

URINE SEDIMENT



Specimen of choice

- First/second-morning, midstream and clean-catch urine.
- First-morning miction is the most concentrated but for the microscopical examination of cells: imagine cells incubated overnight in a 37°C urine. Cytologists prefer the second-morning specimen but, the aim of the tests is quite different.
- urine volume – 6-12 mL

Sample preparation

- Centrifugation (5 minutes at 400 RCF)
- Supernatant aspiration
- Resuspension
- Staining
 - Sternheimer
 - Alcian blue - mucopolysaccharides
 - Pyronin B – red colour of other sediment components, mainly cell cytoplasm, matrix of waxy casts...)
 - Gram-staining
 - Sedi-Stain

Common Crystals

A number of *in vivo* and *in vitro* factors influence the types and numbers of urinary crystals in a given sample:

- **In vivo factors:**

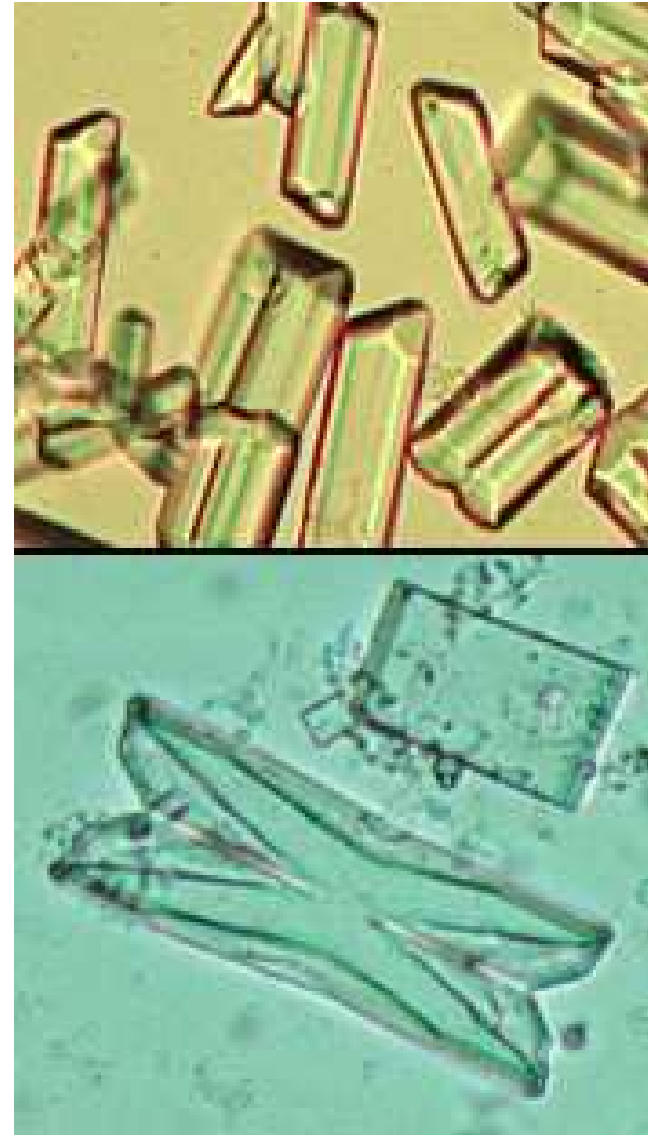
- concentration and solubility of crystallogenic substances contained in the specimen,
- urine pH, and
- excretion of diagnostic and therapeutic agents.

- **In vitro factors :**

- temperature (solubility decreases with temperature),
- evaporation (increases solute concentration), and
- urine pH (changes with standing and bacterial overgrowth).

Struvite

- Struvite crystals (magnesium ammonium phosphate, triple phosphate) usually appear as colorless, 3-dimensional, prism-like crystals ("coffin lids"). Occasionally, they instead resemble (vaguely) an old-fashioned double-edged razor blade (lower frame).
- often seen in urine from clinically normal individuals.
- Though they can be found in urine of any pH, their formation is favored in neutral to alkaline urine.
Urinary tract infection with urease-positive bacteria can promote struvite crystalluria (and urolithiasis) by raising urine pH and increasing free ammonia.



Calcium Carbonate

- Calcium carbonate crystals usually appear as large yellow-brown or colorless spheroids with radial striations. They can also be seen as smaller crystals with round, ovoid, or dumbbell shapes.



Calcium oxalate dihydrate

- colorless squares whose corners are connected by intersecting lines (resembling an envelope)
- urine of any pH.
- The crystals vary in size from quite large to very small. In some cases, large numbers of tiny oxalates may appear as amorphous unless examined at high magnification.
 - Urolithiasis due to calcium oxalate
 - Ethylene glycol intoxication



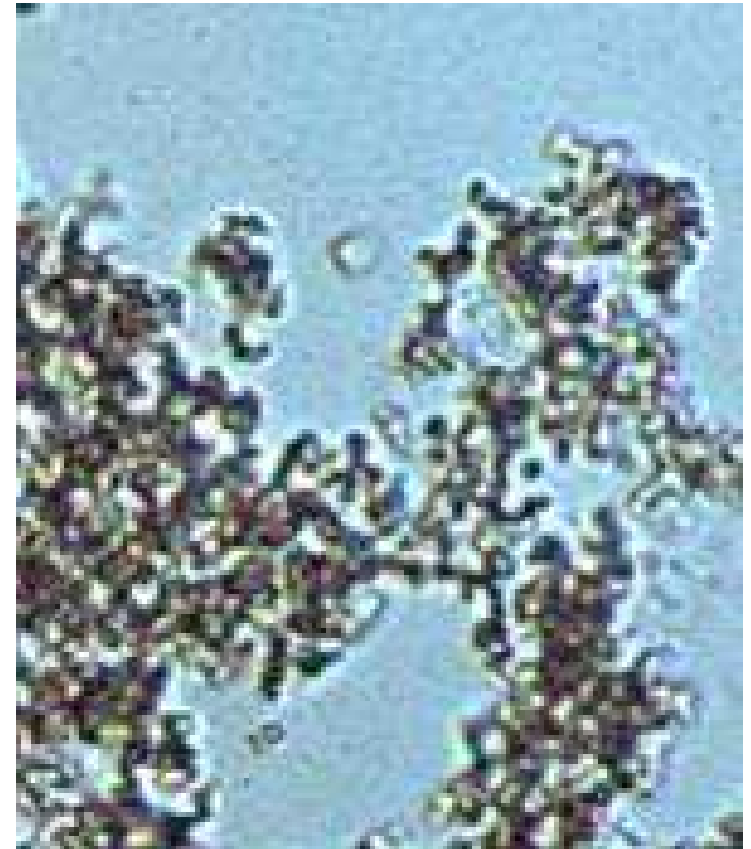
Bilirubin

- Bilirubin crystals tend to precipitate onto other formed elements in the urine. In the top picture, fine needle-like crystals have formed on an underlying cell. This is the most common appearance of bilirubin crystals. In the lower two pictures, cylindrical bilirubin crystals have formed in association with droplets of fat, resulting in a "flashlight" appearance. This form is less commonly seen.



