BBGuy Essentials 051:
"Product Choice and Compatibility Testing (Pretransfusion Testing 2) with Sue Johnson"
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Joe Chaffin: Hi, everyone, and welcome to Blood Bank Guy Essentials, the podcast designed to help you learn the essentials of transfusion medicine. I am Joe Chaffin, and I'm your host, and I'm very glad you're here! Today's episode is the conclusion of a two-part interview I did with the magnificent Sue Johnson on pretransfusion testing. Together today, we are going to discover how to select products for transfusion to a patient, how to test those products for compatibility, and talk a little bit about how to label the products.

Before we start, you should know that this is NOT a continuing education episode! Those continuing education episodes are brought to you by TransfusionNews.com, and Transfusion News is brought to you by Bio-Rad (who has no editorial input for this podcast). If you would like to find episodes that do offer credit (for physicians and laboratorians), just look for episodes ending with the letters "CE" at BBGuy.org/podcast, or visit wileyhealthlearning.com/TransfusionNews.

Last episode, we had a contest to give away three copies of the 31st edition of AABB Standards. We did have three lucky winners: Maha AlJohani, Lissa Shirley, and Emmanuel Fadeyi. Congrats to all three! We hope to have more giveaways in the future; I think it would be fun! Thanks to AABB and Wiley Publishing for providing the copies of Standards for the contest.

On to today's interview: On episode 050CE, I started a two-part interview with my friend Susan Johnson from BloodCenter of Wisconsin. In the first part, Sue discussed sample requirements and serologic testing on patient samples (antibody detection tests, in other words). I realized when I was editing the last episode that there was just TOO MUCH good stuff in there, and I had to cut some things that I didn't really want to cut. There was one discussion, though, that I just couldn't get out of my head, so I decided to include it here at the start of part 2 of the interview. We'll just jump right in to the section about testing our patients for antibodies:

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Joe: Sue, this really seems kind of obvious, but I think maybe we need to really specify it. We've talked about the laboratory testing that we do, and we talked about the antibody screen. We don't have time in this podcast, nor is it my intent to talk in detail about antibody identification, but just bottom line this for me: If the antibody screen is positive, you go and do the antibody identification process, the "panels," as we as we talk about them, and you identify a particular antibody. What does that mean for the patient in terms of future decisions?

Sue Johnson: So, when we identify an antibody, first thing we'll always look at is, is this an antibody that's known to cause hemolytic transfusion reactions or hemolytic disease of the fetus and newborn. In regard to transfusion, if we determine that this is what we think is a "significant" antibody, we will provide blood that is antigen-negative or lacks the antigen to which the patient has an antibody to. So, we've been talking about anti-Jk^a. If my patient has now a newly identified anti-Jk^a, we will give Jk^a-negative units henceforth. Now the patient will

always need to receive Jk^a-negative. And that should be indicated, for any nurses and physicians who might be listening, that should be indicated on a separate tag, or on the compatibility tag; that information will be there if your patient has an antibody. We want you to look for that information, from our perspective.

Joe: Absolutely. And that's true from now until forever, right? Regardless of whether we can still identify the antibody?

Sue: Right. And actually, that's a question sometimes we get, too. You had a previous anti-Fy^a, but that antibody is not detectable now. We still are going to provide Fy^a-negative blood for any future transfusion. So, once you have an antibody, from henceforth, again, even if it's not detectable, you'll receive antigen-negative blood.

Joe: That's hugely important, and again, for those of you that are nurses or physicians listening to this, if your patient discloses to you that in the past, they've had "trouble getting blood," or they "have an antibody," or something, it's important to explore that, and to let us know in the blood bank, because, if we haven't tested the patient, we may not know that information, and you can really save your patient some trouble by helping us to investigate that.

Sue: Great point! Absolutely.

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Joe: OK, now we will pick up the interview where we left off at the end of episode 050CE. Sue and I have discussed sample requirements and patient serologic testing, and now we are ready to roll on. Be sure to listen all the way to the end, because Sue takes on 6 cases that really illustrate some tough issues that can come up in compatibility testing. Enjoy!

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Joe: We've got three more to go, and we can do these fairly quickly, I think. The first is "product selection." And a lot of this, I mean it goes back to "Blood Bank 101," but why don't you just quickly take us through it in terms of, for red cells, how do we select products in terms of in terms of ABOs, Rhs, etc.?

