and a dye specific to nucleic acids in WBCs is used to treat aliquots of the CSF specimen.[10] Digital imaging is used to count the cells as they are illuminated with a semiconductor laser. The instrument automatically enumerates each cell type and displays the stained cells on a screen.[10]

Beckman Coulter's iQ200

The iQ200 (Iris Diagnostics – Beckman Coulter, Brea, CA) can be used for body fluid analysis using the iQ200 Body Fluids Module. The Body Fluid menu includes such fluids as CSF, synovial, pleural, peritoneal, peritoneal dialysate, peritoneal lavage, pericardial, and general serous. Two dilutions of the body fluid specimen are analyzed; one tube is diluted with Iris Diluent, and the other with the iQ Body Fluids Lysing Reagent. The instrument provides counts for total cells, nucleated cells, and RBCs. As with urine microscopy, results are either reviewed by a trained operator or auto-released to the LIS based on user-defined-parameters.

Table 2–3 Body Fluids Analyzed on the Sysmex XN-Series[10]

	XN-10	XN-11	XN-20
CSF	Yes	No	Yes
Peritoneal fluid	Yes	Yes	Yes
Pleural fluid	Yes	Yes	Yes
Pericardial fluid	No	No	No
Synovial fluid	Yes	Yes	Yes

CSF, Cerebrospinal fluid.

Visit www.fadavis.com for Video 2-4 (Body fluid cell count automated analyzer).

For additional resources please visit **www.fadavis.com**

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11. Clinical and Laboratory Standards Institute: Body Fluid Analysis for Cellular Composition: Approved Guideline. CLSI Document H-56A. CLSI, Wayne, PA, 2006.

Additional Information Sources

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DIRUI, Changchun, China: en.dirui.com.cn

77 Elektronika Kft, Budapest, Hungary: en.e77.hu/products/urine-analyzer
Iris Diagnostics – Beckman Coulter, Brea, CA: www.beckmancoulter.com
Roche Diagnostics, Indianapolis, IN: www.roche.com/products
Siemens Healthcare Diagnostics Inc., Deerfield, IL: www.usa.siemens.com/diagnostics
Sysmex Corporation, Kobe, Japan: www.sysmex.com/usa

Study Questions

1. The principle commonly used to measure the concentration of a particular analyte in the chemical examination of urine is:
   A. Reflectance photometry
   B. Digital imaging
   C. Flow cytometry
   D. Auto particle recognition
2. In automated urinalysis, the specific gravity is measured by:
   A. Light transmittance
   B. Light scattering
   C. Refractometry
   D. Turbidity
3. All of the following are true concerning fully automated urine chemistry analyzers, except:
   A. They are designed for a high-volume urinalysis laboratory.
   B. The reagent strip is dipped into the well-mixed urine.
   C. The urine tube moves through the instrument.
   D. A sample probe aspirates the urine.
4. The advantages of an automated urine microscopy analyzer over manual microscopy includes:
   A. Cost-effective
   B. Centrifugation not required
   C. Standardized results
   D. All of the above
5. Which of the following is a complete urinalysis automated urinalysis system?
   A. AUTION ELEVEN AE 4022
   B. Clinitek Atlas
   C. iQ200 Automated Urine Microscopy
   D. Clinitek AUWi Pro System
6. What two technologies are used for urine sediment analysis?
   A. Light scattering and refractometry
   B. Light scattering and flow cytometry
   C. Flow cytometry and digital imaging
   D. Digital imaging and refractometry
7. Which automated urine particle counter combines urine flow cytometry with digital image analysis?
   A. UN-2000
   B. iRICELL
   C. UF-1000i
   D. iQ 200
8. Which of the following urine sediment particles cannot be autovalidated but will be flagged and must be reviewed by laboratory personnel?
   A. RBCs
   B. WBCs
   C. RTEs
   D. Squamous epithelial cells
9. Which of the automated body fluid analyzers does not need to dilute or pretreat body fluids before analysis?
   A. ADVIA 2120i
   B. XN Series
   C. iQ 200
   D. None of the above
10. What is a disadvantage of counting body fluid cells using an automated instrument versus a Neubauer hemocytometer?
    A. Less labor-intensive and time-consuming
    B. More precise
    C. Unable to count low WBC numbers and malignant cells
    D. Able to perform a WBC differential

Case Studies and Clinical Situation

1. A medical laboratory scientist student is being trained on the laboratory automated urinalysis system that performs both the chemical and microscopic examination of urine. The student poured 12 mL of urine into the urine tube that was labeled correctly and placed it on the instrument. The reagent strip results indicated that there was a large amount of blood and leukocytes; however, the microscopic results reported 0 to 2 RBC/hpf and 0 to 2 WBC/hpf.
   a. Do the reagent strip and microscopic results correlate?
   b. What mistake in the procedure may have caused these results?
   c. What should be performed to ensure that the instrument was functioning properly?

2. A patient in the emergency department (ED) presented with a high temperature, headache, and stiff neck. The doctor suspected meningitis and performed a lumbar puncture to obtain a cerebrospinal fluid (CSF) specimen for analysis in the laboratory.
   a. What instruments would be used to count the number of white blood cells in the CSF?
   b. Which instrument would require that the specimen be pretreated?
   c. How long is the prepared CSF specimen stable?

CHAPTER 3

Introduction to Urinalysis

LEARNING OUTCOMES

Upon completing this chapter, the reader will be able to:

3-1 List three major organic and three major inorganic chemical constituents of urine.

3-2 Describe a method for determining whether a fluid in question is urine.

3-3 Recognize normal and abnormal daily urine volumes.

3-4 Describe the characteristics of the specimen containers recommended for urine.

3-5 Describe the correct procedure for labeling urine specimens.

3-6 State four possible reasons why a laboratory would reject a urine specimen.

3-7 List the changes that may take place in a urine specimen that remains at room temperature for more than 2 hours.

3-8 Discuss the actions of bacteria on a urine specimen that is unpreserved.

3-9 Briefly discuss five methods for preserving urine specimens, including the advantages and disadvantages of each.

3-10 Instruct a patient in the correct procedure for collecting the following specimens: random, first morning, 24-hour timed, catheterized, midstream clean-catch, suprapubic aspiration, three-glass collection, four-glass collection, and pediatric. Identify a diagnostic use for each collection technique.

3-11 Describe the type of specimen needed for optimal results when a specific urinalysis procedure is requested.

KEY TERMS

Albuminuria
Anuria
Catheterized specimen
Chain of custody (COC)
First morning specimen
Midstream clean-catch specimen
Nocturia
Oliguria
Polydipsia
Polyuria
Random specimen
Suprapubic aspiration
Timed specimen

History and Importance

Analyzing urine was actually the beginning of laboratory medicine. References to the study of urine can be found in the drawings of cavemen and in Egyptian hieroglyphics, such as the Edwin Smith Surgical Papyrus. Pictures of early physicians commonly showed them examining a bladder-shaped flask of urine (Fig. 3-1). Often these physicians never saw the patient, only the patient's urine. Although these physicians lacked the sophisticated testing mechanisms now available, they were able to obtain diagnostic information from such basic observations as color, turbidity, odor, volume, viscosity, and even sweetness (by noting that certain specimens attracted ants or tasted sweet). These same urine characteristics are still noted by laboratory personnel. However, modern urinalysis has expanded beyond the physical examination of urine to include chemical analysis and microscopic examination of urinary sediment.

Many well-known names in the history of medicine are associated with the study of urine, including Hippocrates, who, in the 5th century BCE, wrote a book on "uroscopy." During the Middle Ages, physicians concentrated their efforts very intensively on the art of uroscopy, receiving instruction in urine examination as part of their training (Fig. 3-2). By 1140 CE, color charts had been developed that described the significance of 20 different colors (Fig. 3-3). Chemical testing progressed from "ant testing" and "taste testing" for glucose to Frederik Dekkers's discovery in 1694 of **albuminuria** by boiling urine.[1]



Figure 3–1 Physician examines urine flask. (Courtesy of National Library of Medicine.)



Figure 3–2 Instruction in urine examination. (Courtesy of National Library of Medicine.)

The credibility of urinalysis became compromised when charlatans without medical credentials began offering their predictions to the public for a healthy fee. These charlatans, called "pisse prophets," became the subject of a book published by Thomas Bryant in 1627. The revelations in this book inspired the passing of the first medical licensure laws in England—another contribution of urinalysis to the field of medicine.

The invention of the microscope in the 17th century led to the examination of urinary sediment and to the development by Thomas Addis of methods for quantitating the microscopic sediment. Richard Bright introduced the concept of urinalysis as part of a doctor's routine patient examination in 1827. By the 1930s, however, the number and complexity of the tests performed in a urinalysis had reached a point of impracticality, and urinalysis began to disappear from routine examinations. Fortunately, the development of modern testing techniques rescued routine urinalysis, which has remained an integral part of the patient examination.

Two unique characteristics of a urine specimen account for this continued popularity:

1. A urine specimen is readily available and easily collected.

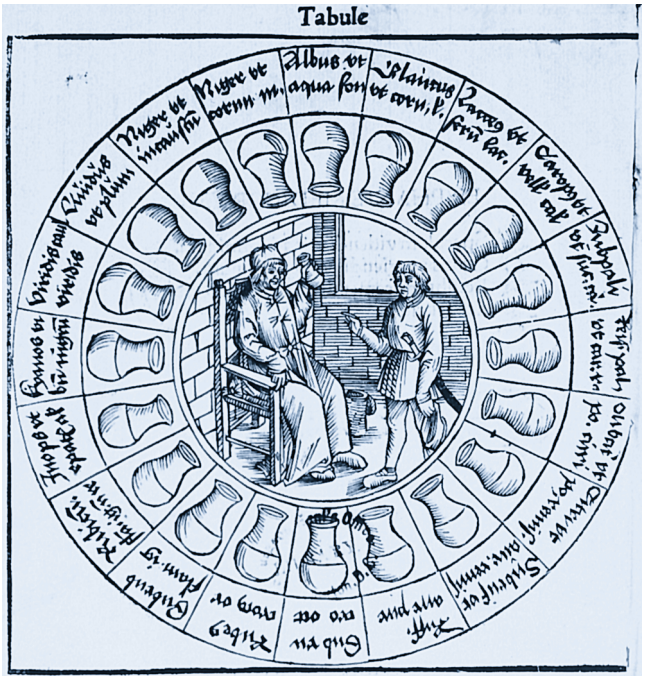

Figure 3–3 A chart used for urine analysis. (Courtesy of National Library of Medicine.)

2. Urine contains information, which can be obtained by inexpensive laboratory tests, about many of the body's major metabolic functions.

These characteristics fit in well with the current trends toward preventive medicine and lower medical costs. In fact, the Clinical and Laboratory Standards Institute (**CLSI**) defines urinalysis as "the testing of urine with procedures commonly performed in an expeditious, reliable, accurate, safe, and cost-effective manner." The reasons for performing urinalysis identified by CLSI include aiding in the diagnosis of disease, screening asymptomatic populations for undetected disorders, and monitoring the progress of disease and the effectiveness of therapy.[2]

Urine Formation

The kidneys continuously form urine as an 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, depending on fluid intake. (Refer to Chapter 4.)

Urine Composition

In general, urine consists of urea and other organic and inorganic chemicals dissolved in water. Urine is normally 95% water and 5% solutes, although considerable variations in the concentrations of these solutes can occur due to the influence of factors such as dietary intake, physical activity, body metabolism, and endocrine functions.

Urea, a metabolic waste product produced in the liver from the breakdown of protein and amino acids, accounts for nearly half of the total dissolved solids in urine. Other organic substances include primarily creatinine and uric acid. The major inorganic solid dissolved in urine is chloride, followed by sodium and potassium. Small or trace amounts of many additional inorganic chemicals are also present in urine (Table 3-1). Dietary intake greatly influences the concentrations of these inorganic compounds, making it difficult to establish normal levels. Other substances found in urine include hormones, vitamins, and medications. Although not a part of the original plasma filtrate, the 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.

Sometimes it is necessary to determine whether a fluid is urine. The best way to do so is to consider the components of the specimen. Creatinine, urea, sodium, and chloride are significantly higher in urine than in other body fluids. Protein and glucose are not present in a normal urine specimen.

Urine Volume

Urine volume depends on the amount of water that the kidneys excrete. Water is a major body constituent; therefore, the amount excreted is usually determined by the body's state of hydration. Factors that influence urine volume include fluid intake, fluid loss from nonrenal sources, variations in the secretion of antidiuretic hormone (**ADH**), and need to excrete increased amounts of dissolved solids, such as glucose or salts. Taking these factors into consideration, although the normal daily urine output is usually 1200 to 1500 mL, a range of 600 to 2000 mL is considered normal.

> ***Technical Tip 3-1.*** Should it be necessary to determine whether a particular fluid is urine, the specimen can be tested for its urea and creatinine content. Because both these substances are present in much higher concentrations in urine than in other body fluids, a fluid that is high in urea and creatinine content can be identified as urine.

Oliguria, a decrease in urine output (less than 1 mL/kg/hr in infants, less than 0.5 mL/kg/hr in children, and less than 400 mL/day in adults), is seen commonly when the body enters a state of dehydration as a result of excessive water loss from vomiting, diarrhea, perspiration, or severe burns.

Oliguria leading to **anuria**, cessation of urine flow, may result from any serious damage to the kidneys or from a decrease in the flow of blood to the kidneys.

The kidneys excrete two to three times more urine during the day than during the night. An increase in the nocturnal excretion of urine is termed **nocturia**. **Polyuria**, an increase in daily urine volume (greater than 2.5 L/day in adults and 2.5 to 3 mL/kg/day in children), is often associated with diabetes mellitus and diabetes insipidus; however, it may be induced artificially by diuretics, caffeine, or alcohol, all of which suppress the secretion of ADH.

Table 3–1 Primary Components in Normal Urine[3]

Component	Comment
Urea	Primary organic component. Product of metabolism of protein and amino acids
Creatinine	Product of metabolism of creatine by muscles
Uric acid	Product of breakdown of nucleic acid in food and cells
Chloride	Primary inorganic component. Found in combination with sodium (table salt) and many other inorganic substances
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

Diabetes mellitus and diabetes insipidus produce polyuria for different reasons, and analysis of the urine is an important step in the differential diagnosis (Fig. 3-4). Diabetes mellitus is caused by a defect either in the pancreatic production of insulin or in the function of insulin, which results in an increased concentration of body glucose. The kidneys do not reabsorb excess glucose, necessitating excretion of increased amounts of water to remove the dissolved glucose from the body. Although appearing to be dilute, a urine specimen from a patient with diabetes mellitus has a high specific gravity because of the increased glucose content.

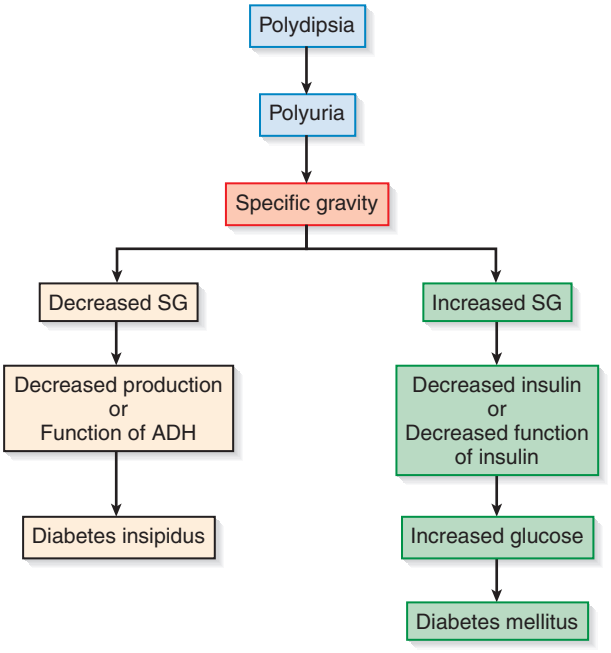

Figure 3–4 Differentiation between diabetes mellitus and diabetes insipidus.

Diabetes insipidus results from a decrease in the production or function of ADH; thus, the water necessary for adequate body hydration is not reabsorbed from the plasma filtrate. In this condition, the urine is truly dilute and has a low specific gravity. Fluid loss in both diseases is compensated by increased ingestion of water (**polydipsia**), producing an even greater volume of urine. Polyuria accompanied by increased fluid intake is often the first symptom of either disease.

Specimen Collection

As discussed in Chapter 1, urine is a biohazardous substance whose handling requires the observance of Standard Precautions (SP). Gloves should be worn at all times when in contact with the urine specimen.

The type of urine collection container, as well as specimen preservation and storage, will affect the quality of urine test results. Written criteria for the collection, preservation, handling, storage, and labeling of urine must be available.

Containers

Specimens must be collected in clean, dry, leakproof containers. Disposable containers should be used because they eliminate the chance of contamination due to improper washing. These disposable containers are available in a variety of sizes and shapes, including bags with adhesive for the collection of pediatric specimens and large containers for 24-hour specimens. Properly applied screw-top lids are less likely to leak than are snap-on lids.

Containers for routine urinalysis should have a wide mouth to facilitate collections from female patients and a wide, flat bottom to prevent overturning. They should be made of a clear material to allow for determination of color and clarity. The recommended capacity of the container is 50 mL, which allows 12 mL of specimen needed for microscopic analysis, additional specimen for repeat analysis, and enough room for the specimen to be mixed by swirling the container.

Individually packaged sterile containers with secure closures should be used for microbiological studies of urine. Sterile containers are also suggested if more than 2 hours elapse between specimen collection and analysis.[2]

Specially designed sterile containers are available that have a screw top containing an integrated transfer device. A BD Vacutainer Urine Transfer Straw is a nonsterile, plastic holder device that contains a needle with a straw attachment that can be used with the collection container to fill evacuation tubes. This device allows for the sterile transfer of urine to tubes containing preservatives for microbiology testing and tubes with conical bottoms for sediment analysis or round bottoms for automated reagent strip testing.[4] Additional information and pictures can be found at https://www.bd.com/en-us/offerings/capabilities/specimen-collection/urine-collection/bd-vacutainer-collection-and-transfer-products.

Labels

All specimens must be labeled immediately after collection with the patient's last and first name and identification number; the date and time of collection; and additional information such as the patient's age and location, the health-care provider's name, and the preservative used, if any, as required by facility protocol. Labels must be attached to the container, not to the lid, and should not become detached if the container is refrigerated or frozen.

Requisition Form

A requisition form (manual or computerized) must accompany specimens delivered to the laboratory. The information on the form must match the information on the specimen label. Additional information on the form can include method of collection or type of specimen, possible interfering medications, and the patient's clinical information. The time the specimen is received in the laboratory should be recorded on the form.

Specimen Rejection

The laboratory should reject specimens that are improperly labeled and/or collected, and appropriate personnel should be notified to collect a new specimen. Situations leading to specimens that should be rejected can include:

1. Specimens in containers that are unlabeled or improperly labeled
2. Labels and requisition forms that do not match
3. Specimens contaminated with feces or toilet paper
4. Containers with contaminated exteriors
5. Specimens of insufficient quantity
6. Specimens that have been transported improperly
7. Specimens that have not been preserved correctly during a time delay
8. Specimens for urine culture collected in a nonsterile container
9. Inappropriate collection for the type of testing needed (for example, midstream clean-catch specimen for bacterial culture)

Technical Tip 3-2. Never discard a specimen before checking with a supervisor.

Laboratories should have a written policy detailing their conditions for specimen rejection.

Visit www.fadavis.com for Video 3-1 (Preparing urine for urinalysis).

Specimen Handling

The fact that a urine specimen is so readily available and easily collected often leads to laxity in the treatment of the specimen after its collection. Changes in urine composition take place not only in vivo but also in vitro, requiring correct handling procedures.

Specimen Integrity

After collection, specimens should be delivered to the laboratory promptly and tested within 2 hours. A specimen that cannot be delivered and tested within 2 hours should be refrigerated or have an appropriate chemical preservative added. Table 3-2 describes the most significant changes that may occur in a specimen allowed to remain unpreserved at room temperature for longer than 2 hours. Notice that most of the changes are related to the presence and growth of bacteria.

Table 3–2 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 ammonia smell	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/lyse in dilute alkaline urine
Bacteria	Increased	Multiplication
Trichomonas	Decreased	Loss of motility, death

These variations are discussed again under the individual test procedures. At this point, it is important to realize that improper preservation can seriously affect the results of a routine urinalysis.

Specimen Preservation

The method of preservation used most routinely is refrigeration at 2°C to 8°C, which decreases bacterial growth and metabolism. If the urine is to be cultured, it should be refrigerated during transit and kept refrigerated until cultured, up to 24 hours.[2] The specimen must return to room temperature before chemical testing by reagent strips. Refrigeration also can cause precipitation of amorphous urate and phosphate crystals.

When a specimen must be transported over a long distance and refrigeration is impossible, chemical preservatives may be added. Commercially prepared transport tubes with a lyophilized preservative are available that allow for the transport, testing, and storage of the urine specimens. The ideal preservative should be bactericidal, inhibit urease, and preserve formed elements in the sediment. At the same time, the preservative should not interfere with chemical tests. Unfortunately, as seen in Table 3-3, the ideal preservative does not exist; therefore, a preservative that best suits the needs of the required analysis should be chosen.

Technical Tip 3-3. Specimens must be returned to room temperature before chemical testing by reagent strips because the enzyme reactions on the strips perform best at room temperature.

Technical Tip 3-4. When preserving specimens that will be transported to another laboratory, confirm that the appropriate preservative is used.

Types of Specimens

To obtain a specimen that is representative of a patient's metabolic state, regulation of certain aspects of specimen collection is often necessary. These special conditions may include time, length, and method of collection, as well as the patient's dietary and medicinal intake. It is important to instruct patients when they must follow special collection procedures. Frequently encountered specimens are listed in Table 3-4.

Random Specimen

This is the specimen received most commonly because of its ease of collection and convenience for the patient. The **random specimen** may be collected at any time, but the actual time of voiding should be recorded on the container.[2] The random specimen is useful for routine screening tests to detect obvious abnormalities. However, it also may show erroneous results resulting from dietary intake or physical activity just before collection. Then the patient will be requested to collect an additional specimen under more controlled conditions.

First Morning Specimen

Although it may require the patient to make an additional trip to the laboratory, this is the ideal screening specimen. It is also essential for preventing false-negative pregnancy tests

Table 3–3 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[2]
Acids (boric acid, HCL, acetic acid, tartaric acid)	Prevents bacterial growth and metabolism	Interferes with analysis of drugs and hormones	Keeps pH at about 6.0 Can be used for transport of urine cultures
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	Good preservative for drug analyses	Inhibits reagent strip tests for glucose, blood, and leukocytes	
Commercial preservative tablets	Convenient when refrigeration not possible Have controlled concentration to minimize interference	Check tablet composition to determine possible effects on desired tests	
Urine Collection Kits[4] (Becton, Dickinson, Rutherford, NJ)	Contains collection cup, transfer straw, culture and sensitivity (C&S) preservative tube, or UA tube		

Table 3–3 Urine Preservatives—cont'd

Preservatives	Advantages	Disadvantages	Additional Information
Light gray and gray C&S tube	Specimen 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	Specimen 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

Table 3–4 Types of Urine 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
Four-glass collection	Prostatic infection

and for evaluating orthostatic **proteinuria** (see Chapter 6). The **first morning specimen** is a concentrated specimen, thereby assuring detection of chemicals and formed elements that may not be present in a dilute random specimen. The patient should be instructed to collect the specimen immediately on arising and to deliver it to the laboratory within 2 hours or keep it refrigerated.

24-Hour (or Timed) Specimen

Measuring the exact amount of a urine chemical is often necessary instead of just reporting its presence or absence. A carefully **timed specimen** must be used to produce accurate quantitative results. Many solutes 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.[2] When the concentration of the substance to be measured changes with diurnal variations and with daily activities, such as exercise, meals, and body metabolism, 24-hour collection is required. If the concentration of a particular substance remains constant, the specimen may be collected over a shorter period. Care must be taken, however, to keep the patient adequately hydrated during short collection periods. Patients must be instructed on the procedure for collecting a timed specimen.

To obtain an accurate timed specimen, the patient must begin and end the collection period with an empty bladder. The concentration of a substance in a particular period must be calculated from the urine volume produced during that time.

On its arrival in the laboratory, a 24-hour specimen must be mixed thoroughly and the volume accurately measured and recorded. If only an aliquot is needed for testing, the amount

HISTORICAL NOTE

Glucose Tolerance Specimens

Glucose tolerance specimens were sometimes collected to correspond with the blood specimens drawn during an oral glucose tolerance test (OGTT). The number of specimens varied with the length of the test. GTTs included fasting, half-hour, 1-hour, 2-hour, and 3-hour specimens and possibly 4-hour, 5-hour, and 6-hour specimens. The urine was tested for glucose and ketones, and the results were reported along with the blood test results as an aid to interpreting the patient's ability to metabolize a measured amount of glucose. The results are correlated with the renal threshold for glucose. Collection of these specimens was a facility option.[5]

saved must be adequate to permit repeat or additional testing. If a specimen is collected in two containers, the contents of the containers should be combined and thoroughly mixed before aliquoting. Consideration also must be given to the preservation of specimens collected over extended periods. All specimens should be refrigerated or kept on ice during the collection period and may require addition of a chemical preservative. The preservative chosen must be nontoxic to the patient and should not interfere with the tests to be performed. Appropriate collection information is included with test procedures and should be read before issuing a container and instructions to the patient. Box 3-1 lists the most common errors associated with timed urine collections.

Box 3–1 Common Errors Associated With Timed Urine Collections

- Loss of urine specimen
- Inclusion of two first morning specimens
- Inaccurate measurement of total urine volume
- Inadequate urine preservation
- Transcription error

PROCEDURE 3-1

24-Hour (Timed) Urine Specimen Collection Procedure

Equipment

Requisition form

24-hour urine specimen container with lid

Label

Container with ice, if required

Preservative, if required

Procedure

1. Provide the patient with written instructions, and explain the collection procedure.
2. Provide the patient with the collection container and preservative, if required.
3. Day 1: 7 a.m.: Patient voids and discards specimen; collects all urine for the next 24 hours.
4. Day 2: 7 a.m.: Patient voids and adds this urine to the previously collected urine.
5. Specimen is transported to the laboratory, where the entire 24-hour specimen is thoroughly mixed and the volume is measured and recorded.
6. The required amount of urine (approximately 50 mL) is aliquoted for testing.
7. The remaining specimen is discarded.

Technical Tip 3-5. Adding urine formed before the start of the collection period will falsely elevate the results, and failure to include the urine produced at the end of the collection period will falsely decrease the results.

Catheterized Specimen

This specimen is collected under sterile conditions by passing a hollow tube (catheter) through the urethra into the bladder. Urine passes from the bladder through the catheter into a plastic bag, where it accumulates. Urine specimens then can be collected from this urine bag. The test requested most commonly on a **catheterized specimen** is a bacterial culture.

Midstream Clean-Catch Specimen

As an alternative to the catheterized specimen, the **midstream clean-catch specimen** provides a safer, less traumatic method for obtaining urine for bacterial culture and routine urinalysis. It provides a specimen that is less contaminated by epithelial cells and bacteria and therefore is more representative of the actual urine than the routinely voided specimen. Patients must be provided with appropriate cleansing materials, a sterile container, and instructions for cleansing and voiding. Strong bacterial agents, such as hexachlorophene or povidone-iodine, should not be used as cleansing agents. Mild antiseptic towelettes are recommended. Some urine collection transfer kits contain Castile Soap Towelettes. Procedures 3-2 and 3-3 provide instruction details for male and female patients.

Suprapubic Aspiration

Occasionally urine may be collected by external introduction of a needle through the abdomen into the bladder. Because the bladder is sterile under normal conditions, **suprapubic aspiration** provides a specimen for bacterial culture that is completely free of extraneous contamination, particularly in infants or children. The specimen also can be used for cytological examination.

Prostatitis Specimen

Several methods are available to detect the presence of prostatitis.

Three-Glass Collection

Before collection, the area is cleansed using the male midstream clean-catch procedure. Then, instead of discarding the first urine passed, it is collected in a sterile container. Next, the midstream portion is collected in another sterile container. Then the prostate is massaged so that prostate fluid will be passed with the remaining urine into a third sterile container. Quantitative cultures are performed on all specimens, and the first and third specimens are examined microscopically. In prostatic infection, the third specimen will have a white blood cell/high-power field count and a bacterial count 10 times that of the first specimen. Macrophages containing lipids also may be

PROCEDURE 3-2

Clean-Catch Specimen Collection: Female Cleansing Procedure[2]

Equipment

Requisition form

Sterile urine container with label

Sterile antiseptic towelettes

Written instructions for cleansing and voiding

Procedure

Instruct the patient to:

1. Wash her hands.
2. Remove the lid from the sterile container without touching the inside of the container or lid.
3. Separate the skin folds (**labia**).
4. Cleanse from front to back on either side of the urinary opening with an antiseptic towelette, using a clean one for each side.
5. Hold the skin folds apart and begin to void into the toilet.
6. Bring the urine container into the middle 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.
7. Finish voiding into the toilet.
8. Cover the specimen with the lid. Touch only the outside of the lid and container.
9. Confirm the container is labeled correctly with the patient's first and last name and time of collection, and place it in the specified area, or follow facility policy.

PROCEDURE 3-3

Clean-Catch Specimen Collection: Male Cleansing Procedure[2]

Equipment

Requisition form

Sterile urine container with label

Sterile antiseptic towelettes

Written instructions for cleansing and voiding

Procedure

Instruct the patient to:

1. Wash his hands.
2. Remove the lid from the sterile container without touching the inside of the container or lid.
3. Cleanse the tip of the penis with antiseptic towelette and let it dry. Retract the foreskin if uncircumcised.
4. Void into the toilet. Hold back the foreskin if necessary.
5. Bring the sterile urine container into the middle 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.
6. Finish voiding into the toilet.
7. Cover the specimen with the lid. Touch only the outside of the lid and container.
8. Confirm the container is labeled correctly with the patient's first and last name and time of collection, and place it in the specified area, or follow facility policy.

present. The second specimen is used as a control for bladder and kidney infection. If it is positive, the results from the third specimen are invalid because infected urine has contaminated the specimen.[6]

Technical Tip 3-6. When both a routine urinalysis and a culture are requested on a catheterized or midstream collection, the culture should be performed first to prevent contamination of the specimen. A collection transfer kit also can be used.

Pre- and Postmassage Test

In the pre- and postmassage test (**PPMT**), a clean-catch midstream urine specimen is collected. A second urine sample is collected after the prostate is massaged. A positive result is significant bacteriuria in the postmassage specimen of greater than 10 times the premassage count.[7]

Stamey-Meares Test for Prostatitis

The traditional four-glass urine collection technique, as described by Meares and Stamey,[7,8] includes examination of four urine specimens as follows:

- The first urine specimen is voided bladder (**VB1**), which is the first 10 mL of urine and represents the urethral specimen.
- Then the patient voids another 100 to 150 mL of urine.
- The second specimen, voided bladder 2 (**VB2**), is collected, which is another 10 mL of urine and represents the bladder specimen.
- The third specimen is the expressed prostatic specimen (**EPS**), which is the fluid collected during prostatic massage.