„Amorphous" crystals

- aggregates of finely granular material without any defining shape at the light microscopic level.
- urates (Na, K, Mg, or Ca salts) tend to form in acidic urine, and may have a yellow or yellow-brown color.
- phosphates are similar in general appearance, but tend to form in alkaline urine and lack color.
- Calcium oxalate dihydrate crystals sometimes also can present as "amorphous" when the individual crystals are very small. Examination at higher magnification will reveal the typical "envelope" appearance.
- Xanthine crystals are usually in the form of "amorphous" crystals.
- Generally, no specific clinical interpretation can be made based on the finding of amorphous crystals. Small amorphous crystals can be confused with bacterial cocci in some cases, but can be distinguished by Gram-staining.

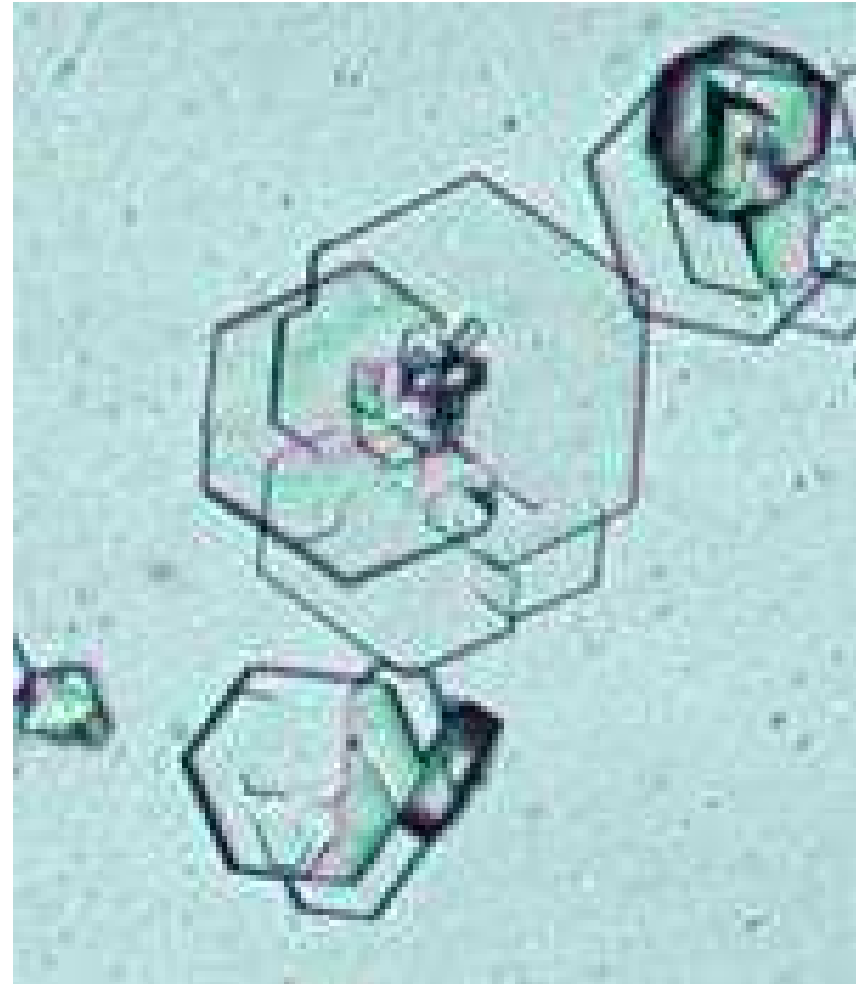


Less Common Crystals

- Cystine
- Biurates
- Drug Crystals
- Tyrosine
- Ca Oxalate Monohydrate

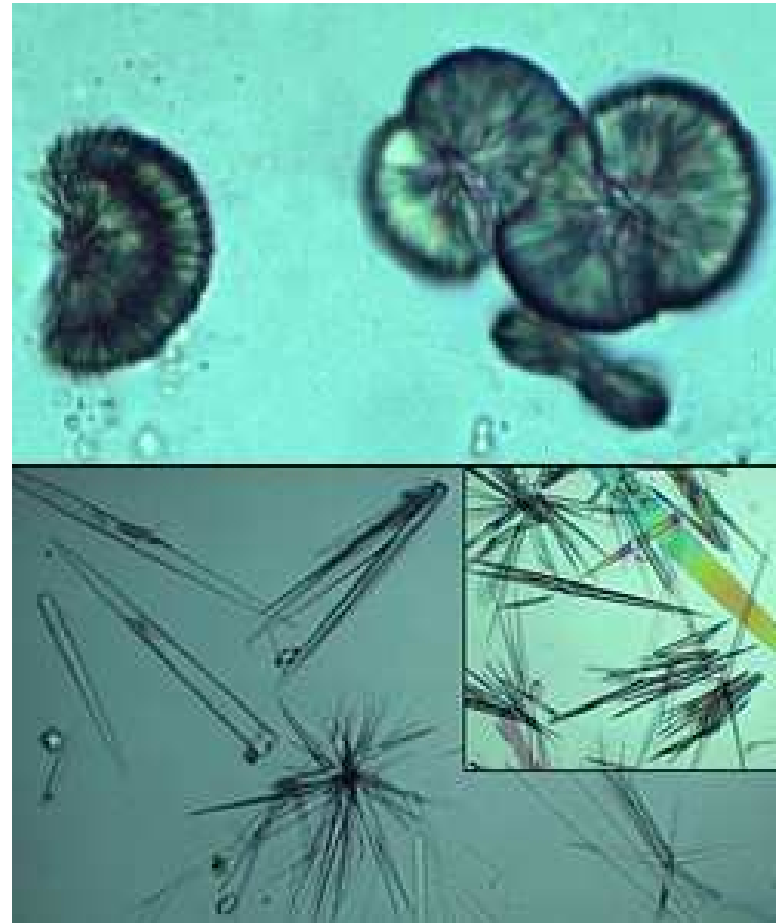
Cystine

- Cystine crystals are flat colorless plates and have a characteristic hexagonal shape with equal or unequal sides. They often aggregate in layers. Their formation is favored in acidic urine.
- Cystine crystalluria or urolithiasis is an indication of cystinuria, which is an inborn error of metabolism involving defective renal tubular reabsorption of certain amino acids including cystine.



