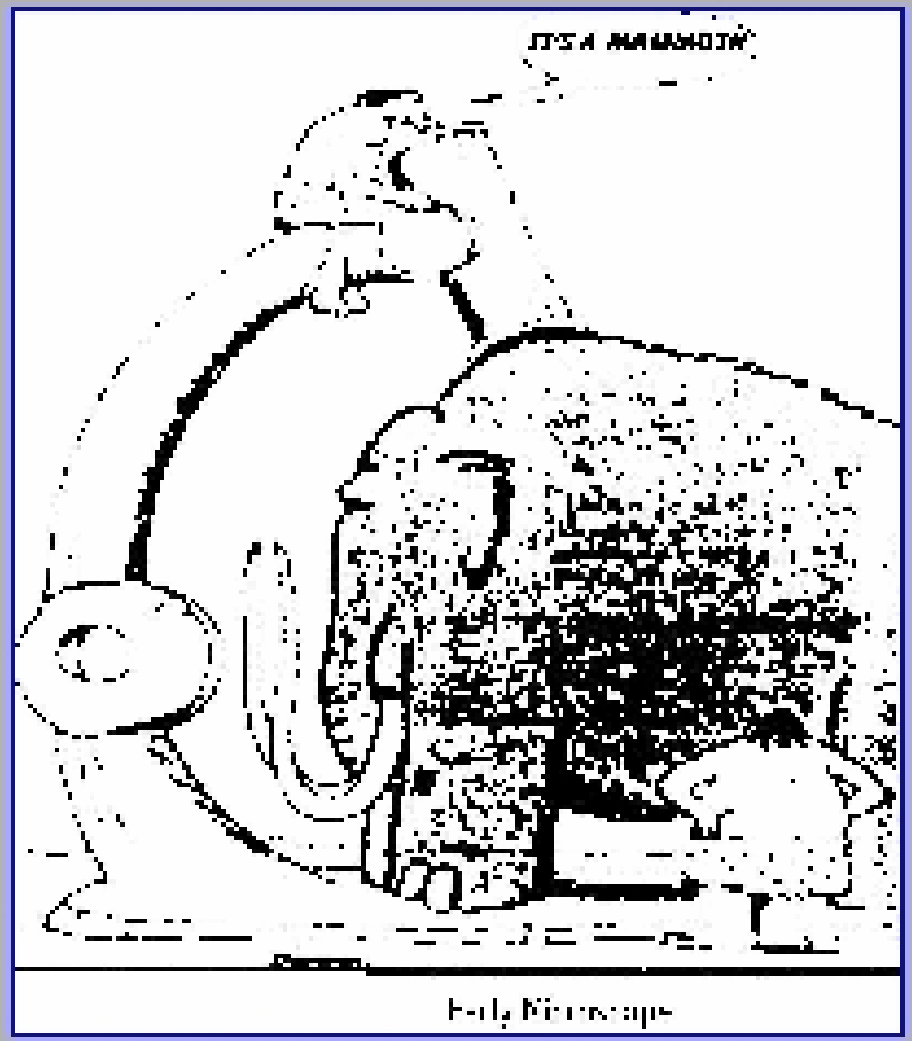
The First Microscope

"It's a Mammoth"



Early Microscope

Microscopic Examination of Urine

- ***Download http://www.vetlab.com/kova.htm***
- Definition of urine sediment: all solid materials suspended in the urine
  - - a semiquantative evaluation of the urine sediment
- Significance of formed elements in the urine
  - ***Well performed microscopic exam can provide information nearly equivalent to a biopsy.***
  - ***Most time consuming part of UA & until recently the least standardized.***
  - ***Ongoing controversy as to when / if to perform the microscopic exam.***

Microscopic Examination of Urine

- Not on lecture guide. Review info in Table 6-1
- Correlation of findings from physical & chemical analysis with expectations in microscopic.

Screening Test	Significance (or what to look for)
Nitrite positive	WBCs / bacteria
Leukocyte esterase pos	WBCs, WBC casts, bacteria
Glucose positive	yeasts

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

- Obj.35. List the correct steps in the collection and preparation of a urine sample for microscopic exam.
- Preparation of specimen ***need to standardize as much as possible***
  - ***Sources of Variation*** ***(not on lecture guide)***
    - ***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 (show video)
  - 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

