MICROSCOPIC EXAMINATION OF URINE

Objectives: Upon completion of this lecture the Physician Assistant student will be able to:

1. Assess the steps used to standardize the microscopic analysis of urine.

2. Analyze the use of the Bright field microscope when examining urine sediment:

3. Discuss the formation and composition of urinary casts.

4. Identify the following structures found in urine by microscopic exam, photographs, digital pictures or word descriptions:
   a. Red blood cells
   b. White blood cells
   c. Epithelial cells
      1) Squamous
      2) Transitional (bladder)
      3) Renal
   d. Casts
      1) Hyaline
      2) Red blood cell
      3) White blood cell
      4) Renal tubular epithelial cell
      5) Granular
      6) Waxy
   e. Crystals
      1) Normal acid
         a) Calcium oxalate
         b) Uric acid
         c) Amorphous urates
      2) Normal alkaline
         a) Triple phosphate
         b) Ammonium biurate
         c) Amorphous phosphates
      3) Pathologic
         a) Cystine
         b) Tyrosine
         c) Leucine
         d) Cholesterol
         e) Bilirubin
      4) Drug induced
         a) Sulfonamides
         b) Radiographic dye
   f. Bacteria
   g. Yeast
   h. Parasites
      1) Trichomonas vaginalis
i. Spermatozoa
j. Mucus
k. Extracellular fat droplets
l. Artifacts (e.g. starch, fibers, glass)

5. Assess the clinical significance of the microscopic structures listed in Objective #4.

6. Correlate physical and chemical urinalysis results with microscopic observations.
I. Standardization
   A. Ensures accuracy and precision of examination

   B. **There are 7 factors that should be standardized in the performance of the microscopic examination of urine:**

   1. Proper specimen
      a. Proper collection
      b. Rapid transport and prompt examination

   2. Standard volume of urine evaluated = 12mls

   3. Proper Centrifugation
      a. Speed = 400-450 g
      b. Length of time = 5 minutes
      c. No brake (disrupts sediment)

   4. Standard volume of urine to resuspend sediment = 1 ml
      (ideal 12:1 ratio of urine to sediment)

   5. Standard volume of resuspended urine examined
      a. Glass slide with coverslip
      b. Commercial system: Kova, Count-10

   6. Consistent Examination: Field of View (FOV)
      a. Must evaluate a **minimum** of 10 representative fields
         1) Increasing the number of fields evaluated increases the validity of the value reported
         2) Elements must be evenly distributed throughout the slide, else prepare a new wetmount

         - Low power:
           Examine coverslip perimeter

         - High power:
           Examine center
7. Standardized report format and terminology
   a. Low power field (LPF): objective = _____10X____
      1) Squamous epithelial cells
      2) Casts
      3) Mucus
   b. High power field (HPF): objective = _____40X____
      1) RBC, WBC, transitional and renal epithelial cells
      2) Bacteria, crystals
      3) Yeast, sperm, trichomonads

C. To ensure accuracy in reporting, the 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</td>
<td>+ blood</td>
<td>Number, hemolysis</td>
</tr>
<tr>
<td>White blood cells</td>
<td>clarity, color</td>
<td>+ protein, leukocytes, nitrite</td>
<td>Number, type of WBC and bacteria</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>clarity</td>
<td>+ protein</td>
<td>Number</td>
</tr>
<tr>
<td>Casts</td>
<td>clarity</td>
<td>pH</td>
<td>Number</td>
</tr>
<tr>
<td>Crystals</td>
<td>clarity, color</td>
<td>+ nitrite, pH</td>
<td>Number, type</td>
</tr>
<tr>
<td>Bacteria</td>
<td>clarity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