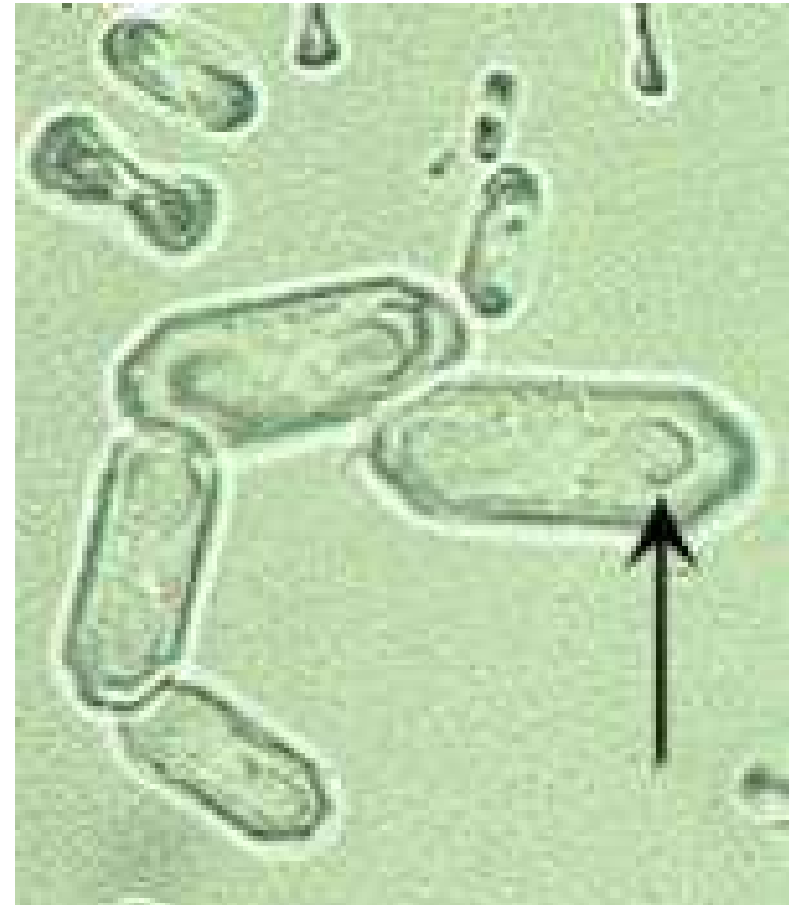
Drug Crystals

- Many drugs excreted in the urine have the potential to form crystals. Hence, a review of the patients drug history is prudent when faced with unidentified urine crystals.
- Most common among these are the sulfa drugs. Both panels on the right are from patients receiving trimethoprim-sulfadiazine. The differing appearance may relate to variation in drug concentration, urine pH, and other factors. The upper panel is from a feline case, the lower from a horse. The inset in the lower panel shows the crystals as they appeared when polarized.
- Other examples include radiopaque contrast agents (Hypaque, Renografin) and ampicillin which may precipitate in acid urine as fine needle-like crystals (not shown).



Calcium Oxalate Monohydrate

- Calcium oxalate monohydrate crystals vary in size and may have a spindle, oval, or dumbbell shape. Most commonly, they appear as flat, elongated, six-sided crystals ("fence pickets") such as shown to the right. The arrow in the photo indicates a "daughter" crystal forming on the face of a larger underlying crystal.
- virtually always associated with ethylene glycol intoxication.



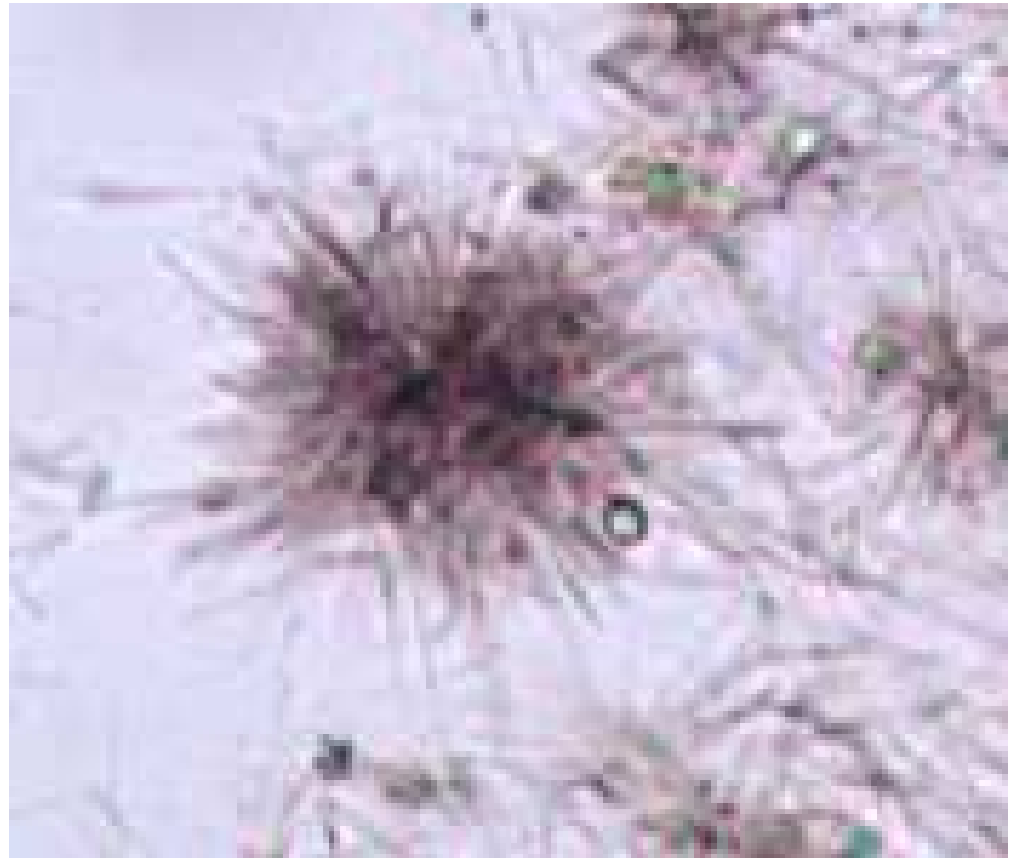
Biurates

Ammonium urate (or biurate) crystals generally appear as brown or yellow-brown spherical bodies with irregular protrusions ("thorn-apples"). Though possible in urine of any pH, their formation is favored in neutral to alkaline urine.



Tyrosine

Tyrosine crystals are usually seen as fine brownish needles. These can be associated with severe liver disease

