BBGuy Essentials 050CE:
Pretransfusion Testing 1 with Sue Johnson
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Joe: Hi, welcome to episode 050CE of Blood Bank Guy Essentials, the podcast designed to help YOU learn the essentials of blood banking and transfusion medicine. My name is Joe Chaffin, and I am as always, your host. 50 episodes! I can't believe it! When I started this podcast in 2016, I don't think I had a clue I would be able to keep this up, and that so many of you all around the world would listen. I want you to know, I'm very grateful to each of you for listening! I've got a special gift for you, so keep listening.

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Now, the giveaway! I am truly grateful that we have three copies of the 31st edition of AABB Standards to give away, that's GIVE AWAY, to three lucky winners. There is only one way to become eligible to win: Sometime in the next two weeks, before May 11, 2018, simply complete the continuing education activity at wileyhealthlearning.com/transfusionnews and you will be entered into the drawing. I will announce the winners on the next episode. If you are listening after May 11, you can still complete the continuing education for credit, but there will be no more books to give away! Finally, if you are a student or a trainee, and you don't need continuing education, you can still do the activity as if you did, discard the certificate at the end, and you will also be eligible. So, once again, that's wileyhealthlearning.com/transfusionnews!

Today I get to interview one of my favorite people! For my landmark 50th episode, I wanted to have the brilliant and awesome Susan Johnson from BloodCenter of Wisconsin here. Sue has been on the podcast before (episode 28), and today, she is starting with part 1 of a two-part interview on Pretransfusion Testing. Sue is going to guide us through the maze of regulations and processes that we simply HAVE to get right! Enough introduction: Here's my interview with Sue Johnson.

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Joe: All right, everyone, I can't tell you how excited I am to have Sue Johnson back on the podcast! Sue is the director of clinical education for BloodCenter of

Wisconsin. She was with me for my most popular podcast so far, which was Episode 28, on the DAT. Sue, you're here!

Sue: Yes! I'm excited to be here!

Joe: Whoo! The crowd goes wild! I'm so happy to have you back. As I said, I think what you did for us before with the DAT was super-popular, and it shows just, a) How good you are (and I don't want to embarrass you... Yeah I do!), and b) Just how much people really want to hear the basics and the essentials of what we're doing. So we are doing SUCH an important topic today, and that is pretransfusion testing. And we want to walk everybody all the way through the process of what we do in blood banks, what should be done at the bedside in terms of getting a patient ready for transfusion, but before we do that, Sue, I think it's really important that we keep all this in context. So, a lot of people that aren't necessarily laboratorians listen to this podcast. And you know, you and I have both been around, we can we can speak frankly about this. I would say that a lot of people that aren't laboratorians have a tendency to think of the attention that we pay to detail and all the little nuances of "oh if I don't get this right. Oh I have to redraw," all that stuff. They may have a tendency to think, or ask, "Why does the blood bank get so worried about little things in this process?" Can you give us some context on that?

Sue: Oh, sure, I talk about that all the time, and absolutely we've had feedback on that. It's like, "You guys in the lab, you know, you're too anal! Why do you care so much about this?" And I have friends that are nurses, RNs, that will go around in circles on things. But, the thing is, the reason that we have to pay attention to that is because when a sample comes into the lab into our hands, we have to ensure that everything that's within our control is accurate. That we are believing that the right patient was drawn. So we're going to be very particular on ensuring that everything that we can see matches, that it IS the right sample, the right labeling and everything. And then from there, our testing we'll do meticulously, and that's part of good laboratorians, that we pay attention to all of those details in the way that we do the testing. And then when it comes time to the labeling, exactly the same kind of process and steps that we go through. And you know, blood bankers are, I think a special breed as far as, their true attention to detail is probably second to none. Somebody might disagree with me, however...[laughs]

Joe: I'm with you!

Sue: But there are people, right, that are laboratorians that are like, "I don't want to do blood banking! That's not for me." But I think, from our perspective, we work diligently to ensure that everything is accurate that's within our control, and if it's not accurate and we can see that, we're going to be "bulldogs" in ensuring that everything is accurate that we can see.

Joe: Absolutely! So are you saying it's not because we... You know, if you're a nurse, and you send a sample, and we make you redraw, it's not because we don't like you? Is that what we're saying? [laughs]

Sue: Exactly! [laughs]

Joe: OK, all you nurses and clinicians out there, mark my words: It's not that we don't like you! We like you guys, I promise you that!

Sue: Absolutely. I have great friends that are RNs and clinicians, absolutely!

Joe: I think that clinicians and clinical staff sometimes have a little bit of a misperception of what we do. But, let me ask you: When we talk about pretransfusion testing, we have a decent number of organizations that are looking over our shoulder with that, right? Can you tell us about, just real quickly, some of the organizations that want to make sure that we're doing things right?

Sue: Oh absolutely. So from the hospital perspective, The Joint Commission. The Joint Commission, every year for I don't know how many years now, has had patient safety goals around sample identification, patient identification, and they'll specifically even highlight blood transfusion. In addition, we have the FDA who's watching and has standards around the testing that we do for pretransfusion testing. And then, most of the labs are accredited, either through the College of American Pathologists (CAP) or the AABB. And again, very strict standards, essentials, elements that we're required to follow. So, it's not just that we're worried about making sure that all our details are in order and everything's accurate, it's that all of these organizations and accrediting bodies and regulators are very interested in how we do these things and what we do.