II. Microscopic Enhancing Techniques
   A. Microscopic Techniques: refractive index of urine and many components in urine are similar and lack sufficient contrast for optimal viewing
      1. Bright-field: hyaline casts, mucous threads, bacteria difficult to see
      2. Phase Contrast Microscopy
         a. Enhances viewing of translucent or low-refractile elements by converting variations in refractive index to variations in contrast
         b. Microscopic evaluation of sediment easier and faster compared to brightfield
      3. Polarizing Microscopy: most often used to confirm presence of fat (will show characteristic ‘maltese cross’ pattern) and crystals (uric acid)

III. Formed Elements in Urine Sediment
   A. Where do they come from?
      1. Anywhere throughout the urinary tract (from glomerulus to the urethra)
      2. Result from damage to basement membrane, infection, disease
      3. Contamination: menstrual/hemorrhoid blood, sperm, fibers, starch
      4. Identifying and enumerating components provides diagnostic tools
   B. Red blood cells (erythrocytes)
      1. Significance: Increased amount of intact red blood cells in the urine is called hematuria
         a. Associated with vascular injury or glomerular membrane damage
         b. Also seen with menstruation or strenuous exercise
2. Normals: 0-3 RBC/hpf (identified on **high power** due to their small size)

3. Abnormals: glomerulonephritis, pyelonephritis, cystitis, toxic and immunologic reactions, malignancies, renal calculi, trauma

4. Identification: appearance is affected by the specific gravity and pH (dilute urine ~ low specific gravity; conc urine ~ high specific gravity)
   a. Smooth biconcave discs (~ 7 microns), no nucleus
   b. Cells crenate in hypertonic (concentrated) urine
   c. Cells swell and lyse in hypotonic (dilute), alkaline urine

5. RBCs can resemble
   a. **Yeast**: tend to be spherical or ovoid, vary in size, often show ‘budding’; add 2% acetic acid to differentiate
      (RBC hemolyze, yeast will not hemolyze)
   b. **Oil droplets or air bubbles**: variations in size
      (RBC uniform in appearance)
   c. **Oval form of calcium oxalate crystal**: add 2% acetic acid, RBC will hemolyze
   d. **WBC in hypertonic (concentrated) urine**: WBC lose water, become smaller; add 2% acetic acid to enhance nucleus (in hypertonic urine, crenated RBC)

6. Correlate with Physical and Chemical Exam:
   a. Color
   b. Clarity
   c. Reagent test strip (blood)
      1) Ascorbic acid (vitamin C): false negative
      2) Myoglobin (muscle protein): false positive

C. **White blood cells (leukocytes)**
1. Significance: An increase in urinary white blood cells is called **leukocyturia**
   a. Indicates infection or inflammation in the genitourinary system: both bacterial and non-bacterial infections
   b. Almost all renal diseases show increased WBC in urine
   c. WBC migrate through tissues to the site of infection/inflammation and can enter the urinary tract at any point

2. Normals: 0-8 WBC/hpf (identified on **high power** due to their small size)
   There are different types of WBC found in plasma, thus different types of WBC can be found in urine. Since neutrophils are the predominant WBC in plasma, neutrophils are the predominant cell in urine
3. Abnormals:
   a. Bacterial infections: pyelonephritis, cystitis, prostatitis, urethritis
   b. Non-bacterial: glomerulonephritis, chlamydia, yeast, trichamonads, lupus erythematosus, tumors

4. Identification of Neutrophils
   a. Spherical, granules in cytoplasm, lobed (segmented) nucleus
   b. ~10-14 microns in diameter (roughly 2x larger than RBC)
   c. Can be found singly or aggregated in clumps (hard to enumerate)
   d. Hypotonic (dilute) urine
      1) WBC swell and become spherical balls that lyse rapidly (50% in 2-3 hours at room temperature)
      2) "Glitter cells" - sparkling appearance, granules moving in swollen cells (brownian movement of granules)
   e. Hypertonic (concentrated) urine: become smaller, but do not crenate (use 2% acetic acid to differentiate from crenated RBCs)
   f. ‘Blebs’ (vacuoles) and finger/wormlike projections (myelin forms) reflect degenerative changes as cell membrane breaks down

