Urine and Stool Examination

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Components of basic routine urinalysis

A) SPECIMEN EVALUATION:

- **Clean, dry, leak proof container (sterile for C/S)**
- Labelling (Name, date & time of collection)
- Visible signs of contamination
- Transportation delays causing deterioration.
- ***First voided morning urine*** which is the most concentrated is best for routine analysis.
- If a single specimen is submitted for multiple measurements, bacteriological examination should be performed first
- Occasionally when very less quantity of urine is received, measurements most pertinent to diagnosis should be made first after consulting the clinician.
- ***Ideally test within 2 h*** of collection

Specimen Rejection Criteria:

- **Unlabeled** containers
- **Nonmatching labels** and requisition forms
- **Contaminated specimens** with feces or toilet paper
- Containers with **contaminated exteriors**
- Insufficient volume of urine
- **Improperly transported or preserved** specimens
- **Transport delays**

Gross/physical examination

- **Colour:**

  Straw yellow color of urine is due to **pigment urochrome.** Increased during fever, thyrotoxicosis, & starvation.

  Pale urine may be seen in high fluid intake and dark urine in dehydration.

Colour changes with drugs:

- Red: Chlorzoxazone, Desferoxamine, L –dopa
- Brown: Furazolidone
- Brown yellow: Nitrofurantoin
- Dark yellow: B –complex vitamin
- Bright orange red: Rifampicin
- Orange yellow: Sulfasalazine
- Pale blue: Triamterene
- Blue green: Amitryptiline
- Black: Metronidazole

Appearance	Cause	Remarks
Colourless	Very dilute urine	Polyuria, Diabetes insipidus, overhydration
Cloudy	Phosphates, carbonates Urates, uric acid Leucocytes Red cells Bacteria, yeasts Prostatic fluid, spermatozoa.	
Milky	Pyuria Fat – lipiduria chyluria	 Nephrosis, crush injury Lymphatic obstruction
Yellow -orange	Concentrated urine Urobilinogen in excess Bilirubin	Dehydration, fever No yellow foam Yellow foam
Yellow Green	Bilirubin- Biliverdin	Yellow foam
Yellow brown	Bilirubin- Biliverdin	“Beer” brown , Yellow foam
Red	Hemoglobin RBCs Myoglobin Porphyrin/aniline dyes/bee	+ Rgt strip for blood + Rgt strip for blood + Rgt strip for blood Neg. Rgt strip for blood

Recommendations for Reagent Strips

Storage:

- Store in ***cool, dry area but not in a refrigerator***. Protect from moisture, heat
- *Check for discoloration* with each use; discoloration may indicate loss of reactivity.
- Keep container tightly stoppered.

Testing:

- Test urine ***as soon as possible after receipt.***
- Remove only enough strips for immediate use; recap tightly.
- Test a ***well-mixed, unspun*** urine sample.
- Urine samples must be at ***room temperature*** before testing.
- Do not touch the test area with fingers.
- Dip reagent strip into urine **briefly—no longer than 1 second.**
- **Drain excess urine off**—run edge of strip along rim of tube, or blot edge on absorbent paper.
- Do not lay reagent strip directly on workbench surface.
- ***Follow exact timing recommendations for each chemical test. (usually 60 sec)***
- **Hold reagent strip close to the colour chart, and read under good lighting.**
- Know sources of error, sensitivity, and specificity of each test on the reagent strip.
- Make correlations between patient history and individual test.

Urine volume:

Average adult: 600- 2000 ml urine per day

- Polyuria; > 2L urine/24h. excessive water intake, diuretucs, iv solutions etc
- Oliguria: less than 500 ml urine/24h; prerenal, Post renal, Renal causes

Specific gravity:

Specific gravity indicates the relative proportion of dissolved solids to total volume of the specimen. ***Urea (20%), NaCl (25%), sulfates & phosphates*** contribute most to the specific gravity of normal urine. **(N: 1.003- 1.030). Usually 1.016-1.022**

Reagent strip: ***Polyelectrolyte, indicator substance & buffer***. Changes in ionic conc changes the pKa (inverse relation) of the polyelectrolytes which show colour change with the indicator.

Refractometers and urinometers can also be used for specific gravity determination.

Low specific gravity (hypostenuria): Diabetes insipidus, pyelonephritis, GN etc

High Specific gravity (hypersthenuria) : dehydration, adrenal insufficiency, hepatic disease etc

Protein.... Methods

- Reagent strip method:

  Reagent strip contains **tetrabromophenol blue** buffered to an acid pH of 3. In the absence of protein the strip is yellow, , variable shades of green develop depending on the nature and quantity of protein. **Most strips will detect a minimum of 5-20mg/dl of albumin.**

- Sulfosalicylic acid method: centrifuge urine, use supernatant. To 3ml of supernatant urine in t.t, add equal amt of SSA. Invert and let it stand for 10min. Invert again twice and observe the degree of turbidity.
  - Negative- No turbidity (5mg/dl or less)
  - Trace- perceptible turbidity (20 mg/dl)
  - 1+ Distinct turbidity , no granulation (50mg/dl)
  - 2+ turbidity with granulation, no flocculation (200mg/dl)
  - 3+ turbidity with granulation and flocculation (500mg/dl)
  - 4+ clumps of ppt protein or solid ppt (1000mg/dl or more)

Chemical screening

pH:

- Ranges from **4.6 to 8.**
- **Acid urine:** meat protein, fruits like cranberries, NH_4Cl, methanamine mandalate, metabolic or respiratory acidosis
- **Alk urine:** citrus fruits and vegetables, $NaHCO_3$, acetazolamide, metabolic alkalosis,
- Methods: Indicator strips contain ***methyl red and bromothymol blue*** gives a range of colors as the pH rises.

Protein:

- Normally upto 150mg of protein is excreted in urine daily with the average urine protein conc from 2-10 mg/dl.
- Detection of abnormal amounts of protein in urine is an imp indicator of renal disease.
- Heavy, Moderate, Minimal.

Glucose

- When hyperglycemia is present, glycosuria usually occurs when blood level **is > 180-200 mg/dl.**
- **Glycosuria with hyperglycemia:** DM, Pituitary & adrenal disorders (acromegaly, cushings, hyperthyroid etc), CNS disorders like tumors, haemorrhage, metabolic disorders associated with infections, burns, uremia, liver disease etc.
- **Glycosuria without hyperglycemia:** Renal tubular dysfunction due to any cause.
- **Reagent strip method:** Based on specific ***Glucose oxidase and peroxidase method***, a double sequential enzyme reaction, reagent strips differ only in the chromogen used. Method is specific for glucose

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{chromogen} \xrightarrow{\text{peroxidase}} \text{oxidised chromogen} + H_2O$$

Chromogens used are o-toluidine, KI, aminopropyl carbazol chromogens

Copper Reduction method:

This method will detect sufficient quantities of ***any reducing substances in urine, including lactose, galactose, fructose, maltose*** etc. Useful in young pediatric patients.

- Clinitest methods
- Benedicts copper reduction method.

Ketones in urine:

- Diabeteic ketonuria
- Non diabetic ketonuria:

Methods:

- Because acetone, acetoacetic acid, 3- hydroxybutyric acid are all present in urine with ketonuria, ***detection of any of these is generally satisfactory.***
- Nitroprussside tests based on ***Rothera's method*** detect acetoacetic acid and acetone.
- Ferric chloride ***(Gerhardt's test)*** detects acetoacetic acid.

Reagent strip method:

Generally based on nitroprusside reaction for ketones (generally acetoacetic acid and acetone) giving red or violet colors.

Parameter	Range of detection	Practical Detection limit	Accuracy
Specific gravity	1.000-1.030		≥ 85% with refractometer
pH	4.5—9		≥ 95% with pH meter
Leucocytes	Neg — ≈ 500/µL (3+)	10—25 WBC/µL	≥ 90% with counting chamber
Nitrite	Neg – Pos (1+)	0.05mg/dl	
Protein	Neg—500 mg/dl (3+)	Albumin 6 mg/dl	≥ 90% with radial immunodiff
Glucose	Normal—1000mg/dl	40mg/dl	≥ 90% with hexokinase method
Ketone bodies	Neg—150 mg/dl	5mg/dl acetoacetic acid	≥ 85% with photometric enzymatic determination
Urobilinogen	Normal –12 mg/dl	0.4mg/dl	≥ 95% with Watson & Henry method
Bilirubin	Neg – ≈ 6 mg/dl	0.5mg/dl	≥ 85% with Jendrassik's method
Blood & Hb	Neg— ≈ 250/µL	Intact RBCs- 5/µL Hemolysed RBCs corr. To 10RBC/µL	≥ 90% with counting chamber

Bilirubin in urine

- **Normal adult urine** contains only **0.02 mg of bilirubin /dl.**
- **Bilirubinuria**: Yellow brown / greenish brown ± yellow foam.

- **Only conjugated bilirubin appears in urine**. **Conjugated bilirubinuria** occurs in:
  - Hepatocellular disease due to any cause (alcohol, viral hepatitis etc)
  - Obstruction to bile flow due to any cause (tumor, stone etc)

- Unconjugated bilirubin is water insoluble and unable to pass through the glomerular barrier.

- ***Urinary bilirubin + / urobilinogen –*** is indicative of intra or extrahepatic biliary obstruction.

- **Principle**: The test is based on the coupling reaction of bilirubin with a diazonium salt in acid medium.

Urine Dipstick Visual Quality Control Log (Outpatient sites)

Multistix Strip Lot # _____ **Expiration:** _____ **Site/Location:** _____

Sentry Urine Dipstick Control **NORMAL CONTROL** Lot #_____ Exp _____	Sentry Urine Dipstick Control **ABNORMAL CONTROL** Lot # _____ Exp _____

Posted Range:																			
Date tested	Glucose	Ketone	Specific Gravity	Blood	p H	Protein	Nitrite	Leukocytes	Glucose	Ketone	Specific Gravity	Blood	p H	Protein	Nitrite	Leukocytes	Corrective Action	Operator

If one or more of the tests listed exceed the posted range, repeat the test. If the repeat test result also exceeds the range, refer to the troubleshooting table on this page and enter the Corrective Action (CA) Code in the CA column above.

CA Codes:
R = Retested within range
O = Retest still outside
S = New test strips or lot
C = New controls
T = Adjusted technique to reflect procedure
M = QC/strip lot not matched
E = Expiration dates exceeded
P = Contacted POCT Coordinator

Basic Urine Microscopy procedure

- It is recommended that examination take place when the **sample is fresh. Cells and casts begin to lyse within 2 h of collection.**
- **Refrigeration (2- 8°C) helps prevent lysis** of these but increase the pptn of various amorphous and crystalline material. Midstream urine is ideal in case of females.
- Pour 10-15ml of well mixed urine into a graduated centrifuge tube. Centrifuge at 1500 rpm for 5 minutes.
- Pour out the supernatant & prepare slides from deposit.
- Examine under LP & HP
- Report RBCs, WBCs, squamous epithelial cells ***per HPF*** (reasonable ranges)
- Report casts as number of casts ***per LPF***
- (reasonable ranges – 0-5; 5-10; 10-20; many/numerous etc)
- Comment on:
  - *Transitional cells if present as sheets/large numbers*
  - *Bacteria, yeast, microorganisms.*
  - *Crystals*

Microscopy

Leucocytes:

- The PMN leucocytes is the predominant type of WBC that appears in urine.
- They appear as granular spheres about 12 µm in diameter with multilobated nuclei
- ***Normally 0 -5 cells/hpf may be present in urine.***
- WBCs increases in Infection, pregnancy, blockage of urinary tract.

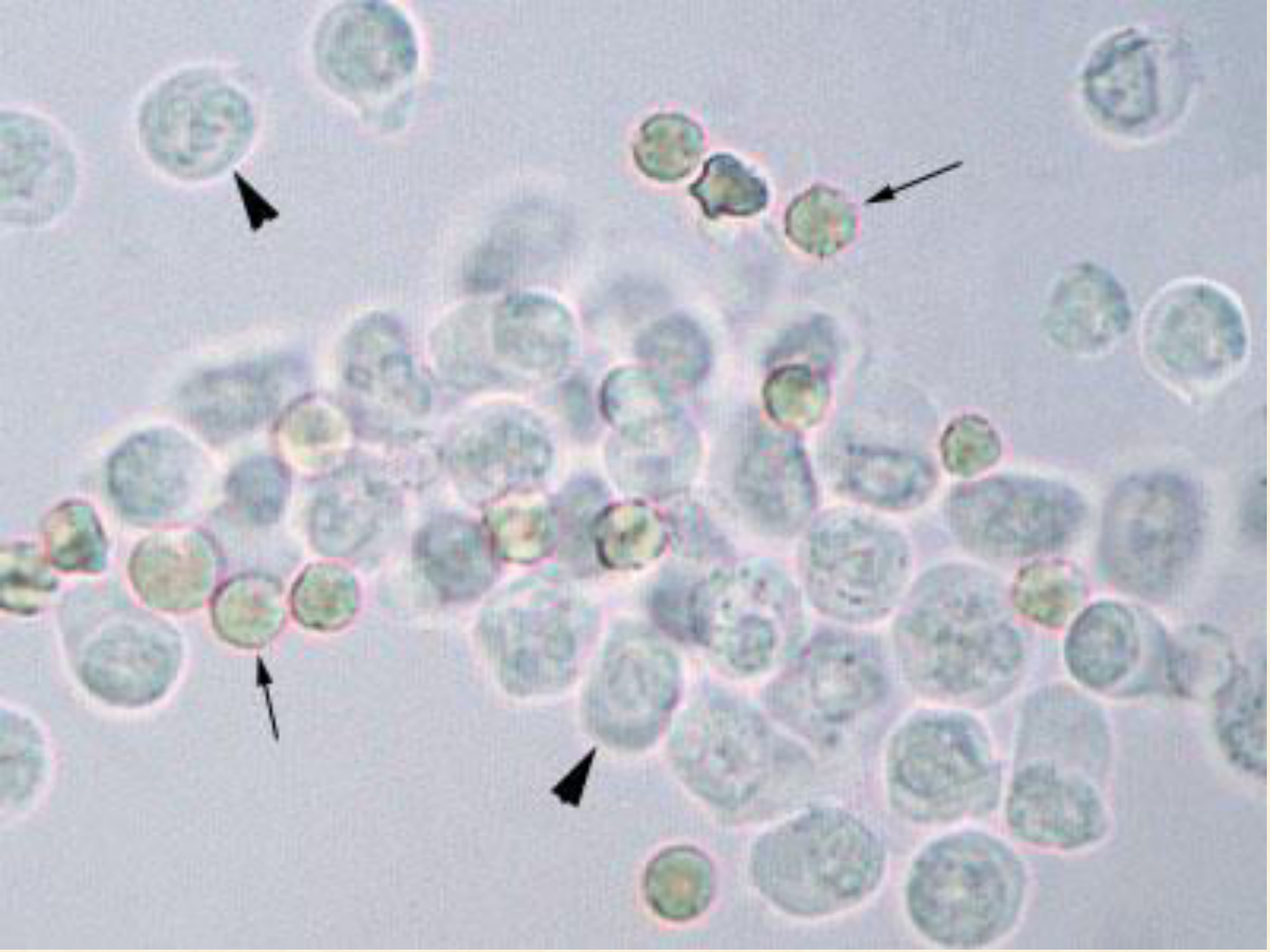
RBCs:

- **Biconcave discs** usually about 7µ in diameter.
- May appear as **faint colorless circles or shadow cells** in urine that is not fresh.
- ***Crenated*** in hypertonic urine and ***ghost cells*** in hypotonic cells.
- **Don't confuse RBCs with yeast cells or oil droplets.**

RBCs… cont

- RBCs are found in small numbers in ***normal urine (0-2/hpf).***

- If ***>3 RBCs/hpf, it is abnormal***. Seen in a variety of urinary & systemic conditions.
  1. Renal disease: GN, lupus nephritis, interstitial nephritis asso with drug, calculus, tumor, infections, infarction, trauma, hydronephrosis etc.
  2. Lower urinary tract disease: Infection, calculus, tumor, stricture, etc
  3. Extra-renal disease: Acute febrile episodes, malaria, SBE, PAN, blood dyscrasias, tumors of colon, rectum, pelvis etc.
  4. Toxic reactions: due to drugs- sulfonamides, salicylates, anticoagulants etc.
  5. Physiologic causes including exercise.



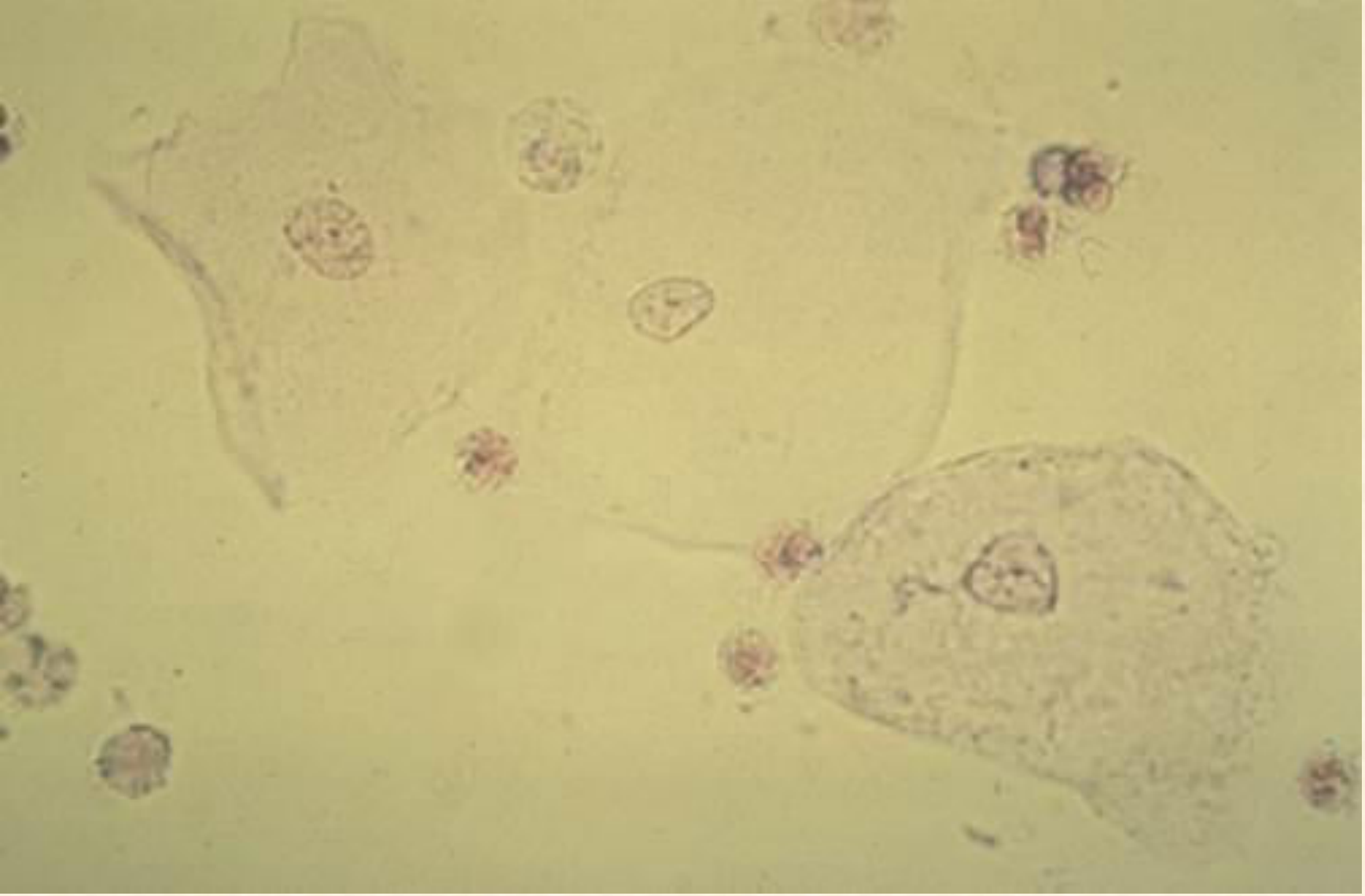
WBC (arrowheads) are larger, colorless and more granular than the round smaller RBC (arrows), which have no internal texture (although appear slightly biconcave).

