Cytology in Clinical Practice
Dr. Tracy Stokol
Video Transcript

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Introduction: So I'd like to give an announcement, for launch announcements, just regarding the specialty of shelter medicine. So almost ten years ago, the Association of Shelter Veterinarians started a lengthy process to establish shelter medicine as a boarded specialty, as many of you know. Due to an incredibly – due to a lot of hard work from many shelter veterinarians, this past April shelter medicine was approved by the AVMA for provisional recognition as a board specialty under the American Board of Veterinary Practitioners.

So this is a truly exciting team for shelter medicine, and the creation of a formally recognized shelter medicine specialty will increase the number of highly trained individuals available to provide communities and the animals they shelter with the exceptional veterinary care that they deserve. So, veterinarians who are interested in becoming board certified should look up the website for ABVP.

This lecture I also will be passing around the CE. If you're interested in taking this for CE, just go ahead and sign your name. We don't need your email for that lecture. And, once again, this is Dr. Tracy Stokol. She will be giving the lecture "Cytology in Clinical Practice" today and –

Dr. Tracy Stokol: Great. And?

Introduction: For those of you who weren't here on the *[inaudible]* board, just so that you know. She has a veterinary degree and a PhD from the University of Melbourne. She did two years of practice in Australia. She came to Cornell in 1993. And she has been a boarded clinical pathologist since 1995, so we're very lucky to have her.

Dr. Tracy Stokol: Thank you. Thank you for the nice introduction. She's going to want something from me in a couple of months, so she's going to be nice to me.

Wine will work, you know. Just kidding. Okay, so this part of the lecture is going to follow pretty much the same track as the hematology lecture – and I'm carrying around my glass case for some reason – in that I'm really going to talk about tips of creating good cytologies and what we recommend with cytology.

And, again, we've got some really great information on our website in the Animal Health Diagnostic Center, and we're going to be expanding over the next 20 years or so our teaching website, which I've giving you the URL for – current URL as well as the one that's going to be launching in 2014, in August.

So someone came up to me after the lecture and reminded me of two things that I should have said about hematology slides. Remember everything is a snapshot in time, and hematology changes pretty darn quick. So, honestly, if you've got a critical patient and you're monitoring them, I would recommend at minimum 24 hour, because we've seen hemograms change pretty much like that, particularly with leukocytes and platelets. Obviously, a red cell is going to take a lot longer, but you can see immediate changes in those with resolving inflammation, worsening inflammation.

The other thing and the other caveat is you don't treat the lab data; you treat the patient, okay? And that's always first and foremost. I say that to students. I say that to anyone who calls asking me about results. The first thing I'm going to ask is tell me more about the case so that I can put the data in context of the case, because everything is context dependent and everything is case dependent. And what we do as clinical pathologists is you really try and make a story of what we're seeing. Then we can help you. And that story means that we're kind of making things fit into a picture and we're using our best judgment to do that, and that's what we do as veterinarians all the time, is we try and let the animal tell us a story and then we treat what we think is going on.

Okay. So, again, the outline is smear preparation and basic cytology principles. Again, I'm not here to teach you how to tell a round cell tumor from an epithelial tumor. I have given you in the notes some guidelines on the things that we look for. And I can also show you pictures of ones that we sit there and we take half an hour looking at the smear because they're challenging cases. And with Cornell now being really a tertiary referral institution, we see some pretty challenging cases. And even the ones – because we read outside cytologies now – we're getting some cases that we scratch our heads about. And they're always interesting and frustrating, particularly if we don't get follow-up, because we want to learn from them so that we can do better next time.

But, essentially, our interpretation can only be as good as what we're looking at, so if a slide is a poor quality slide, we really struggle with those. So what I'm going to work through now is just how to make a good slide and the things that we've learned about making good smears. And, again, I'm also going to talk quickly about the processes that we identify, and for the students that we teach, they will struggle will what do we mean by disease process.

Because ClinPath is all about pattern recognition and disease processes. So, if you've got a high urea and creatinine and we're after azotemia, and then we've got three types. And so, for me, azotemia is a disease process. It tells us that either there's dehydration or something going on in the urinary system. And so like inflammation is a disease process. And, certainly, what we look for is hemorrhage inflammation in cancer and then we get fixated on these small, little necrosis and all these other cool things.

And, again, in cytology more than hematology we have to tell fact from fiction, and there are things that mimic infectious agents all the time. There are things in urine that mimic infectious agents. So we're like, "Oh, my God, what are you?" And a lot of it is match the picture or, you know, what I recall having seen a case ten years ago and what did I do with it that time.

Okay. So, with cytology, what you want to do is make the most appropriate smear for the sample that you have. And, really, this is dictated by what you have. And if it's a fluid sample, there's different things that we do. If it's a solid tissue aspirate or you take a chunk of tissue that you make impression smears, it changes what you do. And if you're sending slides into a lab, they're going to want everything. Yes, we know you're going to keep a slide and I encourage you guys to look at your own cytologies, but there's nothing that we hate more than Diff-Quick. I hate to say that.

