Well, it seems appropriate to start with the basics. And urinalysis is certainly the basic tool of our subspecialty. So I'm going to spend the next 25 minutes talking about urinalysis, and hopefully give some practical pearls and pitfalls, and learn more about how we consider urinalysis today.
The routine urinalysis is one of our best noninvasive diagnostic tools. It allows us to find diseases that may otherwise go unnoticed. Some of those are kidney diseases like glomerulonephritis. And we may not have any idea there’s anything going on until there’s a screening urinalysis for a school physical, or a camp physical, or an athletic physical that shows hematuria and proteinuria. Sometimes we only know that there’s going to be renal failure there because a urinalysis shows proteinuria, and we get a subsequent set of chemistry labs and find out that the kidneys aren’t working. The same is true of hypertensive nephropathy. We might not know it’s there until we see the protein in the urine. And of course the urine is a great screen for undetected diabetes mellitus.
The other nice thing about the urinalysis is it’s something that can easily be done in the office. It does require a few tools. First of all you have to have some collection devices. And those include, for the pediatrician, a variety of containers. You have to have containers for the older kids, and a good supply of bags for the younger kids. And you need mild antiseptics to clean off to get a clean catch midstream. There are occasions when you need to do an in and out cath, and there are a nice number of prepackaged kits that allow you to do that easily, and they’re not that expensive. If you’re going to do urinalysis in the office, you need a refrigerator because you’re going to need to store the urine samples there unless you are willing to look at every one of them completely fresh within an hour. And then you need dipsticks. And the dipsticks come in a variety of sizes and a variety of numbers on the dipstick. And these days you also need to have controls to be sure your dipsticks have not gone out of function. And then, if you’re only going to do those things, that’s fine. But you may want to do a microscopic exam, and that requires a little bit more equipment and may not be necessary if you are just using the urinalysis as a screening tool. If you’re going to do a microscopic exam, you’re going to have to have some real equipment. A centrifuge, and the supplies to put the urine in to spin it. You’re going to need slides, and cover slips, and of course you are going to need a microscope. It doesn’t have to be elaborate as this one. It can be a basic simple microscope.
Now the key to really having a good urinalysis result is having a good urinalysis collection of the specimen. And that means that you really ought to get a freshly voided specimen. And that’s very easy for me to say, but very hard to get in some pediatric patients, especially the younger kids who really don’t want to void on command. The voided specimen is best because there are formed elements that may be important that you’re not going to be able to see if the specimen has been sitting around for a while, especially if the pH is greater than 7, or the specific gravity is less than 1.015. Furthermore, if there is any bacterial contamination in that specimen, the bacteria tends to overgrow at room temperature. And that can metabolize any glucose present, to give a false negative diabetic screen. It can metabolize urea to ammonia, which gives you a very high urine pH and can give you a false positive protein screen. And the bacteria can also produce peroxidases that will interfere with the blood test, and you’ll get a false positive blood test. So the pearl is, get a new sample if the specimen is at room temperature for longer than hour, or in the refrigerator for more than four hours.
Now the best sample to get is a clean catch midstream. It’s really the method of choice even for a routine urinalysis because it avoids contamination with elements outside the bladder and the urinary tract. Those can be skin cells, vaginal cells, bacteria, or protein-containing secretions. This sample can also be used for a bacterial culture, so if you actually have a child with a fever and you get a urinalysis, you can use that for a culture, if you need to, as well. And sometimes it’s very difficult to get the second sample. It also saves time and money for repeating samples that you have false positives in, for some of the things on the screening test. In order to obtain the sample, you need to cleanse the external genitalia with a mild antiseptic. You need to discard the first portion of the void and collect the mid-portion. And this should be done while the urine is still going on. It should not start and stop the stream. My husband is a urologist, and he tells all his adult patients that if your hand didn’t get wet, you didn’t do it right. Now that leads to one of the pitfalls for us because, especially in adolescent girls or any obese girls or boys, it’s a problem to get a good sample. And the obese adolescent girls, the labia really has to be spread apart for the clean collection. And most adolescent girls won’t or don’t do this.
Now specimen collection is also a problem for infants and toddlers. It’s very difficult to get a clean catch midstream because they just won’t go most of the time. And squeezing diapers is not acceptable. So we’re often reduced to a bag specimen, which is actually not a bad thing for screening. It’s just a bad thing if you want to use it for a culture. When you need a culture, if you have an uncircumcised young boy, one you can’t retract the foreskin in, or newborn infants, you really need to do a suprapubic tap. When you do that, you may get a contamination with blood from actually doing the needle stick, so you can’t believe the blood on that specimen. And sometimes it’s useful to do an in and out cath, especially for a culture and urinalysis for toddler girls who just won’t go. The kids who are between the ages of 18 months and four years, who are in toilet training, really have a problem giving you a sample because they know they’re not supposed to go in strange places and strange containers. And they just don’t want to do it.