Epithelial cells

- **Squamous epithelial cells**: large flat with abundant cytoplasm & small round central nuclei. Not significant

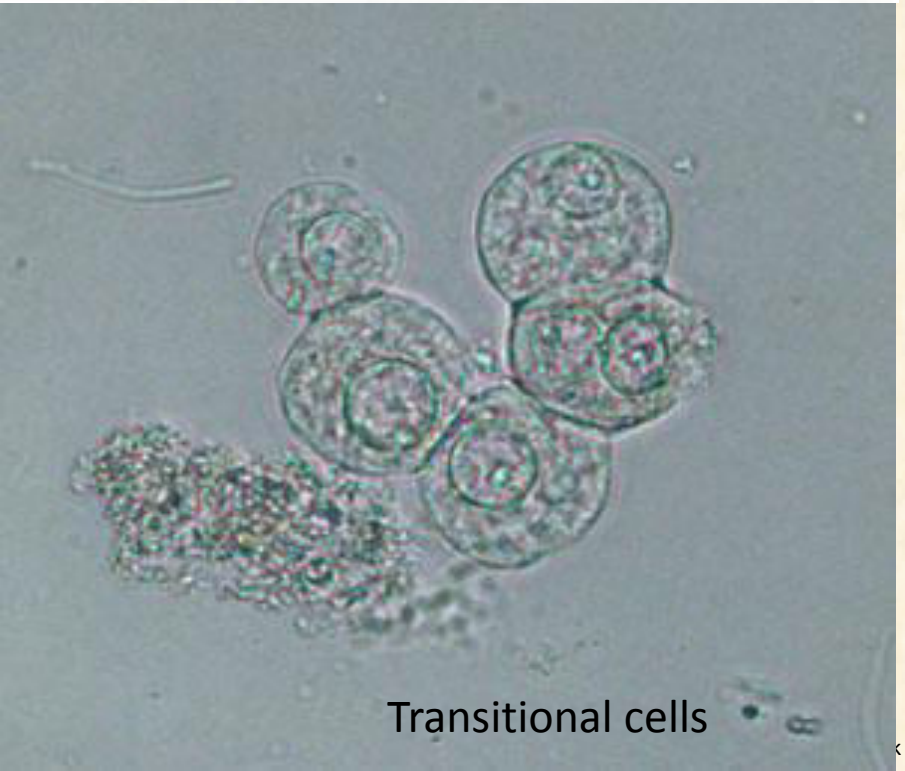
- **Transitional (urothelial) epithelial cells**:
  - Smaller than epithelial cells.
  - ***Round or pear shaped with round central nucleus.***
  - Few urothelial cells are present in normal urine reflecting normal desquamation.
  - ***Presence of large clumps or sheets of urothelial cells*** in the absence of catheterization necessitates cytological examination to evaluate for TCC.

- **Renal tubular epithelial cells:** Most significant type of epithelial cells in urine because *the finding of ↑ numbers indicates tubular damage.*

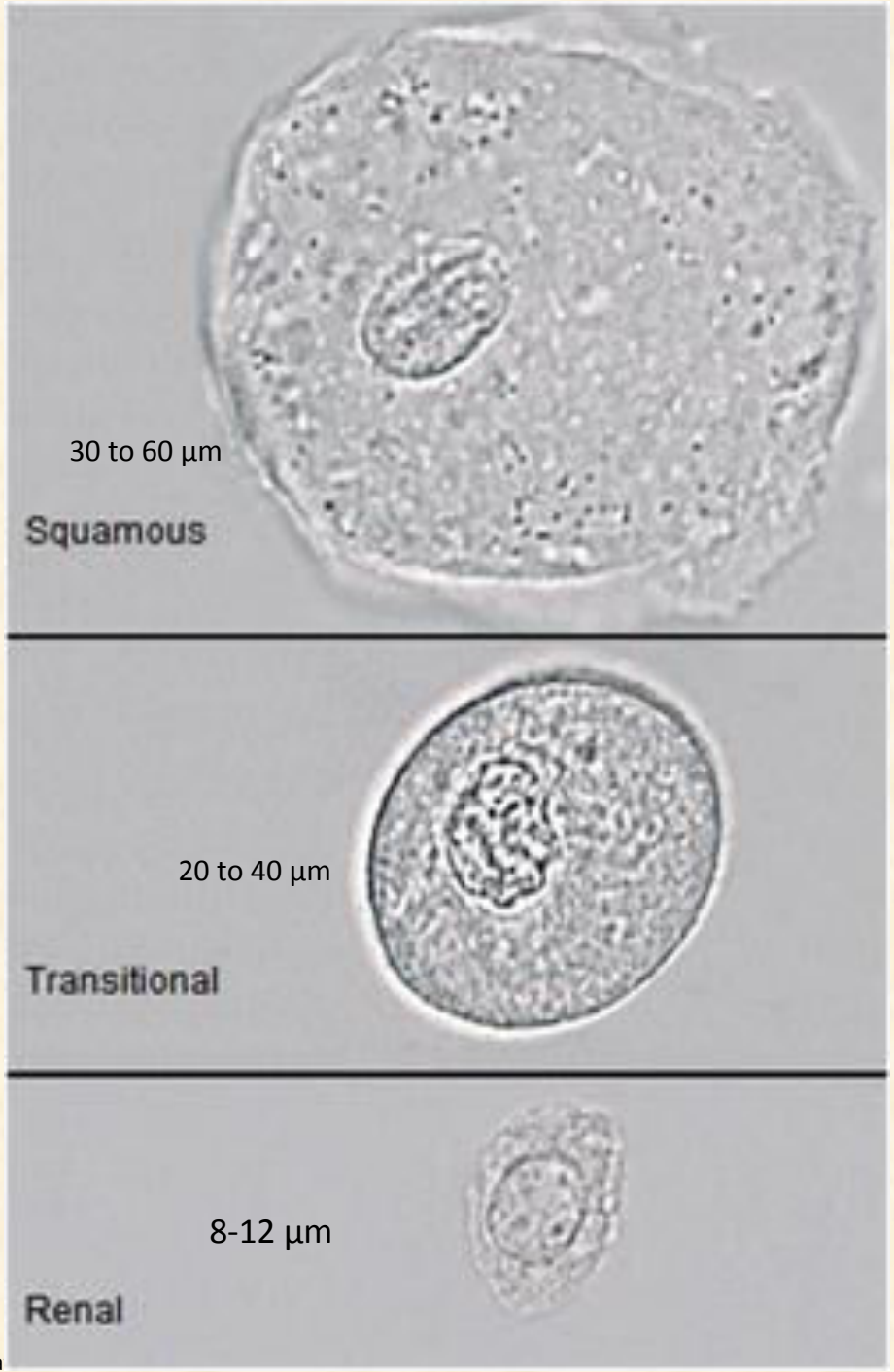


Large polygonal squamous epithelial cells with small nuclei are seen here. A few sq epithelial cells may normally be excreted in urine esp females

Renal tubular cells (8 -12 µ)



Transitional cells



Casts

- The sole source of origin of casts is the kidney.
- Tamm- Horsfall protein is ***the glycoprotein secreted by the thick part of the ascending loop of Henle*** (constitutes one third of urine total protein)
- ***Tamm – Horsfall protein forms the matrix of all casts.***
- Casts may be short & stubby or long & convoluted.

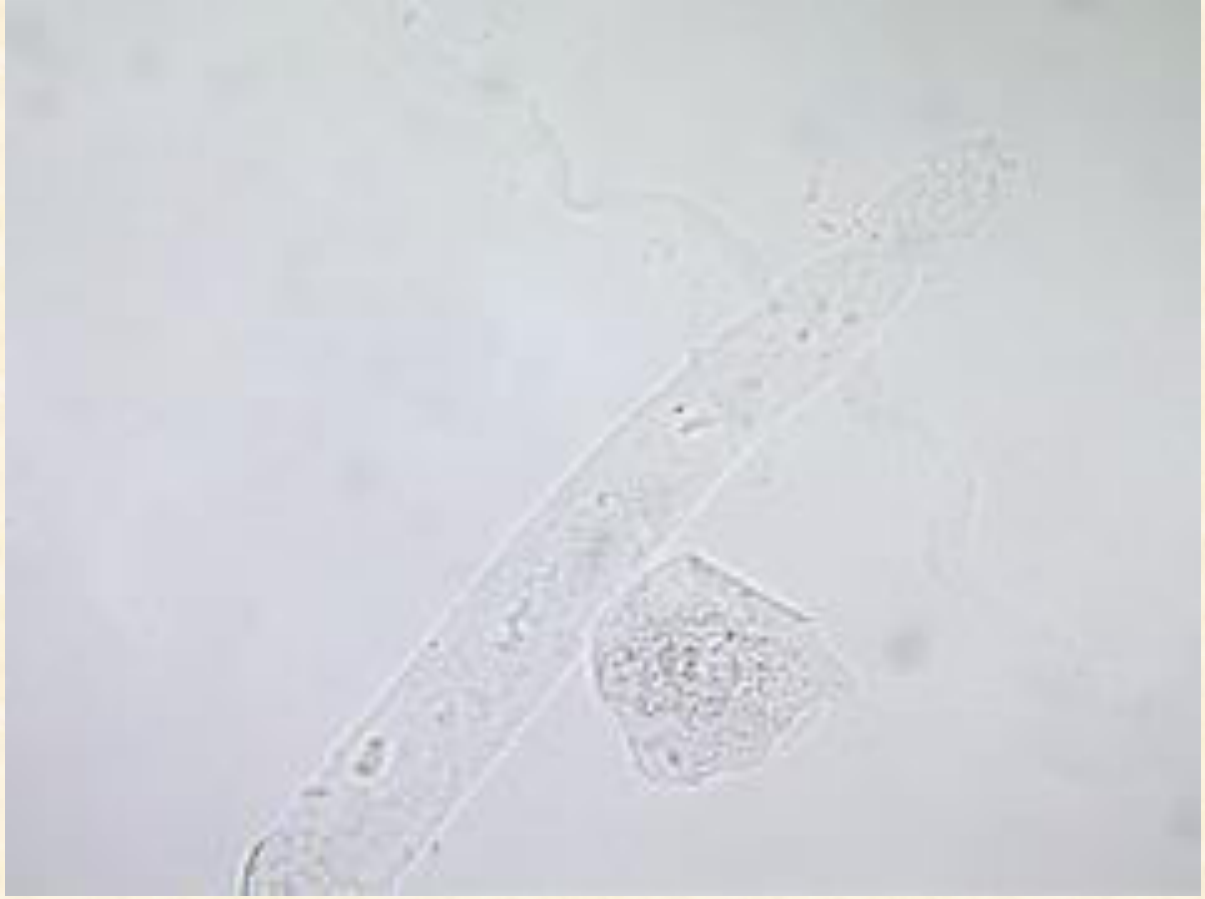
- **Hyaline casts:** 0-2/lpf hyaline casts are normal.

- **Waxy casts**: seen in CRF; acute & chronic renal allograft rejection.

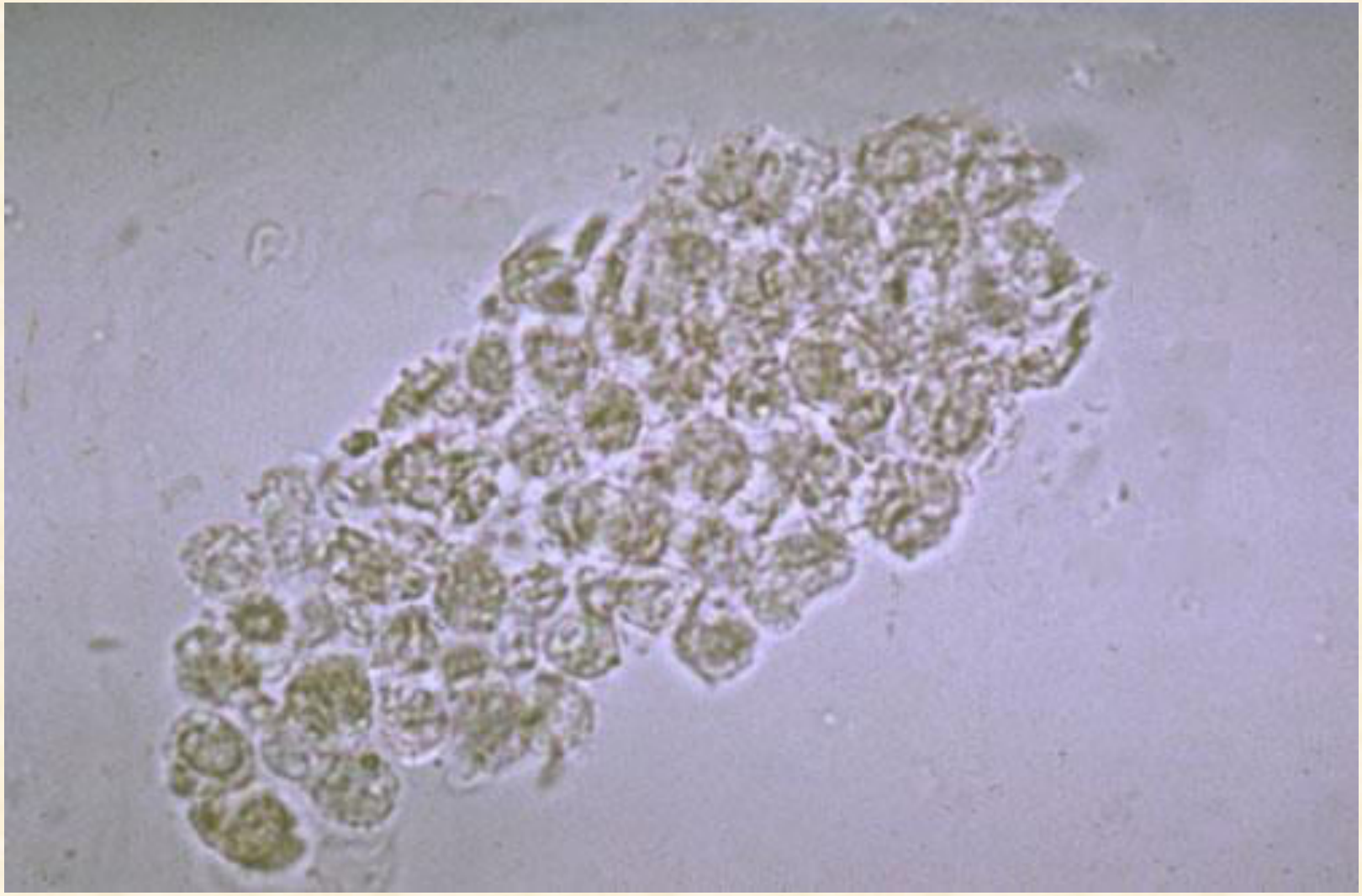
- **Erythrocyte (RBC) casts:** Significant becoz they indicate bleeding in the nephron. Seen in acute GN, IgA nephropathy, lupus nephritis, SBE & renal infarction

Casts…cont

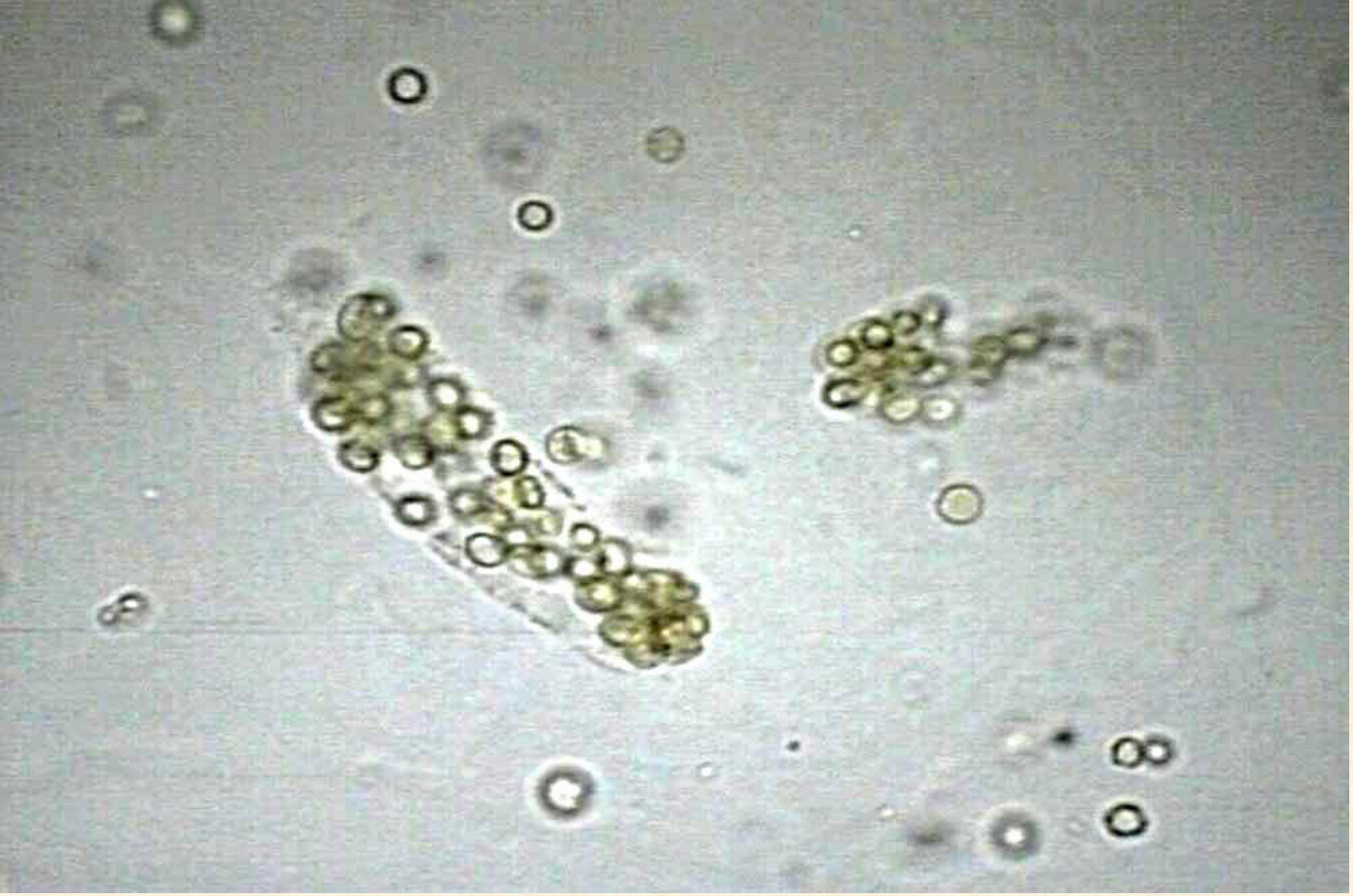
- **Renal tubular epithelial cell casts**: Seen in acute tubular necrosis, viral disease (CMV), drug toxicity, cute allograft rejection in transplant pts.
- **Granular casts:**
  - fairly common and may *be seen in both pathologic and non pathologic states.*
  - They originate from plasma protein aggregates (fibrinogen, immune complexes, globulins) that pass into tubules from damaged glomeruli, & from cellular remnants of leucocytes, erythrocytes or damaged renal tubular cells.
  - Seen in glomerular & tubular diseases, tubulointerstitial disease & renal allograft rejection, pyelonephritis, viral inf etc
- **Fatty casts**: Seen commonly in nephrotic syndrome.
- Rarely crystal casts, Hb, hemosiderin casts, myoglobin casts my be seen.



Hyaline casts can be present in low numbers (0-1/LPF) in concentrated urine of otherwise normal patients and are ***not always associated with renal disease.***
Generally, hyaline casts have parallel sides with clear margins and blunted ends,
Greater numbers of hyaline casts may be seen in association with proteinuria of renal (e.g., ***glomerular disease***) or extra-renal (e.g., overflow proteinuria as in myeloma) origin.
In such cases it has been proposed that the presence of excessive serum protein in the tubular lumen promotes precipitation of the Tamm-Horsefall mucoprotein.