But Diff-Quick is really challenging, so send us everything you have because you may think you've sent us the best slides, but you actually may have the only diagnostic slide in your hand, and then you've wasted for us maybe $40.00, sending a slide in that we say, "I'm sorry, it's not diagnostic," and then you call us up and say, "But we saw cells." And then we're like, "Well, send that slide in," and then there's a week delay. So send us what you have, and if you want it back, just let us know, and if we can we'll give it back to you. But, obviously, we have to keep it for legal reasons.

Give us a history because, if not, we'll definitely make up a really big story or make two and two equals ten. And keep out of contact with formalin if you're taking a surgical biopsy because this is what happens

when you try and stain it. This is actually a lymphoma, but I wouldn't have any clue what this cell is. I can tell it's a neutrophil, but formalin gives a blue background and it prevents the cells from being stained. So make your lives easy and keep it out of formalin.

So if you're collecting – and for a lot of you in shelter medicine or emergency medicine, you guys are going to be collecting potentially some fluid samples, like ascites, possibly a pleural effusion if an animal comes in that's dyspneic. I don't know if you do tracheal washes and stuff, but you might do those. But certainly, if you're collecting a body cavity fluid, you should collect into EDTA because that preserves cell morphology better. If it's a pretty bloody fluid, I recommend collecting into a red top, because if it clots immediately you know that it's acute – either blood contamination or acute hemorrhage or for abdominal tap you may have hit the spleen. If it doesn't clot, you know that bleeding is preexisting because blood clots and difibrinates pretty quickly in the abdominal cavity.

If you anticipate a need for culture, we can do it off a non-anticoagulant tube, but better to put it in culture transport media. And always make fresh smears and submit with it too, because we try and avoid some of these artifacts which I'm going to go through that occur with sample storage. And just like we do with hematology, refrigerate the tubes and not the slides to prevent or delay these artifactual changes.

So, really for body cavity fluids, that's where we have the most choices. And, for us, it really depends on cell counts unless we get a fluid from a cystic lesion on the skin where cell counts aren't required. Then we're pretty much using these same principles that I'm telling you. So if we get the fluid and it kind of looks like this, you make a direct smear, you're going to see nothing. That's a pretty poorly cellular cell count. Essentially, we go by the turbidity of the sample to see is it a highly cellular sample, not understanding that milky fluids could be actually of low cellularity, but you're not going to be able to tell because it's going to look like milk.

If you've got fibrin flecks or tags of mucus such as a trach wash, we actually want that mucus because that's where the cells are. So we actually pick up bits of mucus. If there's a clot in the fluid, we will pick up the clot and we'll look at the clot as well as the other smears that we make from the fluid. So if it's clear, we actually spin a portion of the fluid down and decant it and leave as little liquid as you can in the bottom. We suspend it and make a smear, and we call those sedimental concentrated smears.

If it's really poorly cellular, in our lab we actually do a cytospin smear, and that's a specific centrifuge that none of you are really going to have,

and that concentrates it. And with urine, we do a technique called the line smear, and you can also do this with poorly cellular fluids that look like this, because it concentrates the cells on a line. But it's a line smear of the sediment, so you first do a sediment and then you make a specific smear type which I'm going to show you.

Now, I've added "add protein to CSF." I don't know if any of you guys collect CSF. I actually encourage people not to add protein, but you're going to read the literature. You're going to find that everyone says add protein. The reason we don't do it is that we're concerned that people will add the wrong volume of protein; the protein's going to dilute the counts; it's going to affect the accuracy of the counts. Yes, it will help the cells be preserved better, but it's also going to affect everything, and if you throw a protein in everything, guess what, your protein concentration measure of the fluid is inaccurate.

So keeping two separate portions, one with and without protein, is just too complicated, so I just say, "Send in the CSF. We'll do what we can." And, usually, if you've got information in the CSF, we're going to pick it up. Those borderline ones we may miss because of deterioration, because CSF's a very low protein fluid, which is why the cells die there so quickly.

So these are the hair dryers we have in the lab and, seriously, I go – oh, sorry; I'm moving around – and I go there and I do this with the back of the slide, and it's on high. And I burn my fingers, but I dry the fluids really quickly. Why is that important? Because the cells spread; you can see what they are, and you can look inside them if they're neutrophils and macrophages, the things like infectious agents. And I will show you the difference between slow drying and not drying, and this is really critical. Joint fluids, viscous fluids, trach washes, everything rapidly air dries. Every time I hear that hair dryer on in the lab, I know I'm getting more cytologies to read out because that's how you tell. You will not hurt the cells. Trust me.

So the smears that we make from fluids in the lab are our classic blood smears, which are our wedge smears, because we like to have that feathered edge because that's where big cells, such as cancer cells, go. So we really definitely need that. This is a line smear which is actually a variant of the wedge smear. And instead of going all the way through the end of the slide and keeping going so you get that feathered edge, you actually stop three-quarters of the way and lift it up. And then what I do is I actually put my finger under the slide and tilt it up so that this line kind of goes back on itself a little bit, so it spreads it out a little bit so it's not too thick. And then I rapidly air dry that line first, and then the rest of it.