5. WBCs can resemble
   a. Renal tubular epithelial cells: nucleus to cytoplasm ratio ~ 1:1
   b. Small WBC ~ crenated RBC: add 2% acetic acid to define nuclear structure of WBC

6. Correlate with Physical and Chemical Exam:
   a. Odor: depends on extent of infection
   b. Clarity
   c. Reagent strip test (leukocyte esterase)
      1) Lymphocytes: false negative

7. Other white blood cells
   a. Lymphocytes: rare in normal urine; most often seen with renal graft rejection
   b. Monocytes and macrophages (histiocytes)
   c. Eosinophils

D. Epithelial Cells
1. Significance: seen in urine due to sloughing of old cells from the lining of the genitourinary system or from damage caused by inflammatory process or renal disease
   a. Squamous cells: Originate in the superficial lining of the urethra and vagina; most common type of epithelial cell found in urine. Increased numbers may be seen as a contaminant in females indicating improper collection technique. This type of epithelial cell is ‘not exclusively renal’, and usually is diagnostically insignificant.
   b. Transitional (urothelial) cells: Originate in the lining of the renal
pelvis, ureters, bladder and upper urethra. Normal to see rare to few in urine as a result of normal sloughing. Seldom pathologically important unless large numbers exhibiting unusual morphology is seen: UTI, post-catheterization (cells will be found ‘stuck together’ in sheets), malignancy. If sheets of transitional cells are seen but is not a result of catheterization, then pathology is indicated.

c. **Renal tubular cells**: Originate in the linings of the renal tubules. Renal tubular cells are the most significant as increased numbers indicate tubular necrosis (pathology). Normal to see rare to few in urine as a result of normal sloughing. Newborn infants have more renal tubular cells in urine as compared to older children and adults.

2. Normals: rare to few

3. Abnormals: pyelonephritis, toxic reactions, viral infections, transplant rejection, secondary effects of glomerulonephritis, UTI

4. Identification:
   a. Squamous epithelial cells
      1) Largest epithelial cell (40-60 microns) thus identified on **low power**.
      2) Thin, flat having ‘flagstone’ appearance, distinct edges
      3) Small centrally located nucleus with small amount of cytoplasm (fried egg appearance); can be a-nucleated
      4) Fine granulation in cytoplasm which becomes more dense with degeneration
      5) Clue Cells:
         a) Squamous epithelial cells from vaginal mucosa with large numbers of bacteria adhering to them.
         b) Clue cells appear soft and finely granular with distinct ‘shaggy edge’ borders (bacteria adhering to edge)
         c) Indicates bacterial vaginosis, a synergistic infection most often with *Gardnerella vaginalis*, resulting in a foul smelling vaginal discharge
   b. Transitional epithelial cells
      1) Vary in size depending upon their location in urinary tract; evaluated using **high power**
      2) Most common type originates in the uppermost or superficial layer of epithelium in bladder
         a) 30-40 micron in size (a little bit larger than a WBC)
         b) Round or pear-shaped; dense oval-to-round nucleus about the same size of a RBC or WBC.
         c) **Abundant cytoplasm**: nucleus to cytoplasm ratio is approximately 1:5
         d) The peripheral borders of the nucleus and cytoplasm are distinctly outlined.
3) Cells originating from intermediate layers of epithelium appear smaller, rounder (20-30 micron)
4) Cells originating from the single basal layer of epithelium tend to be elongated or columnar

c. Renal tubular epithelial cells
1) Vary in shape depending upon location in urinary tract; only epithelial cell that is renal in origin; evaluated using high power
2) May need cytological evaluation to identify source location (PCT, DCT, collecting ducts)
3) Generally, cells are round and slightly larger than WBC
4) Nucleus with dense chromatin pattern, usually eccentric; can be multinucleated
5) Nucleus to cytoplasm ratio approx 1:1

d. Oval fat bodies (OFB)
1) Renal tubular epithelial cells that are ‘engorged’ with absorbed lipids (fat).
2) Oval fat bodies are highly refractile due to absorbed lipids. The amount of absorbed lipids per cell can vary considerably
3) Positively identified using polarized microscopy: look for maltese cross formation.
4) Can be seen along with ‘free floating’ fat droplets which also will show maltese cross using polarized light
5) Indicate pathologic condition: glomerular dysfunction with renal tubular cell death and leakage of plasma components (protein and fat) into the urine.

