BBGuy Essentials 102CE:
"Learn to Speak 'Reference Lab'" with Jan Hamilton
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Jan: Hi, I'm Jan Hamilton. I am the manager of the Reference Laboratory at the Red Cross in Detroit, Michigan, and this is the Blood Bank Guy Essentials Podcast.

Joe: Hey everybody. Welcome back to Blood Bank Guy Essentials, the podcast designed to help you learn the essentials of transfusion medicine. My name is Joe Chaffin, and I'm thrilled to be your host for today's episode, which is 102CE. This is an episode... I hardly even know where to start. It's very exciting to me because this is an episode that gets to the core of what I'm trying to do with the overall podcast in general. When I started this podcast, the whole idea was to teach the essentials of transfusion medicine to learners everywhere. That's my motto. That's what I'm really trying to do. That's always my aim. And in this episode, I'm speaking with Jan Hamilton, who's the manager of the Immunohematology Reference Laboratory at the American Red Cross Southeastern Michigan region in Detroit, my hometown, which is, you know, another added benefit. Anyway, this interview with Jan really gets to the core of what I'm trying to do.

Jan has so much to share about some of the basics of the more, well, somewhat more advanced testing that we do in terms of helping to identify antibodies and help make blood transfusions safer. She has just a wealth of knowledge to share, and I can't wait for you to hear it.

But first, this IS a continuing education episode. The free continuing education credit is provided by TransfusionNews.com, and Transfusion News is brought to you by Bio-Rad, who has no editorial input into the podcast. This podcast offers a continuing education activity where you can earn two different types of credit: One AMA PRA Category 1 Credit™, or one contact hour of ASCLS P.A.C.E.® program credit. This activity also may be used to fulfill Lifelong Learning Continuing Certification requirements for the American Board of Pathology. To receive credit for this activity, to review the accreditation information and related disclosures, you just need to visit www.wileyhealthlearning.com/transfusionnews. Finally, don't forget: The continuing education credit is no longer available for this episode two years after the date it was released. In other words, if you are listening to this episode later than June 22, 2025, the continuing education credit will have already expired.

When you first get started in blood banking, you hear terms thrown around that can be hard to understand at times. You hear funny-sounding stuff like "autoadsorption" or "elution" or that someone is treating SOMETHING with "enzymes," and it's easy to get lost! Today's episode is designed to help you get past that, to help you learn how to "speak reference lab," if you will, because many of these techniques are performed in advanced immunohematology laboratories we call "reference labs." Jan Hamilton, today's guest, leads one of the American Red Cross' regional reference labs in Detroit, as I mentioned, and she is here to help guide us and learn the meanings of some terms that might seem unfamiliar.

Jan brings a wealth of experience and teaching credentials to this interview. She is a past chair of the AABB Immunohematology Reference Laboratory Accreditation Program Unit and the ACSP Board of Registry Blood Bank Examination Committee. She is also an associate editor for the journal “Immunohematology.” And, she's the author of an incredible book that you're going to get the chance to win by doing the continuing education for this episode; that book is called “Antibody Identification: Art or Science?” Please stick around to the end where I'm going to tell you exactly how you can win a free copy of that book. Jan and I will talk about that book in just a moment, at the start of the interview.

So, let’s get to it! Here is my interview with Jan Hamilton, "Learn to Speak Reference Lab".

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Joe: Hey Jan, welcome to the Blood Bank Guy Essentials Podcast!

Jan: Hey Joe, thanks for asking me. I'm so excited to do this with you.

Joe: It's only been what, forever and a day since we first talked about this, so I'm super glad that we are finally able to connect. I'm actually going to start by maybe embarrassing you slightly, but I'm going to do something that I actually don't normally do, and I'm surprising you with this, you're not aware that this is coming. I'm going to do something I don't normally do in this podcast and that's plug something, and there's a really important reason for that.

If you guys that are listening could look at the video, you would see that I'm holding up what I consider to be one of the greatest tools for learning about antibody identification that I've ever seen. It's a spiral bound book from AABB that Jan, my wonderful guest today, along with friend of the podcast, Sue Johnson and Sally Rudmann, put together called "Antibody Identification: Art or Science? A Case Study Approach."

And I've got to tell you guys, as someone that that's put out videos on the internet for years, including one that's been watched by a lot of people on basics of how to do antibody ID, I will tell you right now, this book is magical. It is tremendous. It takes you through from start to finish how to evaluate antibody ID panels if working with antibody ID is something that you as a learner don't feel super strong with.

Again, I don't normally plug books and this is not meant to be a commercial, Jan, but I got to tell you, you guys did a wonderful, wonderful job on this book, so congratulations on it. It's not new. I know it's been out for quite a while, close to 10 years I think now, but it's terrific.

Jan: Maybe, is it really that long? Wow. Well thanks a lot, Joe.

Joe: Can you just share with us what was the motivation for doing this book? How did this book come about?

Jan: Well, the motivation was really exactly what you said you found the use, is to just break things down and the title is Art or Science? Well, we learn the science, we learn how to

do the tests, we learn how to do a panel, we learn those mechanics, so the art part is just being able to put all the pieces together and to know what's next. And the three of us strongly felt that we could teach a little bit of that by very systematic working through problems and the increase in difficulty as you would go through the book, and just thought that there was a lot that we could put down and make it in a way that a learner, and all levels of learners, and all types of learners could really hopefully learn something. I mean, obviously learn something from it, but get a sense that this is not just all hocus-pocus that somebody that has done it for 20 years can do and nobody else can. And I'm glad to hear that you feel that we've met that goal.

