Hematology in the Emergency Setting: The Value of the Blood Smear Exam
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[Beginning of Audio]

Introduction: All right. Can everybody hear me? All right. I'm just the introducer, so – okay, there, I'm going to stand close. So this lecture is on Hematology in the Emergency Setting, the value of the blood smear exam. This is Tracy Stokol, Dr. Tracy Stokol from Cornell, and she's going to be doing this lecture in place of Heather Priest who was prior scheduled to do the presentation.

So, Tracy received her veterinary degree and PhD from the University of Melbourne in 1987 and 1993 respectively. After two years in veterinary practice in Australia, she came to Cornell University in 1993 and became board certified in veterinary clinical pathology in 1995. Her clinical pathology interests include hemostasis, focusing on the role of tissue factor in thrombosis and neoplasia of animals, hematopoietic neoplasia, particularly acute leukemia, hematopoietic disorders such as immune-mediated hemolytic anemia, and validation of clinical pathology tests.

We will be passing around a CE sign-up sheet, so if you're interested in doing this first CE, just sign your name to the sheet.

Dr. Tracy Stokol: Well, good morning, everyone, and thank you for coming to my talk. So this talk is going to be kind of a bit of a mix. Clinical pathologists love hands-on slides. We love to look at slides, so you're going to get to look at some digital images which we'll provide to you as, hopefully – they're huge files; they're like 180 megabytes apiece – we'll hopefully provide to you as part of this conference, and you can download the free software, and there are instructions for downloading the software to view that. Unfortunately, I'm a Mac girl and it doesn't work on the Mac, which is annoying, but most people are PC people.

So, for me, blood is a window into the animal. Animals can't speak for themselves, so we let their blood do the talking for them. And so really what we need is to get the best quality blood smears so that we can learn the most from what their blood is trying to tell us.

Okay, so the outline of this talk is just basic principles, the blood collection. And, obviously, we all know that, for hematology, we're going to connect blood into a purple top or EDTA tube. And then I'm going to go through techniques for blood smear preparation because really making a good quality blood smear is key to interpreting and seeing all the findings that you should be seeing.

And then I'm going to go through a really systematic way of how we clinical pathologists look at a blood smear and how we teach our residents to look at blood smears. And then just common things to look out for which can really trick you, and I call this "telling fact from fiction," and I actually think this is one of the hardest things that we do as a clinical pathologist, is knowing what to ignore and what that we need to pay attention to, which takes time and practice.

Okay, so why are we looking at a blood smear? Well, as I said, it's the animal speaking for itself. But we can tell if there's an anemia and the cause of the anemia, such as this cat here, which has a toxin induced hemolytic anemia with the centrocytes, which are these red cell shapes right here, and the Heinz bodies, which we all know some cats can have normally without any evidence of anemia, but these are pretty huge and this is a cat with acetaminophen toxicosis.

We can see evidence of inflammation, neoplasia, and actually I collected blood samples of the shelter mets and crew collected blood samples from cats for me from the shelter for our reference intervals now probably 10 to 12 years ago. It may be coming up again unfortunately, because it's a lot of work. And I found a cat with leukemia, as a clinically healthy cat apparently, but that had leukemia. So you will see these occasionally through the shelter. Luckily now, with feline leukemia virus kind of not as prevalent as it used to be, we don't see these frequently. I mean we like seeing them, but it's not good for the animal, of course. Don't let your clinical pathologist get excited about something in a blood smears. It's usually not good for the animal.

This is an infectious agent, *Babesia canis*. Haven't being seen really too much of this up here, but we certainly see *Babesia gibsoni*, and we're starting to see those lovely tick-borne diseases with global warming. So we can certainly look for infectious agents.

So the main thing is to always do everything with fresh blood. So the minute you get the blood, seriously just make a blood smear. It's really easy to do. I'm going to walk you through our technique and just some tips to help you make a really good quality blood smear. And why do we emphasize this is because you get artificial changes that occur with storage, and this can start occurring within about four hours of collection and is going to be accelerated if you keep the blood in your pocket, close to your body or – so you want to keep the blood, obviously, at four degrees Celsius.

So what we're seeing here is a fresh blood smear and this animal – and it is not a cat – has an inflammatory leukogram because there is a band neutrophil here and here's the seg, and here's some lymphoid cells. And this is actually a cat with leukemia because these are neoplastic lymphocytes. But, here, this is the blood that we got smears of, but the veterinarian had prep smears, which made our lives a lot easier because here is what I would call a false band. It could be a real band. Without having seen that in the first smear, I would be reluctant to say this is a true band. Plus you now have Döhle bodies forming, which – so you get even toxic change forming in all blood smears.

So always make a blood smear. And here you have cells that are dead, cells that are ruptured, and cells that I would have a real problem identifying. And so we would have had a lot of trouble making a diagnosis of leukemia with a secondary inflammation in this cat without having a fresh blood smear that the vet sent to us.

The other things that we see are dead and dying cells. This is an apoptotic cell. I have no idea what that was. I can guess it was probably a neutrophil in its previous life, and that's because it's got neutrophil looking granules, but who knows what it is. These are frequently mistaken for nucleated red cells when they just have a pyknotic nucleus. And, of course, we have a *[inaudible]* formation, and this is blood smear from a dog, and this can occur pretty quickly. Again, definitely within 24 hours you start seeing those changes and accelerated if the blood's not kept at four degrees.