- The fourth specimen, voided bladder 3 (**VB3**), consists of the first 10 mL of urine collected after EPS; it contains any EPS trapped in the prostatic urethra.
- All four specimens are sent for culture.
- The three urine specimens are centrifuged, and the sediment is examined for white blood cells/aggregates, macrophages, oval fat bodies, bacteria, and fungal hypha.

Urethral infection or inflammation is tested for by the VB1, and the VB2 tests for urinary bladder infection. The prostatic secretions are cultured and examined for white blood cells. Having more than 10 to 20 white blood cells per high-power field is considered abnormal.[7]

Pediatric Specimens

Collection of pediatric specimens can present a challenge. Soft, clear plastic bags are available for collecting routine specimens; these bags have hypoallergenic skin adhesive to attach to the cleaned genital area of both boys and girls. Sterile specimens may be obtained by catheterization or by suprapubic aspiration. Care must be taken not to touch the inside of the bag when applying it to the patient's skin.

For routine specimen analysis, ensure the area is free of contamination. Attach the bag firmly over the cleaned genital area, avoiding the anus. A diaper is placed over the collection bag. When enough specimen has been collected, remove the bag and label it, or pour the specimen into a container and label the container following facility policy.

For microbiology specimens, clean the area with soap and water and sterilely dry the area, removing any residual soap residue. Firmly apply a sterile bag. Sterilely transfer the collected specimen into a sterile container and label the container.[2]

Technical Tip 3-7. Check the applied bags approximately every 15 minutes until the needed amount of sample has been collected.

Drug Specimen Collection

Urine specimen collection is the most vulnerable part of a drug-testing program. Correct collection procedures and documentation are necessary to ensure that the results are those of the specific individual submitting the specimen. The **chain of custody** (**COC**) is the process that provides this documentation of proper specimen identification from the time of collection to the receipt of laboratory results. The COC is a standardized form that must document and accompany every step of drug testing, from collector to courier to laboratory to medical review officer to employer.

For urine specimens to withstand legal scrutiny, it is necessary to prove that no tampering of the specimen occurred, such as substitution, adulteration, or dilution. All personnel handling the specimen must be noted. The specimen must be handled securely, with a guarantee that no unauthorized access to the specimen was possible. Proper identification of the individual whose information is indicated on the label is required. Either photo identification or positive identification by an employer representative with photo ID is acceptable.

Urine specimen collections may be "witnessed" or "unwitnessed." The decision to obtain a witnessed collection is indicated when it is suspected that the donor may alter or substitute the specimen or when it is the policy of the client ordering the test. If a witnessed specimen collection is ordered, a same-gender collector will observe the collection of 30 to 45 mL of urine. Witnessed and unwitnessed collections should be handed to the collector immediately.

The urine temperature must be taken within 4 minutes from the time of collection to confirm the specimen has not been adulterated. The temperature should read within the range of 32.5°C to 37.7°C. If the specimen temperature is not within range, the temperature should be recorded and the supervisor or employer contacted immediately. Urine temperatures outside of the recommended range may indicate specimen contamination. Re-collection of a second specimen as soon as possible will be necessary. The urine color is also inspected to identify any signs of contaminants. The pH and specific gravity of the specimen also may be tested. A urine pH of greater than 9 suggests adulteration of the urine specimen and requires that the specimen be re-collected if clinically necessary. A specific gravity of less than 1.005 could indicate dilution of the urine specimen and requires re-collection. The specimen is labeled, packaged, and transported following laboratory-specific instructions. A typical urine drug specimen collection is described in Procedure 3-4.

PROCEDURE 3-4

Urine Drug Specimen Collection Procedure

Equipment

Requisition form
Gloves
Bluing agent (dye)
Specimen container
Label
COC form
Temperature strip

Procedure

1. The collector sanitizes his or her hands and wears gloves.
2. The collector adds bluing agent (dye) to the toilet water reservoir to prevent an adulterated specimen.
3. The collector eliminates any source of water other than toilet by taping the toilet lid and faucet handles.
4. The donor provides photo identification or positive identification from the employer's representative.

PROCEDURE 3-4—cont'd

5. The collector completes step 1 of the COC form and has the donor sign it.
6. 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.
7. The donor sanitizes his or her hands and receives a specimen cup.
8. The collector remains in the restroom but outside the stall, listening for unauthorized water use, unless a witnessed collection is requested.
9. The donor hands the specimen cup to the collector. The transfer is documented.
10. The collector checks the urine for abnormal color and for the required amount (30 to 45 mL).
11. 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.
12. The specimen must remain in the sight of the donor and collector at all times.
13. 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.
14. The donor initials the specimen bottle seals.
15. The collector writes the date and time on the bottle seals.
16. The donor completes step 4 on the COC form.
17. The collector completes step 5 on the COC form.
18. 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.
19. The collector follows laboratory-specific instructions for packaging the specimen bottles and laboratory copies of the COC form.
20. The collector distributes the COC copies to appropriate personnel.



For additional resources please visit
www.fadavis.com

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Study Questions

1. The primary inorganic substance found in urine is:
   A. Sodium
   B. Phosphate
   C. Chloride
   D. Calcium
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, which substances 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 average daily output of urine is:
   A. 200 mL
   B. 500 mL
   C. 1200 mL
   D. 2500 mL

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 before 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 form states the specimen is catheterized
   B. Specimen contains toilet paper
   C. Label and requisition form do not match
   D. Outside of the container has contamination from fecal material

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 specimen collected most frequently 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, then the:
    A. Specimen must be re-collected
    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 before 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

Case Studies and Clinical Situations

1. A patient brings a first morning specimen to the laboratory at 1:00 p.m.
    a. How could this affect the urinalysis results?
    b. If the patient is a known diabetic, what results would be most suspect?
    c. What is the best course of action?
    d. What could the patient have said that would have made the specimen satisfactory for testing?
2. A patient collecting a midstream clean-catch specimen voids immediately into the container.
    a. How could this affect the clarity of the specimen?
    b. How could this affect the microscopic examination?
3. A patient brings a 24-hour-timed specimen to the laboratory and reports that he or she forgot to collect a specimen voided during the night.
    a. How will this affect the results of a quantitative test for creatinine?
    b. What should the patient be told to do?
4. The laboratory receives a urine preservative tube for culture containing a volume of specimen that is considerably below the minimum fill line.
    a. Could this affect the culture?
    b. Why?
5. A worker suspects that he or she will be requested to collect an unwitnessed urine specimen for drug analysis. He or she carries a substitute specimen in his or her pocket for 2 days before being told to collect the specimen. Shortly after the worker delivers the specimen to the collector, he or she is instructed to collect another specimen.
    a. What test was performed on the specimen to determine possible specimen manipulation?
    b. How was the specimen in this situation affected?
    c. If a specimen for drug analysis tests positive, state a possible defense related to specimen collection and handling that an attorney might employ.
    d. How can this defense be avoided?

CHAPTER 4

Renal Function

LEARNING OUTCOMES

Upon completing this chapter, the reader will be able to:

4-1 Identify the components of the nephron, kidney, and excretory system.

4-2 Trace the flow of blood through the nephron, and state the physiological functions that occur.

4-3 Describe the process of glomerular ultrafiltration.

4-4 Discuss the functions and regulation of the renin–angiotensin–aldosterone system (RAAS).

4-5 Differentiate between active and passive transport in relation to renal concentration.

4-6 Explain the function of antidiuretic hormone in the concentration of urine.

4-7 Describe the role of tubular secretion in maintaining acid–base balance.

4-8 Identify the laboratory procedures used to evaluate glomerular filtration, tubular reabsorption and secretion, and renal blood flow.

4-9 Describe the creatinine clearance test.

4-10 Given hypothetical laboratory data, calculate a creatinine clearance and determine whether the result is normal.

4-11 Discuss the clinical significance of the glomerular filtration rate tests.

4-12 Describe and contrast the Modification of Diet in Renal Disease (MDRD), cystatin C, and beta$_2$-microglobulin tests for performing estimated glomerular filtration rates (eGFR).

4-13 Define *osmolarity,* and discuss its relationship to urine concentration.

4-14 Describe the basic principles of freezing-point osmometers.

4-15 Given hypothetic laboratory data, calculate a free-water clearance and interpret the result.

4-16 Given hypothetic laboratory data, calculate a PAH clearance and relate this result to renal blood flow.

4-17 Describe the relationship of urinary ammonia and titratable acidity to the production of an acidic urine.

KEY TERMS

Active transport
Afferent arteriole
Aldosterone
Antidiuretic hormone (ADH)
Beta$_2$-microglobulin (B2M)
Clearance tests
Collecting duct
Concentration tests
Countercurrent mechanism
Creatinine
Creatinine clearance
Cystatin C
Density
Distal convoluted tubule
Endogenous procedure
Efferent arteriole
Exogenous procedure
Fenestrated endothelium
Free water clearance
Glomerular filtration barrier
Glomerular filtration rate (GFR)
Glomerulus
Juxtaglomerular apparatus
Loops of Henle
Macula densa
Maximal reabsorptive capacity (T_m)
Metabolic acidosis
Nephron
Osmolality
Osmolar clearance

Continued

KEY TERMS—cont'd

Osmolarity
Passive transport
Peritubular capillaries
Podocytes
Proximal convoluted tubule
Renal plasma flow
Renal threshold
Renal tubular acidosis
Renin
Renin–angiotensin–aldosterone system (RAAS)
Shield of negativity
Titratable acidity
Tubular reabsorption
Tubular secretion
Vasa recta
Vasopressin

Introduction

This chapter reviews nephron anatomy and physiology and discusses their relationship to urinalysis and renal function testing. A section on laboratory assessment of renal function is included.

Renal Physiology

Each kidney contains approximately 1 to 1.5 million functional units called **nephrons**. As shown in Figure 4-1, the human kidney contains two types of nephrons. Cortical nephrons, which make up approximately 85% of nephrons, are situated primarily in the cortex of the kidney. They are responsible primarily for removal of waste products and reabsorption of nutrients. Juxtamedullary nephrons have longer loops of Henle that extend deep into the medulla of the kidney. Their primary function is concentration of the urine.

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 is controlled in the nephron by the following renal functions:

- Renal blood flow
- Glomerular filtration
- **Tubular reabsorption**
- **Tubular secretion**

The physiology, laboratory testing, and associated pathology of these four functions are discussed in this chapter.

Renal Blood Flow

The renal artery supplies blood to the kidney. The human kidneys receive approximately 25% of the blood pumped through the heart at all times. Blood enters the capillaries of the nephron through the **afferent arteriole**. It then flows through the **glomerulus** and into the **efferent arteriole**. The varying sizes of these arterioles help create the hydrostatic pressure differential that is important for glomerular filtration and to maintain consistency of glomerular capillary pressure and renal blood flow within the glomerulus. Notice the smaller size of the efferent arteriole in Figure 4-2. This increases the glomerular capillary pressure. Renal blood flow is outlined in Box 4-1.

Before returning to the renal vein, blood from the efferent arteriole enters the **peritubular capillaries** and the **vasa recta** and flows slowly through the cortex and medulla of the kidney close to the tubules. The peritubular capillaries surround the proximal and distal convoluted tubules, providing for the immediate reabsorption of essential substances from the fluid in the **proximal convoluted tubule** and final adjustment of the urinary composition in the **distal convoluted tubule**. The vasa recta are located adjacent to the ascending and descending **loops of Henle** in juxtamedullary nephrons. In this area, the 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, which is necessary for renal concentration. Box 4-2 outlines the urinary filtrate flow.

Based on an average body size of 1.73 m^2 of surface, the total renal blood flow is approximately 1200 mL/min, and the total **renal plasma flow** ranges from 600 to 700 mL/min. Normal values for renal blood flow and renal function tests depend on body size. When dealing with body sizes that vary greatly from the average 1.73 m^2 of body surface, a correction must be calculated to determine whether the observed measurements represent normal function. This calculation is covered in the discussion on tests for **glomerular filtration rate** (**GFR**) later in this chapter. Variations in normal values have been published for different age groups and should be considered when evaluating renal function studies.

Glomerular Filtration

The glomerulus consists of a coil of approximately eight capillary lobes, the walls of which are referred to as the **glomerular filtration barrier**. The glomerulus is located within **Bowman capsule**, which forms the beginning of the renal tubule. Although the glomerulus serves as a nonselective filter of plasma substances with molecular weights less than 70,000, several factors influence the actual filtration process. These include the cellular structure of the capillary walls and Bowman capsule, **hydrostatic pressure** and **oncotic pressure**, and the feedback mechanisms of the **renin–angiotensin–aldosterone system** (**RAAS**).

Cellular Structure of the Glomerulus

Plasma filtrate must pass through three glomerular filtration barrier cellular layers: the capillary wall membrane, the basement membrane (basal lamina), and the visceral epithelium of the Bowman capsule. The endothelial cells of the capillary wall

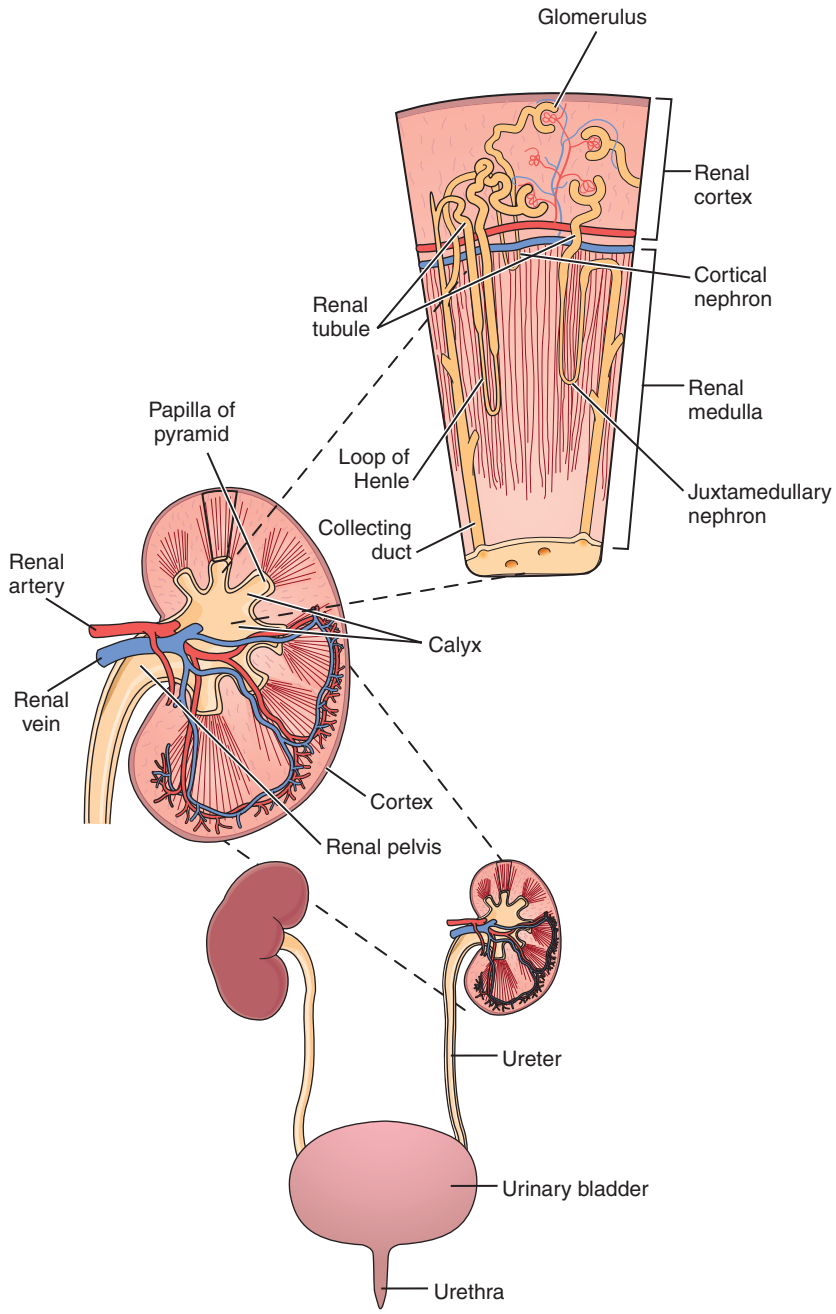

Figure 4–1 The relationship of the nephron to the kidney and excretory system. (From Scanlon, VC, and Sanders, T: Essentials of Anatomy and Physiology, ed 3. FA Davis, Philadelphia, PA, 1999, p 405, with permission.)

differ from those in other capillaries by containing pores and are referred to as **fenestrated endothelium**. The pores increase capillary permeability but do not allow the passage of large molecules and blood cells. Further restriction of large molecules occurs as the filtrate passes through the basement membrane and the thin membranes covering the filtration slits formed by the intertwining foot processes of the **podocytes** of the inner layer of the Bowman capsule (Fig. 4-3A).

In addition to the structure of the glomerular filtration barrier that prohibits the filtration of large molecules, the barrier contains a **shield of negativity** that repels molecules with a negative charge even though they are small enough to pass through the three layers of the barrier. The shield is very important because it is the place where albumin (the primary protein associated with renal disease) has a negative charge and is repelled (Figs. 4-3B and 4-3C).

Glomerular Pressure

As mentioned previously, the presence of hydrostatic pressure, resulting from the smaller size of the efferent arteriole and the glomerular capillaries, enhances filtration. This pressure is necessary to overcome the opposition of pressures from the fluid within the Bowman capsule and the oncotic pressure of unfiltered plasma proteins in the glomerular capillaries. By increasing or decreasing the size of the afferent and efferent arterioles,

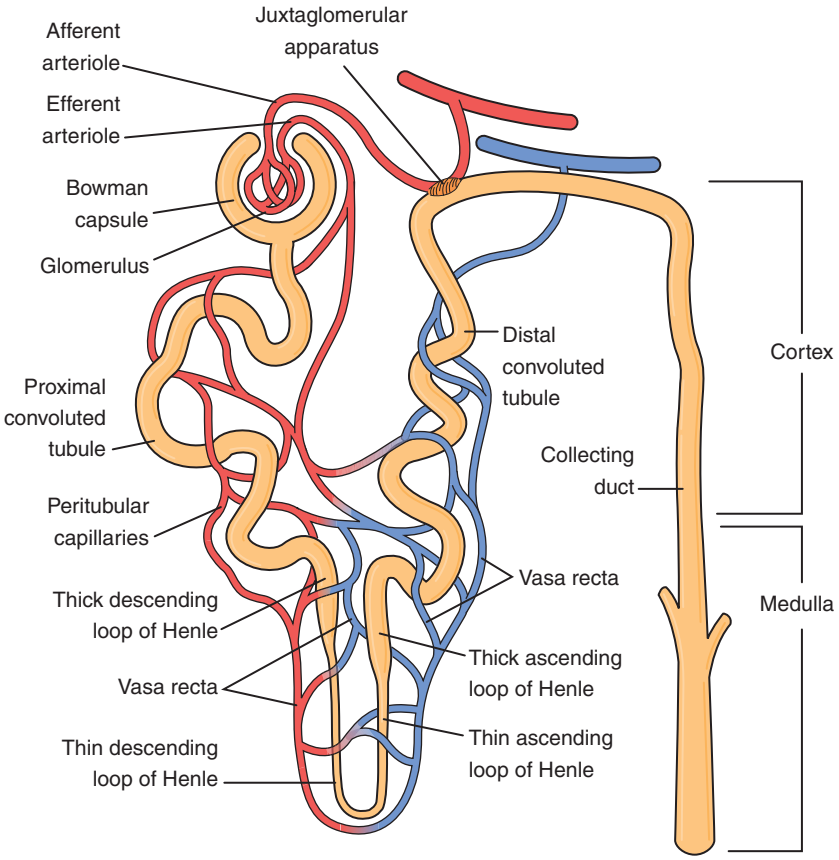

Figure 4–2 The nephron and its component parts.

Box 4–1 Renal Blood Flow

1. Renal artery
2. Afferent arteriole
3. Glomerulus
4. Efferent arteriole
5. Peritubular capillaries
6. Vasa recta
7. Renal vein

Box 4–2 Urinary Filtrate Flow

1. Bowman capsule
2. Proximal convoluted tubule
3. Descending loop of Henle
4. Ascending loop of Henle
5. Distal convoluted tubule
6. Collecting duct
7. Renal calyces
8. Ureter
9. Bladder
10. Urethra

an 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 prevent a marked decrease in blood flowing through the kidney, thus preventing an increase in the blood level of toxic waste products. Likewise, an increase in blood pressure results in constriction of the afferent arterioles to prevent overfiltration or damage to the glomerulus.

Technical Tip 4-1. If it were not for the shield of negativity, all routine urines would have positive readings on reagent strips for protein and albumin.

Renin–Angiotensin–Aldosterone System

The RAAS regulates the flow of blood to and within the glomerulus. The system responds to changes in blood pressure and plasma sodium content that are monitored by the juxtaglomerular apparatus, which consists of the juxtaglomerular cells in the afferent arteriole and the **macula densa** of the distal convoluted tubule (Fig. 4-4). Low plasma sodium content decreases water retention within the circulatory system, resulting in a decreased overall blood volume and subsequent decrease in blood pressure. When the macula densa senses such changes, a cascade of

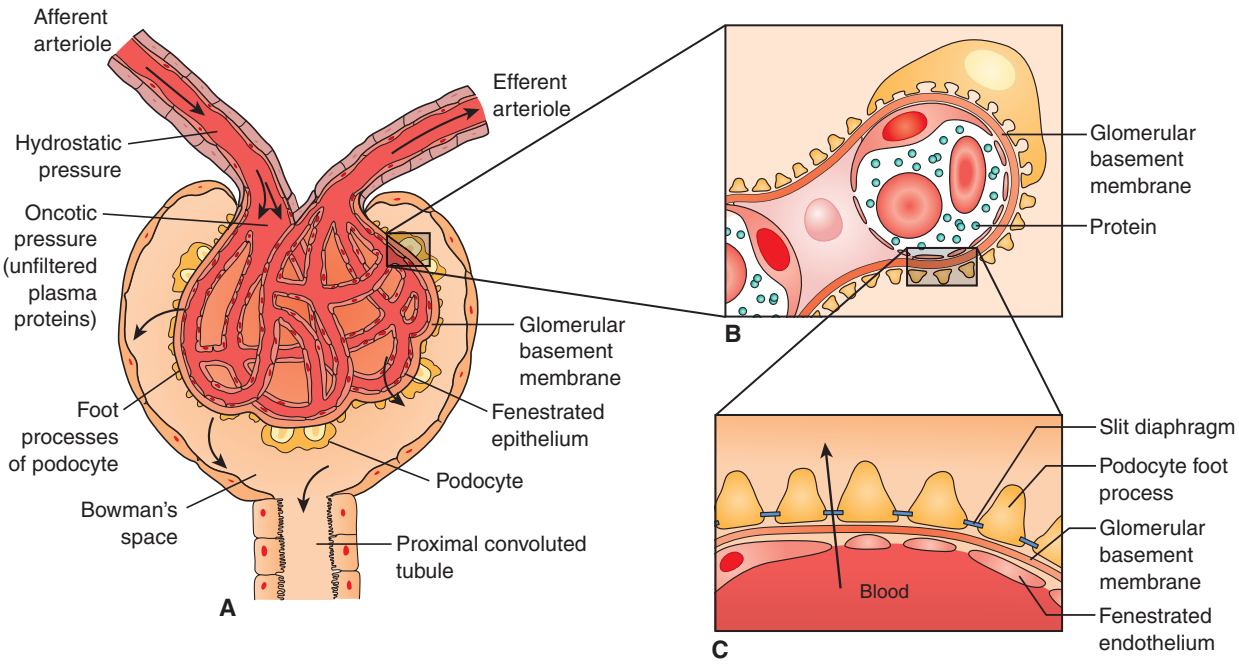

Figure 4–3 Factors affecting glomerular filtration in the renal corpuscle **(A)**. Inset **B,** glomerular filtration barrier. Inset **C,** the shield of negativity.

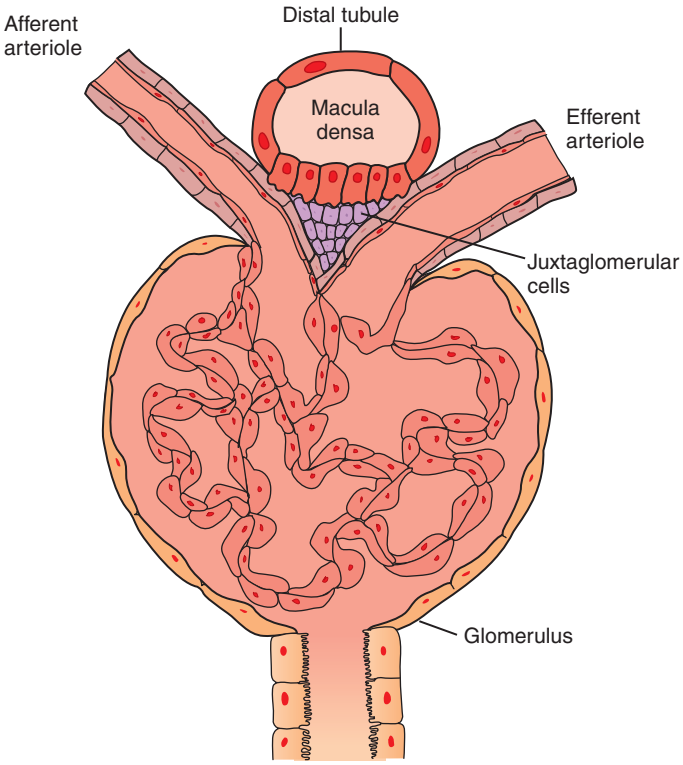

Figure 4–4 Close contact of the distal tubule with the afferent arteriole, macula densa, and the juxtaglomerular cells within the juxtaglomerular apparatus. Note the smaller size of the afferent arteriole indicating increased blood pressure.

reactions within the RAAS occurs (Fig. 4-5). **Renin**, an enzyme produced by the juxtaglomerular cells, is secreted and reacts with the bloodborne substrate angiotensinogen to produce the inert hormone angiotensin I. As angiotensin I passes through the alveoli of the lungs, angiotensin-converting enzyme (**ACE**) changes it to the active form angiotensin II. Angiotensin II corrects renal blood flow in the following ways:

- Causing vasodilation of the afferent arterioles and constriction of the efferent arterioles
- Stimulating reabsorption of sodium and water in the proximal convoluted tubules
- Triggering the release of the sodium-retaining hormone **aldosterone** by the adrenal cortex and antidiuretic hormone by the hypothalamus (Box 4-3).

As systemic blood pressure and plasma sodium content increase, the secretion of renin decreases. Therefore, the actions of angiotensin II produce a constant pressure within the nephron.

As a result of the glomerular mechanisms just discussed, every minute, approximately 2 to 3 million glomeruli filter approximately 120 mL of water-containing low-molecular-weight substances. Because this filtration is nonselective, the only difference between the compositions of the filtrate and the plasma is the absence of plasma protein, any protein-bound substances, and cells. Analysis of the fluid as it leaves the glomerulus shows the filtrate to have a specific gravity of 1.010 and confirms that it is chemically an ultrafiltrate of plasma. This information provides a useful baseline for evaluating the renal mechanisms involved in converting the plasma ultrafiltrate into the final urinary product.

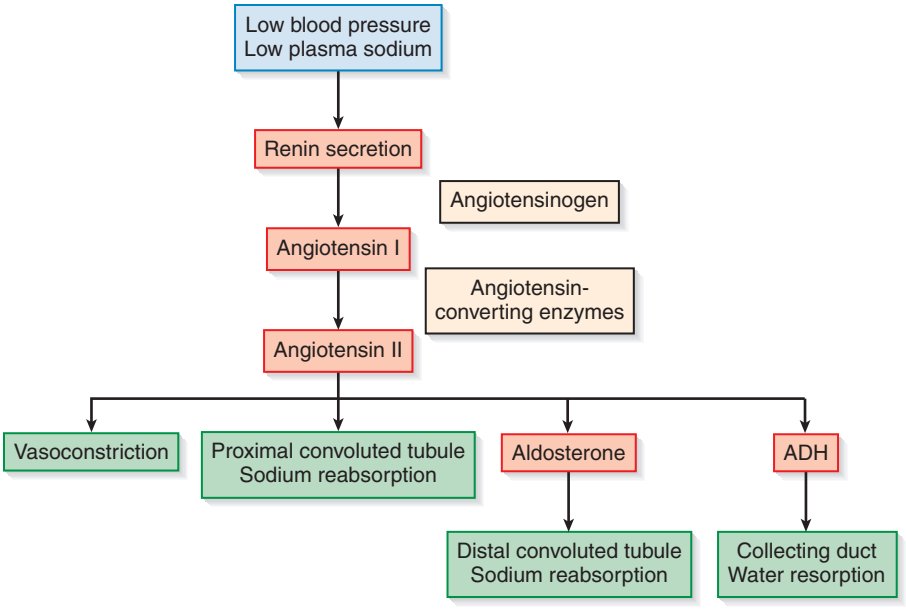

Figure 4–5 Algorithm of the renin–angiotensin–aldosterone system.

Box 4–3 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 the hypothalamus to release antidiuretic hormone to stimulate water reabsorption in the collecting duct

Tubular Reabsorption

The body cannot lose 120 mL of water-containing essential substances every minute. Therefore, when the plasma ultrafiltrate enters the proximal convoluted tubule, the nephrons, through cellular transport mechanisms, begin reabsorbing these essential substances and water (Table 4-1).

Reabsorption Mechanisms

The cellular mechanisms involved in tubular reabsorption are termed **active transport** and **passive transport**. For active transport to occur, the substance to be reabsorbed must combine with a carrier protein contained in the membranes of the renal tubular epithelial cells. The electrochemical energy created by this interaction transfers the substance across the cell membranes and back into the bloodstream. Active transport is responsible for the reabsorption of the following substances:

- Glucose, amino acids, and salts in the proximal convoluted tubule
- Chloride in the ascending loop of Henle
- Sodium in the distal convoluted tubule

Table 4–1 Tubular Reabsorption

	Substance	Location
Active transport	Glucose, amino acids, salts	Proximal convoluted tubule
	Chloride	Ascending loop of Henle
	Sodium	Proximal and distal convoluted tubules
Passive transport	Water	Proximal convoluted tubule
		Descending loop of Henle
		Collecting duct
	Urea	Proximal convoluted tubule
		Ascending loop of Henle
	Sodium	Ascending loop of Henle

Passive transport is the movement of molecules across a membrane as a result of differences in their concentration or electrical potential on opposite sides of the membrane. These physical differences are called gradients. Passive reabsorption of water takes place in all parts of the nephron except the ascending loop of Henle, the walls of which are impermeable to water. Urea is passively reabsorbed in the proximal convoluted tubule and the ascending loop of Henle, and passive reabsorption of sodium accompanies the active transport of chloride in the ascending loop.

