BBGuy Essentials 028:
What the DAT?! with Sue Johnson
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Joe: You are listening to the Blood Bank Guy Essentials Podcast, Episode 28.

Sue: We know that in several studies that have been done and I did one of them actually a long time ago that about 1% of blood donors have a positive DAT. I mean they're perfectly healthy, right? Their hemoglobins are fine and they're donating blood. You know, so that's one of those where you just say, "What the DAT?"

Joe: Thank you for that.

Sue: You're welcome. I had to work at it.

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Joe: Well, hello and welcome to the Blood Bank Guy Essentials Podcast. My name is Joe Chaffin, and I am your host. You know, when I started this podcast in 2016, there were a few people in blood bank world that I thought, "Wow, if I could just get THAT person on the podcast, it would really, really help people." I am so happy to tell you that I have one of those "dream guests" with me today. Sue Johnson is here from the BloodCenter of Wisconsin, Marquette University, and the Indian Immunohematology Initiative.

Sue is just a champion immunohematologist, and I have heard her speak many times over the years. She is awesome at explaining concepts, and in fact I've invited her here today to discuss the magical and wonderful direct antiglobulin test. Now that's the test that goes by several names. I'm sure you've heard them, such as the "Direct Coombs Test," or just the abbreviation "D-A-T" (that's how I tend to say it), or to some the actual acronym "DAT." Anyway, whatever you call it, people learning blood banking everywhere struggle with what it is, how to use it, and really what to do with the results. So Sue is here to be your guide to understanding the DAT, and I am so incredibly honored to have her. So here we go. Here's my interview with Sue Johnson.

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Joe: Well, hi Sue, and welcome to the Blood Bank Guy Essentials Podcast.

Sue: Hi. Glad to be here.

Joe: Well Sue, thank you so much for being here. I want to tell everyone about you, and I'm just warning you in advance, I am going to brag on you just a little bit. So everyone, Sue Johnson is the Director of Clinical Education at

the BloodCenter of Wisconsin, and she is also the director of the Specialist in Blood Banking program there. In fact, to tell you the truth, she is taking time away from her SBB students this morning to be with us here on the podcast. So thank you for that Sue. Her influence in teaching efforts extends way beyond Wisconsin as she's taught in numerous settings throughout the United States and around the world. There's a lot of things that she's done, so much that I really just have to give you the short version. I don't think Sue will mind that.

Sue was a winner of the Sally Frank Memorial Award and Lectureship at the AABB Annual Meeting a few years ago, and that honors her dedication to and her prowess in immunohematology. Sue has also served numerous roles within the AABB. In fact, she just completed a term on the AABB board. You may know her if you've ever attended the AABB annual meeting, by the name "Top Gun," for the sessions, the Top Gun sessions that she did at the AABB Annual Meeting for 10 years. Wow! Well, the bottom line is that Sue, she's gonna hate me saying this, but Sue is a "big deal," and I am just excited beyond belief to have her with me on the podcast today. So Sue, did I embarrass you enough there?

Sue: Yes. Good thing you can't see me right now.

Joe: That is a good thing. All right. Well, so Sue, I really like to, with every guest that I have, because so many people that are listening to this podcast are people that are just getting started in our field, I'm always curious with people that are as accomplished as you are, how did this start for you? How did you get involved in blood banking? What was it that kind of triggered your desire to be involved as a blood banker, and especially what was it that triggered your obvious love for immunohematology?

Sue: You know, that's a great question and it actually goes back to when I was in my clinical year as a medical technologist at the time and I had this amazing instructor who had tons of energy, tons of passion about blood banking that when I graduated, that's what I knew I wanted to be. I wanted to be a blood banker and I only looked for blood banking positions actually. Yeah, it was crazy and when I graduated, there weren't so many openings like there are today. So I actually held out a little bit to be a blood banker.

Joe: That's interesting. The magic of having a passionate mentor, I think is underappreciated, and that's really something that I hear a lot from people that enjoy what they do, is that at some point in their lives there was someone that really kind of spoke into it and said, you know, this is cool, this is fun, and that passion rubs off. Doesn't it?

Sue: Yeah. It sure does. It's amazing. It is and I was fortunate, you know, over the years to, you know, find those mentors along the way. So, and maybe I sought them out a bit as well, but it still, it definitely made a difference.

Joe: I think at this point you're serving that role for your students. That's fantastic. I mean, I mentioned to you off mic before we started this that I've heard you speak many times and your obvious love for immunohematology just comes roaring out of everything that I've seen you do and that's a testament to that passion that you have.

Sue: Oh, thank you.

Joe: Again, still trying to embarrass you.

Sue: You are.

Joe: I'm working on that. The whole "red face thing," we just don't have it. I don't have the picture, but that's all right. Well we're going to talk today with Sue everyone about a topic that is, I know near and dear to her heart as it is to mine. I will tell you by the way, Sue, that I had a similar experience. Back in my training days as a young pathologist, I had a couple of mentors at Walter Reed Army Medical Center, both in the physician side as well as the SBB side. Two SBBs that really, I say to this day, taught me most everything that I know about immunohematology and their passion shone through as well, and this topic, what to do with a DAT.