Joe: Well, I will tell you that I use it and the techniques that are talked about in it with my residents, with my learners in the lab science program at the hospital where I work and the program where I work all the time, it is that good. So again, wonderful, wonderful work and just so everybody knows, I make not one single penny if everybody goes out and buys this book. So Jan might make some pennies, I don't know what Jan's contract is, but the point is, this is a great, great book, "Antibody Identification: Art or Science? A Case Study Approach." You can get that from the AABB Press, and I'm not sure if it's sold on Amazon or other places, Jan, do you know by any chance?

Jan: I do not know. I've only seen it through AABB. And our companion volume... Antibody ID: Art or Science? is all autocontrol [and] DAT-negative situations. The companion volume on the DAT workups is all different reasons why you might have a positive DAT, so...

Joe: That one is excellent too. Both are wonderful, wonderful teaching tools. So now that I've embarrassed you a little bit, and helped to plug something that you didn't know I was going to plug...

Jan: Thanks. Thanks a lot.

Joe: There is a reason that we're here today aside from just talking about this tremendous book, and that is, Jan, I wanted to chat with you because as I saw certainly in this book, and as I've heard talks that you've given over the years, and seen some of the materials that you've put out there, it became clear to me that you are someone that I could really connect with in terms of helping people that are just getting going, people that are learning, and even some people that are perhaps a little bit more advanced in blood banking and transfusion medicine, to understand some of the tools that we use primarily in reference labs, but also to an extent in some hospital transfusion services to help us work through some of these problems and some of the challenges that come up when we're doing serologic workups for patients.

I wanted to get into some of those details with you, but before I did, there's something I think that I wanted to clear up with you. And I don't know if we can "clear it up," but it's definitely something that I get asked about a lot. I got asked a lot about it when I was a medical director for a reference lab, and I still get asked about it a lot through the Blood Bank Guy website. And let me just summarize one of the emails that I got recently that just talks about that scenario. I won't tell you what their name is, but this is someone who wrote, "I used to work in a hospital that used solid phase to do our antibody screens and antibody identification. I've recently moved to a hospital that does gel and

what I'm seeing is that..." I'm going to paraphrase here because I don't want to give specific details of the facility. "...what I'm seeing is that we're missing antibodies, and I'm concerned that patients are not being taken care of appropriately." Again, paraphrasing the email.

I get things like that all the time, Jan, both directions, from people that go from places that use are using solid phase to using column agglutination or gel testing and vice versa, every now and then from places that still use tube testing to do their antibody screens. I don't get that many of those anymore. But what I wanted to throw out to you and open the floor to you to discuss is, how do you respond to scenarios like that? What does the data show, and what's your opinion and your experience on answering that question about what's best?

Jan: Boy, that's certainly a really tough one, and yes, we get it, and we get in our reference lab, samples sent in or work sent in because of these reactions in gel or the reactions in solid phase, and then we're tube users, so we may not see anything. And I can certainly understand the frustration and the exasperation of some people that are working this way.

First of all, I think the main thing that I want to emphasize and everybody knows is that no method is going to pick up every single antibody, whether we understand why some things reacted better in one system than another, but as long as... And we have to preface it by the test is being properly performed, the people that are looking at the results know how to interpret the results and how to look at various panel reactions and make something out of them.

Not every method is going to pick up every antibody, but certainly, like you say, they're both gel and solid phase, both well established methods for antibody, clinically significant antibody, alloantibody detection, so it's very difficult to be able to sort out when somebody thinks they're missing an antibody and not, I really question. Certainly, there's not massive antibodies being missed or these methods wouldn't be allowed to be out there. People wouldn't use them and so it is really a challenge.

What sometimes I have more frustration with is with some of the unwanted positives. Now when you're saying you're missing antibodies, you're talking about you're missing wanted positives.

Joe: Right.

Jan: I think that there is much more likelihood with these methods that are so sensitive, they're very, very sensitive, they're more sensitive than tube in many instances, you're getting unwanted positives.

What could that mean? Both of those methods are more sensitive to warm autoantibodies. Gel methods can be, you can pick up cold autos in there. You can pick because of the way that the cold auto causes the red cells to be agglutinated in that incubation chamber in their reservoir and then those agglutinates don't go through the gel evenly, easily, and it looks like you've got positives in there. Those kinds of things I think are also challenging. Things like gel is because of the direct agglutination and because of the pH of the test system, which is a little bit lower than in tubes. You you're

picking up lots more anti Ms, those kinds of things that you may never have even seen in a tube test and you probably don't care. Solid phase picks up warm autos incredibly well as well as kind of, I'm not going to say the word "junk," so you didn't hear it, but...

Joe: I heard nothing! Nothing…

Jan: ... things that really you can't make into anything that ever turns into something clinically significant.

There is one exception though, and I think you have to call that out, is solid phase is extremely sensitive to Kidd system antibodies and that is one place where you might say this method may pick up antibodies that may not easily be seen in a gel method or a tube method. And we have seen some on, like I said, we're tube, we're PEG tube users, but I will look at a solid phase panel that might have been submitted with a case to the reference lab and scrutinize that panel to make sure I don't see any hint of a Kidd system antibody particularly Jka and I've seen somewhere we couldn't find it but there were, boy, I could kind of feel it being in there and we may have recommended to transfuse Jka or Jkb negative units.

Joe: For those that are just kind of going in our specialty. Jan, why are Kidd antibodies so important? Why? So wha?t Kidd antibodies, big deal! Why do we care?

