The Complete Urinalysis

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Frequently Asked Questions

Q: How do you make a smear from urine?

A: Ideally, the smear should be made from the sediment after centrifugation of the urine sample. It is best to make at least 2 smears using 2 different techniques. Make one smear by placing a small drop of urine sediment near one end of a glass slide and spread the urine as if making a blood smear. Then make another smear by the line smear technique. This is similar to the blood smear technique except you spread the drop only a short distance and then lift the spreader slide directly up to create a line at the end. This helps to concentrate cells and casts along the line to more easily evaluate low cellularity samples. After the slides have dried, they should be heat fixed as this will increase the number of cells that stay on the slide during staining (but do not over heat fix and cook the cells!!!!). The smear may be stained with Diff-Quik or a similar stain or sent to the lab unstained. Do not stain a slide with New methylene blue, Sedi-Stain or any stain that needs to be wet under a cover slip and then send that slide in for evaluation. These are not permanent stains and they will dry out, crystallize and be unusable.

Q: At what speed and how long should urine be centrifuged?

A: This is somewhat of a difficult question to answer because speed and time are dependent on the length of the radial arm of the centrifuge (the longer the radial arm the slower the centrifuge should spin to get the same gravitational force). Urine should be spun at a slow speed. Most centrifuges have a radial arm of ~14.5 cm and should be spun at ~1,500 rpm (with a range of 1,000–2,000 rpm) for ~5 minutes (with a range of 3–5 minutes, longer time for slower speeds). Centrifuging the urine at too high of a speed or for a prolonged amount of time can cause artifacts such as cell rupture and cast fragmentation.

Q: Should the specific gravity reading be performed on noncentrifuged urine or on the supernatant from centrifuged urine?

A: Generally, a refractometer is used to estimate the urine specific gravity (USG) and the USG is an estimate of the osmolality of the urine. We are interested in the specific gravity of the particles in solution. Many urine samples have few to moderate numbers of cells and crystals in suspension, so the USG from noncentrifuged urine would be similar to the USG from the supernatant of centrifuged urine. So in most cases it will make little or no difference. However, some samples have many cells and crystals in suspension which can make the “line” difficult to read on the refractometer. While cells and crystals generally have a minimal effect on USG, their influence can be eliminated by centrifugation..

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Q: Discuss struvite versus triple phosphate crystals and how to differentiate from calcium oxalate monohydrate crystals.

A: Struvite and triple phosphate are names that are often used interchangeably. However, struvite crystals are made up of magnesium, ammonium, phosphate (MAP) and triple phosphate crystals are made up of calcium, magnesium, ammonium, phosphate (cMAP). The two names are used interchangeably because we called these crystals triple phosphate for a long time before we realized the calcium was a contaminate from the urine and not actually part of the crystal. So these crystals are actually struvite crystals. Struvite crystals generally appear "coffin lid-like" since the ends of the crystal tend to point or fold to the center. However, occasionally the ends will point out and the crystal will appear "pickett fence-like." These are differentiated from calcium oxalate monohydrate crystals by the three-dimensional appearance of the struvite crystal and the flat appearance of the calcium oxalate monohydrate crystal. Also, budding crystals are often seen on the flat surface of calcium oxalate monohydrate crystals.

Q: How do I adjust my microscope to look at unstained urine?

A: When looking at unstained samples, the substage condenser needs to be lowered or the diaphragm on the substage condenser needs to be adjusted to allow for light scattering. If the light is polarized, substances with a low refractive index will be difficult to see. By adjusting the microscope, light will contact cells and casts at an angle, which will increase visibility of objects with a low refractive index.

Q: How are renal tubular cells differentiated from WBCs?

A: Renal tubular epithelial cells and WBCs are similar in size and may be difficult to differentiate in unstained urine. Renal tubular cells have a round nucleus and may contain fat (especially in cats because they have fatty renal tubular epithelial cells). WBC, are typically neutrophils and have a lobulated nucleus. It is easiest to differentiate these in fresh, stained urine.

Q: How can we eliminate stain precipitate and/or bacteria in our Sedi-Stain?

A: Stain precipitate is a common problem with new methylene blue type stains. Bacterial contamination is an occasional problem but is much less common than precipitate formation. You can eliminate both of these problems by filtering the stain (with a very small pore filter) and returning the stain to a clean bottle, but it is usually not worth the effort and generally it is best to get a new bottle of stain.

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