5. Correlation with Physical and Chemical Exam
a. Clarity
b. Reagent strip: if RTE or OFB are present, then protein and casts should also be present

<table>
<thead>
<tr>
<th>Type of epithelial cell</th>
<th>Matching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous (lpf)</td>
<td>a. Most common epithelial cell found in urine</td>
</tr>
<tr>
<td></td>
<td>b. Originates in lining of renal tubules</td>
</tr>
<tr>
<td>Transitional (hpf)</td>
<td>c. Often seen as ‘sheets’ of cells from catheterized urine</td>
</tr>
<tr>
<td></td>
<td>d. Originates in superficial lining of urethra and vagina</td>
</tr>
<tr>
<td>Renal tubular (hpf)</td>
<td>e. May indicate improper collection technique if increased numbers found in urine</td>
</tr>
<tr>
<td></td>
<td>f. Originates in upper urethra, bladder, ureters, renal pelvis</td>
</tr>
<tr>
<td>Oval fat body (hpf)</td>
<td>g. RTE with absorbed fat</td>
</tr>
</tbody>
</table>
E. **Casts**: Enumerated using **low power** and identified/classified using high power

1. Significance: Casts reflect status of renal tubules; extent and severity of renal disease correlates with type and number of casts present. Increased numbers usually seen with increased urinary protein levels (due to a glomerular problem) or urinary stasis (due to a blockage or disease)

2. Normal: few hyaline or granular casts can be seen in normal individuals

3. Abnormal: increased **number and type**: certain casts are always pathologic
   a. Renal disease
   b. Can be associated with diuretic therapy
   c. Athletic pseudonephritis: Can occur post-exercise, post marathon running (30-50 hyaline or granular casts/lpf) but will go away quickly (24-48 hrs); does not indicate renal disease

4. Structural makeup of casts
   a. Structure consists of a uromodulin matrix (a glycoprotein formerly called **Tamm-Horsfall protein**) that is secreted by the ascending loop of henle, distal and collecting tubules
   b. Casts are **formed only in the kidney**
   c. **Dipstick protein detects mainly albumin**; it DOES NOT detect the protein making up the cast (uromodulin protein)
   d. Matrix forms fibrin strands in tubules that hold cast in place and trap elements forming the cast; eventually cast detaches and is sloughed into urine

5. **Factors that enhance cast formation**:
   a. Acid pH urine
   b. Increased solute concentration
   c. Decreased rate of urine flow (urinary stasis)
   d. Presence of increased plasma proteins (particularly albumin)

6. Characteristics of casts
   a. Since casts are formed in the tubules, casts are cylindrical with parallel sides, vary in length and width (cigar, submarine shape)
   b. Ends may be round, blunt or broken
   c. May have cellular components included **inside the cast**
   d. Wider if formed in collecting ducts: called broad casts
   e. **Casts can be confused with mucus threads, fibers**

7. Identification:
   **Enumerated using low power, identified/classified using high power**

   Classified by matrix composition and type of substances inside the cast
   Must make sure the ‘substances’ are **inside the cast** and not sitting on top of a hyaline cast or mucus thread.
Can see casts containing more than one formed element or can be of two different matrix types. Identify this type of cast using the term that has the most clinical significance