Jan: Well, those are the ones that can be very clinically significant. They can cause severe transfusion reactions, particularly delayed transfusion reactions and that's because they so quickly drop off in antibody titer and so their detectability in different test systems may be somewhat compromised because the titer has fallen so low, but man, when they get a exposed to and that patient gets exposed to say a Jka positive unit, boom, that antibody comes right back and can cause destruction of transfuse cells and an antibody like that, the solid phase method might pick it up just a little bit before a gel or a tube method might actually. That's the only place where I really hang my hat on there being significant differences in what you detect in the different test methods.

Joe: And I think that leads to, oh, and by the way, just so you know, the silly thing that I use with my residents to help them remember Kidd antibodies. The Kidd antibodies do that is that I'm like, okay, think about kids. What's a game that kids play? Hide and seek. Hide and seek! That's what the antibodies do, too, all the time. Anyway.

Jan: Yup. Yup. I think it... Yeah.

Joe: Just my silly little contribution to the world there. But Jan, that leads me to what I think is a really important question and I'm not sure that this is necessarily as widely understood as it could be. So why, if this is the case, if... Yeah, okay. So you get some increased sensitivity but maybe you get some stuff that you didn't necessarily want. What has been the big push over the years for hospitals in particular to move to using one of these methods like gel or solid phase? I mean why doesn't everybody just do like you and most reference lab I'm aware of use tube testing potentiated with PEG. Why don't hospitals do that anymore?

Jan: Well, hospitals are busy places there, Dr. Joe, and even though it's been a long time since I've worked in one, I'm sure I could never keep up these days with doing all the

testing in tubes. And so the fact that these methods allow for automation largely, other benefits, benefits more stability, less subjectivity to the results, the results made more objective, the results, especially like gel cards can be saved and reexamined by someone at a later time, a supervisory review. So a lot of the reasons that people switched to these methods, yes, some increased sensitivity, but a lot of them had to do with workflow and effective use of personnel time and just being able to keep up with the workload in their hospitals in these days. So there's a lot of other reasons besides they were afraid they were missing antibodies back in the day.

Joe: Right. I think that's a hundred percent, I'm right there with you. That is definitely the motivation in the places that I've worked that switched from tube to one of those two other platforms, there was no question about it. The automation is a big thing. The ability for someone to later review someone's work is another really big thing that we saw. It's tough to review something that, I'm going to use a blood banker phrase Jan, get ready, when a technologist looks at something and says, "Well, it looks a little scratchy." It's tough to review that later on, right?

Jan: Yes, it certainly is. Even moments later somebody says, "Oh, what do you think of this?" I'm like, "Well, it looks okay to me. I would've need to see it coming off the button," and know all those kinds of things. There's just hemagglutination certainly is a gold standard, but there's just so much, so many you have to perform it exactly well and there's a degree of subjectivity to the readings.

Joe: So I can't quite leave this until I ask you about one last thing, and again, I think this is just so important because in my experience I've seen a lot of this and I'm guessing you have too, the scenario where a hospital has a positive on one of those two platforms, gel or solid phase, they send it to you in the reference lab, your lab works it up using the platform that you primarily use as you said tube with PEG and you don't see anything and you're not seeing anything at all. How do you handle those conversations with hospitals that say, "Wait, what? How is that possible?"

Jan: Boy, that's such a challenging one. We have to reassure them that our tube PEG testing is being done... It is a sensitive test for detecting clinically significant alloantibodies and that we feel confident in the training and abilities of our techs to do that test well. You also have to look at things like what's the patient's history? I mean, have they just been transfused maybe two or three or four weeks ago? How likely is it that this really represents a change in their immune response that you're picking up? Have you seen this patient before? What's going on with them? What's their diagnosis? What's their medication history? You really look at all of those things to evaluate whether the more sensitive test methods might be giving you a hint of something that you need to watch.

Okay. “Right now I don't see anything in my tubes, my PEG tubes, but let's keep an eye on this patient and see whether it's going that reactivity you see in gel or solid phase is going to turn into something.” It's a challenging one and they saw what they saw and that's what you start working with.

Joe: For the listeners that work in reference labs, I think it's so important not to, what I always used to tell the people that worked with me in reference lab world was never minimize what other people are seeing. Never say, "Well, we're the reference lab, you must be

wrong." That's not the message that we want to send. And I think what you just said, a cooperative message of let's watch this patient together and let's see, let's evaluate the whole big picture is such an important tack to take. It's easy to get into where the reference lab, what the hospital did must be wrong type of attitude. I'm not saying... Obviously, I'm not suggesting you guys have that Jan, but I think it's easy to get lulled into that. And as you said, they saw what they saw and looking at things collaboratively moving forward I think is massively important. So I like your approach.

Jan: Good, good. Served us well. It's still frustrating for both sides. Definitely.

Joe: Right.

Jan: It's frustrating on the reference lab side also.

Joe: Yes, agreed. It is not a fun situation for anyone when that happens, for sure.

Jan: Right.

Joe: Okay. Well Jan, thank you for your thoughts on that. I really appreciate you kind of walking us through that. I wanted to, for the rest of our time together, just take a little bit of a tour through some of the things that reference labs do primarily, and I will grant that some larger, more complex hospital transfusion services will do some of these tests as well or some of these techniques as well.