Joe: Sure. So here are the five basic steps to pretransfusion testing. We want to take you through this in pretty decent detail, though, obviously, because we're covering a lot, we have to stay pretty high level. But those five steps are:

1. The request and sample process
2. Testing that we do in the laboratory
3. Product selection (covered in part 2 of this interview)
4. Compatibility testing (covered in part 2 of this interview)
5. Product labeling (covered in part 2 of this interview)

So, Sue, let's jump into it. You ready to go?

Sue: I'm ready.

Joe: All right! Here we go. So, step one: The request and sample process. So let's just start off with the basics of someone making a request for a transfusion. What do they have to do?

Sue: All right. So, from the perspective of that first request, we NEED a request! It can be oral, it can be electronic, or written. However, even if it's an oral request, it has to be documented. And I think the majority of labs now, or hospitals, will have electronic ordering, which is wonderful. But if not, there has to be some documentation. The information, then, within that order, that request, must include information to identify the patient. And this is one of those things that the regulators, accreditors are looking at, and that is, we have to have two patient identifiers. It should include patient's first and last name, but then, some sort of medical records number, or separate blood bank number, or any variation of that. Each hospital, each healthcare facility, is going to define what those patient identifiers are for their facility. And then, the order also needs to include the product requested. What blood product? Is it a leukocyte-reduced red blood cell, a platelet order, fresh frozen plasma, what have you. Also needed is special requests, special requirements. So does the blood need to be irradiated, or is CMV-negative desired, or leukocyte-reduced if you don't have 100% leukocyte-reduced. And then, finally, we need to have the ordering physician. That is really important, especially when it comes from the CLIA perspective. We need to have documented, "Who's ordering this?" Very important!

Joe: Right. Okay. So, from the perspective of the requests--a documented request that includes the two patient identifiers, as you said, and tell us what you want, basically, right?

Sue: Exactly. Yep.

Joe: So the next step, generally speaking in that request and sample process, is someone going and drawing some blood from the patient. Is that a process that's---I don't want to say it isn't important because it's obviously important---but why do we care so much about that? Why do we care so much about the steps that people should take around the actual drawing and labeling process of the blood?

Sue: That's a super-important question, or the reasoning for that is super-important, because we know every year, if you have a fatality within your facility due to transfusion, we're required to report that to the FDA. And every year there are reports that there are fatal transfusion reactions that are due to patient misidentification or sample labeling errors. Because of that, it's really important that we have a process that is really precise in the way that we get the samples labeled.

Joe: So has anyone---well you mentioned Joint Commission earlier. Have they weighed in on this process at all?

Sue: Oh, absolutely. The Joint Commission has National Patient Safety Goals, one is just around identifying patients, correctly just in general. And they say, "Use at least two patient identifiers when providing care treatment or services." And then, also, labeling containers used for blood and other specimens IN THE PRESENCE of the patient. And I know I've had lots of conversations with nurses, and you know, been on little audits and things where they'll walk out the door and then label the sample, and you're like, "No, no, no! In the presence..." "Well, I can see the patient." "Yeah, but you left the room!" (laughs).

Joe: Yeah. You know, that one is one that I have, as a blood bank physician a lot with my friends in anesthesiology, who like to tell me when they send an unlabeled sample to the blood bank. "Well, I can tell you what sample it was. I was there." I'm like, "You can look at a BLANK blood tube and identify that as the tube you drew? Really? Seriously?"

Sue: Right. [laughs]

Joe: Well, amazingly enough, they believe they can! So we'll just go on from that [laughs]. I'll leave that uncommented upon! So one thing that you mentioned, I want to make sure that people are clear on. So, generally speaking, well, pretty much universally I would say, people in the hospital that are going to have their blood drawn are going to have an I.D. wristband on. They're going to have something around their wrists that identifies them. How does that play into the process of when the phlebotomist comes in to draw? I mean, if they got they've got their wristband on, that's great. That's all they need, right? They just need to check the wristband and go.

Sue: Yeah. Well they should! Right, so what they should...[laughs]

Joe: [laughs] Little bit tongue in cheek there!

Sue: Exactly! So, that is really important that they are matching the request with the patient, so that they're looking at the wristband to ensure that patient name, first name and last name, match their request. That they have a second identifier, whether again, it's the medical records number or a separate blood bank band number. And you know, if they had the date of birth, that's another possibility. But it's really important that they check that with the patient at that time. The wristband, if the patient is aware, it's also good practice to ask the patient, "State your first name, last name, date of birth," at least, because they can tell you that.

Joe: When the phlebotomist draws that tube from the patient they do all their identification and identification confirmation, they're using the two patient identifiers. What needs to go ON the actual tube?

Sue: They need to then label the tube, at the bedside, with again, the patients first and last name, their medical records number, blood bank number (if they have it), the date of birth, any sort of barcode labels or RFID tags or whatever they might be using within their system. And there also needs to be an identifier, at least a way for the phlebotomist to be identified. So it could be initials, it could be an employee number. That needs to be on the label. And then the other thing that needs to be there is the date of collection. And some people also put the time or that will also be recorded, but the date for sure.

Joe: Well, maybe I should have asked this previously, but if you're a phlebotomist and you're getting ready to go into somebody's room to draw them for a "blood sample," how do they know what kind of a tube to pick up? Red top? Purple top? What do they do?