Now if you’re looking for cells or nitrites, it’s best to get a first morning urine. But that has to be collected at home when you first arise in the morning. That is the most concentrated. It may be inadequate, though, for cells and casts, so you really need to do a fresh specimen for that in the office. Often when you refrigerate that for transport to the center – and it should be refrigerated because bacteria will overgrow and give you all those false positives – when that happens, sometimes the urine turns cloudy from crystal precipitation, which scares everybody. But you just rewarm it to room temperature and the urine clears up. In and out catheterization is not routine, but it is certainly useful when you need urine from a patient who cannot and will not void, and in an adolescent female who cannot or will not do a clean catch.
Now for the routine urinalysis, we do three kinds of exams. A gross exam. A dipstick exam. And a microscopic exam. And I’m going to talk a little in detail about all of those.
The first thing I want to talk about is the gross appearance. And most of us skip that line on the written urinalysis sheet, but it’s often very helpful. We can look at color and turbidity. And in the old days before there were dipsticks, odor was very important because sometimes that was the only way you could tell the difference in a urinary tract infection and not.
The color of the urine is very important to us. The normal color is various shades of yellow. And it’s everything from a more amber to a lighter yellow. If the urine is amber to reddish brown, you need to suspect red cells in the urine, hemoglobin, myoglobin, or hemosiderin. If the color is bright red, that means fresh blood; and you’re more likely to have a traumatic injury, a lesion that’s bleeding in the bladder or in the kidney. Or, in the case of infant diapers, urates are pink, and they may make you think you have red urine, but it’s really just urates. Other things that can give you a bright red color that we see often, we don’t see porphyrins or pyridinium, what we usually see is food coloring and beets. And that makes everybody very nervous. Very infrequently you’ll see a brown or black urine. And the only time I have seen it has been with infant diapers. And the infants that I had with hypertension, who were taking the drug, methyldopa. And then when the diaper was exposed to air, the methyldopa excreted metabolites that turned black. And that does give everybody a bad scare. Bright orange is the color of urine in patients who are taking rifampin, and dark orange is the color of urine in kids who eat too many carrots or who have high bilirubin. Blue-green urine can be seen if you’re getting methylene blue, and that is in some of the medicines used for anesthetizing the urinary tract for pain from urinary tract infection. It contains methylene blue, and it will turn the urine blue. And the main culprit is a drug called Urised.
Now it’s also important to look at the turbidity of the urine. When you have a cloudy urine, you can have all kinds of things. It could be bacteria. It could be leukocytes. Or it could just be crystals. As I said before, urine that’s in the refrigerator may turn cloudy because of the precipitation of crystals. If that’s the case, recentrifuging the urine, acidifying it, or rewarming it to room temperature will clear the urine. It will not clear a urine full of white cells and bacteria. When there are crystals, most of the time it’s the amorphous crystals that precipitate out and give this cloudy color. And if it’s an alkaline urine, that’s going to be phosphates. Or if it’s an acid urine, it’s going to be urates. A hazy urine often is one that has a lot of mucus in it. And the mucus can be from vaginal secretions. It can be from mucus threads in the urine. We see a variety of children who have bladder augmentations with intestinal cells that produce mucus, and we can see a thick mucus plugs in those urine. It’s rare for us to see a milky urine, but if there are a lot of fat globules in the urine, you will see a milky color. And that’s probably only going to be seen in our population in someone with very severe nephrotic syndrome that’s uncontrolled, and they have a high cholesterol and a lot of oval fat bodies in their urine. The most common thing we see is the smoky colored urine. It’s usually red-brown, and it’s full of red blood cells.
I mention the odor here because most of us don’t get that information. It’s not reported on the routine anymore. But it does give valuable information for common and rare conditions. And most notably, a foul-smelling ammonia-smelling urine has either bacterial contamination or a lot of bacteria from infection. A fruity urine is usually full of ketones, and that can be from diabetes with ketoacidosis or from really severe starvation. If you have any kids on the Atkins diet, they might have a fruity-smelling urine. Then for rare diseases, a urine that smells like maple syrup is maple syrup urine disease. Very cleverly named. Musty urine can be seen in patients with phenylketonuria. And sometimes, ingested foods give odd odors, and probably the one we all know the best is asparagus. And there are also certain excreted drugs that give an unusual odor, and some of the antibiotics do that.
