Routine Urinalysis - Microscopic Examination of Urine

Objectives: Upon completion of this unit, the participant will:

• Describe routine UA microscopic exam
• Correlate common microscopic findings with possible clinical conditions
• Identify conditions that enhance urine cast formation
• Describe sources of error

Standardization of Microscopic Exam

• Strict adherence to laboratory protocol using the same:
  – Supplies
  – Sequence of procedural steps
  – Timing intervals
  – Equipment

• Ensures accuracy and precision of microscopic analysis of urine sediment

Commercial Systems

• Produce the same concentration of urine or sediment volume
• Present the same volume of sediment for microscopic analysis
• Control microscopic variables such as focal planes and optical properties of slides

7 Factors to Standardize

1. Specimen collection and handling
2. Specimen volume
3. Centrifugation
4. Sediment concentration
5. Volume of sediment examined
6. Consistent examination procedure
7. Reporting format and terminology
1. Proper Specimen
   • Collection
     – Patient prep may or may not be done
     – Random, clean catch, catheterized, etc
   • Rapid transport to lab
     – Room temperature, refrigerated specimen
   • Prompt examination
     – Within 30 minutes of collection ideal

2. Volume of urine evaluated
   • Well-mixed urine
   • 12 milliliters (10-15 mL range) routine volume analyzed

3. Centrifugation
   • Speed: 400-450 g
     – Relative centrifugal force (g)
     – Independent of centrifuge used and rotor size
     – Speed (RPM) required to attain 450g will vary in different centrifuges
     – Ensures optimal sediment concentration without disruption of fragile formed elements
   • Time: 5 minutes
   • No brake: will resuspend pellet of sediment

4. Urine Volume to resuspend Sediment
   • 12:1 ratio desired:
     – Centrifuge 12 mL of urine
     – Decant urine using pipette, leaving 1 mL urine in bottom of test tube
     – Resuspend sediment in bottom of test tube with the 1 mL urine volume retained

5. Volume of resuspended sediment to examine
   • Glass slides with coverslip: 15 microliters using calibrated pipette
   • Commercial slide: viewing chamber fills by capillary action
   • No bubbles, do not overfill

Commercial Systems
Example: KOVA slide system
6. Consistent examination

- **Minimum** ‘10-12 representative fields’
- Elements must be evenly distributed throughout slide
- If not, prepare another wet prep
- What is a Field of View (FOV)?

**Field of View (FOV)**

FOV: what you see through the ocular lens

---

**Low Power Objective**

- 10x objective
- Examine perimeter of cover-slip
- Look for
  - Squamous epithelial cells
  - Casts
  - Mucus

**Scanning the Microscope Slide**

- Use 10X objective: scan the coverslip perimeter for squamous epithelial cells, casts and mucus
- Example: begin in the upper right hand corner, down the right side then the bottom, up and over
- Remember to scan a MINIMUM of 10 FIELDS

---

**High Power Objective**

- 40x objective
- Examine center area of cover-slip
- Look for
  - RBC and WBC
  - Transitional and renal tubular epithelial cells
  - Bacteria
  - Yeast
  - Sperm
  - Trichomonads
  - Crystals

**Scanning the Microscope Slide**

- Use 40X objective, scan the interior of the slide
- Look for all other cellular elements
- Remember to scan a MINIMUM of 10 FIELDS
7. Report format and terminology

- Number per low power field:
  - 20-100 squamous epithelial cells/lpf
  - 0-3 hyaline casts/lpf
  - 5-10 granular casts/lpf

- Number per high power field:
  - 10-25 RBC/hpf
  - 5-10 renal tubular epithelial cells/hpf

Ensure Accuracy in Reporting

Microscopic results should be correlated to the physical and chemical results.