The ‘youngest’ cast is the hyaline; ‘oldest’ is the waxy cast

a. **Hyaline Casts**
   1) Low refractive index makes this cast very hard to see using bright-field microscopy; increase contrast with phase microscopy
   2) Homogenous matrix, acellular (or may have few granules) 
   Cylindroid = hyaline with a tail at one end, not significant
   3) Can be confused with mucus thread
   4) Most commonly observed cast: normally 0-2/lpf
   5) Increased numbers seen after strenuous exercise, dehydration, fever, emotional stress. Will see increased hyaline casts along with pathological casts as in renal disease

b. **Red Blood Cell Casts**
   1) Contain clearly discernible RBC's.
   2) Some casts will be tightly packed with RBC's, others may have only several RBC’s contained in protein matrix (hyaline)
   3) Cast color may appear yellow to red-brown (due to degeneration or lysing of RBC as cast ages)
   4) As cast ages it becomes granular in appearance
   5) RBC casts in the urine usually indicate bleeding from within the nephron (intrinsic renal disease); **pathologic**
   a) Primary cause is glomerulonephritis (RBC pass across glomerular membrane)
   b) Tubular damage (interstitial nephritis)
   c) Can be seen following contact sports, post strenuous exercise (athletic pseudonephritis)
   d) Incompatible blood transfusions (hemoglobin cast:

c. **White Blood Cell Casts**
   1) Consist of white blood cells in the protein matrix (hyaline)
   2) Readily identifiable, unless WBC are degenerating; then can be misidentified as renal tubular epithelial cell cast.
3) The presence of WBC, free-floating or in clumps, in the urine can suggest strongly that the cast is a WBC cast. Look for lobed nucleus for WBC cast. Can be confused with RARE RTE cast: look for nucleus to cytoplasm ratio 1:1 for RTE cast.

4) Present in infection or inflammation: pathologic
   a) Pyelonephritis: bacteria also present (recall WBC migrate to site of infection)
   b) Glomerulonephritis: RBC cast and protein present

d. Epithelial Cell Casts (rare)
   1) Consist of renal tubular cells in the protein matrix (never squamous or transitional)
   2) Can be misidentified as WBC cast; staining and phase microscopy needed to look at the nucleus to distinguish if RTE or WBC cast
   3) Look for nucleus to cytoplasm ratio 1:1 for RTE cast, lobed nucleus for WBC cast
   4) Epithelial cell cast appears when tubular damage is present (glomerulonephritis, pyelonephritis), often accompanied by proteinuria and granular casts. pathologic

e. Granular Casts
   1) May appear as finely or coarsely granular, no intact cells are visible.
   2) Seen in renal disease; cause of granular cast formation varies: pathologic
      a) Cellular cast that has remained in tubule due to urine stasis (cells degenerate to granules)
      b) Degeneration of RTE cells that have released intracellular components that become enmeshed into cast matrix
      c) Can see occasional fine granular cast in normal individuals, following exercise or stress. These granular casts are unrelated to cellular casts

f. Waxy Casts
   1) Highly refractile, with a homogenous texture, well defined edges, and blunt, uneven ends
   2) Often see cracks along the length of the cast
   3) Appear yellow, grayish or colorless
4) Can be confused with fiber; glass and plastic artifacts

5) Indicate tubular obstruction with prolonged stasis (48 hrs+) Thought to be advanced stage of other casts that transform to waxy during urine stasis. **pathologic**
   a) Tubular obstruction, prolonged stasis
   b) Chronic renal failure
   c) Acute renal disease: glomerulonephritis, nephrotic syndrome
   d) Malignant hypertension, renal allograft rejection.