Sue: Right. You know that's interesting, too, because every hospital will have their specimen requirements for the tests that will be performed. Sample requirements for blood banks have kind of changed over the years. So, with manual test tube methods, it was a red top clotted tube, and at least one EDTA tube. Now, I know, some people are moving away from the clotted sample, the red top tube, and using more EDTA because of the automation we have available. And then, you know, so we just may have to make sure that we match the requirements that are necessary for the lab doing the testing. But the other thing is, when they draw the samples, they have to ensure as best as possible that it's a good draw. That the sample's free of hemolysis, for sure. Because, the reason that we want it free from hemolysis is, if the patient would have a reaction after transfusion, that's one of the things we check is to see if there's the presence of hemolysis. So, we would want to ensure that if we see hemolysis, it actually is from the transfusion reaction versus a difficult draw.

Joe: So, let's move on to talk a little bit about something that, I'll be honest, I just had a question about this two days ago from one of the hospitals that I work with, and that's the whole question of, "How long is this specimen "valid" or "good?" It's such an important thing, and honestly, I fear that there's still some confusion about it. So help us understand this, Sue. How long can you use this specimen that you've gone to all the trouble of drawing, how long can you use it to do all of the work that we're going to describe in a few minutes for that patient?

Sue: Sure. So it's defined really by a number of our standards again, the AABB standards, CAP. They state that if the patient has not been transfused or pregnant in the last three months, there's no requirement on the age of the sample. However, practice is, if there's any uncertainty (which is pretty much the norm, right? I mean, we don't know for sure, absolutely), then the sample must be less than three days old. Now the tricky part comes in, is that the day of collection is considered "day 0."

So, if I had a sample of John today on the 15th, I actually will have until---today is Thursday, right? So I would have until midnight on the 18th, So...which is Thursday, Friday, Saturday, Sunday. So I actually have almost four days of time on that sample.

Joe: So it's like *bonus* time, right? It's not really like 72 hours and that's part---I'm sorry to interrupt you, Sue---but that's part of the confusion that people have. I've had clinicians say to me, "Well why does it expire at midnight? That's so artificial." And I try to explain to them, "We're actually HELPING you by having it expire at midnight!" And that's just kind of, let me make sure I understand that, Sue. That's just kind of come into play as a relatively standard practice. That no matter what time of day it's drawn, that first day is day 0 and it expires on MIDNIGHT of Day 3, right? Pretty much everyone uses that?

Sue: Yep! Pretty much everybody does. I mean, I know of some labs that will expire IT at a different time in the day, just because it's better for their staffing, or when they want to do their inventory (their daily inventory). I mean, I've known of labs that have actually expired cross matches at 6:00 in the morning because they want to do their inventory. But then to me, they just lost like what? 13 hours that they could have had on a cross-match. But yeah, I would say the great majority is midnight of the 3rd day, with day 0 being data collection again. So it is almost four days that you have.

Joe: The question that people ask about this, Sue, of course, and I'm sure you know what's coming, is, "What's so magical about three days? Why the heck did we pick that?"

Sue: Yeah, exactly.

Joe: And again, just to be clear, I'm sorry for that, Sue. Just to be clear, what we're talking about is someone who has been pregnant or transfused in the last three months or if you don't know. Again, why is 72 hours or three days magical?

Sue: Interestingly in my career, I actually remember when it was 48 hours or two days! I remember us being super-excited when we got to go to the third day, because we could get through the weekend. Especially being in an immunohematology reference lab, if a sample was drawn on Friday it would get us to Monday. The days, historically, come from the fact that if somebody is to make an antibody to a transfusion or to pregnancy, that would give an individual enough time to produce an antibody to a level where we could detect it. As far as I know, though, there is no study that actually has ever been done to validate that, the 48 hours (or the two days) vs. three days. It kind of reminds me of the "30-minute rule"...

Joe: The "magical" 30-minute rule?

Sue: Yeah, from when you have to return blood to the blood bank. It really comes down to, you want to test AFTER the patient makes an antibody. Now, is three days a time when you are going to see a patient making a new antibody? There is one study that actually looked at... it was a retrospective study that was done, and I'm blanking on the author right now, but they looked to see how fast did the antibody level drop, or how many antibodies could we potentially be missing? Looking at when we usually test patients after transfusion, and I think the number was like 113 days, which means that we would only detect 30% of antibodies, which is interesting.

Joe: As I recall, Sue, the only thing I have in my files about this is, I think it's a retrospective study that Ira Shulman published in 1990 in Transfusion suggesting the 72-hour thing, but beyond that, I can't find anything, as you said, that prospectively studies it to see that we're still doing the right thing! But we're doing it! That's what we do, right?

Sue: That's what we do, and that's what the regulators, and that's what the accreditors say we have to do!

Joe: That's the key thing! OK, so we've gone through that, the "72-hour rule" (which is really "72 hours PLUS"), so let's ask you this: What about...oh, I have to ask you this question because you mentioned that without that pregnancy or transfusion thing, there's no specific upper limit. So I get this question all the time from hospitals: "Well, if there's no upper limit, how far should I set it? Should I set some specific time, or should I just let it go on for months?" How do you answer that question?