Sue: Okay. So, I think the most important thing to remember is our definitions of "universal donor" and "universal recipient," because we all learned this, right? And we're looking to ensure that we're ABO-compatible. And we all learned that a group O is a universal donor, because there's no A or B antigens on those red cells, so we can give it to anybody. But the important thing that we all remember (and I always stress this, especially with my RN friends that don't have to think about this all the time) but the fact that it only applies to red cell products. Because when we talk about plasma, it's totally flipped around, which we can get to. So, group O can go to the A, the B, or the AB, and it's all good. But if we look at other products, it's going to be different. So, when we select, from a practical point of view, if my patient's an A, my first choice should always be A (or B to a B). But if we have to, or there's a special need, we can give group O. And then, of course, on the other side, AB's have always been considered (we all learned) "universal recipients," but again only from the red cell perspective, because they have no antibodies. They have both antigens on their cells, they can get blood from any type.

Joe: So Sue, sticking with ABO for just a second (we'll get to Rh in just a moment), sticking with ABO, you mentioned that the rules are somewhat "flipped" with plasma. Can you outline that for us?

Sue: So, for plasma, when we talk about plasma compatibility, now we're thinking about, "What antibodies does the DONOR have in their plasma?" So, we need to ensure that the antibodies that are present in the plasma will be compatible with the patient's red cells, what antigens are present on the red cells. So, in the case of "universal donor/universal recipient," an AB is the universal donor because they have no antibodies. They have both A and B on their red cells, but they don't have anti-A or anti-B. So that plasma can be given to anybody, As, Bs, or ABs, and from the blood center perspective, we feel that, because we're usually looking for ABs. And by the way I am an AB and I donate plasma! [LAUGHS]

Joe: Nice! So, I knew there's a reason we're friends. You're a special donor and so am I. You're AB and I'm O neg.

Sue: There it is!

Joe: So we both had to be blood bankers. There was no choice for us!

Sue: Exactly! Perfect. So AB can go to anybody. And then the other thing we have to think about is that A plasma and B plasma actually can go to group Os, which is a little bit confusing, because we have to remember that an O has anti-A and anti-B, so they can get plasma from an A, because they have anti-B, no big deal; or they can get plasma from a B, because they have anti-A. Again, no big deal.

Joe: Let's save our discussion of ABO with platelets until just a little bit later, because things get even more interesting there, and I want to talk about ABO and Rh of platelets together. For now, lets just discuss Rh selection requirements for red cells and plasma. In fact, **l**et's get this out of the way first: What about Rh with plasma? Do we need to worry about Rh with plasma at all?

Sue: No. No problem with plasma. I mean, it's labeled with the Rh type, but we don't have to worry about it.

Joe: OK, good deal. So, we DO, obviously, have to worry about it with red cells. So take us through the thought processes with red cells and Rh.

Sue: OK. So with the red cells, the reason it's important is because if you're Rh-negative and you're exposed to Rh-positive units or red cells, there's a good chance you can make an antibody. And studies are different, now "volunteers" (volunteers, I like that) from the studies that were done...

Joe: Quote-unquote.

Sue: Yeah, quote-unquote, but they showed that, the numbers say 50 to 80% of these volunteers that were Rh-negative receiving Rh-positive blood would make anti-D. And lots of

studies actually have been done in that area because of producing Rh Immune Globulin, actually. It's kind of interesting to look at all that data. But on a PATIENT perspective, there's a couple different studies that have been done looking to see if you're Rh-negative and receive Rh-positive blood you're more likely in about the 30% range or so to make anti-D, for many reasons, probably (you know, the patient's immunosuppressed, or their disease state, or whatever). But nonetheless, there's a good chance that if you are transfused with Rh-positive blood, you can make anti-D. Now in a guy, it's not as big of a deal, but in a woman that's Rh-negative, then we're going to worry about the potential of her making the antibody, and then having a baby that would be affected with Hemolytic Disease of the Fetus and Newborn (HDFN) later on. So, when we look at compatibility then, we're always going to...**if we have an Rh-positive person, our first choice is Rh-positive**. We don't want to waste those rare donors like Joe [LAUGHS] with Rh-positive [patients]. Our second choice though, however, is Rh-negative. And we really should be reserving those Rh-negative units for emergency situations. And, absolutely, when we don't know, that's the product, Rh-negative is the product of choice. But, whenever we know, we should give Rh-pos to Rh-pos. Then if we look at the Rh-negative compatibility, or the individual that's Rh-negative, really our only choice for women, and the standard, some people have different ages, but **generally it's women under 50, should only receive Rh-negative blood**. I've heard people go up to 55 so I guess...

Joe: Yeah, me too.

Sue: It's possible...

Joe: It would be impressive, though, I have to say...[LAUGHS]

Sue: It WOULD be impressive [LAUGHS]!