But I wanted to just walk through some of them with you to take a little bit of the mystery out of them for people that are just trying to figure all this out. And I wanted to start with one technique that's very... It's fairly common I guess, but it's really very widely misunderstood, I think among people that are trying to figure out how we do our testing and that's the use of enzymes. How enzymes fit into the big picture of antibody workups. If you could, why don't you just start with just giving us us an overall big picture of enzymes. What are we doing that for? What are we mixing with what? What are we testing the plasma with enzymes? Are we testing the red cells with enzymes? How do we go about doing this? What's the purpose?

Jan: Okay, so when we're using enzymes in our serologic study, what we're doing is taking fairly common proteases, usually ficin or papain, both of them are commercially available as individual enzymes and also manufacturers in some cases offer a pretreated panel, so you can purchase your panel untreated and treated. So the enzymes have actually removed a lot of the negative charge, the sialoglycoproteins off of the red cell, and that takes a lot of the negative charge off the red cell. Well, what's the negative charge for? The negative charges keep the red cells apart from each other. So when we have something that shaves off these structures that are carrying much of the negative charge, then it allows the red cells to get closer together. And this can help in cases where then the antibodies that can then bind onto the red cells more easily.

In some cases they allows them to cross-link, and so you can get some antibodies showing up a little bit stronger because the antibody and antigen reaction can take place. The red cells aren't being held apart by the negative charges. We see this a lot of benefit in the tube test because then we can actually make some antibodies agglutinate that normally only react by the indirect antiglobulin test. So that's a benefit to tubes, but

it's still, it can enhance reactivity of some antibodies. Antibodies in the Rh system, the Kidd antibodies, other antibodies that you may not want so much like Lewis's and P1s, cold autos. All of those things can be enhanced on those enzyme-treated cells and you recognize some you want and some you don't want. So we can use that to enhance antibody reactivity.

The other thing that enzymes, ficin and papain, can be used for is to destroy antigens. So there are some antigens that if the antigen is on a structure that is cleaved off by the enzyme treatment, then that antigen is going to go away. So you can use that to help look at an unknown plasma, and if you have a reactivity with an untreated cell and then you test the same cell enzyme treated and the reactivity goes away, you might think of an antibody in the Duffy system or an MNS antibody, sometimes s. Those kinds of antibodies might be present in your plasma.

So we're treating the red cell, testing the plasma versus comparing an untreated cell and an enzyme-treated cell. Have you enhanced reactivity? Have you decreased reactivity? And those can give you clues as to what kind of antibodies might be present in your sample.

Some cautions are that enzymes ficin and papain mostly can greatly increase warm autoantibodies also. So you may be getting an increase of that reactivity. It can enhance cold autos. It's very important to perform autologous controls, ficin-treated autologous controls, if you're going to use this technique because you want to make sure that any increase in reactivity is related to an alloantibody and that it's not just increasing the reactivity of an autoantibody also.

Joe: Okay.

Jan: So there's some cautions and some tricks you have to keep in mind, but they can be very helpful in either enhancing an Rh antibody in particular, probably most common in routine work. And I agree. I think it's maybe an underutilized technique.

Joe: Yes.

Jan: Especially when you can purchase cells that are already pretreated.

Joe: Just to kind of reemphasize, we're treating the red cells with these enzymes and we're anticipating some fairly predictable impacts on different blood group systems, as you mentioned, some enhanced, some decreased, maybe some like Kell that don't do anything but-

Jan: Correct.

Joe: ... some variations there that can help us sort out. Am I hearing you correctly that one of the places that you use it often is when you have multiple antibodies and you're trying to gauge the pre enzyme and post enzyme to see which goes up and which goes down?

Jan: Exactly. And kind of seeing does the reactivity go away and then you think Duffy, MNS systems, is it enhanced? You think Rh system very frequently. Kidd, as long as it doesn't look like all of a sudden everything's positive and you've enhanced maybe a cold or warm auto.

Joe: Right. And that's what you were saying, the reason that you want to look at the autologous control that's enzyme treated because that will, my assumption is, let me again, I'm making sure I'm summarizing you correctly, that if all of a sudden everything is, you're not seeing a whole lot of positives, all of a sudden everything lights up and the autologous control also lights up, that's probably enhancing a warm auto.

Jan: Exactly.

Joe: Or cold auto.

Jan: Or cold. It's just giving you a point of comparison to evaluate what's happening if you get increased reactivity with your enzyme treated cells. Thanks for clarifying.

Joe: I'm hopeful that I'll get a chance to talk to you again about and dive into some more antibody ID techniques at another time, Jan, but it's my belief that especially when people are learning antibody ID, that the autocontrol is one of the most forgotten things that people just forget to look at it when they're especially on exams and they just don't look at it and evaluate it when can there be, and there can be so much useful information there.

Jan: Oh, I totally agree. And we could. We could do a whole episode on the value of the autocontrol and what you can get out of it and how you compare it to a DAT to understand what it might be actually going on. So yeah, let's talk about that in the future.

Joe: All right. I'm marking a note to self, “talk to Jan about that later on,” so I'm going to hold you to that. All right, so enzymes that... Thank you. I think that really helps. Let's move on a little bit and talk about another technique that reference labs use a lot and I think it holds a lot of mystery to folks in most transfusion services and that is adsorption. They hear us talking about things like autoadsorption and oops, we can't do autoadsorption, we have to do alloadsorption and their hair catches on fire because they're not sure exactly what we're talking about.

Jan: Yeah.

Joe: So let's just kind of run through it a little bit from the start. What's the basic overriding principle with adsorption? What are we trying to do when we say we need to do an adsorption?