Active transport, like passive transport, can be influenced by the concentration of the substance being transported. When the plasma concentration of a substance that is normally completely reabsorbed reaches a level that is abnormally high, the filtrate concentration exceeds the **maximal reabsorptive capacity** (T_m) of the tubules, and the substance begins appearing in the urine. The plasma concentration at which active transport stops is termed the **renal threshold**. For glucose, the plasma renal threshold is 160 to 180 mg/dL, and glucose appears in the urine when the plasma concentration reaches this level. Knowledge of the renal threshold and the plasma concentration can be used to distinguish between excess solute filtration and renal tubular damage. Active transport of more than two-thirds of the filtered sodium out of the proximal convoluted tubule is accompanied by the passive reabsorption of an equal amount of water. Therefore, as seen in Figure 4-6, the fluid leaving the proximal convoluted tubule still maintains the same concentration as the ultrafiltrate.

Technical Tip 4-2. Glucose appearing in the urine of a person with a normal blood glucose level is the result of tubular damage and not diabetes mellitus. A nonfasting patient with high glucose intake would not have a normal blood glucose. The plasma glucose would reach the renal threshold and appear in the urine.

Tubular Concentration

Renal concentration begins in the descending and ascending loops of Henle, where the filtrate is exposed to the high osmotic gradient of the renal medulla. Water is removed by osmosis in the descending loop of Henle, and sodium and chloride are reabsorbed in the ascending loop. Excessive reabsorption of water as the filtrate passes through the highly concentrated medulla is prevented by the water-impermeable walls of the ascending loop. This selective reabsorption process is called the **countercurrent mechanism** and serves to maintain the osmotic gradient of the medulla (see Fig. 4-6). The sodium and chloride leaving the filtrate in the ascending loop prevent dilution of the medullary interstitium by the water reabsorbed from the descending loop. Maintenance of this osmotic gradient is essential for the final concentration of the filtrate when it reaches the **collecting duct**.

In Figure 4-6, the actual concentration of the filtrate leaving the ascending loop is quite low due to the reabsorption of salt and not water in that part of the tubule. Reabsorption of sodium continues in the distal convoluted tubule, but now it is under the control of the hormone aldosterone, which regulates reabsorption in response to the body's need for sodium (Fig. 4-5).

Collecting Duct Concentration

The final concentration of the filtrate through the reabsorption of water begins in the late distal convoluted tubule and continues in the collecting duct. Reabsorption depends on the osmotic gradient in the medulla, as well as the hormone **vasopressin** (**antidiuretic hormone** [**ADH**]) that is released by the posterior pituitary gland when the amount of water in the body decreases. One would expect that as the dilute filtrate in the collecting duct comes in contact with the higher osmotic concentration of the medullary interstitium, passive reabsorption of water would occur. However, the process is controlled by the presence or absence of ADH, which renders the walls of the distal convoluted tubule and collecting duct permeable or impermeable to water. A high level of ADH increases permeability, resulting in increased reabsorption of water, and a low-volume concentrated urine. Likewise, absence of ADH renders the walls impermeable to water, resulting in a large volume of dilute urine. Just as the production of aldosterone is controlled by the body's sodium concentration, the production of ADH is determined by the state of body hydration. Therefore, the chemical balance in the body is actually the final determinant of urine volume and concentration. The concept of ADH control can be summarized as follows:

↑ Body Hydration = ↓ ADH = ↑ Urine Volume
↓ Body Hydration = ↑ ADH = ↓ Urine Volume

Tubular Secretion

In contrast to tubular reabsorption, in which substances are removed from the glomerular filtrate and returned to the blood, tubular secretion involves the passage of substances from the blood in the peritubular capillaries to the tubular filtrate (Fig. 4-7). Tubular secretion serves two major functions: eliminating waste products not filtered by the glomerulus and regulating the acid–base balance in the body through the secretion of hydrogen ions.

Many foreign substances, such as medications, cannot be filtered by the glomerulus because they are bound to plasma proteins. When these protein-bound substances enter the peritubular capillaries, they develop a stronger affinity for the tubular cells and dissociate from their carrier proteins, which results in their transport into the filtrate by the tubular cells. The major site for removal of these nonfiltered substances is the proximal convoluted tubule.

Acid–Base Balance

To maintain the normal blood pH of 7.4, the blood must buffer and eliminate the excess acid formed by dietary intake and body metabolism. The buffering capacity of the blood depends on bicarbonate (HCO_3^-) ions, which are readily filtered by the glomerulus and must be returned to the blood expediently to maintain the proper pH. As shown in Figure 4-8, the secretion of hydrogen ions (H^+) by the renal tubular cells into the filtrate prevents the filtered bicarbonate from being excreted in the urine and causes the return of a bicarbonate ion to the plasma. This process, which occurs primarily in the proximal convoluted tubule, provides for almost 100% reabsorption of filtered bicarbonate.

As a result of their small molecular size, hydrogen ions are readily filtered and reabsorbed. Therefore, the actual excretion of excess hydrogen ions also depends on tubular secretion.

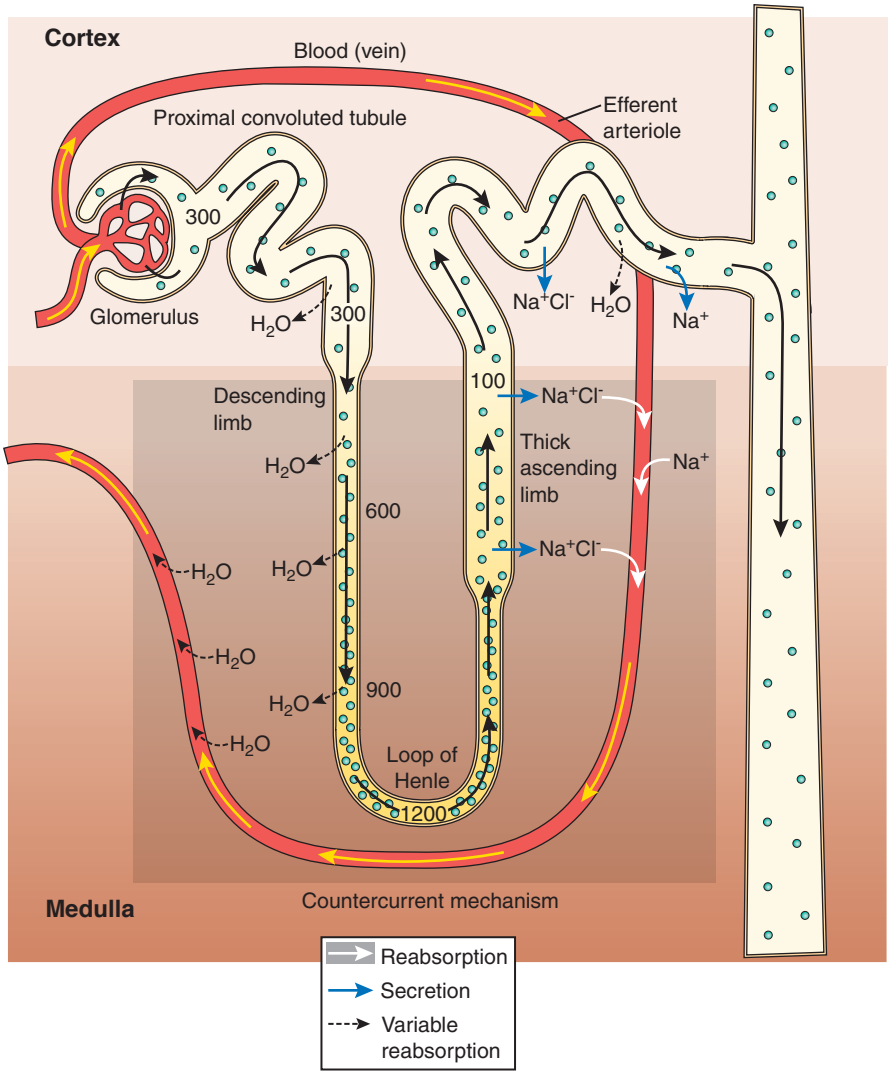

Figure 4–6 Renal concentration.

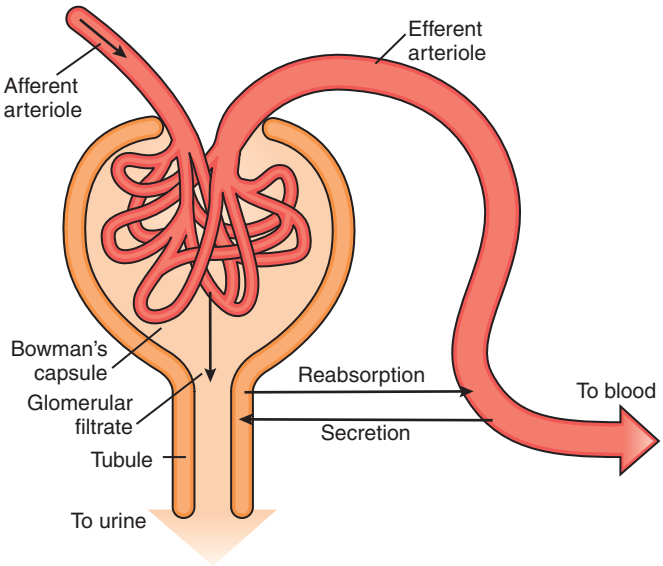

Figure 4–7 Summary of movement of substances in the nephron.

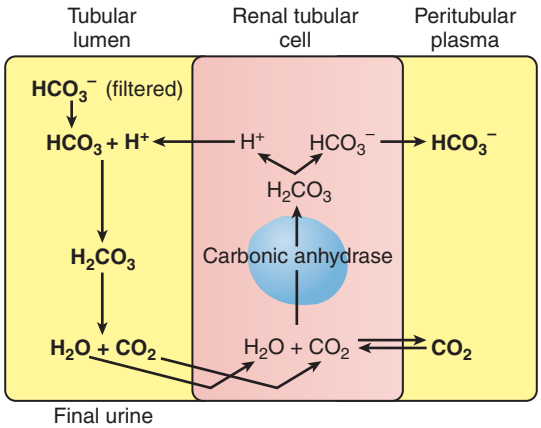

Figure 4–8 Reabsorption of filtered bicarbonate.

Figures 4-9 and 4-10 are diagrams of the two primary methods for the excretion of hydrogen ions in the urine. In Figure 4-9 the secreted hydrogen ion combines with a filtered phosphate ion instead of a bicarbonate ion and is excreted rather than reabsorbed. In the proximal convoluted tubule, ammonia is produced from the breakdown of the amino acid glutamine.

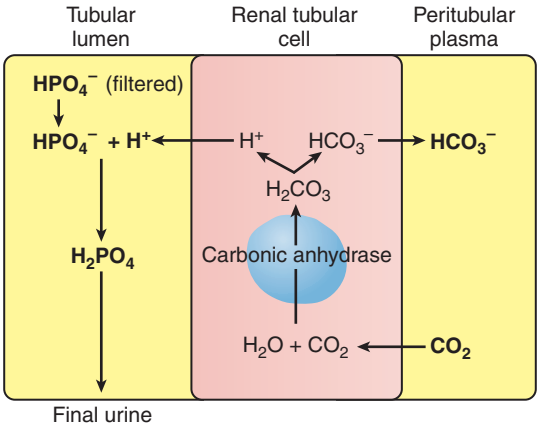

Figure 4–9 Excretion of secreted hydrogen ions combined with phosphate.

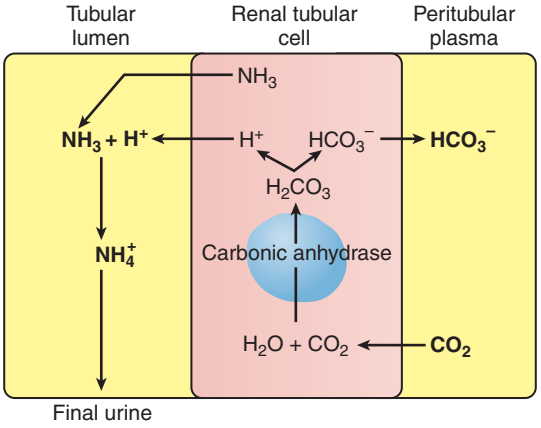

Figure 4–10 Excretion of secreted hydrogen ions combined with ammonia produced by the tubules.

The ammonia reacts with the H^+ to form the ammonium ion (NH_4^+) (Fig. 4-10). The resulting ammonium ion is excreted in the urine. Should there be additional need for the elimination of hydrogen ions, the distal convoluted tubule and the collecting duct are also able to produce ammonium ions.

All three of these processes occur simultaneously at rates determined by the acid–base balance in the body. A disruption in these secretory functions can result in **metabolic acidosis** or **renal tubular acidosis**, the inability to produce an acid urine.

Renal Function Tests

This brief review of renal physiology shows that there are many metabolic functions and chemical interactions to be evaluated through laboratory tests of renal function. In Figure 4-11, the parts of the nephron are related to the laboratory tests used to assess their function.

Glomerular Filtration Tests

The standard tests used to measure the filtering capacity of the glomeruli are termed **clearance tests**. As its name implies, a clearance test measures the rate in milliliters per minute at which the kidneys are able to remove (to clear) a filterable substance from the blood. To ensure that glomerular filtration is being measured accurately, the substance analyzed must be one that is neither reabsorbed nor secreted by the tubules. Other factors to consider in selecting a clearance test substance include the stability of the substance in urine during a possible 24-hour collection period, the consistency of the plasma level, the substance's availability to the body, and the availability of tests to analyze the substance.

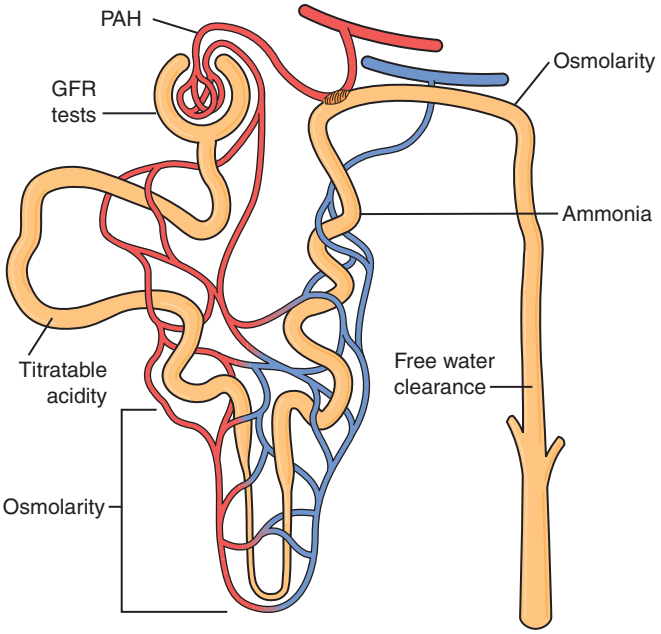

Figure 4–11 The relationship of nephron areas to renal function tests.

Clearance Tests

A variety of substances has been used to measure the GFR. Newer methods that eliminate many of the problems mentioned previously have replaced some of these tests. They are summarized as Historical Notes.

At present, **creatinine**, **beta$_2$-microglobulin** (**B2M**), **cystatin C**, and, possibly, radioisotopes are the primary substances used in clearance tests. Each procedure has its advantages and disadvantages.

HISTORICAL NOTE

Urea Clearance

The earliest glomerular filtration tests measured urea because of its presence in all urine specimens, as well as the existence of routinely used methods of chemical analysis. Because approximately 40% of the filtered urea is reabsorbed, normal values were adjusted to reflect the reabsorption, and patients were hydrated to produce a urine flow of 2 mL/min to ensure that no more than 40% of the urea was reabsorbed.

HISTORICAL NOTE

Inulin Clearance

Inulin, a polymer of fructose, is an extremely stable substance that is neither reabsorbed nor secreted by the tubules. It is not a normal body constituent, however, and must be infused by IV at a constant rate throughout the testing period. Therefore, although inulin was the original reference method for clearance tests, current methods are available that are endogenous and can provide accurate GFR results.

A test that requires an infused substance is termed an **exogenous procedure** and is seldom the method of choice if a suitable test substance is already present in the body (**endogenous procedure**).

Creatinine Clearance

Creatinine is a waste product of muscle metabolism that is produced enzymatically by creatine phosphokinase from creatine, which links with adenosine triphosphate (**ATP**) to produce adenosine diphosphate (**ADP**) and energy. Because creatinine is normally found at a relatively constant level in the blood, it provides the laboratory with an endogenous procedure for evaluating glomerular function. The use of creatinine has several disadvantages, and careful consideration should be given to them:

1. Some creatinine is secreted by the tubules, and secretion increases as blood levels rise.
2. Chromogens present in human plasma react in the chemical analysis. Their presence, however, may help counteract the falsely elevated rates caused by tubular secretion.
3. Medications, including gentamicin, cephalosporins, and cimetidine (Tagamet), inhibit tubular secretion of creatinine, thus causing serum levels that are falsely low.[1]
4. Bacteria will break down urinary creatinine if specimens are kept at room temperature for extended periods.[2]
5. 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. The increased intake of meat can raise the creatinine levels in urine and plasma during the 24-hour collection period.
6. Measurement of **creatinine clearance** is not a reliable indicator in patients suffering from muscle-wasting diseases or those involved in heavy exercise or athletes supplementing with creatine.
7. Accurate results depend on the accurate completeness of a 24-hour collection.
8. Creatinine clearance values must be corrected for body surface area, unless normal is assumed, and must always be corrected for children.

Newer methods that do not require the collection of timed (24-hour) urine specimens have been developed using just the serum creatinine, cystatin C, or B2M values. The results of these tests are reported as estimated glomerular filtration rate (**eGFR**). The traditional procedure for creatinine clearance is included here because it is still being performed, and its principles apply to other clearance procedures using urine.

Procedure

By far, the greatest source of error in any clearance procedure using urine is the use of urine specimens that are improperly timed. The importance of using a specimen that is accurately timed (see Chapter 3) will become evident in the following discussion of the calculations involved in converting isolated laboratory measurements to the GFR. The GFR is reported in milliliters cleared per minute; therefore, it is necessary to determine the number of milliliters of plasma from which the clearance substance (creatinine) is removed completely during 1 minute. To calculate this information, one must know urine volume in mL/min (V), urine creatinine concentration in mg/dL (U), and plasma creatinine concentration in mg/dL (P).

The urine volume is calculated by dividing the number of milliliters in the specimen by the number of minutes used to collect the specimen.

EXAMPLE

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

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

The concentrations of plasma and urine 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:

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

EXAMPLE

Using urine creatinine of 120 mg/dL (U), plasma creatinine of 1.0 mg/dL (P), and urine volume of 1440 mL obtained from a 24-hour specimen (V), calculate the GFR.

$$V = \frac{1440 \text{ mL}}{60 \text{ minutes} \times 24 = 1440 \text{ minutes}} = 1 \text{ mL/min}$$

$$C = \frac{120 \text{ mg/dL} \times 1 \text{ mL/min (V)}}{1.0 \text{ mg/dL (P)}} = 120 \text{ mL/dL}$$

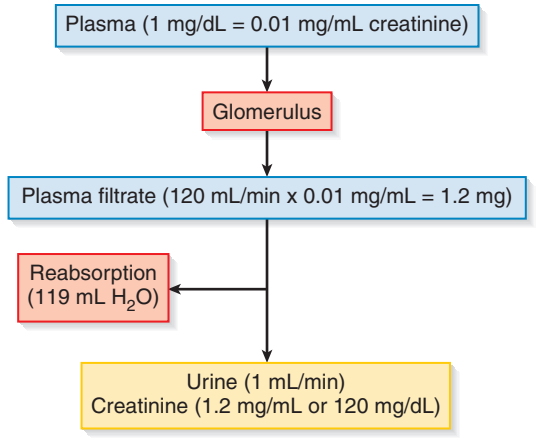

Figure 4–12 Creatinine filtration and excretion.

By analyzing this calculation and referring to Figure 4-12, at a concentration of 1 mg/dL, each milliliter of plasma contains 0.01 mg creatinine. Therefore, to arrive at a urine concentration of 120 mg/dL (1.2 mg/mL), it is necessary to clear 120 mL of plasma. Although the filtrate volume is reduced, the amount of creatinine in the filtrate does not change because the creatinine is not reabsorbed.

Knowing that in the average person (1.73 m^2 body surface), the approximate amount of plasma filtrate produced per minute is 120 mL, it is not surprising that normal creatinine clearance values approach 120 mL/min (men, 107 to 139 mL/min; women, 87 to 107 mL/min). The normal reference range of plasma creatinine is 0.6 to 1.2 mg/dL. These reference values consider variations in size and muscle mass. Values are considerably lower in older people; however, an adjustment also may have to be made to the calculation when dealing with body sizes that deviate greatly from 1.73 m^2 of surface, such as with children. To adjust a clearance for body size, the formula is:

$$C = \frac{UV}{P} \times \frac{1.73}{A}$$

with A being the actual body size in square meters of surface. The actual body size may be calculated as:

$$\log A = (0.425 \times \log \text{weight}) + (0.725 \times \log \text{height}) - 2.144$$

or it may be obtained from the nomogram shown in Figure 4-13.

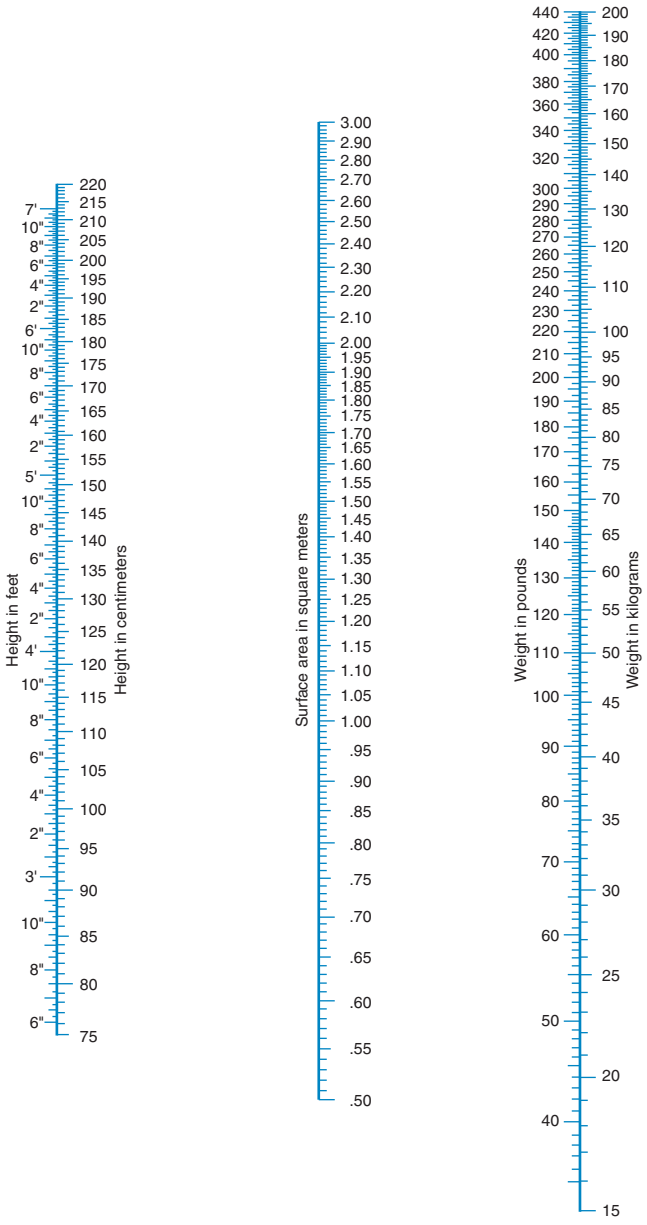

Figure 4–13 A nomogram for determining body surface area. (From Boothby, WM, and Sandiford, RB: Nomogram for determination of body surface area. N Engl J Med 185:227, 1921, with permission.)

Estimated Glomerular Filtration Rates

In the past years, a variety of formulas for eGFR have been used, and those formulas continue to be revised. Because the formulas can be programmed into automated instruments, estimated clearances can be used for routinely screening patients as part of a metabolic profile and to monitor patients already diagnosed with renal disease or at risk for renal disease. In addition, the formulas are valuable when there is a need to prescribe medications that require adequate renal clearance.

The formula used most frequently is called the Modification of Diet in Renal Disease (**MDRD**) study. The formula has been modified several times to make it more accurate and standardized. At present, the formula recommended by the National Kidney Disease Education Program (**NKDEP**) is called the MDRD-IDMS-traceable formula. A primary discrepancy in the previous formulas was found to be the methods used to measure serum creatinine. eGFR equations are superior to serum creatinine alone because they include variables for race, age, and gender.[3] Current laboratory methods primarily use creatinine assays, such as enzyme assays, that do not have the same interference as the original Jaffe chemical method. These methods correspond more closely to the isotope dilution mass spectrophotometry (**IDMS**) reference method.

The MDRD-IDMS traceable formula is:

$$GFR = 175 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times 0.742 \text{ (if patient is female)} \times 1.212 \text{ (if patient is black)}$$

The formula is designed to essentially equal the results that compare to the reference body size of 1.73 m^2.

Because eGFRs are calculated for an average body size, they are not accurate for pediatric patients. They also have been shown to be most accurate when results are lower than 60 mL/min.[4] It is recommended that results be reported with numerical values below 60 mL/min (for example, 59 mL/min) and higher values reported as equal to or greater than 60 mL/min (for example, ≥60 mL/min).

The formula recommended for use when serum creatinine methods do not compare to the IDSM standard is provided as a Historical Note.

The eGFR is classified into the following stages based on the kidney disease. The Improving Global Outcomes (**KDIGO**) stages of chronic kidney disease (**CKD**) are[3]:

- Stage 1 GFR greater than 90 mL/min/1.73 m
- Stage 2 GFR between 60 and 89 mL/min/1.73 m
- Stage 3 GFR between 45 and 59 mL/min/1.73 m
- Stage 4 GFR between 15 and 29 mL/min/1.73 m
- Stage 5 GFR less than 15 mL/min/1.73 m (end-stage renal disease)

Cystatin C

The measurement of serum cystatin C has been shown to be a good procedure for screening and monitoring GFR. Cystatin C is a small protein (molecular weight 13,359) produced at a constant rate by all nucleated cells. It is filtered readily by the glomerulus and reabsorbed and broken down by the renal tubular cells. Therefore, no cystatin C is secreted by the tubules, and the serum concentration can be related directly to the GFR. Immunoassay procedures are available for measuring cystatin C.[5] Monitoring levels of cystatin C is recommended for pediatric patients, people with diabetes, the elderly, and patients who are critically ill.[6] An advantage of cystatin C is that it is independent of muscle mass.

Recent studies also have shown that measuring both serum or plasma cystatin C and creatinine can provide even more accurate information on a patient's GFR.[7]

HISTORICAL NOTE

Original MDRD Calculation

The formula for MDRD calculation of GFR when the serum creatinine method is not standardized to IDMS.

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

Beta$_2$-Microglobulin

B2M (molecular weight 11,800) dissociates from human leukocyte antigens at a constant rate and is removed rapidly from the plasma by glomerular filtration. The B2M may be used to distinguish disorders of the kidney as either glomerular or tubular. Its excretion in urine is normally low, but when the renal tubules become damaged or diseased, the B2M concentration increases due to the decreased ability to reabsorb this protein. By measuring B2M concentrations of both blood and urine to evaluate kidney damage or disease, disorders that affect the glomerulus and renal tubules may be distinguished.[8] The concentration of B2M in serum is normal, and its concentration in urine increases when tubular reabsorption is reduced. Increased levels of B2M in the blood combined with low levels in the urine indicate that the disorder is associated with glomeruli disorders. The B2M also is used to identify end-stage renal disease and early rejection of a kidney transplant. Sensitive methods using enzyme immunoassay are available for the measurement of B2M. A rise in the plasma level of B2M has been shown to be a more sensitive indicator of a decrease in GFR than creatinine clearance. However, the test is not reliable in patients who have a history of immunologic disorders or malignancy.[9]

Radionucleotides

Although it is an exogenous procedure and more labor intensive and costly, injecting radionucleotides such as ^{125}I-iothalamate 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.[10] This procedure can be valuable to measure the viability of a transplanted kidney. Other exogenous markers used are radioisotopes, such as chromium-51 ethylene-diamine-tetra-acetic acid (**^{51}Cr-EDTA**), and technetium-99-labeled diethylene-triamine-pentaacetate (**^{99}Tc-DTPA**). A nonradioactive contrast agent, iohexol, is used for children.[3]

Clinical Significance

When interpreting the results of a creatinine clearance test, the GFR is determined not only by the number of functioning nephrons but also by the functional capacity of these nephrons. In other words, even though half of the available nephrons may be nonfunctional, a change in the GFR will not occur if the remaining nephrons double their filtering capacity. This is evident in people who lead normal lives with only one kidney. Therefore, although the GFR is a frequently requested laboratory procedure, its value does not lie in the detection of early renal disease. Instead, it is used to determine the extent of nephron damage in known cases of renal disease, to monitor the effectiveness of treatment designed to prevent further nephron damage, and to determine the feasibility of administering medications, which can build up to dangerous blood levels if the GFR is markedly reduced.

Tubular Reabsorption Tests

As discussed, although measurement of the GFR is not a useful indication of early renal disease, the loss of tubular reabsorption capability is often the first function affected in renal disease.

This is not surprising when one considers the complexity of the tubular reabsorption process.

Tests to determine the ability of the tubules to reabsorb the essential salts and water that have been nonselectively filtered by the glomerulus are called **concentration tests**. As mentioned, the ultrafiltrate that enters the tubules has a specific gravity of 1.010; therefore, after reabsorption, one would expect the final urine product to be more concentrated. However, many urine specimens do not have a specific gravity higher than 1.010, yet no renal disease is present. This is 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.

As seen in Figure 4-14, both urine specimens contain the same amount of solute; however, the urine density (specific gravity) of patient A will be higher. Therefore, control of fluid intake must be incorporated into laboratory tests that measure the concentrating ability of the kidney.

Throughout the years, various methods have been used to produce water deprivation, including the Fishberg and Mosenthal concentration tests, which measured specific gravity. In the Fishberg test, patients were deprived of fluids for 24 hours before specific gravity was measured. The Mosenthal test compared the volume and specific gravity of urine specimens collected during the day and at night to evaluate concentrating ability. Neither test is used now because the information provided by specific gravity measurements is most useful as a screening procedure, and quantitative measurement of renal concentrating ability is best assessed through osmometry.

Currently renal concentrating testing is performed after various periods of fluid deprivation, measuring urine, and often serum **osmolality**. Controlled intake procedures can include after-dinner overnight deprivation of fluid for 12 hours followed by collection of a urine specimen. A urine osmolality reading of 800 mOsm or higher is normal, and the test can be discontinued. If the urine test is abnormal, the fluid is restricted for another 2 hours, and specimens of both urine and serum are collected for osmolality testing. A urine-to-serum ratio (**U:S ratio**) of 3:1 or greater or a urine osmolality of 800 mOsm or greater indicates normal tubular reabsorption.