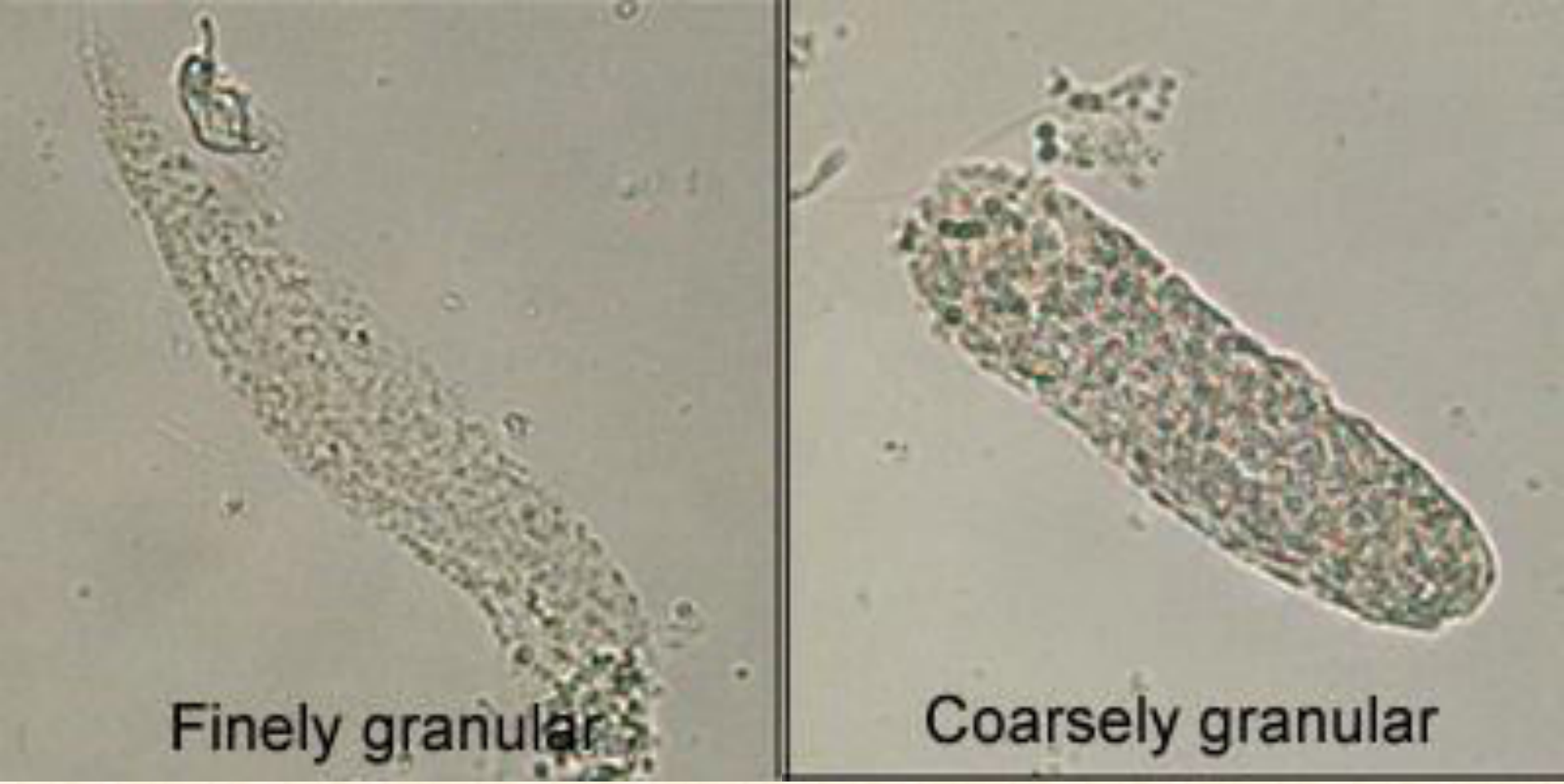
Leukocyte (WBC) casts: Leucocytes enter tubular lumen from the intersitium and usually reflect tubulointerstitial disease with neutrophilic exudates & interstitial inflammation. **Seen in pyelonephritis, interstitial & lupus nephritis.**



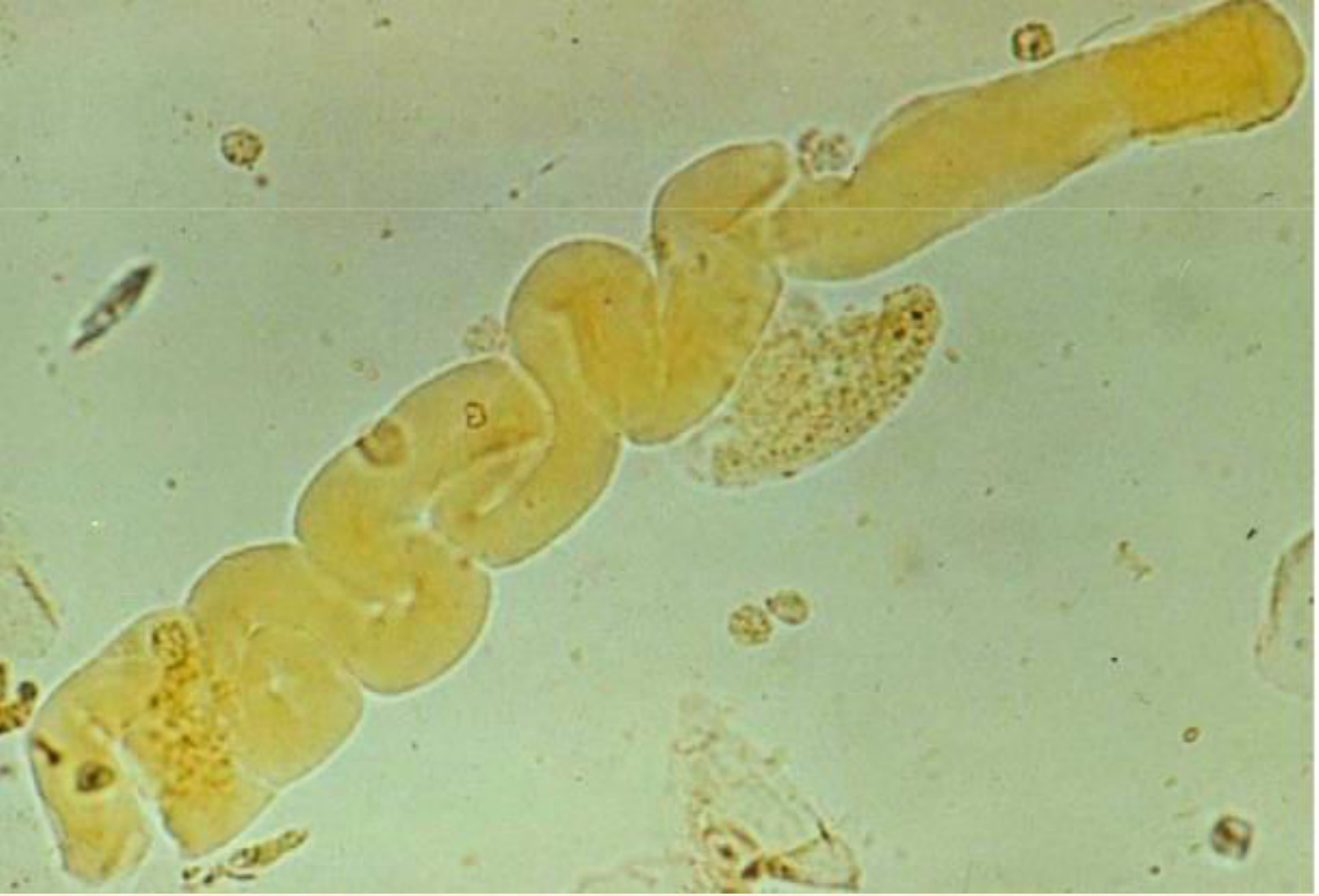
RED CELL CASTS

means renal hematuria, always pathologic

useful to diagnose glomerular diseases: acute glomerulonephritis, lupus nephritis, Goodpastures, SBE, renal trauma renal infarction, severe pyelonephritis, RCHF, renal vein thrombosis, polyarteritis nodosa may appear brown or colorless may contain only a few RBC's in a prot matrix or be solid packed RBCs RBCs are smaller than ECs RBC casts can degenerate to hemoglobin or blood cast, brown-red in color



Granular casts are generally the result of degeneration of cells in cellular casts. Their significance lies with the cast from which they were formed. In general, the presence of granular casts suggests stasis in the nephron. The casts are associated with **tubulointerstitial disease**

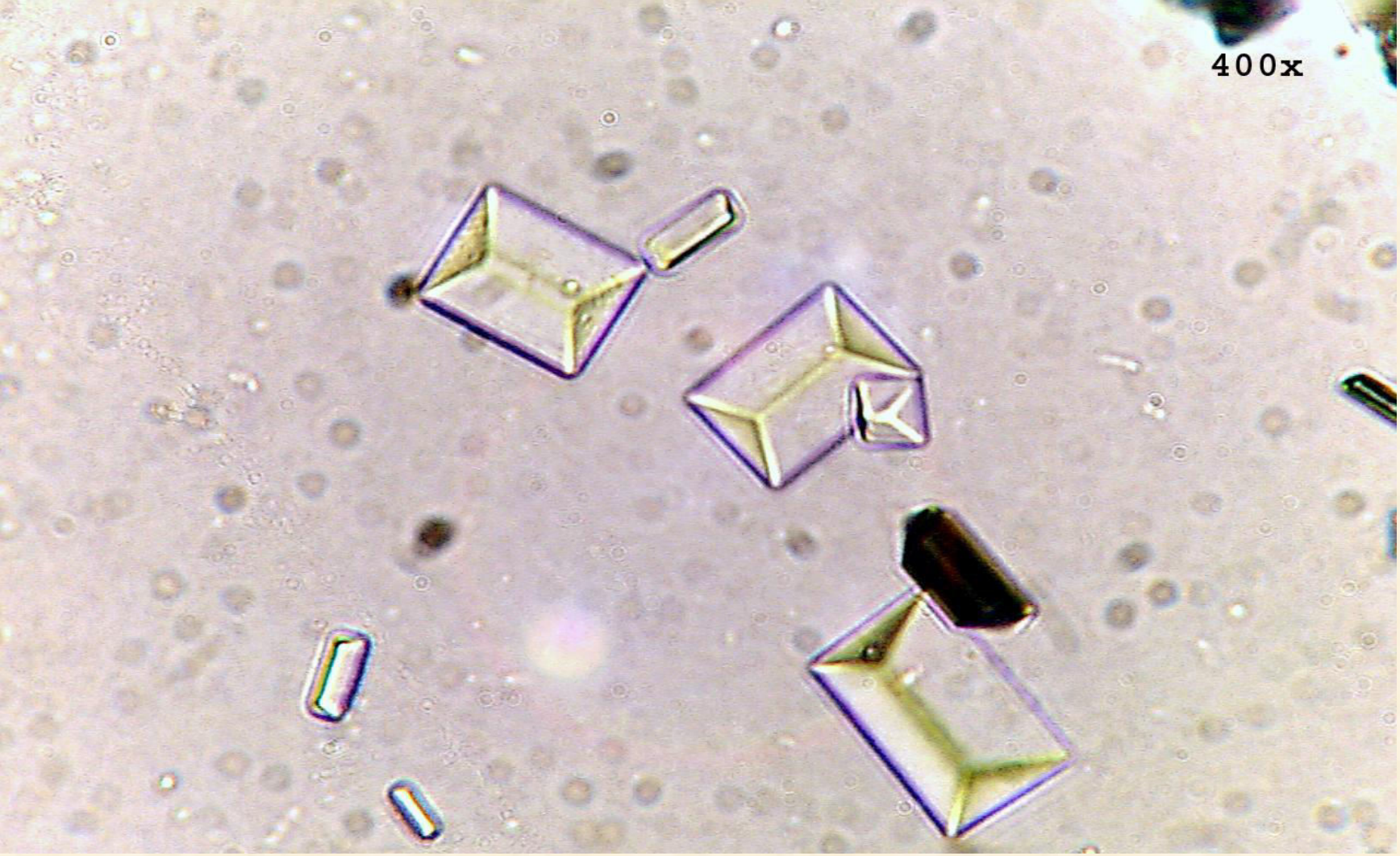


Waxy cast- pathologic; seen in severe progressive renal disease

Crystals:

- ***Crystals form by precipitation of urinary salts due to ↑ solute concentration.***

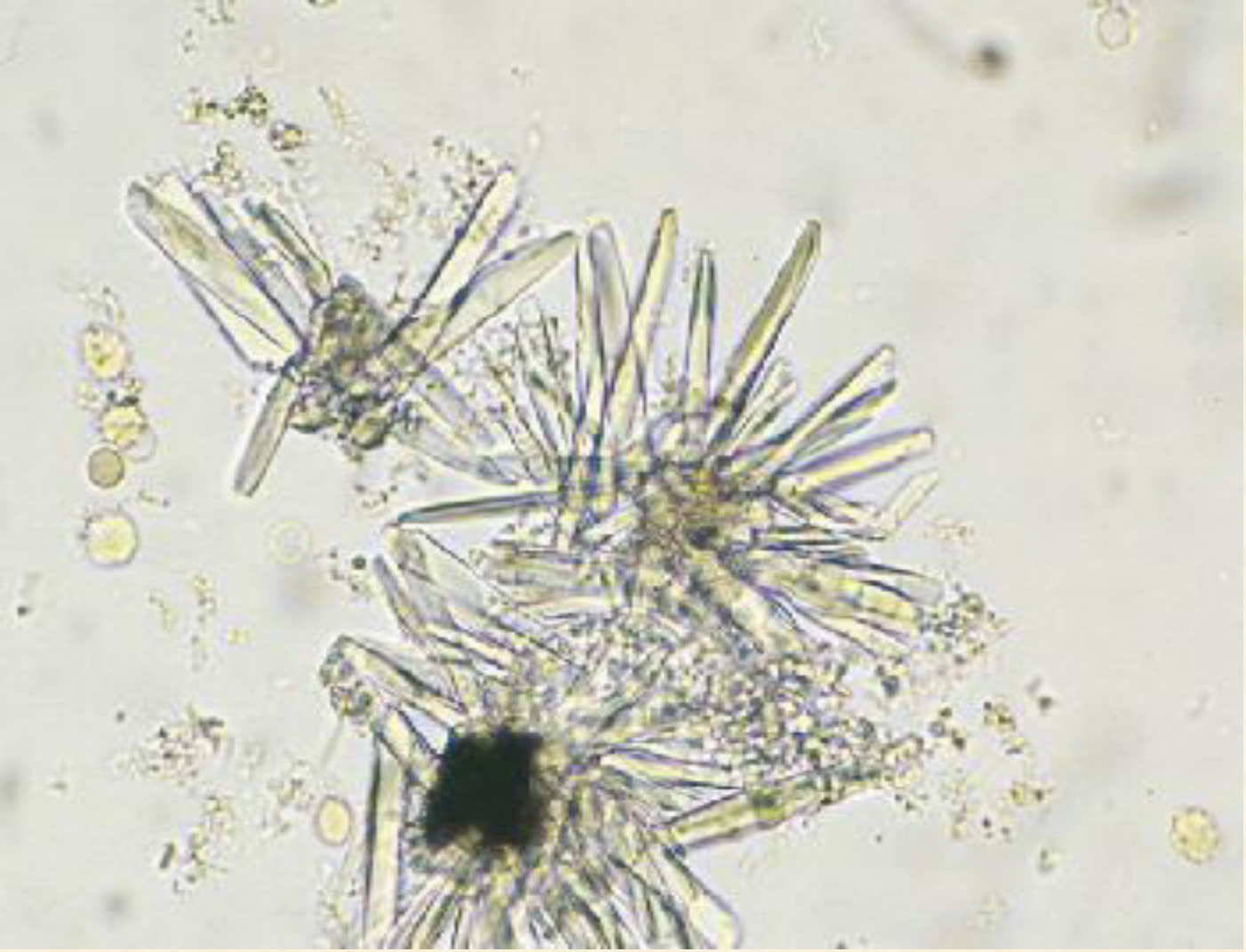
- ***Majority of crystals in urine are of limited clinical significance*** but proper identification of crystals is essential so as not to miss the few abnormal crystals associated with certain pathological conditions.



Triple Phosphate crystals (NH_4 Mg phosphate – struvite) are <u>normal</u> in urine

<u>Appearance:</u> *typically appear in "**coffin-lid" form**. May also appear as "fern-leaf" shape if freshly formed*.

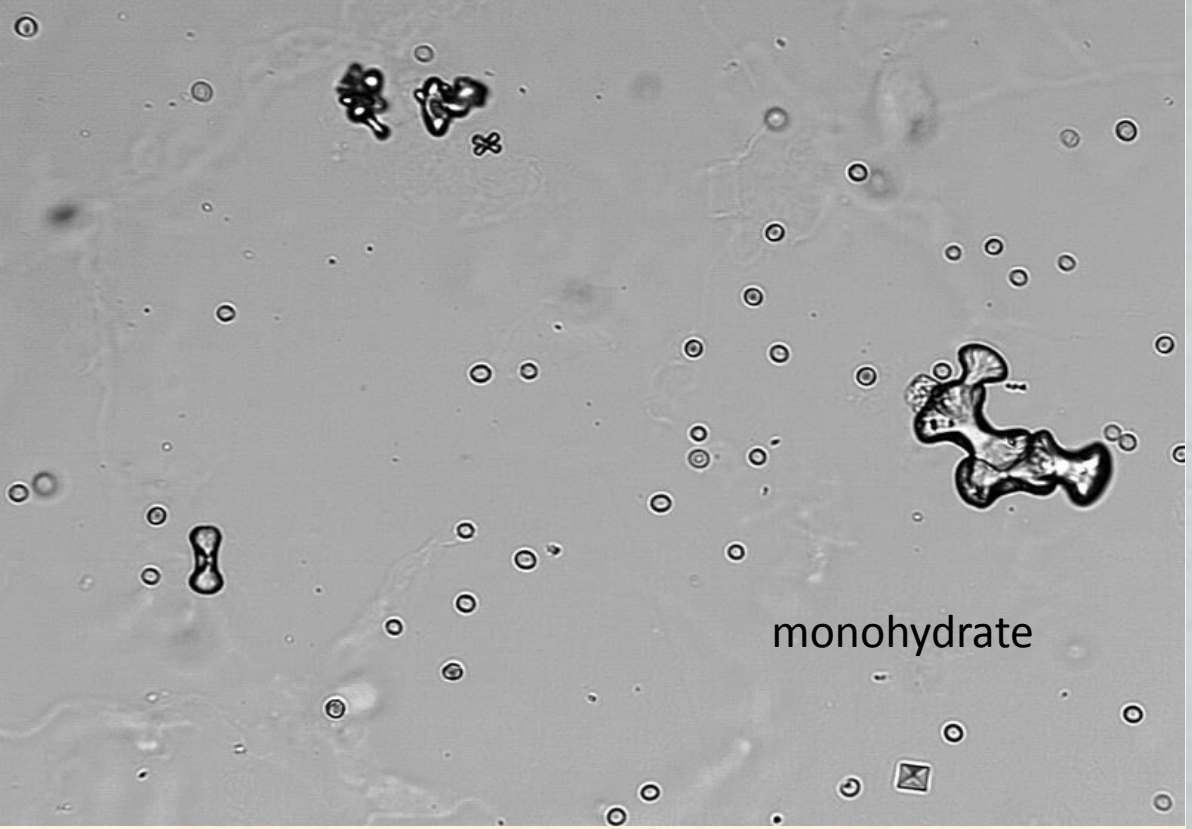
Although considered normal they may also be associated with **kidney stone** formation



Calcium phosphate crystals are <u>normal</u> in urine

<u>Appearance:</u> ***Large flat-shaped plates or wedge-shaped prisms****. The prisms often appear in rosettes. Single prisms are usually blunt on one end and pointed on the other end.*

Although considered normal they may also be associated with **kidney stone** formation.



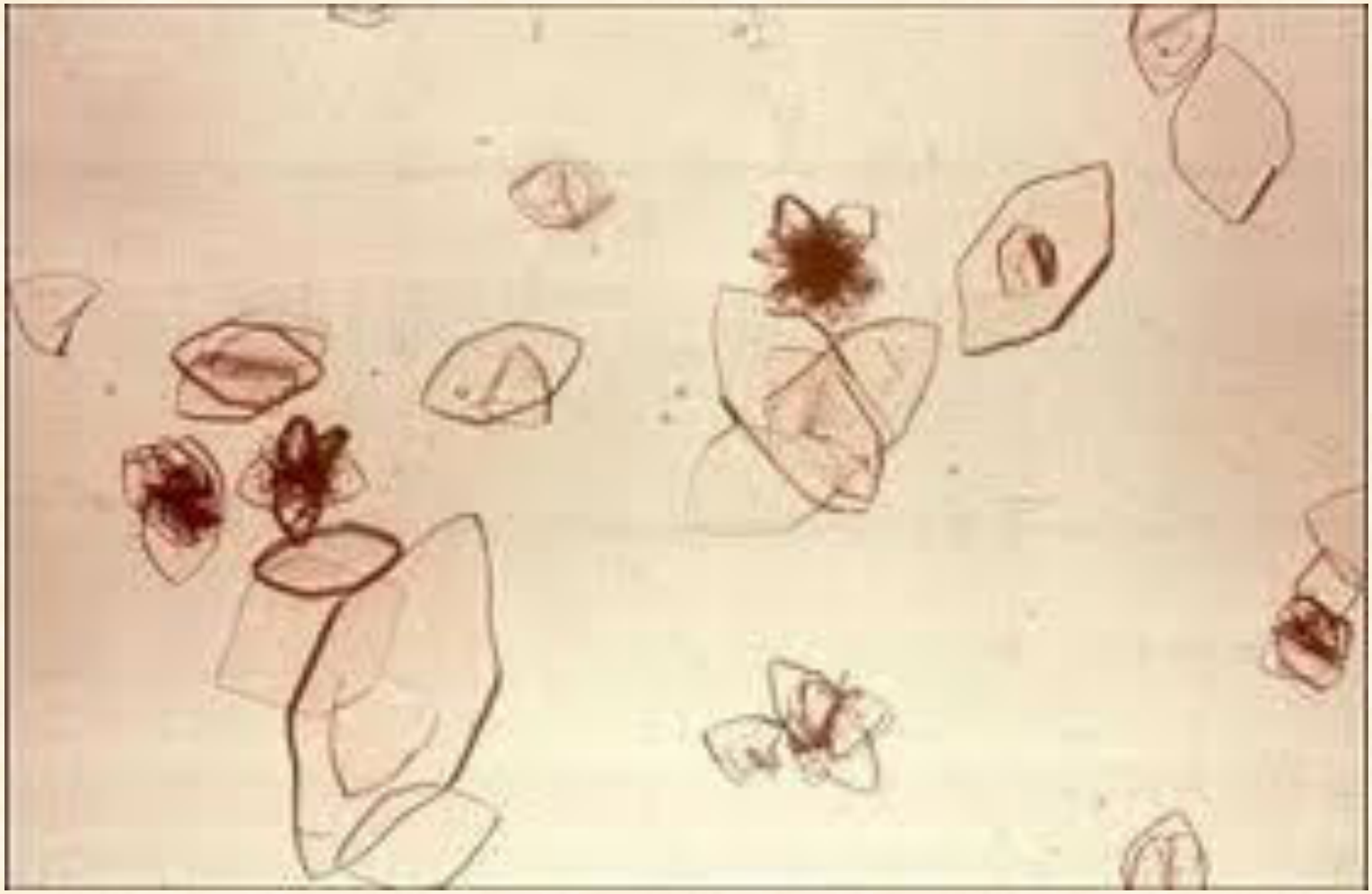
Calcium Oxlalate crystals are <u>normal</u> in urine.