Microscopic Examination of Urine

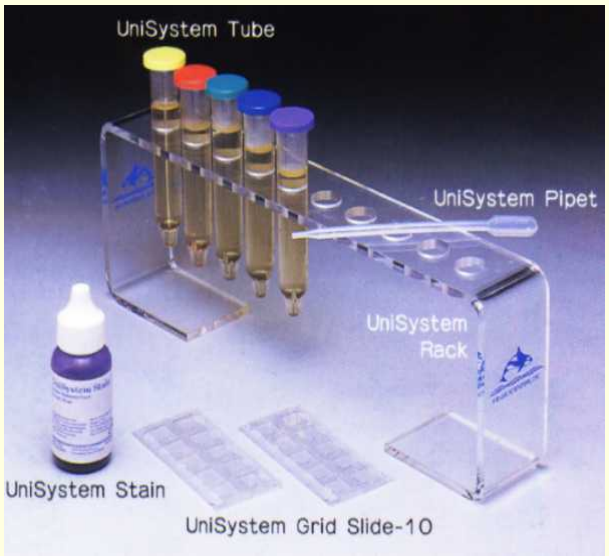
- Pour off supernatant ***- except last .5-1 mL. have pipettes that assist***
- Re-suspend sediment ***- mix well, tap, or use pipette provided***
- Evaluate sediment in a chamber standardized for given volume and depth of field. ***- "In-house methods = Mount a <u>small</u> drop on a clean slide, cover-slip - or use commercial materials such as Count 10***
- Use standardized reporting format consistent with other techs in the institution

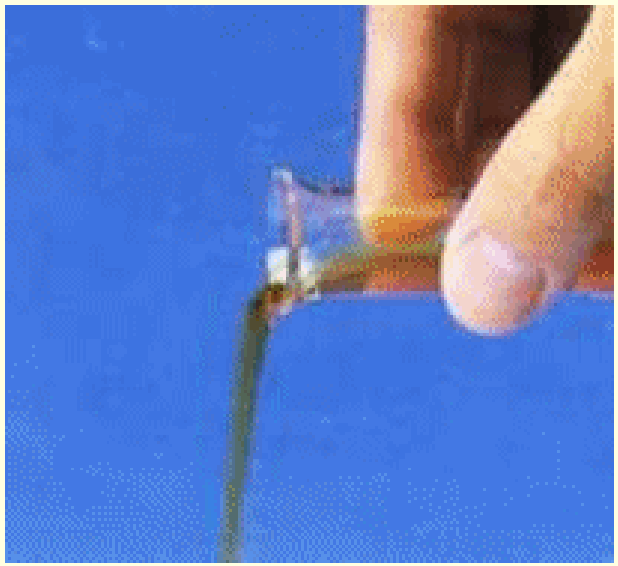
Microscopic Examination of Urine

- Commercial systems
  - UriSystem – slide to follow
  - KOVA System – video or several slides to follow
  - 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'***