I’d like to move now on to talk about the dipstick. This is the best bang for your buck. You can get a lot of information in a two-minute period. You need to read it by the times that are on the dipstick that you’re using. So if it says, “Glucose 30 seconds,” you need to have a watch with a second hand, and you need to be reading that at 30 seconds. If you don’t read it at 30 seconds, it may turn a very positive color and give you a false positive. You need to read ketones at 40 seconds. You need to read the specific gravity at 45 seconds. And at 60 seconds you need to multitask. You need to do pH, protein, blood, and urobilinogen. You also do nitrates at 60 seconds, but you don’t do leukocytes until two minutes. And it’s important to wait for those times to get accurate results. If you’re going to get accurate and useful information from the dipstick, you need to use a fresh, well-mixed, unspun urine sample. The dipstick bottle that you’re using should be kept closed and dry and out of the sun, and at a reasonable room temperature, not a Houston summer room temperature. And it should be used within its expiration date. It’s very important that you not remove the desiccant from the bottle, or the dipstick pads will lose their accuracy. And then you need to do quality control with a new bottle and periodically, if you keep a bottle around for many days or many weeks. Those kind of quality control pads can be bought at the same places that you buy the dipstick supplies.
Now what can you learn from what’s on the dipstick? Well, urine pH has a very widely normal range. Anything from 4.5 to 8.5. And the actual dipstick reads 5 to 8. If the pH is greater than 7.5, that’s not really very normal, and we only usually see that in someone who is taking bicarbonate or alkali supplements. If the pH is 8 or higher, you have to suspect that there are urea splitting bacteria present in the urine, either as contaminants or with a urinary tract infection, and giving you a really falsely high pH because of the creation of ammonia in that sample. If the patient has systemic acidosis, and the urine pH is greater than 6, you have to worry about renal tubular acidosis. The pearl for the pH is that the pH determines what kind of amorphous crystals you might have. And if it’s an acid pH, it will be urates; and if it’s an alkaline pH, it will be phosphates. One pitfall of the pH dipstick is that if you have excess urine on the dipstick, the urine will run over from the protein reagent, and it will falsely lower your pH result. And so you have to be very careful how much urine you put on the dipstick sample.
The urine specific gravity is very helpful to us. It’s a guide to urine concentration. But one pitfall you have to avoid is thinking that the specific gravity can tell you about concentration, urine concentration, and about the ability of the kidney to concentrate in dehydration, and tell you something about the state of hydration of the patient — if the patient has renal failure or some disease that causes you to lose concentrating ability, you can feel falsely secure when the specific gravity is 1.010 on a dehydrated child with renal failure because they just can’t concentrate the urine anymore. The urine specific gravity dipstick estimates the osmolality, but it’s not the same measurement. And it’s close enough that it helps you with a variety of other conditions that you’d like to estimate the osmolality. The only proviso is that the specific gravity is falsely increased above the osmolality in the presence of increased protein. And since I see a lot of patients with increased protein, I have to look at the specific gravity with a grain of salt, if you will. The dipstick method, however, is not affected by glucose, x-ray contrast, as was the old refractometer that we used to use for this purpose. The dipstick specific gravity reading is from 1.000 to 1.030, and it correlates very well with the refractometer, which is a very accurate way to do specific gravity but requires a lot more equipment and skill, and it’s not quite as accurate in the high pH range.
Just a few words about the urine protein. The dipstick is more sensitive to albumin than other forms of protein that appear in the urine, such as globulins, mucoproteins, and Bence-Jones proteins. Which is very good for what we do because in most of our patients, we’re looking for albumin. Any time you see protein of 1+ or more in the urine, that’s significant. And if the specific gravity is really low, even trace might be significant in some conditions. But it’s important to remember that sometimes you are getting the urinalysis at a time the child is sick, and so you are going to get transient proteinuria often in the presence of fever, stress, or exercise or congestive heart failure, which we don’t see very often. So if you get a positive in one of those circumstances, just repeat the urine when the child is well before you start working them up for renal disease. There are a variety of things that can give you a false positive for protein, and they include some of the things we use in the antiseptics to clean off for the clean catch specimen. So you need to be careful that these are not in the antiseptic that you use. The most common problem we see in our adolescent patients is vaginal or prostatic secretions in kids who don’t clean off very well, and that may give us a higher protein when we don’t expect protein.
Blood on the dipstick usually detects a very small amount of blood, 3 to 4 red cells per high powered field. And it can also detect hemoglobin or myoglobin without the presence of intact red cells. So you need a microscopic exam to distinguish those things. There are a variety of cases that you have decreased reactivity or false positives.
Urine Dipstick: Glucose & Ketones