<u>Appearance:</u> *colorless, many forms:*

Dihydrate: **octahedral ("envelope")** is **most common**

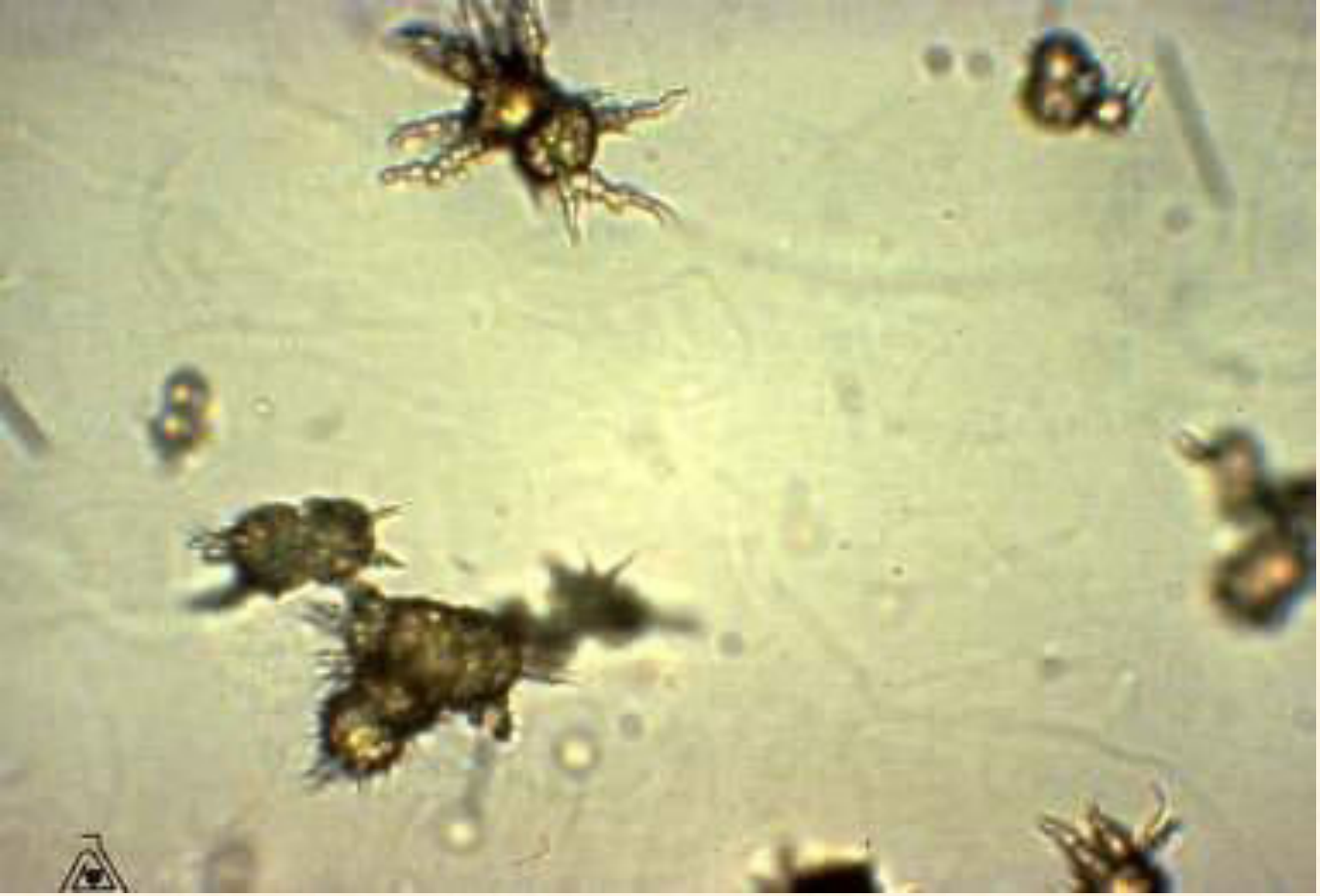
Monohydrate: ***dumbbell, ovoid,*** or rectangular in shape.

Associated with food high in oxalate (tomatoes, asparagus, ascorbic acid), Calcium oxalate is the **major component of renal calculi**. Large nos of crystals my be seen in chronic renal disease. Monohydrate calcium oxalate crystals are often seen in **ethylene glycol poisoning**.



Uric acid crystals are <u>normal</u> in urine.

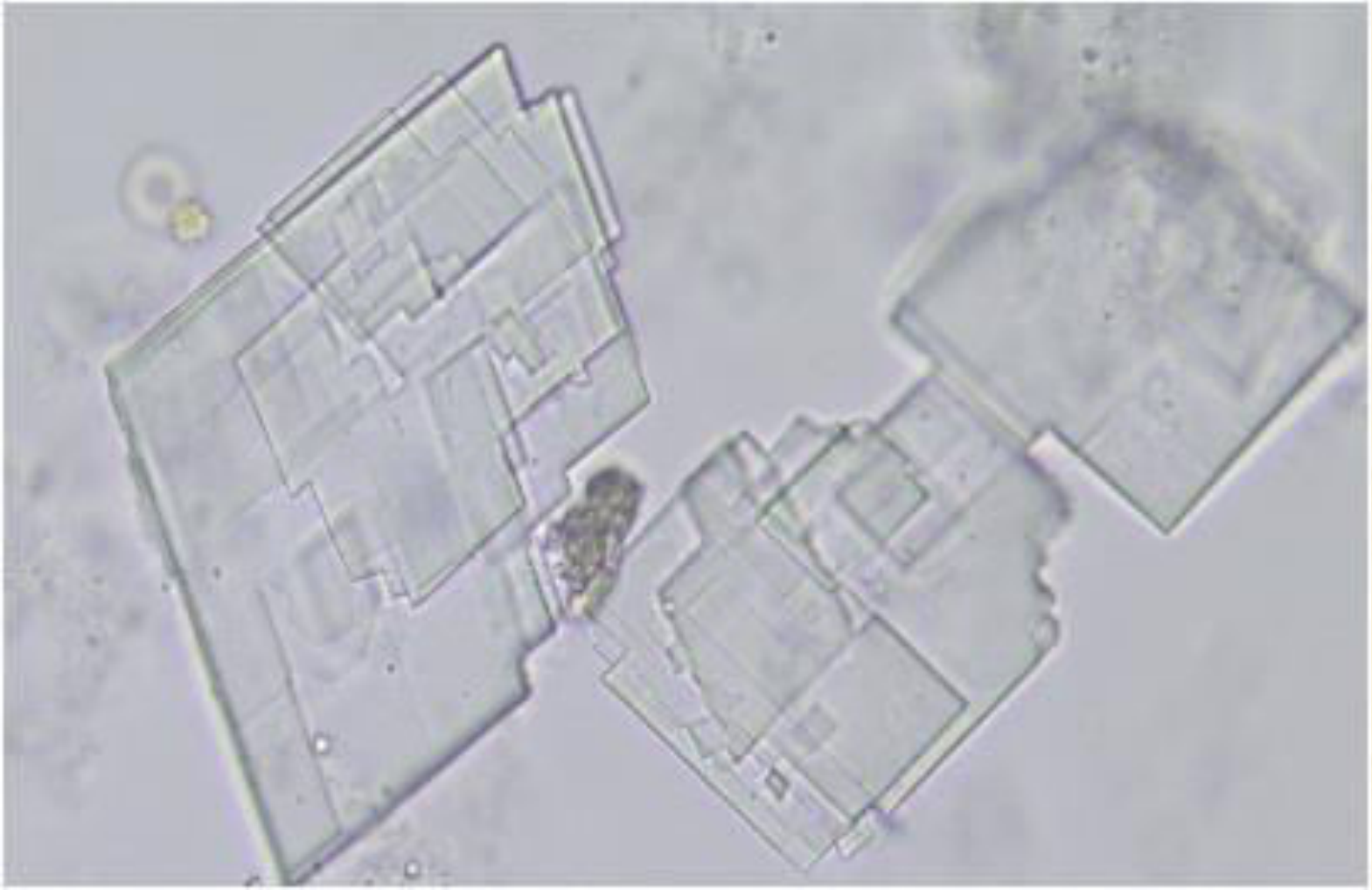
<u>Appearance:</u> *Found in multiple forms. They are often yellow/brown in color.* ***Rhombic or four sided flat plates, prisms, oval forms with pointed ends****, wedges & irregular plates. Large numbers may be seen with ↑nucleoprotein turnover, esp during chemotherapy of leukemia or lymphomas.* Their appearance may be associated with kidney stone formation. They are also seen in patients with **Gout, Lesch-Nyhan syndrome, and leukemia.**



Ammonium biurate crystals are <u>normal</u> in urine.

<u>Appearance:</u> *yellowish-brown, can be seen in a* ***"thorn apple" shape*** *(****round with thorny projections)*** *or in spherical form.*

Ammonium biruate crystals can be seen in normal urine. However, the presence of ammonium biurate crystals especially in combination with a urine pH 9.0 or higher usually indicates an **old or poorly preserved specimen**. Best practice is to **NOT report** any urinalysis results. **A recollect should be requested**.

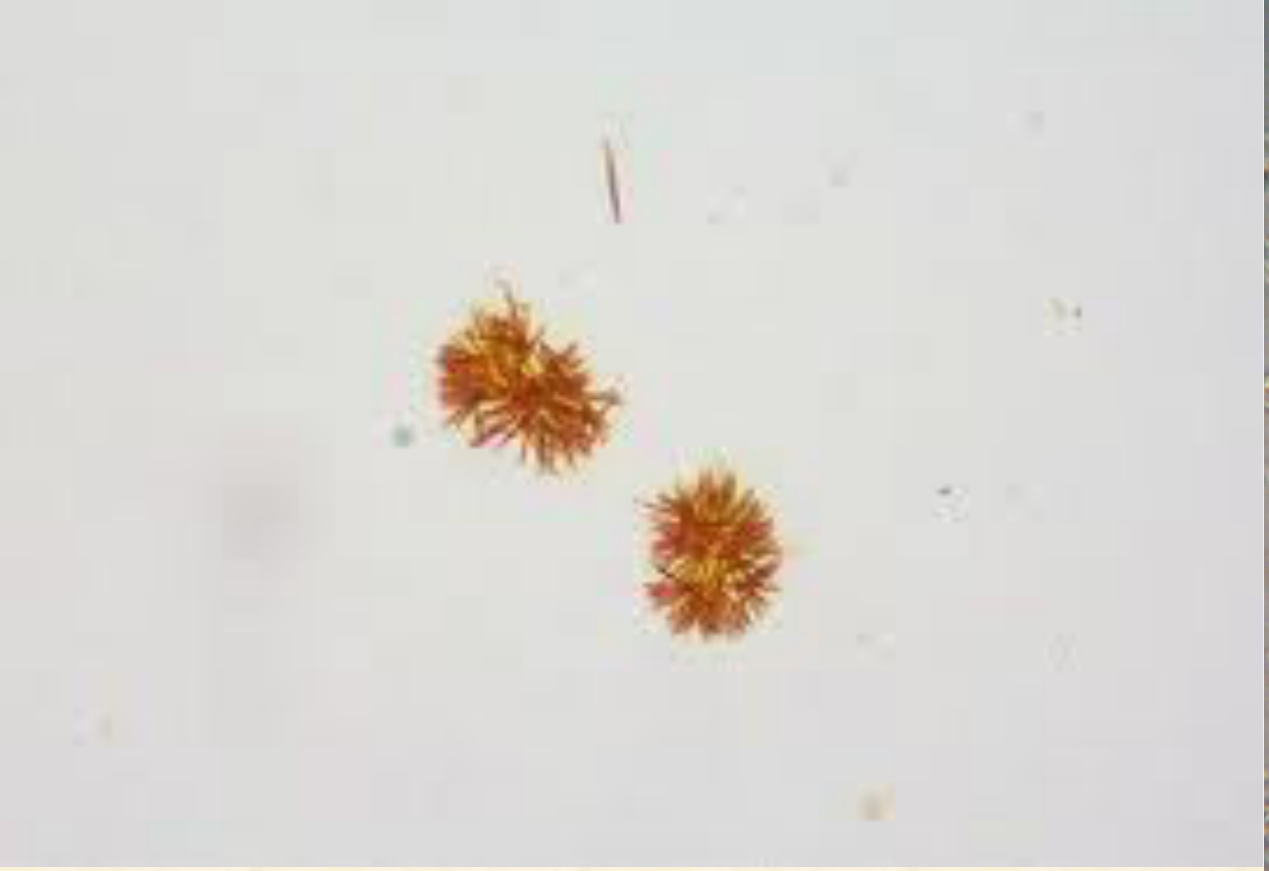


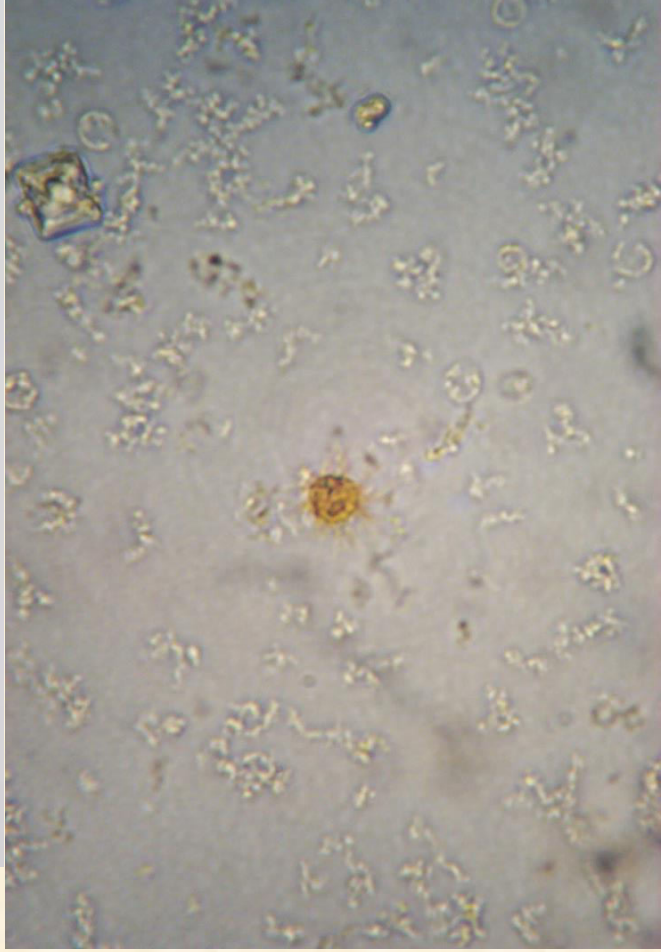
Cholesterol crystals are <u>abnormal</u> in urine

<u>Appearance:</u> *clear,* ***flat plates with notched corners.***

The appearance of cholesterol is associated with the **Nephrotic Syndrome**.

Cholesterol crystals are accompanied by a positive biochemical test for **protein**. They usually appear after the urine sample has been refrigerated and may be accompanied by **oval fat bodies, fatty casts, and free fat droplets** in the sediment.



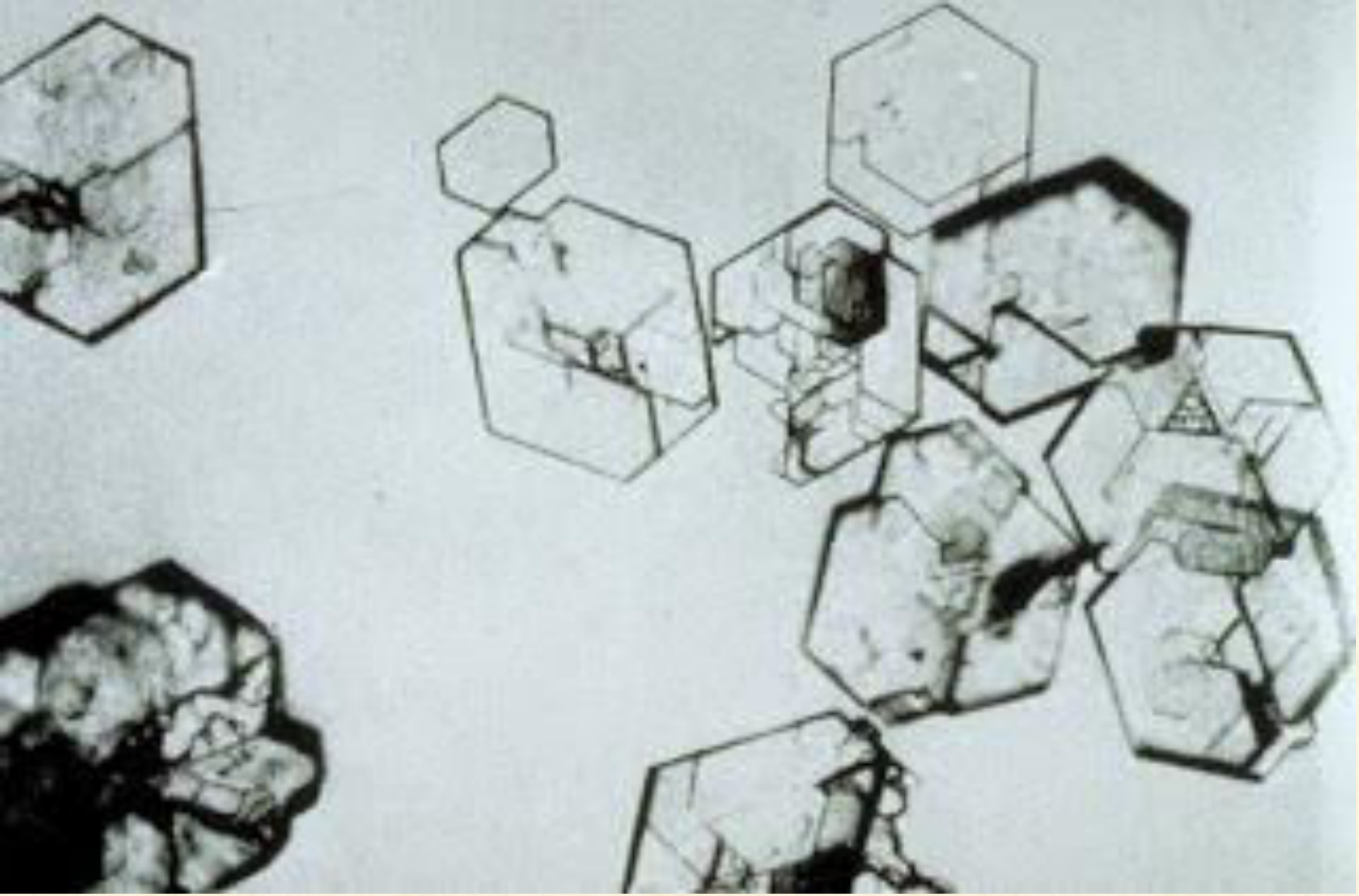


Bilirubin crystals are <u>abnormal</u> crystals in urine.

<u>Appearance:</u> ***Yellow-brown needles*** *or granules. They are frequently attached to the surface of cells.*

Bilirubin crystals are seen in several **hepatic disorders**.

The appearance of bilirubin crystals should be accompanied by a positive biochemical test for **bilirubin** (reagent test pad and Ictotest).