Microscopic Examination of Urine

- UniSystem Standardization of Urine Sediment





KOVA Urine Sediment System

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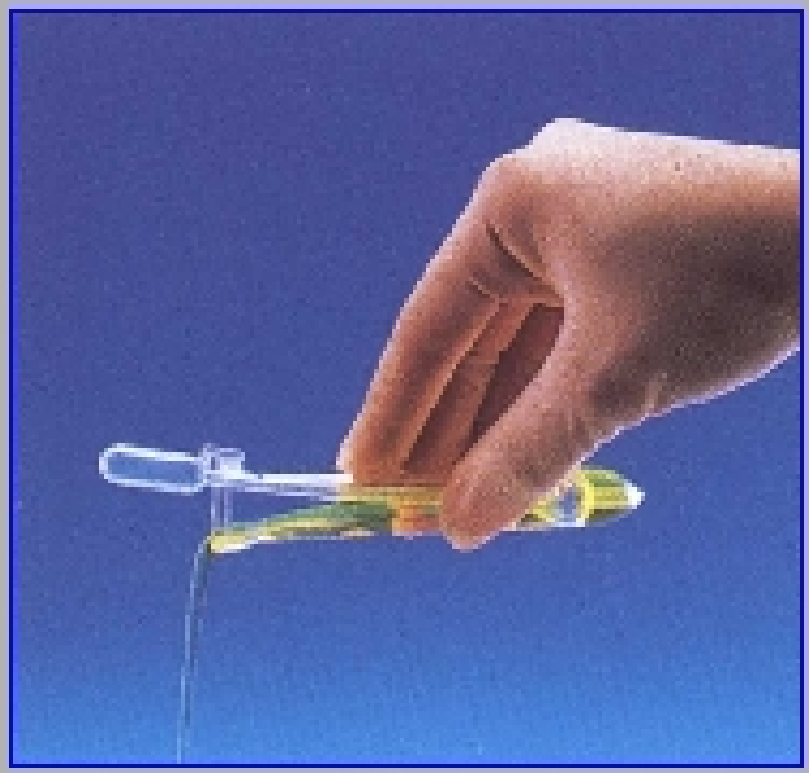
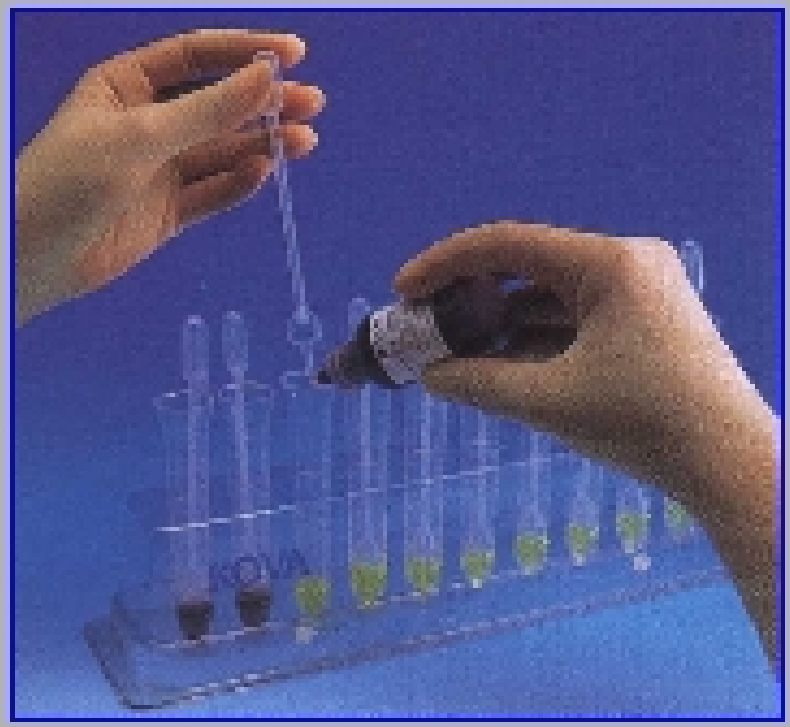
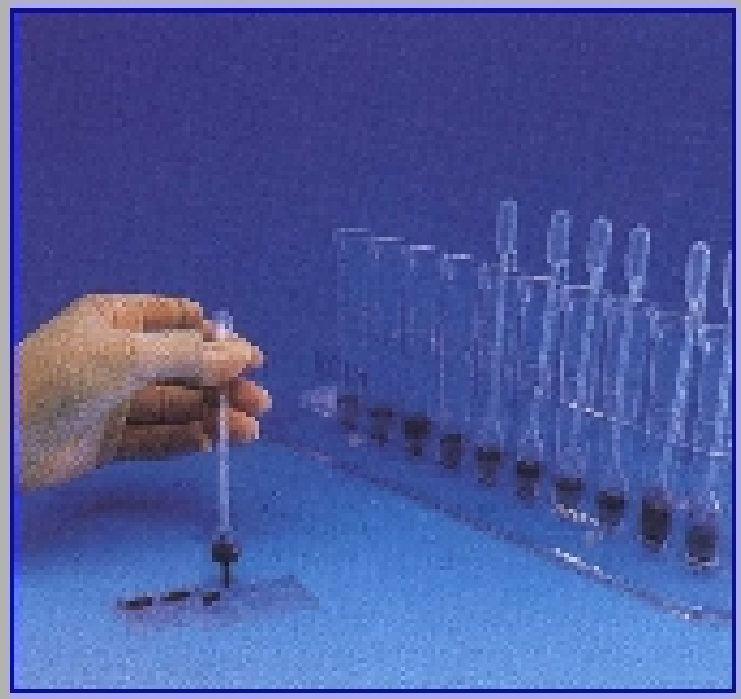


KOVA Urine Sediment System



KOVA Urine Sediment System



KOVA Urine Sediment System

Microscopic Examination of Urine

- Sedi-Stain (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

- Not on lecture guide – Table 6-3
  - Sediment stain characteristics
- Toluidine blue – nuclear structure
  - Assists in differentiating WBC from renal epith.
- 2% acetic acid - removes interfering RBCs and enhances nuclei of WBC
- Lipid stains - Oil Red O, Sudan III - stains triglycerides and neutral fats orange-red to ID lipid containing cells.

Microscopic Examination of Urine

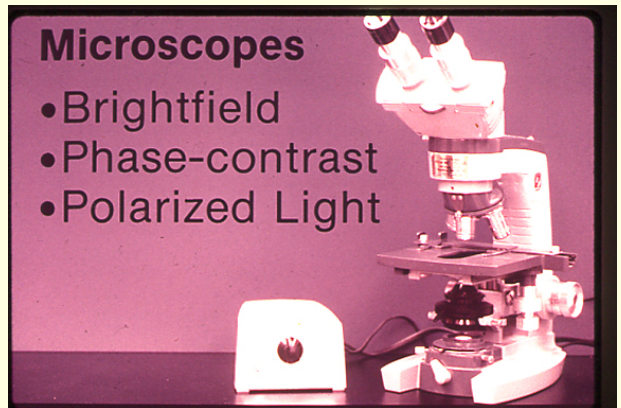
- Gram stain - to assist in ID of gram reaction of bacteria.
- Hansel stain - methylene blue and eosin Y stains eosinophilic granules - ID eosinophils
- Prussian blue reaction - makes iron granules blue in color (hemosiderin granules appear yellow until stained)