If the test continues to be abnormal, additional testing is performed to determine whether the failure to concentrate the urine is caused by diabetes insipidus that occurs as the result of a problem with the production of or the response of the kidney to ADH. The patient is injected with ADH, and specimens of serum and urine are collected in 2 and 4 hours. Normal test results indicate that the patient is not capable of producing ADH (neurogenic diabetes insipidus). If the test is abnormal, then the renal tubules are not responding to ADH (nephrogenic diabetes insipidus). See Figure 4-15.

Osmolality

As will be discussed in Chapter 5, osmolality measures only the number of particles in a solution, whereas specific gravity is influenced by both the number and **density** (molecular weight) of the particles. Renal concentration is concerned with small particles, primarily sodium and chloride molecules. Large-molecular-weight molecules, such as glucose and urea, do not contribute to the evaluation of renal concentration. Therefore, osmolality is performed for a more accurate evaluation of renal concentrating ability.

Freezing-Point Osmometers

Measurement of freezing-point depression was the first principle incorporated into clinical osmometers, and many instruments employing this technique are available now. These osmometers determine the freezing point of a solution by supercooling a measured amount of sample to approximately 27°C. The supercooled sample is vibrated to produce crystallization of water in the solution. The heat of fusion produced by the crystallizing water temporarily raises the temperature of the solution to its freezing point. A temperature-sensitive probe called a thermistor, in which resistance decreases as temperature increases, measures this temperature increase, which corresponds to the freezing point of the solution, and the information is converted into milliosmoles. Conversion is

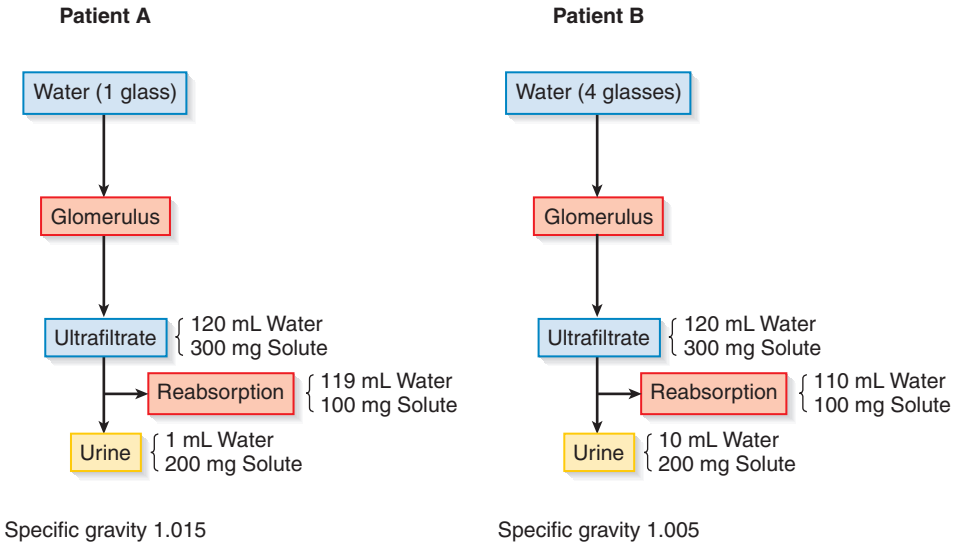

Figure 4–14 The effect of hydration on renal concentration. Notice the decreased specific gravity in the more-hydrated Patient B.

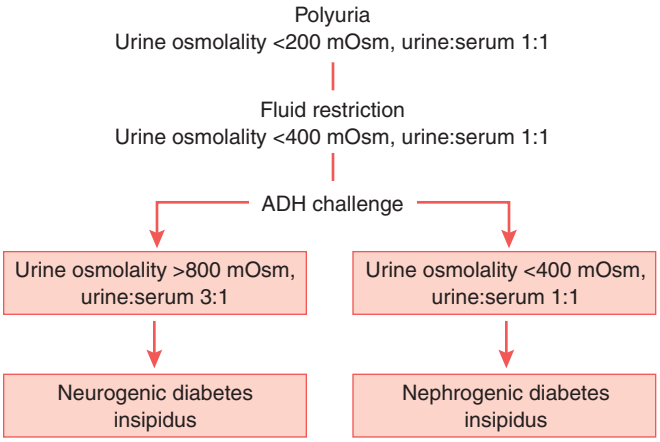

Figure 4–15 Differentiation of neurogenic and nephrogenic diabetes insipidus.

made possible by the fact that 1 mol (1000 mOsm) of a nonionizing substance dissolved in 1 kg of water is known to lower the freezing point 1.86°C. Therefore, by comparing the freezing-point depression of an unknown solution with that of a known molal solution, the **osmolarity** of the unknown solution can be calculated. Clinical osmometers use solutions of known NaCl concentration as their reference standards because a solution of partially ionized substances is more representative of urine and plasma composition.

Vapor Pressure Osmometers

The other instrument used in clinical osmometry is called the vapor pressure osmometer. The actual measurement performed, however, is that of the dew point (temperature at which water vapor condenses to a liquid). The depression of dew point temperature by solute parallels the decrease in vapor pressure, thereby providing a measure of this colligative property.

Samples are absorbed into small-filter paper disks that are placed in a sealed chamber containing a temperature-sensitive thermocoupler. The sample evaporates in the chamber, forming a vapor. When the temperature in the chamber is lowered, water condenses in the chamber and on the thermocoupler. The heat of condensation produced raises the temperature of the thermocoupler to the dew point temperature. This dew point temperature is proportional to the vapor pressure from the evaporating sample. Temperatures are compared with those of the NaCl standards and converted into milliosmoles. The vapor pressure osmometer uses microsamples of less than 0.01 mL; therefore, care must be taken to prevent any evaporation of the sample before testing. Correlation studies have shown more variation with vapor pressure osmometers, stressing the necessity of careful technique.

Technical Factors

Factors to consider because of their influence on true osmolarity readings include lipemic serum, lactic acid, and volatile substances, such as ethanol, in the specimen.

- In lipemic serum, the serum water displacement by insoluble lipids produces erroneous results with both vapor pressure and freezing-point osmometers.
- Values that are falsely elevated due to lactic acid formation also occur with both methods if serum specimens are not separated or refrigerated within 20 minutes.
- Vapor pressure osmometers do not detect the presence of volatile substances, such as alcohol, as they become part of the solvent phase; however, measurements performed on similar specimens using freezing-point osmometers will be elevated.

Clinical Significance

Major clinical uses of osmolarity include initially evaluating renal concentrating ability, monitoring the course of renal disease, monitoring fluid and electrolyte therapy, establishing the differential diagnosis of **hypernatremia** and **hyponatremia**, and evaluating the secretion of and renal response to ADH. These evaluations may require determination of serum in addition to urine osmolarity.

Reference serum osmolality values are from 275 to 300 mOsm. Reference values for urine osmolality are difficult to establish because factors, such as fluid intake and exercise, can greatly influence the urine concentration. Values can range from 50 to 1400 mOsm.[2] Determining the ratio of urine to serum osmolality can provide a more accurate evaluation. Under normal random conditions, the ratio of urine to serum osmolality should be at least 1:1; after controlled fluid intake, it should reach 3:1 (Fig. 4-15).

Technical Tip 4-3. Vapor pressure osmometers are used primarily to analyze serum and sweat microsamples for disorders not related to renal function, such as cystic fibrosis. They are used primarily in the chemistry department.

The ratio of urine to serum osmolality, in conjunction with procedures such as controlled fluid intake and injection of ADH, is used to differentiate whether diabetes insipidus is caused by decreased ADH production or inability of the renal tubules to respond to ADH. Failure to achieve a ratio of 3:1 after injecting ADH indicates that the collecting duct does not have functional ADH receptors. In contrast, if concentration does take place after ADH injection, an inability to produce adequate ADH is indicated. Tests to measure the ADH concentration in plasma and urine directly are available for difficult diagnostic cases.[11]

Free Water Clearance

The ratio of urine to serum osmolarity can be expanded further by performing the analyses using water deprivation and a timed urine specimen and then calculating the **free water clearance**. The free water clearance is determined by first calculating the **osmolar clearance** using the standard clearance formula:

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

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

EXAMPLE

Using a urine osmolality of 600 mOsm (U), a urine volume of 2 mL/min (V), and a plasma osmolality of 300 mOsm (P), calculate the free water clearance:

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

$$C_{H2O} = 2\ (V) - 4.0\ (C_{osm}) = -2.0\ (\text{free water clearance})$$

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. By comparing the osmolar clearance with the actual urine volume excreted per minute, it can be determined whether the water being excreted is more or less than the amount needed to maintain an osmolality that is the same as that of the ultrafiltrate.

The calculation in the previous Example shows a free water clearance of –2.0, indicating that less than the necessary amount of water is being excreted: a possible state of dehydration. If the value had been 0, no renal concentration or dilution would be taking place; likewise, if the value had been +2.0, excess water would have been excreted. The calculation of the free water clearance is used to determine the ability of the kidney to respond to the body's state of hydration.

Tubular Secretion and Renal Blood Flow Tests

Tests to measure tubular secretion of nonfiltered substances and renal blood flow are closely related in that total renal blood flow through the nephron must be measured by a substance that is secreted rather than filtered through the glomerulus. Impaired tubular secretory ability or inadequate presentation of the substance to the capillaries due to decreased renal blood flow may cause an abnormal result. Therefore, an understanding of the principles and limitations of the tests and correlation with other clinical data are important in test interpretation.

The test most commonly associated with tubular secretion and renal blood flow is the *p*-aminohippuric acid (**PAH**) test.

PAH Test

To measure the exact amount of blood flowing through the kidney, it is necessary to use a substance that is completely removed from the blood (plasma) each time it comes in contact with functional renal tissue. The principle is the same as in the clearance test for glomerular filtration. However, to ensure measurement of the blood flow through the entire nephron, the substance must be removed from the blood primarily in the peritubular capillaries rather than being removed when the blood reaches the glomerulus.

Although it has the disadvantage of being exogenous, the chemical PAH meets the criteria needed to measure renal blood flow. This nontoxic substance is loosely bound to plasma proteins, which permits its complete removal as the blood passes through the peritubular capillaries. Except for a small amount of PAH contained in plasma that does not come in contact with functional renal tissue, all the plasma PAH is secreted by the proximal convoluted tubule. Therefore, the volume of plasma flowing through the kidneys determines the amount of PAH excreted in the urine. The standard clearance formula can be used to calculate the effective renal plasma flow:

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

Based on normal hematocrit readings, reference values for the effective renal plasma flow range from 600 to 700 mL/min, making the average renal blood flow about 1200 mL/min. The actual measurement is renal plasma flow rather than renal blood flow, because the PAH is contained only in the plasma portion of the blood. Also, the term "effective" is included because approximately 8% of the renal blood flow does not come into contact with the functional renal tissue.

The amount of PAH infused by IV must be monitored carefully to ensure accurate results; therefore, usually the test is performed by specialized renal laboratories. Nuclear medicine procedures using radioactive hippurate can determine renal blood flow by measuring the plasma disappearance of a single radioactive injection and, at the same time, provide visualization of the blood flowing through the kidneys.[11]

Titratable Acidity and Urinary Ammonia

The ability of the kidney to produce an acid urine depends on the tubular secretion of hydrogen ions, as well as production and secretion of ammonia by the cells of the distal convoluted tubule. A typical person excretes approximately 70 mEq/day of acid in the form of titratable acid (H^+), hydrogen phosphate ions ($H_2PO_4^-$), or ammonium ions (NH_4^+). In typical people, a diurnal variation in urine acidity, consisting of alkaline tides, appears shortly after arising and postprandially at approximately 2 p.m. and 8 p.m. The lowest pH is found at night.

The inability to produce an acid urine in the presence of metabolic acidosis is called renal tubular acidosis. This condition may 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.

Urine pH, **titratable acidity**, and urinary ammonia measurements can be used to determine the defective function. The tests can be run simultaneously on either fresh urine specimens or those preserved with toluene and collected at 2-hour intervals from patients who have been primed with an acid load consisting of oral ammonium chloride. By titrating the amount of free H^+ (titratable acidity) and then the total acidity of the specimen, the ammonium concentration can be calculated as the difference between the titratable acidity and the total acidity.

HISTORICAL NOTE

Phenolsulfonphthalein Test

Historically, excretion of the dye phenolsulfonphthalein (PSP) was used to evaluate these functions. Standardization and interpretation of PSP results are difficult, however, 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. Therefore, the PSP test is not currently performed.



For additional resources please visit www.fadavis.com

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Study Questions

1. The type of nephron responsible for renal concentration is the:
   A. Cortical
   B. Juxtaglomerular
   C. Efferent
   D. Afferent
2. The function of the peritubular capillaries is:
   A. Reabsorption
   B. Filtration
   C. Secretion
   D. Both A and C
3. Blood flows through the nephron in the following order:
   A. Efferent arteriole, peritubular capillaries, vasa recta, afferent arteriole
   B. Peritubular capillaries, afferent arteriole, vasa recta, efferent arteriole
   C. Afferent arteriole, efferent arteriole, peritubular capillaries, vasa recta
   D. Efferent arteriole, vasa recta, peritubular capillaries, afferent arteriole
4. Filtration of protein is prevented in the glomerulus by:
   A. Hydrostatic pressure
   B. Oncotic pressure
   C. Renin
   D. The glomerular filtration barrier
5. The renin–angiotensin–aldosterone system is responsible for all of the following *except:*
   A. Vasoconstriction of the afferent arteriole
   B. Vasoconstriction of the efferent arteriole
   C. Reabsorbing sodium
   D. Releasing aldosterone
6. The primary chemical affected by the renin–angiotensin–aldosterone system is:
   A. Chloride
   B. Sodium
   C. Potassium
   D. Hydrogen

7. Secretion of renin is stimulated by:
- A. Juxtaglomerular cells
- B. Angiotensin I and II
- C. Macula densa cells
- D. Circulating angiotensin-converting enzyme

8. The hormone aldosterone is responsible for:
- A. Hydrogen ion secretion
- B. Potassium secretion
- C. Chloride retention
- D. Sodium retention

9. The fluid leaving the glomerulus has a specific gravity of:
- A. 1.005
- B. 1.010
- C. 1.015
- D. 1.020

10. For active transport to occur, a chemical must:
- A. Combine with a carrier protein to create electrochemical energy
- B. Be filtered through the proximal convoluted tubule
- C. Be in higher concentration in the filtrate than in the blood
- D. Be in higher concentration in the blood than in the filtrate

11. Which of the tubules is impermeable to water?
- A. Proximal convoluted tubule
- B. Descending loop of Henle
- C. Ascending loop of Henle
- D. Distal convoluted tubule

12. Glucose will appear in the urine when the:
- A. Blood level of glucose is 200 mg/dL
- B. T_m for glucose is reached
- C. Renal threshold for glucose is exceeded
- D. All of the above

13. Concentration of the tubular filtrate by the countercurrent mechanism depends on all of the following *except:*
- A. High salt concentration in the medulla
- B. Water-impermeable walls of the ascending loop of Henle
- C. Reabsorption of sodium and chloride from the ascending loop of Henle
- D. Reabsorption of water in the descending loop of Henle

14. ADH regulates the final urine concentration by controlling:
- A. Active reabsorption of sodium
- B. Tubular permeability
- C. Passive reabsorption of urea
- D. Passive reabsorption of chloride

15. Decreased production of ADH:
- A. Produces a low volume of urine
- B. Produces a high volume of urine
- C. Increases excretion of ammonia
- D. Affects active transport of sodium

16. Bicarbonate ions filtered by the glomerulus are returned to the blood:
- A. In the proximal convoluted tubule
- B. Combined with hydrogen ions
- C. By tubular secretion
- D. All of the above

17. If ammonia is not produced by the distal convoluted tubule, the urine pH will be:
- A. Acidic
- B. Basic
- C. Hypothenuric
- D. Hypersthenuric

18. Place the appropriate letter in front of the following clearance substances:
- A. Exogenous
- B. Endogenous

_____ $beta_2$-microglobulin
_____ creatinine
_____ cystatin C
_____ ^{125}I-iothalmate

19. The largest source of error in creatinine clearance tests is:
- A. Secretion of creatinine
- B. Improperly timed urine specimens
- C. Refrigeration of the urine
- D. Time of collecting blood specimen

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:
- A. Not filtered by the glomerulus
- B. Completely reabsorbed by the proximal convoluted tubule
- C. Secreted in the distal convoluted tubule
- D. Neither reabsorbed nor secreted by the tubules

22. Performing a clearance test using radionucleotides:
- A. Eliminates the need to collect urine
- B. Does not require an infusion
- C. Provides visualization of the filtration
- D. Both A and C

23. Variables that are included in the MDRD-IDSM estimated calculations of creatinine clearance include all of the following *except:*
- A. Serum creatinine
- B. Weight
- C. Age
- D. Gender

24. An advantage to using cystatin C to monitor GFR is that:
- A. It does not require urine collection
- B. It is not secreted by the tubules
- C. It can be measured by immunoassay
- D. All of the above

25. Solute dissolved in solvent will:
- A. Raise the vapor pressure
- B. Lower the boiling point
- C. Decrease the osmotic pressure
- D. Lower the freezing point

26. Substances that may interfere with freezing-point measurement of urine and serum osmolarity include all of the following *except:*
- A. Ethanol
- B. Lactic acid
- C. Sodium
- D. Lipids

27. Clinical osmometers use NaCl as a reference solution because:
- A. 1 g molecular weight of NaCl will lower the freezing point 1.86°C
- B. NaCl is readily frozen
- C. NaCl is partially ionized, similar to the composition of urine
- D. 1 g equivalent weight of NaCl will raise the freezing point 1.86°C

28. The normal serum osmolarity is:
- A. 50 to 100 mOsm
- B. 275 to 300 mOsm
- C. 400 to 500 mOsm
- D. 3 times the urine osmolarity

29. After controlled fluid intake, the urine-to-serum osmolarity ratio should be at least:
- A. 1:1
- B. 2:1
- C. 3:1
- D. 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

Case Studies and Clinical Situations

1. A 44-year-old man diagnosed with acute tubular necrosis has a blood urea nitrogen of 60 mg/dL and a blood glucose level of 100 mg/dL. A 2+ urine glucose is also reported.
   a. State the renal threshold for glucose.
   b. What is the significance of the positive urine glucose and normal blood glucose?
2. A patient develops a sudden drop in blood pressure.
   a. Diagram the reactions that take place to ensure adequate blood pressure within the nephrons.
   b. How do these reactions increase blood volume?
   c. When blood pressure returns to normal, how does the kidney respond?
3. A physician would like to prescribe a nephrotoxic antibiotic for a 60-year-old white man. The patient has a serum creatinine level of 1.5 mg/dL.
   a. How can the physician determine whether it is safe to prescribe this medication before the patient leaves the office?
   b. State two additional blood tests that the physician could use to continue monitoring this patient.
   c. If the patient has a history of prostate malignancy, would both of the methods listed in answer choice 3b provide reliable results? Explain your answer.
4. A laboratory is obtaining erratic serum osmolarity results on a patient who is being monitored at 6 a.m., 12 p.m., 6 p.m., and 12 a.m. Osmolarities are not performed on the night shift; therefore, the midnight specimen is run at the same time as the 6 a.m. specimen.
   a. What two reasons could account for these discrepancies?
   b. What substance is causing the erratic results?
   c. If a friend were secretly bringing the patient a pint of whiskey every night, would this affect the results? Explain your answer.
5. After overnight (6 p.m. to 8 a.m.) fluid deprivation, the urine-to-serum osmolarity ratio in a patient who is exhibiting polyuria and polydipsia is 1:1. The ratio remains the same when a second specimen is tested at 10 a.m. Then ADH is administered subcutaneously to the patient, and the fluid deprivation is continued until 2 p.m., when another specimen is tested.
   a. What disorder do these symptoms and initial laboratory results indicate?
   b. If the urine-to-serum osmolarity ratio on the 2 p.m. specimen is 3:1, what is the underlying cause of the patient's disorder?
   c. If the urine-to-serum osmolarity ratio on the 2 p.m. specimen remains 1:1, what is the underlying cause of the patient's disorder?

PART TWO

Urinalysis

Chapter 5: Physical Examination of Urine

Chapter 6: Chemical Examination of Urine

Chapter 7: Microscopic Examination of Urine

Chapter 8: Renal Disease

Chapter 9: Urine Screening for Metabolic Disorders

CHAPTER 5

Physical Examination of Urine

LEARNING OUTCOMES

Upon completing this chapter, the reader will be able to:

5-1 List the common terminology used to report normal urine color.

5-2 Discuss the relationship of urochrome to normal urine color.

5-3 State how the presence of bilirubin, biliverdin, uroerythrin, and urobilin in a specimen may be suspected.

5-4 Discuss the significance of cloudy red urine versus clear red urine.

5-5 Name two pathological causes of black or brown urine.

5-6 Discuss the significance of phenazopyridine in a specimen.

5-7 State the clinical significance of urine clarity.

5-8 List the common terminology used to report clarity.

5-9 Describe the appearance and discuss the significance of amorphous phosphates and amorphous urates in urine that was freshly voided.

5-10 List three pathological and four nonpathological causes of cloudy urine.

5-11 Define *specific gravity,* and tell why this measurement can be significant in the routine analysis.

5-12 Describe the principles of the refractometer, reagent strip, and osmolality for determining specific gravity.

5-13 Given the concentration of glucose and protein in a specimen, calculate the correction needed to compensate for these high-molecular-weight substances in the refractometer reading of specific gravity.

5-14 Name two nonpathogenic causes of abnormally high readings of specific gravity using a refractometer.

5-15 Describe the advantages of measuring specific gravity using a reagent strip and osmolality.

5-16 State possible causes of abnormal urine odor.

KEY TERMS

Clarity
Hypersthenuric
Hyposthenuric
Isosthenuric
Refractive index
Refractometry
Specific gravity
Urobilin
Urochrome
Uroerythrin

Introduction

The physical examination of urine includes the determination of the urine color, **clarity**, and **specific gravity**. As mentioned in Chapter 3, early physicians based many medical decisions on the color and clarity of urine. Today, observation of these characteristics provides preliminary information concerning disorders, such as glomerular bleeding, liver disease, inborn errors of metabolism, and urinary tract infection. Measurement of specific gravity aids in the evaluation of renal tubular function. The results of the physical portion of the urinalysis also can be used to confirm or to explain findings in the chemical and microscopic areas of urinalysis.

Color

The color of urine varies from almost colorless to black. These variations may be due to normal metabolic functions, physical activity, ingested materials, or pathological conditions. A noticeable change in urine color is often the reason that a patient seeks medical advice; then it becomes the responsibility of the laboratory to determine whether this color change is normal or pathological. The more common normal and pathological correlations of urine colors are summarized in Table 5-1.

Normal Urine Color

The terminology used to describe the color of normal urine may differ slightly among laboratories but should be consistent within each laboratory. Common descriptions include pale yellow, yellow, and dark yellow. See Figure 5-1 for examples of normal urine color. Care should be taken to examine the specimen under a good light source, looking down through the container against a white background. The yellow color of urine is caused by the presence of a pigment, which Thudichum named **urochrome** in 1864. Urochrome is a product of endogenous metabolism, and under normal conditions, the body produces it at a constant rate. The actual amount of

Table 5–1 Laboratory Correlation of Urine Color[1,4]

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
	Sulfasalazine (Azulfidine)	Anti-inflammatory drug
Yellow-green	Bilirubin oxidized to biliverdin	Colored foam in acidic urine and false-negative chemical test results for bilirubin
Green	*Pseudomonas* infection	Positive urine culture
	Asparagus	None
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
	Propofol	Anesthetic
	Familial hypercalcemia	"Blue diaper syndrome"
	Indomethacin (Indocin, Tivorbex)	Nonsteroidal anti-inflammatory drug

Table 5–1 Laboratory Correlation of Urine Color[1,4]—cont'd

Color	Cause	Clinical/Laboratory Correlations
Pink Red	RBCs	Cloudy urine with positive chemical test results for blood and RBCs visible microscopically
	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 people who are genetically susceptible
	Rifampin	Tuberculosis medication
	Menstrual contamination	Cloudy specimen with RBCs, mucus, and clots
Port wine	Porphyrins	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 Black	Homogentisic acid (alkaptonuria)	Seen in alkaline urine after standing; specific tests are available
	Malignant melanoma Melanin or melanogen	Urine darkens on standing and reacts with nitroprusside and ferric chloride
	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
	Chloroquine and primaquine	Antimalarial drugs
	Methocarbamol	Muscle relaxant
	Fava beans, rhubarb, or aloe	None

urochrome produced is dependent on the body's metabolic state, with increased amounts produced in patients with thyroid conditions and/or those in fasting states.[2] Urochrome also increases in urine that stands at room temperature.[3]

Because urochrome is excreted at a constant rate, the intensity of the yellow color in a fresh urine specimen can give a rough estimate of urine concentration. A dilute urine will be pale yellow, and a concentrated specimen will be dark yellow. Remember that due to variations in the body's state of hydration, these differences in the yellow color of urine can be normal.

Two additional pigments, **uroerythrin** and **urobilin**, are also present in the urine in much smaller quantities, and they contribute little to the color of normal, fresh urine. The presence of uroerythrin, a pink pigment, is most evident in specimens that have been refrigerated, resulting in the precipitation of amorphous urates in an acid urine. Uroerythrin attaches to the urates, giving a pink color to the sediment. Urobilin, an oxidation product of the normal urinary constituent urobilinogen, imparts an orange-brown color to urine that is not fresh.

Abnormal Urine Color

As seen in Table 5-1, abnormal urine colors are as numerous as their causes. Certain colors, however, are seen more frequently and have a greater clinical significance than do others.

Dark Yellow/Amber/Orange

Dark yellow or amber urine may not always signify a normal concentrated urine but can be caused by the presence of the abnormal pigment bilirubin. If bilirubin is present, it will be detected

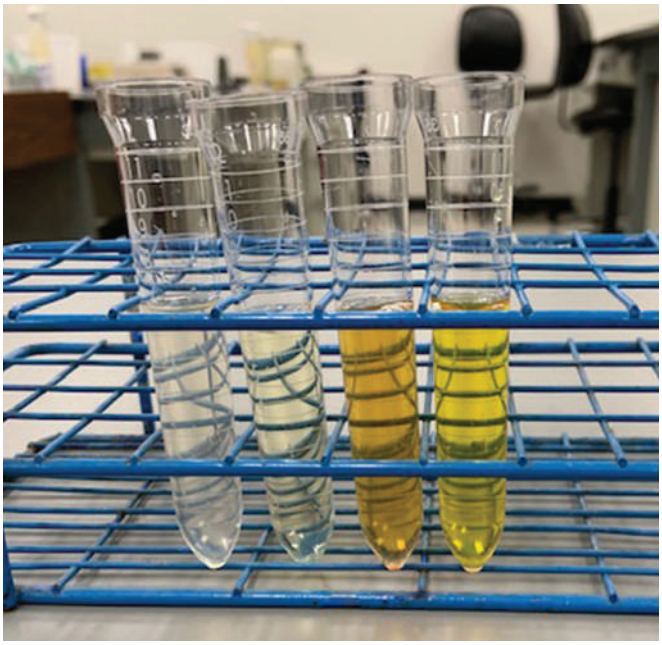


Figure 5–1 Examples of normal urine color ranging from light yellow to dark yellow.

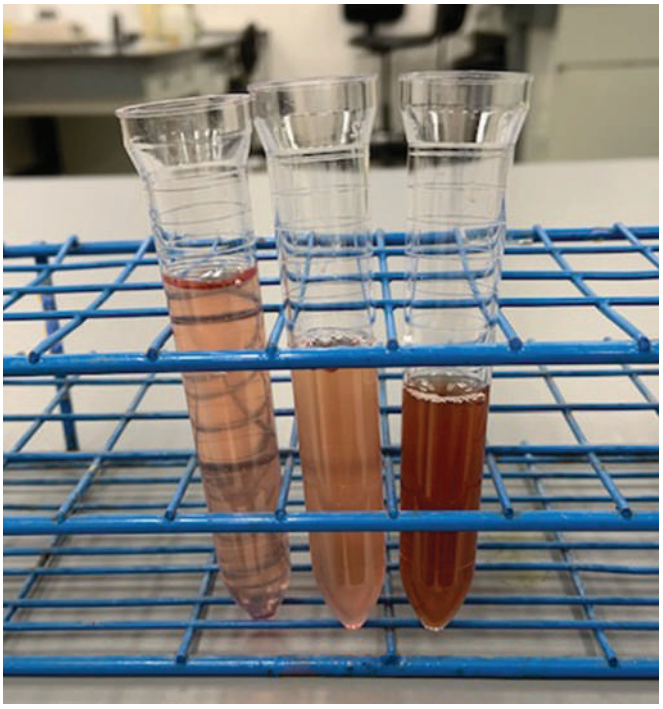


Figure 5–2 Examples of blood in the urine.

during the chemical examination; however, its presence is suspected if yellow foam appears when the specimen is shaken. Normal urine produces only a small amount of rapidly disappearing foam when shaken, and a large amount of white foam indicates an increased concentration of protein. A urine specimen that contains bilirubin also may contain hepatitis virus, reinforcing the need to follow standard precautions. The photo-oxidation of large amounts of excreted urobilinogen to urobilin also produces a yellow-orange urine; however, yellow foam does not appear when the specimen is shaken. Photo-oxidation of bilirubin imparts a yellow-green color to the urine caused by the presence of **biliverdin**.

Also frequently encountered in the urinalysis laboratory is the yellow-orange specimen caused by the administration of **phenazopyridine** (brand name Pyridium) or azo-gantrisin compounds to people who have urinary tract infections. This thick, orange pigment not only obscures the natural color of the specimen but also interferes with chemical tests that are based on color reactions. It is important to recognize the presence of phenazopyridine in a specimen so that laboratories can use alternative testing procedures. Specimens containing phenazopyridine produce a yellow foam when shaken, which could be mistaken for bilirubin.