<table>
<thead>
<tr>
<th>Microscopic Elements</th>
<th>Physical (color/ clarity)</th>
<th>Chemical (dipstick)</th>
<th>Exceptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
<td>clarity, color: + blood</td>
<td>+ protein, leukocytes, nitrite</td>
<td>N/Ob, hemolysis, N/Ob, type, or WBC and bacteria</td>
</tr>
<tr>
<td>White blood cells</td>
<td>clarity</td>
<td>+ protein</td>
<td>Number</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>clarity</td>
<td>+ protein</td>
<td>Number</td>
</tr>
<tr>
<td>Casts</td>
<td>clarity, color: pH</td>
<td>+ protein</td>
<td>Number</td>
</tr>
<tr>
<td>Crystals</td>
<td>clarity</td>
<td>+ protein</td>
<td>Number</td>
</tr>
<tr>
<td>Bacteria</td>
<td>clarity, color: + esculin, pili</td>
<td>+ protein</td>
<td>Number, type</td>
</tr>
</tbody>
</table>

If red blood cells are present, the color and clarity are affected (Red/Hazy), and the dipstick should show positive blood. The exceptions are: if there are not enough RBCs or ascorbic acid is present (Red/Hazy, negative blood); or if the RBCs are lysed (Red/Clear, positive blood).

Microscopic Techniques

- Brightfield vs Phase Contrast

Red Blood Cells

- Intact RBC: hematuria
- Normal: 0-3/hpf
- Abnormal: damage to basement membrane of glomerulus, kidney infection, kidney stones, trauma

Formed Elements

- Originate throughout urinary tract
- Can be a result of damage to basement membrane, infection, disease
- Contaminant
RBC: smooth biconcave discs, no nucleus

Concentrated urine: RBCs crenate

Dilute urine: RBCs will swell

Ghost RBC

RBC's showing rouleaux

RBC can resemble:

- Yeast
- Oil droplets, air bubbles
- Calcium oxalate crystals, oval form
- In concentrated urine, RBC will crenate and resemble WBC

Air bubbles
RBC Correlation

- Correlate microscopic evaluation with
  - Physical exam
    - Color
    - Clarity
  - Chemical exam
    - Positive reagent strip
    - Ascorbic acid: causes false negative result
    - Myoglobin: causes false positive result

WBC: leukocytes

- WBC in urine: leukocyturia
- Indicates infection (bacterial, non-bacterial)
- Normal: 0-8 WBC/hpf
- Neutrophil predominant type of WBC found in urine

WBC: leukocytes

- Spherical
- Contain nucleus, granules, etc
- Approximately 2x larger than RBC
- Can be found singly or in clumps

WBC: leukocytes

- Cytoplasm contains granules
- Nucleus is segmented (lobed)
Hypotonic (dilute) Urine

- Dilute urine: WBC swell and then lyse
- Glitter cells: swollen WBCs showing brownian movement

Hypertonic (concentrated) Urine

- WBC become smaller, due to water moving out of the WBC
- Unlike RBC, WBC do not crenate

WBCs can resemble:
Renal tubular epithelial cells
Crenated RBC

WBC Correlation:

- Correlate microscopic evaluation with
  - Physical exam
    - Odor (infection)
    - Clarity
  - Chemical exam
    - Positive reagent strip for leukocyte esterase
    - Non-granular WBC (lymphocytes) will not react with reagent strip reaction (false negative)

Epithelial Cells

- Found in urine due to
  - Normal sloughing of old cells from lining of genitourinary system
  - Inflammation of the lining
  - Renal disease
  - Common contaminant in female specimens
**Epithelial Cells**

- **Three types:**
  - Squamous epithelial cells
  - Transitional epithelial cells
  - Renal tubular epithelial cells

- Normal: small amount

- Abnormal: infections, disease

**Squamous Epithelial Cells**

- Most common type of epithelial cell found in urine

- Originates in superficial lining of urethra and vagina

- Increased numbers may indicate poor collection technique

**Transitional Epithelial Cells**

- Originates in lining of renal pelvis, ureters, bladder, upper urethra

- Increased numbers may indicate urinary tract infection (UTI), collection by catheterization, malignancy