The way you title it when you give this presentation is, "Who DAT?" I love that! "Who DAT? Investigating the positive DAT." The Direct Antiglobulin Test is a test that is on its surface very simple, but there's a lot of complexities to it and a lot of, what I know you want to discuss with us today is, to take us through some of the details on what it means, what do we do with it, and how it all works. So the way that you break this down, when you do this, is into three ways or three different objectives, summarized in a way that I just love. So we're going to do "What's DAT?", "Why DAT?", and then at the end, "Who DAT?"

I can't even help myself. You have to smile when you say that. So let's start at the beginning. What's DAT? What is the Direct Antiglobulin Test, Sue?

Sue: All right. So the Direct Antiglobulin Test is, it's just an exquisitely simple test to do. From the point of view that we are taking red cells from a sample, from the patient directly or the donor, whoever it may be, and we're washing those red cells and then adding anti-IgG and anti-C3 to detect IgG and or complement binding to the patient's red cells. So it is a "direct" test because we're using their red cells and we're testing them with the antibody. Exquisitely sensitive test considering that the majority of DATs performed are done manually in a test tube method. You know, we know we can detect a small amount, I mean, probably in experienced laboratory scientists' hands, down to 100 molecules of IgG. It's pretty amazing.

Joe: Wow. So, I don't want to leave that before we really make sure that people have a really clear picture on what you just said. So let's go over that just

quickly, one more time. So these are red cells that you've just taken straight from somebody's body and all you're doing is checking to see that they have something on their surface, specifically IgG or C3?

Sue: Well, basically. However, what we do is we do take a fresh sample of the red cells right from the EDTA tube (the preferred sample) and then what we'll do is we'll take a drop of cells, wash those four times with saline and prepare cell suspension, wash it four times. You can do it two ways, but basically it's the patient red cells, washing them, and then directly adding the anti-IgG and anti-C3. It could be that it's a polyspecific anti-human globulin reagent that we use initially. Meaning that it has both the anti-IgG and anti-C3 on it and then if it's positive, we want to know is it IgG coating the cells or complement C3 coating the cells? If the poly's positive, then we're going to go ahead and test with what we call the "monospecific" anti-human globulins, which are the two separate reagents, anti-IgG alone and anti-C3 alone.

Joe: Okay. Okay. A couple things that pop up in that description, Sue. So you mentioned that we take those cells and we wash them. Well, why? What's the big deal about washing? Why do we have to wash the cells first?

Sue: So washing, basically what it'll do is remove any free antibody that might be surrounding the red cells because the red cells are always in plasma, so you know that there'll be potentially antibody in the plasma, the same antibody that could be coating the patient's red cells. So we want to make sure that whatever we detect is really antibody that is actually bonded to the red cell versus floating around.

Joe: I see. So if we didn't wash, would that make us at risk of a false positive or a false negative?

Sue: Yeah. Yeah. So interestingly, it's a false negative most often, because the antibody would actually neutralize, any free antibody would neutralize the anti-IgG component of the anti-human globulin reagent that we use, so it would bind to the anti-IgG that we're adding and give us a false negative reaction. You know, the other crucial thing in the test that is the fact that the lab scientists, when they do the testing, should be making a fresh cell suspension directly from the patient's sample, versus one that's been sitting on, you know, sitting on the bench top.

Joe: Right, right. Excuse me. Okay. One other thing that pops up in your description there. You threw out a couple of words, "polyspecific" and "monospecific." Can we just go over that again for just a second, because I think that sometimes when we're talking about antibodies people confuse that with polyclonal and monoclonal. I've heard people struggle with that a little bit. So tell us again what you mean when you say you're using a polyspecific anti-human globulin or a monospecific anti-human globulin.

Sue: Sure. So a polyspecific, by definition we mean it's got a combination of anti-IgG and an anti-C3. So both antibodies are in the one reagent. So, and then the monospecific reagent is kind of what it stands for. The mono is one, right? So it's separate, it's anti-IgG in one bottle, anti-C3 in the second bottle. In some labs, actually, it depends on your laboratory, but some labs will automatically just do the monospecific reagents so that they know right away. Traditionally, I think most labs will start with the polyspecific and then if there's a positive, go to the monospecific, the separate reagents.

Joe: Got it. So it's kinda, the poly is almost, you could think of it almost like as a screen?

Sue: Yeah.

Joe: If it's negative, then there's no reason to do the anti-IgG or C3. Is that a fair way to put it?

Sue: Yep. Perfect.

Joe: Okay. You also mentioned that it's an exquisitely sensitive test, which is obviously super-important to remember, and you said as few as like 100 molecules of IgG. That's incredible, but I want to make sure that people get this point. If the DAT is negative, that doesn't necessarily mean that there's NOTHING on the red cells, but there is a limit to the sensitivity of the test, right?

Sue: Correct. So there are some individuals that we define as having a "DAT-negative immune hemolytic anemia." So they have either a very low-level amount of antibody that is attached to the red cells that only by using enhanced DAT methods that are we able to see the antibody. Or some people have very low affinity antibody and again, we have to use an enhancement method to, you know, allow the antibody to stay on the red cells so that we can see it. There's one other point. So I was just thinking, the other thing just to mention is the fact that, you know, why is complement binding? You know, why are we looking for complement?

Joe: Sure. Yeah. Go.