Joe: Sorry.

Sue: But, then you'd have those children for a long time. And then, as far as Rh-positive is our second choice to an Rh-negative male or female. And, we can give Rh-positive the first time. We have to remember that; that's important: We can give Rh-positive blood to anybody the first time, before they've been immunized, and there's going to be no problem at all. And, in an emergent situation, it's better than not giving blood. But, in guys, we're not as concerned if we need to go to the Rh-positive blood. I can tell you my experience is, we do everything possible to give women Rh-negative. In the guys a little bit less lenient, but we have wonderful Rh-negative donors, so we're kind of spoiled.

Joe: Yeah, but I think that's also, that can sometimes be, I don't want to say "geographic," because that's not completely accurate, but depending on the time of year, depending on... Well, again we're recording this in February. We've just been through and are in the midst of a horrible flu season and horrible weather in places like where you live, for example, that has decreased somewhat our O-negative "cushion" of supply. And there have had to be some really hard choices made about giving Rh-negative people Rh-positive blood, because there's not as much Rh-negative blood to be had in certain situations.

Sue: Right. Right. Absolutely, and we're all looking at that, too. You know, how is O negative blood being used?

Joe: For sure. And that's totally a topic for another day. But man, that's why we get upset. I don't use the word "upset" very often, but we do get upset when people waste Rh-negative blood, and O negative blood in particular, when something else could be used, because there is such a dramatic need! And as an O negative donor (again I'm on the "soapbox"!), as an O negative donor, use it when it's appropriate, and let us use it for someplace else where it's needed if you're going to use it otherwise.

Sue: Absolutely. Actually, and I always, when I meet somebody, and they tell me they're O negative, I always apologize, because I know they get called all the time [LAUGHS]

Joe: That is for sure. All right Sue, for platelets, you know, there's a lot that we could say about ABO and Rh compatibility for platelets, but that in and of itself is probably another episode, because there's a lot that we could go into, and honestly, there is enough disagreement about this that I'm probably going to get blasted no matter what I say. I do just want to talk about general philosophies, general practice, anyway. From your experience and mine, I guess the most basic question I would ask you is, you've just talked about the rules for ABO compatibility for transfusing red cells. How do you feel about, or what you see in practice, for how people cross those ABO boundaries with platelet transfusions?

Sue: That's, yeah, it is a tough question. So, what I see, and what everybody's procedures will say is **always give ABO-specific as your first choice**. So, if your patient's an A, give an A, an O, give an O, etc. However, we all know the challenges in keeping our adequate platelet inventory. So, when you look practically, there are lots of places that will give ABO-incompatible if they absolutely have to. So, of course, you can give an 0 to an A, but you have the plasma that's there. Some would encourage volume-reducing the platelets, so trying to remove some of the plasma from the platelets before you give those to a patient that needs a platelet product. You know, I guess in pediatrics they wouldn't cross that; they would try very hard to give type-specific, because the patient's blood volume is so much smaller. But, in adults, practically speaking, they're not going to let a platelet go to waste if there's a patient in need and they need those platelets.

Joe: And you mentioned volume-reducing, there are obviously other things that you can do as well. One of the things I see a lot (and interestingly enough, I'm speaking just from the U.S. perspective, so those of you that are international, I'd be interested in hearing your opinions on this as well), but in the U.S., what I see a lot is, more on the eastern side of the country from my conversations with people like Connie Westhoff in New York, if they're going to do that, that O platelet to an A recipient, they're pretty much, well maybe not universally, but most of them are doing titers to make sure there's not "high-titer" anti-A or anti-B (whatever that means to the individual facility). Out here on the west coast, I see that a lot less, but people do do it sometimes. How are things for you? You're kind of in the Midwest, so how are things for you guys? Do you guys do a lot of titers on those?

Sue: No! Actually, we don't do any! It's one of those where there's been talk about it, but we have avoided that. We're also fortunate that we have wonderful platelet donors that are so committed to coming in every two weeks. So, we probably haven't had quite the platelet shortages that are on the coasts. I know there's always conversation about it. You know, like you already said though: What does the titer mean? What does that result mean? And there's

also the conversation around whole blood and trauma, and A plasma after trauma. All those things, right? There's a lot we don't know about what the best practice is!

Joe: That's for sure, and I would be remiss if I didn't mention that there are some facilities that believe anytime you do something like that, volume reduction is one option, and even washing is another option that some places will do. You know, again, there's a lot of opinions on this, and I know that you and I can't say what THE right thing to do is, because there's a lot of debate and discussion. I think we should leave that there and just let people know, hey look, you need to see what's going on in your local facility, because there are a lot of different opinions on this. The one thing you said at the beginning, though, Sue, that we can 100% agree on is that ABO-identical is always choice 1.