   g. **Fatty Casts**
   1) Highly refractile due to fat content
   2) Contain free fat globules (and sometimes OFB) inside cast matrix (hyaline, granular)
   3) Fat globules often appear light yellow to brown
   4) Polarized microscopy to identify: fat will polarize; cholesterol will form characteristic maltese cross (triglyceride will not form maltese cross)
   5) Can use Sudan III or Oil Red O stain (triglycerides stain orange-red; cholesterol does not)
   6) Found in numerous renal diseases, especially **nephrotic syndrome** and significant proteinuria; severe crushing injury with disruption of body fat. **pathologic**

   h. **Broad Casts**
   1) Identification: all types of casts may occur in this wider, much larger form
   2) Significance: formed in the collecting ducts; **pathologic**
      a) Urine flow is severely compromised (stasis); presence of many broad casts = poor prognosis
      b) Occur in renal failure, glomerulonephritis, nephrotic syndrome

8. **Correlation with Physical and Chemical Exam:**
   a. Clarity
   b. Reagent strip: proteinuria

   | 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 (40x) |
   | True / False: Hyaline and RBC casts are not pathologic |
F. **Crystals**: evaluated using **high power**

1. **Significance**:
   a. Crystals are usually not clinically significant
   
   b. Normally not found in **freshly voided urine**; will precipitate as urine cools
   
   c. When found in freshly voided urine, indicates in-vivo formation and is always clinically significant **pathologic**
   
   d. All clinically significant crystals are found in acidic urine; the relatively few abnormal types represent such disorders as liver disease, inborn errors of metabolism or renal damage

2. **Factors contributing to crystal formation**
   a. Increased concentration of solute in urine
   b. **pH** of urine
   c. Flow of urine through tubules (stasis) allowing time for formation
   d. Temperature (**in vitro**: room temperature or refrigerator)

3. Crystals are formed by the precipitation of urine solutes: urine becomes concentrated as it flows through tubules (this is a normal process). If increased amount of solute is present due to dehydration, dietary excess, or medication, the urine becomes supersaturated resulting in crystal formation

4. Identified by microscopic appearance and **pH** of urine

5. **Correlation with Physical and Chemical Exam**:
   a. **Color**
   b. **Clarity**
   c. **Reagent strip**: **pH**

6. **Normal crystals found in acid urine**
   
   a. **Amorphous urates**
      
      1) Urate salts (sodium, potassium, magnesium, calcium) can precipitate in amorphous (non-crystalline) forms
      
      2) Microscopically appear as small ‘sand-like’ granules
      
      3) Uroerythrin (pigment) readily deposit on these granules giving sediment a pink-orange (‘brick-dust’) color
      
      4) Formation enhanced when urine refrigerated; readily dissolve at alkaline pH or heating to 60°C
      
      5) Similar to amorphous phosphates: **use pH to differentiate**
b. **Uric acid**
   1) Occur in several forms (pleomorphic), singly or clusters and layer or laminate on top of one another
   2) Yellow to orange-brown color with intensity depending on thickness of crystal; **multicolored when polarized**.
   3) Diamond shape most common form; rhombic plates, rosettes, wedges, needles, barrels; can be misidentified as cystine crystals when they have six sides
   4) Uric acid is a normal urine solute that originates in plasma as a result of purine nucleoside (adenosine and guanosine from RNA and DNA) catabolism
   5) Usually considered a ‘normal crystal’ but can be associated with gout and chemotherapy

c. **Calcium oxalate**
   1) Most frequently observed crystal in urine; most common form is octahedral or envelope shape; size varies
   2) Small, ovoid or dumbbell shape is less common; can be misidentified as RBC (add 2% acetic acid to differentiate, RBC will hemolyze); multicolored when polarized
   3) Foodstuffs high in ascorbic acid: tomatoes, asparagus, spinach, rhubarb and citrus fruits
   4) **Seen in ethylene glycol poisoning (ovoid form)**