And what that does with a poorly cellular fluid is it concentrates the cell on the line, and that's where I go to. And we do that for urines all the time. We also do it with other poorly cellular fluids if we don't have enough to do a cytospin, and it's a really good technique. It's also more gentle on the slide than doing other smears because the cells are just being drawn out gently. Often with the wedge smear, the regular blood smear type prep, the cells are ruptured out of the feathered edge. So this tends to preserve the cells a little bit better. But it can be thick, so you don't want to do it for a very highly cellular *[inaudible]*. Excuse me.

So I really hate the term "squash smear," because people think you have to squash. So I want you guys to think about it as a contact smear. All you want, just like I mentioned with the blood, making the blood smear, is you want contact. You don't want pressure because pressure is your enemy. You're going to rupture all the cells with pressure, and particularly fragile cells to begin with, such as those pesky lymph node aspirates with lymphoma.

So even though it's called a squash smear, think of it as a contact smear, and this is essentially how I do it. I use the spreader slide at 90 degree angle. Other people do it parallel to each other. That's fine. There's multiple techniques. You put a drop or you put an aspirate – this applies to aspirates as well – close to the frosted edge, not right on the frosted edge. I put the contact slide on top, so that it spreads between, and then I very gently move them apart, maintaining that contact.

Now, I don't do this, because in my mind – and I was bad at physics, so I could be wrong – but in my mind that creates double the pressure. It's just keeping this steady and moving that. But, again, I'm not putting pressure. I'm just putting gentle contact. So the one exception to that where I do put pressure is when I take a scalpel blade scraping, which is when you actually have tissue. And that's because it's naturally going to be thicker – now, even lymph nodes aspirates.

The other thing is don't put all your eggs in one basket. Give us ten slides and not one slide, okay? Because we can do additional stains. We're now doing all these special diagnostic techniques to differentiate lymphomas, the type of lymphoma. We can do other immunohistochemical stains and cytology smears. So you give us more slides; we can do a lot more with them. So make more slides than fewer. There's one vet who's great. She sends us 20 slides. We kind of sigh every time we get them, but we know we've got plenty to work with if we need them. But 20 is a lot.

So the solid tissue is where I make the exception. So if you're taking a biopsy and you want to get better at cytologies, the best way is to read your cytologies and see what the histopath comes back. That will make

you a better cytologist almost immediately, other than the things they can't see on histopath.

But what you want to do is take a fresh cut surface and avoid ulcerated areas where there's going to be a lot of artifacts, superficial infections, as well as dysplasia because, secondary to that, superficial inflammation which can mimic cancer. So take the deeper tissue. You make imprint spheres and always blot it with a bit of Kimwipe or tissue paper or gauze, just to get most of the fluid off so that the cells exfoliate. And remember that mesenchymal tissue with a lot of fibrous connective tissue will not exfoliate well.

And what I actually do is, instead of actually putting the imprints like this, I actually roll the slide over the imprints to kind of roll it. And I do about three imprints per slide, three to five. If you do 20 imprints per slide you're going to kill us because we have to look at every little dot and it's really, really tiring. So it's better to give us 5 slides with 5 imprints per slide than 1 slide with 20 little dots on them.

If I get a big enough piece of tissue and it comes down as tissue to compact, what I usually do is take a scalpel blade, one of those – any one – and I take a fresh-cut surface, and then I put the scalpel blade just like that, and I scrape. And then I'll wipe the edge of the scalpel blade on the slide, and that's when I put a little bit more pressure. So I know that areas are going to be squished to glory, but other areas are going to be beautifully well-preserved and other areas are going to be too thick.

And that's also what we have to do as a cytologist, is we have to find the right area to look at, and that's also quite key, and there's also an art to it, which you learn. And I'm going to go through that.

So these are the stains that we use for both hematology and cytology. Obviously, we use a beautiful automated stainer. You want to take proper care of them. You want to top them up. You don't want to let them dehydrate. You want to make sure that there's no floaties or disgusting fungi growing in there, which I've certainly seen. We had bacteria growing in the stain. And, ideally, you should put it back in the primary container, or if you want to keep that primary container for when you're replacing the stain, you can put another secondary sealed container that's not going to dehydrate in use, and then just take it out when you need it.

And these are Coplin jars, which we use, which are really easy to stain with, but they do dehydrate if they're left in there, and that gets pretty grungy after some time, so it's a good idea to change it frequently, particularly with fresh samples. And, you know, if you're doing a lot of cytology, you'll need to buy Diff-Quick every 6 to 12 months at least.

And I think at least 12 months should be fine. So, common artifacts. You see stain precipitate, particularly with the blue one, and bacterial overgrowth, which I showed you a picture of in the hematology section.

And lymph nodes always need longer, so we actually put lymph nodes and bone marrows through our stainer twice to get them adequately stained. So the first 10X overview is your first. Is the stain adequate? And if we oil it, we're done, because then it's going to take another at least six hours for us to get that oil off and re-stain it, so our residents aren't allowed to put any oil on it until they think it's adequately stained. And when in doubt, put it through the stainer twice.

So with Diff-Quick, it's a little different, so what I usually do is I usually do a little bit of a longer dunk for lymph nodes in the blue because it's usually under stained in the blue, so I usually double the staining time in the blue for lymph nodes. And double – you know, you don't want to live by any rules. You want to kind of get a feel for what works. But I usually do the blue a bit longer than the red, for lymph nodes or thicker tissues like that. And you don't want to do that for thin samples like fluids because it's going to be over stained, and that's not good either.