- **Glucose**
  - Specific for glucose
  - glucose oxidase reagent
  - Need clinitest tablet to test for other sugars (galactose, fructose, lactose)
  - Need concomitant blood glucose to distinguish hyperglycemia from renal glucosuria

- **Ketones**
  - Pearl: Dipstick reacts with acetoacetic acid, not acetone or β-hydroxybutyric acid
  - False positives:
    - highly pigmented urine, mesna, levodopa metabolites

The urine glucose and ketones are important to remember that in the glucose, the test is specific for glucose and not for other sugars. So that if you want to screen for other sugars, you must do a clinitest. And the key for the ketones is that the dipstick really reacts just with acetoacetic acid and not with acetone or beta-hydroxybutyrates, so it may not give you a completely accurate assessment of what’s going on.
I want to move on and talk just a little bit about urobilinogen, which is that thing on the dipstick that we nephrologists like to ignore, and that many people do ignore. And the question is, “What does it mean when it comes up positive?” Well, urobilinogen is usually made in the intestine from bilirubin. It’s excreted primarily in the stool, but less than 5% is excreted in the urine, and the urobilinogen dipstick is very sensitive for that. So it’s normal to see trace amounts of urobilinogen in the urine. And that means you’ll see a .2 to 1mg/dl reading. So if you see anything up to 1, don’t worry about it. Just ignore it and move on. It’s when it’s 2 or above that you need to think there might be something there. And an abnormally high urobilinogen in the urine is usually found in our population in the presence of hemolytic anemia or in the presence of intestinal obstruction. The absence of urobilinogen on the dipstick does not mean there isn’t any urobilinogen because urobilinogen is very sensitive to the air. Once it’s exposed, it’s converted to other metabolites that don’t react with the dipstick.
Microscopic Exam

- Preparation of the sediment
  - Place 10 ml well-mixed fresh urine in a conical centrifuge tube
  - Centrifuge at 2000-3000 rpm for 5 min
  - Discard all but 0.5-1.0 ml supernatant
  - Resuspend the sediment
  - Transfer 20 µl to glass slide and cover with a glass cover slip
  - View under microscope with reduced light