Okay. So good hematologic technique, it's all about making a good quality blood smear. So we usually use a microhematocrit tube versus a posture pipette that I'm showing here, because then you can really control the size of the drop, and it's all about the size of the drop and being comfortable. And the thing is, glass slides are cheap, relatively. Practice, practice, practice until you get it. And once you get it, you will always have it, hopefully. It's like riding a bike – so they tell me because I don't ride a bike very well. But, really, once we tell you the tricks of the trade of making a good smear, you can really do it, and it's practicing.

So this is actually on our website, which is here, so you can access this at any time and you can see instructions that are written. But, essentially, you want to put a drop of the smear near the frosted end, and how this illustration of the person holding the blood smear at the very end is critical because you want to get your hands out of the way, but you also want to stabilize that blood smear that you're making the smear on. And you can see that the hand here is really out of the way of the spreader hand. You put the blood – the spreader slide in front of the drop. You pull it back into the drop so that it spreads nicely along the edge of that spreader slide. And then with a smooth, confident, firm stroke, you go forward and you create a beautiful looking smear just like this.

And we would do that probably eight out of ten times, and two times we're not that happy and we'd redo it again – well, probably one time out of ten. And you would need to practice to get this. But this is a really beautiful slide because the feathered edge is three-quarters of the way along the slide, so if you send it to us, we have an automated stainer and it won't stain anything there or there, so it's really nice if the entire smear is in the first two-thirds of that glass, beyond the frosted end, which is right here.

So what are the things that I see people doing? I see sometimes people holding it – and this is how I used to do it till the techs in the lab told me how to do this better. And I used to hold with my hand like that on that edge, and I would come towards my hand. I'd go, "Oh, my God, I'm going to hit my hand and hurt myself, so I'm going to lift up." And lifting up is not a good idea. So this way you can keep going all the way and just keep hitting off the edge of the slide. And that means you don't lift up, because lifting up means you don't get a feathered edge.

The other thing that is key is this angle. This angle is really important. So that's about a 45 degree angle, roughly, to the slide. That's really key. If you increase your angle, you make a shorter slide. If you decrease your angle, you make a longer slide. So we pretty much 45, 45, 45, and you want to maintain that 45 degree angle entire way you go across the smear. So what I see particularly students doing is they start off great and then they go like that as they go along, and that's actually going to make a really long slide. So keep that angle and really smooth motion and even contact.

So I like to say it's contact. You don't want to be pressing down with that spreader slide. You just want to be contacting. You want to feel a glass contacting from the spreader slide as well as your blood smear slide. And, really, if you follow those tips and you don't make too big a drop of blood – you want a drop of blood that's around five millimeters wide, just like a small drop of blood. If you have a huge drop of blood, which you

obviously will get often with a posture pipette, it's going to be too big to handle. You're going to go way off the edge of the slide because it's going to be too much blood.

And if you have too little blood, that can also really affect your results because you may start to make the wrong decisions about is this animal anemic, has this animal got a normal white count, what the platelet count. And we can actually look at a blood smear and go, okay, there's way too little blood here. And I've got just this blood smear to exam. I don't have counts because we just had the smear. All bets are off. I'm not going to even estimate anything. And we provide estimates if we just have a blood smear of pretty much everything.

And, really, just practice. Glass slides are cheap. Also, you want clean, good quality glass slides. If you pick them out of that box and they're all stuck together and you're scrooching them apart, and you're putting your hands all over them. Avoid your hands; they're greasy. And that really impacts how you can make a good blood smear. So get clean slides. I wipe slides on my clothing. Hopefully it's clean. I haven't seen my horse that morning. So, essentially, nice clean slides free of lint and just make everything easy for you to make a blood smear. You want good equipment. And if it doesn't work, you can always blame the glass slide.

Okay. Then the next key is you've made a good quality slide. You need to stain it. And I'm going to talk a little bit more about staining in the hematology lecture, but most of you – in the cytology lecture – but most of you work with Diff-Quick, right? You usually use Diff-Quick? Any other rapid stains you guys use? No. So most of the slides I'm going to show you here are unfortunately a Wright stain, which is very different from Diff-Quick. And I was looking yesterday for Diff-Quick blood smears images, and I'm going to take some because we didn't have any, because it does make it a little more challenging.

But, essentially, once you've stained the slide, you want to develop a systematic way of approaching the slides so you don't miss anything. And you want to be thorough and you want to be consistent. And the very first thing you do is you check the quality of the smear. So these are some images of the hall of shame, which are slides that we've received at Cornell. And so you guys aren't going to make a slide that looks like that, okay?

Okay, we do have kind of attempt at a feathered edge, but it's hitting the frosted edge here. And this is really, really, really thick. So this is going to be really hard for me to find even a thin area for me to look at where I can actually see what's going on. So this slide would be tough for even us to examine.

This slide, someone tried twice on the exact same slide. And, you know, but this one we can deal with. We can go – right there is where I'd go, or maybe right here where it's thin enough to deal with. And you can see this is just a huge drop of blood that they put on that slide. And, of course, that's perfect, which is what our techs make in the lab.

Okay, so then what I do is at low power I look at the quality of the stain, because this is your chance to make it a better stain if it's not quite right. So the red cells should be red, and I like to see the white cells popping out at me so you can kind of see their nuclei because they're purple versus red on over here. And Diff-Quick they're not going to pop as much, but you should still see them pretty easily, so if the red cells are too blue, it's going to be really hard to see those white cells. And if it's under stained, you're not going to see very much at all. So look at 10X, and then you can fiddle with the stain to make it bluer or maybe a little more red, if you can. Usually, with Diff-Quick, if you've over stained it, you're done, essentially. So make more than one slide and you can stain up another one.