And that's just a picture of stain precipitate again, which mimics cocci just beautifully. You know, one trick is you can Gram stain that because that will be red on a Gram stain, whereas cocci just jump out at you. Well, they should jump out at you with a Gram stain.

So, again, know your stain and I guess this is a Diff-Quick stain and this is our Wright stain. This is a round cell tumor because they're discrete individualized cells. They've got nice round nuclei and they look like fried eggs, and when we put our stain on it, the mast cell granules lit up. And those lymphoma of granule lymphocytes, those granules really stain up very poorly with Diff-Quick, which I discovered to my horror once.

And so, now, which is why whenever I get intestinal aspirate from a cat, I'm like, "You've got to give me unstained slides because I could miss this diagnosis even if I'm looking for it." So that kind of freaks me out, not having my own stain to double check. So what we always do is we look at your slides first, and then we stain up ours if we need it. And it's kind of just a good security check for us because we definitely like our own stain.

So with cytology, again like hematology, you want to be consistent, you want to be thorough, and you also want to remember here blood sampling is great – you get the blood, you've got a snapshot of the blood – but cytology you could be anywhere. So you may not even be in the lesion that you think you're trying to sample. And this is the lymph node, for instance, and you go straight into that general follicle, you're going to get

more than 50 percent blast and don't make a mistake I made, calling that lymphoma.

And I've certainly done that and I won't do that again because now I know better, but the first time you did. Luckily the clinician was good enough not to believe me. So he called and said, "Ah, this doesn't fit." And I'm like, "Oh, damn it. Okay." So we don't like to make mistakes, but we do know our limitations, and this is one of them.

I've certainly seen an aspirate of a lymph node going straight into the medullary sinus. Plasma cells and histiocytes – that's all you're going to see, and erythrophagocytic macrophages. And you're like, "Oh, my God." And then you get the cortex and you get lymphoid tissue, and then you go straight into a hyperplastic general follicle and it looks like lymphoma. So you've got to kind of know what the tissue is and what the architecture of the tissue is that you're aspirating in order to recognize what's normal and what's not. Again, consistency.

So I tell our residents 10X is the most crucial view. That's where I'm already starting to work through what I think it is. And the 10X view is what I want them to give me a description, and in that first sentence I should be able to tell what they're looking at. So this is really important, and what we're looking for at the 10X view is – again, it's all pattern recognition – but we're also looking for the good areas to look at.

So this is an example of a tracheal wash stained with Diff-Quick. So which of these is the best area? So I'm going to tell you right now this is the best area because here I can actually see that the cells of the wall spread, and that's a neutrophil because I can tell it a by its segment.

Here's mucus. This is really thick. I'm not going to see diddly in there, right? I will look in there, but I'm not going to spend a lot of time looking for bacteria in there because I don't even know what the cells are, let alone looking inside of them. And right there is under stained, okay, so I'm not even going to bother looking there because that's wasting my time. So that's good stain, and that's the perfect area because I can see that these are neutrophils, and they spread well enough that I can look inside their cytoplasm for something. I come in here; those are also neutrophils, but I can't look at them because they're scrunched up, and I can't look for bugs in them. And this is separate of inflammation, and I'm going to be looking for bugs.

And at 10X, I'm like, "Okay, what am I seeing?" And then suddenly I come across this, and I'm like, "Okay, that's a little funky. I want to look at that a little closer at 10X." So I'm going to go and look at this, and this is one of the cases I'm going to show you on the digital slide. So we've

got all this stuff in the background, which I'm going to ask you what it is in a sec, but that is something that we'll clue me in that I need to pay attention to that. Whoops.

So then, again, you can either use 40X, and I use 50X oil immersion and I go to 100X to look for bugs, but 50's what I live by and I love that lens. It's just great. It's $1,000.00 but it's awesome. And what are we looking for? We're looking for blood and then we are "is the blood contamination or is the blood part of the lesion?" So is there hemorrhage or, in the case of a body cavity fluid, a diapedesis?

So what we look for is erythrophagia, and this is a macrophage phagocytizing erythrocytes. And that tells us that there's either been prior hemorrhage, or diapedesis if it's a body cavity fluid. Now, that's if it's a fresh sample. If it's a stored sample, macrophages in a tube can start to phagocytize erythrocytes within two hours of collection. And, in fact, bacteria can be phagocytized by neutrophils and macrophages within 30 minutes of collection. So the sooner you make your slides, the better.

Then I look for inflammation and the type and the cause. So this is a lymph node aspirate from a dog – actually it's a cat because there's cat eosinophils. And there's a ton of eosinophils here. There's a mast cell here with a purple granule. And eosinophilic inflammation, I'm going in one direction. And I know some cancers in cats and dogs and cause eosinophilia, but generally I'm steering towards allergic or hypersensitivity diseases, and this is a cat with an allergic dermatitis.