Well, now I want to go on in the last few minutes of this talk and talk about the microscopic exam. The first thing you have to do for the microscopic exam is prepare the sediment well. You need to start with 10ml of well-mixed fresh urine. It should be in a conical tube. It should be centrifuged between 2,000 and 3,000 for five minutes. And once you've decided what your tabletop centrifuge level is closest to in that 2,000 to 3,000 range, you should stay there forever. You should not be trying to go back and forth at different levels so you have some consistency in your reading. Once you have centrifuged it for five minutes, then you discard all but about .5 to 1ml, resuspend the sediment, transfer it to a glass slide, cover it with a cover slip, and then be sure to look at it under a microscope where you've put the condenser way down and turned the light down so that you have the best contrast to see what you're going to see.
And here’s the variety of things you can see with a microscopic exam. But, one picture, or several pictures, may be worth a thousand words, so let’s go to the pictures.
These are what red blood cells actually look like when you're looking through low power. There's no way you can tell those are red blood cells. You can only tell there are a lot of cells there. So you must go to high power to see that these are red blood cells. These are normal. If you see those that are just what we call dysmorphic-looking, those that have come in funny shapes and are all varied, they are more likely to be from glomerular disease than from some other source. And I had a very difficult time trying to find any pictures of those. The red blood cells can be confused with other things on the slide. If you weren't noticing very carefully, you might think that oxylate crystals at low power were red cells. But I think you can see, if you look closely, that these have sort of a cross in them, and they are actually double pyramids. And even yeast, especially budding yeast, can look like red cells. This is at high power, and this looks like a red cell on its side, where it gives you that sort of biconcave disk look.
Urinalysis: "White" Cells

Leukocytes

Renal Tubular (RTE) Cells

The thing to remember when looking at white cells on the urine is that not all white cells are leukocytes. And the dipstick picks up leukocytes, but it doesn’t pick up lymphocytes, renal tubular epithelial cells, macrophages, and other things that might be in the urine of patients with pathologic conditions. And here you can see the difference between what a renal tubular cell looks like and what white cells look like.
You can also see a variety of epithelial cells. These are transitional bladder epithelial cells. They look similar to the renal tubular epithelial cells. And here are a variety of squamous cells, usually from vaginal source in most of our patients, although you can see a variety of squamous cells from the foreskin area in uncircumcised males. And just from skin, in any patient that you're getting a urine on.
This is a very specialized cell that we see in nephrotic syndrome patients, and it’s a renal tubular cell that’s loaded with fat globules. And so we call it an oval fat body. When you see it, it means you’ve got nephrotic syndrome. And this is that same thing under polarized light.
Microscopic Exam: Casts

* Formation: molded in renal tubules
  - Mostly formed in distal and collecting tubules
  - Precipitation of Tamm-Horsfall mucoprotein (hyaline casts)
  - Clumping of cells within protein matrix (RBC, WBC, RTE or granular casts)
  - Conglutination of luminal proteins, including abnormally filtered serum proteins and cells (waxy or broad casts)
* Quantitated by number per low powered field (lpf)
  - Cells quantitated by number per high powered field (hpf)
* Pearls:
  - Look at edge of cover slip to screen for casts.
  - Hyaline and granular casts may be present in normal urine
  - All other casts or large numbers of hyaline or granular casts indicate renal pathology
  - Waxy or broad casts usually present only in advanced renal failure

Now the microscopic exam is also very good for casts. And casts are molded in the renal tubules under a variety of circumstances. The place to look for casts on the sample is at the edge of the cover slip. And it’s important to note that some casts in the urine are normal. Hyaline and granular casts are a normal variant, and you see it in normal patients, and you shouldn’t worry about it. If you see more than five to ten hyaline casts per low powered field, you have to worry that you have a condition of heavy proteinuria. Waxy and broad casts are only seen in those patients that have severe renal disease and usually have renal failure.
Here are the common casts that we see. The granular cast. And this hyaline cast, you can barely see; and I call that the ghost of a cast.
This is a red blood cell cast, and you can see the individual red blood cells in it, and that’s to be distinguished from a myoglobin cast or a hemoglobin cast in which you see just the pigmented substance without the cells.
This is a white blood cell cast with white blood cells in it. And this is a waxy cast.
Microscopic Exam: Microbes