Sue: Well, one of the ways that I answer it is, "What does your package insert say for the reagents that you use?" Actually, if you really look at the package inserts, and it's an EDTA tube, it's 48 hours from when you should test! I mean it's kind of scary, actually. And then the second part of that is then, well how long would a sample actually be good for to test for the presence of antibody? I mean that's really the thing to me that would be the most likely to deteriorate. So, if you do your crossmatch on day 21 after draw, is an antibody that was there earlier really going to last that long? I mean, you don't know for sure. Or, if it's complement-dependent, any complement, at least in a serum tube, would be totally denatured by that point. So there isn't a good answer, but I think most people are in the... We actually asked this question last year at the AABB meeting to see what people said about presurgical samples. When you want to hold them? Patient comes in ahead of time, and then how long are you going to use your sample for? And it was anywhere from 7 days to 30 days were the responses. If I'm remembering correctly, most were in the 14 day or so range.

Joe: Yeah, and so it sounds like there is... I generally say what you said about package insert as well, but also, you know, it's one of those kind of locally set and locally done things. There's no national standard for that for sure.

Sue: Correct.

Joe: OK. So one more quick question before we from the specimen stuff and that's this: How long are we required to keep that sample AFTER the transfusion occurs?

Sue: So after, we are required to keep for at least 7 days. Just because, again, if there's a transfusion reaction that's called, or another sample is drawn, and we see something new, we want to be able to go back and test that sample from the transfusion.

Joe: Actually the last question before I leave this, is what do the folks in the blood bank have as their responsibility when the sample comes in, the request comes in, what kind of things do the people in the blood bank have to do immediately, just in terms of that sample?

Sue: Right. So, immediately when it comes in, they're going to, again they're going to check their request for transfusion to verify patient name, first and the last name, they're going to look at the sample and make sure it's not hemolyzed or overly lipemic (I know if the patient's lipemic, you can't do a lot about that). But, they will look at that and make sure that they have the right samples, and that they look good. But then, the other thing is really just that all the identifiers are accurate and they match the request for transfusion: Patient first and last name, medical record number, blood bank number, is the date there, identity of who drew the sample (or a way to identify who drew the sample). But they will be, they should be, they're trained to be meticulous on accepting the sample. And if there's any discrepancy, they're going to have procedures in which they're going to ensure that they check to see why there's a discrepancy in any of that identifier information.

Joe: Got it. And that's the point where sometimes, well, let's just say, "Disagreements can occur." Right? (I'm trying to be polite)

Sue: Absolutely. And I always tell the students, the new grads, or the soon to graduate medical lab science students that, "OK, they're going to know you're new! Don't buckle!" [laughs]

Joe: Right! [laughs] It's true!

Sue: Be strong!

Joe: Blood in the water. New blood...I hear you. OK. So we have taken a pretty deep dive into the request and sample process. That was step one. Step two has a lot of stuff to cover as well, and that's lab testing. OK, so we have our sample, we have our properly labeled and identified sample with our request and everything that comes into the blood bank. Why don't you just take us quickly through what are the things that the blood bank does in terms of testing those samples?

Sue: Sure. So the testing is pretty straightforward. I mean the most important thing is that ABO type, and we will always do a "forward type," so we're going to test with anti-A and anti-B that's known, so we can determine if the patient's an A, an O, or a B [or AB]. And then we'll also include in that ABO type, a look at the patient's plasma or serum, and make sure that they have a corresponding anti-A or anti-B (which comes into play later when we talk about compatibility). But we just have to make sure that that forward and reverse type match, or at least we can explain why we might see a discrepancy in the typing (because of a transplant or something like that). The other piece that goes right with the ABO type is an Rh type looking for the presence or absence of RhD. So determining whether you're Rh positive (you'll have the D antigen present), or Rh negative (and you don't). And we're only required for pretransfusion testing to do a direct test or an "immediate spin" test. We know that individuals can have varying degrees of D antigen, and we are okay in pretransfusion testing to be a little more lenient and maybe call an individual "Rh negative" when they might actually have some weak antigen present, and that's a whole 'nother podcast to talk about! .

Joe: It is, absolutely!

Sue: So we stop there. And then finally we do an antibody detection test or what everybody calls an "antibody screen." So what we're doing is looking at the patient's plasma to determine if they have any additional antibodies beyond their "normal" ABO antibodies. So we know there's over 300 antigens on the red blood cells, and so there's lots of different possible antibodies an individual could make.

Joe: OK, we're going to spend a decent amount of time on the antibody detection test and what that means. But before we leave ABO and Rh, there's something that has come up, well, very recently, this podcast will be released in April. We're recording it in late February, and Sue, at this point, there's a whole lot of discussion and angst and hand-wringing and concern over a standard which will be in place from AABB by the time this podcast is released. But it is specifically Standard 5.14.5., which states, and I've covered this in a previous podcast that was just released (with Pat Ooley, the chair of the Standards Committee, everyone, so there's more on that), but I wanted to get your take on it Sue. It basically says, that new standard essentially says, that there have to be two determinations of the recipient's ABO group (and this is new for AABB). And you can either do that by testing a second current sample (in other words, a separately drawn current

sample), comparison with previous records, or retesting of the same sample under certain conditions. I want to get your take on it, Sue. Again people will already have to have this in place by the time this podcast is released, but how do you feel about how people might meet this standard when it's going to be a change for them?