So also know the limitations and also the virtues of your stain. You know, clinical pathologists and academics hate Diff-Quick and there's a variety of reasons for it. But Diff-Quick has its virtues; they're just few in number. So Diff-Quick tends to make nucleoli more prominent than they should be, so you can be misled. We like a lot of colors. We like our purples. We like our magentas. We like our pinks. We need red, and we like to kind of demonstrate all those colors in our write-up. But Diff-Quick's kind of purple and purple. It's light purple, dark purple. So that kind of makes it a little more challenging.

We really look for chromatin pattern, looking for neoplastic cells in blood as well as in cytology, and Diff-Quick kind of ruins the chromatin patterns. That makes it tough. As you all know, it will not stain up mast cell granules, and I'll come back to that in a sec. And lymphocyte granules – and this is actually a granular lymphocyte in a cat. If I see this in blood, it means it's got lymphoma – this particular lymphocyte. There are others that it doesn't mean that. But this particular one means it has lymphoma, and unfortunately these granules are just like mast cell granules. They do not stain with Diff-Quick. So you can miss this diagnosis. It's a particular nasty, nasty lymphoma and we can actually give you a clue from blood that it's there, if we see these cells.

But Diff-Quick is actually awesome for distemper inclusions, which hopefully you guys won't see too much, but potentially you could. And this is our lovely stain, our Wright stain here, and there's this really pale inclusion here, and there's another pale inclusion here, but you're kind of

guessing. Whereas you put on Diff-Quick and you can see these lovely pink inclusions in these red cells there and there, and in these neutrophils there's actually a couple of them. So if we get a history of suspect distemper, we Diff-Quick it because we have Diff-Quick in the lab.

Then at a 10X power view you want to check the feathered edge, and I'll tell you why you're checking the feathered edge, and right here is the feathered edge. And what I do is I check that entire feather, except I usually start here and then I go there and then I have to go back, which is kind of annoying, but it's just the way I do things. I mean I've been doing this for God knows how many years – too many – and I still start here versus over there for some reason. I like to find the tip of that feathered edge to start the blood smear.

So what are we looking at the feathered edge? We're looking for platelet clumps, and this is the platelet clumps which we see all the time in cats, as you know. This is also in this pale, fibrillar material, which is actually fibrin, and sometimes this fibrin can mimic a platelet clump, so you actually think it's a platelet clump from a high power, but it's not. And, as you know, platelet clumps are going to affect our estimate of the platelet count. And we obviously are going to look for thrombocytopenia when we're looking and estimating platelets from a blood smear when we're looking at a smear.

We'll see microfilariae out there. And microfilariae are usually used to behind the feathered edge, actually, rather than at the feathered edge, particularly when they're low in number. We can also see other infectious agents such as some of these protozoal diseases that we don't tend to see up here so much, adding leukocytes in the feathered edge. So we always try in our resonance to actually look within the cells at the feathered edge, not just at 10X.

We're also looking for large things, so we often see reactive lymphocytes which we get to review. If our tech sees some big blue cells, they'll give it to us to review to make a decision as to what they are. And this is a neoplastic red blood cell in a feathered edge of a cat with feline leukemia virus infection. We see other neoplastic cells such as acute leukemia cells or lymphoma that may be out there. We can see megakaryocytes out there. The first time I saw it, I was like, "Oh, my God, what's that big cell? This is diseased." And then I'm like, "Oh, God. You're an idiot. It's an akaryocyte." And that's just normal and that's fine, but it's like this huge, multi-globulated, telling me like, "Oh, my God. It has to be cancer." And it's not.

And then we can see macrophages, which we call histiocytes, and this one's erythrophagocytic. They're not always erythrophagocytic, but this

was a dog with immune-mediated hemolytic anemia, so you can see these. We also see these circulating in dogs with histiocytic sarcoma, but they're usually more reactive than neoplastic. And, of course, we see mast cells, and these are the variants in mast cells that we can see at the feathered edge. See it more commonly in animals with inflammation or cancer than we see it in animals, and not necessarily mast cell tumors. Dogs with allergic skin disease can get it. Cats usually means neoplasia, but not always. Whereas the dog I usually think of non-neoplasia before I think of neoplasia and I see mast cells in blood.

So then you find the monolayer, and the monolayer is going to depend on the hematocrit. If the animal's anemic, you're going to make a longer smear. If the animal is polycythemic, the smear's going to be shorter. But, essentially, the monolayer is usually one or two fields back from the feathered edge, and that really is the monolayer area right there, before it starts to get too thick.

And what you want to see is you want to see the red cells disperse very nicely. And if the animal's anemic, they're going to be way more dispersed than this. And you want to be able to see the leukocytes really nicely spread so you can identify them very easily. And that's a neutrophil and that's a monocyte, just by a quick look. That's really nice. You can see platelets and everything's there. And this is a blood smear from a dog because it's got *[inaudible]*.

This is too close to the feathered edge, so it's too thin. And what the cells do there, they distort very easily, so they're hard to identify. And this is way too thick, where who the hell knows what these cells are. You know you're going to be making life difficult if you look there, and that's one of the biggest mistakes students make when they first looking at blood smears; they're always like way too far back. So they ask us what the cell is and we're like, "Nah, we don't know, and now you need to be here." And they're like, "Oh, my God. Look. I can see." So that makes a big difference.

So then we go – once we've found the monolayer, I actually scan the slide, and I've learnt to do this over the years. I scan just to kind of look for things that shouldn't be there, and I've found neoplastic cells just on scanning that aren't at the feathered edge. I've found mast cells just in scanning. I've found other cells that I kind of want to take a closer look at. So I kind of just do a quick scan before I just jump down at the feathered edge.