Sue: Absolutely. Absolutely.

Joe: I don't think anybody would argue with that! I hope not, anyway!

Sue: And you know the tricky part is that you are worrying about the antibodies in the plasma for the most part. But there are also those situations where we know that ABO-incompatible platelets have some A and B antigens on them, too. You know, there's some situations where those don't survive as well in patients.

Joe: You're completely right. Well, what about Rh with platelets? So, I very well remember when I was very early in my transfusion medicine career, when we used a ton of whole blood-derived platelets, and giving an Rh-negative person Rh-positive platelets pretty much always came with either (depending on the place where I was working) an "OK, here's your platelets and here's your vial of Rh Immune globulin," or at least a discussion about doing that. And I'm not sure that recommendation is quite as strong anymore, but I wanted to get your take on it. What about the scenario where you have an Rh-negative patient who needs platelets, and all you've got available is Rh-positive?

Sue: Right. That HAS changed over the years, because I remember the same when it came to whole blood-derived platelets. Now, it's not seen as as big of a deal. I think **you can give Rh-positive to Rh-negative**, because most of the platelets transfused are from apheresis donors, and those platelets have very, very few red cells present in them. The other thing to keep in mind is that the Rh protein is NOT present on platelets. So it doesn't matter, from the platelet perspective, what you're worrying about is are there any contaminating red cells that could elicit an immune response in an Rh-negative patient.

Joe: OK Sue, thank you for taking us through that. We've spent some time on product selection as well as lab testing and the whole request and sample process. We need to we need to close this out by talking a little bit about compatibility testing. And just some definitions; clinicians, I think, get confused about this, and I think "learners" in blood banking can sometimes get a little lost in the terminology of the "serologic crossmatch," the "immediate-spin crossmatch," the "AHG crossmatch," the "computer crossmatch." Shed some light on this for us, Sue: What are our options?

Sue: All right, when it comes to the compatibility test or what we usually call a "crossmatch," we have those possibilities that you just mentioned, but it comes down to the method that we

choose (and I'll go through them), but **the method that we choose has to be able to detect ABO incompatibility**. Really important, right? It's the last step, the last thing that we're going to do before we...we've done the ABO, we've done the screen, we've done the Rh; now, is that unit REALLY compatible with my patient, and ABO compatible?

So, the **serologic crossmatch** is generally what we would call our "**immediate-spin**" crossmatch. For the grand majority of people that don't have antibodies, we can do immediate-spin. So basically, it's just taking the red cells from the unit from one of those segments that's attached and mixing it with the patient's serum or plasma and ensuring that we get no reactivity, that it's negative and compatible. And that should detect most ABO incompatibilities (I can never say "always" in blood banking, but we should detect *most*, and that's what's accepted). **If our patient has an antibody, or a history of a significant antibody like an anti-Jk^{a}, then we are required to do the testing that includes an antiglobulin test**. And again, because these antibodies that we consider "significant" are usually IgG, in order to see the IgG antibody, we have to do that antiglobulin test. So, immediate-spin crossmatch goes really fast. We're done in 5 minutes. The antiglobulin crossmatch takes about 30 to 45 minutes (when everything goes smoothly).

So that's the methodology for the serologic crossmatch. But the other thing that we can do now is what's considered or called a **"computer" crossmatch or an "electronic" crossmatch**. This is where we totally rely on our computer system to ensure that ABO compatibility is occurring. The trick here is you have to have a computer system that is validated to ensure that it can verify, "Yes, I picked an A-positive, leukocyte-reduced red cell off the shelf." I can scan it with the barcode reader, it says, "Yep, you're an A pos," and then it's going to match that to my patient to ensure that, yes my patient's an A pos, and then within the computer system there are logic tables that are built they'll tell us, "Yep, A to A is good, or "An O to an A is good." And all that logic is built into the system. So that the computer crossmatch, I mean, it will YELL at you, it'll beep and whatever, it won't let you go any farther if it doesn't match. The caveat here is you must have two ABO/Rh types, and now with the new Standards, everybody will. And it has to again tell us that there's a mismatch. But actually, we've done some studies looking at different methodologies, and the immediate-spin crossmatch, intentionally testing incompatible units. And our final conclusion was that we actually felt that the computer crossmatch was the best option.

Joe: Wow. So Sue (and you may have said this, forgive me if I missed it), but to use a computer crossmatch, you mentioned the two ABO types; anything about antibody history required there?