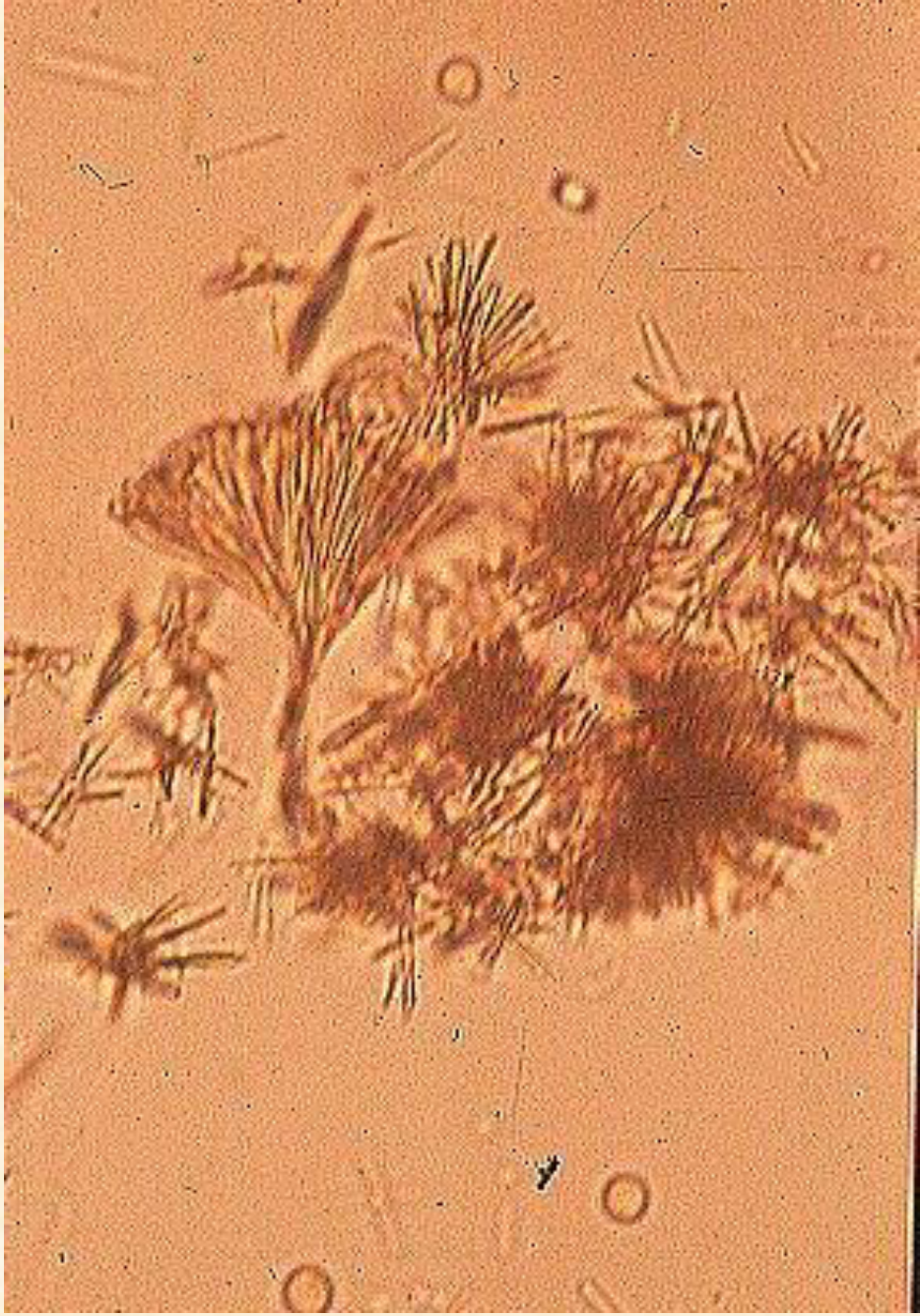
Cystine crystals: Abnormal. Though uncommon, cystine stones (seen in the genetic condition cystinosis) are ***hexagonal-shaped crystals***. This is pathognomonic

Tyrosine crystals are <u>abnormal</u> in urine

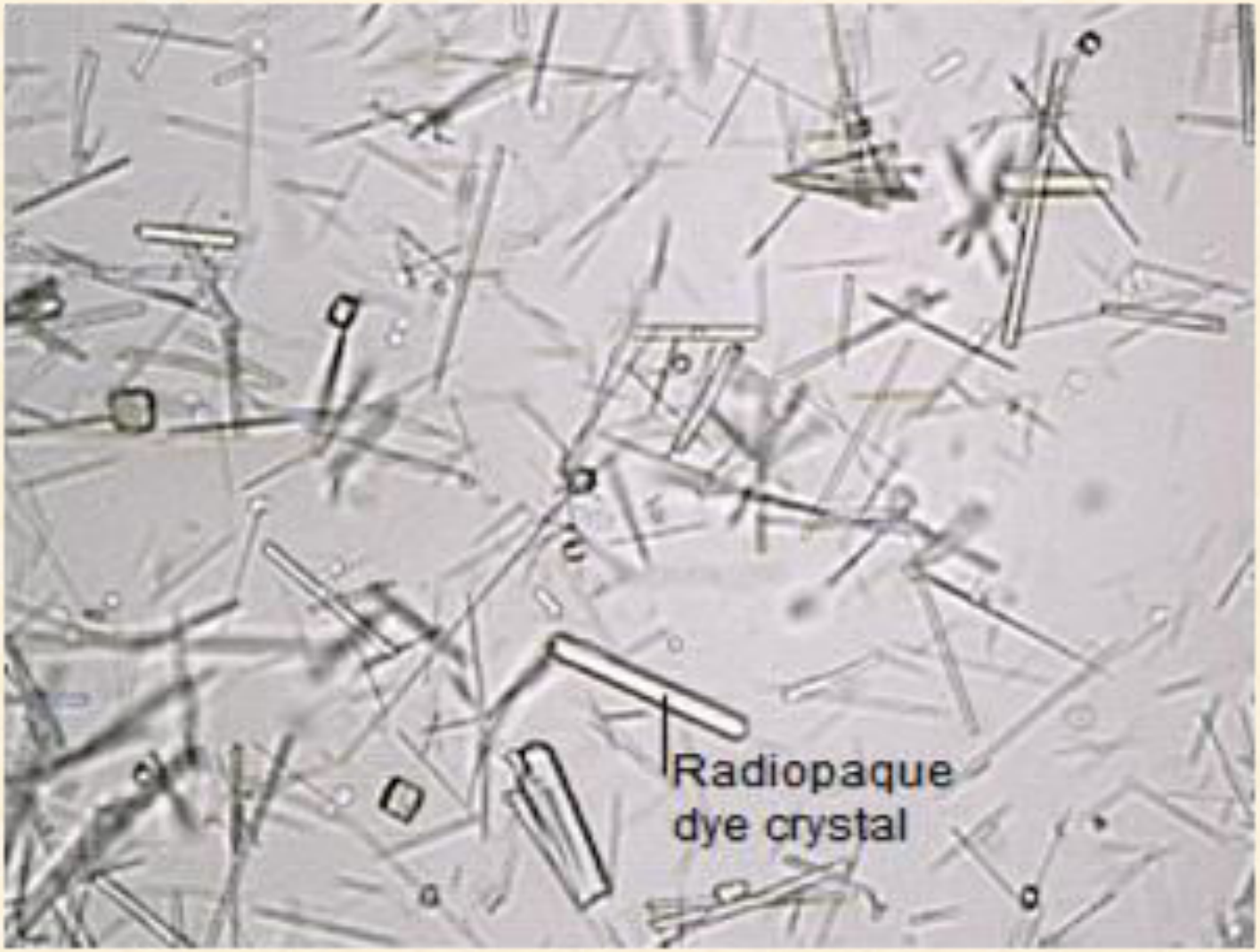
<u>Appearance:</u> *colorless to **yellow-brown single needles**. Also seen as **sheaves or rosettes.***

Tyrosine crystals may be seen in **tyrosinemia** and in certain liver disorders in which amino acid metabolism is impaired.

The presence of tyrosine crystals is usually accompanied by a positive biochemical test for **bilirubin** and are often accompanied by the **presence of leucine crystals** in the sediment.



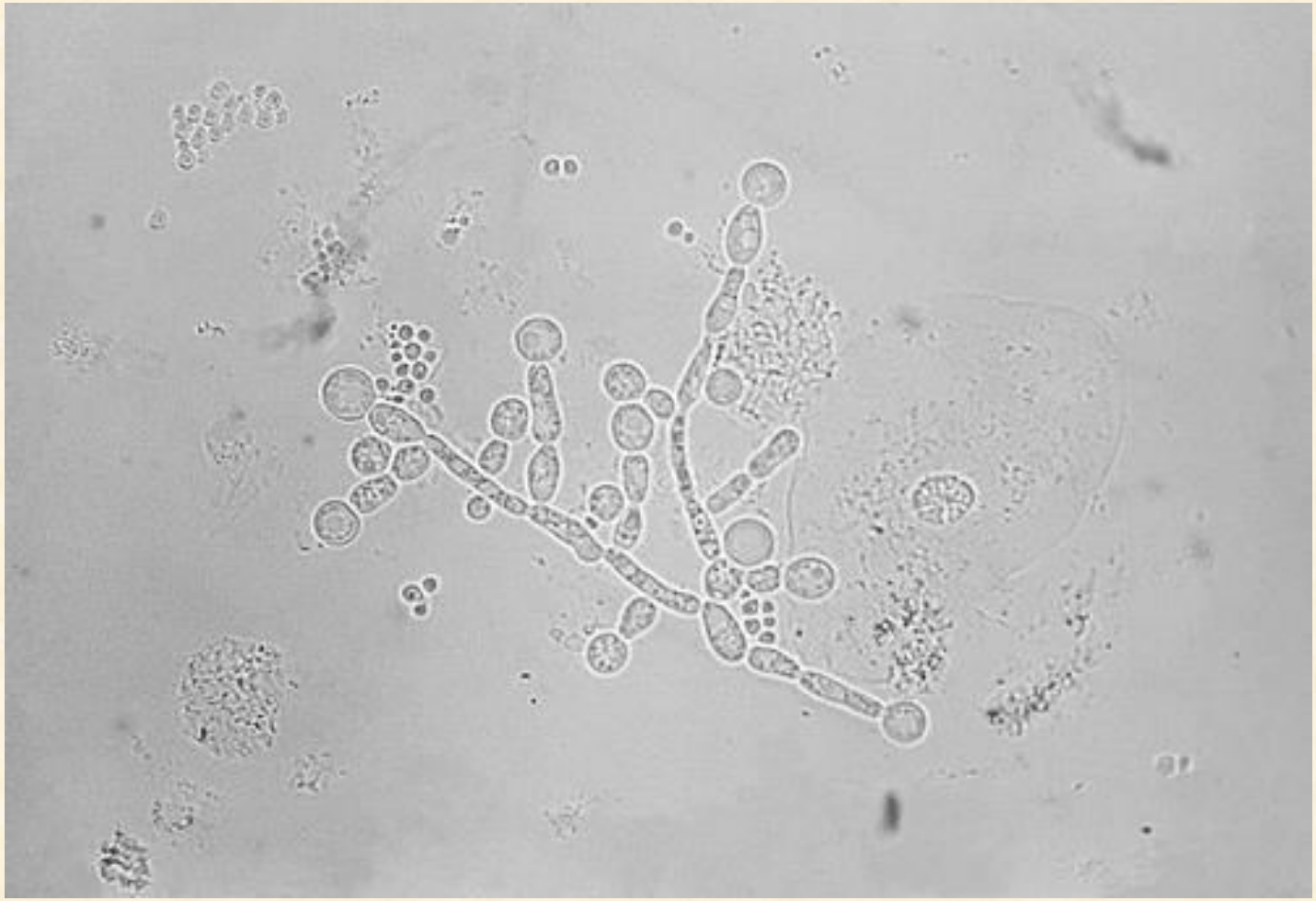
Sulfanomide crystals are considered <u>abnormal</u> in urine.
<u>Appearance:</u> *flat needles,* ***sheaves of small needles*** *or as spheroids. Often brown color.*
The presence of sulfanomide crystals usually indicates administration of the drug and not necessarily a pathological condition.
However, their presence is also associated with **kidney stone formation**.



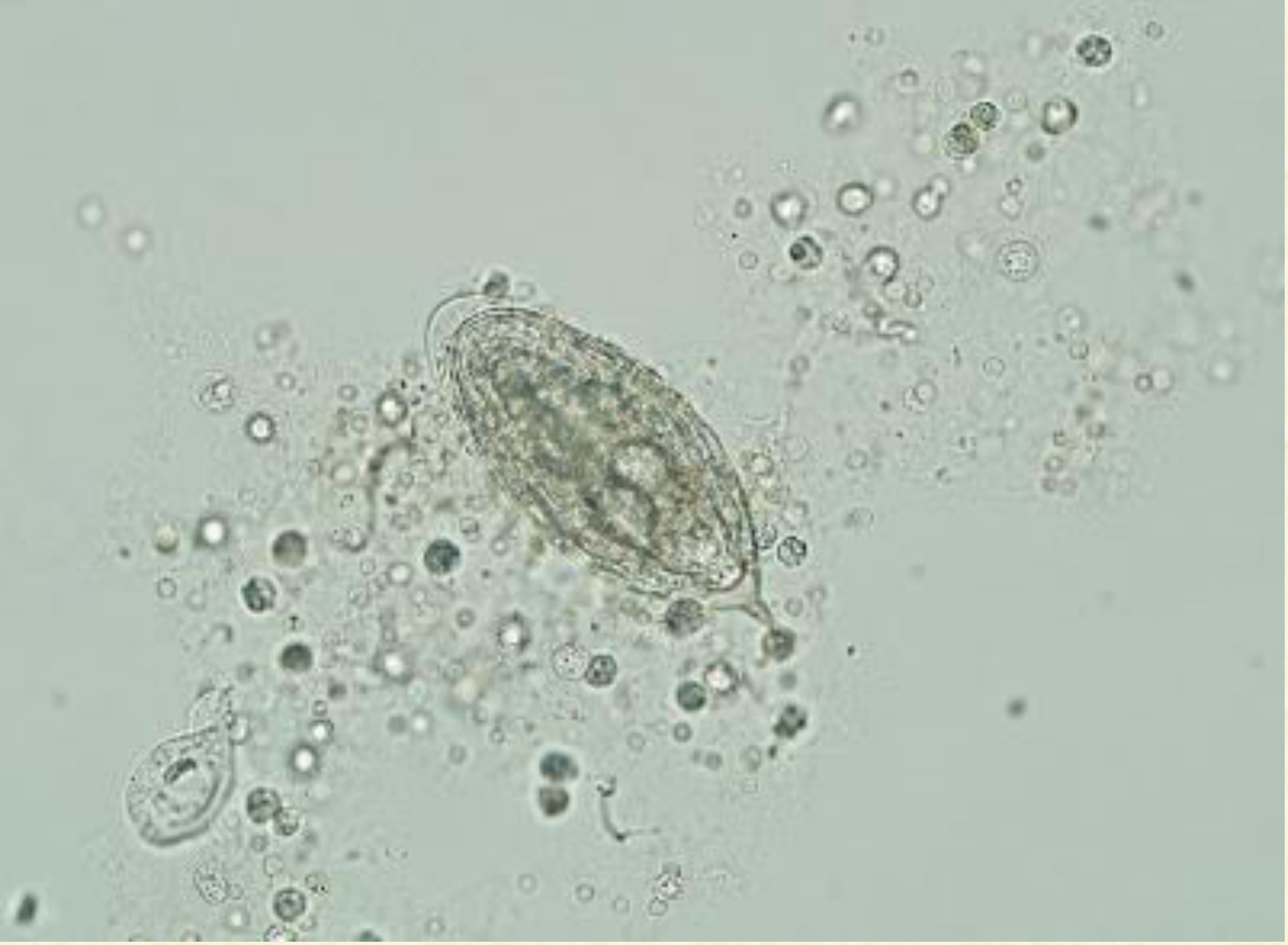
Radiopaque dye is considered <u>abnormal</u> in urine

<u>Appearance:</u> *flat needles or sheaves accompanied by round globules, variable form*.

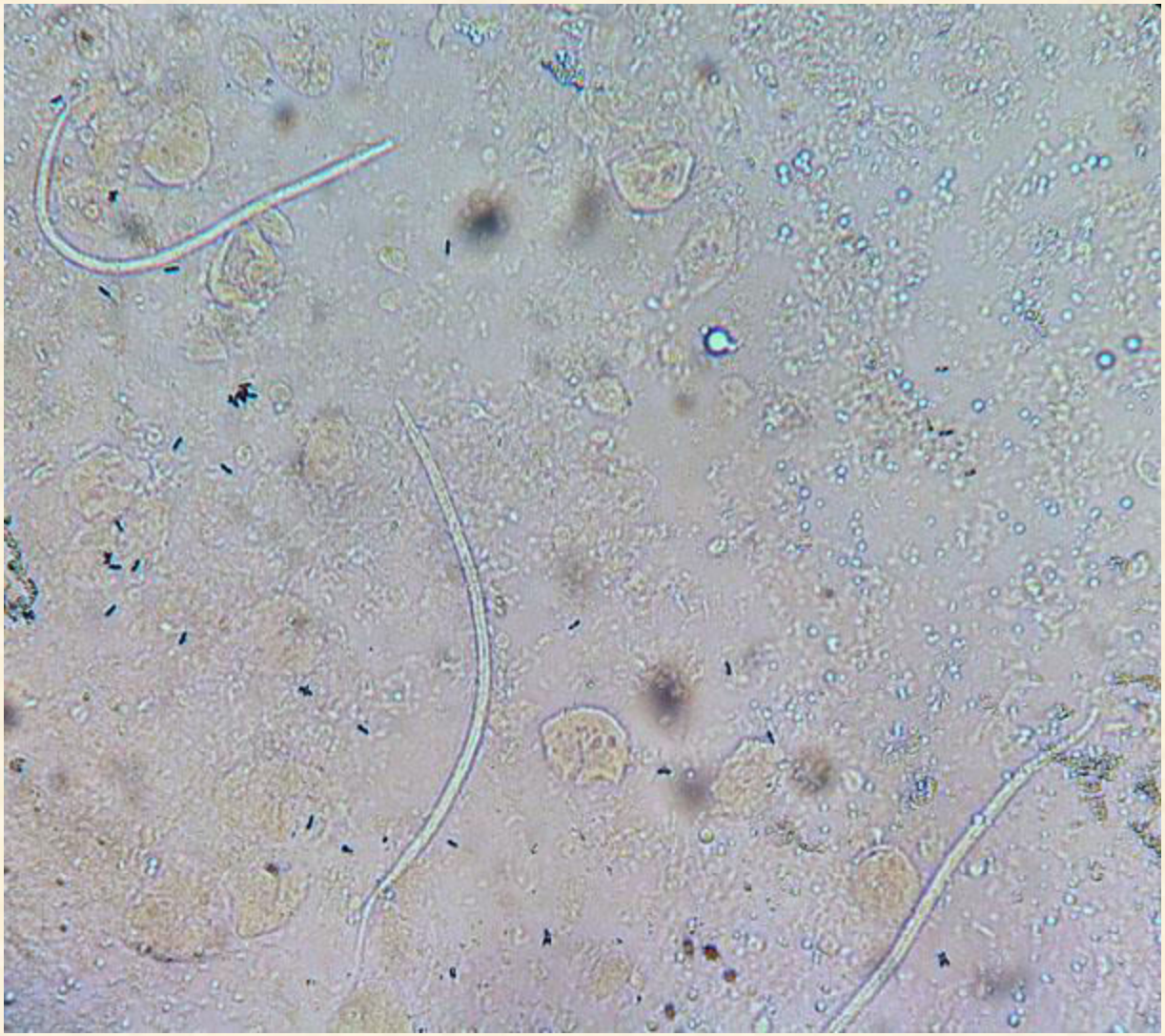
When the presence of radiopaque dye crystals is suspected, the ordering location should be consulted to confirm administration of contrast media.



The presence of yeast in the urine sediment may indicate an infection. A frequently seen yeast in urine is **Candida**. Identification of this organism is relatively easy because of its usual club shape. In the majority of cases, only the isolated cells are seen but, in some cases, budding pseudohyphea may be observed . Most often they are Candida, which may colonize bladder, urethra, or vagina.



