

Urinalysis and Body Fluids CP9

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

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

Microscopic Examination of Urine

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

Test	What to look for,
Color & Clarity red, pink / hazy or cloudy White / hazy or cloudy	RBCs WBCs
Positive nitrite	WBCs / bacteria
Positive Leukocyte esterase	WBCs, WBC casts, bacteria
Positive Glucose	yeast

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.

Microscopic Examination of Urine

- O&T Summarize the correct steps in the collection and preparation of a urine sample for microscopic exam.
 - Sources of Variation
 - Collection method
 - Centrifugation time and speed
 - Re-suspension of sediment
 - Type of microscope slide
 - Viscosity of specimen
 - Reporting of the results

Microscopic Examination of Urine

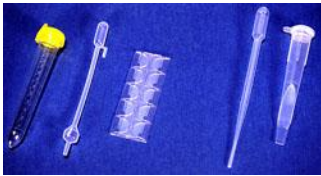
- Preparation of specimen (standardized)
 - Mix specimen well
 - Pour specified volume into urine centrifuge tube
 - Not enough specimen? (VARIES - FOLLOW PROTOCOL)
 - If < 1 mL - perform microscopic on unspun sample and note the report form
 - If 1 - 6 ml - spin down entire sample and note on report 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 μ L
 - 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

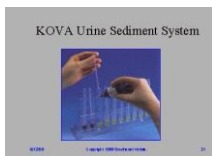
Commercial systems

- Evaluate sediment in a chamber standardized for given volume and depth field
 - UriSystem - slide on right
 - KOVA System - slide below
 - Count -6 or Count 10
- 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



Microscopic Examination of Urine

- 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 - oranges cytoplasm & dk purple nuclei) from transitional epithelial (which are more over-all blue)



Microscopic Examination of Urine

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

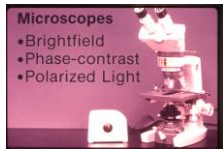
Microscopic Examination of Urine

- Brightfield binocular microscope
 - Adjustable condenser and iris diaphragm to provide Köhler 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, mucous, 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
 - Squamous epithelial cells
- Quantitate
 - mucous using semi-quantitative terms



Microscopic Examination of Urine

- Examining the Urine Sediment
- 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

Microscopic Examination of Urine

- 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 disintegrate in alkaline urine
- Bacterial growth - increased alkalinity
- Increased precipitation of crystals, especially amorphous (as the urine cools off the crystals begin to precipitate).