7. **Normal crystals found in alkaline urine**

   a. **Amorphous phosphates**
      1) Non-crystalline form of phosphates resemble fine, colorless grains of sand.
      2) Sediment appears white; soluble in acid, does not dissolve at 60°C
      3) Very similar to amorphous urates; use pH to differentiate

   b. **Triple phosphate**
      1) Most common crystal seen in alkaline urine
      2) Colorless, 4 to 6-sided prisms resembling “coffin lids”; not all perfectly formed, and size varies
      3) Upon standing will begin to dissolve and appear feathery, resembling fern leaf
4) Generally not clinically significant; associated with UTI (alkaline pH) and calculi formation

c. **Calcium phosphate**
   1) Distinctly different crystalline shapes: colorless thin wedge-like prisms arranged in rosettes or thin, long needles arranged in bundles or irregular, granular sheets/flat plates, latter form sometimes seen floating on top of urine specimen.
   2) Not clinically significant

d. **Ammonium biurate**
   1) Yellow-brown spheres with striations on surface and irregular spicules (projections) giving a ‘thorny apple’ appearance
   2) Most often seen in urine improperly handled (prolonged storage) and clinically not significant
   3) Clinically significant if found in fresh urine (iatrogenically induced alkalinization) because in vivo precipitation causes renal tubular damage; presence indicates inadequate hydration of patient
   4) Can resemble some forms of sulfonamide crystals (urine pH to differentiate)

e. **Calcium carbonate**
   1) Very small colorless granules, slightly larger than amorphous material; often found in pairs giving them dumbbell shape
   2) Can be confused with bacteria, amorphous material
   3) Multicolored when polarized

8. **Abnormal crystals found in acid or neutral urine** (usually not alkaline)

   a. **Cystine**
      1) Colorless hexagonal plates, often layered or laminated; do not polarize
      2) Can confuse with uric acid (uric acid polarize)
      3) **Clinically significant:** indicates congenital cystinosis or cystinuria. (associated with inborn errors of metabolism) Crystals deposit in renal tubules as calculi causing renal tubular damage
b. **Cholesterol**  
1) Clear, flat, large, rectangular plates with notched corners; multicolored when polarized.  
2) Accompanied with proteinuria and lipiduria (free floating fat, oval fat bodies, fatty casts)  
3) Can confuse with radiographic dye (also polarize)  
   Differentiate by specific gravity (radiographic dye >1.040) and presence of proteinuria and lipiduria  
4) **Clinically significant:** seen in nephrotic syndrome  
c. **Leucine**  
1) Yellow-brown spheres with concentric circles or radial striations on surface; highly refractile  
2) Can resemble fat globules (leucine does not stain with a fat-specific stain, and does not show maltese cross formation)  
3) Amino acid crystal is **clinically significant**  
   a) Indicates rare inherited metabolic disorder (inborn error in metabolism = IEM)  
   b) Severe liver disease  
   c) Must confirm before reporting  
d. **Tyrosine**  
1) Colorless or yellow fine delicate needles; often form clusters but can appear singly  
2) Amino acid crystal is **clinically significant**  
   a) Indicates rare inherited metabolic disorder (inborn error in metabolism = IEM)  
   b) Severe liver disease  
   c) Must confirm before reporting  
e. **Bilirubin**  
1) Yellow-brown, small clusters of fine needles or granules  
2) Crystals indicate large amount of bilirubin present in urine  
3) Must confirm bilirubin with positive dipstick or ictotest  
4) Associated with severe liver disease  
f. **Drug-induced crystals**  
1) Iatrogenic cause: induced in patient as a result of treatment, medications  
2) Identification important because if in-vivo crystal formation, can result in tubular damage
a) Obtain list of patient medications  
b) Determine if recent infusion of medications or dyes has occurred  

3) **Sulfonamides**  
a) Form varies and is dependent upon the type of drug administered. Recent sulfa drugs are more soluble than past drugs, thus are not seen as much  
b) Yellow to brown, bundles of needles that resemble sheaves of wheat  
c) Brown rosettes or spheres with irregular striations  
Can resemble ammonium biurate crystals  
d) Acetylsulfadiazine, Bactrim, Septra  