Sue: Oh, yes! Thank you, sorry. Yes, **if our patient has a history of an antibody, then we are required to do a serologic crossmatch**. Then we have to go back, and we do one of those crossmatches that would include the antiglobulin test. That's a requirement. Yeah definitely.

Joe: So, functionally speaking, the people that are eligible for an immediate-spin crossmatch and the people that are eligible for a computer crossmatch overlap pretty significantly, right?

Sue: Yeah, absolutely. Pretty much, we're going to do the crossmatch on any of those samples that are within that sample collection dates of three days. And either is...pretty much

if a facility is licensed or has validated their system to be able to do computer crossmatches, that's what they do. I mean that's what they do, and only do the serologic crossmatch if the patient has an antibody or a history of an antibody.

Joe: Got it. And time wise for doing a computer crossmatch?

Sue: Oh, it's fast! [LAUGHS]

Joe: How long does it take your computer to think, right?

Sue: Exactly! It's wonderful for inventory management, as well, because if you crossmatch serologically, you pretty much want to do that ahead of time, so that when the need is there you can just issue the blood. Whereas, with a computer crossmatch, it's just all part of the system, right? Boom, boom, boom! You're done.

Joe: All right, Sue, with compatibility testing, one thing that I get asked about often that I think there's some confusion about is, what if the patient is only going to be getting, say, a platelet transfusion, or less commonly, a plasma-only transfusion? What are the compatibility testing requirements in those situations?

Sue: Interestingly, there's **no crossmatch that's required. All that we need to do is ensure ABO compatibility**. So, we need to have a current ABO, or a record of a patient's ABO/Rh type on file, and then we select based on that patient history. So, no crossmatch. And it kind of makes sense, because a crossmatch, the way that we perform it today, is taking the patient's plasma and testing it against the red cells in the unit that we're going to transfuse. In this case, we're transfusing plasma (no red cells present) or platelets (again, we just talked about, no red cells present). So, there's really no reason to do a crossmatch (at least the crossmatch that we do). I mean the crossmatch that would be required in this case would be a "minor crossmatch" and we haven't done one of those in... Forever! Not even in MY career, and I've been doing this a long time! [LAUGHS]

Joe: Well, you and me both, and I have never seen one done either, but just for people that are wondering what the heck is a minor crossmatch. You're right, we don't even use that terminology, "major crossmatch" or "minor crossmatch," but what we've been talking about is technically a major crossmatch, that thing that you just described, checking for the patient's plasma against the donor red cell. So what's a minor crossmatch, Sue?

Sue: So, a **minor crossmatch is taking the donor's plasma and testing it against the patient's red cells**. So, looking for, is there anybody in the donor that would be incompatible with the patient?

Joe: And believe it or not, I get questions about that a lot. People write me on the Blood Bank Guy site and say, "Why don't we do a minor crossmatch?", or "Why don't we worry about, say, a donor who has anti-K? How would we detect that?" How do you answer that question, Sue? What about the donor who has an anti-K. How would we know?

Sue: So, the reason, I believe, that we quit doing minor crossmatches was when all the donor centers went to doing antibody screens on every donor. So we know what antibodies the

donor will have, and in fact, you would never see a plasma, for example, that would have an anti-K in it in your hospital. The only way you might see it is if it was being used for competency or training, where you asked for that unit for that purpose. So, we do that testing routinely on all of our donors. So it's not an issue. You never see that.

Joe: When we talk about doing serologic crossmatches, Sue, I think most everyone has a picture in their head of the blood banker sitting there with tubes, shaking tubes, and checking those out. And obviously that's pretty classically the way that it's done. But I wanted to ask you: You talked earlier about the other platforms like gel or solid phase (and I think we need to restrict this discussion to the U.S., because I'm not sure of what the rules are elsewhere), but in the U.S., can you DO serologic cross matches with for example gel or solid phase?

Sue: Well, you COULD do the crossmatch with gel or solid phase. However, it is not meeting the requirements of the FDA to detect ABO incompatibility. We know that gel and solid phase methods aren't as effective in detecting when you have, for example, a group B plasma that is crossmatched with an A. Then there's been studies that have been done that just have shown it just doesn't detect ABO antibodies, ABO incompatibility, as well as the test tube methods do. And you know I guess people could try to validate it, but I don't think they'll be successful. There's enough data out there that just shows it's not effective.

Joe: But I mean, certainly, it's marketed as something that can be done for certain types of crossmatches. Are we talking about maybe doing the AHG portion with gel or solid phase and doing the ABO check with either immediate-spin or computer is that what we're talking about?