But then what you do is, once you've found the monolayer and you've done your scan, then we have 50 oil immersion, which I love to death, or 100 oil for the fine detail. But if you don't have oil, you can use 40X, but

you are going to miss stuff at 40, and you need to add a cover slope if you're looking at – or using a 40X lens.

So what do we look at a high power? Well, we've got all those cells to look at. We've got white cells, including doing a differential cell count. And this is kind of how I tend to do it. You can go back and forth like that in the monolayer. This is getting a little too thick here. But you can just figure out a system. I usually go there, there, there, there. That's just me. But there's no one perfect way to do it. Find a technique that works for you. We do a 200-cell diff if there's more than 20,000 to 30,000 – I think it's 30,000 cells in blood. And, obviously, if you have a very leukopenic animal, you're going to be struggling to reach 100 cells, and you won't be in the monolayer either. You'll have to review the whole slide.

Then we look in detail at their features. And I'm going to just highlight a couple of things that we look for. Red blood cells. Obviously, we're looking at their features and platelets number and morphologic features. But what do we also do from 10Xs? We estimate a white count and the R formula. They don't always work that great, so we just teach our residents to estimate the white counts by looking at the feather, by looking at the body of the smear. And you can only estimate a white count if there's no leukocyte clumping and if there's even distribution of the cells in the smear. So if they're all at the feather and there's none in the body, then all bets are off.

But we do this because we occasionally get samples where the sample's clotted and we're trying to give the vet the most information that we can, so we'll estimate a while count, estimate whether the animal's anemic or not, based on the density of red cells in the monolayer, and also estimate a platelet count. And, of course, if you make a thin blood smear from too little blood, all those estimates are going to be way off.

Okay, so what are we looking for? With the leukocytes, we're obviously looking for a left shift, and this is blood from a cat with a severe inflammatory leukogram. This is a neutrophil, because it's nice and segmented, but it's got toxic change which is diffuse cytoplasmic basophilia as well as this indistinct vacuolation. This is a beautiful band with lovely toxic change, including *[inaudible]* in these Döhle bodies. And this is between a band and a seg, so this series more of a band in here, but it's too constricted. So greater than 50 percent constriction is what we call a seg.

There's always going to be subjectivity, but I think we'd all recognize that this cat has got pretty toxic neutrophils. This would be moderate to severe for us. And it's got a left shift, so we'd all interpret this as an inflammatory leukogram. Whether we get 10 bands and you get 15, it's

not going to make a huge difference as long as you recognize those key features in inflammation. And certainly you are going to be looking for that in shelters if you have sick animals. You're going to be wanting to identify bands and you're going to be wanting to look for toxic change.

If you think you're seeing severe toxic change, you should have a pretty decent left shift. Mild toxic change, particularly cats, can have a couple of Döhle bodies even when they're healthy, so we let them have a couple of Döhle bodies. So if you're seeing what you think is toxic change without a left shift, you're probably not seeing toxic change. That's kind of our general rule. You can, however, see a left shift with no toxic change. Okay? So… And every rule is made to be broken.

So we also see some big blue cells, and then the question is are they reactive lymphocytes or are they neoplastic cells. And neoplastic cells could be myeloid or lymphoid in origin, so we just give them a generic term of blasts if we see them. And, essentially, this just is a compilation of images from three patients with leukemia and three patients with reactive lymphocytes. And this is just to show how difficult it is to distinguish between them. And because I took the pictures, I know what they are, so that's what they ended up being in these animals.

But you can actually see reactive lymphocytes alongside neoplastic cells in blood, so you can actually see both of them in the same sample. And this is probably one of the hardest distinctions you have to make, and I would recommend you always verify what you think because this could be the life or death of an animal if you're going to call it cancer, particularly hematopoietic neoplasia such as acute leukemia. You don't want to be misdiagnosing that.

Then, obviously, we're going to look for inclusions such as infectious agents, and we're definitely seeing way more of this. This is *Anaplasma phagocytophilum*. This is actually in a horse, but we're starting to see this more in dogs. And in the dog this could be a *Ehrlichia ewingii* or *Anaplasma phagocytophilum*, which is now – the *Ehrlichia ewingii* is picked up on the 40X plus but not the old 40X test. And associated hematologic findings, the most common hematologic findings you're going to have is thrombocytopenia and maybe anemia. But thrombocytopenia is pretty classic for these infectious agents.

Other things that we see are infections agents, obviously, are *Mycoplasma haemofelis*, which we all know what it looks like. You can see abnormal granulations, and this is blood from a Birman cat and you can see these lovely pink granules in here. Granules could be a whole variety of things. They could be mast cell granules in an animal undergoing acute

anaphylaxis. They could be a storage disease, which is incredibly rare. If you think you see it, send it to me because they're always fun.

This is actually an inherited abnormality in these cats, and it mimics toxic change, but it is of no known pathologic relevance. It's just kind of cool. And then we can see, as you saw, erythrophages and actually hemosiderin within neutrophils and macrophages in blood or monocytes in blood in an immune-mediated hemolytic anemia, usually when it's intravascular hemolysis.

So what do we look for in red cells? I can give an entire lecture on just each of these topics, so this is just a really brief overview. So the very first question I'll ask our residents is, if they say there's an anemia, the next question is it regenerative or not. So what are we looking for with regenerative anemia? And you can start getting a feel for this at 10X. We look for polychromasia in particular because you can see nucleated red cells in animals with heat stroke, where there is no regeneration there. You can see Howell-Jolly bodies in animals with a whole variety of conditions – splenic dysfunction, corticosteroid therapy.

