Unit 2; Session 1

Urine Microscopic Examination

Microscopic Examination of Urine

- The Complete Urinalysis
  - Physical properties
    - already covered
  - Chemical analysis
    - in the next unit
  - Microscopic
    - our current focus

Microscopic Examination of Urine

- Urine sediment
  - all of the solid / insoluble materials suspended in the urine
  - Blood cells - Red and White
  - Epithelial cells
  - Casts
  - Bacteria & Yeast parasites
  - Spermatozoa
  - Mucus
  - Crystals & Artifacts
- Least standardized, most time-consuming
Microscopic Examination of Urine

- **Significance of formed elements in the urine**
  - Well performed microscopic exam can provide information nearly equivalent to a biopsy.
  - Ongoing controversy as to when / if to perform the microscopic exam.
- **To qualify for microscopic, the urine must meet specific standards:** based on physical properties or chemical results
  - Color, clarity, blood, protein, nitrite, leukocyte esterase, and possibly glucose
  - Special populations: pregnant women; pediatric, geriatric, diabetic, immunocompromised, renal patients

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Microscopic Examination of Urine

- **Clinical and Laboratory Standards Institute (CLSI)**
  - Requested by the physician
  - Laboratory-specified population
  - Any abnormal physical or chemical result
    - Laboratory criteria are programmed into automated instrumentation

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Microscopic Examination of Urine

- **Macroscopic Screening & Chemical Sieving**
  - Correlation of findings from physical & chemical analysis with expectations in microscopic.

<table>
<thead>
<tr>
<th>Test</th>
<th>What to look for</th>
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<tbody>
<tr>
<td>Color &amp; Clarity</td>
<td></td>
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<tr>
<td>red, pink / hazy or cloudy</td>
<td>RBCs</td>
</tr>
<tr>
<td>White / hazy or cloudy</td>
<td>WBCs</td>
</tr>
<tr>
<td>Positive nitrite</td>
<td>WBCs / bacteria</td>
</tr>
<tr>
<td>Positive Leukocyte esterase</td>
<td>WBCs, WBC casts, bacteria</td>
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<tr>
<td>Positive Glucose</td>
<td>yeast</td>
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Microscopic Examination of Urine

- **Specimen requirements**
  - **Collection of specimen**
    - Prefer the concentrated first morning specimen, collected = mid-stream, clean catch
    - first morning most concentrated and will be able to demonstrate the most abnormalities.
    - Mid stream, clean catch technique will eliminate fecal & vaginal contamination
  - Container must be clean and free of lint / debris
    - usually disposable plastic, must be sure no soap residue
  - Fresh - tested within 2 hours of voiding, or refrigeration needed.

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Microscopic Examination of Urine

- Sources of Variation
  - Collection method
  - Centrifugation time and speed
  - Re-suspension of sediment
  - Type of microscope slide
  - Viscosity of specimen
  - Reporting of the results

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Microscopic Examination of Urine

- **Preparation of specimen (standardized)**
  - Mix specimen well
  - Pour specified volume into urine centrifuge tube
  - Not enough specimen? (VARIES - FOLLOW PROTOCOL)
    - If < 1 mL – perform microscopic on unspun sample and note the report form
    - If 1 – 6 ml – spin down entire sample and note on report form
    - If 6-11.5 mL – add saline and account for dilution
Microscopic Examination of Urine

• Preparation of specimen
  • Mix specimen well
  • Pour 12 ml into urine centrifuge tube
  • Centrifuge five minutes, 1200-2000 RPM (speed varies depending on the centrifuge's characteristics)
    • Speed and time should be consistent. The "relative centrifugal force" is important.