Sue: So I think the Standard is a good change actually. And the reason that I believe that is because of the fact that I know many transfusion services have been moving toward having a second sample draw when you have no record of the patient. And there's a number of studies now that have shown that, and reports that have shown that, it's greatly reduced the "wrong blood in tube" (the wrong patient being drawn), because, again, as meticulous as the lab is, we don't control the phlebotomy. And this is a way for us to ensure, "Oh wait, this patient typed as an A, and now we got a second brand new-drawn sample, and they're really an O!," or whatever it might be. So I think the second sample draw has been implemented in many organizations. I'm sure it's going to cause angst in the ones that haven't. But I think what the other thing people are seeing is the fact that there were a lot of times when there were near misses, like the wrong sample was drawn, but we were fortunate, because the ABO types were OK, (you know, they were compatible). It wasn't an issue. And I think the other thing, too, is it's only on the patients that we have no record of. It's not every patient.

Joe: That's really important. I agree.

Sue: Yeah, yeah. I mean it's going to take... I know it's going to take people time to implement. But, for those that have implemented it, I know there's pain points. But it happens, I mean you get through it.

Joe: So let's move on to talk about the antibody detection test or the "antibody screen," as people will also call it. What are we trying to do with that, Sue? What's the goal of doing an antibody detection test?

Sue: Well, the goal is to detect as many clinically significant antibodies as possible. And "clinically significant," generally we mean those that are reactive at 37 degrees, at body temperature, and those that we have history on, that we know about that are ones that have caused transfusion reactions. Another goal, then, is to detect this as few of the INsignificant antibodies as possible, which gets to sensitivity of a test. We don't really care about detecting "cold-reactive antibodies" that many people have, because they aren't going to cause any trouble in the body when a transfusion occurs because we're at body temperature. And then the other goal for any antibody detection test is that we can do it quickly, that it gets done in a timely manner, because everybody needs their blood!

Joe: Sure, of course. OK, so detect as many important ones as we can, avoid the unimportant ones, and do it as fast and as accurately as possible. I guess I'll throw in the accurate part, which I knew was implied.

Sue: Absolutely!

Joe: So just functionally, from the practical perspective, how do we DO that? What do we add to what to try and detect these significant antibodies?

Sue: OK, so, when we're going to get our patient sample then, we've worked with the red cells doing the ABO and the Rh type, and a little bit of the plasma to look for those "expected" ABO antibodies, but now we're also going to then take the patient's serum or plasma, and we're going to test them with additional cells that we call "screening cells." And they're usually either from a set that has two donors where we've defined the phenotype or we know the phenotype of the donor, so for example we know they have the D antigen and maybe K, and Jk^a, and listing off, of course, all the antigens that we test for. So that would be one donor, but then we need a second donor in the set that would be D negative (Rh negative). So this set matches very nicely in the fact that some of the cells from a donor will be positive for one thing, and negative for some, and then the next donor we test will be negative for some things, and positive for others, so that we can ensure that we're going to be able to detect any or most unexpected antibodies that a patient might have. And usually, again, it's two or three cells, cells from two or three donors. And the FDA actually tells us (or tells the manufacturers of our "screening cell sets" is what we call them), they tell us, "You must include certain antigens on those cells, including the common D, C, E, c, e, and so on."

Joe: What we're talking about here is detecting non-ABO antibodies, right?

Sue: Correct.

Joe: So how do we KNOW that ABO is not an issue with these screening cells that we're using?

Sue: So yeah, that's a good question. So these screening cells are ALL GROUP O...I know, so we don't have to worry, because we all know group O red cells are the "universal donor." So we can test any patient with these group O cells.

Joe: OK, Sue, so you mentioned the antigens that the FDA requires to be represented in the screening cells in an antibody detection test. I think we need to just mention them real quick, because you know people that are taking exams, and you train SBB's, I train pathology residents, they may get asked this, so break them down for us real quickly. What kind of antigens are we we talking about?

Sue: So in the Rh blood group system: D, C, E, c, and e, that's what's required. In MNS, it's: M, N, S, and s. The P1/Pk system, we need to know about P1. Lewis system: Le^a and Le^b. And the Kell system: K, k. Duffy blood group system, antigens we need to know about are Fy^a, Fy^b, and Kidd system: Jk^a and Jk^b.

Joe: But just for clarity, Sue, the antibodies associated with all those would not all be considered clinically significant, would they?

Sue: Yeah that's a good question. So there are several here that are not ALWAYS clinically significant, but there are rare examples where they are. So the ones would be like anti-M, anti-Le^a, anti-Le^b, generally not significant (-Le^b, I don't think has EVER been reported in an acute transfusion reaction situation). Anti-P1, another one: Super, super-rare to be significant, but there's a possibility. And there are also antibodies that we see. You also should err on the side of caution.

Joe: Okay, Sue, so those 18 that you've just that you've just mentioned are obviously important, and obviously the FDA feels strongly about them and we've talked about them. But I'm curious: I look at a panel (I have a panel up on my computer screen in front of me right now); there's WAY more than 18 on this thing, Sue! Come on! What's going on? Why do we have to have so darn many of these things on the panel? What's the deal?

Sue: That's a great question. Sometimes I ask that myself! Because sometimes you find things, you're like, "Ugggghh!" So for example, there are some antigens in the Rh blood group system: Cw, f, that are often or always represented on an antibody identification panel. Another in the Rh system is V and Vs. And the reason that those are indicated is that we see patients that make antibodies to those, and they can be significant especially, for example, anti-Cw. However we don't go out of our way looking for anti-Cw, but if we find it, we're going to respect it. And you know there are other antigens on the panels as well, and as good blood bankers in the laboratory, we want to be complete and thorough in our investigation, and sometimes we do find people that make antibodies to these other antigens. I mean, like we talked about before, there's over 340 now "officially recognized antigens," and that's crazy! And the reason they're recognized as a "blood group system" is the fact that somebody has made an antibody to them. So we do our best to detect as many as possible and to identify as many as possible.