We obviously look for anisocytosis because immature red blood cells are bigger than normal red cells. And you may see basophilic stippling, which you also know can be seen in dogs with lead poisoning, as well as nucleated reds that are disproportionate to the degree of polychromasia anemia. And this is an anemic dog, and these are polychromatophils here, here, and here.

And then you go to say, "Are there enough polychromatophils for the degree of anemia to make this a regenerative anemia?" And in this dog, it's got plenty. Even though it's pretty severely anemic, as you can see by all the white space between the cells, there's at least eight to ten percent polychromatophils, which would be roughly an eight to ten percent retic count, and so this dog would be regenerative anemia. He has a beautiful band. Here's a seg and a seg. Then we look for abnormalities in shape, and you can see some keratocytes here and – schistocytes; actually it's more of a schistocytes than a keratocyte. Here's a schistocyte there too, and there's a Howell-Jolly body on that cell there.

And so the next thing I'm going to say: okay, the animal is in anemia and it's regenerative. Can I identify cause on this blood smear so I that I can tell clinician what's going on? And, unfortunately in this case, I can just see some fragmentation, so I'd be worried about potentially concurrent DIC or vasculitis. But this is pretty non-specific, so I can't give a specific diagnosis. And this actually was just a dog with a blood loss anemia because of being hit by a car. So, actually, it didn't – I wouldn't have seen anything. And, of course, we can't tell if the dog's bleeding from a blood

smear, but you can tell by looking at it. We look for abnormal color, which is hypochromasia, for iron deficiency. And, obviously, we look for inclusions and infectious agents, Heinz bodies, anything like that.

So platelets – what we do in the monolayer of the smear to estimate it, you need 100X oil immersion fields. You can probably do it at 40, but we never do it on 40 because truly identifying platelets can be tough just at 40. And one platelet per 100X oil immersion field is equivalent to 15,000 per micro liter. So if you're seeing one platelet per field in a dog, it's a severe thrombocytopenia. Two would be 30,000, and 1 is 15,000. And if you're not seeing any and there's none out at the feathered edge, then you've got a pretty severe thrombocytopenia, as long as you don't have any in the sample that's impartially clotted.

So these are different samples from different animals. This is a dog here. This is a cat with a really huge platelet, which normally cats have big platelets just to make our lives more challenging. And here's a lovely fibrin clump, which can look like degranulated platelets. And, of course, we look for inclusions in these, and this is actually an animal with *Anaplasma platys*, which is an *Ehrlichia* that – or bacteria – that infects platelets and causes a thrombocytopenia.

You can see this is a dog and that some of the platelets are huge, and that is a suggestion that the animal is potentially producing more platelets. The big platelets are like big purple red cells. They're like polychromatophils. They usually mean a regenerative response, but they can also mean abnormal platelet production, so there's something wrong with it even – so it's not necessarily a regenerative response to a thrombocytopenia. It could be a platelet abnormality, so myelodysplastic syndrome or leukemia. And the Cavalier King Charles Spaniels have lovely big platelets because of a tubulin defect, so defective production.

So then we want to tell fact from fiction, and this is some of the hardest things that we have to deal with. So you're not going to make your life easy if you've under or over stained the slide, because it's going to be really hard to see things that you need to see. Stain precipitate looks like bacteria, particularly Mycoplasma haemofelis, and this is a huge chunk of it, so we're all going to say that's way too many cells to be Mycoplasma. But for some reason – I don't know if you guys have seen this – stain precipitate hangs around the edges of the red cells. It likes to do this, so it likes to mimic *Mycoplasma*. So it's kind of irritating.

This was bacteria that was actually in the Diff-Quick stain. If you're seeing a bacterium and the animal should have a severe inflammatory leukogram and trying to die in front of you. So if you don't have a

severely sick animal and your inflammatory leukogram and you see a bacteria, you've probably added it there. So keep your stains pretty clean.

Water artifact is the most challenging, and this is blood from a cat. And look at these things on the edges of the red cell. This is all water artifact. This cat did not have *Mycoplasma haemofelis*. It's a little bit more refractile than *Mycoplasma*, and every time a clinician has come down and said, "I think there's *Mycoplasma*," it's usually been either stain precipitate or water artifact. And, of course, *[inaudible]*, which is great. But, you know, in the old days, you just rallied on. And then, for us, we have issues if there's too much EDTA – cats that you can't get blood from. It tends to cause artifacts in our results that we send out because it causes changes in the red cell volume.

Some artifacts we can't do anything about. Those we can do things about. The artifacts you can't do anything about is an animal that's lipemic because of disease. It distorts the red cells and you get rupturing of the white cells. And intravascular hemolysis, this actually is an artifact that happened. This blood was mailed in during a real cold winter. I think it was below freezing for two weeks here in Ithaca, and the blood froze in transit, and that's kind of what happened to all the red cells. So those are really difficult to examine because these are all ruptured red cells and there's a platelet here, and even the leukocytes tend to get a bit trashed.

Okay, so those are just some useful resources. We're revamping our ClinPath teaching site and it's going to be launching August 2014. It's http://www.eclinpath.com, so easy to see it, where we have a lot of this information. And then on our website with the Animal Health Diagnostic Center, we have all the things I pretty much told you about – making your blood smears as well as that image.

Okay. So I'm going now go over some digital slides and we're going to look in there and try and figure out what's going on with these animals, so this is going to be a question and answer type session. But before I do that, do you guys have any questions? Yes?

Question: How do you recommend *[inaudible]*?