Microscopic Examination of Urine

- Table 6-5 – ***page 73 provides information on types of microscopic techniques that have application in UA***

Brighfield microscope – very subdued light: lowered condenser, closed iris diaphragm, use filters

- ***Continuously focus up and down with fine adjustment as you learned in hematology.***
- Polarized light - may use to ID crystals, lipids



Microscopic Examination of Urine

- Types of Sediment
  - As one author puts it:
    - ***Cells***
    - ***Casts***
    - ***Crystals***
    - ***Critters***

Microscopic Examination of Urine

- Types of Sediment
  - Organized – biological part
    - RBC
    - WBC
    - Casts
    - Epithelial cells
    - Bacteria, parasites, yeast and fungi
  - Unorganized
    - Crystals
    - Amorphous crystalline matter.

Microscopic Examination of Urine

- Examination
  - ***- should correlate with physical and chemical dipstick, may need to recheck***

- Scanning - – 10-15 fields using low power (10X). Look for casts, mucous, and squamous epithelial cells ***in general getting an overall feel***
- ***Report squamous epithelial cells, crystals, mucous, etc. using semi-quantitative terms such as rare, few, moderate, or many (or trace, 1+,2+,3+, & 4+) according to lab protocol.***

Microscopic Examination of Urine

- Enumeration ***- quantitate. Method may vary from lab to lab***
  - Average number of RBC/hpf
  - Average number of WBC/hpf
  - Average number of any renal tubular or transitional epithelial cells /hpf.

Microscopic Examination of Urine

Average number ***(and type)*** of casts/__***average # of casts /hpf***_____

- ***authors have varied back and forth as whether low or high power should be reported... use low power to locate and enumerate the various types , but may need to switch to high power to identify the type...***
- ***Strasinger says report / lpf (use hpf to ID)***

- Unorganized sediment – few, moderate, many, packed; kinds seen

- Note presence of bacteria, yeasts, crystals, epithelial cells (***covered***), etc.
  - ***quantitate these also***

Microscopic Examination of Urine

- .Changes in urine sediment when allowed to stand
  - ***important to keep in mind the changes in <u>microscopic structures</u> 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

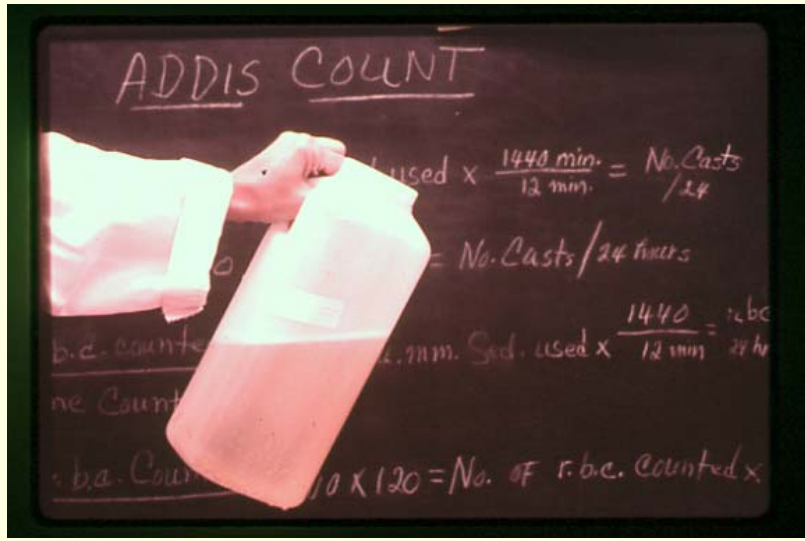
Microscopic Examination of Urine

- Microscopic sediment
  - Red Blood Cells
  - White Blood Cells
  - Epithelial Cells
  - Casts
  - Crystals
  - Miscellaneous structures

    - Students go to end of area's lecture guide. Continue to next slide.

Microscopic Examination of Urine

- Addis Count – Strasinger page 68
  - ***Early way of accurately enumerating urine sediment.***
  - Actual enumeration of casts, RBC, WBC, using a hemacytometer
  - ***developed as a way to standardize urine microscopics to monitor known cases of renal disease.***
  - ***Rarely done today as most urine microscopic systems produce standardized results if manufacture directions are followed.***



- Binocular
- Mechanical Stage
- Adjustable Abbe Condenser
- Built-in light source, preferably halogen
- 10X Widefield eyepieces
- 4X, 10X, 40X and 100X oil