**Renal Tubular Epithelial Cells**

- Originates in lining of renal tubules

- Increased numbers indicate tubular necrosis

**Squamous Epithelial Cells**

- Largest epithelial cell found in urine

- Enumerate using low power objective

- Cells are thin and flat; central nucleus

- Fine granulation in cytoplasm that becomes dense as cell degenerates

**Clue Cells**

- Squamous epithelial cells with large amount of bacteria adhering to them giving them a 'shaggy' appearance

- Originates in vaginal mucosa, so considered vaginal contaminant; presence indicates bacterial vaginal infection
Squamous Epi vs Clue Cell

Transitional Epithelial Cells
- Size varies dependent upon location in urinary tract
- Most common type seen in urine originates in the bladder
- Generally much larger than WBC with abundant cytoplasm; **nucleus to cytoplasm ratio ~ 1:5**
- Nucleus generally centrally located
- Borders of nucleus and cytoplasm distinct

Transitional Cells
- Evaluate and enumerate using high power objective

Renal Tubular Epithelial (RTE) Cells
- Shape varies dependent upon location in urinary tract
- Cells usually are round and **slightly larger** than WBC
- Nucleus is eccentric; can be multinucleated
- Nucleus to cytoplasm ratio ~ 1:1

RTE Cells
- Evaluate and enumerate using high power objective
- Notice the ‘hard outline’ of the nucleus

Oval Fat Bodies (OFB)
- Renal tubular epithelial cell with absorbed fat
- Highly refractile due to absorbed fat; amount of absorbed fat varies
- Laboratory must see maltese cross using polarized microscopy to verify cell is OFB
- May also see free floating fat droplets
- Indicates pathology: evaluate and enumerate using high power objective
Epithelial Cell Correlation:

- Correlate microscopic evaluation with:
  - Physical exam
    - Clarity
  - Chemical exam:
    - Protein reagent strip reaction usually positive when RTE or OFB present

Type of Epithelial cell:  
- Characteristic:
  - Squamous (lpf)
    - a. Most common epithelial cell found in urine
  - Transitional (hpf)
    - b. Originates in lining of renal tubules
    - c. Often seen in ‘sheets’ of cells from catheterized urine
  - Renal tubular (hpf)
    - d. Originates in superficial lining of urethra and vagina
  - Oval fat body (hpf)
    - e. May indicate improper collection technique if increased numbers found
    - f. Originates in bladder

Casts

- Presence of casts reflect health status of renal tubules
  - Number and type of cast is significant
  - Increased amounts of casts: usually also have a positive protein dipstick

- Enumerated: lpf  →  Identified: hpf

Casts

- Normal: few hyaline or few granular casts
- Abnormal: increased number and type of cast significant
- Certain types of casts are always pathologic: RBC, WBC
- Casts are only formed in the kidney tubules: not the bladder, not urethra, not ureters
Structural Makeup of Casts

- Consists of a uromodulin matrix
- Uromodulin is a glycoprotein formerly called the Tamm-Horsfall protein
- This protein matrix does not react with the protein reagent strip test

Cast Formation Enhanced By:

- Acidity of urine
- Increased solute concentration
- Decreased urine flow rate (urine stasis)
- Presence of plasma proteins (albumin, globulins, hemoglobin, myoglobin)

Characteristics of Casts

- Cylindrical, cigar shape, parallel sides
- Vary in length and width
- Mucus and fibers can be misidentified as casts

Cast Identification/Classification

- Enumerate using low power objective;
- Identify using high power objective
- Classified by substance incorporated into cast matrix

Hyaline Cast

- Low refractive index and homogeneous matrix makes this cast very hard to see using bright field microscopy
- Phase microscopy used to enhance visualization

Adjust condenser to enhance visualization
Lower the condenser to enhance visualization of casts and mucus

Hyaline Cast
- Most common cast seen in normal individuals
- Normal: 0-2 hyaline casts/lpf
- Increased amounts seen with dehydration, fever, emotional stress, strenuous exercise