Sue: Right, exactly. I mean you could do an IgG gel card to detect, for example, an ABO incompatibility, however, not all individuals will have a high titer IgG antibody. You could use a buffered gel (which, there's no diluent in the gel), but again, the studies that have been done still show it's not as effective at detecting ABO incompatibility. So I think ultimately... I actually had a student do this comparison at one point, and the best thing was at the very end when she said, "I believe the best crossmatch is as the electronic crossmatch!" Let the computer do the checking! [LAUGHS]

Joe: Music to my ears! There is obviously a lot more we could go into with how the crossmatch can go wrong and what positive crossmatches mean, but we will leave that for another time, because we need to finish with our very last one and that is the labeling of the product. What kind of things are blood banks required to put on the product before it leaves the blood bank?

Sue: So, when we look at labeling, we are required to have what they call a "compatibility label" or you know some sort of label like that. But the label has to include (again, it's going to sound familiar), **patient's first and last name** (so we're going back to the beginning, when we drew the sample), the **medical records number** (and/or blood bank number), the **crossmatch results** (is it compatible or incompatible? Sometimes there's reasons for that), the **ABO/Rh of the patient** and the **ABO/Rh of the product**, and then **when does the crossmatch expire**? Those things have to be attached to the unit somewhere. It either could be a tie tag or a label, and I'm sure anybody listening has seen it. I mean that's really important. And, as that label...we should talk about the fact that it's really important that the lab scientist the blood banker that generates that label then PUTS that label on the right unit.

So that they're saying, "Yes this is unit [13-digit number], that number matches the label. My patient name is accurate, that matches the sample that I was just working on, that ABO/Rh is correct on the unit, and that the ABO that's on the label, that's the ABO that I just did. The type all matches." All of that should be verified as the label is put on the product. We know of times when two units are being labeled, and the labels get put on the wrong units. So…

Joe: I just broke out in hives! Just, literally broke out in hives. OK maybe not literally; figuratively I broke out in hives!

Sue: Figuratively, definitely! It drives you crazy. But yeah, I know of issues like those "near-miss" kind of things or what have you. This is often an area...a sticky point.

Joe: Yes, for sure. Sue, this has been a great discussion! We've hit a lot of stuff with compatibility testing, but I think I would like to kind of make this practical a little bit, and maybe hit you with a few scenarios and to illustrate kind of some classic points in compatibility testing, and just get your thoughts. So, I have like six different "case-like things" that I want to hit you with. You ready to do this? Can you handle it?

Sue: Yeah! I love cases!

Joe: I know you can handle it. I'm not worried about that. All right, so these aren't necessarily well-defined cases, but let me just describe the scenario for you. So, **here's the first one**: You have a patient who comes in for pretransfusion testing. They have a negative antibody screen, and you are in a facility that happens to use the immediate-spin crossmatch for cases like that, where the antibody screen is negative, and there's no other indication for a "full" or "anti-human globulin" crossmatch. So, you do this immediate-spin crossmatch, and "UH-OH!" The immediate-spin crossmatch is incompatible. So negative antibody screen, incompatible immediate-spin crossmatch: What kind of things does that bring up in your head as possibilities?

Sue: Okay, the first thing I'd think about is ABO incompatibility. Did I grab the right unit? Like we talked about before, our anybody screen's negative, so we don't THINK there's an antibody there. So, that would be my first thought: ABO. Did I get the right unit? My second thought, after I verified that, is just thinking about the fact, again, that what antibody screening method am I'm using? Is it a method that's designed to detect IgG only, like the column or solid phase? Then I'm thinking it is probably some sort of IgM antibody we didn't detect on antibody screen, like an anti-M, -P1, or a cold autoantibody. Those would be the things that would come to mind right away. And those are pretty common antibodies that will cause that incompatible crossmatch while the screen's negative. You could be an antibody to a "low" [NOTE: Antibody to a low-frequency red blood cell antigen not represented on the antibody screen], but I'm still thinking more IgM type, like anti-M or -P1.

Joe: Right. Because we're talking specifically about immediate-spin, something that reacts at room temperature or colder would kind of jump to the top of your list, right?

Sue: Yep, absolutely.

Joe: So just, again, we want to stay high level here, but if you've ruled out ABO and you're thinking it might be one of those cold or room temperature-reacting antibodies, what might you do next to try and work that out?