Then you're going to see tissue cells. So if you see blood, then is there inflammation? If there's inflammation, what type of inflammation – neutrophilic, lymphocytic? And, again, I've got guidelines in the notes as to the kind of inflammation that we look for and we categorize. And then I'm going to say, "Is there something else other than inflammatory cells?" Are there tissue cells? And the first question is are there tissue cells that should be there.

So if you aspirate a lymph node and you see epithelial cells, clearly they should not be there, right? So either it's a metastatic tumor or you have an aspirated lymph node. And so we get that a lot with mandibular lymph nodes that people think are enlarged. They often will aspirate the salivary gland instead. And they won't be lymphoid cells, but there will be lots of beautiful salivary gland cells.

So should it be there? And if it should be there, is it normal? Or is it hyperplastic or dysplastic because of inflammation? And if it's not normal, is it neoplastic? And then what kind of neoplasm is it? And generally we break it into four categories, and I put the fourth under

"other" because it's much less common, but there's round discrete cell neoplasms, epithelial, mesenchymal, and other, which are the endocrine, neuroendocrine, primitive neuroectodermal tumors, and all these new things that we're discovering.

So that's a mast cell tumor, which is categorized under the round or discrete cell tumor. Beautiful purple granules. This is a Wright stain, so that's why they're so beautiful and purple. And they're individual discrete round cells. Other ones, the histiocytoma, plasmacytoma, lymphoma of course, and transmissible venereal tumors. And there are carcinomas that mimic these, so you got to love the ones that mess with your head.

Then this is a beautiful cluster of cells. They are roundish and quite large, but they want to stick together. They want to be together. If they want to be together and they're not being held together by matrix, which means that they're epithelial in origin. And, again, is that normal for the site or is this abnormal? And this is obviously a fluid and we should not see carcinoma cells in a fluid, but the first question is are they mesothelial cells or carcinoma cells. And in this case, this was cancer because it had cytologic criteria of malignancy.

And then we have the mesenchymal tumors, and this is a sarcoma. How do I tell this? Because there are red cells all between these cells, so even though they look like they're hanging together, they're not. And if you look at their individual shapes, they're more spindled than they are anything else. And this was a hemangiosarcoma, and it's showing gorgeous cytologic criteria of malignancy, huge nucleus here compared to this, and there was lots of other cytologic criteria of malignancy that I can't show you in the slide.

So, facts from fiction. So, as I mentioned, an erythrophage – and this is in a body cavity fluid – could be storage in a sample. So if we see erythrophagia in a mailed-in sample and they haven't made smears for us, and if we see them, then we're going to be fence sitting. We're going to be saying the erythrophagia could have occurred in vitro, or there is truly hemorrhage or diapedesis into this cavity, so we fence it. If you give us slides and they're good quality, and we can examine them, and we got enough cells to look at, and we don't see erythrophagia, we're going to default to it being an in vitro artifact. So that's where your slides help us.

Again, you'll see phagocytosis of bacteria, which could be sepsis or contamination. And, yeah, you have these neutrophils, which aren't looking that degenerate; they're looking okay. There's tons of bugs in the background and there's phagocytosis of these bugs in here. And this is actually a mailed-in sample where the bacteria contaminated the sample. They overgrew in transit and were phagocytized – and, as I said, within 30

minutes this can occur. And so we look for intracellular bacteria to confirm sepsis, and in this case we're going to fence it unless we have your smear to look at and see them in there.

You also get cell lysis, as I showed you with the hematology. And samples collected post mortem are invariably frustrating and often not helpful unless you're actually imprinting masses, because particularly body cavity fluids you get clostridial overgrowth and you get exfoliation of mesothelial cells and pretty much cell death, so you don't even know what you're looking at.

So other things that make our lives more difficult is understanding – and this is a Diff-Quick slide and this is clearly under stained, so this needed longer in the blue. When we put this longer in the blue – I actually put it in our stain after it had been Diff-Quicked – it popped up and this is lymphoma. So I couldn't make a diagnosis of that, but could off that. And this occurs if there's insufficient time in the stain or the smear's too thick and just needs double staining.

This is a trach wash that was poorly dried. I have no idea what these cells are. They could be macrophages. They could be neutrophils. I assume these are macrophages because they're bigger. That one and that one? Who the hell knows? I have no idea. So this an area that I would have to look very closely in the slide to find a thin area that quickly dried. Otherwise, we're just going to say this is not diagnostic.

This is mucus and ruptured cells. And that's a slowly dried slide that was viscous to begin with. And these are cell ruptured, so this is a lymph node. And, yeah, I'm suspicious of lymphoma, but I can't give you that diagnosis, and that's really frustrating when I've stained up five of these slides and they're all the same. And that happens with squash smears, when you truly squash it, and also some lymphoid cells are really fragile and they're going to break even with a little bit of pressure. So what I usually recommend for people to do there: if you get a good aspirate, make one a squash and then do a wedge smear for one, because the wedge smear is going to be much more gentle. Blood smear technique on a lymph node aspirate.

And cell rupture. Again, these are often what we see in lymph node aspirates. They're just ruptured. This is nuclear streaming, and these can start mimicking fungal organisms and strands of bacteria, so that adds an additional complication.