4) **Radiographic contrast media/dyes**  
a) Shape varies: colorless, long pointed needles or flat, long rectangular plates resemble cholesterol crystals  
b) **Multicolored when polarized**  
c) Contrast media specific gravity >1.040  
d) Renografin, Hypaque, Cystografin, Renovist  

| 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 often indicates the urine is old |
| True / False: Presence of bilirubin crystals indicates severe liver disease |

F. Other microscopic elements  
1. **Bacteria**  
a. Most often see rod-shaped (bacilli) but can also see coccid forms; vary in size and shape; identified using **high power**  
b. Motility often differentiates bacteria from amorphous material  
c. May be due to contamination from vagina or GI tract; or may indicate poor specimen handling (usually no WBC)  
d. Normal healthy urine is sterile; presence of bacteria implies UTI (usually WBC also present) or contamination
e. Correlate with other results:
   1) Reagent strip test: nitrites
   2) pH often alkaline: urea → ammonia (urease)

2. **Yeast**
   a. Ovoid, colorless cells; can resemble RBC (add 2% acetic acid to lyse RBC; **reported as present**)
   
   b. Yeast are more refractile than RBC, and often show budding forms and pseudohyphae (referred to as mycelial elements)
   
   c. Presence of yeast implies vaginal yeast infection with contamination of urine occurring during urine collection
   
   d. Yeast can be the primary cause of UTI (although rare)
   
   e. Pregnancy, use of oral contraceptives, diabetes mellitus promote development of vaginal yeast infections. Immunosuppressed patients often have systemic yeast infections
   
   f. Most common is *Candida albicans* (show budding and pseudohyphae, referred to as mycelial elements)

3. **Fat, lipid**
   a. Highly refractile globules or spheres, vary in size and can appear colorless, yellow-green or brownish
   
   b. Found in urine in 3 forms: free-floating fat globules, within oval fat bodies, within cast matrix (fatty cast)
   
   c. Type of fat present can also vary:
      1) Triglycerides: stain orange-red with Sudan III or Oil Red O
      2) Cholesterol: show birefringence; maltese cross pattern using polarized microscopy
   
   d. **Lipiduria is clinically significant:** **need to also see proteinuria**
   
   e. Starch, RBC can resemble fat
      1) Starch: not spherical, has central dimple, maltese cross
      2) RBC do not vary in size, are not birefringent and will not stain with fat specific stains
   
   f. Can be contaminant: catheter lubricants, topical ointments, creams, lotions, immersion oil (from oil objective)
4. **Trichomonas vaginalis**  
   a. Lemon/pear shaped protozoan flagellate  
   b. Undulating membrane and flagella  
   c. Characteristic flitting or jerky motion; once in urine, trichomonads begin to die, need fresh urine to identify; **reported as present**  
   d. Similar in size: WBC, RTE  
   e. Sexually transmitted; most common parasitic gynecological infection in females; can be seen in male urine

5. **Spermatozoa**  
   a. May be seen in female and male urine  
   b. Usually not clinically significant when found in urine (rape, child)

6. **Mucus**  
   a. Wavy, delicate, ribbon-like strands with irregular or serrated ends. Can be mistaken for hyaline cast  
   b. Low refractive index makes them difficult to see with Brightfield microscopy; identified on **low power**  
   c. Produced in renal tubular epithelium, genitourinary tract and vaginal epithelium

7. **Starch**  
   a. Vary greatly in size and shape, **centrally located dimple**; not perfectly round, have scalloped or faceted edges  
   b. Maltese cross (with polarized microscopy) is less defined when compared to maltese cross seen with fat  
   c. **Contaminant** from body powders, protective gloves (PPE):

8. **Fibers**  
   a. Hair, cotton, fabric threads; fecal contaminant (vegetable fibers, muscle fibers from ingested food)  
   b. **Contaminant** that is multicolored when polarized  
   c. Large with distinct edges, moderately refractile  
   d. Misidentified as cast: fibers tend to be fat and thicker at the margins

9. **Glass:** contaminant

10. **Plastic:** contaminant