Other medications that can cause an orange-colored urine include the anti-inflammatory drug sulfasalazine (Azulfidine), some laxatives, and certain chemotherapy drugs.[4]

Red/Pink/Brown

One of the most common causes of abnormal urine color is the presence of blood. Red is the usual color that blood produces in urine, but the color may range from pink to brown, depending on the amount of blood, the pH of the urine, and the length of contact (Fig. 5-2). Red blood cells (**RBCs**) remaining in an acidic urine for several hours cause the urine to turn brown due to the oxidation of hemoglobin to methemoglobin. A fresh urine containing blood that is brown also may indicate glomerular bleeding, resulting from the conversion of hemoglobin to methemoglobin.[5]

Besides RBCs, two other substances, hemoglobin and myoglobin, produce a red urine and result in a positive chemical test result for blood (Fig. 5-3). When intact RBCs (hematuria) are present, the urine is red and cloudy; however, if hemoglobin or myoglobin is present, the specimen is red and clear. Distinguishing between hemoglobinuria and myoglobinuria may be possible by examining the patient's plasma. Hemoglobinuria resulting from the in vivo breakdown of RBCs is accompanied by red plasma. Breakdown of skeletal muscle produces myoglobin. Myoglobin is cleared more rapidly from the plasma than is hemoglobin and therefore does not affect the color of the plasma. Fresh urine containing myoglobin frequently exhibits a more reddish-brown color than does urine containing hemoglobin. The possibility of hemoglobinuria being produced from the in vitro lysis of RBCs also must be considered. Chemical tests to distinguish between hemoglobin and myoglobin are available (see Chapter 6).

Urine specimens containing porphyrins also may appear red, resulting from the oxidation of **porphobilinogen** to **porphyrins**. They are often referred to as having the color of port wine.

Nonpathogenic causes of red urine include menstrual contamination or ingestion of highly pigmented foods and/or medications. In people who are genetically susceptible, eating fresh beets causes a red color in alkaline urine.[6] Ingestion of blackberries can produce a red color in acidic urine. Many medications,

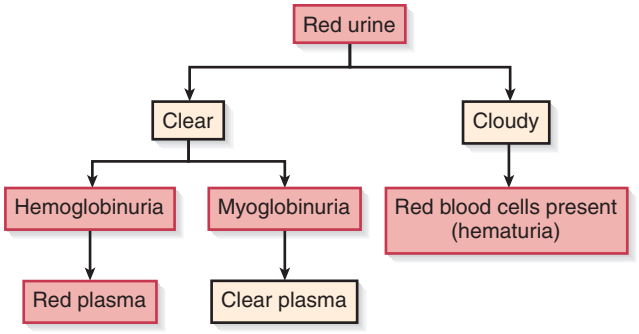


Figure 5–3 Differentiation of red urine testing chemically positive for blood.

including rifampin, phenolphthalein, phenindione, and phenothiazines, produce red urine.

Brown/Black

Additional testing is recommended for urine specimens that turn brown or black on standing and have negative chemical test results for blood, inasmuch as they may contain melanin or homogentisic acid. Melanin is an oxidation product of the colorless pigment melanogen, which is produced in excess when a malignant **melanoma** is present. Homogentisic acid, a metabolite of phenylalanine, imparts a black color to alkaline urine from patients with the inborn error of metabolism, called **alkaptonuria**. These conditions are discussed in Chapter 8.

Medications producing brown/black urines include antimalarial drugs (chloroquine and primaquine, levodopa, methyldopa, phenol derivatives, and the antibiotics metronidazole [Flagyl] and nitrofurantoin [Furadantin]); laxatives containing cascara or senna; and methocarbamol, a muscle relaxant.[4] Liver and kidney disorders and muscle injury from extreme exercise can result in a dark-brown urine.[4]

Nonpathogenic causes of a dark-brown (cola-colored) urine can include ingesting large amount of fava beans, rhubarb, or aloe.[4]

Blue/Green

Pathogenic causes of blue/green urine are limited to bacterial infections, including urinary tract infection by *Pseudomonas* species, and intestinal tract infections, resulting in increased urinary indican. A familial benign hypercalcemia, a rare inherited disorder, is sometimes called "blue diaper syndrome" because children with the disorder will have blue urine.[4]

Ingestion of breath deodorizers (Clorets) can result in a green urine.[7] Brightly colored food dyes, B vitamins, and asparagus also can cause a green urine.[4] The medications methocarbamol (Robaxin), methylene blue, indomethacin (Indocin, Tivorbex), amitriptyline (Elavil), and propofol (Diprivan) may cause blue urine.[4]

Observation of specimen collection bags from patients who are hospitalized frequently reveals urine that is abnormally colored. This may signify either a pathological condition that requires the urine to stand for a period of time before color development or the presence of medications. Phenol derivatives found in certain IV medications produce green urine on oxidation.[8] A purple staining may occur in catheter bags and is caused by indican in the urine or a bacterial infection, frequently caused by *Klebsiella* or *Providencia* species.[9]

Clarity

"Clarity" is a general term that refers to the transparency or turbidity of a urine specimen. In routine urinalysis, clarity is determined in the same manner that ancient physicians used: by visually examining the mixed specimen while holding it in front of a light source. The specimen should, of course, be in a clear container. Color and clarity are routinely determined at the same time. Common terminology used to report clarity includes clear, hazy, cloudy, turbid, and milky. As discussed in the previous section on urine color, terminology for clarity should be consistent within a laboratory. A description of urine clarity reporting is presented in Table 5-2.

Normal Clarity

Freshly voided, normal urine is usually clear, particularly if it is a clean-catch midstream specimen. Precipitation of amorphous phosphates and carbonates may cause a white cloudiness in an alkaline urine (Fig. 5-4).

Nonpathological Turbidity

The presence of squamous epithelial cells and mucus, particularly in specimens from women, can result in urine that is hazy but normal.

Specimens that are allowed to stand or that are refrigerated also may develop turbidity that is nonpathological. As discussed in Chapter 3, improper preservation of a specimen results in bacterial growth; this increases specimen turbidity but is not representative of the actual specimen.

Refrigerated specimens frequently develop a thick turbidity caused by the precipitation of amorphous phosphates, carbonates, and urates. Amorphous phosphates and carbonates produce a white precipitate in urine with an alkaline pH, whereas amorphous urates produce a precipitate in acidic urine that resembles pink brick dust due to the presence of uroerythrin.

Additional nonpathological causes of urine turbidity include mucus, normal urine crystals, semen, fecal contamination, radiographic contrast media, talcum powder, and vaginal creams (Box 5-1).

Table 5–2 Urine Clarity

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

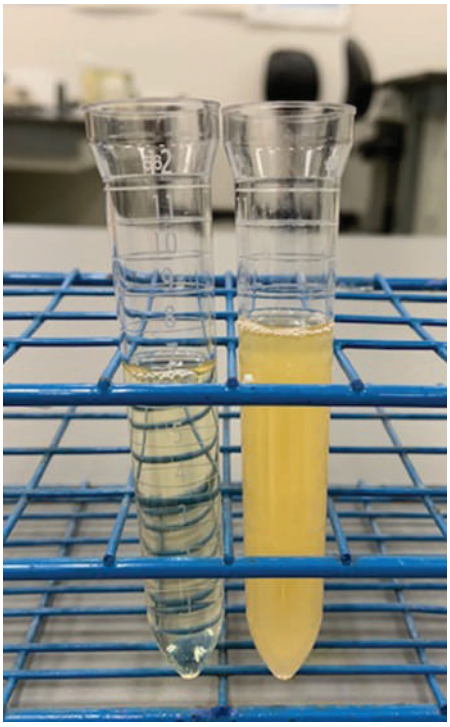


Figure 5–4 Examples of urine clarity. The urine in the left tube is clear. The urine in the right tube is turbid.

PROCEDURE 5-1

Urine Color and Clarity Procedure

Procedure

1. Evaluate an adequate volume of specimen.
2. Use a well-mixed specimen.
3. View the urine through a clear container.
4. View the urine against a white background using adequate room lighting.
5. Maintain adequate room lighting.
6. Evaluate a consistent volume of urine:
   - Determine the urine color.
   - Describe the urine clarity (Table 5-2).

Pathological Turbidity

The pathological causes of turbidity in a fresh specimen that are encountered most commonly are RBCs, white blood cells (WBCs), and bacteria caused by infection or a systemic organ disorder. Other causes of pathological turbidity that are encountered less frequently include abnormal amounts of nonsquamous epithelial cells, yeast, trichomonads, abnormal crystals, lymph fluid, and lipids (Box 5-2). See Figure 5-5 for examples of abnormal urine turbidity.

The clarity of a urine specimen certainly provides a key to the microscopic examination results because the amount of turbidity should correspond with the amount of material observed under the microscope.

Clear urine is not always normal. However, with the increased sensitivity of the routine chemical tests, most abnormalities in clear urine will be detected before the microscopic

Box 5–1 Nonpathological Causes of Urine Turbidity

- Squamous epithelial cells
- Mucus
- Amorphous phosphates, carbonates, urates
- Semen, spermatozoa
- Fecal contamination
- Radiographic contrast media
- Talcum powder
- Vaginal creams

Box 5–2 Pathological Causes of Urine Turbidity

- RBCs
- WBCs
- Bacteria
- Yeast
- Trichomonads
- Nonsquamous epithelial cells
- Abnormal crystals
- Lymph fluid
- Lipids

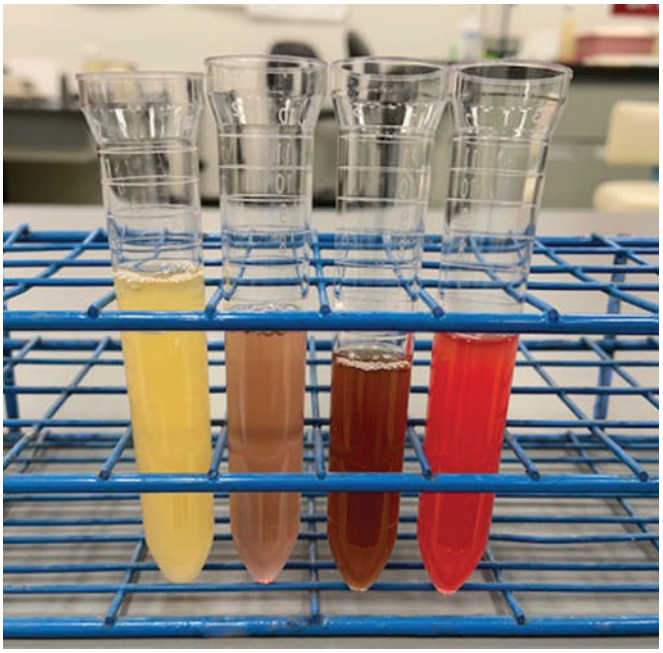


Figure 5–5 Examples of urine specimens with abnormal clarity and color.

analysis. Current criteria used to determine the necessity of performing a microscopic examination on all urine specimens include both clarity and chemical tests for RBCs, WBCs, bacteria, and protein.

Specific Gravity

As discussed in Chapter 4, the kidney's ability to concentrate the glomerular filtrate by selectively reabsorbing essential chemicals and water from the glomerular filtrate is one of the kidney's most important functions. The evaluation of urine concentration is included in the routine urinalysis by measuring the specific gravity of the specimen. Including specific gravity in the routine urinalysis provides an additional function, which is to determine whether specimen concentration is adequate to ensure the accuracy of chemical tests.

The specific gravity of the plasma filtrate entering the glomerulus is 1.010. The term **isosthenuric** is used to describe urine with a specific gravity of 1.010. Specimens below 1.010 are **hyposthenuric**, and those above 1.010 are **hypersthenuric**. One would expect urine that has been concentrated by the kidneys to be hypersthenuric, but this is not always true. Normal random specimens may range from approximately 1.002 to 1.035, depending on the patient's amount of hydration. Specimens measuring lower than 1.002 probably are not urine. Most random specimens fall between 1.015 and 1.030.

Specific gravity is defined as the density of a solution compared with the density of a similar volume of distilled water (SG 1.000) at a similar temperature. Because urine is actually water that contains dissolved chemicals, the specific gravity of urine is a measure of the density of the dissolved chemicals in the specimen. As a measure of specimen density, specific gravity is influenced not only by the number of particles present but also by their size. Therefore, large molecules contribute more to the reading than do small molecules. This may require the need to correct for the presence of substances that are not normally seen in urine, such as glucose and protein in the specimen. Currently the only method in use in routine urinalysis that requires correcting is the refractometer. The other two methods in use are chemical reagent strips and osmolality. The principles behind current specific gravity measurement techniques are presented in Table 5-3.

Table 5–3 Current Urine Specific Gravity Measurements

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

Refractometer

Refractometry determines the concentration of dissolved particles in a specimen by measuring refractive index. **Refractive index** is a comparison of the velocity of light in the 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. Clinical refractometers make use of these principles of light by using a prism to direct a specific (monochromatic) wavelength of daylight against a manufacturer-calibrated scale of specific gravity. The concentration of the specimen determines the angle at which the light beam enters the prism. Therefore, the specific gravity scale is calibrated in terms of the angles at which light passes through the specimen.

The refractometer provides the distinct advantage of determining specific gravity using a small volume of specimen (one or two drops). Temperature corrections are not necessary because the light beam passes through a temperature-compensating liquid before being directed at the specific gravity scale. Temperature is compensated between 15°C and 38°C. Corrections for glucose and protein must be calculated by subtracting 0.003 for each gram of protein present and 0.004 for each gram of glucose present. The amount of protein or glucose present can be determined from the chemical reagent strip tests.

When using the refractometer, a drop of urine is placed on the prism, the instrument is focused at a good light source, and the reading is taken directly from the specific gravity scale. The prism and its cover should be cleaned after each specimen is tested. Figure 5-6 illustrates the use of the refractometer.



Visit www.fadavis.com for Video 5-1 (Specific gravity via refractometer).

The refractometer is calibrated using distilled water that should give a reading of 1.000. If necessary, the instrument contains a zero setscrew to adjust the reading for distilled water (Fig. 5-7). The calibration is further checked using either 5% NaCl, which, as shown in the refractometer conversion tables, should read 1.022 ± 0.001, or 9% sucrose, which should read 1.034 ± 0.001. Urine control specimens representing low, medium, and high concentrations also should be run at the beginning of each shift. Calibration and control results are always recorded in the appropriate quality control records.

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 (protein) = 1.027 − 0.004 (glucose) = 1.023 corrected specific gravity

Results that are abnormally high—above 1.040—are seen in patients who have recently undergone an IV pyelogram. This is caused by the excretion of the injected radiographic contrast media. Patients who are receiving dextran or other high-molecular-weight IV fluids (plasma expanders) also produce urine with an abnormally high specific gravity. Once the foreign substance has been cleared from the body, the specific gravity returns to normal. In these circumstances, urine

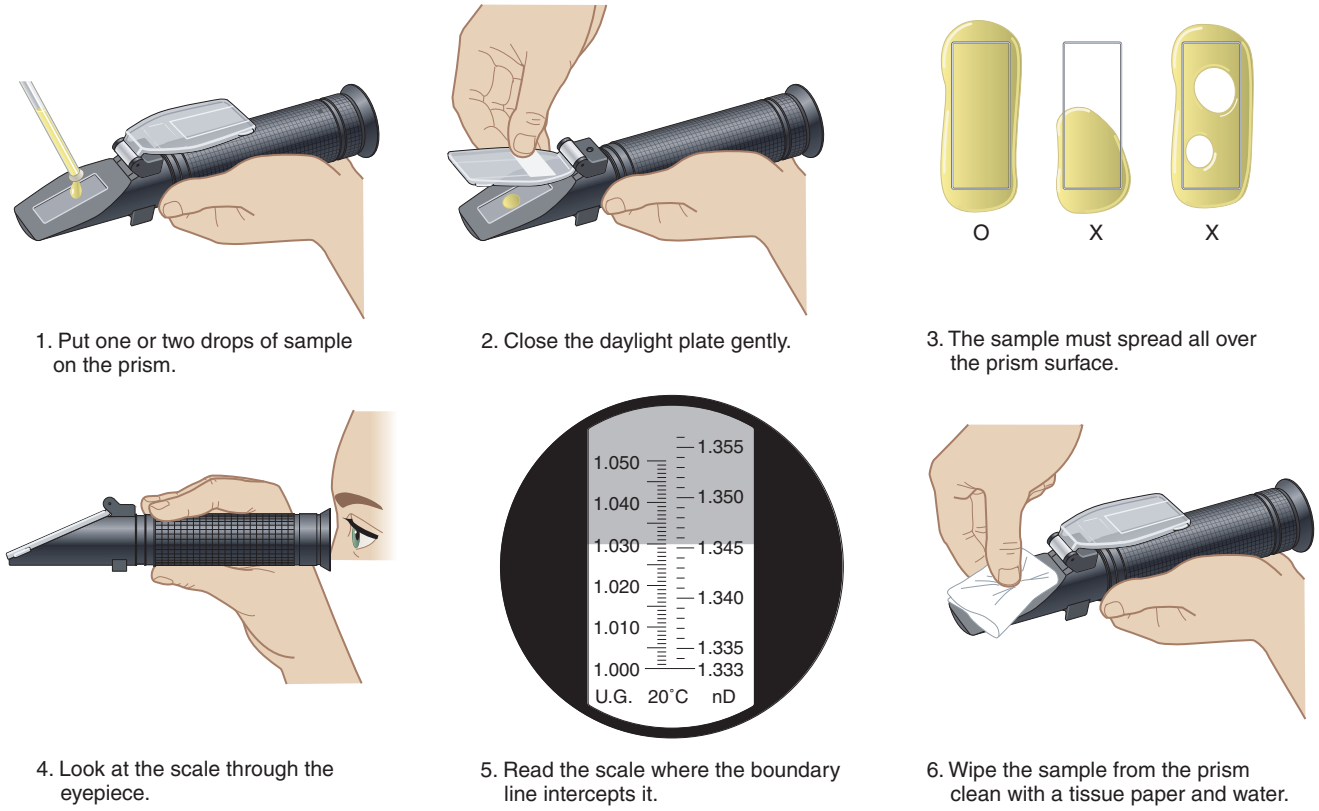


Figure 5–6 Steps in the use of the urine specific gravity refractometer. (Courtesy of NSG Precision Cells, Inc., 195G Central Ave., Farmingdale, NY, 11735.)

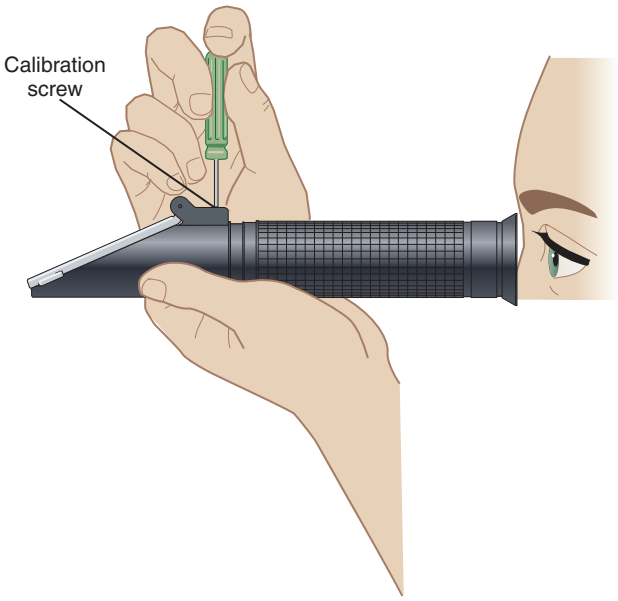


Figure 5–7 Calibration of the urine specific gravity refractometer. (Courtesy of NSG Precision Cells, Inc., 195G Central Ave., Farmingdale, NY, 11735.)

concentration can be measured using the reagent strip chemical test or osmometry because they are not affected by these high-molecular-weight substances.[10]

Osmolality

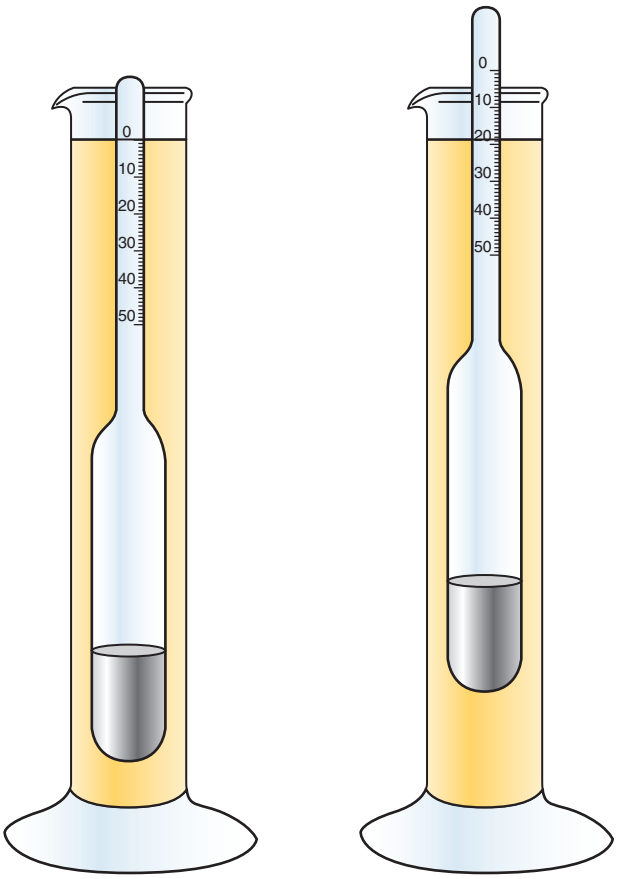
As stated previously, specific gravity depends on the number of particles present in a solution, as well as the density of these particles; in contrast, osmolality is affected only by the number of particles present. When evaluating renal concentration ability, the substances of interest are small molecules, primarily sodium (molecular weight 23) and chloride (molecular weight 35.5). However, urea (molecular weight 60), which is of no importance to this evaluation, will contribute more to the specific gravity than will the sodium and chloride molecules. Because all three molecules contribute equally to the osmolarity of the specimen, a more representative measure of renal concentrating ability can be obtained by measuring osmolarity (see Chapter 4).

An osmole is defined as 1 g molecular weight of a substance divided by the number of particles into which it dissociates. A 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. Just like molality and molarity, there are osmolality and osmolarity. An osmolal solution of glucose has 180 g of glucose dissolved in 1 kg of solvent. An osmolar solution of glucose has

HISTORICAL NOTE

Urinometry

The 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. The additional mass provided by the dissolved substances in urine causes the float to displace a volume of urine smaller than that of distilled water. The level to which the urinometer sinks, as shown in the figure, represents the specimen's mass or specific gravity.



Urinometry is less accurate than the other methods currently available and is not recommended by the Clinical and Laboratory Standards Institute (**CLSI**).[11]

180 g of glucose dissolved in 1 L of solvent. The unit of measure used in the clinical laboratory is the milliosmole (**mOsm**), because it is not practical when dealing with body fluids to use a measurement as large as the osmole (23 g of sodium per kilogram).

The osmolarity of a solution can be determined by measuring a property that is mathematically related to the number of particles in the solution (**colligative property**) and comparing this value with the value obtained from the pure solvent. Solute dissolved in solvent causes the following changes in colligative properties: lower freezing point, higher boiling point, increased osmotic pressure, and lower vapor pressure (see Table 5-4).

Because water is the solvent in urine, the number of particles present in a sample can be determined by comparing a colligative property value of the sample with that of pure water. To measure osmolality in the urinalysis laboratory requires special equipment referred to as an osmometer and, therefore, an additional step in the routine urinalysis procedure.

The A_2O Advanced Automated Osmometer (Advanced Instruments, Inc., Two Technology Way, Norwood, MA 02062) uses freezing-point depression to measure osmolality, providing a more automated method for measuring both urine and serum osmolality. (The principles and uses of the freezing-point and vapor pressure osmometers currently in use in the clinical laboratory are covered in Chapter 4.)

Technical Tip 5-1. The term "molality" is used most commonly because both the solute and the solvent are expressed in the same units of measure.

Technical Tip 5-2. Additional information on osmometry can be found at https://www.aicompanies.com/osmometers/a2o-advanced-automated-osmometer/ (accessed March 11, 2020). A video also can be accessed there.

Reagent Strip Specific Gravity

The addition of a specific gravity testing area to urinalysis chemical reagent strips has provided a convenient way to perform the routine urinalysis by eliminating the need for an additional procedure.

The reagent strip reaction is based on the change in pK_a (dissociation constant) of a polyelectrolyte in an alkaline medium. The polyelectrolyte ionizes, releasing hydrogen ions in proportion to the number of ions in the solution. The higher the concentration of urine, the more hydrogen ions are released, thereby lowering the pH. Incorporation of the indicator bromothymol blue on the reagent pad measures the

HISTORICAL NOTE

Harmonic Oscillation Densitometry

Harmonic oscillation densitometry is based on the principle that the frequency of a sound wave entering a solution changes in proportion to the density of the solution. This technique was originally used in early automated urinalysis instruments. The addition of reagent strip analysis for specific gravity has replaced this technique in automated systems.

Table 5–4 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

change in pH. As the specific gravity increases, the indicator changes from blue (1.000 [alkaline]), through shades of green, to yellow (1.030 [acid]). Readings can be made in 0.005 intervals by careful comparison with the color chart. A diagram of the specific gravity reaction is shown in Chapter 6, Figure 6-6.

Odor

Although it is seldom of clinical significance and is not a part of the routine urinalysis, urine odor is a noticeable physical property. Freshly voided urine has a faint aromatic odor. As the specimen stands, the odor of ammonia becomes more prominent. The breakdown of urea is responsible for the characteristic ammonia odor. Causes of unusual odors include bacterial infections, which cause a strong, unpleasant odor similar to ammonia, and diabetic ketones, which produce a sweet or fruity odor. A serious metabolic defect results in urine with a strong odor of maple syrup and is called, appropriately, maple syrup urine disease. This and other metabolic disorders with characteristic urine odors are discussed in Chapter 9. Ingestion of certain foods, including onions, garlic, and asparagus, can cause an unusual or pungent odor in urine. Studies have shown that although everyone who eats asparagus produces an odor, only certain people who are genetically predisposed can smell it.[12] Common causes of urine odors are summarized in Table 5-5.

Technical Tip 5-3. Because ions, such as Na^+, Cl^-, and NH_4^+ are important in evaluating renal concentrating ability, the reagent strip method provides additional information and is not affected by nonionizing substances, including urea, glucose, protein, and contaminating substances such as radiographic dye.

Table 5–5 Possible Causes of Urine Odor[1]

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



For additional resources please visit www.fadavis.com

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Study Questions

1. The concentration of a normal urine specimen can be estimated by which of the following?
   A. Color
   B. Clarity
   C. Foam
   D. Odor
2. The normal yellow color of urine is produced by:
   A. Bilirubin
   B. Hemoglobin
   C. Urobilinogen
   D. Urochrome
3. The presence of bilirubin in a urine specimen produces a:
   A. Yellow foam when shaken
   B. White foam when shaken
   C. Cloudy specimen
   D. Yellow-red specimen
4. A urine specimen containing melanin will appear:
   A. Pale pink
   B. Dark yellow
   C. 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 urine that is pale yellow?
   A. 1.005
   B. 1.010
   C. 1.020
   D. 1.030
11. A urine specific gravity measured by a 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 a 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 examination 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 point 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 1 liter of solvent
B. One gram molecular weight of solute dissolved in 1 kilogram of solvent
C. Two gram molecular weights of solute dissolved in 1 liter of solvent
D. Two gram molecular weights of solute dissolved in 1 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

Case Studies and Clinical Situations

1. Given the following physical urinalysis results, determine additional urinalysis results that may be abnormal.
   a. A green specimen with a strong foul odor of ammonia
   b. A pale yellow urine with a specific gravity of 1.030
   c. A dark yellow specimen with yellow foam
   d. A cloudy red urine
2. The urology clinic questions a urinalysis report from the laboratory.

   The laboratory report states a reagent strip reading of a specific gravity of 1.020, protein 3 g/dL, and glucose 2 g/dL. The specific gravity in the urology clinic was greater than 1.035.
   a. Correct the refractometer reading to account for the concentrations of protein and glucose. What is the corrected specific gravity?
   b. Do the specific gravities correlate?
   c. If the specific gravity was also checked using osmometry, should the result agree with the laboratory or the urology clinic results? Why or why not?
3. State two pathological causes of a clear red urine.
   a. State a method that could distinguish between the two causes that does not require laboratory testing.
4. A woman frequently shops at the farmer's market near her home. She notices her urine has a red color and brings a specimen to her physician. The specimen tests negative for blood.
   a. What is a probable cause of the woman's red urine?
   b. The woman collects a specimen at the physician's office. The color is yellow, and the pH is 5.5. Is this consistent with the previous answer? Why or why not?
5. Is a clear urine always normal? Explain your answer.

CHAPTER 6

Chemical Examination of Urine

LEARNING OUTCOMES

Upon completing this chapter, the reader will be able to:

6-1 Describe the proper technique for performing reagent strip testing.

6-2 List four causes of premature deterioration of reagent strips, and describe how to avoid them.

6-3 List five quality-control procedures routinely performed with reagent strip testing.

6-4 List the reasons for measuring urinary pH, and discuss their clinical applications.

6-5 Discuss the principle of pH testing by reagent strip.

6-6 Differentiate among prerenal, renal, and postrenal proteinuria, and give clinical examples of each.

6-7 Explain the "protein error of indicators," and list any sources of interference that may occur with this method of protein testing.

6-8 Discuss microalbuminuria, including significance, reagent strip tests, and their principles.

6-9 Explain why glucose that is normally reabsorbed in the proximal convoluted tubule may appear in the urine, and state the renal threshold levels for glucose.

6-10 Describe the principle of the glucose oxidase method of reagent strip testing for glucose, and name possible causes of interference with this method.

6-11 Describe the copper reduction method for the detection of urinary-reducing substances, and discuss the current use of this procedure.

6-12 Name the three "ketone bodies" appearing in urine and three causes of ketonuria.

6-13 Discuss the principle of the sodium nitroprusside reaction to detect ketones, including sensitivity and possible causes of interference.

6-14 Differentiate among hematuria, hemoglobinuria, and myoglobinuria with regard to the appearance of urine and serum, as well as the clinical significance of each.

6-15 Describe the chemical principle of the reagent strip method for blood testing, and list possible causes of interference.

6-16 Outline the steps in the degradation of hemoglobin to bilirubin, urobilinogen, and urobilin.

6-17 Describe the relationship of urinary bilirubin and urobilinogen to each of the following diagnoses: bile duct obstruction, liver disease, and hemolytic disorders.

6-18 Discuss the principle of the reagent strip test for urinary bilirubin, including possible sources of error.

6-19 State two reasons for increased urine urobilinogen and one reason for a decreased urine urobilinogen.

6-20 Discuss the principle of the nitrite reagent strip test for bacteriuria.

6-21 List five possible causes of a false-negative result in the reagent strip test for nitrite.

6-22 State the principle of the reagent strip test for leukocytes.

6-23 Discuss the advantages and sources of error of the reagent strip test for leukocytes.

6-24 Explain the principle of the chemical test for specific gravity.

6-25 Compare reagent strip testing for urine specific gravity with osmolality and refractometer testing.

6-26 Correlate physical and chemical urinalysis results.