Sue: How you could do your screening cell set, you could do tube test, looking at immediate-spin and taking it through 37C and antiglobulin test with LISS or PEG (or no additive, actually. No additive would be absolutely fine, just to see what you're dealing with). And most likely, it's going to be positive at immediate-spin. And then, you'd want to go to an antibody identification panel, running, again, pretty much the same methodology, wherever that antibody reacted best, maybe it's immediate-spin, and seeing what kind of reactivity you get. If it is an IgM antibody, maybe doing a room temperature incubation. And include, most importantly, include an autocontrol so you can differentiate "auto" from "allo."

Joe: So, I 100% agree with everything that you just said. The only thing I would add to it (and it's not really an "add," it's just a "note"), in my experience as a transfusion medicine doc, the places this comes up, it has a tendency to be really inconvenient! It's the scenario where you've talked to the clinicians about how, "You know what? You don't need to do a type and cross on everybody! We just do the antibody screen and then when we're ready to transfuse, we just do this magical immediate-spin cross match: Bada-bing, bada-boom, the unit's out the door!", and, "Oh, darn, we've got an incompatible immediate-spin crossmatch." So, from the physician side, docs often have to get involved in this discussion to help our blood bank staff understand the priority of, the significance of what the patient needs, and sometimes we have to make tough decisions in cases like that. God knows, we want to get it all worked up, but sometimes, if the patient is in desperate need, you may have to make a choice that may not be easy.

Sue: That's a great example.

Joe: My usual reaction when I have a situation like that is, well, my words are typically censored, let's just put it that way. [high-pitched] "OH NO!" That's the nice version of it. [LAUGHS] All right. So that's the negative antibody screen/incompatible immediate-spin crossmatch scenario. Let's talk about **the next one**, which is the same kind of thing: Person comes in for pretransfusion testing, everything through the antibody screen is A-OK, no issues with ABO and Rh. The antibody screen is completely negative. However, this is a facility that likes to use "full" or "anti-human globulin" crossmatches for every transfusion. And in this case, the anti-human globulin crossmatch comes back incompatible. So, negative screen, incompatible AHG crossmatch; what kind of things does that make you think of, Sue?

Sue: Okay, so the first thing I think about is, doing a direct antiglobulin test, a DAT on the DONOR unit, because you wonder, does that unit have a positive DAT? Because we know that up to 1% of blood donors will have a positive DAT, and you just had bad luck. You grabbed the wrong unit!

Joe: Wait, now just for clarity, Sue, blood donor centers don't do DATs on all their donors?

Sue: Correct. They will do ABO/Rh typing, they'll run a control line, and if that control is negative, and they can get a valid ABO, then the unit is labeled. And the DAT is only rarely, rarely done, so it's not a routine thing for sure.

Joe: Okay. I hope everyone can kind of get the visual picture of that. So, if you're doing an anti-human globulin crossmatch, and you're checking a unit where the red cells are ALREADY coated with antibody, obviously, that's going to come up incompatible, right? I mean it's just inherent in the test.

Sue: Absolutely, because we are using anti-IgG in our antiglobulin test, so that anybody is going to detect the antibody already present on those donor red cells.

Joe: Got it. Okay. Anything else that scenario makes you think of? Go ahead, I'm sorry...

Sue: No, no it's OK. The second thing I would think about, then, would be our patient has an antibody to a low-prevalence antigen that's present on the donor red cells. So, previously we talked about Cw. Maybe that unit is Cw-positive, and your patient happens to have an anti-Cw that we wouldn't see in a regular antibody detection method. So those are the two scenarios: Positive DAT or an antibody to a "low," that I would think of right away.

Joe: And potentially two dramatically different potential outcomes there, right? I mean with a positive DAT in the donor, OK, you find it, you let your blood center know that that's the case, OK, no big deal. But if it was a low-incidence, that actually has potential clinical significance.

Sue: Oh, absolutely! Right. Now you find an antibody, then we're going to go ahead and we want to try to identify what that antibody is, and running a panel, and sometimes, depending on how "low-frequency" the antigen is, you may have to have a conversation with your medical director, "Do you want to send it out an immunohematology reference lab to try and identify this?", so that you know what to do for future transfusions. Although, it should be pretty easy to find compatible blood because it's such low-prevalence, low-frequency.

Joe: Right, in the end, oftentimes you just end up pulling another unit and crossmatching it, right?

Sue: Right. Yeah. Exactly.

Joe: All right, so that's scenario two. **Let's do another one that also includes a negative antibody screen**. A patient has that negative antibody screen, and this is a facility that uses the electronic or computer crossmatch. It is completely compatible; however, the patient gets transfused, and a couple days later, a few days later, you see evidence that this patient might have hemolysis. How can that be, Sue? How is that possible?