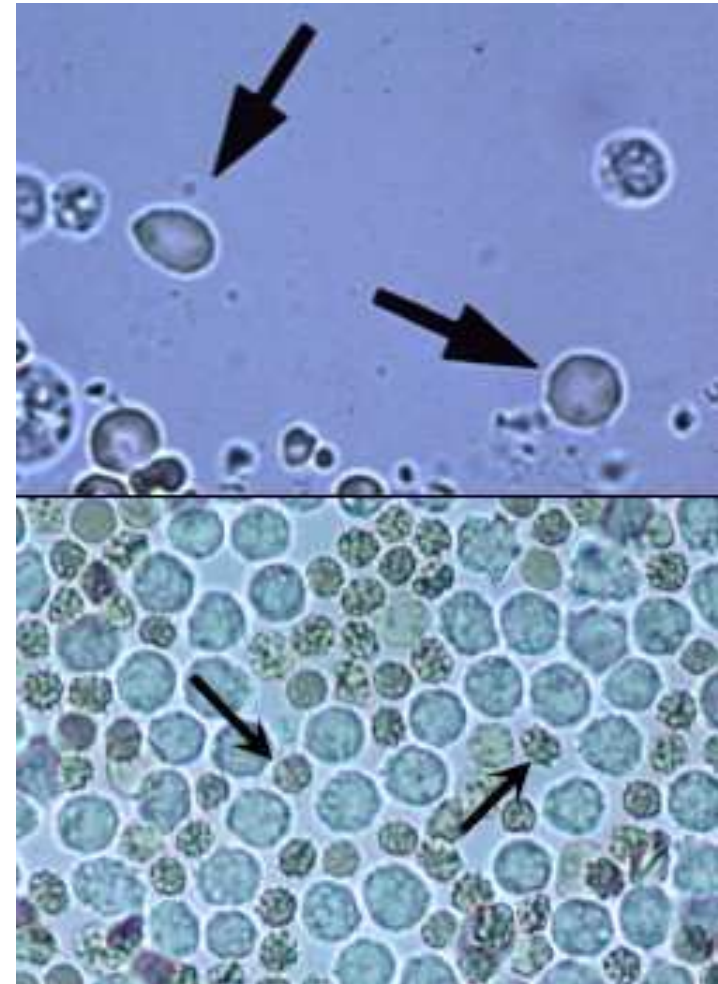


Cells in Urine Sediment

- Urine is a hostile environment for cells since they encounter abnormal osmotic pressures, pH changes, and exposure to toxic metabolites. For these reasons, post-collection delay of examination should be minimized. If delay is unavoidable, refrigeration will slow degeneration of cells.
- For routine purposes, cells are examined as unstained wet-mounts of sedimented urine. Under some circumstances, air-dried smears are prepared and stained with hematologic stains.
- Red blood cells and leukocytes are quantified as cells/HPF (High Power Field - 40x objective). Other cell types are usually subjectively listed as "few, moderate, or many".

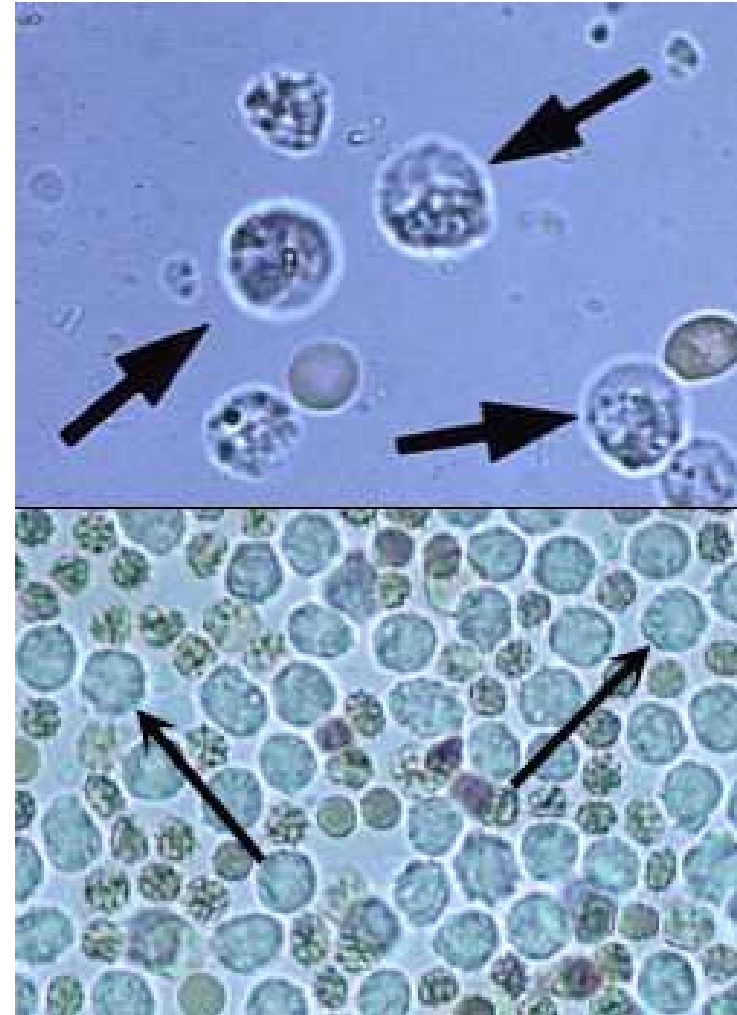
Red Blood Cells

- The appearance of red blood cells (RBC) in urine depends largely on the concentration of the specimen and the length of time the red cells have been exposed.
- Fresh red cells tend to have a red or yellow color (lower panel). Prolonged exposure results in a pale or colorless appearance as hemoglobin may be lost from the cells (upper panel).
- In fresh samples with S.G. of 1.010-1.020, RBC may retain the normal disc shape (upper panel). In more concentrated urines (>1.025), red cells tend to shrink and appear as small, crenated cells (lower panel). In more dilute samples, they tend to swell. At urine S.G. <1.008 and/or highly alkaline pH, red cell lysis is likely. Lysed red cells appear as very faint "ghosts", or may be virtually invisible.
- Red blood cells up to 5/HPF are commonly accepted as normal. Increased red cells in urine is termed hematuria, which can be due to hemorrhage, inflammation, necrosis, trauma, or neoplasia somewhere along the urinary tract (or urogenital tract in voided specimens).
- The method of collection must be considered in interpreting hematuria to aid in localizing the source, and because catheterization, cystocentesis, and manual compression can induce hemorrhage.