And this is stain precipitate, huge chunks of it. And this is beautiful ultrasound gel. So when you guys are ultrasounding, make sure you wipe it off, because you stain and *[inaudible]* put them through the stainer ten

times and the stuff just soaks up that stain, and all your cells are under stained around it. So make sure that you wipe off the ultrasound gel. And we know when we get new radiology residents because they always have that to start off with, and then we start writing comments, and then it disappears.

So those are some things that we have to live with. And, again, these are the useful resources that we hope will be just going to get better over time. Okay. So questions on cytology, and I'll go through a couple of cases. What I've done is kind of focus on something that you might see in shelter medicine, so infectious diseases more than lumps and bumps. Yeah.

Question: The ____ that don't need ____. Is it – is there any way *[inaudible]*?

Dr. Tracy Stokol: You can't do anything. You're done. It's Diff-Quick. You cannot – there are some that will stain, and if you look hard enough you will find granules in Diff-Quick, but there's really – the type of stain. There is a variant of the Diff-Quick stain that was written up in Vet ClinPath. They will stain it with, but there really is nothing you can do. So that's just a issue with Diff-Quick that you're going to have to live with.

Again, these granules in these lymphocytes – you look long and hard enough, you'll find them, but are you going to be confident when you're only finding one or two cells? You could be looking at residual cells and not the tumor population. So just know it's a limitation, but then also learn to recognize mast cells, what they look like without Diff-Quick, and they kind of look like fried eggs. But so do histiocytoma sometimes and so do plasma cell tumors. So just, you know, but look hard for the granules. You'll probably find them, except in these poorly granulated grade 3 tumors that actually look like lymphomas sometime do. They're tough for us too.

And I hate to say it, but I've sat on the fence and gone, "I don't know if this is lymphoma – or a lymphoma of granular lymphocytes or it's a mast cell tumor." And we've ended up having to do special stains *[inaudible]* and immunocytochemistry to differentiate them. We don't like fence sitting on those things, but we do. Discrete cell tumor you should diagnose every time. No, no, no. Any other questions? Yep?

Question: Do you have a resource – I don't know if the new online site is going to have this – with example pictures of common things? Like I know you've got the hematology atlas, *[inaudible]* now, but for other organs. I was trying to look at an *[inaudible]*, and I had no idea what I was – you know, I thought it was abnormal, but *[inaudible]*.

Dr. Tracy Stokol: So, yes, it will be up there eventually. If you can get me two other clinical pathologists that can write and want to just focus on this full time, we can get it up in a year. It's going to be a work in progress. We've added some info on cytologies, mostly things that we've had on our lectures. But my goal is to have a hematology-cytology atlas. Right now we've got like an atlas of urine crystals, urine – what else do I have? Artifacts in urine. I've got some more images, that aren't on our current site, on blood and inflammation. And it's going to be populated over time. But there are chunks of time I can't spend on it, so – and it's really just me working on it, so it's going to take some time.

But there are some really good ClinPath textbooks, so there's – *Urinalysis* is the Osmond textbook, but I think it's out of print, so that's going to be hard to come by. There are some good human urinalysis textbooks that you can buy from the American Association of Clinical Chemistry. So you can always email me and I can – we go to that textbook a lot, but remember human and human versus veterinary.

There's *Diagnostic Cytology of the Dog and Cat* by Cowell and Tyler. There's a new one coming. I think it's just come out. There's Rose Raskin from Florida, a book on cytology which is excellent. But, yeah, they have limited images, and my goal eventually is to have a lot of images. We've got a huge data bank of images which I'm going to put up.

And Diff-Quick. I plan to put up Diff-Quick because I can show you a beautiful Wright stain, which is what all these atlases show, and they don't look the same on Diff-Quick. So we're compiling Diff-Quick, which are definitely going to go up. And the goal is to make them eventually into apps so that you can just have it on your iPhone. I've got lots of plan. Give me people. I don't have the time to do it all.

So I would go to those. But you could buy those books because they're very good. And we go to them if we don't know what it is, and we match the picture, and we Google if we think it's spleen. You can just Google images. But you're not going to get very many veterinary images, unfortunately.

Question: When you have a sample with a lot of mucus, how do we handle that?

Dr. Tracy Stokol: Okay, when I have a sample with a lot of mucus. So if we get a trach wash, we want mucus, right? So what we do with a trach wash, we will look at it and go –

So I'm going to go to the first one that I showed you what was – let's look at that a little closer. So let's go to Case 6. And, again, you're going to get a different thing coming up if you do this. So this is a really highly

cellular sample. So this is a pleural fluid from a cat that was severely dyspneic and febrile, had a beautiful inflammatory leukogram. And, again, we've only taken a snapshot of this slide.

But what I do when I scan at 10X, I scan the entire slide from start to finish. And, basically, what I'm looking for is the right area to go down on. Where's the stain? The first order, does it need re-staining? And then, if it doesn't, what am I seeing? And from 10X, I'm already going inflammation. I'm suspicious of cancer; I'm suspicious of this type of cancer. And then you go down and you confirm your suspicions from 10X.

But the 10X view is where we start recognizing the patterns. If I'm seeing a lot of blood, my next thought is, "Is there inflammation along with the blood, or are all these leukocytes blood associated?" And, yes, I need to look for platelets and I need to look for erythrophages. And there's a lot of inflammation. I'm looking at the types of inflammatory cells and I'm looking for a cause. Sometimes you can see that at 10X. And, of course, we always look for cancer and then we always go down higher.