Jan: So an adsorption is used to take an antibody out of the plasma. So adsorption means that you have a, let's say, a solid and you're going to stick something to it. So we have our red cells, whether they're the patient's own cells in an autologous adsorption or someone else's red cells in an alloadsorption. And we're going to mix the plasma that contains an antibody in it that we want to get out of that plasma.

Joe: Got it.

Jan: And so we mix that red cell sample and this plasma together and we want an antibody, hopefully, it's one we actually know what's going to happen, but we want something to get stuck onto that red cell because it's an antigen antibody reaction. So you're coating a red cell with an antibody from the plasma and then if we centrifuge that mixture and take off the supernatant plasma, the absorbed plasma, we would hope that that

antibody is gone. It's a technique we use to take something out of the plasma by sticking it on- taking an antibody out of the plasma by sticking it on to the red cell and then-

Joe: Like soaking it up with a sponge…

Jan: Let's not go soaking.

Joe: Okay.

Jan: Because that's *absorption*.

Joe: Ah. I see. I'm glad you said that.

Jan: When you do a sponge kind of thing, the antibody's not going into the red cell like water would go into a sponge.

Joe: Got it.

Jan: That's absorption. *Adsorption* is when you put something on the outside of a surface.

Joe: Perfect.

Jan: So we're taking the surface of our red cell, putting our antibody from our plasma coating the surface of that red cell with that antibody and then it's a way to separate antibodies out of a mixture taking or take antibody out. We most frequently use the technique to remove autoantibody, warm autoantibody could be cold but frequently warm from a patient's plasma. So then we can look like underneath that warm autoantibody once we've gotten rid of it and see if there's any alloantibodies that might be present that would affect what kind of red cells need to be transfused to the patient.

Joe: And for the learners, why do autoantibodies need to be removed to kind of see what's underneath? Why do autoantibodies confuse the picture?

Jan: Well, autoantibodies are reacting with antigenic components that are basic to virtually all red cells. And so that's the picture you get when the serologic picture you get is that all of your cells or virtually all of your cells in your routine panel or antibody screen, and then a panel will be positive because that autoantibody is reacting to a target antigen that every red cell carries, including the patient's own. And so you can't see the reactivity that it might be due to an alloantibody that's there at the same time, especially with very strong autoantibodies. And so you have to do something to remove that reactivity. You want to take away some of those layers of reactivity that the autoantibody might be causing. Remove it from your plasma and then test that plasma again to look for an anti-E or little c, or whatever might also be there, but you just can't see it because the warm autos, you can't see the reactivity caused by the anti-c, let's say, because the reactivity caused by the autoantibody is just covering it up.

Joe: Yeah. That makes total sense and I think that again, not just for learners but really just to reemphasize that for everybody is so important because obviously we see autoantibodies and as you put it, they obscure things behind it, but the stuff behind it can be in terms of the clinical importance to the patient, far more important than

transfusing someone with a warm autoantibody in most cases, right? Somebody with a warm auto and an anti-c or an anti-K or something in the background, that background thing is going to be way more important to them clinically usually than the autoantibody.

Jan: Certainly, that's going to affect the type of cells that you select for transfusion.

Joe: Right.

Jan: You still need antigen-negative units even though the autoantibody might make them incompatible because you're definitely going to have those units cleared in a way and the clinicians still have to deal with the autoantibody present in the patient and what else might be going on there, but we want to prevent destruction of any transfused red cells by alloantibodies.

Joe: And I wasn't meaning to imply that warm autos are unimportant clinically, obviously they are important clinically and in patients that have warm autoimmune hemolytic anemia, that's obviously an important clinical thing, but for us in terms of choosing the red cells I guess is what I was going for, that's massively important.

Okay. So somebody hears, we have a, let's just say we're working in a hospital transfusion service. We identify what looks to be a warm autoantibody, all the cells are positive. We have a panagglutinin with a positive autocontrol, send it off to the reference lab. From there, I think from the hospital perspective, things can get a little mysterious and they often are wondering, well why the heck does it take so long to do these adsorptions for crying out loud? So can you just, high level, walk us through what does a reference lab have to do an adsorption and further as part of that, how do reference labs decide whether they can do an autoadsorption versus having to do an alloadsorption?

Jan: Well, that's the first question the reference lab will always have to address. And that's why your reference lab may be so demanding to say, "Are you sure? Check the transfusion history" because if there are circulating donor cells in that patient sample from transfusions that have happened in the last three or four months, those donor cells have antigens on them also.

Joe: Yes.

Jan: Let's say if you have circulating E-positive cells, so you've got your autologous cells, you've got circulating donor cells that are E-positive and let's say your patient has a warm autoantibody and an allo anti-E. Okay, if I do an autoadsorption, air quotes here, because it's really not autologous cells, it's a mixture of donor cells and autologous cells. If I put those two pieces together, the donor E-positive cells could actually bind the allo anti-E that's in the patient's plasma as well as binding the autoantibody.

So the autologous cells will never bind an alloantibody, that's by definition. They're always antigen negative, but circulating antigen positive donor cells might bind alloantibody and you would never know it's there. So that's one reason why in a patient that's been transfused, we can't use the patient's blood sample to absorb out the autoantibody. If the patient has not been transfused, we can use that cell, that

autologous patient sample. However, if those cells are coded with antibody, because it's an autoantibody, they've got a positive DAT.

Joe: Right.