- **Bacteria**
  - Pearls
    - If even a few seen on unspun urine, means UTI
    - If a "few" seen on spun urine, means nothing (probable contamination)
  - Yeast
    - May be round (confused with RBC) or budding
    - **Pitfall:** Contamination by yeast from diaper rash may be mistaken for UTI with yeast
  - Parasites
    - Pinworm eggs
    - Trichomonas vaginalis

And finally, or almost coming to a close, you can also see bacteria on the urine. And the pearl here is that if you see even a few bacteria on an unspun urine, you probably have a urinary tract infection. But if you see a few on a spun urine, don't worry about it. It means nothing. It's probably just contamination.
Microscopic Exam: Microbes

Bacteria

Pearls: If even a few seen on unspun urine, means UTI.
If a “few” seen on spun urine, means nothing but probable contamination.

This is a sample of a spun urine, and it’s got tons of rods in it, and this someone who had a urinary tract infection.
You can also see unusual microbes in the urine. You can see yeast forms, and most often we see these in kids who have a concomitant diaper rash, and we have a bag specimen. And many times I've diagnosed pinworms by seeing pinworm eggs in the urinalysis samples in our clinics and this is the typical appearance.
**Microscopic Exam: Crystals**

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<th>Acid Urine (pH &lt;6)</th>
<th>Alkaline Urine (pH &gt;6)</th>
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<tr>
<td>• Uric acid</td>
<td>• Phosphates</td>
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<tr>
<td>• Rhombic prism form</td>
<td>• Triple phosphate</td>
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<tr>
<td>• Sodium urate</td>
<td>• Calcium phosphate</td>
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<td>• Amorphous urate</td>
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<td>• Calcium oxalate</td>
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And finally, I just want to show that there are different kinds of crystals.
Pearl: Hang one on the wall of your lab space, if you do microscopic exam.

You usually should have a reference to look at in your office. You know which crystals are which. And the most important things there to remember are that crystal often appear after the urine stands for a while.
Microscopic Exam: Crystals

Pearls
• Crystals often appear after the urine stands for a while, but not in fresh voids.
• Crystals usually of no clinical significance
  - Calcium oxalate, urates, phosphates
• Pathologic crystals
  - Cystine, leucine, tyrosine, cholesterol, sulfa

That most of the crystals are of no clinical significance. And that the few pathologic crystals that we see, we don’t see very often in pediatric population.
Here are the crystals we see the most. This is an acid urine, and the various forms of urate. I probably see these every week in my clinic. I occasionally see these rhomboid forms. I occasionally see these little spicule forms. I almost never see those sort of sea urchin looking forms.
We see a lot of oxalate crystals in the urine, especially people who drink a lot of iced tea. And it doesn't mean anything. It's not even very helpful for predicting calcium oxalate stones. Cystine crystals are very distinctive, very rare, almost never seen in pediatric patients except that rare teenager who gets diagnosed with cystinuria.
The alkaline pH crystals that we see a lot of are the phosphate crystals. We see amorphous crystals. We see the calcium phosphorus spicules. And we see a lot of the sort of envelope looking crystal shapes of the triple phosphates. And we see those every week in clinic.
And finally, we see lots of artifacts in the urine, the most common being this fiber. And that fiber comes from the gauze we use to have the patient clean off with; and if you don’t know, you might want to call that a cast. But I think you can see a granular cast here, and it’s totally different looking from this fiber. Starch crystals that used to be seen a lot when people used cornstarch for babies’ diapers. We see mostly talcum powder now, which looks a little bit different than this. And you can also be confused by an air bubble in the urine, which makes you think you have something awful, some crystal, and it really is nothing but air in the urine.
And I just want to leave you with the thought that urine is beautiful. And hopefully that will set the tone for the rest of this afternoon’s presentation.