Schistosoma hematobium egg

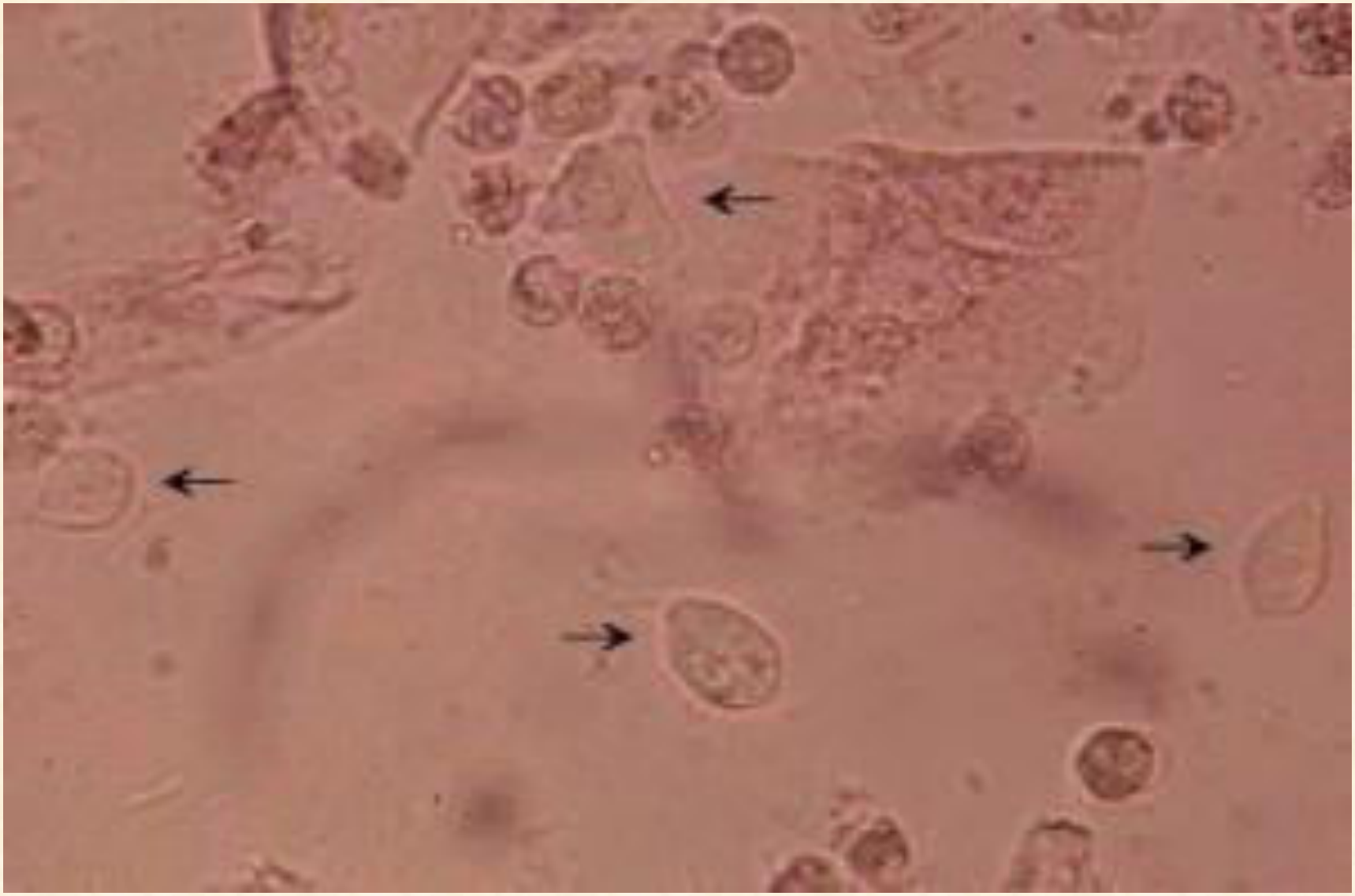


Microfilariae:

Worm-like unsheathed, with sharply pointed, curved tails. Are 150 to over 350 µm long, and 5-9 µm in diameter.

Microfilariae exit the nodules created around the adult worms and migrate actively through the dermis and connective tissues, not only in the vicinity of the nodules, but also at some distance from them.

Rarely, microfilariae may be found in urine, blood, sputum, and the eye (during heavy infections)



Trichomonas vaginalis

Stool examination

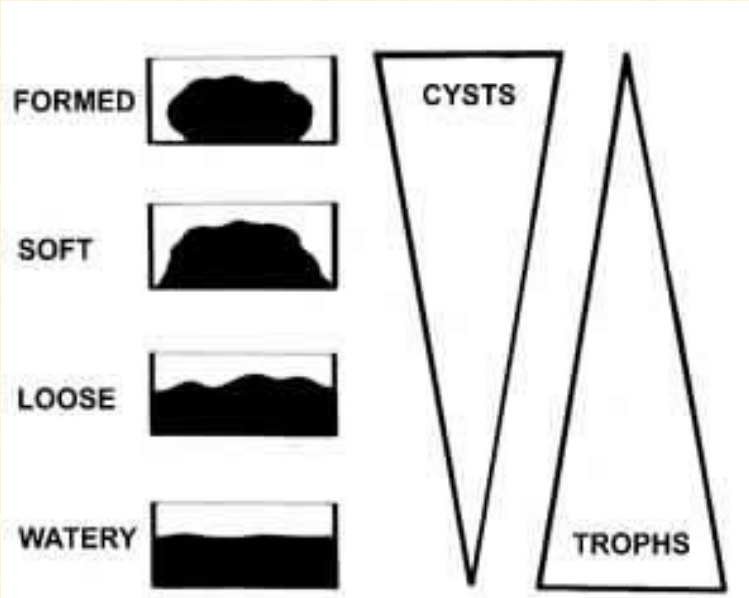
Stool collection

- **Collect the stool in a dry, clean, leakproof (screw-capped) container. Make sure no urine, water, soil or other material gets in the container.**
- Fresh stool should be examined, processed, or preserved immediately.
- If delay anticipated, add one volume of the stool specimen to three volumes of the preservative. Do not freeze.
- Refrigeration is not ideal.
- Ensure that the specimen containers are sealed well.
- Contamination with mineral oil, barium, bismuth, antibiotics, anti-malarials, or non-absorbable antidiarrheal agents can prevent parasite recovery for one to several weeks.
- Transport to the laboratory ASAP.
- **Handle all samples as Biohazard.**

•Fresh stool specimen is good for processing for culture & O&P.

• If delay is expected then faeces can be stored at **4˚C for C/S**

•For O&P, use preservative-formalin/PVA



Preservatives for stool exam

Preservative	*Advantages*	*Disadvantages*
10% Formalin	➢All purpose fixative ➢Easy to prepare ➢Long shelf life ➢***Good preservation of morphology of helminth eggs, larvae, protozoan cysts, and coccidia*** ➢Suitable for concentration procedures and UV fluorescence microscopy ➢Suitable for acid-fast, safranin, and chromotrope stains ➢Compatible with immunoassay kits and UV fluorescence microscopy	❖Not suitable for some permanent smears stained with trichrome ❖Inadequate preservation of morphology of protozoan trophozoites ❖Can interfere with PCR, especially after extended fixation time
LV-PVA (low viscosity polyvinyl-alcohol)	➢**Good preservation of morphology of protozoan trophozoites and cysts** ➢Easy preparation of permanent smears stained with such as trichrome (solution both preserves organisms and makes them adhere to slides) ➢Preserved samples remain stable for several months	❖Inadequate preservation of morphology of helminth eggs and larvae, coccidia, and microsporidia ❖Contains mercuric chloride ❖Difficult and expensive to dispose of ❖Difficult to prepare in the laboratory ❖Not suitable for concentration procedures ❖Cannot be used with immunoassay kits ❖Not suitable for acid-fast, safranin and chromotrope stains

Physical examination

- Consistency: **formed, unformed (soft), loose or watery**. The cysts have been mostly found in the formed stools, while trophozoites have been most abundantly found in watery stools.

- The **presence of blood, mucus or pus**.

- The **presence of worms,** e.g. Enterobius Vermicularis, Ascaris, tapeworm segments, e.g. Taenia species.

- **Colour** (white, yellow, brown or black).

- Normal faeces appear brown and formed or semiformed. Infant faeces are yellow-green and semiformed

Formalin - ethyl acetate sedimentation concentration method

- Formalin - ethyl acatate sedimentation leads to recovery of all protozoa, eggs and larvae present. This method is recommended as being the easiest to perform, allowing recovery of the broadest range of organisms, and being the least subject to technical error.
- Specimen must be fresh or formalin- PVA preserved stool.
- Transfer ½ tsp fresh stool into 10 ml of 5 or 10% formalin in around bottomed tube. Mix thoroughly and let the mixture stand for 30 min for fixation. If the received specimen is already in formalin, restir the mixture.
- Depending on the amt & viscosity of the specimen, strain a sufficient quantity thro wet guaze, into a conical 15ml centrifuge tube to give the desired amt of sediment (1ml).
- Add 0.85% saline, or 5-10% formalin, almost to the top of the tube, & centrifuge for 10min at 500g.
- Decant the supernatant, and resuspend the sediment in saline or formalin; add saline/formalin to the top of the tube & centrifuge again for 10 min at 500g. This step may be omitted if the supernatant after the first wash is light tan or clear.

- Decant the supernatant fluid & resuspend the sediment in 5-10% formalin. Fill only half the tube. If the amount of sediment left in the bottom of the tube is very small or the original specimen contained a lot of mucus, do not add ethyl acetate at this point; merely add the formalin, resuspend the specimen, centrifuge, decant, and examine the remaining sediment. Otherwise, add 4 to 5 cc. of ethyl acetate. Stopper the tube, and shake it vigorously for at least 30 seconds, exerting pressure on the stopper throughout to prevent accidental release of specimen. Perform this step behind the protection of a bench safety shield. Hold the tube so that the stopper is directed away from your face. After a 15 to 30 second wait, remove the stopper.
- Centrifuge for 10 minutes at 500 g. Four layers should result: a small amount of sediment (containing any parasites) in the bottom of the tube; a layer of formalin; a plug of fecal debris on top of the formalin layer; and a layer of ethyl acetate at the top.
- Free the plug of debris by ringing the plug with a wooden applicator stick; decant all of the supernatant fluid. After proper decanting, a drop or two of fluid remaining on the side of the tube may run down into the sediment. Mix this fluid with the sediment. If the sediment is still somewhat solid, add a drop or two of saline to the sediment, mix, and add a small drop to a slide, add a cover slip, and examine.
- Systematically scan with the 10X objective. The entire cover slip should be examined. If you see something suspicious, use the 40X objective for a more detailed study. Even if nothing suspicious is observed, approximately one third of the cover slip should be examined with the 40X objective. Addition of Lugol's iodine can be used to enhance morphological detail such as the inner structures of cysts of Entamoebae.

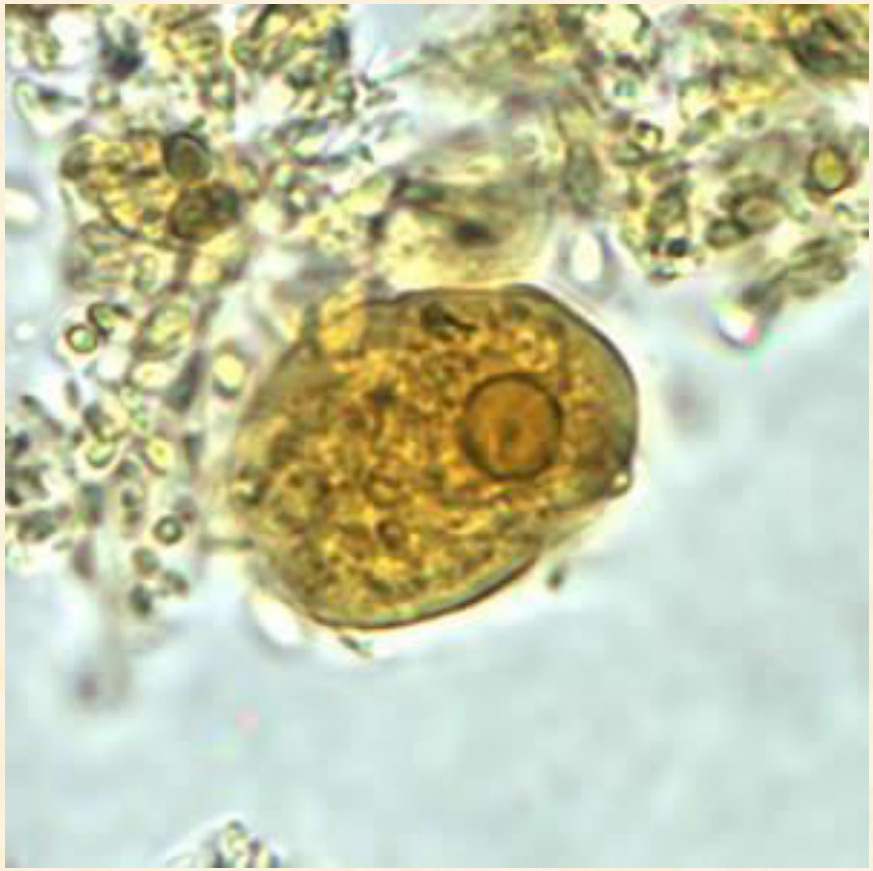
Stool examination... cont

Microscopy: Wet mounts and Iodine mounts:

- **Pus cells and RBCs:**
  - ***clumps of pus cells of > 50 cells per high power field*** along with macrophages and erythrocytes are typical of shigellosis.
  - ***A smaller number of pus cells of <20 per high power field*** are found in salmonellosis and in infections which are caused by invasive E.coli.
  - ***Few leucocytes (< 5 cells per high power field)*** are present in cholera, EPEC and ETEC and viral diarrhoea.
  - Note number of RBCs, if any per hpf.

- Undigested material, starch, pollen, Charcot -Leyden crystals may be seen.

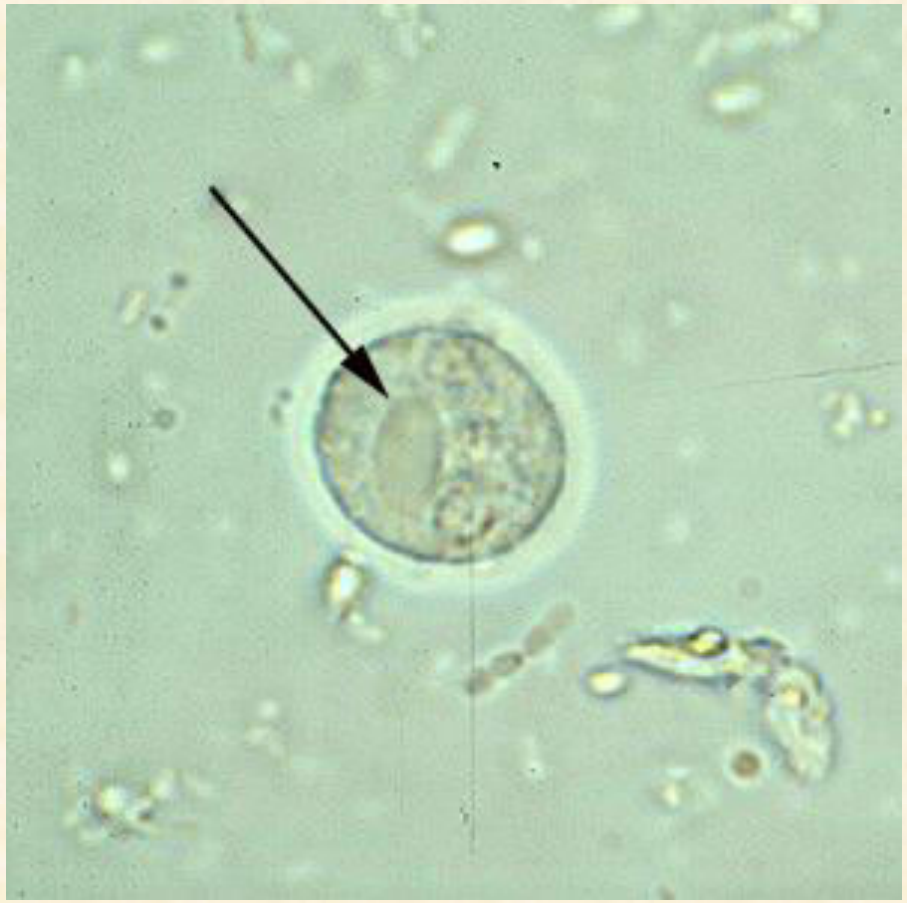
- Parasitic ova, cysts and trophozoites



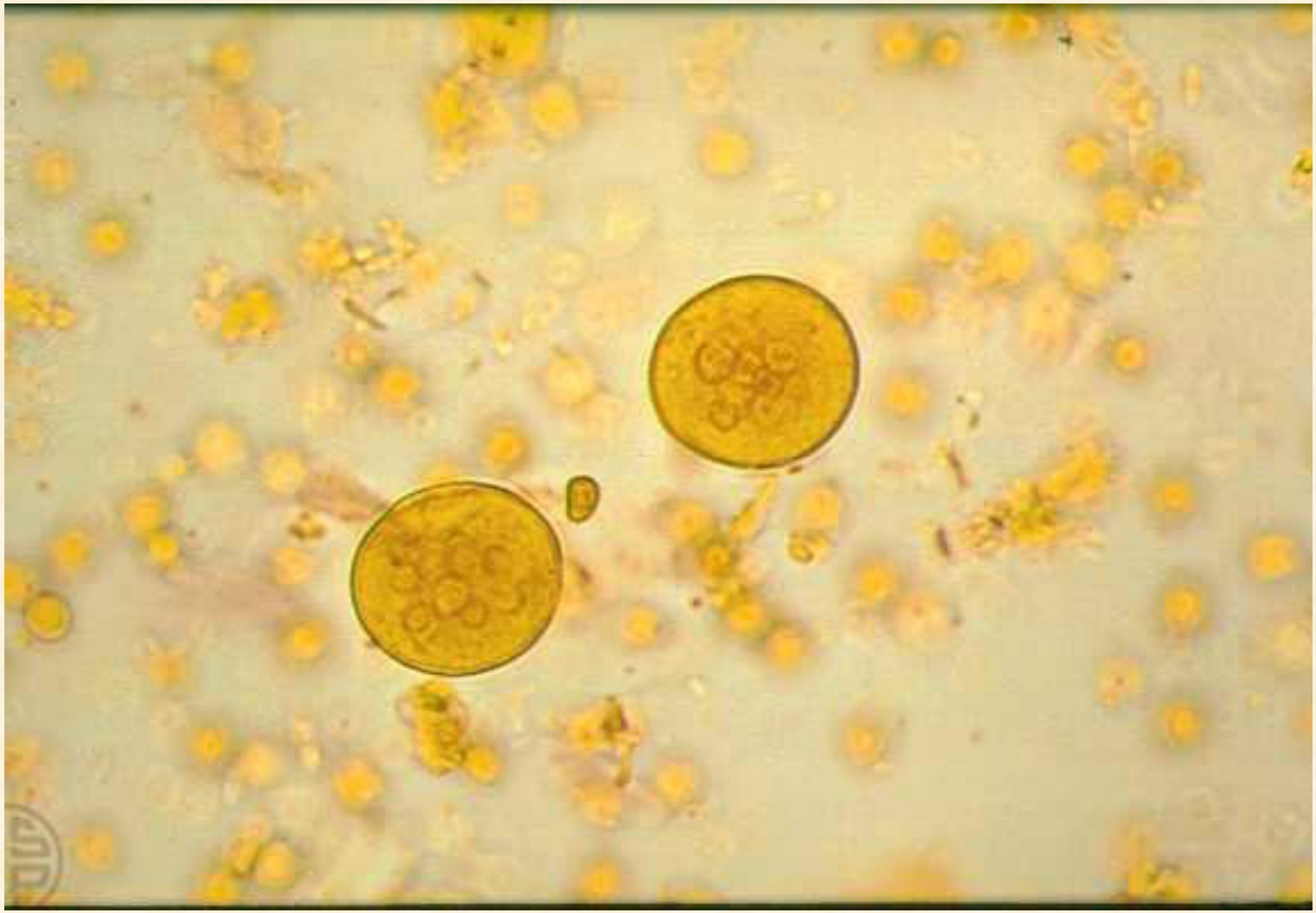
A, **B:** Trophozoites of *E. histolytica*/*E. dispar* in a direct wet mount stained with iodine.