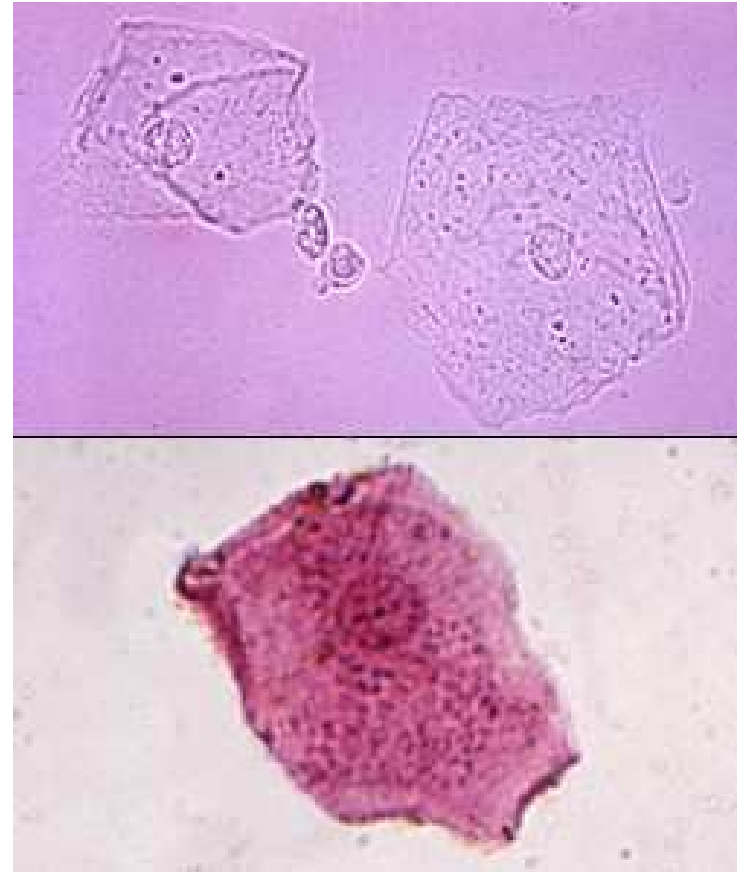
White Blood Cells

- White blood cells (WBC) in unstained urine sediments typically appear as round, granular cells which are 1.5-2.0 times the diameter of RBC. The details of nuclear shape often are difficult to discern, especially if the specimen is not fresh. WBC in urine are most commonly neutrophils. Staining of air-dried sediment smears with a hematologic stain sometimes is useful for more specific identification. Like erythrocytes, WBC may lyse in very dilute or highly alkaline urine.
- WBC up to 5/HPF are commonly accepted as normal. Greater numbers (pyuria) generally indicate the presence of an inflammatory process somewhere along the course of the urinary tract.
- Pyuria often is caused by urinary tract infection, and many times bacteria can be seen on sediment preps. Depending on clinical signs, pyuria may be an indication for culture of urine even if no bacteria are seen. Non-septic causes of inflammation, such as uroliths and tumors, also must be considered.



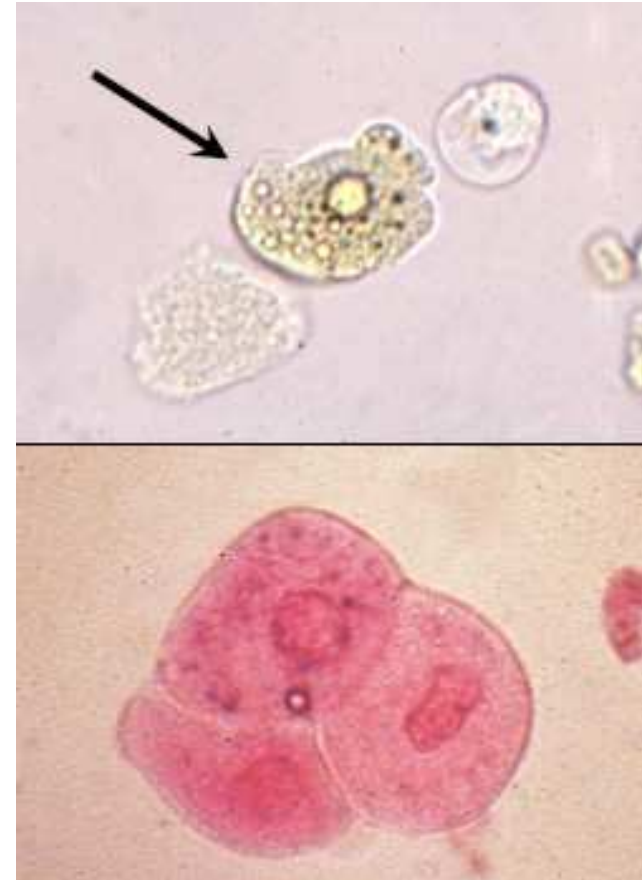
Squames

- Squamous epithelial cells are the largest cells which can be present in normal urine samples.
- They are thin, flat cells, usually with an angular or irregular outline and a small round nucleus. They may be present as single cells or in variably-sized clusters. Those shown in the upper panel are unstained; that in the lower panel was prepared using Sedi-Stain.
- Squamous cells are common in low numbers in voided specimens and generally represent contamination from the genital tract. Their main significance is as an indicator of such contamination.



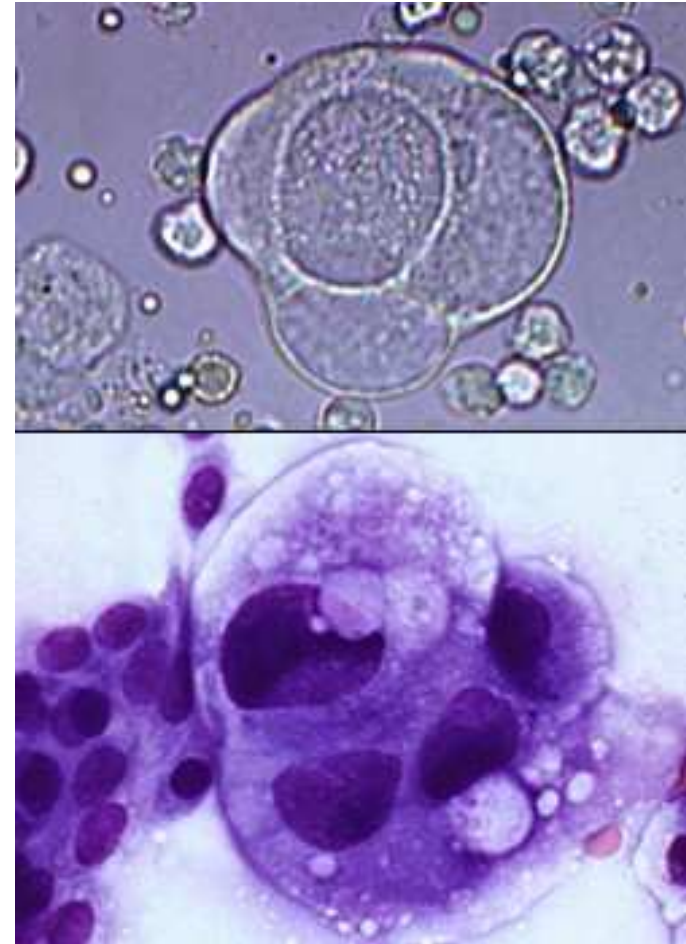
Transitional Cells

- Transitional epithelial cells originate from the renal pelvis, ureters, urinary bladder and/or urethra. Their size and shape depends on the depth of origin in the mucosa. Most often they are round or polygonal; less commonly pear-shaped, tailed, or spindle-shaped. They are generally somewhat smaller and smoother in outline than squamous cells, but larger than WBC. They may develop refractile, fatty inclusions as they degenerate in older specimens (arrow, upper panel).
- In cleanly-collected normal samples, transitional cells are few, and present as single cells or small clusters (arrow, lower panel, Sedi-Stain). Specimens collected by catheter sometimes contain large sheets of cells scraped off during passage of the catheter. In inflammatory conditions causing hyperplasia of the urinary mucosa, larger numbers/clusters may exfoliate. In such cases, differentiation from neoplastic transitional cells may be difficult.



