Microscopic Examination of Urine

Roxanne Alter MS MT(ASCP)
ralter@unmc.edu

Objectives:

- Review the objectives on page 1 and 2 of the lecture handout
- Objectives marked with ‘*’ will not be tested over during student lab rotation

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

Commercial Systems

KOVA slide system

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. Volume of urine 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
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, cast 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

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

High Power Objective
• 40x objective
• Examine center area of coverslip
• Look for
  – RBC and WBC
  – Transitional and renal tubular epithelial cells
  – Bacteria
  – Yeast
  – Sperm
  – Trichomonads
  – Crystals

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/shape)</th>
<th>Chemical (digests)</th>
<th>Exceptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells</td>
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<tr>
<td>White blood cells</td>
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<tr>
<td>Epithelial cells</td>
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<td>Casts</td>
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<td>Crystals</td>
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<td>Proteins</td>
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Techniques Used to Enhance Visualization
• Stains
• Microscopic techniques
Acetic Acid, 2%
- Enhances nuclear structure of WBC
- Differentiates RBC from yeast (RBC will hemolyze)

Before Acetic Acid was added
After Acetic Acid was added

Microscopic Techniques
- Brightfield vs Phase Contrast

Microscopic Techniques
- Polarization vs Brightfield

Formed Elements
- Originate throughout urinary tract
- Can be a result of damage to basement membrane, infection, disease
- Contaminant

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

RBC: smooth biconcave discs, no nucleus
Concentrated urine: RBCs crenate

Dilute urine: RBCs will swell

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

Add 2% acetic acid: RBC will lyse, yeast will remain intact

Air bubbles
Crenated RBC can resemble WBC

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
- Approximately 2x larger than RBC

WBC: leukocytes

- Cytoplasm contains granules
- Nucleus is segmented (lobed)
- Can be found singly or in clumps
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

Hypertonic (concentrated) Urine
- Use 2% acetic acid to differentiate WBC from crenated RBC
- RBC will lyse, WBC nuclear structure accentuated

WBC: degenerative changes

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

**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; presence indicates bacterial vaginal infection

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

Casts
- Presence of casts reflect health status of renal tubules
- Normal: few hyaline or few granular casts
- Abnormal: increased number and type of cast significant

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
Cast Identification/Classification

- Youngest cast is the hyaline, oldest is waxy
- Cast becomes waxy as the cast ages and substances inside the cast degenerate

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

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)

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

Phase

Hyaline cast

RBC

WBC
Granular Cast
• Aged cellular cast: fine or coarse granules
• Significance: pathologic

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)

Waxy Cast

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

Fatty Cast

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

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

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

Oval form, can be confused with RBC (RBC do not polarize light)
Normal Alkaline pH Crystals
- Amorphous phosphates
- Triple phosphate
- Ammonium biurate
- Calcium carbonate

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

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

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

Calcium Carbonate Crystals
- Alkaline pH urine; very small colorless granules, slightly larger than amorphous material
- Multicolored when polarized
- Easily confused with bacteria
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

<table>
<thead>
<tr>
<th>Cystine Crystal</th>
<th>Uric Acid Crystal</th>
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<tbody>
<tr>
<td>Acid pH urine</td>
<td>Acid pH urine</td>
</tr>
<tr>
<td>Do not polarize</td>
<td>Multicolored when polarized</td>
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</tbody>
</table>

Cholesterol Crystals

- Clear, large, flat, rectangular plates with notched corners
- Multicolored when polarized
- Can be confused with radiographic dye crystals

Leucine Crystals

- Yellow-brown spheres with concentric circles on surface
- 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 ictotest
- When present in urine, indicates large amount of bilirubin is present

Bilirubin vs Tyrosine Crystals

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

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
- Correlate with
  - Nitrite reagent strip

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

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

Early microscopes