Dr. Tracy Stokol: How frequently do I recommend changing the stains? You know, to be honest with you, what we do at Cornell is we've had the same stain for probably three years because we hardly ever use Diff-Quick, right? We only use it if the slides are broken. But if you're using them pretty regularly and you're putting them in jars that are open to the air, I would recommend at the end of the day you put them back in their containers. Okay?

And if they're in frequent use, I really don't know if I can answer that. I think you're going to need to judge. If you're getting a lot of stain precipitate, I would clean out your jars because it's usually the gunky stain, and those can be pretty darn hard to clean out. If it starts to really get down because it's evaporating, it's really going to affect it, which is why I recommend putting it back.

And, I don't know, the Diff-Quick that we've had has been around for probably three years. I think we recently changed it because it we were really unhappy with it, so it might have been around for five years and we just recently bought some new one. So I would probably, if you're using it every day, I would change it every 6 to 12 months, depending on the frequency of use. As long as you put it away and you don't keep it in those jars where it evaporates, you're not putting really disgusting, dirty slides that liberate bacteria in there. And if you have a problem, just change it. That's the thing. Yeah?

Question: For making fresh blood smears, what's your time window?

Dr. Tracy Stokol: So making fresh blood smears, what is your time window? I would say we know we can see artifactual changes within four hours of collection, so I would say the sooner the better. But I think you've got at least an hour to two hours, particularly if you've kept it cold. Now, you don't want to refrigerate the slides because they'll lysis when water accumulates on them when you take them out of the refrigerator. And those slides are good for a month at least, once – and we don't heat fix. We just take a hair dryer and rapidly air dry them.

And when you've got really humid 90-degree days in the summer and there's a lot of humidity in the air, we found that we get this staining artifact where the red cells are distorted, and we air dry with a hair dryer all our slides. And I've burnt my hand putting slides on that nozzle, but you put it on the back of the slides. You aren't going to hurt the cells. You'll hurt your hand but not the cells. So we have hair dryers everywhere in our lab. Yeah?

Question: *[Inaudible]*. Is there a way to *[inaudible]* adequate *[inaudible]*?

Dr. Tracy Stokol: That's a great question. So this is something we struggle with all the time. So your machine count – and there's different machines. If you guys use the QVC machines, when they clumps it usually overestimates the count, most like the Hemo Vets and all those other point-of-care tests. They tend to underestimate a count because they're excluding the big platelets. They don't see the clumps as a platelet. They see it, actually, in some as a white cell, so they could artifactually increase your white count.

And so what happens if you get a low count and you're checking your smear? That's awesome, that you checked the smear to verify that count. We verify every single count from a machine. We do not trust the machine. I know that sounds terrible, but we verify everything. We verify the white count, the red count, everything by looking at a smear. I trust my eyes more than I trust a machine, to some extent. Sometimes the machine is right, though.

So what we do – so this is something I've had to really – we've had to really educate most of the technicians in most of the places I've worked. The assumption is that if they clump they're adequate. That is a totally wrong assumption. So it depends on the degree of clumping, so how big are those clumps, how many platelets are in those clumps in each, and how many of them are dispersed around the slide.

So you have a couple of small clumps of maybe 10 to 15 platelets. We don't count them. I'm just estimating here. You have three to four platelet clumps and there's only very small clumps, and then you go in the body of the smear and you see nothing, you've got a low platelet count, regardless of those clumps. Now, what it is, your guess is as good as mine. It's probably not 15,000 per micro liter, but is it 45, is it 70, is it 100? Who the hell knows, right?

If you've got a really huge platelet clump and you're seeing still quite a few in the body, it's probably adequate. And there are some that we go, "You know, the platelet clumps aren't that big; I'm not seeing that many in the body of the smear. I'm just not certain." And we go low question mark because, as I say, it could be normal or it could be low. And the reason why we do that is because I've seen it assay adequate one day and then the next day it's low. And then I go back and I look at the smear and I'm going, "It probably was low," and I'd rather it run on the side of being cautious than saying "yeah, it's definitely fine" when it may not be.

So those are challenging for everyone and I think it really just depends on that. There's also studies that have shown that, making ten different blood smears from the same sample, you can have different sizes of clumps and different numbers of platelet clumps in the smears across those ten. We look at one slide and we make our judgment from there. So you do the best you can. But don't assume the count is adequate based on the clumps, and also make sure they are platelet clumps and not fibrin, because that can then make a platelet clump. Great question. Any other questions?

Okay, well, I'm going to now ask you guys the questions because you're going to help me work through these blood smears. And, hopefully, this will work.

So they're irritating. So we're going to do – I think I want to do *[inaudible]* cure first. I want to do cure first. Yeah, I want to do cure first. Okay. So this is this program that we told you about, and it's pretty cool. This gives you the overview of where you are in the slide, and this gives you a magnification of that image. And that's annoying, so go away. Go away. If I can't go away, I'm just going to ignore it, so hopefully you can too.

So this tells you it's a 4.2X image and this is giving you a snapshot of where you are. And you can kind of move that around just like you would be scanning a slide. So these images are huge, so unfortunately on this one we don't have a feathered edge. I do have a normal blood smear and I could go through that technique, but I figured that I would just jump to the cool stuff. But if you want me to do that, I can definitely do that.

So about an 8.3X is roughly a 10X *[inaudible]* magnification view. So I'm scanning this cat's blood, and you can see you can move it and kind of scan it. There's a nice big scratch, but that happens when – and then that's the edge of the smear. Because if we scanned in this whole blood smear, it would take about half an hour and the size of the file would be about 400 megabytes.