Jan: So part of the process is to treat the cells with frequently a reagent called WARM. It's a mixture of enzymes and DTT and that will strip off the autoantibody from the patient's red cells and give more antigen sites available for us to do this adsorption technique and put that antibody back on the patient cells in vitro so that when we perform our separation centrifugation that the autoantibody can be gone.

Now, it sounds like it's a one-time-one and done thing, but frequently you have to do this, you take an aliquot of patient cells, put the plasma on them, incubate them together, centrifuge them. The autoantibodies, some might be gone but maybe not all of it. So you have to take another aliquot of patient sample, do it again, incubate it again for another half an hour or so. So there's lots of centrifugation, there's lots of incubation, and then there's the testing that has to come along. So certainly a warm autoantibody workup, even when you're doing an auto absorption can take well into the six or eight hour timeframe potentially depending upon how strong the warm auto is, how many cycles of adsorption you have to do to remove it, so.

Joe: Yeah, not just a five-minute process for sure. Right?

Jan: It definitely isn't.

Joe: Yeah. One thing I just want to make sure that the audience is clear on, when you say recently transfused, is there a cutoff for that? How recent the transfusion had to have been?

Jan: We usually use three months. Some places I know go four months based on the length you would expect a donor cell to circulate in the patient's be in the patient's circulation, but those donor cells aren't always the freshest you've got.

Joe: Makes sense.

Jan: A variety of spread of age of cells. And so we usually use three months as a cutoff.

Joe: If you are in a situation where you have a patient who has been recently transfused within that three month timeframe and you have to do alloadsorptions as opposed to autoadsorptions. Are there any, again, just for interest of time, we probably don't have a time for a ton of detail on this. I know there's a lot of pitfalls in doing alloadsorptions and I know reference lab techs when they find out they have to do an alloadsorption go, "[Horrified sound]." So what are, I guess, the big things that you worry about when you're having to do an alloadsorption opposed to autoadsorption?

Jan: Oh, boy. Well, one of the things and let's clarify for the listeners that alloadsorptions are using somebody else's red cells and so they carry not only the target antigen for the autoantibody, but they, like the one that's common to everybody's red cells, they also carry all of those antigens. They've got a red cell phenotype. They may be C-positive and Jka-positive, K-negative.

So in addition to a adsorbing out an autoantibody, those cells can actually absorb out alloantibodies that the patient may have. So you really can't use just one cell, one adsorbing cell because you might be- you know, you have got to know what that donor cell that allo adsorbing cell phenotype is. So you can anticipate what alloantibodies might be removed. And so then we have to absorb with a second example of an allogeneic red cell from a donor that would leave that alloantibody.

It's very difficult to explain in just words and my hands that nobody can see, but you have to consider that each of those adsorbing cells will remove autoantibody but also has the potential to remove alloantibody. So we actually will do three separate adsorptions, three separate adsorbing cells, which is an adsorption sequence in itself and then have to test each of those alloadsorbed plasmas.

Joe: Right. For people that are learning, the take home message is it's not something you're going to get within a few moments once you send it to the reference lab, it takes a while. Especially in a recently transfused patient, it's a process and in the interpret... We didn't even get started on the interpretation, which is challenging mentally as you do the gymnastics of this antigen that's on these cells and that's my hair goes on fire when I start doing that.

Jan: I will tell you that doing alloadsorptions and for a number of purposes we usually use alloadsorptions for removal of warm autoantibody, but they can be used in many different ways. That process is the absolute last thing that I teach reference lab tests is because-

Joe: I believe it.

Jan: Because your brain will explode otherwise.

Joe: And you might lose them during the training if you teach it first.

Jan: Yeah. Yes, exactly.

Joe: Okay, well so thank you. Thank you so much for that, Jan. Let's move on and talk about another really big one. And it's a technique that often goes along with adsorption, but that people, especially as they're learning, have a tendency to mix up and that is the wonderfully named, I love saying "elution." Elution just makes me happy to say it. I don't know why. I'm silly that way, but what about elution? How does elution fit in? How is illusion different from adsorption?

Jan: Well, I'd have to say there is different this night as day because adsorption is putting something on a cell, a red cell, and elution is taking something off of a red cell. So we use this in the hospital setting. It would be most frequently used if patient has a positive DAT and then you say, well, what's causing that positive DAT? You would look at then the monospecific antiglobulin reagents to tell whether it's complement coating the cells or IgG antibody coating the cells.

And then if there's IgG antibody on those cells, you would proceed then to wonder, okay, let's take it off, the red cells and see what it is. So that's exactly what an elution procedure does. It removes the antibody off. The red cell then allows you to take that

eluate is the sample that you're testing to see what reactivity might you see in that antibody or in that eluate. And where did it come from? It came off of the red cells in that blood sample you're testing.

Joe: Yes.

Jan: So if you have an autoantibody, if your positive DAT is being caused by an autoantibody, you would expect to see all of the cells when tested with your eluate to be reactive because again, that autoantibody is directed at an antigen that is present on virtually all normal red cells.

On the other hand, if your patient might be having some kind of a transfusion reaction, maybe that antibody that's causing the positive DAT is only coming off of the circulating donor cells. Patient cells are E-positive. They are, excuse me, the patient's cells are E negative, they've made an allo anti-E circulating E-positive donor cells are present. And so when you make this eluate off of this sample, then you might detect the anti-E coming off of the donor cells that is frequently then used to help substantiate a diagnosis of a transfusion reaction.

And that being the reason why the DAT is positive, the patient may be clearing red cells. So that technique is used to see what's coating red cells.

Joe: Yeah.