KEY TERMS

Ascorbic acid
Bacteriuria
Bilirubin
Diazo reaction
Ehrlich reaction
Fanconi syndrome
Ferritin
Glucosuria
Glycosuria
Greiss reaction
Hematuria
Hemoglobinuria
Hemosiderin
Jaundice
Ketonuria
Leukocyturia
Microalbuminuria
Myoglobinuria
Orthostatic proteinuria
Postrenal proteinuria
Prerenal proteinuria
Protein error of indicators
Proteinuria
Renal proteinuria
Stercobilinogen
Urobilinogen
Uromodulin

Introduction

Routine chemical examination of urine has changed dramatically since the early days of urine testing due to the development of the reagent strip method for chemical analysis. Commercial reagent strips currently provide a simple, rapid means for performing medically significant chemical analysis of urine, including pH, protein, glucose, ketones, blood, **bilirubin**, **urobilinogen**, nitrite, leukocytes, and specific gravity. The two major types of reagent strips are manufactured under the trade names Multistix (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY) and Chemstrip (Roche Diagnostics, Indianapolis, IN). These products are available with single- or multiple-testing areas, and the brand and number of tests used are a matter of laboratory preference. Certain variations relating to chemical reactions, sensitivity, specificity, and interfering substances occur among the products and are discussed in the following sections. Reagent strip brands are also specified by instrumentation manufacturers.

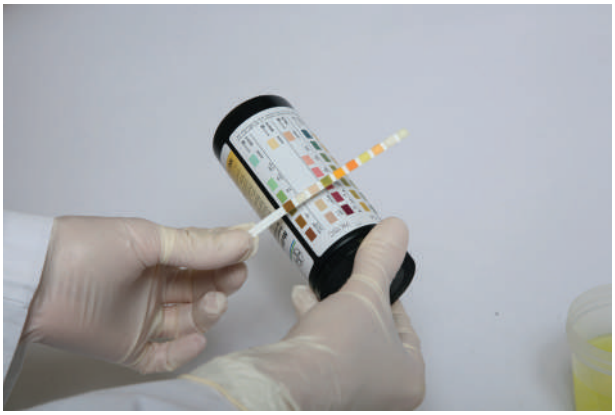


Figure 6–1 Chemical examination of urine comparing reagent strip color reactions using reagent strips consisting of chemical-impregnated absorbent pads attached to a plastic strip. (From Strasinger, SK, and DiLorenzo, MA: The Phlebotomy Textbook, 4th ed. FA Davis, Philadelphia, 2019.)

Reagent Strips

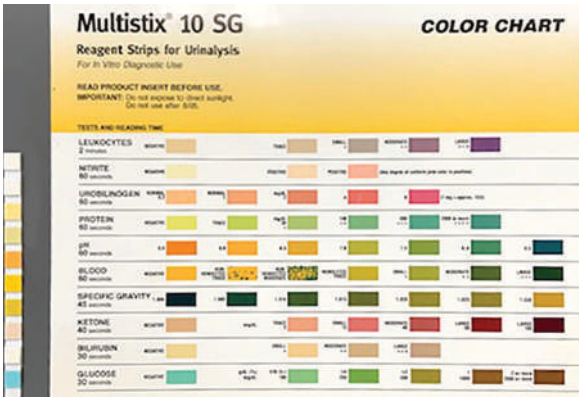
Chemical reagent strips consist of chemical-impregnated absorbent pads attached to a plastic strip (Fig. 6-1). A color-producing chemical reaction takes place when the absorbent pad comes in contact with urine. The reactions are interpreted by comparing the color produced on the pad within the required time frame with a chart supplied by the manufacturer (Fig. 6-2). Several colors or intensities of a color for each substance being tested appear on the chart. By careful comparison of the colors on the chart with the strip, a semiquantitative value of trace, 1+, 2+, 3+, or 4+ can be reported. An estimate of the milligrams per deciliter present is available for appropriate testing areas. Automated reagent strip readers also provide Système International (SI) units.

Reagent Strip Technique

The testing methodology includes dipping the reagent strip completely, but briefly, into a well-mixed specimen at room temperature, removing excess urine from the strip by running the edge of the strip on the container when withdrawing it from the specimen, blotting the strip horizontally on an absorbent medium, waiting the specified length of time for reactions to take place according to the manufacturer, and then comparing the colored reactions against the manufacturer's chart using a good light source.

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. Allowing excess urine to remain on the strip after its removal from the specimen can produce a runover between chemicals on adjacent pads, producing distortion of the colors. To ensure against runover, blotting the edge of the strip on absorbent paper and holding the strip horizontally while comparing it with the color chart is recommended.



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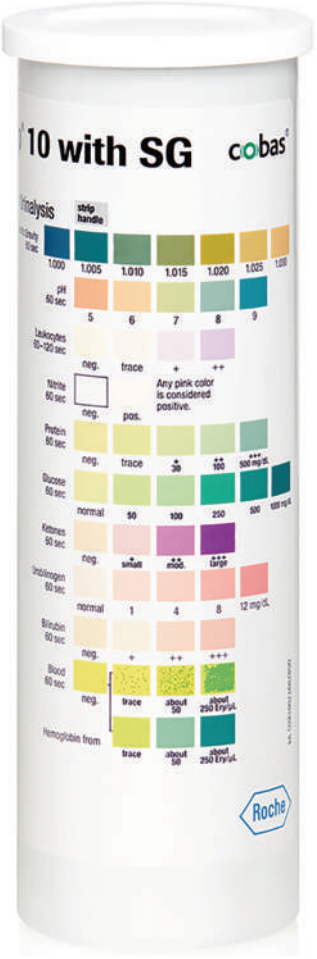

B

Figure 6–2 **(A)** Siemens Multistix 10 SG Reagent Strips Color Chart for Urinalysis. **(B)** Roche Chemstrip 10SG Reagent Strips Color Chart. (Courtesy of Roche Diagnostics Corporation.)

4. The timing for reactions to take place varies between tests and manufacturers and ranges from 30 seconds to 120 seconds for leukocyte esterase (LE). 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 LE 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. Manufacturers vary in the direction of the reagent strip to the color chart on the container when reading results. 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 (Chapter 2).
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 before reagent strip testing, as the enzymatic reactions on the strips are temperature dependent.

Handling and Storing Reagent Strips

In addition to using the correct testing technique, reagent strips must be protected from deterioration caused by moisture, volatile chemicals, heat, and light. Reagent strips are packaged in opaque containers with a desiccant to protect them from light and moisture. Strips are removed just before testing, and the bottle is tightly resealed immediately. Bottles should not be opened in the presence of volatile fumes. Manufacturers recommend that reagent strips be stored at room temperature below 30°C (but never refrigerated). All bottles are stamped with an expiration date that represents the functional life expectancy of the chemical pads. Reagent strips must not be used past the expiration date. Care must be taken not to touch the chemical pads when removing the strips. A visual inspection of the strip should be done each time a strip is used to detect deterioration, even though the strips may still be within the expiration date.

PROCEDURE 6-1

Reagent Strip Technique[1,2]

Visit www.fadavis.com for Video 6-1 (Chemical testing of urine).

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.
6. Read the results at the correct time, and record the results.

Quality Control of Reagent Strips

Reagent strips must be checked with both positive and negative controls according to the frequency established by the laboratory policy. Many laboratories perform this check at the beginning of each shift. Testing is also performed when a new bottle of reagent strips is opened, questionable results are obtained, or there is concern about the integrity of the strips. All quality control results must be recorded following laboratory protocol. Several companies manufacture both positive and negative controls. Distilled water is not recommended as a negative control because reagent strip chemical reactions are designed to perform at ionic concentrations similar to urine. All readings of the negative control must be negative, and positive control readings should agree with the published value. Results that do not agree with the published values must be resolved through the testing of additional strips and controls (see Chapter 1).

Demonstration of chemically acceptable reagent strips does not entirely rule out the possibility of inaccurate results. Interfering substances in the urine, technical carelessness, and color blindness also produce errors. Reagent strip manufacturers have published information concerning the limitations (e.g., interfering substances, sensitivities) of their chemical reactions, and laboratory personnel should be aware of these conditions. As mentioned in Chapter 5, a primary example of reagent strip interference is the masking of color reactions by the orange pigment present in the urine of people taking phenazopyridine compounds. If laboratory personnel do not recognize the presence of this pigment or other pigments, they will report many erroneous results.

Confirmatory Testing

Confirmatory tests are procedures using different reagents or methodologies to detect the same substances as detected by the reagent strips with the same or greater sensitivity or specificity.[3]

Nonreagent strip testing procedures using tablets and liquid chemicals may be available when questionable results are obtained or highly pigmented specimens are encountered. In the past, many of these procedures were used routinely to confirm positive results. Increased specificity and sensitivity of reagent strips and the use of automated strip readers have reduced the need for routine use of these procedures.[3] The chemical reliability of these procedures also must be checked using positive and negative controls.

Specific confirmatory tests are discussed in this chapter under their specific sections or the Historical Notes devoted to the chemical parameters for which they are used. The principles and procedures for these tests are included to provide additional information on the principles of the reagent strips and to provide the methodology to perform these tests if necessary. Facility protocol will determine the situations when they should be performed.

SUMMARY 6-1 Reagent Strip Testing

Care of Reagent Strips

1. Store with desiccant in an opaque, tightly closed container.
2. Store below 30°C; do not freeze.
3. Do not expose to volatile fumes.
4. Do not use past the expiration date.
5. Do not use if chemical pads become discolored.
6. Remove strips immediately before use.

Technique

1. Mix specimen well.
2. Let refrigerated specimens warm to room temperature before testing.
3. Dip the strip completely, but briefly, into the specimen.
4. Remove excess urine by withdrawing the strip against the rim of the container and by blotting the edge of the strip.
5. Compare reaction colors with the manufacturer's chart under a good light source at the specified time.
6. Perform confirmatory tests when indicated.
7. Be alert for the presence of interfering substances.
8. Understand the principles and significance of the test; read package inserts.
9. Relate chemical findings to each other and to the physical and microscopic urinalysis results.

Quality Control

1. Test open bottles of reagent strips with known positive and negative controls per facility protocol.
2. Resolve control results that are out of range by further testing.
3. Test reagents used in confirmatory tests with positive and negative controls.
4. Perform positive and negative controls on new reagents and newly opened bottles of reagent strips.
5. Record all control results and reagent lot numbers.

pH

Along with the lungs, the kidneys are the major regulators of the acid–base content in the body. They do this through the secretion of hydrogen in the form of ammonium ions, hydrogen phosphate, and weak organic acids and by the reabsorption of bicarbonate from the filtrate in the convoluted tubules (see Chapter 3). A healthy individual usually produces a first morning specimen with a slightly acidic pH of 5.0 to 6.0; a more alkaline pH is found after meals (alkaline tide). The pH of normal random specimens can range from 4.5 to 8.0. Consequently, no normal values are assigned to urinary pH, and it must be considered in conjunction with other patient information, such as the acid–base content of the blood, the patient's renal function, the presence of a urinary tract infection (**UTI**), the patient's dietary intake, and the age of the specimen (Table 6-1).

Table 6–1 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 (*Escherichia coli*)	Old specimens
High-protein diet	
Cranberry juice	
Medications (methenamine mandelate [Mandelamine], fosfomycin tromethamine [Monurol])	

Clinical Significance

The importance of urinary pH is primarily as an aid in 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. In respiratory or metabolic acidosis not related to renal function disorders, the urine is acidic; conversely, if respiratory or metabolic alkalosis is present, the urine is alkaline. Therefore, a urinary pH that does not conform to this pattern may be used to rule out the suspected condition, or, as discussed in Chapter 4, it may indicate a disorder resulting from the kidneys' inability to secrete or to reabsorb acid or base.

The precipitation of inorganic chemicals dissolved in the urine forms urinary crystals and renal calculi. This precipitation depends on urinary pH and can be controlled by maintaining the urine at a pH that is incompatible with the precipitation of the particular chemicals causing the calculi formation. For example, calcium oxalate, a frequent constituent of renal calculi, precipitates primarily in acidic and not alkaline urine. Therefore, maintaining urine at an alkaline pH discourages formation of the calculi. Knowledge of urinary pH is important in the identification of crystals observed during microscopic examination of the urine sediment. This will be discussed in detail in Chapter 7.

Maintaining an acidic urine can be valuable in treating UTIs caused by urea-splitting organisms because they do not multiply as readily in an acidic medium. These same organisms are also responsible for the highly alkaline pH found in specimens that have been allowed to sit unpreserved for extended periods. Urinary pH is controlled primarily by dietary regulation, although medications also may be used. People on high-protein and high-meat diets tend to produce acidic urine, whereas urine from vegetarians is more alkaline due to the formation of bicarbonate after digestion of many fruits and vegetables. An exception to the rule is in patients who consume cranberry juice or supplements, which produce an acidic urine and have long been used as a home remedy for minor bladder infections because they inhibit the colonization of certain urinary pathogens. People who are prone to frequent UTIs are often advised to drink cranberry juice or take over-the-counter cranberry pills. Medications prescribed for UTIs, such as methenamine mandelate (Mandelamine) and fosfomycin tromethamine (Monurol), are metabolized to produce an acidic urine.

The pH of freshly excreted urine does not reach above 8.5 in normal or abnormal conditions. A pH above 8.5 is associated with a specimen that has been preserved improperly and indicates that a fresh specimen should be obtained to ensure the validity of the analysis.

Technical Tip 6-1. Collecting specimens in containers other than the single-use laboratory-supplied containers can produce a pH above 8.5 if alkaline detergent remains in the container.

Reagent Strip Reactions

The Multistix and Chemstrip brands of reagent strips measure urine pH in 0.5- or 1-unit increments between pH 5 to 8.5 visually and pH 5 to 9 instrumentally.[2] To differentiate pH units throughout this wide range, both manufacturers use a double-indicator system of methyl red and bromothymol blue. Methyl red produces a color change from red to yellow in the pH range 4 to 6, and bromothymol blue turns from yellow to blue in the range of 6 to 9. Therefore, in the pH range 5 to 9 measured by the reagent strips, colors progress from orange at pH 5 through yellow and green to a final deep blue at pH 9.

$$\text{Methyl red} + H^+ \rightarrow \text{bromothymol blue} - H^+$$
$$(\text{Red-orange} \rightarrow \text{yellow})\ (\text{green} \rightarrow \text{blue})$$

No known substances interfere with urinary pH measurements performed by reagent strips. However, bacterial growth by certain organisms in a specimen may cause a marked alkaline shift, usually because of urea conversion to ammonia.[2]

Technical Tip 6-2. Care must be taken to prevent runover between the pH testing area and the adjacent, highly acidic protein testing area on Multistix, as this may produce a reading that is falsely acidic in an alkaline urine.

Protein

Of the routine chemical tests performed on urine, the most indicative of renal disease is the protein determination. Often **proteinuria** is associated with early renal disease, making the urinary protein test an important part of any physical examination. Normal urine contains very little protein: usually, less

SUMMARY 6-2 Clinical Significance of Urine pH

- Respiratory or metabolic acidosis/ketosis
- Respiratory or metabolic alkalosis
- Defects in renal tubular secretion and reabsorption of acids and bases—renal tubular acidosis
- Renal calculi formation and prevention
- Treatment of UTIs
- Precipitation/identification of crystals
- Determination of unsatisfactory specimens

SUMMARY 6-3 pH Reagent Strip

Reagents	Methyl red, bromothymol blue
Sensitivity	Multistix: 5.0–8.5 in 0.5 increments visually; 5.0–9 instrumentally
	Chemstrip: 5.0–9.0 in 1.0 increments
Sources of error/ interference	No known interfering substances
	Runover from adjacent pads
	Old specimens
	Bacterial growth
Correlations with other tests	Nitrite
	Leukocytes
	Microscopic

than 10 mg/dL or 100 mg per 24 hours is excreted. This protein consists primarily of low-molecular-weight serum proteins that have been filtered by the glomerulus, as well as proteins produced in the genitourinary tract. Due to its low molecular weight, albumin is the major serum protein found in normal urine. Even though it is present in high concentrations in the plasma, the normal albumin content in urine is low because the majority of albumin presented to the glomerulus is not filtered, and much of the filtered albumin is reabsorbed by the tubules. Other proteins include small amounts of serum and tubular microglobulins; **Tamm-Horsfall protein** (**THP**), also known as **uromodulin**, produced by the renal tubular epithelial cells; and proteins from prostatic, seminal, and vaginal secretions. Uromodulin is a more recent name for THP. Uromodulin, a glycoprotein, is produced routinely in the ascending loop of Henle.[4] As will be discussed in Chapter 7, uromodulin forms the matrix of casts.

Clinical Significance

Demonstration of proteinuria in a routine analysis does not always signify renal disease; however, its presence does require additional testing to determine whether the protein represents a condition that is normal or pathological. Clinical proteinuria is indicated at 30 mg/dL or greater (300 mg/L).[2,5] The causes of proteinuria are varied and can be grouped into three major categories: **prerenal**, renal, and **postrenal**, based on the origin of the protein.

Prerenal Proteinuria

As the name implies, prerenal proteinuria is caused by conditions affecting the plasma before it reaches the kidney and therefore is not indicative of actual renal disease. Often this condition is transient and caused by increased levels of low-molecular-weight plasma proteins, such as hemoglobin, myoglobin, and the **acute-phase reactants** associated with infection and inflammation. The increased filtration of these proteins exceeds the normal reabsorptive capacity of the renal tubules, resulting in an overflow of the proteins into the urine. Because reagent strips detect primarily albumin, prerenal proteinuria is usually not discovered in a routine urinalysis.

Bence Jones Protein

A primary example of proteinuria due to increased levels of serum protein is the excretion of Bence Jones protein by people with **multiple myeloma**, a proliferative disorder of the immunoglobulin-producing plasma cells. In multiple myeloma, the serum contains levels of monoclonal immunoglobulin light chains (Bence Jones protein) that are markedly elevated. This low-molecular-weight protein is filtered in quantities exceeding the tubular reabsorption capacity and therefore is excreted in the urine. Suspected cases of multiple myeloma must be diagnosed by performing serum electrophoresis and immunoelectrophoresis. The screening test for Bence Jones protein is not performed routinely, as cases of multiple myeloma are easily detected by chemical methods (see the Historical Note, Screening Test for Bence Jones Protein).

Renal Proteinuria

Proteinuria associated with true renal disease may be the result of damage to the glomerular membrane or tubular dysfunction.

Glomerular Proteinuria

When the glomerular membrane is damaged, selective filtration is impaired and increased amounts of serum protein and,

HISTORICAL NOTE

Screening Test for Bence Jones Protein

Unlike other proteins, which coagulate and remain coagulated when exposed to heat, Bence Jones protein coagulates at temperatures between 40°C and 60°C and dissolves when the temperature reaches 100°C. Therefore, a specimen that appears turbid between 40°C and 60°C and clear at 100°C can be suspected of containing Bence Jones protein. Interference due to other precipitated proteins can be removed by filtering the specimen at 100°C and then observing the specimen for turbidity as it cools to between 40°C and 60°C.

eventually, red and white blood cells pass through the membrane and are excreted in the urine. Conditions that present the glomerular membrane with abnormal substances (e.g., **amyloid material**, toxic substances, and immune complexes found in lupus erythematosus and streptococcal glomerulonephritis) are major causes of proteinuria due to glomerular damage.

Increased pressure from the blood entering the glomerulus may override the selective filtration of the glomerulus, causing increased albumin to enter the filtrate. This condition may be reversible, such as occurs during strenuous exercise and dehydration or is associated with hypertension. Proteinuria that occurs during the latter months of pregnancy may indicate a preeclamptic state and should be considered by the physician in conjunction with other clinical symptoms, such as hypertension, to determine whether this condition exists.

The discovery of protein, particularly in a random specimen, is not always of pathological significance because several benign causes of **renal proteinuria** exist. Benign proteinuria is usually transient and can be produced by conditions such as strenuous exercise, high fever, dehydration, and exposure to cold.

Microalbuminuria

The development of diabetic **nephropathy** leading to reduced glomerular filtration and eventual renal failure is a common occurrence in people with both type 1 and type 2 diabetes mellitus. Onset of renal complications can be predicted first by detection of **microalbuminuria** (albumin levels in the urine are 20 to 200 mg/L), and the progression of renal disease can be prevented through better stabilization of blood glucose levels and control of hypertension. The presence of microalbuminuria also is associated with an increased risk of cardiovascular disease.[6,7]

Orthostatic (Postural) Proteinuria

A persistent benign proteinuria occurs frequently in young adults and is termed **orthostatic proteinuria**, or postural proteinuria. It occurs after periods spent in a vertical posture and disappears when a horizontal position is assumed. Increased pressure on the renal vein when in the vertical position is believed to account for this condition. Patients suspected of orthostatic proteinuria are 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. Both specimens are tested for protein, and if orthostatic proteinuria is present, a negative reading will be seen on the first morning specimen, and a positive result will be found on the second specimen.

Tubular Proteinuria

Increased albumin is also present in disorders affecting tubular reabsorption because the albumin that is normally filtered can no longer be reabsorbed. Other low-molecular-weight proteins that are usually reabsorbed are also present. Causes of tubular dysfunction include exposure to toxic substances and heavy metals, severe viral infections, and **Fanconi syndrome**. The amount of protein that appears in the urine after glomerular damage ranges from slightly above normal to 4 g/day, whereas protein levels that are markedly elevated are seldom seen in tubular disorders.

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). Bacterial and fungal infections and inflammations produce exudates containing protein from the interstitial fluid. The presence of blood as the result of injury or menstrual contamination contributes protein, as does the presence of prostatic fluid and large amounts of spermatozoa.

HISTORICAL NOTE

Microalbuminuria Testing

Before the development of current reagent strip methods that are specific for albumin, detection of microalbuminuria required collection of a 24-hour urine specimen. Specimens were tested using quantitative procedures for albumin. Results were reported in mg of albumin/24 hours or as the albumin excretion rate (**AER**) in µg/min. With these methods, microalbumin was considered significant when 30 to 300 mg of albumin were excreted in 24 hours or the AER was 20 to 200 µg/min.

SUMMARY 6-4 Clinical Significance of Urine Protein

Prerenal	Tubular Disorders
Intravascular hemolysis	Fanconi syndrome
Muscle injury	Toxic agents/heavy metals
Acute-phase reactants	Severe viral infections
Multiple myeloma	
Renal	**Postrenal**
Glomerular disorders	Lower urinary tract infections/inflammation
Immune complex	Injury/trauma disorders
Amyloidosis	Menstrual contamination
Toxic agents	Prostatic fluid/spermatozoa
Diabetic nephropathy	Vaginal secretions
Strenuous exercise	
Dehydration	
Hypertension	
Preeclampsia	
Orthostatic or postural proteinuria	

Reagent Strip Reactions

Traditional reagent strip testing for protein uses the principle of the **protein error of indicators** to produce a visible colorimetric reaction. Contrary to the general belief that indicators produce specific colors in response to particular pH levels, certain indicators change color in the presence of protein even though the pH of the medium remains constant. This is because protein (primarily albumin) accepts hydrogen ions from the indicator. The test is more sensitive to albumin because albumin contains more amino groups to accept the hydrogen ions than other proteins. Depending on the manufacturer, the protein area of the strip contains either tetrabromophenol blue (Multistix) or 3′,3″,5′,5″-tetrachlorophenol, 3,4,5,6-tetrabromosulfonphthalein (Chemstrip) and an acid buffer to maintain the pH at a constant level. At a pH level of 3, both indicators appear yellow in the absence of protein; however, as the protein concentration increases, the color progresses through various shades of green and, finally, to blue. Readings are reported in terms of negative, trace, 1+, 2+, 3+, and 4+ or the semiquantitative values of 30, 100, 300, or 2000 mg/dL corresponding to each color change. Trace values are considered to be less than 30 mg/dL. Interpretation of trace readings can be difficult. Reporting of trace values may be a laboratory option.

$$\underset{\text{(Yellow)}}{\text{Indicator + protein}} \xrightarrow{\text{pH 3.0}} \underset{\text{(blue-green)}}{\text{protein + H}^+\text{ indicator – H}^+}$$

Reaction Interference

The major source of error with reagent strips occurs with highly buffered alkaline urine that overrides the acid buffer system, producing a rise in pH and a color change unrelated to protein concentration. Likewise, a technical error of allowing the reagent pad to remain in contact with the urine for a prolonged period may remove the buffer. False-positive readings are obtained when the reaction does not take place under acidic conditions. Highly pigmented urine and contamination of the container with quaternary ammonium compounds, detergents, and antiseptics also cause false-positive readings. A urine that is visibly bloody may cause results that are falsely elevated.[2] A false-positive trace reading may occur in specimens with a high specific gravity.

Sulfosalicylic Acid Precipitation Test

The sulfosalicylic acid (SSA) test is a cold precipitation test that reacts equally with all forms of protein. Various concentrations and amounts of SSA can be used to precipitate protein, and methods vary greatly among laboratories. All precipitation tests must be performed on centrifuged specimens to remove any extraneous contamination. Based on the protocol of the laboratory, an SSA test may be performed as a confirmatory test in certain situations. This test may not be relevant to current laboratory practice; however, the procedure is included in this section to serve as a reference if needed (Procedure 6-2).[3]

PROCEDURE 6-2

Sulfosalicylic Acid Precipitation Test

Procedure

1. Add 3 mL of 3% SSA reagent to 3 mL of centrifuged urine.
2. Mix by inversion, and observe for cloudiness.
3. Grade the degree of turbidity (see the following table).

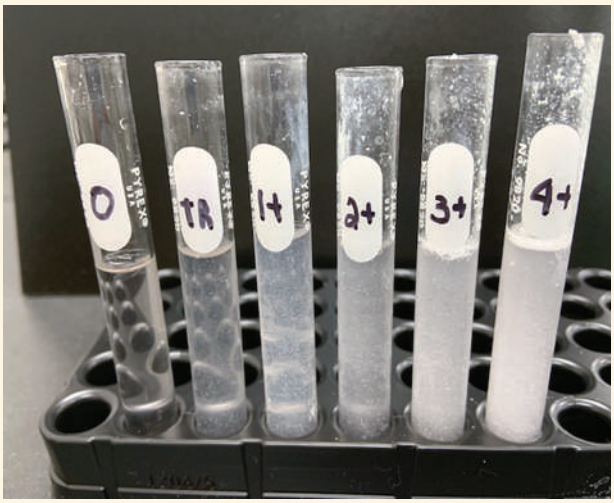

Table Reporting SSA Turbidity

Grade	Turbidity	Protein Range (mg/dL)
Negative	No increase in turbidity	Less than 6
Trace	Noticeable turbidity	6–30
1+	Distinct turbidity, no granulation	30–100
2+	Turbidity, granulation, no flocculation	100–200
3+	Turbidity, granulation, flocculation	200–400
4+	Clumps of protein	Greater than 400

Technical Tip 6-3. The specific gravity of the urine specimen should be considered in evaluating urine protein, because a trace protein in a dilute specimen is more significant than in a concentrated specimen.

Testing for Microalbuminuria

Several semiquantitative reagent strip methods have been developed so that patients at risk for renal disease can be monitored using random or first morning specimens. These methods

are based on immunochemical assays for albumin or albumin-specific reagent strips that also measure creatinine to produce an albumin:creatinine (A:C) ratio.

Immunochemical assays include the Micral-Test (Roche Diagnostics, Indianapolis, IN) and the ImmunoDip (Sekisui Diagnostics, Framingham, MA). Both reagent strips are read visually, and first morning specimens are recommended.

Micral-Test reagent strips contain a gold-labeled antihuman albumin antibody–enzyme conjugate. Strips are dipped into the urine up to a level marked on the strip and held for 5 seconds. Albumin in the urine binds to the antibody. The bound and unbound conjugates move up the strip by wicking action. Unbound conjugates are removed in a captive zone by combining with albumin embedded in the strip. The urine albumin–bound conjugates continue up the strip and reach an area containing enzyme substrate. The conjugated enzyme reacts with the substrate, producing colors ranging from white to red. The amount of color produced represents the amount of albumin present in the urine. The color is compared with a chart on the reagent strip bottle after 1 minute. Results range from 0 to 10 mg/dL.

The ImmunoDip reagent strip uses an immunochromographic technique. Strips are packaged individually in specially designed containers. The container is placed in the urine specimen for 3 minutes. A controlled amount of urine enters the container through a vent hole. The urine encounters blue latex particles coated with antihuman albumin antibody. Albumin in the urine binds with the coated particles. The bound and unbound particles continue to migrate up the strip. The migration is controlled by the size of the particles; unbound particles do not migrate as far as the bound particles. First a blue band is formed by the unbound particles. The bound particles continue to migrate and form a second blue band farther up the strip. Therefore, the top band represents the bound particles (urine albumin), and the bottom band represents unbound particles. The color intensity of the bands is compared against the manufacturer's color chart. A darker bottom band represents less than 1.2 mg/dL, equal band colors represent 1.2 to 1.8 mg/dL, and a darker top band represents 2.0 to 8.0 mg/dL of albumin. A darker bottom band is negative, equal band color is borderline, and a darker top band represents positive results.

Albumin:Creatinine Ratio

The Clinitek Microalbumin reagent strips and the Multistix Pro reagent strips (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY) provide simultaneous measurement of albumin/protein and creatinine that permits an estimation of the 24-hour microalbumin excretion.[8] As discussed in Chapter 4, creatinine is produced and excreted at a consistent rate for each individual. Therefore, by comparing the albumin excretion to the creatinine excretion, the albumin reading can be corrected for either overhydration or dehydration in a random specimen. In addition to including creatinine on the reagent strip, the 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.

SUMMARY 6-5 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:	**False positive**
	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
	False negative
	Proteins other than albumin
	Microalbuminuria
Correlations with other tests:	Blood
	Nitrite
	Leukocytes
	Microscopic

Reagent Strip Reactions

Albumin

Albumin reagent strips use the dye bis(3',3"-diiodo-4', 4"-dihydroxy-5',5"-dinitrophenyl)-3,4,5,6-tetrabromo sulphonphthalein (DIDNTB), which has a higher sensitivity and specificity for albumin. Whereas conventional protein reagent pads have a sensitivity of 30 mg/dL or greater and may include proteins other than albumin, the DIDNTB strips can measure albumin between 8 and 15 mg/dL (80 and 150 mg/L) without inclusion of other proteins. Reaction interference by highly buffered alkaline urine (always a concern with conventional reagent strips) is controlled by using paper treated with bis-(heptapropylene glycol) carbonate. The addition of polymethyl vinyl ether decreases the nonspecific binding of polyamino acids to the albumin pad. Colors range from pale green to aqua blue. Falsely elevated results can be caused by urine that is visibly bloody, whereas urine that is abnormally colored may interfere with the readings.[2]

Creatinine

The principle of the reagent strip for creatinine is based on the pseudoperoxidase activity of copper–creatinine complexes. The reaction follows the same principle as the reaction for blood on the reagent strips discussed later in this chapter. Reagent strips contain copper sulfate ($CuSO_4$), 3,3',5,5'-tetramethylbenzidine (TMB), and 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.[5]

$$CuSO_4 + CRE \rightarrow Cu(CRE)\ peroxidase$$

$$\underset{(peroxidase)}{DBDH} + \underset{(chromogen)}{TMB} \xrightarrow{Cu(CRE)\ peroxidase} \underset{(orange\ to\ blue)}{oxidized\ TMB} + H_2O$$

Results are reported as 10, 50, 100, 200, or 300 mg/dL or 0.9, 4.4, 8.8, 17.7, or 26.5 mmol/L of creatinine.