So, okay. So there's quite a few white cells in here, so there, there, there, there, there. To me, that's quite a lot of white cells, so if I was estimating a count in a cat, I would say this is probably mildly increased. It's not 50,000 or 60,000. It's not huge, right? But if this is the monolayer of this smear, it seems to be mildly increased. There's a little bit more white space in here than I would like, so I'd be wondering if the animal is mildly anemic.

And, now obviously, when I look at a blood smear, I look at it blinded. In other words, I don't look at the counts or anything first. And I make all these assessments and then we look and see if everything matches. And if it doesn't match, we go back and double check. It's a way to teach yourself. It's also a way of not focusing on something and missing something else, which I've certainly done, so I try and learn from my mistakes. So I would have estimated a high white count through there and maybe a mild anemia. Platelets, I have no idea from this angle.
Okay, so that's a 10X view. So 10X view we want to find near to the feathered edge, so this is getting to the feathered edge because I can see my white space. So there's a nice big hole that I want to avoid there, so we're going to come down here. I think you can. And you can zoom in by just clicking and by just holding the mouse down and going in, or you can use this button. And you can see how, in the lower right hand corner, there's a magnified image.

Okay. So let's go and look around and let's do some cell ID. Oh, I don't want to move in. I want to move up. Whoops. Too far out. Okay. So what do you guys think this particular cell is right here? So is it neutrophil or monocyte? I'll help you out. Neutrophil. Great. And is it a band or is it a seg? Okay, so I heard band and I heard seg.

So this is one that's in between. For me, this is like 50 percent constricted here compared to that bulbous end, but it's got a pretty smooth outline – depending on my mood on the day, honestly. You could call it a seg or you could call it a band, but I would probably call it a seg because it's 50 percent constricted, more than 50 percent narrower here than it is on the edges. But it's got a pretty smooth outline. Well, it's starting to ruffle a little bit, so, yeah, I'd definitely call that a seg. See how we have these little internal fights with each other? And, you know, I'm signing this out, so I'm responsible, so it's my call regardless.

So what do you think about –? Is there any other abnormalities in the cell that you're worried about, or is this a happy, healthy neutrophil/band? Think it's toxic? Yes, and I would agree with it – you; it is toxic. There is lovely Döhle bodies right here. There's cytoplasmic basophilia, which is this streaky blue stuff. And see this indistinct vacuole? It's kind of rarefaction in the cytoplasm. That's toxic change.

So what – one cell? Yes, I know you never make a diagnosis in one cell, but this is a pretty severely toxic neutrophil, so what do you think's going on with this cat? As far as white cells go. Great. Left shift and toxic change. So what's the process that we now learn about? So what's going on in the cat? What does left shift and toxic change tell us? Inflammation. So you're going to go and look for a source of inflammation, right? And here is another beautiful – this is actually between a band and a metamyelocyte because it's looking almost reniform shaped. A beautiful toxic neutrophil.

Okay, so what's this over here? Anyone know what these are over here? Okay, what's that there? And then that is a clump of platelets, right? So you can get platelet clumping not only at the feathered edge, but also in the body of the smear. And if you estimate the platelet count here, it's one, two, three, four, five, six, seven, eight. That's a bit low for a cat, right? Eight in a 100 oil X immersion field is going to be under 150,000. That's because I can multiple 15 by 10, so 80's about 120,000, which means this cat could be thrombocytopenic if there were no clumps at the edge.

But, you know, these clumps make it difficult to estimate a count when they're in the body of the smear, so that's why you don't only look at one section; you want to look at others. Here's another beautiful toxic band

and you can see lovely Döhle bodies. What's the cell here? So that's a normal red cell there, so this is more purple and slightly bigger, so that's a…

And so if I said this cat was mildly anemic, would you say it's trying to regenerate? Yeah, absolutely. We've got echinocytes. We've got what we call echino leptocytes, which are oval shaped echinocytic cells. You see that with liver disease, but not only liver disease. So this cat has a nice inflammatory leukogram. He's got some poikilocytosis. I'm not really seeing anything else. I did see one other finding in his blood smear.

And you can see as I'm scouting around – whoops, and I went too far over there – that there's really not a lot of platelets in each of these fields, so this cat is mildly thrombocytopenic. So he has a macrocyte, which probably corresponds to a punctate reticulocyte, so it was an immature cell. Here's a lovely toxic seg. And let's just zoom out and see if I can find something other than a seg. Yep, I can, so let's zoom in on that cell.

So those are *[inaudible]* toxic neutrophils there and there. And this cell is an entirely different cell, and it's looking kind of funky. And that's a monocyte over there because it's a different cell. But that's not an easy monocyte. This here is a toxic metamyelocyte, so the left shift's extending all the way. Anyone know what this cell is – round nucleus, small amount of blue cytoplasm? Lymphocyte. Great.

And band, band. Let's see what else there is in here. And there's – looks another toxic band, beautiful toxic band. This cat actually had way more bands than segs, so it had a degenerative left shift because there were more bands than segs, and I need to – okay, so what's that cell there? Dark, really dark pyknotic nucleus and kind of reddish purple cytoplasm? Nucleated red. Yeah. You guys rock. So it could be part of the regenerative response, but cats throw nucleated reds out when they feel like it. And the information could be enough to do nucleated reds out.

So now I'm in a bit thicker area of the smear, and you can see the red cells stacking up on each other. That's *[inaudible]* formation. It's normal for cat blood. It could be excessive in this cat. I think it looks okay. But if it was present and we did call it, it would indicate high globulins, which would not be surprising considering this cat has an inflammatory leukogram.