Sue: So the complement binding is indirectly telling us if there, most often, is indirectly telling us if there is IgM antibody present in the patient. Right now, right, we don't have the ability, nobody's been able to design an anti-IgM so that we would be able to directly see IgM patient antibody binding to the red cells. So instead we know IgM antibodies will bind complement so, and we have good anti-C3 reagents. So if the IgM antibody is able to bind complement to the red cells and we do the DAT and add anti-C3, that anti-C3 will detect the complement binding that the IgM antibody basically activated.

Joe: Okay. That's important. So, kind of using the phrase that you used a moment ago, it's kind of an indirect way of detecting that there may be IgM around.

Sue: Exactly.

Joe: Okay and again, just so we can be crystal clear, I'm sure that there are some people out there sitting there right now saying "C3?" Help us with that. What is C3?

Sue: Okay. So interestingly, I get that question a lot because it's actually not really described very well anywhere as it applies to immunohematology, because we all have learned the complement cascade, right? Everybody's learned that and we've had to memorize it and we know it's role, right? That when we get to the membrane attack complex that a C6, you know, 6, 7, 8, 9 form the pore in the cell and, you know, the cell bursts, right, but why it's important in immunohematology and why C3 is the fact that, when complement gets activated, we know that starts with the C1, you know, then C1q, 4 and all that. I didn't say that all right, but it gets ...

Joe: It's okay. Nobody remembers that anyway, Sue.

Sue: No. I know, exactly. So C3 is the point where, when C3 gets deposited or attached to the red cell, it is generally cleaved into C3b and C3d. So many actually of our anti-human globulin reagents will have anti-C3b, C3d or just anti-C3d. So the C3d basically is telling us that an antibody bound but it didn't cause total lysis of the red cells. So if it did continue, if the complement cascade did continue, the C3b actually has a amplification step in the complement cascade and then the red cells would probably get lysed and not as easily detected. You know, we wouldn't be able to detect complement as well on the cells then.

Joe: Sure, sure. Okay. That's important. So see, you're right. It is somewhat imprecisely phrased when we just say "anti-C3," there's more to it than just anti-C3. There's different components, but if I remember right, so the C3d specificity at least is what's required to get licensed as a monospecific anti-C3. Is that correct or do I have that backwards?

Sue: It's C3d. Yeah, it is d.

Joe: That's what I thought. Right. Okay. Well, so going back to the methodology, you were talking about doing this in test tubes and that's the primary, certainly the most classical way of doing it, right?

Sue: Right.

Joe: Are there other options? Are there other ways that this can be done?

Sue: Sure. Another common way that the Direct Antiglobulin Test is performed is using a column agglutination method. In the United States, it's a gel column, but depending on where you're listening from, there are column

methods that actually have the micro beads. So either though are similar from the point of view that when, kind of the same thing, you add the patient's red cells directly to the column, the column has incorporated into it anti-IgG or anti-C3 and then if there's IgG or complement coating the patient's red cells, the antibody in the column will attach and cause agglutination of the red cells. Then instead of seeing the agglutination in a test tube, the agglutination will get hung up. Like, so the agglutinates will stay at the top of, or agglutinates will form and will move through the gel column versus all the cells going to the bottom, which is considered a negative reaction.

Joe: Right, right. Okay. Are there any, I mean obviously it's a different platform and the procedure is different, but aside from that, are there any differences in how sensitive these tests are, or what have you seen in your practice in terms of, is there variation between results that you might expect in a test tube versus the column?

Sue: You know, so at first when we first started to use gel actually, because I've been doing this for awhile, I thought, oh gel is going to be more sensitive. I mean it just seemed to me like, well, because with gel you make your cell suspension, I didn't mention this. You make your cell suspension, you don't wash it, you just add the cells and then it goes, you know, it does it's centrification and you read it. An actual fact, it isn't more sensitive, especially, again, when you're in a reference lab setting, it's very similar reactivity to the test tubes. In fact we always run a gel column on DAT negative hemolytic anemia evaluations and looking at what others have done, it doesn't seem, I mean there are cases where sometimes the gel will be positive. Then there are sometimes cases where the tube is positive and the gel is negative. So I don't think there's overall sensitivity improvement with one method over the other actually.

Joe: That's interesting.

Sue: Yeah, I really thought it would be more sensitive, but I don't think it ...

Joe: Well and that kind of goes to some conversations that I have with people a lot of times about the various testing platforms in that, to go away from DAT, but just people talking about, you know, gel versus solid phase versus tubes versus tubes in PEG etc, and what I always tell people, and I would love your take on this, is that they all have their strengths and weaknesses and there's no perfect platform. There's always potential holes in every platform. Is that a fair way to put that?

Sue: Absolutely. I totally agree with you on that. I mean I always say, you know, if there was one method that was highly sensitive and specific, we would all use it, right? I mean and we just, it's not there. We don't have it.

Joe: Well and so forgive me, that was a little bit of a sidelight, but I get that question a lot, so I just wanted to make sure I got your take on it. So

before we move on into why people might have a positive DAT, you can probably tell, Sue, I'm a total immunohematology nerd. I love the techniques and it makes me happy. So I want to just before we leave the technique, let's go back to tube testing of DAT for just a second and help me understand where the potential pitfalls are. Where are, in your experience and you've taught this for a long time, where are the things that people might mess up and how do we figure out that we've messed up in those settings?