Joe: Sue, one of the things that I hear people talking about when they talk about antigens that need to be represented on the antibody screen is, sometimes you hear people say things about, "Well, such-and-such antigen should be represented in a 'double dose.'" And I think most people know what we mean when we say that, but just for the learners out there that aren't totally clear on that, what do we mean when we talk about a "double dose?"

Sue: So, when we talk about double dose, some people think that in older terminology, and that we want cells from a donor that would have a homozygous expression of the gene, that would then result in double dose the antigen present on the screening cells. I know we've moved to talking about "double-dose"/"single-dose" a lot, and some of the techs learned "homozygous"/"heterozygous" get a little confused about that. Totally understandable, because we've moved that direction. When we talk about double dose, though, the reason we talk about it from a screening cell perspective, or a panel, is the fact that those cells will have the strongest expression of the antigen, or they've inherited two genes that will code for the same thing. So for example, two Jka genes will give us double the amount of Jka antigen or Jka protein on the red cells. The reason we want that is because if my patient makes an anti-Jka, it could be weak. We also know that those are the antibodies that fall below the level of detection. So we would prefer to have screening cells that would have double the dose of the Jka antigen to make sure that we detect the antibody, because those antibodies are famous for showing dosage. So, those antibodies showed dosage, they will react preferentially with somebody that has double the amount of antigen.

Now, the challenge when we look at screening cells and panel cells is for the manufacturers to find a "perfect" set of screening cells that would have double-dose expression of every antigen. And that's what some people feel is like, the best! And I guess I would say, "Yes, that would be wonderful." But I've also had the experience of making my own panel, designing my own panel, and I know realistically, that's not practical. It's really difficult to find a donor or two or three donors that would have the perfect match, the perfect set, where every single antigen is expressed in double-dose. And so what we have to do is think about, "Well, what are the antibodies that are most likely the ones that will show dosage?" And it's, you know, the Kidd (-Jk^a, -Jk^b), -Fy^a can, more commonly antibody, -Fy^b can. But then you also look at, "Well, what are the antibodies I SEE more often?" So Fy^a! You see that way more often than anti-Fy^b. And also, we look at another one that people sometimes ask is anti-K. Anti-K generally doesn't show dosage. It's a strong expressed antibody, it will react very well, and that's one that we usually are very comfortable with saying, "I'm OK" if my cell has a single dose (which means it's K+k+).

Joe: All right, so let's move on. So, we've talked about the theory behind antibody detection. Let's get practical on this. We have, I know, a lot of options for how we do this, and this is going to vary from place to place, but in general the options for the different platforms for how people can do these antibody detection tests.

Sue: I kind of break them up into three different platforms. So, one is by test tube methods, which would include the traditional, "Don't add anything except for the patient's serum with screening cells, and then incubate at 37^{o}C." Or we can add something called "LISS," which is a low ionic strength solution, or "PEG," which is

polyethylene glycol (actually usually in a LISS diluent). But those three things are basically done the same way from the perspective of, the test is in a test tube, and we incubate at 37 degrees, and then followed by an antiglobulin test which we can talk more about...

Joe: Since it's like your favorite topic! Of course we're going to talk about it! [laughs].

Sue: The second method or platform is "column agglutination testing." And in the States, our column agglutination testing is mostly micro gel beads. So mostly it's gel test, but once you get out of the United States, there's a lot more column agglutination testing that's performed using glass beads. Similar set-up, but different in the way that, at least what's in the cassettes, in the cards. And then, the third general method is "solid phase," where the red cells or stroma from the screening cells are actually effaced to the bottom or attached to the bottom of a microplate well. So, it's test tubes, it's a column, or it's the solid phase on a microplate. The beauty of the column and the solid phase assays is that they really lend themselves well to automation.

Joe: I want to make sure before we leave the whole work that we do before we start the testing thing. The steps that blood bankers need to take before they actually start running these tests in terms of in terms of checking what's been going on with the patient previously can you. Can you take us through that very quickly what are the things that we have to do.

Sue: Absolutely. It's a super important step. Before we do anything, well, we do our sample ID, but the next thing is that we compare or look to see if we have any previous records on the patient. So some people call it the "history check." Have we seen the patient before? Then, that helps us verify ABO/Rh type. So if we've seen them before, there's our second ABO, because we're going to do another one now with the new sample that came in. We're looking for the presence of antibodies. Did they have antibody before? Because that's really important, because sometimes they had an antibody, and then it falls below the level of detection in the new sample. And then finally, again really important, is are there any records that indicate the patient has a need for special blood requirements, like irradiated is one that always pops into my mind as being really important. Or age requirement, or less than seven days old or whatever it might be. And the other thing too is just to say, "Did they have any trouble the last time even just doing the ABO type or Rh type. So, really important step, and really helps the lab scientist in evaluating the current sample.