Jan: It's important also, just to make... I didn't want to infer that we only do eluates in the presence of a positive DAT due to IgG because sometimes in a very, very recently transfused patient, if you have an antibody, again, those Kidd antibodies that we talk about most frequently, you might only be detectable on the red cells because of the complement that they bind. Kidds are great complement binding antibodies.

Joe: Right.

Jan: So maybe there's not quite enough IgG anti-Jka to make the DAT with anti-IgG positive. But all you're seeing is the complement that this antibody binds. So then in a real recently transfused patient, complement only is a reason to do an eluate if you don't say, "Oh, I don't need to do an eluate because there's no IgG there."

Joe: Right.

Jan: You still want to do that eluate and on occasion, it's not going to happen all the time, but on occasion, you might pick up an antibody like a Kidd antibody that is only coating the red cells and you can't really quite see that antibody even in the plasma yet.

Joe: Wow.

Jan: So I don't know. I mean I just wanted to clear up by, I said if it's IgG, we're going to do an eluate.

Joe: Yes.

Jan: That's most of the time. But there are certain clinical situations where you want to do an eluate even when you only see complements on the cells.

Joe: So would you say Jan, would you categorize an eluate as concentrating an antibody?

Jan: Yes, because of the way that you're taking it off of a very limited red cell sample. I mean, I don't really understand the mechanics of exactly why it all is considered a concentration technique. But yes, an eluate can concentrate the antibody and you may see it's a very weakly few antibodies coating the red cell surface. You can detect them better in an eluate because of its ability to concentrate antibodies.

Joe: Makes sense. And I guess what we're partly talking about is the DAT reagents, the anti-IgG and a DAT reagent has a threshold of detectability, right?

Jan: Mm-hmm.

Joe: I forget what the number is, but a certain number of antibodies coating the red cells below that, it's not going to be positive, but getting that into the tube, maybe you can see it that way is what you're saying, I think.

Jan: Exactly. Exactly. Yep. I think it's maybe 500-ish or less molecules of IgG on the red cell before you can see it. Depending again on what technique you're using for doing that DAT.

Joe: Okay. One other thing I wanted to hit before we move on to our last topic that we're going to hit together is the kind of weird scenario. And again, go as much or a little detail as you feel like talking about this if you feel like talking about it at all. But what about the scenario where you have a positive DAT and say it's positive DAT with IgG but the eluate is negative. You talked about before the DAT is negative for IgG, but you get, maybe you find a Kidd antibody. What about that confounding situation where the DAT is positive with IgG but the eluate is negative? What do you make of those situations?

Jan: Well again, it could be a concentration, but there's a lot of other things that can stick to red cells besides blood group antibodies. So albumin, you could actually have IgM and IgA sticking to red cells, that depending upon the formulation of your anti IgG reagent, you might pick up a little bit of weak reactivity if it's not heavy chain specific. But there are many things that can stick to red cells in vivo that you will not recover in an antibody area, not recover in an eluate. And that's informative. That's informative in itself, I think.

Joe: Okay. Okay. So obviously possible and it's something we, at another time, could go into greater detail on. But I do want to hit this one last thing and I know you wanted to make sure we talked about this and I think especially because in recent years we've seen the rise of medications like daratumumab, we're seeing a whole lot of more use of DTT, Dithiothreitol, in reference labs and in hospital transfusion services. I'm seeing a lot of hospital transfusion services that over the last few years have said, "okay, I've got to figure out this DTT thing. How do you make this? How do you get it? What do you do?" So why don't you walk us through Jan, a little bit about DTT and if there are any other substances that act in a similar way to DTT, why are we using those, but maybe both historically and nowadays?

Jan: Great. So DTT is one of my favorite blood bank tools because there's so many ways you can use this chemical. I'll tell you. And let's just get right out of the way. I'm only going to say DTT, but there is another chemical that I'm not going to say except in its abbreviation.

Joe: 'Cause it's like that long. It's like super long.

Jan: Yes. 2-AET. And I was really going to try hard to learn how to say it just for you, but I didn't. And so understand that DTT, Dithiothreitol and AET, you can take the information about them pretty much interchangeably. AET smells worse than DTT and DTT smells bad enough. But I'm just going to be talking about DTT and if you are an AET fan, you can substitute things there.

Joe: There you go.

Jan: So DTT, what we term, is a sulfhydryl reagent and it's pretty indiscriminate. What it does is every time it sees a disulfide bond, it breaks it. Okay. And that's all DTT does.

Now there's many places in what we do where these bonds might exist. So we can use DTT to destroy antigens that are on red cells. We would take a test red cell, treat it with DTT and any of the antigens that rely on are any of the structures that rely on disulfide bonds for their confirmation like Kell system antigens. That's a big one we think about. There's some other blood group system antigens, Cartwright system antigens that require these disulfide bonds to be intact for the antigen to be have it shape and they're therefore the antibody react with.

So if we DTT treat red cells, we make those antigens, we destroy those antigens and the antibody can no longer react with it. Another thing that another compound on the red cells or structure is CD38 and we know that's the target antigen for the different dara reagents, daratumumab. And so that's really where the use of this and the awareness of this reagent has just skyrocketed in the last five to seven years is because this circulating anti CD38 is going to react with every red cell basically.

And if we take these red cells and treat them with DTT, then we're destroying the CD38 antigen on the red cells and we can then mitigate this anti-CD38. We make it so that it doesn't react, so that the red cells aren't going to react with it any longer. We're not actually changing the antibody, we're changing the antigen on our test cells and then we can go ahead and use these treated cells to detect many but not all of the antigens that might be or that might be directed that a patient might have antibodies directed at.