Sue: Sure. Okay. So, you know, like I said, the DAT is such a simple test to do, but it's, there's a couple of points that are really important. First thing is that when we start to do the test, we get a fresh drop of red cells directly from the patient sample from the EDTA tube. The reason is, I didn't say this before, but the reason is if you've already made a cell suspension and those cells are sitting in saline, we know that a weak positive DAT where there's just weak binding of IgG antibody, especially IgG, just by its sitting in saline, the antibody can come off the red cells. So you'll get a false negative, right. So first thing, make sure it's a fresh drop of cells from the patient sample. It doesn't take that long, just a quick, get the cells, make your cell suspension, then start washing and the washing in a test tube method should be uninterrupted washing.

So I mean if we have to wash manually, it should be, you know, centrifuge is done, decap the saline, fill it up again and just keep that process going because again, if the red cells sit on saline at all, then that antibody, or if it's a low affinity type antibody, the antibody is going to come off the cells. If it's in a cell washer, it shouldn't be interrupted. Then the key point there is that you, as soon as it's done washing, you add your anti-human globulin reagent, your polyspecific or your monospecific reagents. I mean, I've seen it, I've seen, you know, you get interrupted, you have to go issue blood, the phone rings and the tube will sit there for awhile. I mean that's not a good practice for any test tube method, but especially a DAT on a patient. So and then read immediately. So it's mostly about worrying about false negatives.

Joe: Gotcha. Gotcha. One, and those are awesome tips I love that, one thing that people, I think probably more on the physician trainee side than the lab scientist trainee side struggle with is the whole concept of the so called “Coombs control” or the “check cells.” Can you talk us through what that means?

Sue: Sure. So the Coombs control cells or the checks cells, they're either cells that are IgG-coated or complement-coated cells and what we're doing there is when we have a negative reaction, we want to ensure that the anti-human globulin reagent that we've added is working. So if there's a negative, we don't know for sure until we add those cells that we know are coated with IgG or complement. So when we add the IgG-coated red cells, then we're adding that to the test. We centrifuge it again, and then the anti-human globulin and the anti-IgG that's present in the test will bind to

those check cells, or IgG coated cells. Whatever we call them, right? Coombs control cells, we call them check cells. That's why I keep saying that. Okay?

Joe: No problem.

Sue: Okay. So, when we add those, the anti-IgG will bind. That will give us a positive reaction and it's usually, you know, a one to 3+ positive reaction, but it's a positive reaction. Same thing for the anti-C3. If we have anti-C3 alone in our test, we're going to add complement coated cells to any negative reaction because there was no complement coating the patient red cells. We have to make sure that anti-C3's working. So we add complement coated cells to the test, add those right in, centrifuge, and again, then the anti-C3 should bind to the complement coated cells. Again, proving that it's working.

Joe: So we've talked about the mechanics. So let's go to “why” this happens. What's the cause of a positive DAT? What are some of the possibilities for things that could give us either IgG or C3 on a red cell.

Sue: All right. So why DAT? We're going on. So, you know, what's the cause, right? The cause of a DAT. So we know, right, there are different reasons why antibody or complement coating of the red cells can occur. We know a patient could have an alloantibody. That antibody of course is not going to attach to their own red cells because by definition the patient is antigen negative. They've made the antibody, it's alloantibody but that antibody can bind to transfused red cells. So the antibody will cause a positive DAT when we test the patient because we're detecting that antibody attached to the transfusion or the transfused foreign red cells.

We'll have a positive DAT when a mom has an antibody that is directed against antigens on her baby's red cells, on the fetal red cells. A positive DAT will also occur when we have a patient that has the autoantibody and I think that's probably the one that we all think about right away and we've all learned about, right. You know, that antibody is positive with the autocontrol. It causes a positive DAT because the antibody's against yourself. So it makes sense that the DAT is positive.

Drug dependent antibodies definitely will cause a positive DAT there. It's because the drug that the patient is taking, they've made an antibody to the drug or to some component or element of the drug that creates this new antigen with the red cells. So that antibody can then coat or attach to the patient's red cells because the drug is present.

Then there's some non-immune causes, ones that we probably don't really understand. Where there's nonspecific binding of an antibody that we aren't able to say what causes it, but it might be hypergammaglobulinemia or some other situation that's going on in the patient.

Then the last one I think about is passive antibody, where the patient is receiving maybe ABO platelets that are not their own type, right. Like a patient that's receiving O platelets, for example. Or maybe an intravenous gamma globulin. I mean patients that get that, they're just basically getting a bag of antibodies. So who knows what could coat the patient's red cells from a IVIG treatment.

So there's lots of reasons for positive DATs and then the last, like I said, there's patients that just have a positive DAT. You know, and we don't know the reason, I mean clinically, you know, it's hard to know why it's positive.

Joe: I think that's a hugely important point, Sue and I think we need to emphasize that simply because I'm not sure that that's completely understood and I know you have some statistics for how many people just kind of are walking around or in the hospital just that have positive DATs that don't mean anything. Can you talk about that for just a second?