Microscopic Examination of Urine

• Pour off supernatant - except last .5-1 mL. have pipettes that assist
• Re-suspend sediment - mix gently, but well. tap, or use pipette provided

Microscopic Examination of Urine

• Preparing to view the sediment
  • Glass slide method:
    • 20 μL
    • 22 x 22 mm glass cover slip
    • Do not overflow cover slip
      • Heavier elements (casts) flow outside
      • Increased chances for variability
Microscopic Examination of Urine

- **Commercial systems**
  - Evaluate sediment in a chamber standardized for given volume and depth field
    - UniSystem - slide on right
    - KOVA System - slide below
    - Count 6 or Count ID
  - all have their ‘own brand’ of tubes, pipettes, stain, slides, etc.
  - Authors also mentions several other ‘all in one-type of systems’
  - Use standardized reporting format consistent with other techs in the institution

- **Sternheimer and Malbin - crystal violet, safranin-O**
  - Sedi-Stain & KOVA stain are commercial preparations with addition of stabilizers to prevent precipitation.
  - Supra-vital stain used to increase visibility of structures.
  - Assists greatly in differentiating renal tubular epithelial cells
    - which will take on an eosinophilic - orange cytoplasm & dark purple nuclei) from transitional epithelial (which are more overall blue)

### Enhancement | Purpose
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Toluidine blue</td>
<td>nuclear structure - assists in differentiating WBC from renal epithelial cells</td>
</tr>
<tr>
<td>2% acetic acid</td>
<td>removes interfering RBCs and enhances nuclei of WBC</td>
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<tr>
<td>Hansel stain</td>
<td>methylene blue and eosin Y stains eosinophilic granules - ID eosinophils</td>
</tr>
<tr>
<td>Lipid stains - Oil Red O, Sudan III</td>
<td>stains triglycerides and neutral fats orange-red to ID lipid containing cells</td>
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<tr>
<td>Eosin</td>
<td>Stains RBCs, while yeast do not stain</td>
</tr>
<tr>
<td>Prussian blue reaction</td>
<td>makes iron granules blue in color (hemosiderin granules appear yellow until stained</td>
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<tr>
<td>Gram stain</td>
<td>to assist in ID of gram reaction of bacteria</td>
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Microscopic Examination of Urine

• Brightfield binocular microscope
  • Adjustable condenser and iris diaphragm to provide Koehler illumination
  • Parfocal objectives – to keep object in focus when changing magnification
  • Want subdued light
    • Have light source on low setting
    • Lower condenser
    • Closed iris diaphragm.
    • Use filters
  • Continuously focus up and down with fine adjustment.

Microscopic Examination of Urine

• Viewing urine sediment – with other types of microscopes
  • Phase-contrast microscopes
    • ID of translucent elements such as casts
    • Special condenser and objective alter light causing a halo effect around element
  • Polarized light microscopes
    • to help ID crystals, lipids

Microscopic Examination of Urine

• Viewing urine sediment
  • Want subdued light
    • Keep the light’s setting as low as possible.
    • Partially close the iris diaphragm
    • Adjust the condenser - downward
  • Continuously focus up and down with fine adjustment.
Microscopic Examination of Urine

- **Examining the Urine Sediment**
  - **Start on low power objective (10X ocular x 10X objective = 100X)**
  - **Scanning**
    - Examine 10-15 fields using low power (10X).
    - Look for casts, mucus, and squamous epithelial cells and in general getting an overall feel.
    - Use reporting criteria established by the site.
    - In MLT courses - follow ‘Urinalysis Reporting Standardization Guide’ as published in Microscopic lab.
  - **Enumerate**
    - Casts - use low power to enumerate, but switch to high power to aid in identification.
  - **Quantitate**
    - Mucus using semi-quantitative terms.

- **Switch to high power objective (10X ocular x 40X high-dry obj = 400X)**
  - To identify types of casts.
  - **Enumerating**
    - WBCs
    - RBCs
    - Renal epithelial cells
  - **Quantitate**
    - Crystals (including amorphous crystals)
    - Bacteria, yeast & other parasites
    - Other miscellaneous items
  - Follow protocol of the facility.
  - Correlate microscopic with physical and chemical dipstick results.

- **Changes in urine sediment when allowed to stand**
  - Important to keep in mind the changes in microscopic structures that can occur (don’t forget the other chemical changes ie bilirubin, pH, ketones).
  - RBC distorted - crenation, swelling, disintegration.
  - WBC disintegrates in alkaline urine.
  - Cast disintegrates in alkaline urine.
  - Bacterial growth - increased alkalinity.
  - Increased precipitation of crystals, especially amorphous (as the urine cools off the crystals begin to precipitate).