Joe: OK! So, now Sue, I know that the antiglobulin test is your favorite thing in the world. And everyone, Sue does a magnificent job taking us through how the direct antiglobulin test is done and all of the details of it. That's at BBGuy.org/028. Now,

Sue, I'm going to limit you now! I'm sorry, but I'm going to limit you! So, you've got like a very short time to thumbnail the antiglobulin test, and most importantly... Well actually let me ask you this first: One of the things you did NOT mention when we talked about the list of things we do for pretransfusion testing was the direct antiglobulin test. And that's important, because I think a lot of times people assume that the DAT IS part of routine pretransfusion testing, and we covered this on Episode 28. But, can you tell us, a) Is DAT a routine part of pre transfusion testing? b) If not, why not?

Sue: OK. DAT (direct antiglobulin test) is not, is NOT part of routine pretransfusion testing in MOST facilities. Well, we used to do an "auto control," which was taking the patient's serum or plasma, testing it with the patient's own red cells, and it was kind of indirectly telling us that the patient would have a positive DAT. And so, we would get a positive, we would do a DAT, and then we get this result, and we didn't know what to do with it. Because we know, and I think we talked about this before, that 20-30% of certain hospitalized patient groups have a positive DAT. So, we did a LOT of work, and clinically, it didn't matter to the patient. So, I think it was in the mid 80s, there was a push to say, "Let's quit doing this testing!" Today, it's probably just as, or it's probably more important, as we look at test utilization. So it's not done.

Joe: OK so, the antiglobulin test that we use in pretransfusion testing is actually an INdirect antiglobulin test. And so, again, I have to limit you (sorry), help us understand how the IAT fits into the pretransfusion testing that we do.

Sue: The IAT, the indirect antiglobulin test, is critical and actually REQUIRED to be able to detect these clinically significant antibodies. The test is an "indirect" test because what we are looking for is the presence of IgG antibody that the patient has, that binds to the screening cells, the antigens on the red cells. The problem is we can't visualize IgG antibody, I mean, the test can't see it. If we just centrifuge the red cells like we do with an ABO, we won't get any kind of clumping or agglutination. So in order to visualize the fact that the IgG antibody's bound to the screening cells, we have to add the antihuman globulin reagent, or what many people slang call the "Coombs reagent," to determine if there is IgG antibody that bound to the screening cells. And that's what we call an indirect test, because direct test, we're testing the patient red cells; the indirect test, we're testing the screening cells, looking for the antibody in the plasma attached to the screening cell.

Joe: And is it fair to say with the different platforms that we discussed and the different ways that we do the antibody screen, that no matter what the platform is, essentially the key test there is the IAT?

Sue: It is. Every assay has some form of it, where we're adding an antibody to the IgG antibody to ensure that we can see it. Yeah, it's kind of hard to just talk about it; I'm visualizing it in my brain.

Joe: It's okay. Word pictures! I like it!

Sue: Yeah, exactly! But the key thing is you have an antibody to an antibody, and we need that antibody to an antibody (the antiglobulin reagent) to see the patient antibody stuck to the screening cells.

Joe: Okay so everyone, we don't have time today to go into all the intricate details of how we do the tube testing, how we do the gel testing, how we do the solid-phase testing. However, if you look, you can "YouTube the heck out of this," and you'll see a lot of examples, including something that I did previously on YouTube. Just look at the Blood Bank Guy channel where I talk about some of those methods. We don't have a ton of time to talk about that, Sue, but I would like for you to help us understand some of the advantages and disadvantages of those different platforms. So just in general, for tube testing, gel testing, solid phase testing, can you take us through some of the reasons why labs might choose to do those and might have some concerns about doing those.

Sue: Tube testing is the traditional method, whether you use LISS, or PEG, or no additive. That method is... it still, to me, when you're going to problem-solving is the "gold standard." And the reason for that is because we can test at different phases of reactivity. We can see if there's an IgM antibody versus and IgG antibody, because we can test at immediate spin through to the antiglobulin test. We know how certain groups of antibodies react weaker right in a tube test, whereas some of the other technologies, they might as show it as 2+ or 3+. We don't get the variability. Disadvantage of tube testing, of course, is it's VERY subjective. It is reliant on the laboratory scientist to gently shake a test tube to shake in the tube so that we can get the red cells to dislodge from the bottom and see if there's clumping or agglutination. It's the interpretation of that result. So, you really need well-trained lab scientists doing that testing. But it's sensitive! I believe it is truly sensitive, and it is specific for the most part. I mean, PEG is probably the least specific but most sensitive, which usually goes hand in hand with any test method; the more sensitive it gets the more likely you're going to pick up other junk.

Joe: Well I think the evidence of that in many ways is that in just about every reference lab, in fact, I can say, in EVERY reference lab I've personally been associated with, all my techs LOVE PEG! That's their favorite deal.

Sue: Me too! I love PEG. We've actually always made it in house and it just works wonderfully. I always say, "It'll take REAL antibodies and make them stronger!" Right. Whereas like the ones you're not quite sure about, it kind of doesn't do much to it.

Joe: Yeah okay. Okay so. So that's pros and cons of tube testing. What about gel testing?