Sue: Sure. So we know that, for example, in blood donors, we know that in several studies that have been done, and I did one of them actually a long time ago, that about 1% of blood donors have a positive DAT. I mean they're perfectly healthy, right? Their hemoglobins are fine and they're donating blood. You know, so that's one of those where you just say, “What the DAT?”

Joe: Thank you for that.

Sue: You're welcome. I had to work at it. So, you know, those are just, who knows why? I mean and the DATs can be 1+ to I've seen 4+ positive DATs in normal healthy blood donors. Then if we look at the patient population, different patient groups have been studied, but it's up to, you know, 15% of patients with, for example, sickle cell or thalassemia in a chronic transfusion state that will have positive DATs that clinically don't seem important. At least not that we can, you know, put a definite attribution to causing trouble in the patient. So yeah, there's a lot. I mean we used to do, I mean, in my lifetime of blood banking, we used to do autocontrols all the time and what we found was that we were doing a lot of work for no, you know, no apparently good reason for the patient. Right?

Joe: Sure, sure. Yeah. Tracking down stuff that just doesn't mean anything.

Sue: Exactly. Exactly.

Joe: Yeah but I think that's a really, really important point because you took us through the causes of a positive DAT, like an alloantibody, an antibody, a red cell antibody against someone else's antigens, auto antibodies against your own red cell antigens, I mean a maternal antibody against baby red cells, drug dependence, nonspecifics, and passive antibodies like with out of group platelets and IVIG. So that kind of brings us to the next point, is

why should people do DATs, and I think it kinda goes to what we just talked about, right? In terms of what the causes are.

Sue: Right. I mean, so the reason for doing the DAT, right, is that it helps you explain something that's going on in the patient. I mean as an immunohematologist too, like loving this stuff, right. Is we do a lot of testing on antibodies, right? We're working mostly on antibodies and making sure that we're getting the right blood to the right patient, providing antigen negative, but a Direct Anticoagulant Test, the result can be diagnostic. I mean they're in all these situations, right, almost all of them that I talked about, it's telling us something about the patient that might not have been known yet. You know, that they could have a drug induced hemolytic anemia. Of course, I love that, but it could be a transfusion reaction. It could be HDFN, right. So it is super important and I've had clinicians tell me when we've evaluated those DAT negative patients where, you know, the patients had all the signs and symptoms of hemolysis and they haven't been able to tell the patient there's a positive test result. They said this means all the difference because now I can tell them I have a positive. So I was like, wow. No, that, you know, I see it.

Joe: Absolutely. So it can kind of give the answer where one wasn't present before and that for us in blood bank world, that's a little bit of a unique experience, isn't it?

Sue: It is, it is. It's actually really cool when you can do that.

Joe: I like it. I like it. So again, let's just talk through the settings. You had mentioned acute transfusion reaction investigations. What other kind of things might be going on clinically that might trigger either a doc to order a DAT or a transfusion service or reference lab to say, I need to do a DA?

Sue: Okay, well, right. The transfusion reaction absolutely, in acute transfusion reaction setting, the other place it's common that doesn't usually get ordered by a doc, but in the end will be as, in the case of delayed transfusion reactions, and those are generally where the patient comes in, you know, they got blood two weeks ago and their hemoglobin is back down. So the order comes to the lab for two units or one unit, whatever and you do the workup and now you find there's a new antibody present. The antibody you identify, you find the auto control and/or the DAT are both positive, depends on how somebody approaches the workup, but you see a new antibody. Generally you do a Direct Antiglobulin Test and you find positive result.

Sue: Another scenario would be in those patients that are showing signs and symptoms of hemolysis, you know, hemoglobin of course is down. You know, the LDH is elevated. spherocytes on the smear, bilirubin's elevated. All the other lab tests are pointing to it, those are generally more physician ordered. Hematologists that will order, you know, I put in quotes, "the Coombs panel".

Joe: Yes, of course, of course.

Sue: Which we all know means a Direct and Indirect Antiglobulin Test, or they call it in Coombs direct, an indirect, but in that case then those are physician ordered tests, right. That we're doing and that truly is, they're looking for something to occur or for a reason, for the what looks like hemolysis. Then, again, the same thing would be for a drug induced case, generally. It's either that their ordering blood because their hemoglobin is down to five or they are seeing these signs and symptoms of hemolysis. However, but oftentimes actually, the hemolysis is so brisk that they're ordering blood and then trying to figure out what's going on.

Joe: Right. That's certainly true. One other thing, and forgive me I'm interrupting, but I'm just jumping ahead just a little bit, in terms of when a transfusion service or reference lab might do it, maybe in a antibody identification workup, what might trigger a transfusion service to kind of automatically order or do a DAT.

Sue: So definitely if you're running, say for example, your routine antibody detection method is solid phase, then usually when you have a positive antibody screen or you detect an antibody, you're going to do a panel, you can't run an autocontrol. Well you can, but it's very tedious and most people don't do that manually. They will go automatically to a DAT. In a case where labs are using gel or tube testing, they might do a DAT automatically when they get a positive antibody screen again. Or they see an antibody, run an autocontrol, also then do a DAT. So those would be the scenarios generally and then I guess I didn't mention babies. Cord blood. I forgot it, to mention cord blood. You know, and then everybody's got slightly different rules on when they do their, they'll do a DAT on a cord of blood, but that would be the other scenario, right? That's generally written within the standard guidelines, right, for the hospital on which babies they'll evaluate.