So 10X overview, this is what I'm thinking. I have no idea what they are at this view because it's a little too high. And here I still don't know what they are. Here's something I may come down and look a little closer at. But this is a highly cellular sample, and any area of this smear is good enough. But, usually, I go to edges like this because this is where the cells spread well, so let's go down and look at that area a little closer.

So do you guys know what these are? So I don't know what they are. Well, I do, but that's because I've seen these a lot. So these are actually – looking at them, you don't know what they are because they're blobs of purple, right? They're purple blobs. That's what they are. But, actually, if you look really closely – and can you like imagine a neutrophil nucleus there in there? Kind of imagine this scope? You got to use your imagination a lot when you're doing cytology. So these are ruptured lysis to neutrophils. These are dying, dead neutrophils. And this cat had like a 400,000 nucleated cell count in its pleural fluid, which is never a good thing. It seriously looked like pus, and that's exactly what it is.
So you're looking at this. You're going to go, "I have no idea what these cells are," and neither do I. And I have two choices. This is all necrotic cells, so this is a necrotic, dead tumor; or these are dying neutrophils as part of an abscess. And in this case, I was pretty confident that they were neutrophils for the reasons that I'm pretty sure that's a dying neutrophil. You can kind of see its indented cytoplasm.

So then I'm going to look for a cause. Now, these are not happy, intact neutrophils that you can look inside them for phagocytized bacteria. But,

you know, separate of pleuritis in a cat, there's not that many things. So let's go back to that structure and see if we can find it again. And I need to zoom out, and there. And this is one there, and there's one there. So let's go in here and zoom in.

Okay, so this just looks like a *[inaudible]* to you, right? But if you look really closely, can you see me tracing that filamentous thing coming off here? I'm going to prove to you that this is a sulfur granulin; this is either actinomycosis or nocardia infection. Now, do we always see the neutrophils *[inaudible]*? No, we don't. But whenever I see the neutrophils and macrophages in a mixture of neutrophils and macrophages, like a foreign body type response, I look for these bugs. And cat pleural effusions, I'm always looking for these bugs.

So this is just like pus and dead, dying neutrophils. And here is our cause. And these are filamentous bacteria – and it's now no longer responding, so I'll have to shut it down and start – oh, don't do that. I'll just *[inaudible]* on, and show you the Gram stain, which will *[inaudible]*, I hope.

So immediately you can see that these are – here's the reds. So the neutrophils are red, which they should be because – well, the dead neutrophils are red – because that's – when you do a Gram stain to properly decolorize, if it's properly decolorized, cell nuclei should be red, so that's a good internal control. And if we go down on here, see the nice filamentous Gram positive bacteria. And let me go on – and, see, there was even more that I wasn't picking up, and see how they're forming these beaded filaments. It's really hard to get this in focus in oil, so it's never as good as the microscope, believe it or not, but it's pretty good.

So this is a cluster of these organisms, and these beaded filamentous Gram positive rods mean it's nocardia actinomyces. And these are really difficult organisms to culture, so you often have to put a special request to the laboratory to culture them. And that can take about 10 to 14 days to culture.

Now, this is another thing about a Gram stain that we don't like, is that in the background there are probably some Gram negative rods. I actually don't believe I'm seeing any in here. And it's really hard to see it. Now, here's a beautiful beaded – you can see the beading in that there's dark and light stained areas on this filamentous bacteria.

How much time do I have? Ten. Okay, great. Then I can go through a couple of more cases. And that's pretty typical morphologic features for nocardia and actinomyces, and here's another. See, here you can see the bacteria coming off. They're often accompanied by Gram negative anaerobes, such as *Fusarium* and other species, and those often overgrow

these organisms, and I know they're treated differently, so it's really important to look for them.

And my policy on any suppurative inflammatory action is to Gram stain everything in case you miss something, because cocci can mimic ruptured cells, and ruptured nucleus streaming can mimic the bacteria, and it's always good to Gram stain things. And Gram staining, for me, is really more of a hunt for Gram positives, because there are going to pop out like these, and less for Gram negatives because you can imagine Gram negatives in any Gram stain. You really can. So I use the regular stain, the Diff-Quick, whether I stain to pick up Gram negative bacteria.

So that was that pleural fluid in the cat. And the cat actually did okay, believe it or not. So, which one do I want to do next? Case 1. So this is a pleural fluid from a cat that was the barn cat on a horse farm. And so I can't remember if these were direct or sent-in smears. This is actually a mystery slide case in our annual meeting. We have this really cool session where we get slides that we don't know what it is, and it's a great teaching case for residents. We see some cool stuff in these cases.

And we'd actually not seen this before in this particular species, and it's been published since then in *JAVMA*, which is in the notes that I just sent to Aaron to give to you. So if we just assume this is a direct smear for a fluid, a normal fluid count on a pleural fluid should be less than 5,000 cells, so this is a really cellular smear. So this would be like 80,000 or 90,000 cells in there. So now let's go a little closer and see.