Neoplastic Cells

- Neoplastic cells may be seen in urine sediments of patients with tumors of the urinary tract.
- Transitional cell carcinomas arising in the urinary bladder or urethra are most likely to spontaneously exfoliate, but cannot be ruled out based on a failure to identify malignant cells in urine. Rarely, lymphomas and renal carcinomas also can be diagnosed from urine sediment.
- The pictures shown are from a case of transitional cell carcinoma. Though the presence of neoplastic cells may be suspected on examination of unstained wet-mounts (upper panel), evaluation of air-dried sediment smears or cytocentrifuge preps stained with hematologic stains (lower panel) is necessary for confirmation. In the case shown here, the cytologic criteria of malignancy are clearly fulfilled; in other cases a distinction from hyperplastic cells cannot be made with certainty without a tissue biopsy.

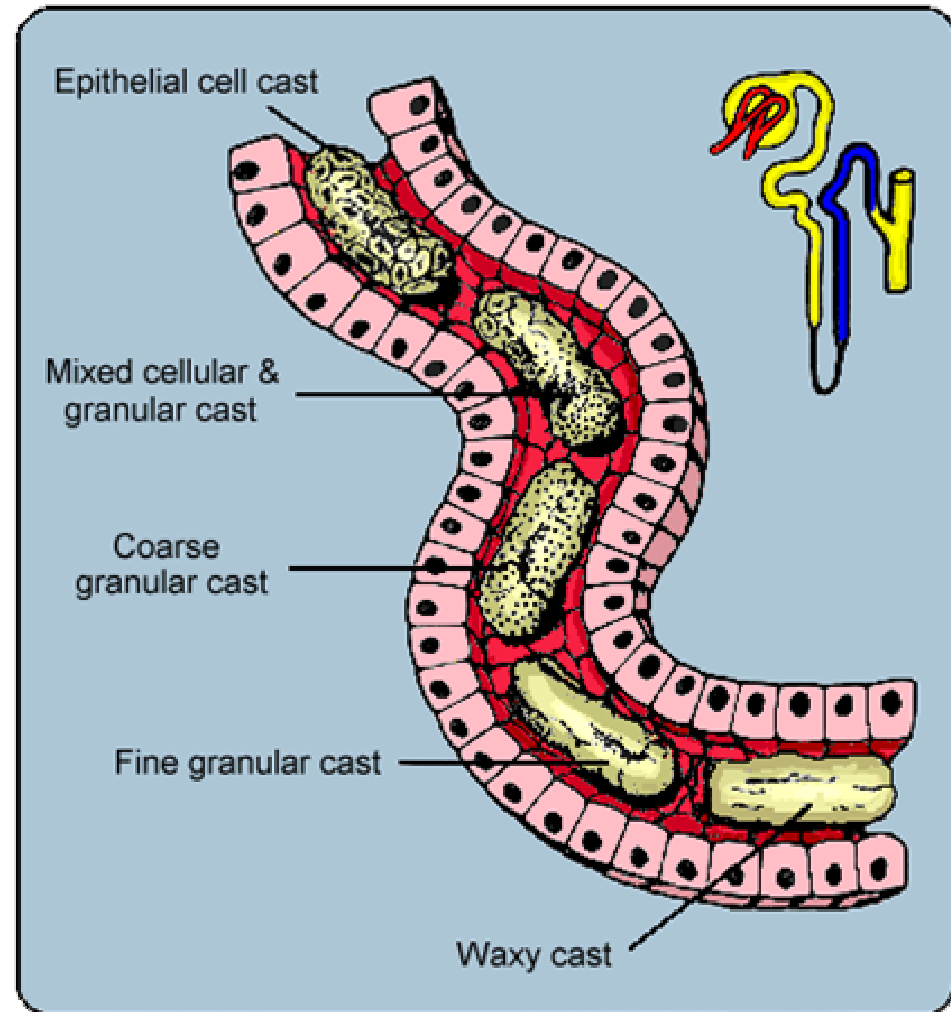


Urinary Casts

Casts are cylindrical structures composed mainly of mucoprotein (the **Tamm-Horsfall mucoprotein**) which is secreted by epithelial cells lining the loops of Henle, the distal tubules and the collecting ducts. The factors responsible for the precipitation of this mucoprotein are not fully understood, but may relate to the concentration and pH of urine in these areas. Casts may form in the presence or absence of cells in the tubular lumen. If cells (epithelial cells, WBC) are present as a cast forms, they may adhere to, and subsequently be surrounded by, the fibrillar protein network

Formation of casts

A commonly-held theory is that cellular, granular, and waxy casts represent different stages of degeneration of cells in a cast. The appearance of a cast observed in a urine sediment depends largely upon the length of time it remained in situ in the tubules prior to being shed into the urine. A cast recognizable as "cellular", for example, was shed shortly after it was formed. A waxy cast, in contrast, was retained longer in the tubular system prior to being released



Schematic illustration of transition between epithelial cell, coarse granular and waxy casts formed in the loops of Henle, distal tubules, and collecting ducts (blue area of nephron in upper right-hand corner). From Osborne, et. al.

General Interpretation of casts-

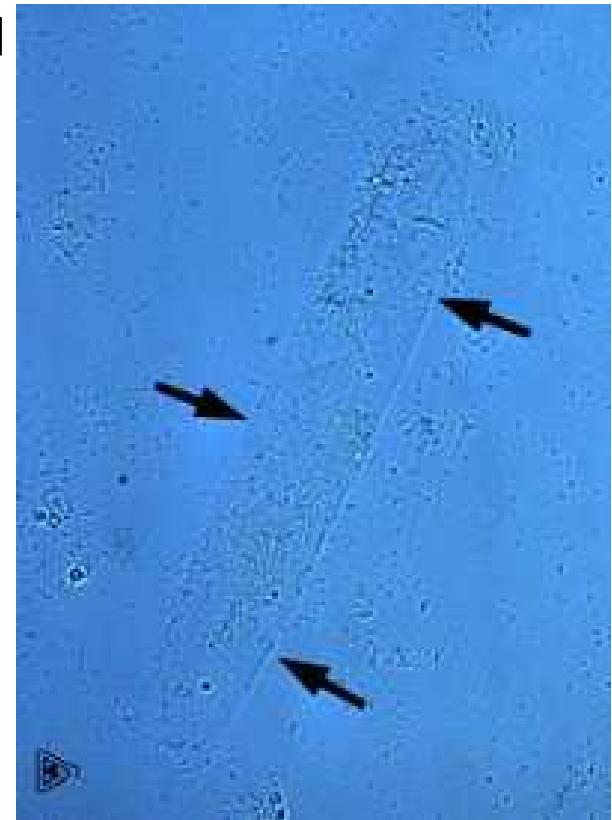
- Casts are quantified for reporting as the number seen per low power field (10x objective) and classified as to type (e.g., waxy casts, 5-10/LPF). Casts in urine from normal individuals are few or none.
- An absence of casts does not rule out renal disease. Casts may be absent or very few in cases of chronic, progressive, generalized nephritis. Even in cases of acute renal disease, casts can be few or absent in a single sample since they tend to be shed intermittently. Furthermore, casts are unstable in urine and are prone to dissolution with time, especially in dilute and/or alkaline urine.
- Although the presence of numerous casts is solid evidence of generalized (usually acute) renal disease, it is not a reliable indicator of prognosis. If the underlying cause can be removed or diminished, regeneration of renal tubular epithelium can occur (provided the basement membrane remains intact).