Joe: Right. Okay. Well, and so speaking of that, in terms of evaluating this and I don't want to leave this point before we make this point crystal clear to people. That's simply this, context matters with the DAT. It matters why it's being ordered, it's extraordinarily important in helping clinicians to understand what it means. You got to know why it's ordered and that's me on my soapbox for a second, Sue, but I know you feel the same way about that.

Sue: Definitely. I mean when we get that positive test result in the lab, if we don't know a history, we don't know what it means, right. We have to get the patient history. What's their age? I mean that tells you a ton right there, right. Yeah. All those things. Super, super important.

Joe: Okay. Well, so with that being said, let's get to how we look at the results because I love the way you look at this. You say some things especially in

terms of the interpretation of strength that I haven't heard a lot of other people saying. So I want to make sure that we get to that.

So let's talk through this. So we've got someone who has a DAT ordered for whatever reason, and we do the test and we get back some results. How do you go about analyzing those results to kind of help point you in the right direction to see what they mean?

Sue: Okay. Well, the first thing we look at is what's the strength of reactivity because, you know, a positive is not a positive, right? I mean to me looking at what the strength is and then considering that in the context of the patient, tells you the story almost always, almost always. So if you look at, we look at what's positive, of course right away. So if the polyspecific is positive, we automatically will go to the monospecific reagents, the anti-C3, anti-IgG. If only complements are on the red cells, then that's putting me in a whole different direction, right, of thinking about what could be on the red cell. So if it's a weak positive with complement only and I know my patient just was transfused, just like currently or 14 days ago, I'm thinking a transfusion reaction. It could be acute or delayed depending on the timing because I wouldn't expect to see a weak complement only with a cold agglutinin disease for example.

So if I compare that then, right, I have a stronger positive C3 on the cell, so a two to 4+ and they usually aren't 4+, but they could be, it's usually more in the two to 3+ range. I think that's because of the you know, avidity of the antibody and how much complement gets onto the red cells. However, if it is stronger, then I'm thinking more and there's no history of transfusion, I'm absolutely thinking more drugs, cold agglutinin disease, or PCH, paroxysmal cold hemoglobinuria. If there's IgG only on the cells, again, the strength, if it's a 1+ or less then, and kind of have to know how your techs, you know, shake a test tube as well but if I'm confident that it's a 1+ or less, then again, I'm thinking it's more likely a transfusion reaction, maybe passive acquired antibody, or if it's an infant, a cord blood, maybe more ABO-HDFN.

If the DAT is stronger, and I usually say 2 to 4+ positive, and you know, you could even argue again, depending on how skilled your laboratory scientists are, but if they're stronger positives, I'm thinking again, warm auto, drugs, and if it's, again, in the case of a cord blood and thinking more of RH hemolytic disease of the fetus and newborn or one of the other types, right, but RH most commonly. Again, because weaker positive, that's telling me the antibody is only binding to a smaller amount of red cells or there isn't as much antibody, right, and that's why I would think of a transfusion reaction. It's only binding to the transfused red cells. Strong positive, well that auto antibody's binding to all my red cells, I should expect to see a strong positive. Same thing with Rh and anti-D crossing the placenta, binding to babies RhD positive red cells, it should be strong. Then finally, if I see both IgG and complement, I kind of use the same rule. Weaker positive, more likely and there's a history of transfusion, I'm

thinking transfusion reaction. Strong positive, I'm thinking drugs or warm auto.

Joe: Sue, that is such a cool way of looking at these things and I know that you and a couple of your SBB students put together a chart about this or a flow chart, a rough kind of a diagram that you have been kind enough to allow me to post on the website. So everyone, what Sue just talked about is such a really, really helpful way of looking at your results and when fitting it together with the context, you can point in the right direction really, really well. So that's great. Sue, thank you for going through that.

Sue: Oh, no problem.

Joe: Okay. So we've got all these possibilities and we now know that it's essential to understand why it's being ordered. We know that it's essential once you know how it's being ordered, what the context is to take a look at what's positive on the test, what the strength is on the test, and how all that works together. So I guess once you get through all that and once you get a positive DAT and you're kind of pointing in the right direction, how do you give feedback to your ordering docs about what this means? Does this mean a direct conversation, do you just put out a report and yay everybody's happy? How do you feel about that interaction?

Sue: You know, that's a great question. You know, I'm fortunate that I work with board certified transfusion medicine physicians that feel very comfortable and actually want to be notified whenever there's a new positive DAT for that reason, right. So that they can communicate or we can communicate, right, the results. So generally the results would get communicated right, right away from our hospital transfusion service perspective, then our physicians would then go ahead and, you know, follow up, make a note, talk to the doc, whatever it might be. I know a lot of times the results are just issued, right, and however, like from a reference lab perspective or even in the transfusion service, whenever you have a new positive DAT, that patient history is so important for that context so that you can evaluate. Otherwise, all you can report is the DAT is positive due to IgG or the DAT is positive due to a complement. If we have history then we can say, well, because the patient was transfused, these results are consistent with right, transfusion action and what have you. So I think it depends on who you work with, but absolutely it should be communicated.