Trophozoite

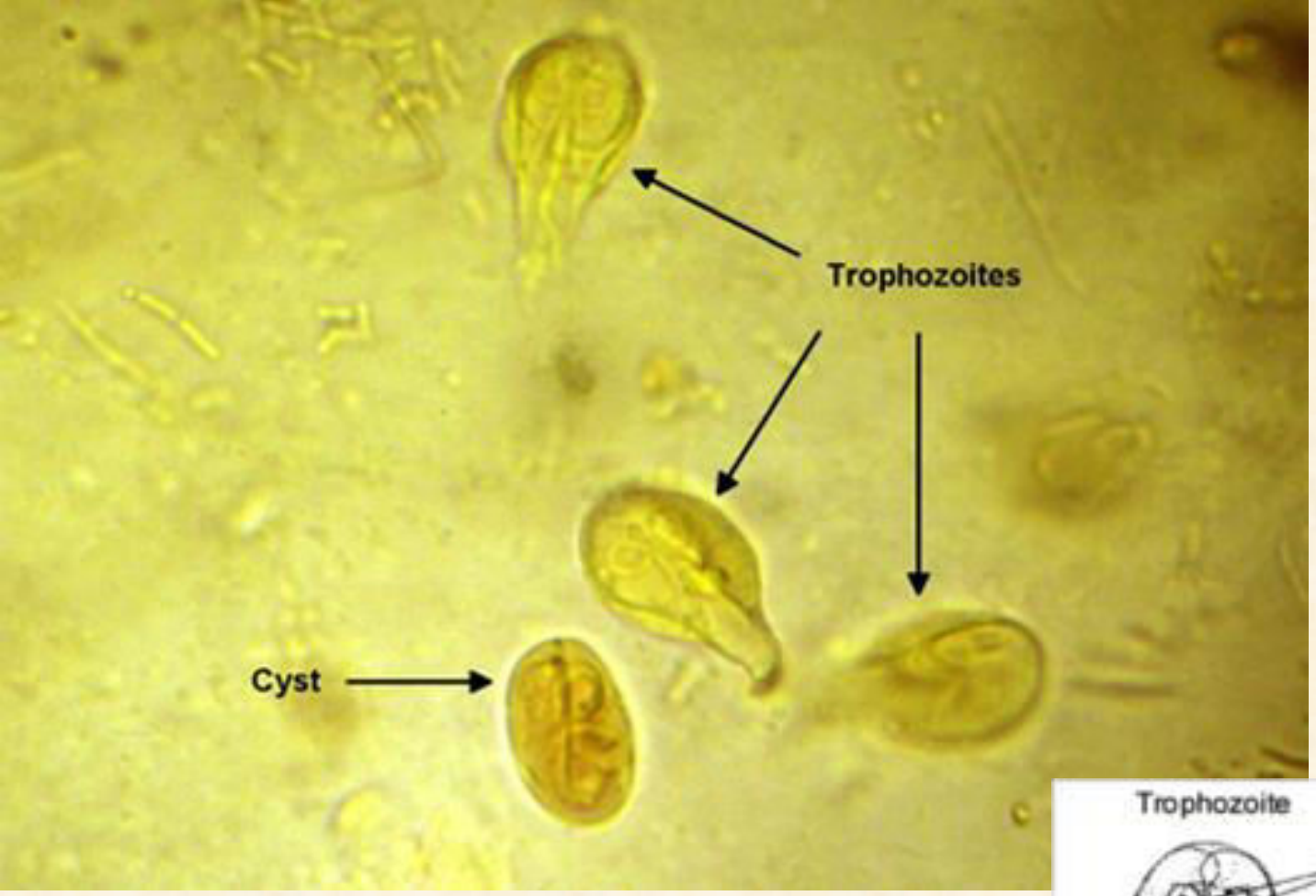
Size: 10-60 µm; usual range, 10-20 µm (>20 µm in the hematophagousform

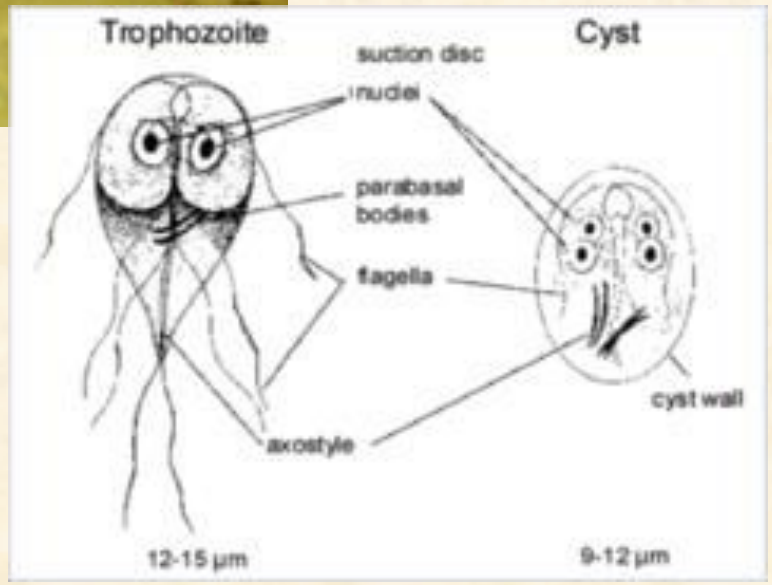


A, **B:** Cysts of *E. histolytica*/*E. dispar* in an unstained concentrated wet mount of stool. Notice the chromatoid bodies with blunt, rounded ends (arrows). **Size**: from 10 to 20 µm (usual range, 12-14 µm). Immature cysts are generally larger.



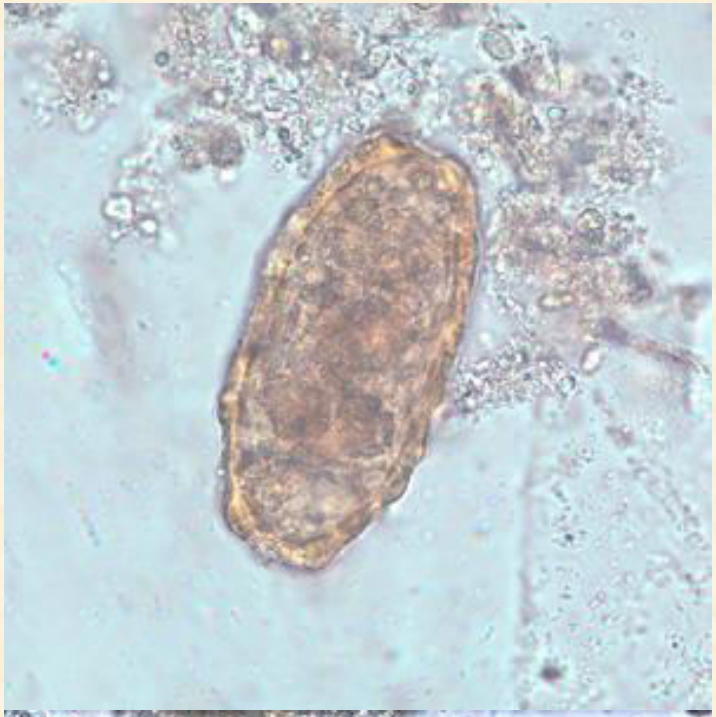
Cysts of *Entamoeba coli*, wet mount in iodine. Mature cysts typically have 8 nuclei, and measure usually 15 to 25 µm (range 10 to 35 µm). The cyst in the figure shows 5 nuclei visible in this focal plane



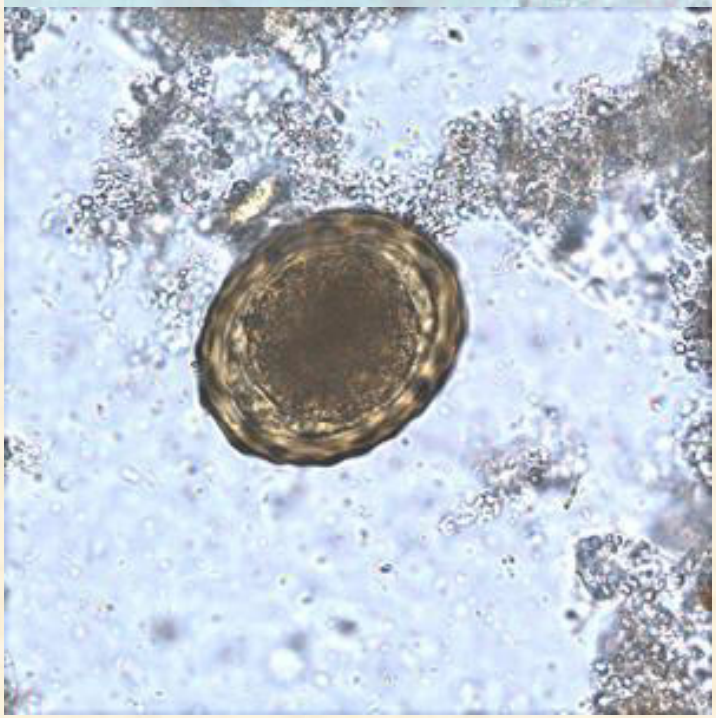


Giardia lamblia: trophozoite and cysts:

Size cyst: 8 - 13 microns x 5-8 microns
Size trophozoite: 10 - 20 microns x 5-9 microns

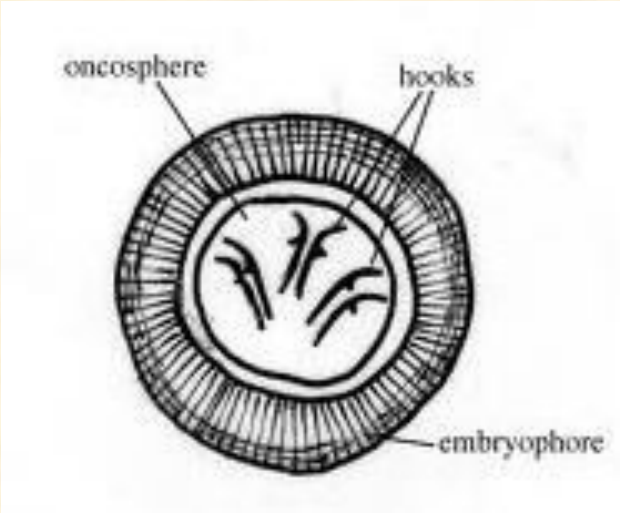


Unfertilized egg of *A. lumbricoides* in an unstained wet mount of stool. Unfertilized eggs are elongated and larger than fertile eggs (up to 90 µm in length x 45 µm).

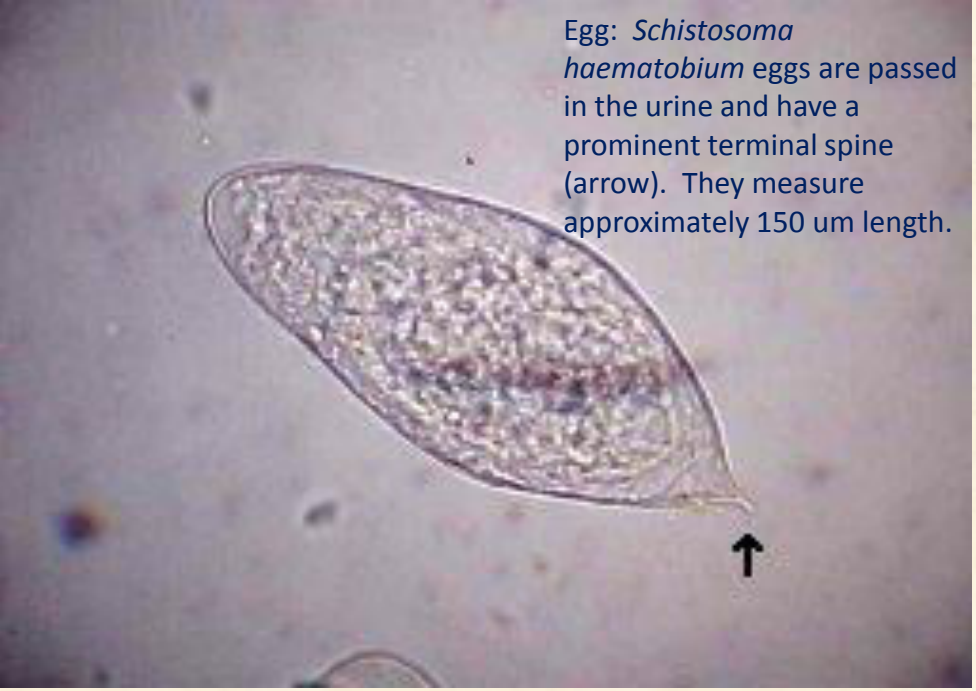


Fertilized egg of *A. lumbricoides* in an unstained wet mount of stool. Fertilized eggs are are rounded and have a thick shell with an external mammillated layer that is often stained brown by bile. In some cases, the outer layer is absent (known as decorticated eggs). Fertile eggs range from 45 to 75 µm in length.

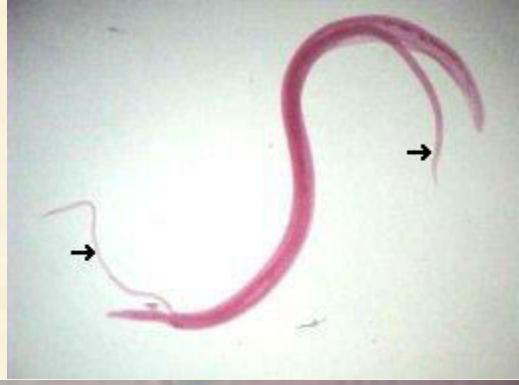
vivek bhat

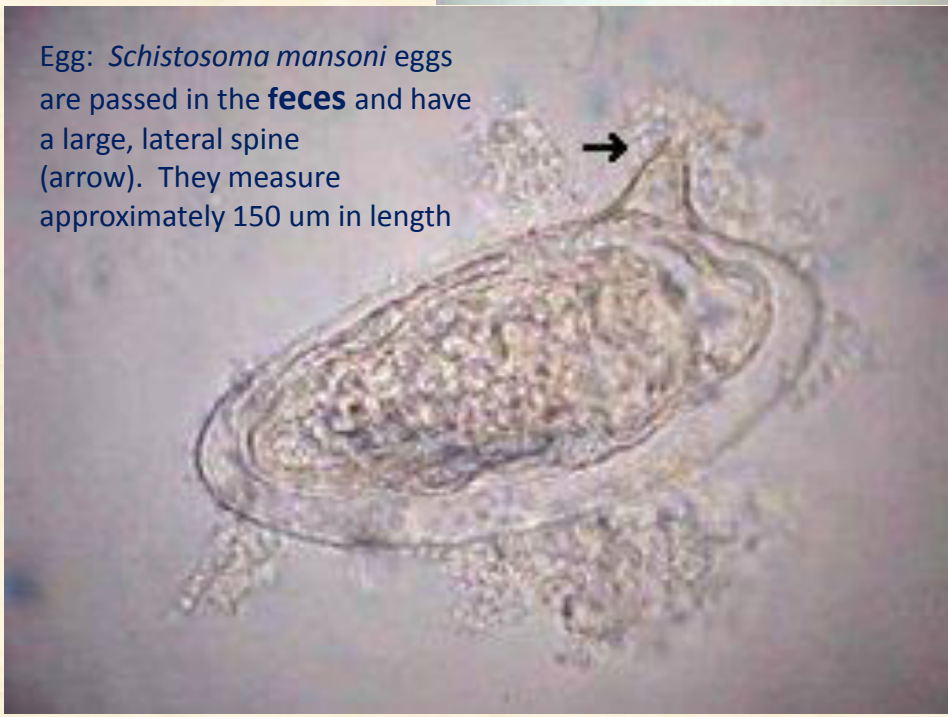


The eggs of Taenia saginata and T. solium are undistinguishable morphologically (morphologic species identification will have to rely on the proglottids or scolices). The eggs are rounded or subspherical, diameter 31 - 43 µm, with a thick radially striated brown shell. Inside each shell is an embryonated oncosphere with 6 hooks.
Worldwide distribution

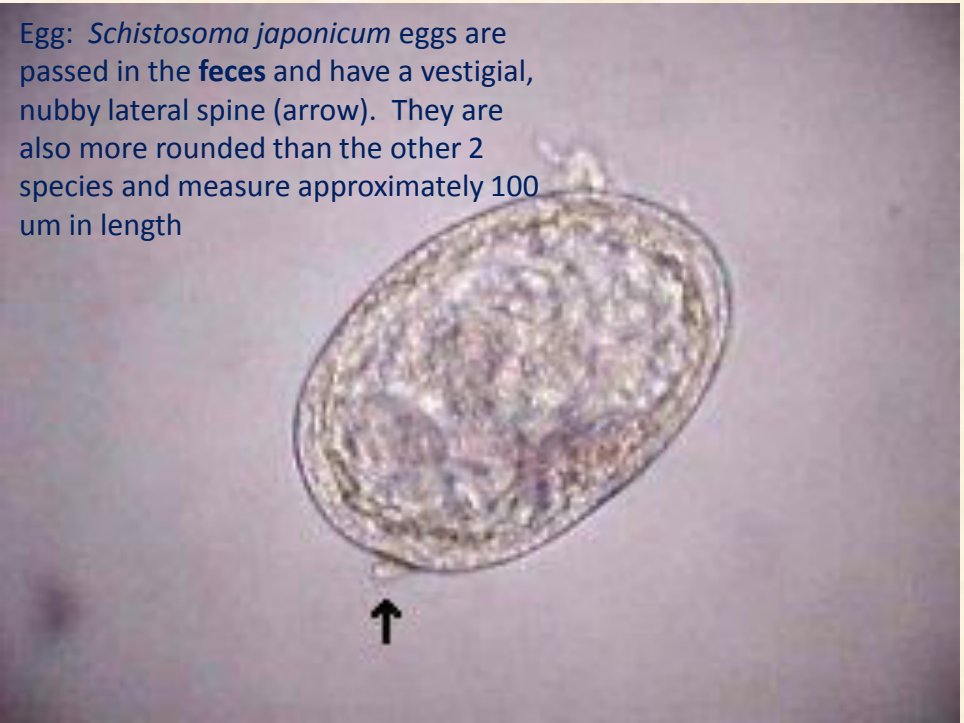


Egg: *Schistosoma haematobium* eggs are passed in the urine and have a prominent terminal spine (arrow). They measure approximately 150 um length.

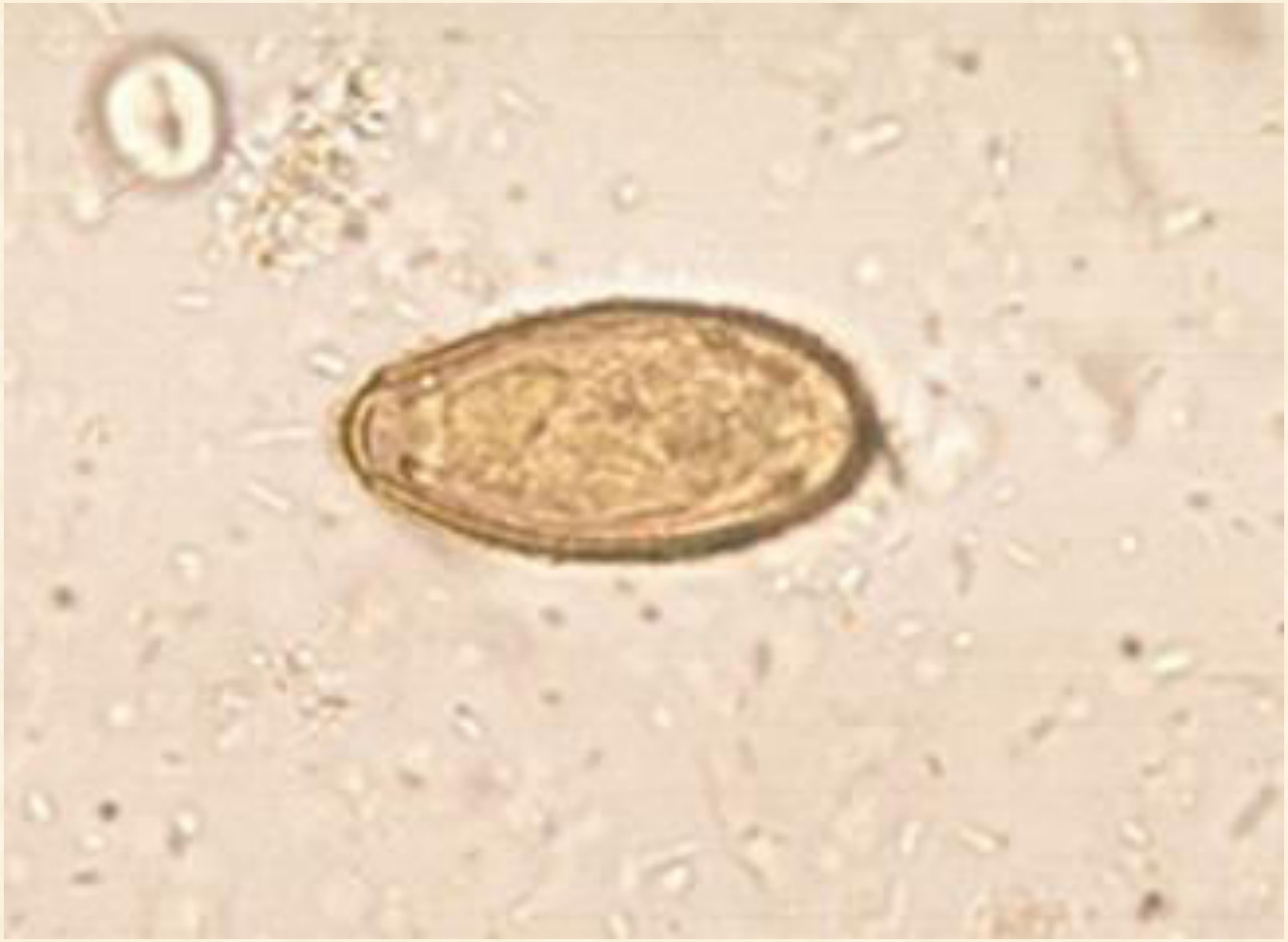




Egg: *Schistosoma mansoni* eggs are passed in the **feces** and have a large, lateral spine (arrow). They measure approximately 150 um in length



Egg: *Schistosoma japonicum* eggs are passed in the **feces** and have a vestigial, nubby lateral spine (arrow). They are also more rounded than the other 2 species and measure approximately 100 um in length



Ova of *Clonorchis sinensis.* Showing the prominent opercular shoulders which makes identifying this trematode easy. They are described as flask shaped, bile stained. **Clonorchis sinensis** eggs are small, ranging in **size** from 27 to 35 µm by 11 to 20 µm. The eggs are oval shaped with a convex operculum. (**seen in far east mainly)**