Reagent strips are unable to detect the absence of creatinine. Results that are falsely elevated can be caused by urine that is visibly bloody, as well as the presence of the gastric acid–reducing medication cimetidine (Tagamet). Urine that is abnormally colored also may interfere with the readings.

No creatinine readings are considered abnormal, as creatinine is normally present in concentrations of 10 to 300 mg/dL. The purpose of the creatinine measurement is to correlate the albumin concentration to the urine concentration, producing a semiquantitative albumin:creatinine ratio (A:C) ratio.

Albumin/Protein:Creatinine Ratio

Both automated and manual methods are available for determining the A:C ratio based on the reactions discussed previously. The Clinitek Microalbumin reagent strips are designed for instrumental use only. Strips are read on Clinitek Urine Chemistry Analyzers. The strips measure only albumin and creatinine, and the analyzer calculates the A:C ratio automatically. Results are displayed and printed out for albumin, creatinine, and the A:C ratio in both conventional and SI units. Abnormal results for the A:C ratio are 30 to 300 mg/g or 3.4 to 33.9 mg/mmol.[8]

The Siemens Multistix Pro 10 reagent strips include reagent pads for creatinine, protein-high, and protein-low (albumin), along with pads for glucose, ketones, blood, nitrite, LE, pH, bilirubin, and specific gravity. Urobilinogen is not included on these strips. The strips can be read manually or on automated Clinitek instruments. The protein-high reaction uses the protein error of indicators principle, whereas the protein-low reaction is the dye-binding method discussed previously. Results are reported as the protein:creatinine ratio, although the protein-low result is used in the calculation. Results from the Clinitek are calculated automatically. Results are reported as normal or abnormal. A result of normal dilute indicates that the specimen should be re-collected, making sure it is a first morning specimen.

When the reagent strip is read manually, a manufacturer-supplied chart is used to determine the ratio based on the results of the readings for protein-high, protein-low, and creatinine. When using this chart, the higher of the protein-low or protein-high result is used (Fig. 6-3).[9]

Glucose

Because of its value in the detection and monitoring of diabetes mellitus, the glucose test is the chemical analysis performed most frequently on urine. Due to the nonspecific symptoms associated with the onset of diabetes, it is estimated that more than half of the cases in the world are undiagnosed. Therefore, blood and urine glucose tests are included in all physical examinations and are often the focus of mass health screening programs. Early diagnosis of diabetes mellitus through blood and urine glucose tests provides a prognosis that is greatly improved. Using reagent strip methods for both blood and urine glucose testing that are currently available, patients can monitor themselves at home and can detect regulatory problems before the development of serious complications.

Clinical Significance

Under normal circumstances, almost all the glucose filtered by the glomerulus is actively reabsorbed in the proximal convoluted tubule; therefore, urine contains only minute amounts of glucose.

SUMMARY 6-6 Microalbumin Testing

Immunological Tests

	Micral-Test	ImmunoDip	Albumin:Creatinine Ratio Clinitest Microalbumin Strips/Multistix-Pro
Principle	Enzyme immunoassay	Immunochromographics	Sensitive albumin tests related to creatinine concentration to correct for patient hydration
Sensitivity	0–10 mg/dL	1.2–8.0 mg/dL	Albumin: 10–150 mg/L Creatinine: 10–300 mg/dL, 0.9–26.5 mmol/L
Reagents	Gold-labeled antibody B-galactosidase Chlorophenol red galactoside	Antibody-coated blue latex particles	Albumin: dye DIDNTB Creatinine: $CuSO_4$, 3,3′,5,5′-TMB, and DBDH
Interference	False negative: Dilute urine	False negative: Dilute urine	Visibly bloody or abnormally colored urine Creatinine: cimetidine: False positive

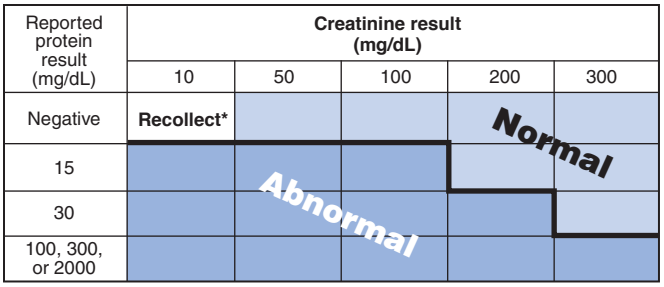

Figure 6–3 A protein-creatinine ratio determination chart. (Image adapted from Bayer HealthCare LLC, Elkhart, IN.)

Tubular reabsorption of glucose is by active transport in response to the body's need to maintain an adequate concentration of glucose. Should the blood level of glucose become elevated (**hyperglycemia**), as occurs in diabetes mellitus, the tubular transport of glucose has reached its renal threshold, and glucose appears in the urine. The blood level at which tubular reabsorption stops (renal threshold) for glucose is approximately 160 to 180 mg/dL. Blood glucose levels fluctuate, and a typical person who is nonfasting may have **glycosuria** after a meal containing a high glucose content. Therefore, the most informative glucose results are obtained from specimens collected under controlled conditions. Fasting before the collection of specimens for screening tests is recommended. For purposes of diabetes monitoring, specimens are usually tested 2 hours after meals. A first morning specimen does not always represent a fasting specimen because glucose from an evening meal may remain in the bladder overnight, and patients should be advised to empty the bladder and collect the second specimen.[2]

Hyperglycemia that occurs during pregnancy and disappears after delivery is called gestational diabetes. The onset of hyperglycemia and glycosuria is normally around the sixth month of pregnancy, although glycosuria may occur sooner. Hormones secreted by the placenta block the action of insulin, resulting in insulin resistance and hyperglycemia. Detection of gestational diabetes is important to the welfare of both the mother and baby because glucose crosses the placenta, whereas insulin does not. The baby develops high glucose levels, causing the baby's pancreas to produce more insulin. The excess glucose transferred to the baby is stored as fat, resulting in a large baby (macrosomia) at risk for obesity and, later, type 2 diabetes. Women who have gestational diabetes also are prone to developing type 2 diabetes mellitus in later years.

Hyperglycemia of nondiabetic origin is seen in a variety of disorders and also produces glycosuria. Many of these disorders are associated with hormonal function and include pancreatitis, acromegaly, Cushing syndrome, hyperthyroidism, pheochromocytoma, and thyrotoxicosis. The hormones glucagon, epinephrine, cortisol, thyroxine, and growth hormone, which are increased in these disorders, work in opposition to insulin, thereby producing hyperglycemia and **glucosuria**. Whereas a primary function of insulin is to convert glucose to glycogen for storage (**glycogenesis**), these opposing hormones cause the breakdown of glycogen to glucose (**glycogenolysis**), resulting in increased levels of circulating glucose. Epinephrine is also a strong inhibitor of insulin secretion and is increased when the body is subjected to severe stress, which accounts for the glucosuria seen in conjunction with cerebrovascular trauma and myocardial infarction.

Glycosuria occurs in the absence of hyperglycemia when the reabsorption of glucose by the renal tubules is compromised. This is frequently referred to as "renal glycosuria" and is seen in end-stage renal disease, cystinosis, and Fanconi syndrome. Glycosuria not associated with gestational diabetes is seen occasionally as a result of a temporary lowering of the renal threshold for glucose during pregnancy.

Reagent Strip (Glucose Oxidase) Reaction

The glucose oxidase procedure provides a specific test for glucose. Reagent strips employ the glucose oxidase testing method by 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.

$$1.\ \text{Glucose} + O_2\ (\text{air}) \xrightarrow{\text{Glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$2.\ H_2O_2 + \text{chromogen} \xrightarrow{\text{Peroxidase}} \text{oxidized colored chromogen} + H_2O$$

Reagent strip manufacturers use several different chromogens, including potassium iodide (green to brown) (Multistix) and tetramethylbenzidine (yellow to green) (Chemstrip). Urine glucose may be reported in terms of negative, trace, 1+, 2+, 3+, and 4+; however, the color charts also provide semiquantitative measurements ranging from 100 mg/dL to 2 g/dL or 0.1% to 2%. The American Diabetes Association recommends semiquantitative reporting.

SUMMARY 6-7 Clinical Significance of Urine Glucose

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

Reaction Interference

Because the glucose oxidase method is specific for glucose, false-positive reactions are not obtained from other urinary constituents, including reducing sugars that may be present. False-positive reactions may occur, however, if containers become contaminated with peroxide or strong oxidizing detergents from disinfectants used on laboratory instruments.

Substances that interfere with the enzymatic reaction or strong reducing agents, such as **ascorbic acid**, that prevent oxidation of the chromogen may produce false-negative results. To minimize interference from ascorbic acid, reagent strip manufacturers are incorporating additional chemicals into the test pads. An example is iodate that oxidizes ascorbic acid so that it cannot interfere with the oxidation of the chromogen. Product literature should be reviewed carefully for current information regarding all interfering substances. High levels of ketones also affect glucose oxidase tests at low glucose concentrations; however, because high levels of ketones are usually accompanied by marked glycosuria, this seldom presents a problem. High specific gravity and low temperature may decrease the sensitivity of the test. By far, the greatest source of false-negative glucose results is the technical error of allowing specimens to remain unpreserved at room temperature for extended periods, subjecting the glucose to bacterial degradation.

Copper Reduction Test (Clinitest)

Measurement of glucose by the copper reduction method was one of the earliest chemical tests performed on urine. The test 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.

$$CuSO_4 \text{ (cupric sulfide)} + \text{reducing substance} \xrightarrow[\text{Alkali}]{\text{Heat}}$$

$$Cu_2O \text{ (cuprous oxide)} + \text{oxidized substance} \rightarrow \text{color (blue/green} \rightarrow \text{orange/red)}$$

The classic Benedict solution was developed in 1908 and contained copper sulfate, sodium carbonate, and sodium citrate buffer.[10] Urine was added to the solution, heat was applied, and the resulting precipitate was observed for color. A more convenient method that employs Benedict's principle is the Clinitest tablet (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY). The tablets contain copper sulfate, sodium carbonate, sodium citrate, and sodium hydroxide. Upon addition of the tablet to water and urine, heat is produced by the hydrolysis of sodium hydroxide and its reaction with sodium citrate, and carbon dioxide is released from the sodium carbonate to prevent room air from interfering with the reduction reaction. Thick-walled tubes should be placed in a heat-resistant rack and not held in the hand because the reaction heat could cause a burn. At the conclusion of the effervescent reaction, the tube is shaken gently, and the color, ranging from blue to orange/red, can be compared with the manufacturer's color chart to determine the approximate amount of reducing substance.

Care must be taken to observe the reaction closely as it is taking place because at high glucose levels, a phenomenon known as "pass through" may occur. When this happens, the color produced passes through the orange/red stage and returns to a green-brown color, and if not observed, a high glucose level may be reported as negative. An alternative method using two drops instead of five drops of urine can minimize the occurrence of "pass through." A separate color chart must be used to interpret the reaction. This chart provides values up to 5 g/dL, whereas the five-drop method is limited to 2 g/dL.

The sensitivity of Clinitest to glucose is reduced to a minimum of 200 mg/dL, so the Clinitest cannot be used as a confirmatory test for glucose. As a nonspecific test for reducing substances, Clinitest is subject to interference from other reducing sugars, including galactose, lactose, fructose, maltose, pentose, ascorbic acid, certain drug metabolites, and antibiotics, such as the cephalosporins.

Clinitest tablets are very hygroscopic and should be stored in their original, tightly closed packages. A strong blue color in the unused tablets suggests deterioration due to moisture accumulation, as does vigorous tablet fizzing.

SUMMARY 6-8 Glucose Reagent Strip

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

Clinical Significance of Clinitest

In addition to glucose, commonly found reducing sugars include galactose, fructose, pentose, and lactose, of which galactose is the most clinically significant. Galactose in the urine of a newborn represents an "inborn error of metabolism" in which a lack of the enzyme galactose-1-phosphate uridyl transferase (**GALT**) prevents breakdown of ingested galactose and results in failure to thrive and other complications, including death. Depending on the laboratory population, the Clinitest was performed on all pediatric specimens from patients up to at least the age of 2 years. However, now all states have incorporated screening for galactosemia into their required newborn screening programs (see Chapter 9) because early detection followed by dietary restriction can control the condition. Because of the increased sensitivity and specificity of the newborn blood screening test for the activity of GALT, the urine screening for carbohydrate metabolism disorders using the Clinitest reagent tablets is no longer recommended.[11] The appearance of other reducing sugars is usually of minimal clinical significance, and lactose is frequently found in the urine of nursing mothers.

Technical Tip 6-4. Keep in mind that table sugar is sucrose, a nonreducing sugar, which does not react with Clinitest or glucose oxidase strips. Therefore, it cannot be used as a control or in preparation of a laboratory exercise for glucose testing.

PROCEDURE 6-3

Clinitest Procedure

Procedure

1. Place a thick glass test tube in a rack; add five drops of urine.
2. Add 10 drops of distilled water to the urine in the test tube.
3. Drop one Clinitest tablet into the test tube, and observe the reaction until completion (cessation of boiling).

   CAUTION: The reaction mixture gets very hot. Do not touch the bottom area of the test tube. Use a thick glass test tube only.
4. Wait 15 seconds after boiling has stopped, and gently shake the contents of the tube.
5. Compare the color of the mixture to the Clinitest color chart, and record the result in mg/dL or percent. Observe for the possibility of the "pass through" phenomenon. If present, repeat the procedure using two drops of urine instead of five drops.

Ketones

The term "ketones" represents three intermediate products of fat metabolism, namely, acetone (2%), acetoacetic acid (20%), and β-hydroxybutyrate (78%). Normally, measurable amounts of ketones do not appear in the urine because all the metabolized fat is completely broken down into carbon dioxide and water. However, when the use of available carbohydrate as the major source of energy becomes compromised, body stores of fat must be metabolized to supply energy. Then ketones are detected in urine.

Clinical Significance

Clinical reasons for increased fat metabolism include the inability to metabolize carbohydrate, as occurs in diabetes mellitus; increased loss of carbohydrate from vomiting; and inadequate intake of carbohydrate associated with starvation and malabsorption.

Testing for urinary ketones is most valuable in the management and monitoring of insulin-dependent (type 1) diabetes mellitus. **Ketonuria** shows a deficiency in insulin, indicating the need to regulate dosage. It is often an early indicator of insufficient insulin dosage in type 1 diabetes and in patients who experience medical problems in addition to diabetes. Increased accumulation of ketones in the blood leads to electrolyte imbalance, dehydration, and, if not corrected, acidosis and eventual diabetic coma.

The use of multiple-test strips in hospital laboratories often produces positive ketone tests unrelated to diabetes because the patient's illness either prevents adequate intake or absorption of carbohydrates or produces an accelerated loss, as in the case of vomiting. Clinics for weight loss and eating disorders can use a practical application of ketonuria produced by avoidance of carbohydrates to monitor patients. Frequent strenuous exercise can cause overuse of available carbohydrates and produce ketonuria that can be toxic to the kidney tubules.

SUMMARY 6-9 Clinical Significance of Urine Ketones

- Diabetic acidosis
- Insulin dosage monitoring
- Starvation
- Malabsorption/pancreatic disorders
- Cold exposure
- Strenuous exercise
- Vomiting
- Inborn errors of amino acid metabolism (see Chapter 9)
- Alcoholism
- Febrile state in children

Reagent Strip Reactions

The three ketone compounds are not present in equal amounts in urine. Both acetone and β-hydroxybutyric acid are produced from acetoacetic acid (Fig. 6-4). The proportions of 78% β-hydroxybutyric acid, 20% acetoacetic acid, and 2% acetone are relatively constant in all specimens.

Reagent strip tests use the sodium nitroprusside (nitroferricyanide) reaction to measure ketones. In this reaction, acetoacetic acid in an alkaline medium reacts with sodium nitroprusside to produce a purple color. The test does not measure β-hydroxybutyrate and is only slightly sensitive to acetone when glycine is also present; however, inasmuch as these compounds are derived from acetoacetic acid, their presence can be assumed, and it is not necessary to perform individual tests. Results are reported qualitatively as negative, trace, small (1+), moderate (2+), or large (3+), or semiquantitatively as negative, trace (5 mg/dL), small (15 mg/dL), moderate (40 mg/dL), or large (80 to 160 mg/dL).

Acetoacetate (and acetone) + sodium nitroprusside

$$\text{+ (glycine)} \xrightarrow{\text{alkaline}} \text{purple color}$$

Reaction Interference

Large amounts of levodopa and medications containing sulfhydryl groups, including mercaptoethane sulfonate sodium (**MESNA**) and captopril, may produce atypical color reactions. Reactions with interfering substances frequently fade on standing, whereas color development from acetoacetic acid increases, which leads to false-positive results from readings that are improperly timed. Values that are falsely decreased due to the volatilization of acetone and the breakdown of acetoacetic acid by bacteria are seen in specimens that have been preserved improperly.

The Acetest tablet test has been used as a confirmatory test for questionable reagent strip results; however, it was primarily used for testing serum and other bodily fluids and dilutions of these fluids for severe ketosis. The Clinical and Laboratory Standards Institute (**CLSI**) states that the confirmatory test for ketones using the Acetest may not be relevant to current laboratory practice.[3] Currently, new methods measuring β-hydroxybutyrate using reagent strips have been developed to provide automated methods for testing serum and other body fluids. Notice in Figure 6-4 the ketone with the highest concentration is β-hydroxybutyrate.

Acetest Tablets

Acetest (Siemens Healthcare Diagnostics, Inc., Deerfield, IL) provides sodium nitroprusside, glycine, disodium phosphate, and lactose in tablet form. The addition of lactose gives better color differentiation. Acetest tablets are hygroscopic; if the specimen is not absorbed completely within 30 seconds, a new tablet should be used. See Procedure 6-4.

SUMMARY 6-10 Ketone Reagent Strip

Reagents	Sodium nitroprusside
	Glycine (Chemstrip)
Sensitivity	Multistix: 5–10 mg/dL acetoacetic acid
	Chemstrip: 9 mg/dL acetoacetic acid; 70 mg/dL acetone
Interference	**False positive:**
	Phthalein dyes
	Highly pigmented red urine
	Levodopa
	Medications containing free sulfhydryl groups
	False negative:
	Improperly preserved specimens
Correlations	Glucose

Blood

Blood may be present in the urine either in the form of intact red blood cells (**RBCs**) (**hematuria**) or as the product of RBC destruction, hemoglobin (**hemoglobinuria**). As discussed in Chapter 5, blood present in large quantities can be detected visually; hematuria produces a cloudy red urine, and hemoglobinuria appears as a clear red specimen. Because any amount of blood greater than five cells per microliter of urine is considered clinically significant, visual examination cannot be relied upon to detect the presence of blood. Microscopic examination of the urinary sediment shows intact RBCs, but free hemoglobin produced either by hemolytic disorders or lysis of RBCs is not detected. Therefore, chemical tests for hemoglobin provide the most accurate means for determining the presence of blood. Once blood has been detected, the microscopic examination can be used to differentiate between hematuria and hemoglobinuria.

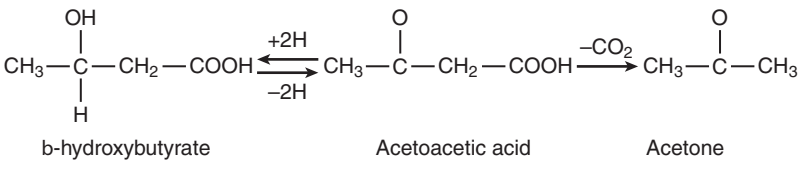

Figure 6–4 Production of acetone and butyrate from acetoacetic acid.

PROCEDURE 6-4

Acetest Procedure

Procedure

1. Remove the Acetest tablet from the bottle, and place it on a clean, dry piece of white paper.
2. Place one drop of urine on top of the tablet.
3. Wait 30 seconds.
4. Compare the tablet color with the manufacturer-supplied color chart.
5. A positive result is indicated by a purple color. Report as negative, small, moderate, or large.

Clinical Significance

The finding of a positive reagent strip test result for blood indicates the presence of RBCs, hemoglobin, or myoglobin. Each of these has a different clinical significance.

Hematuria

Hematuria is most closely related to disorders of renal or genitourinary origin in which bleeding is the result of trauma or damage to the organs of these systems. Major causes of hematuria include renal calculi, glomerular diseases, tumors, trauma, pyelonephritis, exposure to toxic chemicals, and anticoagulant therapy. The laboratory is frequently requested to perform a urinalysis when patients presenting with severe back and abdominal pain are suspected of having renal calculi. In such cases, hematuria is usually of a small to moderate degree, but its presence can be essential to the diagnosis. Hematuria of nonpathological significance is observed after strenuous exercise and during menstruation.

Hemoglobinuria

Hemoglobinuria may result from the lysis of RBCs produced in the urinary tract, particularly in dilute, alkaline urine. It also may result from intravascular hemolysis and the subsequent filtering of hemoglobin through the glomerulus. Lysis of RBCs in the urine usually shows a mixture of hemoglobinuria and hematuria, whereas no RBCs are seen in cases of intravascular hemolysis. Under normal conditions, the formation of large hemoglobin–haptoglobin complexes in the circulation prevents the glomerular filtration of hemoglobin. When the amount of free hemoglobin present exceeds the haptoglobin content—as occurs in hemolytic anemias, transfusion reactions, severe burns, bites from the brown recluse spider, infections, and strenuous exercise—hemoglobin is available for glomerular filtration. Reabsorption of filtered hemoglobin also 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.

Myoglobinuria

Myoglobin, a heme-containing protein found in muscle tissue, not only reacts positively with the reagent strip test for blood but also produces a clear red-brown urine. In **myoglobinuria**, the presence of myoglobin rather than hemoglobin should be suspected in patients with conditions associated with muscle destruction (**rhabdomyolysis**). Examples of these conditions include trauma, crush syndromes, prolonged coma, convulsions, muscle-wasting diseases, alcoholism, heroin abuse, and extensive exertion. The development of rhabdomyolysis has been found to be a side effect in certain patients taking the cholesterol-lowering statin medications.[12] The heme portion of myoglobin is toxic to the renal tubules, and high concentrations can cause acute renal failure. The massive hemoglobinuria seen in hemolytic transfusion reactions also is associated with acute renal failure.

Reagent Strip Reactions

Chemical tests for blood 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.

$$H_2O_2 + \text{chromogen} \xrightarrow[\text{Peroxidase}]{\text{Hemoglobin}} \text{oxidized chromogen} + H_2O$$

Reagent strip manufacturers incorporate peroxide and tetramethylbenzidine into the blood testing area. Two color charts are provided that correspond to the reactions that occur

SUMMARY 6-11 Clinical Significance of a Positive Reaction for Blood

Hematuria
- Renal calculi
- Glomerulonephritis
- Pyelonephritis
- Tumors
- Trauma
- Exposure to toxic chemicals
- Anticoagulants
- Strenuous exercise

Hemoglobinuria
- Transfusion reactions
- Hemolytic anemias
- Severe burns
- Infections/malaria
- Strenuous exercise/RBC trauma
- Brown recluse spider bites

Myoglobinuria
- Muscular trauma
- Crush injuries
- Prolonged coma
- Convulsions
- Muscle-wasting diseases
- Alcoholism/overdose
- Drug abuse
- Extensive exertion
- Cholesterol-lowering statin medications

with hemoglobinuria, myoglobinuria, and hematuria (RBCs). In the presence of free hemoglobin/myoglobin, a uniform color ranging from a negative yellow through green to a strongly positive green-blue appears on the pad. In contrast, intact RBCs are lysed when they come in contact with the pad, and the liberated hemoglobin produces an isolated reaction that results in a speckled pattern on the pad. Reagent strip tests can detect concentrations as low as five RBCs per microliter; however, care must be taken when comparing these figures with the actual microscopic values because the absorbent nature of the pad attracts some urine. The terms trace, small, moderate, and large or trace, 1+, 2+, and 3+ are used for reporting.

Reaction Interference

False-positive reactions due to menstrual contamination may be seen. They also occur if strong oxidizing detergents, such as sodium hypochlorite (bleach), are present in the specimen container.[2] Vegetable peroxidase and bacterial enzymes, including an *Escherichia coli* peroxidase, also may cause false-positive reactions. Therefore, sediments containing bacteria should be checked closely for the presence of RBCs.

Traditionally, ascorbic acid (vitamin C) has been associated with false-negative reagent strip reactions for blood. Both Multistix and Chemstrip have modified their reagent strips to reduce this interference to very high levels (25 mg/dL) of ascorbic acid. Multistix uses a peroxide that is less subject to reduction by ascorbic acid, and Chemstrip overlays the reagent pad with an iodate-impregnated mesh that oxidizes the ascorbic acid before it reaches the reaction pad. False-negative reactions can result when urine with a high specific gravity contains crenated RBCs that do not lyse when they come in contact with the reagent pad. Decreased reactivity also may be seen when formalin is used as a preservative or when the hypertension medication captopril or high concentrations of nitrite (greater than 10 mg/dL) are present. RBCs settle to the bottom of the specimen container, and failure to mix the specimen before testing causes a reading that is falsely decreased.

SUMMARY 6-12 Blood Reagent Strip

Reagents	Multistix: Diisopropylbenzene dihydroperoxide and 3,3′,5,5′-tetramethylbenzidine
	Chemstrip: dimethyldihydroperoxyhexane and tetramethylbenzidine
Sensitivity	Multistix: 5–20 RBCs/mL, 0.015–0.062 mg/dL hemoglobin
	Chemstrip: 5 RBCs/mL, hemoglobin corresponding to 10 RBCs/mL
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

HISTORICAL NOTE

Hemoglobinuria Versus Myoglobinuria

Before the development of sensitive serum immunoassay tests for myoglobin, a procedure was used to differentiate between hemoglobin and myoglobin in the urine. A precipitation test was used to screen for the presence of myoglobin; 2.8 g of ammonium sulfate are added to 5 mL of centrifuged urine. After mixing and allowing the specimen to sit for 5 minutes, the urine was filtered or centrifuged, and the supernatant was tested for a reaction for blood with a reagent strip. The principle of this screening test was based on the fact that the larger hemoglobin molecules were precipitated by the ammonium sulfate and myoglobin remained in the supernatant. Therefore, when myoglobin was present, the supernatant retained the red color and gave a positive reagent strip test for blood. Conversely, hemoglobin produced a red precipitate and a supernatant that tested negative for blood.

Bilirubin

The appearance of bilirubin in the urine can provide an early indication of liver disease. It is often detected long before the patient exhibits **jaundice**.

Bilirubin Production

Bilirubin, a highly pigmented yellow compound, is a degradation product of hemoglobin. Under normal conditions, the life span of RBCs is approximately 120 days, at which time they are destroyed in the spleen and liver by the phagocytic cells of the reticuloendothelial system. The liberated hemoglobin is broken down into its component parts: iron, protein, and protoporphyrin. The body reuses the iron and protein, and the cells of the reticuloendothelial system convert the remaining protoporphyrin to bilirubin. Then the bilirubin is released into circulation, where it binds with albumin and is transported to the liver. At this point, the kidneys cannot excrete the circulating bilirubin because not only is it bound to albumin but also it is water insoluble (unconjugated bilirubin). In the liver, bilirubin is conjugated with glucuronic acid by the action of

glucuronyl transferase to form water-soluble bilirubin diglucuronide (conjugated bilirubin). Usually, this conjugated bilirubin does not appear in the urine because it is passed directly from the liver into the bile duct and on to the intestine. In the intestine, intestinal bacteria reduce bilirubin to urobilinogen, which is oxidized and excreted in the feces in the form of stercobilinogen and urobilin. Figure 6-5 illustrates bilirubin metabolism for reference with this section and the subsequent discussion of urobilinogen.

Clinical Significance

Only conjugated bilirubin can appear in the urine when the normal degradation cycle is disrupted by bile duct obstruction (posthepatic jaundice) (e.g., gallstones or cancer) or when the integrity of the liver is damaged (hepatic jaundice), allowing leakage of conjugated bilirubin into circulation. Hepatitis and cirrhosis are common examples of conditions that produce liver damage resulting in bilirubinuria. Not only does the detection of urinary bilirubin provide an early indication of liver disease, but also its presence or absence can be used in determining the cause of clinical jaundice. As shown in Table 6-2, this determination can be even more significant when bilirubin results are combined with urinary urobilinogen. 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.

Table 6–2 Urine Bilirubin and Urobilinogen in Jaundice

	Urine Bilirubin	Urine Urobilinogen
Bile duct obstruction	+ + +	Normal
Liver damage	+ or –	+ +
Hemolytic disease	Negative	+ + +

SUMMARY 6-13 Clinical Significance of Urine Bilirubin

- Hepatitis
- Cirrhosis
- Other liver disorders
- Biliary obstruction (gallstones, carcinoma)

Reagent Strip (Diazo) Reactions

Routine testing for urinary bilirubin by reagent strip uses the **diazo reaction**. Bilirubin combines with 2,4-dichloroaniline diazonium salt or 2,6-dichlorobenzene-diazonium-tetrafluoroborate

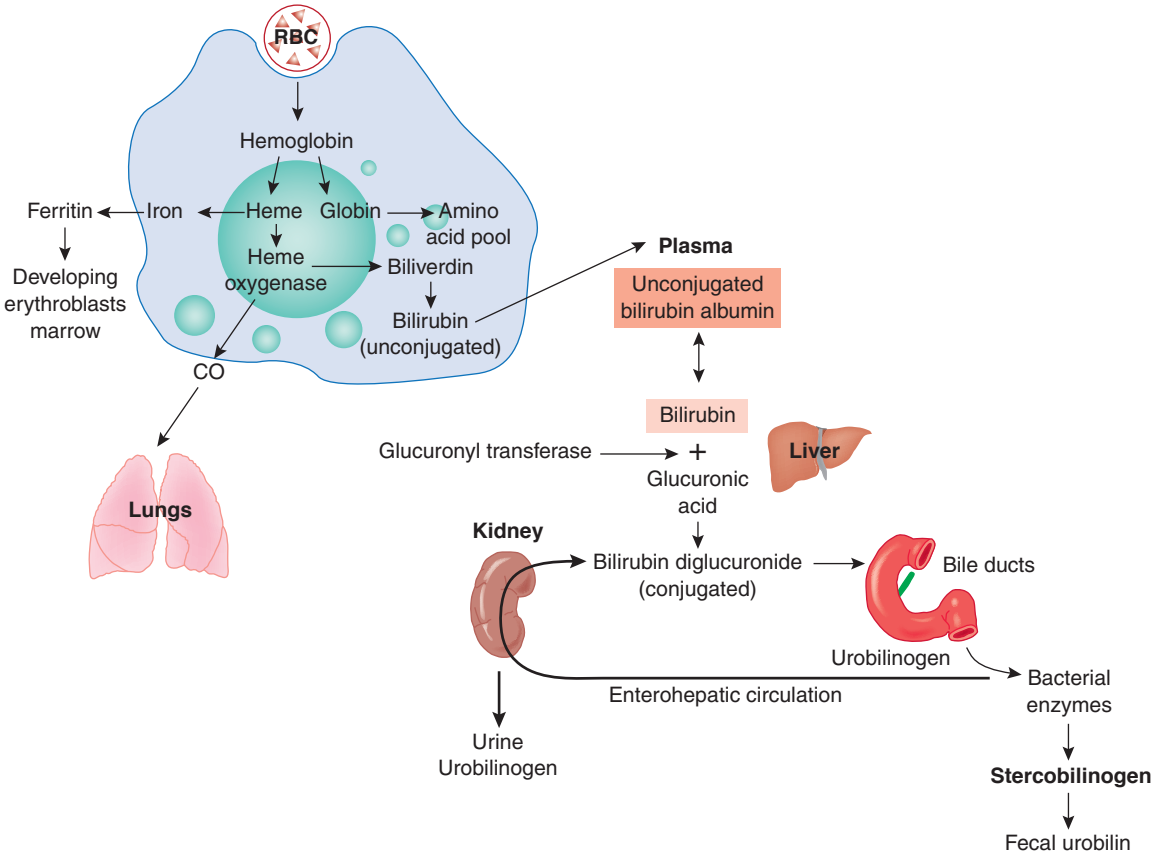

Figure 6–5 Hemoglobin degradation and production of bilirubin and urobilinogen.

in an acid medium to produce an azo dye, with colors ranging from increasing degrees of tan or pink to violet, respectively. Qualitative results are reported as negative, small, moderate, or large or as negative, 1+, 2+, or 3+. Reagent strip color reactions for bilirubin are more difficult to interpret than are other reagent strip reactions, and those reactions are easily influenced by other pigments present in the urine. Atypical color reactions are frequently noted on visual examination and are measured by automated readers. Further testing should be performed on any questionable results.

$$\text{Bilirubin glucuronide} + \text{diazonium salt} \xrightarrow{\text{Acid}} \text{azo dye}$$

Reaction Interference

As discussed previously, false-positive reactions are primarily due to urine pigments. Of particular concern are the yellow-orange urines from people taking phenazopyridine compounds because the thick pigment produced may be mistaken for bilirubin on initial examination. The presence of indican and metabolites of the medication Lodine may cause false-positive readings.