And most notably is the Kell system. All of the Kell system antigens are destroyed by DTT treatment. So that's why in either a reference lab report or in the hospital when you're doing this, if you're in a hospital you're doing this treatment of the red cells, you always, unless you know the patient is K positive, and can't make anti-K, you have to transfuse K negative red cells because on your antibody screen or your panel cells, you will have destroyed that K antigen.

At least one of your cells on your screen is always big K positive.

Joe: Sure.

Jan: You will have destroyed that antigen and therefore those cells will no longer detect an allo anti-K that might be in the patient's plasma. So that's the caveat of using those DTT treated cells.

One thing about DTT is it's not commercially available as a prepared product that I am aware of. You have to buy the powder and make up the solution yourselves. It's very, very easy. Dissolves real quickly. We make a 0.2 molar solution and then use that to treat the patient's red cells. So that's one common use. Aside from the patients who are receiving daratumumab therapy, it's a great tool in antibody identification. If you have an antibody that you don't know where the specificity's going, and we use it most frequently in those antibodies that appear to be directed at an antigen of high prevalence. So it's reacting with all the cells you test except the patient's own cells. Okay, where do I start? There's lots of antigens on those cells.

Joe: Sure.

Jan: I'll take some test cells or a reference labs technology. We'll take the test cells, treat them with DTT and see if the reactivity goes away. A lot of times it doesn't, but if it does, man, you know that you've made your path a lot easier.

Joe: Nice.

Jan: Because it may be in the Kell system. It may be an anti-Cartwright. It may be a Lutheran system antibody, it may be a Dombrock system antibody. So out of all of our blood group systems, we've narrowed it down to just a couple that we might begin to work with. It's frequently paired in the reference lab setting. We'll take cells and we'll treat them with DDT and we'll treat the same cells with enzymes because I spoke earlier about those antigens that might be destroyed by enzymes.

There are some others that are of high prevalence that can be destroyed. Some of the garbage system and gosh, I'm blanking right now, but Cartwright is also destroyed by enzymes. So you put together the information from DTT treated cells with the information from your ficin treated cells and at least it can give you a guidance of a path to go down to resolve this potentially very difficult antibody identification.

Joe: Nice.

Jan: That's probably one of the most common uses of DTT in either a hospital setting or in the reference lab setting.

Joe: Jan, honest to goodness, I can't believe how the time has flown. I'm fascinated with the stories, with the explanations and I just so appreciate you taking the time to help all of our listeners understand a little better some of these special techniques. And I am so looking forward to connecting with you again and doing a little bit more discussion about antibody ID because there's definitely more that we could talk about. So again, thank you so much for doing this.

Jan: My pleasure. There's so much to talk about. It's so fun.

Joe: Thanks so much. Talk to you later.

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Joe: Hi everybody, it's Joe. Thanks for sticking around for just a minute, and I'm really glad that you did, because, as I mentioned at the top of the podcast, I want to give you the opportunity to win a free copy of the book that I mentioned at the top of the interview. The book is called "Antibody Identification: Art or Science." It's written by Jan and two of my other favorite authors in transfusion medicine, Sue Johnson and Sally Rudman. And this is a, just a tremendous book that will help you learn so much about antibody ID, especially if you're someone who's just learning about it. It's a really, really magical book, and I'm thrilled that AABB Press and the AABB, as well working with Transfusion News and WileyHealth.com, are offering this book to you absolutely free for at least two of you.

We're going to do a drawing, and that drawing is gonna happen on July, Friday, July 14, 2023, Friday, July 14, 2023. You may ask, "How can I get this book, Joe, how can I enter this drawing?" Well, the great thing about this is that it's really, really simple. All you have to do is go to Wileyhealthlearning.com/transfusionnews, which is where you normally go to get continuing education. And go ahead and do the continuing education module for this particular episode, 102CE.

Now, I know that some of you're saying, "Oh, wait a minute, Joe, I'm still a student. I'm still a resident. I'm not someone who needs continuing education yet, so I never do the CE!" Well, this time, DO IT! Go ahead and go there. The registration is free. It doesn't cost you anything. You can go ahead and go through as if you were needing the CE.

If you complete the module, you are automatically entered into the drawing. Again, thank you so much to the AABB for allowing us to give away some copies of this truly amazing book.

Again, you can also go to BBGuy.org/102 and take a look at Jan's bio there and some of the resources that have placed on the show page. Also, I always appreciate those of you that go to Apple Podcasts regardless of where you get this, but the rating system on Apple Podcasts is the one that people use the most. So if you go to Apple Podcasts, give this podcast a rating and a review, I see every one of those and I learn from the things that you guys tell me. So thank you so very much for all those of you that have done this already, if you haven't done it for a while, go ahead and do it again. It would really be helpful to me if you, if you wouldn't mind.

I have a lot more fun episodes to release in the near future, including one that was supposed to be released prior to this one, but just had some technical difficulties. That one's called, "So you want to be a blood banker," and I think that's gonna be really helpful for those of you that are interested in blood banking on the laboratory side, potentially as a career. So a lot of great stuff coming. That one coming very soon. I can't wait to share all of that with you, you guys.

But until then, my friends, I hope that you smile, have fun, tell the ones that you love just how much you do, and above all, never, ever stop learning! Thanks so much for hanging out with me. I'll catch you next time on the Blood Bank Guy Essentials Podcast.