I don't see big clusters of carcinoma cells, but what I am seeing are small, little cells in the background, red cells, and these kind of big, vacuolated, voluminous cells which I know are macrophages. So let's go down a little closer and see what these cells really are. And here, yes, that's a macrophage for something in the cytoplasm. Here's a neutrophil next to a macrophage. We also have quite a few lymphocytes, so lymphocytes – and they're small lymphocytes because they're about the same size as these neutrophils over here, and there's another neutrophil.

So if we see lymphocytes that are small in a pleural effusion, we're going to think there may be a *[inaudible]* component to the effusion, i.e., there's some lymphatic or venous hypertension. So here we have more very vacuolated cells which are macrophages. And there's another cell type – and then I moved off. And here's binucleated macrophages. They're allowed to do that. They can be – they can mimic carcinoma, these macrophages. They can have mitotic figures and they can be in big aggregates.

And here are some neutrophils that aren't looking very healthy. They look somewhat degenerate. And this is the kind of stuff that we say, "This is fiction," so we would ignore this stuff. But let's go and look inside these. And I couldn't find that other cell that I was looking for. And, again, this is fiction. This is a ruptured cell.

So you want to find a well-spread area and something that's well spread. So I kind of like that macrophage, and that's the cell I was looking for, and that's a plasma cell – centric nucleus, deep blue cytoplasm. So let's zoom in on that. Okay. Actually, there's even better. So you've got something in there.

So this is not stain precipitate. On our stain bacteria blue and stain precipitate's purple, so there's a definite color differential. The other thing I look for with stain precipitate, it's often out of the plane of focus of the smear. And if it's in focus and it's in the same plane as like everything else is in focus for the cell, then I'm more concerned that it's truly not stain precipitate. The other thing I look for is uniformity. If it's very uniform and equal size, it's more likely to be an infectious agent or something real, than if it's very variable chunks like you've seen on the stain precipitate there.

So these look like coccoid bacteria, right? But they're in a macrophage. If you look around, there are actually some within a neutrophil, so this is an unhappy neutrophil next to a lymphocyte. But there are a couple in a neutrophil that you really have to hunt for. And there's one, a neutrophil jam packed with them, and there's another neutrophil right next to it with the bacteria right in there. You can kind of see it just over there.

And the presence of coccoid bacteria in a macrophage is very abnormal, and when we see that we think of one particular bacteria, and it's *Rhodococcus equi*. And we see that in horses, young foals, three-month-old foals, all the time. We see *Rhodococcus equi* in them and that's what we expect. But this is pretty unusual in cats, but it has been reported, and so it's kind of a newly recognized disease syndrome in some cats, particularly when they're housed with horses that they can get secondary *Rhodococcus equi* infections, because it's not something we typically expect to be a small animal pathogen.

Question: So it's some clinical or – ?

Dr. Tracy Stokol: No, they're clinical. They have a pleuritis for sure. And then the last one is going to real quick because I don't want to hold up you too much for your next one because you don't have a lot of time between cases. And this was Case 2, and this is something you may definitely see in the shelter. This is actually a duodenal imprint, but you may see this in feces.

And so these are imprint smears. And these are the little dots that I was telling you about that we hate because we have to look at every single little dot.

But I'm just going to go down here. And, again, we use first principles, even when we're in an unusual site. So, unfortunately, many of the cells are ruptured in the background, so I have no idea what that cell is, but you can kind of make out a neutrophil nucleus down there, so that's a neutrophil. And this is a slide from a friend of mine. There's lymphocytes here. That's a lymph – I can recognize that. I think these are macrophages. And so there's mixed inflammation. There's definitely a neutrophil here. And then there's this organism here in the background.

And I don't think you're going to see it on here, which is such a pity because it's really pretty, but these – oh, yeah, you can see it. Can you see the flagelli? But does anyone know what this is? Causes diarrhea? Really putrid, foul smelling, disgusting, cow-pie-like diarrhea. It's usually probably a multi-cat household, so it could potentially get in a shelter. This is *Tritrichomonas foetus*. Very cool. The only one I've ever seen, and I was like, "Oh, my God, this is so exciting."

The cat ended up actually having lymphoma as well as inflammation. So it had intestinal lymphoma, probably small cell lymphoma because that's the most common type of – we wouldn't have called lymphoma from the cytology in ten million years, by the way, but we would have said a protozoan infection, suspect *Tritrichomonas foetus*, just knowing what it is at the site. But this three flagelli is pretty characteristic. And there are just a ton of organisms on it.

He's a great source of slides. He just sent me a *Clostridium piliforme* in a dog liver, which is very unusual. That's also a horse pathogen. So, all these horse pathogens coming into dogs.

And I'm out of time, but I just want to share some of the fun stuff that we get to see as clinical pathologists. So you see we get really excited. And some of us really love critters. I'm like a cancer junky, but I do like critters. But we call them our little friends. So any questions? I definitely don't want to hold you up from Dan. He's going to have a fantastic demonstration for you guys, a lot of fun now, so…
And, you know, we're always available for questions, and you can email us, and you can call us. And, of course, you can always send slides to us. We're more than happy to look at stuff. So enjoy the rest of your conference and thank you for your time today.

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