Hyaline Casts

Hyaline casts are formed in the absence of cells in the tubular lumen. They have a smooth texture and a refractive index very close to that of the surrounding fluid. **Reduced lighting is essential to see hyaline casts.** Lower the substage condenser.

When present in low numbers (0-1/LPF) in concentrated urine of otherwise normal patients, hyaline casts are not always indicative of clinically significant renal disease.

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-Horsfall mucoprotein.



Granular Casts

Granular casts, as the name implies, have a textured appearance which ranges from fine to coarse in character. Since they usually form as a stage in the degeneration of cellular casts, the interpretation is the same as that described previously.



Cellular Casts

Cellular casts most commonly result when disease processes such as ischemia, infarction, or nephrotoxicity cause degeneration and necrosis of tubular epithelial cells. The presence of these casts indicates acute tubular injury but does not indicate the extent or reversibility of the injury.

A common scenario is the patient with decreased renal perfusion and oliguria secondary to severe dehydration. Ischemic injury results in degeneration and sloughing of the epithelial cells. The resulting casts often are prominent in urine produced following rehydration with fluid therapy. The restoration of urine flow "flushes" numerous casts out of the tubules.

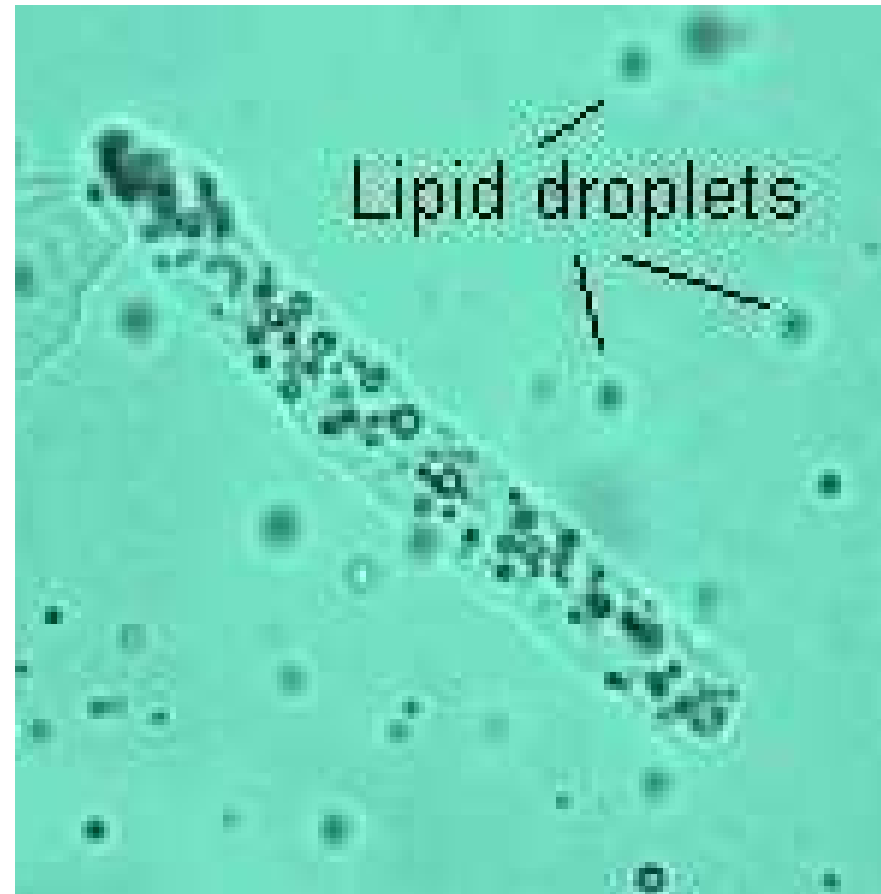
Leukocytes can also be incorporated into casts in cases of tubulo-interstitial inflammation (eg, pyelonephritis). It is rarely possible to distinguish between epithelial casts and leukocyte casts in routine sediment preparations, however, since nuclear detail is obscured by the degenerated state of the cells.



Fatty Casts

Fatty casts are identified by the presence of refractile lipid droplets. The background matrix of the cast may be hyaline or granular in nature. Often, they are seen in urines in which free lipid droplets are present as well. Interpretation of the significance of "fatty" casts should be based on the character of the cast matrix, rather than on the lipid content per se.

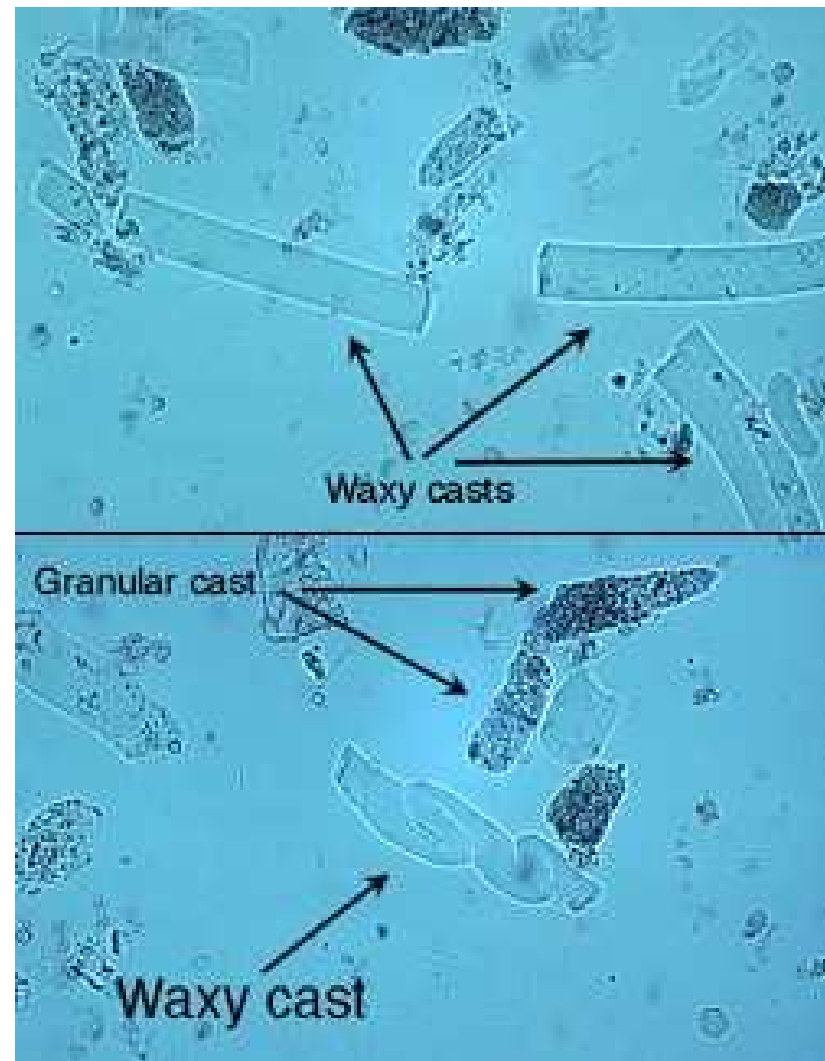
Pictured here is a fatty cast with a hyaline matrix. Also notice the free lipid droplets in the background. Such droplets are most commonly seen. As an isolated finding, lipiduria is seldom of clinical significance.