The false-negative results caused by the testing of specimens that are not fresh are the most frequent errors associated with bilirubin testing. Bilirubin is an unstable compound that is rapidly photo-oxidized to biliverdin when exposed to light. Biliverdin does not react with diazo tests. False-negative results also occur when hydrolysis of bilirubin diglucuronide produces free bilirubin because this is less reactive in the reagent strip tests. High concentrations of ascorbic acid (greater than 25 mg/dL) and nitrite may lower the sensitivity of the test because those substances combine with the diazonium salt and prevent its reaction with bilirubin.

SUMMARY 6-14 Bilirubin Reagent Strip

Reagents	Multistix: 2,4-dichloroaniline diazonium salt
Sensitivity	Chemstrip: 2,6-dichlorobenzene-diazonium salt
	Multistix: 0.4–0.8 mg/dL bilirubin
	Chemstrip: 0.5 mg/dL bilirubin
Interference	**False positive:**
	Highly pigmented urines, phenazopyridine
	Indican (intestinal disorders)
	Metabolites of Lodine
	False negative:
	Specimen exposure to light
	Ascorbic acid greater than 25 mg/dL
	High concentrations of nitrite
Correlations with other tests	Urobilinogen

Ictotest Tablets

A confirmatory test for bilirubin is the Ictotest (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY). Ictotest kits consist of testing mats and tablets containing *p*-nitrobenzene-diazonium-*p*-toluenesulfonate, SSA, sodium carbonate, and boric acid. Ten drops of urine are added to the mat, which has special properties that cause bilirubin to remain on the surface as the urine is absorbed. After the chemical reaction, a blue-to-purple color appears on the mat when bilirubin is present. The Ictotest is four times more sensitive than the reagent strip and will detect 0.05 to 0.1 mg/dL bilirubin. Colors other than blue or purple appearing on the mat are considered to be a negative result. If interference in the Ictotest is suspected, it can usually be removed by adding water directly to the mat after the urine has been added. Interfering substances are washed into the mat, and only bilirubin remains on the surface.

Urobilinogen

As shown in Figure 6-4, 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**. Some of the urobilinogen is reabsorbed from the intestine into the blood, recirculates to the liver, and is excreted back into the intestine through the bile duct. The stercobilinogen cannot be reabsorbed and remains in the intestine, where it is oxidized to stercobilin. The recirculated urobilinogen that reaches the intestine is also oxidized to urobilin. Both stercobilin and urobilin are excreted in the feces and are the pigments responsible for the characteristic brown color of feces. Urobilinogen appears in the urine because as it

PROCEDURE 6-5

Ictotest Procedure

Procedure

1. Place 10 drops of urine onto one square of the absorbent test mat.
2. Using forceps, remove one Ictotest reagent tablet, recap the bottle promptly, and place the tablet in the center of the moistened area.
3. Place one drop of water onto the tablet, and wait 5 seconds.
4. Place a second drop of water onto the tablet so that the water runs off the tablet onto the mat.
5. 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.

circulates in the blood back to the liver, it passes through the kidney and is filtered by the glomerulus. Therefore, a small amount of urobilinogen—less than 1 mg/dL or 1 Ehrlich unit—is normally found in the urine.

Clinical Significance

Increased urine urobilinogen (greater than 1 mg/dL) is seen in liver disease and hemolytic disorders. Measurement of urine urobilinogen can be valuable in the detection of early liver disease; however, studies have shown that when urobilinogen tests are performed routinely, 1% of the nonhospitalized population and 9% of a hospitalized population exhibit elevated results.[13] This is frequently caused by constipation.

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.

The clinical jaundice associated with hemolytic disorders results from the increased amount of circulating unconjugated bilirubin. This unconjugated bilirubin is presented to the liver for conjugation, resulting in a markedly increased amount of conjugated bilirubin entering the intestines. As a result, increased urobilinogen is produced, and increased amounts of urobilinogen are reabsorbed into the blood and circulated through the kidneys, where filtration takes place. In addition, the overworked liver does not process the reabsorbed urobilinogen as efficiently, and additional urobilinogen is presented for urinary excretion.

Although it cannot be determined by reagent strip, the absence of urobilinogen in the urine and feces is also diagnostically significant and represents an obstruction of the bile duct that prevents the normal passage of bilirubin into the intestine. An additional observation is the production of pale stools as the result of the lack of urobilin. Refer back to Table 6-2 for an outline of the relationship of urine bilirubin and urine urobilinogen to the pathological conditions associated with them.

Reagent Strip Reactions and Interference

The reagent strip reactions for urobilinogen differ between Multistix and Chemstrip much more significantly than do other reagent strip parameters. Multistix uses a modification of the **Ehrlich reaction**, in which urobilinogen reacts with *p*-dimethylaminobenzaldehyde (Ehrlich reagent) to produce colors ranging from light to dark pink. Results are reported as Ehrlich units (**EU**), which are equal to mg/dL, ranging from normal readings of 0.2 and 1 through abnormal readings of 2, 4, and 8. Chemstrip incorporates an azo-coupling (diazo) reaction using 4-methoxybenzene-diazonium-tetrafluoroborate to react with urobilinogen, producing colors ranging from white to pink. This reaction is more specific for urobilinogen than the Ehrlich reaction. Results are reported in mg/dL. Both tests detect urobilinogen that is present in normal quantities, and color comparisons are provided for the upper limits of normal as well as abnormal concentrations. Reagent strip tests cannot determine the absence of urobilinogen, which is significant in biliary obstruction.

Multistix:

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

Chemstrip:

Urobilinogen + diazonium salt (4-methyloxybenzene-diazonium-tetrafluoroborate) $\xrightarrow{\text{Acid}}$ red azo dye

Reaction Interference

The Ehrlich reaction on Multistix is subject to a variety of interferences, referred to as Ehrlich-reactive compounds, that produce false-positive reactions. These include porphobilinogen, indican, *p*-aminosalicylic acid, sulfonamides, methyldopa, procaine, and chlorpromazine compounds. The presence of porphobilinogen is clinically significant; however, the reagent strip test is not considered a reliable method to screen for its presence. Porphobilinogen will be discussed in Chapter 9.

The sensitivity of the Ehrlich reaction increases with temperature, and testing should be performed at room temperature. Highly pigmented urines cause atypical readings with both brands of reagent strips. As a result of increased excretion of bile salts, urobilinogen results are normally highest after a meal.

False-negative results occur most frequently when specimens are improperly preserved, allowing urobilinogen to be photo-oxidized to urobilin. High concentrations of nitrite interfere with the azo-coupling reaction on Chemstrip. False-negative readings also are obtained with both strips when formalin is used as a preservative.

Technical Tip 6-5. The urobilinogen test pad on the Multistix Pro11 and Clinitek Microalbumin strips has been replaced by the protein-low test pad.

SUMMARY 6-15 Clinical Significance of Urine Urobilinogen

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

Nitrite

Clinical Significance

The reagent strip test for nitrite provides a rapid screening test for the presence of UTI. The test is designed to detect cases in which the need for a culture may not be apparent; it is not

SUMMARY 6-16	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
Interference	**Multistix:**
	False positive:
	Porphobilinogen
	Indican
	p-aminosalicylic acid
	Sulfonamides
	Methyldopa
	Procaine
	Chlorpromazine
	Highly pigmented urine
	False negative:
	Old specimens
	Preservation in formalin
	Chemstrip:
	False positive:
	Highly pigmented urine
	False negative:
	Old specimens
	Preservation in formalin
	High concentrations of nitrite
Correlations with other tests	Bilirubin

intended to replace the urine culture as the primary test for diagnosing and monitoring bacterial infection. Many UTIs are believed to start in the bladder as a result of external contamination and, if untreated, progress upward through the ureters to the tubules, renal pelvis, and kidney. Infections are caused most commonly by gram-negative organisms, such as *E. coli, Proteus* species, *Enterobacter* species, and *Klebsiella* species. UTIs are eight times more common in women than in men due to a shorter urethra, providing a shorter distance for the bacteria to travel. Patients who are catheterized also have a higher incidence of infection. The nitrite test is valuable for detecting initial bladder infection (cystitis) because often patients are asymptomatic or have vague symptoms that would not lead the physician to order a urine culture. Pyelonephritis, an inflammatory process of the kidney and adjacent renal pelvis, is a frequent complication of untreated cystitis and can lead to renal tissue damage, impairment of renal function, hypertension, and even septicemia. Therefore, detection of **bacteriuria** through the use of the nitrite screening test and subsequent antibiotic therapy can prevent these serious complications. The nitrite test also can be used to evaluate the success of antibiotic therapy and to periodically screen people with recurrent infections, patients with diabetes, and pregnant women, all of whom are considered to be at high risk for UTI. As discussed in the following section, many laboratories use the nitrite test in combination with the LE test to determine the necessity of performing urine cultures.

Reagent Strip Reactions

The chemical basis of the nitrite test is the ability of certain gram-negative bacteria to reduce nitrate, a normal constituent of urine, to nitrite, which normally does not appear in the urine. Nitrite is 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 azo dye. To prevent false-positive reactions in externally contaminated specimens, the sensitivity of the test is standardized to correspond with a quantitative bacterial culture criterion of 100,000 organisms per milliliter. Although different shades of pink may be produced, the test does not measure the degree of bacteriuria, and any shade of pink is considered to represent a number of bacteria that is clinically significant. Results are reported only as negative or positive.

$$\text{Para-arsanilic acid or sulfanilamide} + NO_2 \xrightarrow{\text{Acid}} \text{diazonium salt (nitrite)}$$

$$\text{Diazonium salt} + \text{tetrahydrobenzoquinolin} \xrightarrow{\text{Acid}} \text{pink azo dye}$$

Reaction Interference

Several major factors can influence the reliability of the nitrite test, and tests with negative results in the presence of clinical symptoms that are even vaguely suspicious should always be repeated or followed by a urine culture.

1. 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

SUMMARY 6-17	Clinical Significance of Urine Nitrite
Cystitis	
Pyelonephritis	
Evaluation of antibiotic therapy	
Monitoring of patients at high risk for UTI	
Screening of urine culture specimens	

SUMMARY 6-18 Nitrite Reagent Strip

Reagents	Multistix: *p*-arsanilic acid
	Tetrahydrobenzo(h)-quinolin-3-ol
	Chemstrip: Sulfanilamide, hydroxytetrahydrobenzoquinoline
Sensitivity	Multistix: 0.06–0.1 mg/dL nitrite ion
	Chemstrip: 0.05 mg/dL nitrite ion
Interference	**False negative:**
	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
	False positive:
	Improperly preserved specimens
	Highly pigmented urine
Correlations with other tests	Protein
	Leukocytes
	Microscopic

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.

2. 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 specimens.[2]
3. 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 before testing, the possibility of a false-negative result due to lack of dietary nitrate does exist.
4. Further reduction of nitrite to nitrogen may occur when large numbers of bacteria are present, and this causes a false-negative reaction.
5. 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.

Leukocyte Esterase

Before the development of the reagent strip test for LE, detection of increased urinary leukocytes required microscopic examination of the urine sediment. This can be subject to variation depending on the method used to prepare the sediment and the technical personnel examining the sediment. Therefore, the chemical test for leukocytes offers a more standardized means for the detection of leukocytes. The test is not designed to measure the concentration of leukocytes, and the manufacturers recommend that quantitation be done by microscopic examination. An additional advantage to the chemical LE test is that it detects the presence of leukocytes that have been lysed, particularly in dilute alkaline urine, and that would not appear in the microscopic examination.

Clinical Significance

Normal values for leukocytes are based on the examination of microscopic sediment and vary from 0 to 2 to 0 to 5 per high-power field (**hpf**). Women tend to have higher numbers than men as a result of vaginal contamination. Increased urinary leukocytes are indicators of UTI. The LE test detects the presence of esterase in the granulocytic white blood cells (**WBCs**) (neutrophils, eosinophils, and basophils) and monocytes, but not lymphocytes. Neutrophils are the leukocytes most frequently associated with bacterial infections. Esterases also are present in *Trichomonas* and histiocytes. Lymphocytes, erythrocytes, bacteria, and renal tissue cells do not contain esterases. A positive LE test result is accompanied most frequently by the presence of bacteria, which, as discussed previously, may or may not produce a positive nitrite reaction. Infections caused by *Trichomonas, Chlamydia,* yeast, and inflammation of renal tissues (i.e., interstitial nephritis) produce **leukocyturia** without bacteriuria.

Screening urine specimens using the LE and nitrite chemical reactions to determine the necessity of performing urine cultures can be a cost-effective measure.[14] The LE test contributes significantly more to the reliability of this practice than does the nitrite test.

SUMMARY 6-19 Clinical Significance of Urine Leukocytes

Bacterial and nonbacterial UTI

Inflammation of the urinary tract

Screening of urine culture specimens

Reagent Strip Reaction

The 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. Then the aromatic compound combines with a diazonium salt present on the pad to produce a purple azo dye.

$$\text{Indoxylcarbonic acid ester} \xrightarrow[\text{Esterase}]{\text{Leukocyte}} \text{indoxyl} + \text{acid indoxyl}$$

$$+ \text{ diazonium salt} \xrightarrow{\text{Acid}} \text{purple azo dye}$$

The LE reaction requires the longest time of all the reagent strip reactions (2 minutes). Reactions are reported as trace, small, moderate, and large or trace, 1+, 2+, and 3+. Trace readings may not be significant and should be repeated on a fresh specimen.

Reaction Interference

The presence of strong oxidizing agents or formalin in the collection container causes false-positive reactions. Urines that are highly pigmented, as well as the presence of the antibiotic nitrofurantoin, may obscure the color reaction.

False-negative results may occur in the presence of high concentrations of protein (greater than 500 mg/dL), glucose (greater than 3 g/dL), oxalic acid, and ascorbic acid. In this reaction, ascorbic acid also combines with the diazonium salt. Crenation of leukocytes preventing release of esterases may occur in urines with a high specific gravity. The presence of the antibiotics gentamicin, cephalexin, cephalothin, and tetracycline decreases the sensitivity of the reaction.

SUMMARY 6-20 Leukocyte Esterase Reagent Strip

Reagents	Multistix: Derivatized pyrrole amino acid ester
	Diazonium salt
	Chemstrip: Indoxylcarbonic acid ester
	Diazonium salt
Sensitivity	Multistix: 5–15 WBC/hpf
	Chemstrip: 10–25 WBC/hpf
Interference	**False positive:**
	Strong oxidizing agents
	Formalin
	Highly pigmented urine, nitrofurantoin
	False negative:
	High concentrations of protein, glucose, oxalic acid, ascorbic acid, gentamicin, cephalosporins, tetracyclines; inaccurate timing
Correlations with other tests	Protein
	Nitrite
	Microscopic

Specific Gravity

The reagent strip test for specific gravity is included as part of the physical examination of urine in Chapter 5 and is reviewed here as part of the chemical examination. A summary of the clinical significance is included in this chapter.

Reagent Strip Reaction

The reagent strip reaction is based on the change in pK_a (dissociation constant) of a polyelectrolyte in an alkaline medium. The polyelectrolyte ionizes, releasing hydrogen ions in proportion to the number of ions in the solution. The higher the concentration of urine, the more hydrogen ions are released, thereby lowering the pH. Incorporation of the indicator bromothymol blue on the reagent pad measures the change in pH. As the specific gravity increases, the indicator changes from blue (1.000 [alkaline]), through shades of green, to yellow 1.030 [acid]). Readings can be made in 0.005 intervals by careful comparison with the color chart. The specific gravity reaction is diagrammed in Figure 6-6.

Reaction Interference

The reagent strip specific gravity measures only ionic solutes, thereby eliminating the interference by the large organic molecules, such as urea and glucose, and by radiographic contrast

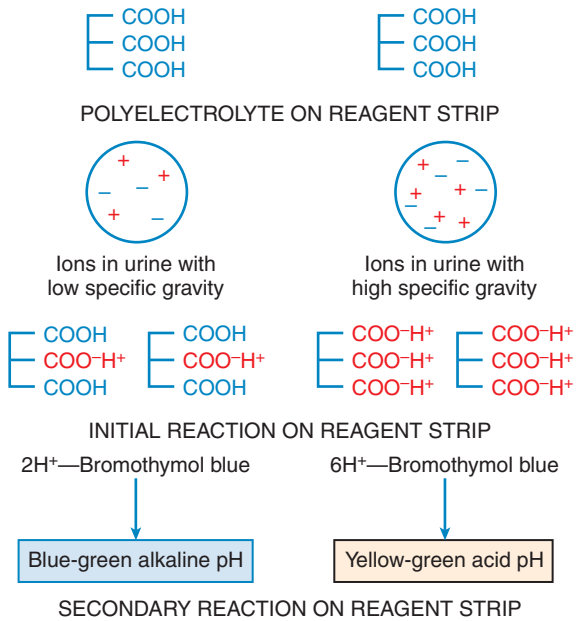

Figure 6–6 Diagram of reagent strip–specific gravity reaction.

SUMMARY 6-21 Clinical Significance of Urine Specific Gravity

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

SUMMARY 6-22 Urine Specific Gravity Reagent Strip

Reagents	Multistix: Poly (methyl vinyl ether/maleic anhydride) bromothymol blue
	Chemstrip: Ethylene glycol diaminoethyl ether tetra-acetic acid, bromothymol blue
Sensitivity	1.000–1.030
Interference	**False positive:** High concentrations of protein
	False negative: Highly alkaline urines (greater than 6.5)

media and plasma expanders that are included in physical measurements of specific gravity. This difference must be considered when comparing specific gravity results obtained by a different method. Elevated concentrations of protein slightly increase the readings as a result of protein anions.

Specimens with a pH of 6.5 or higher have decreased readings caused by interference with the bromothymol blue indicator (the blue-green readings associated with an alkaline pH correspond to a low reading for specific gravity). 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.



For additional resources please visit
www.fadavis.com

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Study Questions

1. Leaving excess urine on the reagent strip after removing it from the specimen will:
   A. Cause runover between reagent pads
   B. Alter the color of the specimen
   C. Cause reagents to leach from the pads
   D. Not affect the chemical reactions

2. Failure to mix a specimen before inserting the reagent strip will primarily affect the:
   A. Glucose reading
   B. Blood reading
   C. Leukocyte reading
   D. Both B and C

3. Testing a refrigerated specimen that has not warmed to room temperature will adversely affect:
   A. Enzymatic reactions
   B. Dye-binding reactions
   C. The sodium nitroprusside reaction
   D. Diazo reactions
4. The reagent strip reaction that requires the longest reaction time is the:
   A. Bilirubin
   B. pH
   C. Leukocyte esterase
   D. Glucose
5. Quality control of reagent strips is performed:
   A. Using positive and negative controls
   B. When results are questionable
   C. Per laboratory policy
   D. All of the above
6. All of the following are important to protect the integrity of reagent strips *except:*
   A. Removing the desiccant from the bottle
   B. Storing in an opaque bottle
   C. Storing at room temperature
   D. Resealing the bottle after removing a strip
7. The principle of the reagent strip test for pH is the:
   A. Protein error of indicators
   B. Greiss reaction
   C. Dissociation of a polyelectrolyte
   D. Double indicator reaction
8. A urine specimen with a pH of 9.0:
   A. Indicates metabolic acidosis
   B. Should be re-collected
   C. May contain calcium oxalate crystals
   D. Is seen after drinking cranberry juice
9. In the laboratory, a primary consideration associated with pH is:
   A. Identifying urinary crystals
   B. Monitoring vegetarian diets
   C. Determining specimen acceptability
   D. Both A and C
10. 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.
    A. _____ Microalbuminuria
    B. _____ Acute-phase reactants
    C. _____ Preeclampsia
    D. _____ Vaginal inflammation
    E. _____ Multiple myeloma
    F. _____ Orthostatic proteinuria
    G. _____ Prostatitis
11. The principle of the protein error of indicators reaction is that:
    A. Protein keeps the pH of the urine constant
    B. 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 values on a reagent strip *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 farther 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. Immunological 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 is frequently performed on a patient with severe back and abdominal pain 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:

A. Binding of heme and a chromogenic dye
B. Peroxidase activity of heme
C. Reaction of peroxide and chromogen
D. Diazo activity of heme

33. A speckled pattern on the blood pad of the reagent strip indicates:

A. Hematuria
B. Hemoglobinuria
C. Myoglobinuria
D. All of the above

34. List the following products of hemoglobin degradation in the correct order of metabolism by placing numbers 1 to 4 in the blank, where 1 indicates the beginning and 4 indicates the end product.

A. _____ Conjugated bilirubin
B. _____ Urobilinogen and stercobilinogen
C. _____ Urobilin
D. _____ Unconjugated bilirubin

35. The principle of the reagent strip test for bilirubin is the:

A. Diazo reaction
B. Ehrlich reaction
C. Greiss reaction
D. Peroxidase reaction

36. An elevated urine bilirubin with a normal urobilinogen is indicative of:

A. Cirrhosis of the liver
B. Hemolytic disease
C. Hepatitis
D. Biliary obstruction

37. The primary cause of a false-negative bilirubin reaction is:

A. Highly pigmented urine
B. Specimen contamination
C. Specimen exposure to light
D. Excess conjugated bilirubin

38. The purpose of the special mat supplied with the Ictotest tablets is that:

A. Bilirubin remains on the surface of the mat
B. It contains the dye needed to produce color
C. It removes interfering substances
D. Bilirubin is absorbed into the mat

39. The reagent in the Multistix reaction for urobilinogen is:

A. A diazonium salt
B. Tetramethylbenzidine
C. p-Dimethylaminobenzaldehyde
D. Hoesch reagent

40. The primary problem with urobilinogen tests using Ehrlich reagent is:

A. Positive reactions with porphobilinogen
B. Lack of specificity
C. Positive reactions with Ehrlich reactive substances
D. All of the above

41. The reagent strip test for nitrite uses the:

A. Greiss reaction
B. Ehrlich reaction
C. Peroxidase reaction
D. Pseudoperoxidase reaction

42. All of the following can cause a negative nitrite reading *except:*

A. Gram-positive bacteria
B. Gram-negative bacteria
C. Random urine specimens
D. Heavy bacterial infections

43. A positive nitrite test and a negative leukocyte esterase test is an indication of a:

A. Dilute random specimen
B. Specimen with lysed leukocytes
C. Vaginal yeast infection
D. Specimen older than 2 hours

44. All of the following can be detected by the leukocyte esterase reaction *except:*

A. Neutrophils
B. Eosinophils
C. Lymphocytes
D. Basophils

45. Screening tests for urinary infection combine the leukocyte esterase test with the test for:

A. pH
B. Nitrite
C. Protein
D. Blood

46. The principle of the leukocyte esterase reagent strip test uses a:

A. Peroxidase reaction
B. Double indicator reaction
C. Diazo reaction
D. Dye-binding technique

47. The principle of the reagent strip test for specific gravity uses the dissociation constant of a(n):

A. Diazonium salt
B. Indicator dye
C. Polyelectrolyte
D. Enzyme substrate

48. A specific gravity of 1.005 would produce the reagent strip color:

A. Blue
B. Green
C. Yellow
D. Red

49. Specific gravity readings on a reagent strip are affected by:

A. Glucose
B. Radiographic dye
C. Alkaline urine
D. All of the above

Case Studies and Clinical Situations

1. A patient taken to the emergency department after an episode of syncope has a fasting blood glucose level of 450 mg/dL. Results of the routine urinalysis are as follows:

COLOR: Yellow	KETONES: 2+
CLARITY: Clear	BLOOD: Negative
SP. GRAVITY: 1.015	BILIRUBIN: Negative
pH: 5.0	PROTEIN-LOW: 15 mg/dL
PROTEIN-HIGH: 30 mg/dL	NITRITE: Negative
GLUCOSE: 250 mg/dL	LEUKOCYTES: Negative
CREATININE: 200 mg/dL	

a. Explain the correlation between the patient's blood and urine glucose results.
b. What is the most probable metabolic disorder associated with this patient?
c. Considering the patient's condition, what is the significance of the reading of the patient's protein-to-creatinine ratio?
d. If the patient in this study had a normal blood glucose level, as well as normal results for protein and creatinine, to what would the urinary glucose level be attributed?

2. Results of a urinalysis performed on a patient scheduled for gallbladder surgery are as follows:

COLOR: Amber	KETONES: Negative
CLARITY: Hazy	BLOOD: Negative
SP. GRAVITY: 1.022	BILIRUBIN: Moderate
pH: 6.0	UROBILINOGEN: Normal
PROTEIN: Negative	NITRITE: Negative
GLUCOSE: Negative	LEUKOCYTES: Negative

a. What would be observed if this specimen were shaken?
b. Explain the correlation between the patient's scheduled surgery and the normal urobilinogen.
c. If blood were drawn from this patient, how might the appearance of the serum be described?
d. What special handling is needed for specimens of serum and urine from this patient?

3. Results of a urinalysis on a patient who is very anemic and jaundiced are as follows:

COLOR: Red	KETONES: Negative
CLARITY: Clear	BLOOD: Large
SP. GRAVITY: 1.020	BILIRUBIN: Negative
pH: 6.0	UROBILINOGEN: 8 EU
PROTEIN: Negative	NITRITE: Negative
GLUCOSE: Negative	LEUKOCYTES: Negative

a. Would these results be indicative of hematuria or hemoglobinuria?
b. Correlate the patient's condition with the urobilinogen result.
c. Why is the urine bilirubin result negative in this jaundiced patient?
d. Would this method also measure urine porphobilinogen? Why or why not?

4. A female patient arrives at the outpatient clinic with symptoms of lower back pain and urinary frequency with a burning sensation. She is a firm believer in the curative powers of vitamins. She has tripled her usual dosage of vitamins in an effort to alleviate her symptoms; however, the symptoms have persisted. She is given a sterile container and asked to collect a clean-catch midstream urine specimen. Results of this routine urinalysis are as follows:

COLOR: Dark yellow	KETONES: Negative
CLARITY: Hazy	BLOOD: Negative
SP. GRAVITY: 1.012	BILIRUBIN: Negative
pH: 7.0	UROBILINOGEN: Normal
PROTEIN: Trace	NITRITE: Negative
GLUCOSE: Negative	LEUKOCYTES: 1+
Microscopic	
8 to 12 RBC/hpf	Heavy bacteria
40 to 50 WBC/hpf	Moderate squamous epithelial cells

   a. What discrepancies exist between the chemical and microscopic test results? State and explain a possible reason for each discrepancy.
   b. What additional chemical tests could be affected by the patient's vitamin dosage? Explain the principle of the interference.
   c. Discuss the correlation between urine color and specific gravity results, and give a possible cause for any discrepancy.
   d. State three additional reasons not previously given for a negative nitrite test in the presence of increased bacteria.

5. Results of a urinalysis collected from a 20-year-old college athlete after practice are as follows:

COLOR: Dark yellow	KETONES: Negative
CLARITY: Hazy	BLOOD: 1+
SP. GRAVITY: 1.030	BILIRUBIN: Negative
pH: 6.5	UROBILINOGEN: 1 EU
PROTEIN: 2+	NITRITE: Negative
GLUCOSE: Negative	LEUKOCYTES: Negative

   The physician requests that the athlete collect another specimen in the morning before classes and practice.
   a. What is the purpose of the second specimen?
   b. What changes would you expect in the second specimen?
   c. Is the proteinuria present in the first specimen of prerenal, renal, or postrenal origin?

6. A construction worker is pinned under collapsed scaffolding for several hours before being taken to the emergency room. His abdomen and upper legs are severely bruised, but no fractures are detected. A specimen for urinalysis obtained by catheterization has the following results:

COLOR: Red-brown	KETONES: Negative
CLARITY: Clear	BLOOD: 4+
SP. GRAVITY: 1.020	BILIRUBIN: Negative
pH: 6.5	UROBILINOGEN: 0.4 EU
PROTEIN: Trace	NITRITE: Negative
GLUCOSE: Negative	LEUKOCYTES: Negative

   a. Would hematuria be suspected in this specimen? Why or why not?
   b. What is the most probable cause of the positive blood reaction?
   c. What is the source of the substance causing the positive blood reaction and the name of the condition?
   d. Would this patient be monitored for changes in renal function? Why or why not?

7. Considering the correct procedures for care, technique, and quality control for reagent strips, state a possible cause for each of the following scenarios.
   a. The urinalysis supervisor notices that an unusually large number of reagent strips are becoming discolored before the expiration date has been reached.
   b. A physician's office is consistently reporting positive nitrite test results with negative LE test results.
   c. A student's results for reagent strip blood and LE are consistently lower than those of the laboratory staff.
   d. One morning, the urinalysis laboratory was reporting results that were questioned frequently by physicians.