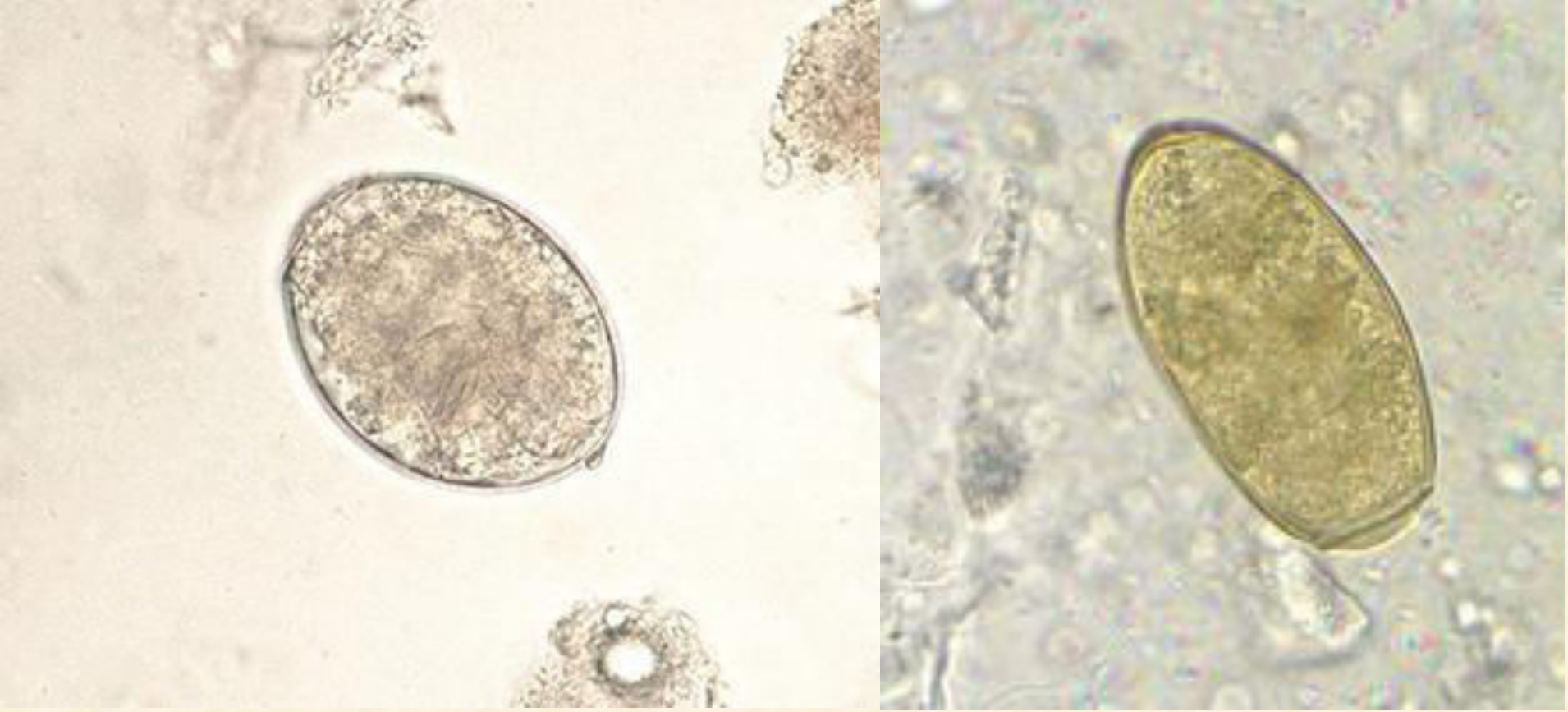
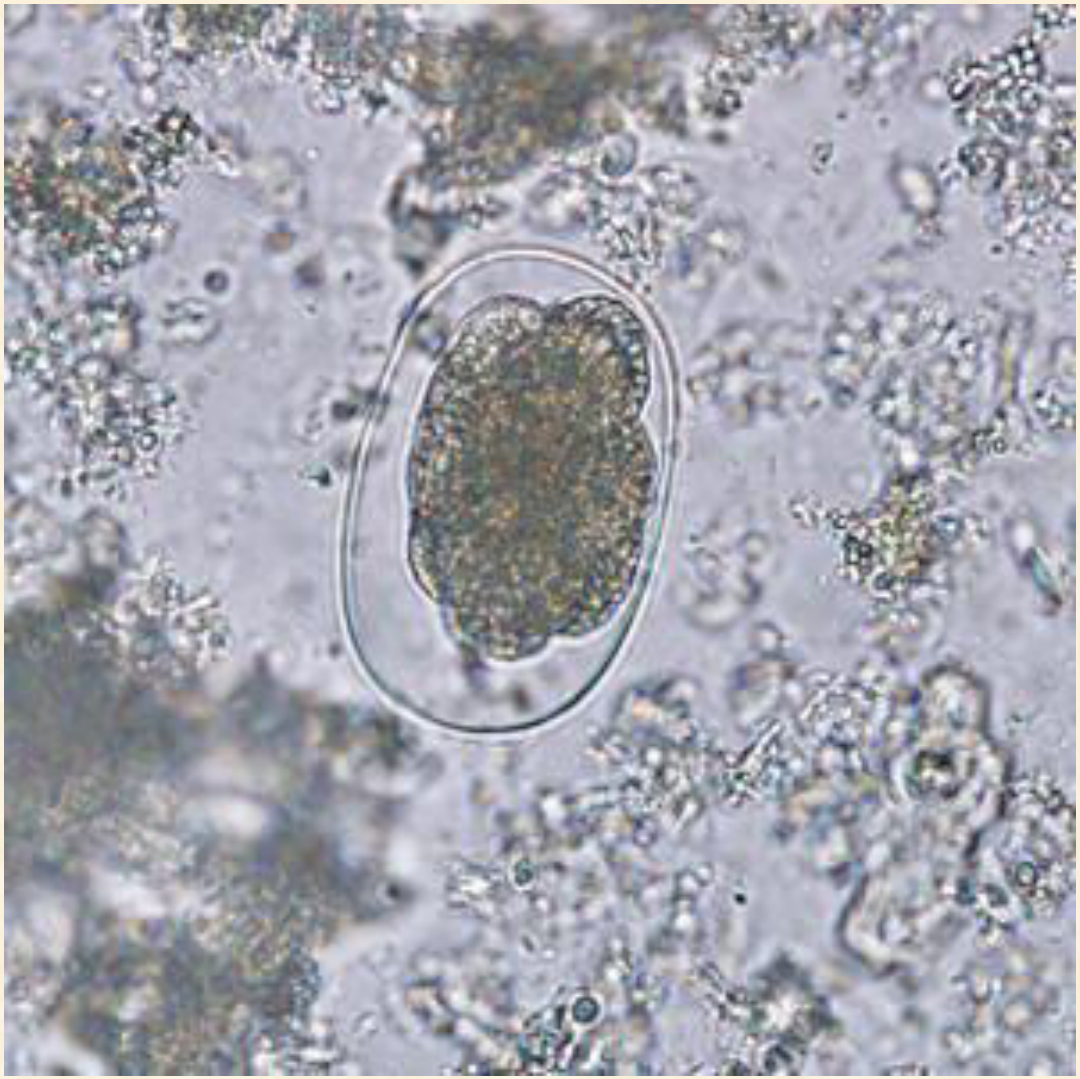
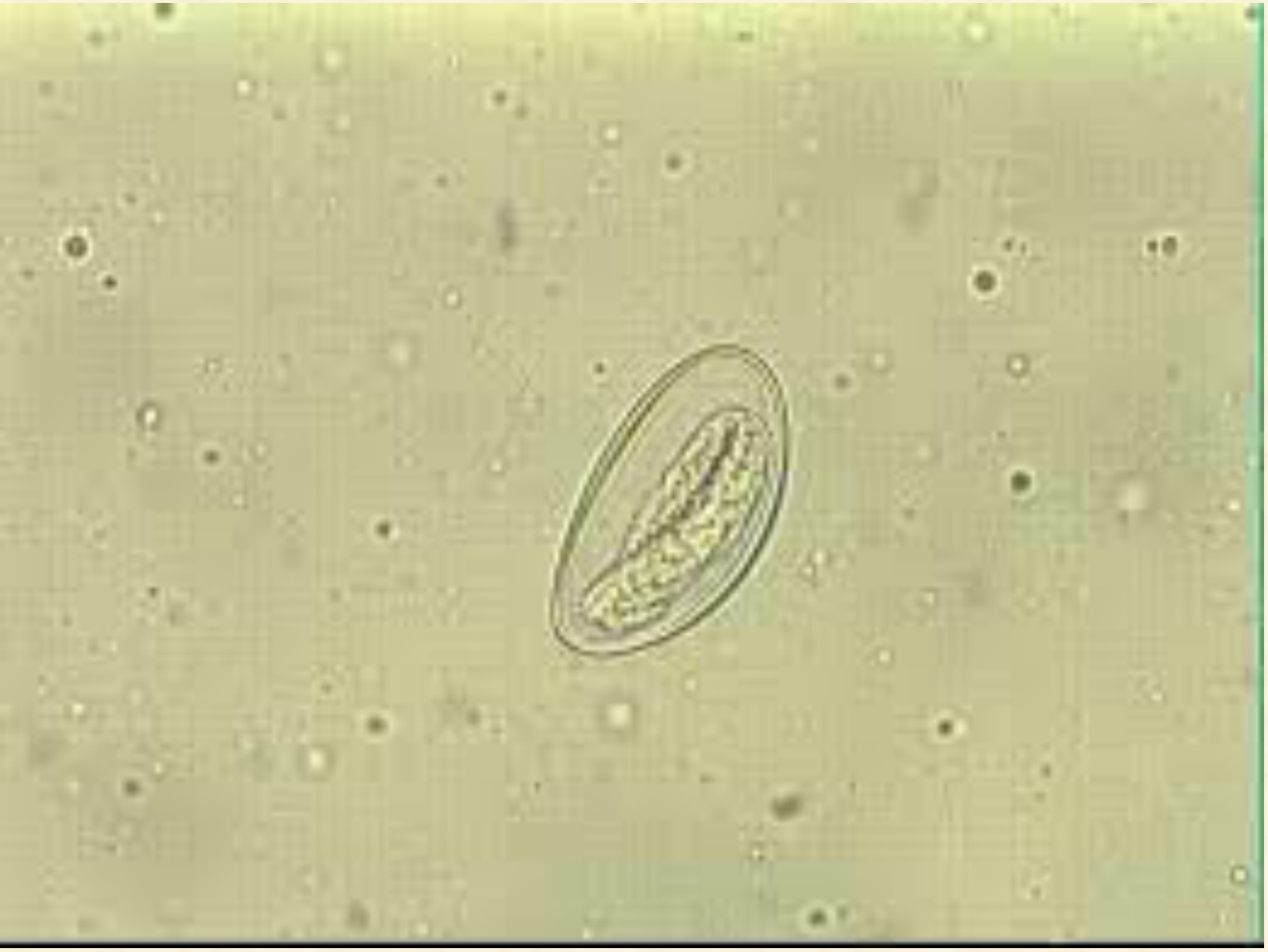


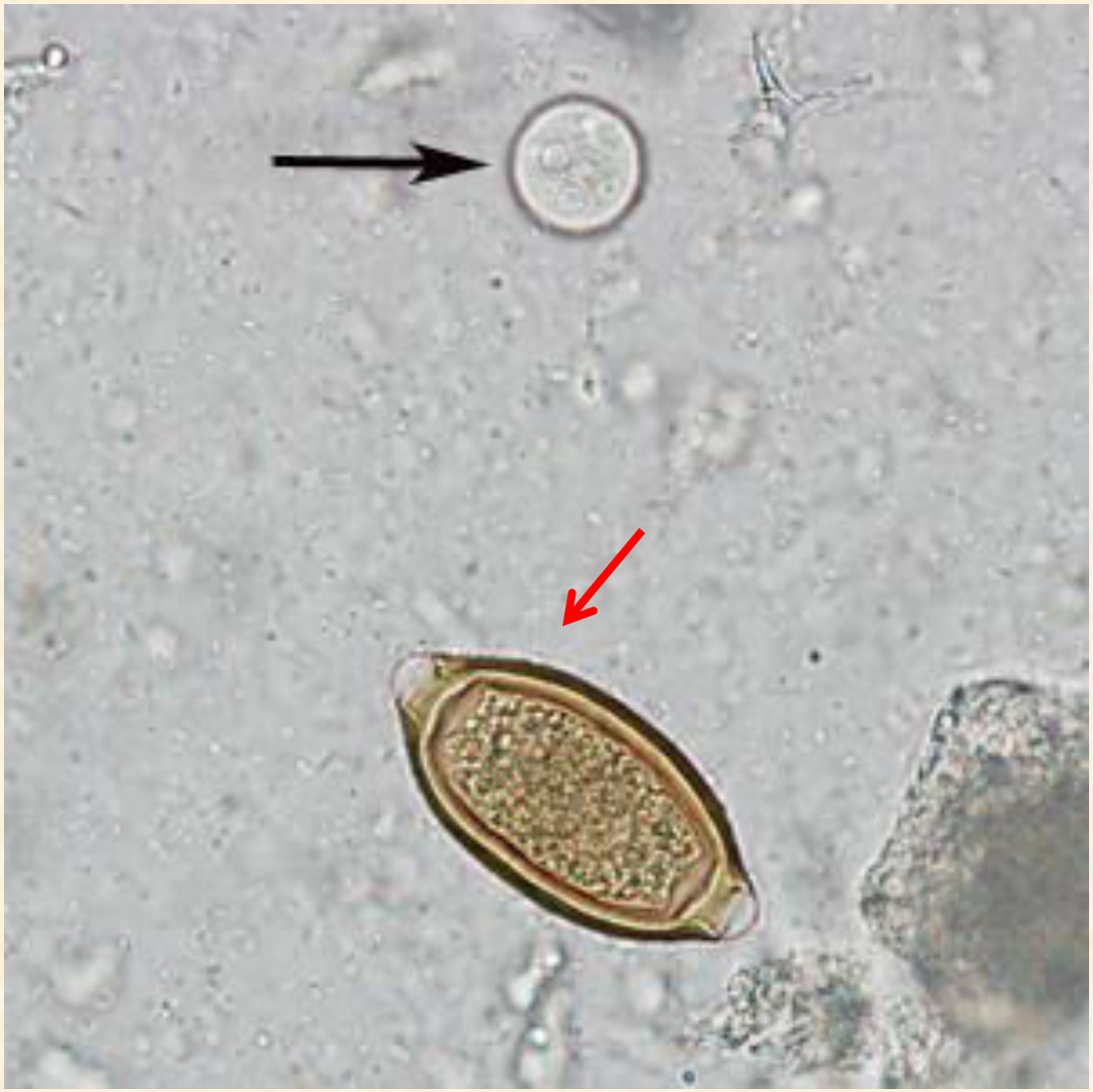
Image 6-6. Saline smear of ***Paragonimus westermani*** **egg**. The egg shells are thick and operculated. 80-100µm by 45-65µm. **Seen in Northeast india**.



Hookworm eggs in unstained wet mounts. The eggs of *Ancylostoma* and *Necator* can not be differentiated microscopically. The eggs are thin-shelled, colorless and measure 60-75 µm by 35-40 µm.
Worldwide distribution

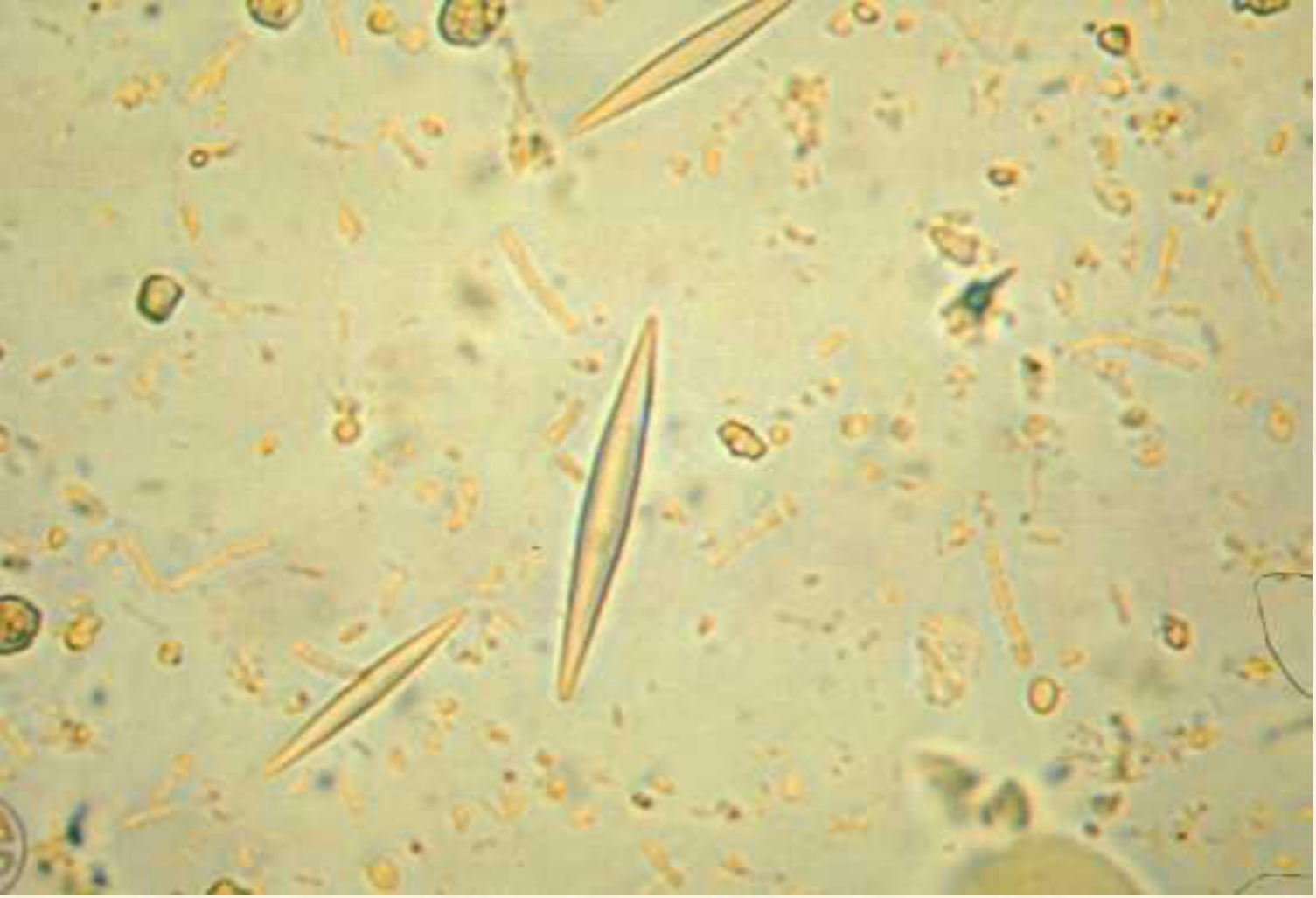


The eggs of *Enterobius vermicularis* measure 50-60 µm by 20-30 µm , are elongate-oval and slightly flattened on one side. They are usually partially-embryonated when shed. Enterobiasis can be diagnosed by applying cellulose tape to the anus of a suspect patient, especially in the morning before the patient's first bowel movement. Eggs will adhere to the tape and can be seen microscopically. **World wide distribution**



Trichuris trichiura eggs are 50-55 micrometers by 20-25 micrometers. They are barrel-shaped, thick-shelled and possess a pair of polar “plugs” at each end. The eggs are unembryonated when passed in stool. **Worldwide distribution**

Strongyloides stercoralis is an intestinal nematode .
Infection occurs when filariform larvae in the soil penetrate the skin and are carried in the blood to the lungs. From the lungs they travel up the trachea and are swallowed. Once in the intestine they develop into mature female worms that begin to produce eggs. These eggs, which are rarely seen, hatch in the intestine into **rhabditiform larvae, the diagnostic stage**. These larvae are 180-380 µm, and typically have a short buccal cavity and a prominent genital primordium. They are excreted into the feces, and once in the soil, develop into both male and female worms to complete the life cycle.



Charcot-Leyden Crystals. Crystals in wet mount preparation from fecal concentration procedure (unstained, high power). Charcot-Leyden crystals are breakdown products of eosinophils and frequently are found in feces; however, they may also occur in sputum and in tissues. Their shape is that of double elongated pyramids with pointed ends. They are often found in people with tissue-invading parasitic infections as well as in individuals with various allergic conditions.

Stool for culture

- Infectious causes of GE
- Collection: Clean, dry, screw capped container; Rectal swabs
- Rectal swabs not acceptable for Rotavirus/ Adenovirus EIA or *C difficile testing*
- Enrichment used for Salmonella (Selenite F), vibrio (Alkaline peptone water)
- Culture on MacConkey, BA, selective medium as per lab policy. (SS agar, TCBS etc)
- Biochemicals
- Agglutination with Antisera.

Thank you