Waxy Casts

Waxy casts have a smooth consistency but are more refractile and therefore easier to see compared to hyaline casts. They commonly have squared off ends, as if brittle and easily broken.

Waxy casts indicate tubular injury of a more chronic nature than granular or cellular casts and are always of pathologic significance.



Infectious Agents

- Infectious agents of various classes can be observed in urine sediments. In most cases, their significance can be properly assessed only in light of the clinical signs, method of collection, post-collection interval, and other findings in the urinalysis.
 - Yeasts
 - Bacteria

Yeasts

- Yeasts in unstained urine sediments are round to oval in shape, colorless, and may have obvious budding (upper panel). They often represent contaminants, and are especially suspect if the sample is voided and/or old.
- In other circumstances, however, their significance should not be discounted. The pictures shown here, for example, are of fresh urine collected from a patient that had been on long-term antibiotic and immunosuppressive therapy.
- The lower photo shows pseudohyphae formation by the yeasts, which were identified on culture as *Candida albicans*.



Bacteria

- Bacteria can be identified in unstained urine sediments when present in sufficient numbers. Rod-shaped bacteria and chains of cocci are often readily identifiable. The images at right show *E.coli* bacilli from a case of cystitis. However, small amorphous crystals, cellular debris, and small fat droplets can either mask or mimic [cocci](#). If there is any doubt about the presence of bacteria, a Gram-stained smear of urine sediment (middle panel) should be examined.
- Urine in the bladder of normal animals is sterile. Though bacteria from the distal urethra and/or genital tract may contaminate voided specimens, they are usually too few to see if a good mid-stream collection was obtained.
- Although phagocytized bacteria cannot be seen in unstained wet mounts of urine sediment, they may be found in stained smears of sediment. The lower panel at the right shows a neutrophil containing phagocytized bacteria. Notice that the nucleus in this cell is round; nuclei tend to become round as neutrophils age in urine.
- Bacteriuria of clinical significance, e.g., bacterial cystitis, is usually accompanied by increased numbers of white cells (pyuria). The presence of a few bacteria without pyuria is very rarely significant of infection.



Contaminants in Urine Sediment

- Extraneous contaminating materials of many types can make their way into urine specimens,.
- Striving for optimal collection and transport of specimens will help maximize useful results and minimize confusing findings.
- Spores and Pollens
- Microbial Overgrowth
- Fibers
- Starch Granules

Spores and Pollen

Mold spores and pollens come in a wide variety of shapes and sizes. Shown here is an *Alternaria* spore surrounded by amorphous crystals and a few lipid droplets.

Pollen grains are generally round to oval and some have a yellow to brown tint. They are most likely to be confused for parasitic ova. A good knowledge of the actual appearance of the few true parasite eggs that can occur in urine is easier to achieve than specific recognition of all types of pollen and mold spores. Again, optimizing specimen collection and handling will reduce the chances of seeing potentially confusing structures.



Overgrowth of Microbes

Specimens mailed to laboratories without refrigeration or preservatives are subject to overgrowth of microbes, whether contaminants or pathogens. Shown here is a dense mat of fungal hyphae which was seen in a sediment prep of a canine urine specimen which had been several days in transit. Since fungal infection of the urinary tract in dogs is quite uncommon, the odds are that this represents overgrowth of contaminants.

Bacteria, whether pathogens or contaminants, also can multiply when analysis is delayed. This often clouds the interpretation of both sediment examination and culture results.

Refrigeration is perhaps the best all-around method for preserving a specimen. Some laboratories also suggest specific transport media or swabs when sending a specimen for culture.



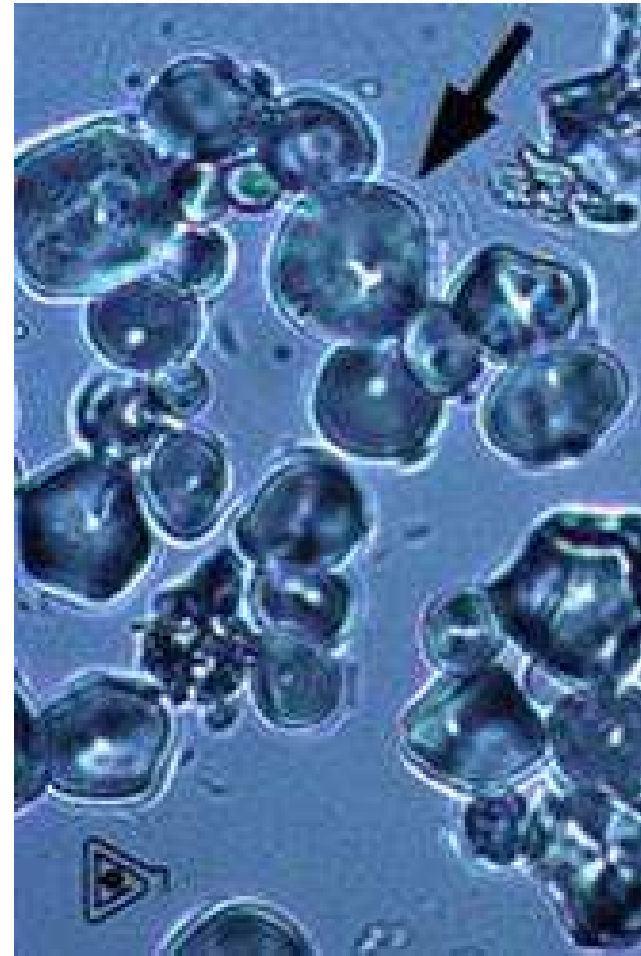
Fibers

Cotton, plant, and paper fibers may be confused for urinary casts. Care in sample collection and handling will minimize the presence of such material.



Starch Granules

Granular starch is used as powder on surgical and exam gloves. These granules are commonly encountered as contaminants not only in urine sediments, but also in cytology smears of various types. They are variable in size, round to polygonal in shape, colorless, and usually have a circular or Y-shaped "dot" in the center.



Miscellaneous

- General "crud" or unidentifiable objects may find their way into a specimen, particularly those that patients bring from home.
- Spermatozoa can sometimes be seen. Rarely, pinworm ova may contaminate the urine. In Egypt, ova from bladder infestations with schistosomiasis may be seen.