Joe: Yeah. Well, so before we go on to do just a couple of quick cases, Sue, I wanted to throw a couple of things at you because people ask us about these things. The first one is this, okay, so you've got a positive DAT. You got a positive DAT with IgG and let's say it's in the setting of a transfusion reaction workup or an autoantibody or something like that. Is there a way, and I'm guessing there is, is there a way for us to determine what the heck that antibody is that's coating the red cells?

Sue: Oh, absolutely.

Joe: Boy that's a softball right there, right?

Sue: Absolutely and I was so focused on the DAT, right? Yeah. What we can do when we have that positive Direct Antiglobulin Test, especially with IgG, but I would not deter either depending on the context, again, with only complement on the cells, that we can get a good idea of what's coating the red cells by doing an elution method, right? So if we do an elution, we can, most commonly today we can treat the red cells with acid, which is a low pH, like pH 3.5 and if we add acid to red cells, the antibody does not like that. The antibody will come off the red cells and we buffer it back to normal so that we can then test what's now called the eluate.

So we have the acid, the antibody comes off into the supernatant, that's the eluate. We can't use that yet, we have to buffer it back to a normal pH so we can test it with normal red cells. So we add a buffering solution back and then we can take that eluate that we just made, which is the antibody that should have come off the patient's red cells. We can test that then against a panel of red cells, like we would do any antibody identification and determine what's coating the red cells. So is there a single antibody, is it an antibody that's reacting with everything and then that helps us determine, put the story together. You know, if it's 4+ DAT, I would expect to see the eluate reacting strongly positive with everything consistent with warm auto antibodies.

Joe: Nice. Okay. Okay. So, and because I know that there are some people out there that are sitting there going, “okay, people often use these words together, they talk about elutions and they talk about adsorptions and my goodness, I can't remember what the difference is.” I know this is going off on a little bit of a tangent, but just help everyone understand real quickly what's the difference between an elution and an adsorption?

Sue: So an elution, right, think of it this way, you're eluding off, so you're pulling an antibody off the red cells. Whereas an adsorptIon is we're taking antibody, usually a know antibody, we have something that is in the plasma that we want to remove from the plasma. So, we're going to take it and we're going to do an adsorption. So we're going to take the plasma, mix it with some red cells, other red cells or the patient's own red cells and the antibody will adsorb from the plasma onto the red cells. So elution is pulling antibody off and adsorption is taking antibody from the plasma and putting it onto red cells.

Joe: Okay. So we just have a few minutes left and I wanted to just kind of put some traction to this and just hit you with just a few quick clinical scenarios. Kind of the who DAT section here and just quick hitters. Just kind of tell me how you would approach situations like this. Okay. So here's the first one. You're working in the blood bank and you get a call from a clinician who says, "hey, I have this patient", doesn't give you a lot of info. He says, "I have this patient that I really need to order a direct and indirect Coombs on and, you know, the patient was just transferred from

another hospital and I have these results that are like, he's got 1+ poly, 1+ IgG. I don't know what any of that means but I know that I need a direct and indirect Coombs". What do you do?

Sue: Okay. So in that case, right, so he knows, or what we know is that it looks like the patient has an antibody, an IgG antibody bound to their red cells. That's all we know in this context, right. So what I would say is well, if he wants it done here, we probably need to do it in our lab. So we would do the direct, again look at that. We would probably do an eluate depending on what the clinical situation is. So and it might be, well, do you have any results yet? Do you have a bilirubin, you know, LDH anything like that. Do you have a transfusion history? What's the patient's diagnosis, right. All of those things again, because like I can't tell much other than the fact that the patient has 1+ positive DAT and it might not be important at all, because we know up to 15% of people can have a positive DAT.

Joe: Yep. Yep. Okay. Okay. Awesome. Okay. That's number one. Like I said, quick hitters. Number two, you've got a patient who's a 45 year old female with acute myelogenous leukemia who's getting a transfusion of red cells and has a fever during the transfusion. So as part of the transfusion reaction workup, everything initially looks fine. There's no clerical errors. All the paperwork checks out. There's no hemoglobin when you spin down the post transfusion sample, but when they do the DAT on that post transfusion sample, they see a 2+ poly and that's all the information you have. What do you do with that?

Sue: Well, first thing is remain calm.

Joe: Yes. Take your own pulse, right?

Sue: Yes, exactly because most often everybody gets a little freaked out when the DAT's positive. Either that or you see hemolysis, one or the other, right? So, then the most important thing at that point is to go ahead and then get a pretransfusion sample because again, that patient could have had a positive DAT before they received that unit of blood, and then really assess at that point, right? If the pre is positive, then there's really not much more. I mean you could do more work but like eluates and things like that to get a handle on it, but for the transfusion reaction investigation you're not gonna be able to tell a whole lot more. If both of the pre and the post are positive.

Joe: Good. I see that question a lot and it's important for people to understand that it should be reflex, right? It should be automatic. You see that positive post, you got to do the pre. One other question related to that though, that sometimes people ask me and they say, okay, well they were both positive. The pre was positive and the post was positive, but the pre was 1+ and the post is 2+. Should I freak out?