Sue: So gel testing is a great method as well (Oh, they're all great methods). The advantage of gel is that we're looking for the agglutination to occur within the beads, and there is no shaking. There's nothing that the technologist, the lab scientist has to do, other than add it, incubate it, and centrifuge. And they're looking, again, they can look to see if the cells are agglutinated within these beads. So there's some subjectivity in the reading, but you don't have any "technique subjectivity", right, the how you do the test. The other advantage is it's very standardized. We don't have to do any washing steps which we do the tube methods. The reactions are stable, which is nice, because the third shift tech could have done the testing and said, "Aah, I'm not sure," and leave it for first shift to take a quick look (as long as the patient doesn't need blood). So, it's stable. And, you know, it does enhance some antibodies, particularly, it's kind of famous for enhancing Rh antibodies, which is good and bad, especially if you have a patient with passive anti-D, but it's good. And then, automated. This is one of those platforms that is, and actually was the FIRST platform that came out licensed, FDA-licensed, that we could put the cards on an instrument, and it would run the whole way. Disadvantage is that it's a long centrifugation time. So, that comes into play when we're talking especially about ABO/Rh typing. A tech, trained lab scientist, can do the testing for an ABO in about five minutes. Whereas gel tests, it's going to take a 10 minute centrifugation, then reading so it's probably 15 minutes has passed, actually. And then, of course, it's got some false positive reactions, and may not detect some weak antibodies. Some of the weaker antibodies that, say for example, PEG would detect.

Joe: OK but it's obviously a very popular platform. I think I'm accurate in saying that it's the most popular current platform in non-reference lab transfusion services and blood banks.

Sue: Yeah I think it keeps shifting, you know the solid-phase, because they had great automation, made a big surge, and now gel/column seems to be coming back more. But, from my experience in traveling internationally, the column agglutination is by far the most popular. By FAR!

Joe: OK well what about solid phase. I am on record as being a long term big fan of solid phase. So tell me what's good and bad about that platform?

Sue: OK so solid phase again, it's absolutely automatable. I mean, actually, that's the BEST way to run it on one of the instruments. So it's got an objective endpoint, it's a standardized procedure again just like column agglutination. It's VERY sensitive. We know that in laboratories that have switched from say a tube method to solid phase or a gel method actually [to] solid phase. It's particularly good at

detecting anti-Jka and anti-Jkb. It's very sensitive to that and probably, now, it's hard to know why. In tube method, you can guess, because they're weak antibodies. In gel, it's just probably the design of the test, who knows? Nobody knows exactly. And, you know, it's fast, the automation has been really solid. Disadvantages is that there are a number of false positives, but there's false positives with column as well.

Joe: Of course!

Sue: I always say that! And it's real sensitive at detecting autoantibodies, like gel, column is. And yeah, there's a number of different... Actually WE did one study looking at solid phase and positive unexplained or unidentified positive reactions to look at demographics of the patient. And the only thing we saw was there was an association with autoimmune disease, and women. Which not surprising, right? More likely immunized as well as their immune system's fired up.

Joe: OK. So, let me ask you for your feelings on this, and I will tell you what I say when people ask me, "Which platform is best? Blah blah blah." I personally say that there are pluses and minuses to each, as you've outlined. And there is NO perfect platform. There are holes, potential holes, in every platform and nothing is absolutely perfect. Am I steering people the wrong way, Sue? hat do you think?

Sue: Right on. Totally, totally say the same thing. In fact, the only other thing I would add is the fact that there is no method that will detect every antibody, and being in a reference lab setting for many years, and seeing it today, there's SO much variability and we always say, "The antibodies don't read the books!" I mean, we say, "Oh -,Jka and -Jkb are great in solid phase." But there's going to be one that reacts best in PEG! And I always say, too, if there was one method that detected everything, we'd all use it! [laughs]

Joe: Absolutely right! For sure. And that and we're not going there any time soon. OK, so we've talked through some of the high-level details on the different platforms that we can use to do this antibody detection. We've talked about the ABO and Rh testing on our recipients. Before we leave lab testing, which we've spent a while on, one of the things I think that people just learning miss is what we have to do to those donor red cell units that come in. So help us with that, Sue. So, you get a shipment of red cells from your blood center, and you're there in the transfusion service. What do you have to do for those?

Sue: So we are required, actually SOMEBODY is required to do an ABO and Rh verification on Rh-negative units. So we're required to do this before the transfusion. The majority of the time, this testing (and we call it the "ABO retype," or "ABO/Rh retype") is done in the transfusion service. And the requirement is that we must take a segment or link or pigtail (people call them all different things), but one

of those segments that is integrally attached to the unit that's labeled, and then verify that it is really an A, B, AB, or an O. We have a little leeway in the way we do our testing (a tiny bit). So, for example, for A's, B's, and AB's, we have to test with anti-A and anti-B, but with a group O, we can type with just anti-A,B, which if it's negative, it's good! That's what we want. And then, for Rh-negative units, we're required to test with anti-D, just to verify that the unit is Rh-negative. Because, you know, everything that the blood centers do, the blood collectors do to ensure that the unit's labeled appropriately, there are periodic times when there is a unit that is mislabeled. I mean there are not many but they happen.

Joe: As a current blood center medical director, I would say, "NOOOOOOO! What are you talking about?"

Sue: NEVER!

Joe: That would never happen! Well, for obvious reasons, it rarely does, but when we got to make sure that we check that.

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Joe: I've kept you longer than I intended, so we are going to break this into two parts. Sue had so much great stuff to share that I just couldn't stop! We will cover the last three parts of the pretransfusion process: Product selection, Compatibility testing, and product labeling, in part two of this interview, coming soon.

Until then, as always, I hope you smile, and have fun, and above all, never, EVER stop learning! See you next time on the podcast!