RBC Cast
- RBC inside a hyaline cast
- Cast may appear yellow to reddish-brown color due to degenerating or hemolyzing RBCs
- Significance: **pathologic** condition (not normal)

WBC Cast
- WBC inside a hyaline cast
- Identify by looking for lobed nucleus
- Significance: **pathologic** condition (not normal)

Epithelial Cell Cast
- Always renal tubular epithelial cells in hyaline matrix
- Can be misidentified as WBC cast; look for 1:1 ratio of nucleus to cytoplasm
- Significance: always **pathologic** (never normal)

Granular Cast
- Aged cellular cast: fine or coarse granules
- Significance: **pathologic** if increased
Waxy Cast
- Highly refractile, homogeneous texture, well defined edges, blunt uneven ends
- May see cracks along the length of the cast
- May appear yellow to gray to colorless
- Significance: **pathologic** (prolonged stasis)

Fatty Cast
- Highly refractile due to fat content
- Fat in the form of free fat droplets or oval fat bodies inside the cast
- Identify using polarized microscopy: look for characteristic maltese cross formation
- Significance: **pathologic** finding, often seen in Nephrotic Syndrome

Broad Cast
- Broad casts are wider than normally seen, since they are formed in the wider collecting ducts
- All types of casts may occur in this wider form
- Significance: **pathologic**

Cast Correlation
- Correlate microscopic evaluation with
  - Physical exam
    - Clarity
  - Chemical exam
    - Protein reagent strip
    - Why?
True / False: Casts are formed in the bladder
True / False: Acid pH and urine stasis are conducive for cast formation
True / False: Casts are enumerated using the high power objective
True / False: Hyaline and RBC casts are not pathologic

Crystals
- Not normally found in fresh urine
- If found in fresh urine, pathologic
- Crystals precipitate as urine cools to room temp or when urine is refrigerated
- All clinically significant crystals are found in acid urine

Crystal Formation Enhanced By
- Increased concentration of solute in urine
- Urine pH
- Urine stasis
- Temperature

Crystal Identification
- Microscopic appearance
- Urine pH

Crystal Correlation
- Correlate microscopic evaluation with
  - Physical exam
    - Color
    - Clarity
  - Chemical exam
    - pH

Crystals
- Normal acid pH crystals
- Normal alkaline pH crystals
- Pathologic crystals found in acid or neutral urine
- Drug induced crystals
Normal Acid pH Crystals

- Amorphous urates
- Uric acid
- Calcium oxalate

Amorphous Urates

- These crystals have no distinct form and appear as sand-like granules microscopically
- Macroscopically appear as a pink sediment after urine centrifugation
- Acid pH urine
- Often seen after urine is refrigerated

Uric Acid Crystals

- Acid pH urine
- Appear in several forms
- Multicolored when polarized
- Diamond shape most common form

Calcium Oxalate Crystals

- Acid pH urine
- Most frequently observed crystal in urine
- Most common form is octahedryl shape, often referred to as an ‘envelope’ shape
- Multicolored when polarized

Calcium Oxalate Crystals

Oval form, can be confused with RBC
(RBC do not polarize light)
Normal Alkaline pH Crystals

- Amorphous phosphates
- Triple phosphate
- Ammonium biurate

Amorphous Phosphates

- These crystals have no distinct form and appear as sand-like granules microscopically
- Macroscopically appear as a white sediment after urine centrifugation
- Alkaline pH urine
- Often seen after urine is refrigerated

Triple Phosphate Crystals

- Most frequently observed crystal in alkaline urine
- Colorless, 4-6 sided prisms
- Referred to as ‘coffin lid crystals’

Triple Phosphate vs Calcium Oxalate

- Alkaline pH urine
- Acid pH urine

Ammonium Biurate Crystals

- Alkaline pH urine
- Yellow spheres with spicules on surface
- Referred to as ‘thorny apple crystals’
- Significant when found in fresh urine
- Presence indicates urine is old