So what did Kyra have wrong with her? That's Molly, which we'll do next. So Kyra had – oh, yeah, she was a surgical case and this was done post-surgery. She did well after initial surgery for a foreign body. She did well initially, and then two days after surgery she spiked a fever and was looking depressed. And they tapped her fluid, her abdominal fluid,

because she *[inaudible]*. She had aseptic peritonitis. They went back in, found devitalized bowel, and she did great after the second surgery.

So the inflammatory leukogram really told them there's a serious issue here, as did the septic peritonitis, but that was the first thing they would have looked at, and they would have probably STATed this blood smear. So that tells us look for a source of inflammation, which with her was devitalized gut.

How much time do I have? Five minutes. Do you want me to do another case in five minutes, or do you have any questions about that case? Another case? Okay. Yeah.

So there's three cases that we guys were going to give you. One's Kyra. And, again, you can look at it, and I will send you the case information on these so that you have it. Close. Why are you not closing now? Not responding. Oh, you got to love this. Close the program. Down to Cancel. I don't want you to check it. Thank you kindly. Go away. Molly.

Okay. So Molly is a dog. So here's the feathered edge. It's not the greatest feathered edge because it's kind of this raggedy feathered edge, so we would have to go up and down, up and down. But we would have rescanned the feathered edge and looked for anything that we saw there. So let's go back, and this is where the monolayer is. So, just from there, do you think Molly's anemic or not? So if this is – actually, we're pretty high up, so this is a 10X view. So do you think Molly is anemic? Yeah. And pretty severe anemia, right, because there's an awful lot of white space.

So next question: do you think Molly is regenerating? This is 40X view. Do you –? Yeah, great. Do you see beautiful polychromasia? And there's quite a lot of it, so she has regenerative anemia.

Now the next question is can we identify a cause, so now we're going to look at the red cells and start to see are there any abnormalities other than these polychromatophils. Spherocytes. Yeah, I heard it. Great. Beautiful, small, round cells and tons of them. So these are spherocytes and there are a lot of them, and a lot of spherocytes give you the diagnosis of –? IMHA. Now, a few spherocytes does not mean IMHA, but a lot, it's IMHA till proven otherwise.

Now, we haven't finished with Molly. There are quite a few other abnormalities in her. So what are these cells here? Anyone know what these are there? Okay. So Molly had brown urine. So what do you think

is going on with her? Intravascular hemolysis. So these ghost cells support it.

Now, you're going to see ghost cells in almost every blood smear you make, just out of artifact. But when there are this many, plus her plasma, when you spin that down and you look for a hematocrit or a *[inaudible]* volume, it's going to be red plasma. So the brown urine, presumptive hemoglobin, *[inaudible]* until proven otherwise. So, obviously, we all know intravascular hemolysis is the prognostic indicator, and I wonder if Molly was a Cocker Spaniel because they love to intravascular hemolyze.

Okay. So we've got a diagnosis, but we know IMHA can be secondary to infectious diseases, so we would always look for Babesia, we'd always look for mycoplasma. Babesia more than mycoplasma, particularly with intravascular hemolysis, because mycoplasma is an epicellular parasite. It doesn't rupture the red cells. But Babesia ruptures the red cells when it replicates, causing intravascular hemolytic anemia.

So now we're going to look at the white cells, so that's a neutrophil, right? Then this is also – what is this? It's a neutrophil and it's got this little, indistinct vacuolation in its cytoplasm over there. And, to me, it's looking a little toxic from here because it seems to be bluer than it should be. It's got maybe some discrete vacuolation. When we see these discrete, clear margin vacuoles in the cytoplasm that could be an artifact. But when it's more indistinct, then it's definitely cytoplasmic vacuolation.

Here's a band. I would definitely call this a band. Here's beautiful ghost cells. So she also has an inflammatory leukogram. So what do you guys think this is? That's what we call a smudge-ocyte or a basket cell. In other words we don't know what it is. And it's not an intact cell, so who knows what it was in its past life. And this guy here looks to me like it's a lymphocyte. And here's a Howell-Jolly body on there, which is kind of mimicking a Babesia gibsoni, and what's this cell here? That's a nucleated red blood cell.

So you guys are pretty good with your – and you can see here that there's way too many leukocytes, but also our eye and the machine is going to count nucleated red blood cells as leukocytes, so you need to correct the count. If you have a lot of nucleated reds, and she had 42, I think, nucleated reds, to 100 whites, but she still ended up having leukocytosis.

And this cell here is – well, we'll just zoom in on it. And that cell here seems to be a totally different lineage from this cell, so what do you guys think this is? Pleomorphic nucleus. A pretty big cell. Monocyte. Great.

So we have one more thing to do because we haven't been consistent. What's the last cell lineage we have to look at? Platelets. And what do you guys think? They're kind of a little low, so this dog was also thrombocytopenic. Actually, these hemolyzed red cells get counted falsely as platelets by analyzers, so that's an artifact that we look out for. And, if necessary, we would cancel accounts and give you an estimate from a smear, which is why learning how to estimate is really important.

So she was just a typical IHA. Well, not typical, but an intravascular hemolyzing IMHA with an inflammatory leukogram, which they often have. And she was also concurrently mildly thrombocytopenic, which they can concurrently be.

Okay. So I think that's all I have time for. Took way too much longer on the preamble. I'll try and speed up this afternoon. But you do all have these slides to play with. As I said, you can download this software. It's going to come up with an irritating sign-in every time. Just ignore it. Any questions? Okay, great. I'm sure you're all starving, so you can go for lunch.

[End of Audio]