Sue: That's a hard one because in most labs, right, if you did like gave the same say set of dilutions to all the laboratory scientists in your lab and had them test the same red cells. Even in the best of labs I would say you always have a plus or minus one reaction grade between the techs. So it's difficult to know one. I mean hopefully it's the same tech that did the pre and the post, but if not even the variability between in the tests that still could happen. So that's still within the normal range to me, you know, not a significant change. However, I mean if I felt really good about it and it was a one or a two, I would probably go ahead and do an eluate and just compare the eluate on the pre with the post, again-

Joe: That's reasonable.

Sue: Yeah. Pull a antibody off the cell, see what you have.

Joe: And certainly you know, if it's 1+ to 4+, which I mean you've already talked about in the setting of a transfusion reaction, that's unlikely, but if you did have a substantial change like that, that's obvious that you got some more work to do.

Sue: Yeah. Absolutely.

Joe: The big kicker that we're looking for is the negative to positive though. That's the flag.

Sue: That is the flag.

Joe: All right, so that's the second one. How about the third one? So we've got a 72 year old male who was admitted with a sky high white count, primarily mature lymphocytes on that white count and he is showing, well he's anemic and, you know, when the chemistry, I'm sorry, when the hematology section of the lab spins down his sample, they say, wow, his serum really looks yellow. What is the deal with this? Someone orders a DAT and it's 4+ poly, 4+ IgG, and 2+ C3. What kind of things might we be thinking about in that scenario?

Sue: So in that scenario, so that's a perfect setup. That's a lot of great information. One, I'd-

Joe: More than we usually get, right.

Sue: More, exactly.

Joe: Sorry. I made it too easy.

Sue: No you, and actually I was laughing to myself when you said anemic, because I can tell you being in a reference lab, I would say half the recs come in with a diagnosis of anemia.

Joe: Yes. You're right. You're right about that.

Sue: And you're always like, “okay, come on!”

Joe: Yeah. Really. Give me more.

Sue: Yes. So CLL, first thing I think of is, and with a strong positive DAT, I'm thinking a patient with a warm autoantibody. We know that a warm auto is not uncommon, secondarily seen to CLL patients. The strength of the positive DAT being 4+, complement positive, very, again, consistent with a warm auto, and the fact that you told me the plasma was yellowish would make me think more warm auto. If it was reddish then I actually, and it was a patient with a hemoglobin of 4 [g/dL], I would have been thinking more a drug case, you know, a drug-induced like anemia. Yeah. I've done this, I've walked into the lab and said, can I see a patient, a sample that has a warm auto? They look pretty normal, like they're just yellowish, but that strong positive DAT is, that's the key thing. Sounds like your warm auto.

Joe: So we have taken a kind of a grand tour through the wonderful world of the Direct Antiglobulin Test and, Sue, you have been a magnificent, magnificent tour guide. I can't tell you how much I appreciate you doing this. Is there anything before we go, any last tips that you would give to people that are learning about the DAT, trying to understand the DAT? Any last thoughts to leave us with?

Sue: Oh, I mean, just that this thing about the Direct Antiglobulin Test is the fact that remembering that we're looking at patient red cells and remembering that it's oftentimes not just their red cells, right? That there could be transfused red cells there as well. Or even I guess baby red cells, but that would be a lot of. That it is patient side of the world, it's the patient red cells versus plasma, right, because normally we're looking at the antibodies in the plasma, in the serum. I guess the other thing is, again, when we're interpreting those results, like I think we've hit on a lot, is that, you know, we look to see that, what's the context, what's the patient history, you know, everything around that. Then the strength of reactivity because if you have all that, you can really do a great job at interpreting the results and providing that information to the patient or to the patient's physician. So usually like we've talked about that, right? The what, you know, what's DAT, why DAT, who DAT. So I always say you gotta end with we love DAT.

Joe: Ah, yes. Yes, we do. Well Sue, you are awesome. Thank you so very much for doing this. I can't tell you how much I appreciate it.

Sue: Oh, you're very welcome. Tons of fun. I hope everybody finds it helpful.

Joe: I'm sure they will. We'll talk to you soon.

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Joe: You know, sometimes I think that when you get to talk with someone that you really admire, that you feel disappointed afterwards. You know, it's

kind of the whole hero thing. Then you find out that your hero is not really all that great. Well, let me just say that that is not how I feel after talking to Sue today. I learn from her every time I hear her speak and today was absolutely no exception. I hope that you enjoyed it. I know I did. I should tell you that when you look on the show page that's on bbguide.org/podcast and you'll find this episode, I've included not only a bunch of slide images that Sue has generously provided, but also a link to a series of videos that Sue did on the Indian Immunohematology Initiative website where she is actually performing the DAT. You actually get to watch her performing a tube DAT as well as a bunch of other basic immunohematology tests. The videos are great and I think you'll learn a lot more by watching them.

So my thanks to Sue Johnson for appearing on the podcast, my thanks to all of you for listening and downloading the podcast and, you know, just thanks for inviting me along in your journey towards mastery of blood banking. Please head on over to bbguy.org/subscribe and get on my email list so that you won't miss any of the cool stuff that's coming down the line and also the next time you're near a computer, please just head on over to iTunes and give this podcast a rating and a review because it really helps others find it, see it, and learn from it too.

So that is it for today. Thanks again, as always, and as we close, I hope that as you go through your day, that you'll smile, and have fun, and above all, never, ever stop learning. Thanks a lot. We'll catch you next time on the podcast.