Pathologic Crystals (acid, neutral pH)

- Cystine
- Tyrosine
- Leucine
- Cholesterol
- Bilirubin
Cystine Crystals
- Colorless hexagonal plates
- Do not polarize
- Can be confused with uric acid crystals

Cystine vs Uric Acid Crystals
- Cystine Crystal: Acid pH urine, Very rare, Do not polarize light
- Uric Acid Crystal: Acid pH urine, Frequently seen, Multicolored when polarized

Cholesterol Crystals
- Clear, large, flat, rectangular plates with notched corners
- Multicolored when polarized
- Can be confused with radiographic dye crystals
- Also should see proteinuria and lipiduria

Leucine Crystals
- Yellow-brown spheres with concentric circles on surface (tree trunk)
- Can resemble free fat globules

Tyrosine Crystals
- Colorless or yellow-brown fine delicate needles
**Bilirubin Crystals**
- Yellow-brown small clusters of needles or granules
- Must confirm with positive icterest
- When present in urine, indicates large amount of bilirubin is present

**Bilirubin vs Tyrosine Crystals**
- Bilirubin: thicker needles, blunt ends
  - Ictotest positive
- Tyrosine: fine needles, pointy ends
  - Ictotest negative

**Drug Induced Crystals**
- Sulfonamides
- Radiographic dye (contrast media)

**Sulfa Crystals**
- Form varies dependent upon the type of sulfa drug administered

**Radiographic Dye Crystals**
- Also referred to as Contrast Media
- Colorless long pointed needles, or flat rectangular plates (resemble cholesterol crystals)
- Multicolored when polarized

**Contrast Media vs Cholesterol**
- Both crystals multicolored when polarized
- Contrast Media: specific gravity > 1.040
- Cholesterol: notched edges
True / False: Crystals are identified by shape and urine pH

True / False: Uric acid crystals are found in acid urine and are shaped like a 'coffin lid'

True / False: Presence of ammonium biurate crystals indicate the urine is old

True / False: Presence of bilirubin crystals indicate severe liver disease

Other Microscopic Elements
- Bacteria
- Yeast, mycelial elements (pseudohyphae)
- Fat
- *Trichomonas vaginalis*
- Sperm
- Mucus
- Starch, talc
- Fibers
- Glass, plastic

Bacteria
- Most often rod-shaped
- Vary in size
- Must use high power objective
- Presence may indicate
  - UTI
  - Contamination

Bacteria
- Correlate with
  - Nitrite reagent strip
- pH often alkaline
  urea $\rightarrow$ ammonia
  ammonia increases the pH

Bacteria DO NOT 'make' the urine alkaline
Bacterial enzyme convert urea to ammonia

Yeast and Mycelial Elements
- Budding forms or singly
- Ovoid and more refractile than RBC
- Will not lyse with acetic acid or KOH
- Note pseudohyphae (mycelial elements)

Fat
- Highly refractile
- Found in 3 forms:
  - Within fatty cast
  - Within oval fat body
  - Free fat droplet
Fat vs Starch

- Both polarize light
- Starch has characteristic central dimples

Trichomonas vaginalis

- Round to lemon-pear shape
- Undulating membrane and flagella provide movement
- Sexually transmitted
- Similar in size with WBC and RTE
- Can be confused with WBC

Spermatozoa

- May be seen in male and female urine
- Usually not clinically significant unless
  - Post vasectomy
  - Rape
  - Child urine

Mucus

- Low refractive index makes it difficult to see
- Wavy, delicate ribbon-like strands or threads
- Can be mistaken for hyaline cast

Starch, talc

- Contaminant
- Varies in size and shape
- Characteristic central dimple

Fibers

- Contaminant
- Large, with distinct edges
- Misidentified as casts
Fibers

Glass, Plastic
- Contaminant from
  - Glass cover slips
  - Plastic cover slips
- Misidentified as a crystal