Sue: That's possible because of those famous antibodies we know can show "evanescence." They'll drop below the level of detection in our antibody screening method. So, we look at, for example, classic are the anti-Jk^a and anti-Jk^b. A few days later, now the patient was exposed from a transfusion to, say, Jk^a-positive cells, and that anybody will come back in large amount, or enough that we can now detect it (usually, anyway; depends on how soon we test). But yeah, those are the classic delayed transfusion reactions that can occur. So, the antibody is too low, we can't detect it, and then the patient gets transfused, it comes back, or causes hemolysis.

Joe: If there are any clinicians still listening to this (this has been pretty technical, so I may have lost most of my clinicians that listen to this podcast), but if there are any clinicians that are listening to this still, important to recognize: What Sue just described, that doesn't mean that anybody's done anything wrong! I mean, it's an inherent part of...we know that when we do pretransfusion testing, that those antibodies that Sue just described (your Kidd antibodies, especially), love to just drop below the levels where we can detect them. Nobody's messed up. It's just a risk of the whole situation. Right, Sue? Is that a fair way to put it?

Sue: That is very fair. It's just, when we look at these, we can't detect them all. And there's a number of antibodies, and there's actually been a study where they did the mathematical modeling to look at how often these antibodies will fall below the level of detection. The one paper that I'm thinking of talked about the fact that we probably only detect about a third of the antibodies that people make.

Joe: UGGH!

Sue: I know! Kinda scary! Well, we're talking about all this work we do, right, to make sure?

Joe: I know. I don't like that paper.

Sue: It's mathematical modeling, however it still sounds pretty good. It makes you think.

Joe: Yes. All right. Well, I suppose, in that scenario, the previous thing that you talked about with low-incidence, that could do that presentation as well, right?

Sue: Right. Yeah, it's possible.

Joe: All right, let's move on and talk about some scenarios with POSITIVE antibody screens. Again, we're just trying to let people know patterns, things to recognize, things to think about when you see these things. So, **here's one that I think is fairly classic**: A patient has a positive antibody screen, and I'll just leave it undescribed, it's positive. There's cells that are reacting, but when they go to do the antibody identification panel, as we like to do when we have a positive antibody screen, it has the pattern that we call "panreactivity," in other words, EVERYTHING is reactive. When we do an autocontrol, the patient's OWN red cells are reacting against the patient's serum. We do a direct antiglobulin test (your favorite test in the world!), which turns out positive with polyspecific and IgG and C3. What's the classic pattern that I'm describing there, Sue?

Sue: The classic is a patient who has a warm autoantibody, reactive in the serum or plasma as well as attached to the patient's red cells. And these are a challenge for the blood bank because these antibodies will react with, as you described, with EVERYTHING. And so it takes additional testing to determine if there are any alloantibodies present, because the autoantibody is "masking" everything, pretty much. And so, there's hours of workup that usually has to be done to be able to sort these kinds of problems out. And the one thing they'll want to do is an eluate, making sure that we remove the antibody from the red cells, and that's just to confirm that it's a warm autoantibody [NOTE: Original recording continued: "…versus the unusual case of a drug-induced antibody."]

Joe: And obviously, there's a lot that we could go into there, but I mean, warm autoantibody is probably a podcast in and of itself, but bottom line is that your work is not done when you get to this point. There's a lot more as you said that you could do. You're talking eluates, you're potentially talking autoadsorptions, you're potentially talking alloadsorptions if the patient has been recently transfused. There's a lot of stuff there. But bottom line, that's a classic pattern for a warm autoantibody and that's what people should recognize.

Sue: Absolutely, and that it's going to take probably four to six hours to do the additional work.

Joe: But you know what? That's a really important statement. Obviously, what you just said, it depends on the capability of your individual transfusion service. I work with hospitals that don't have the capability to do any of that. And when they have to send it to my immunohematology reference lab, "four to six" is optimistic because you got transport time and all that.

Sue: Oh absolutely. That's in the hands of somebody that's actually doing all the work, so right, it could take all that additional time in there. Definitely.

Joe: OK, well maybe another time we can do a warm autoantibody work up discussion, but for today, let's move on to the next case. And this one's a little artificial and a little weird, but I wanted to again give a little bit of a different perspective. So, **case five** is a scenario where the patient, when you look at their antibody screen, you have kind of weak "sporadic" activity. And let's just say that it was a... let's say it was a gel panel. And it's kind of positive, kinda negative, not super strong. But your facility doesn't happen to be one that has a lot of capability to work things up. So, you send it off to your immunohematology reference lab, and the first thing that they do is, they throw it, as reference labs do, they throw it into PEG and they look at the reactions and they see 3+ reactions at immediate-spin. There's no hemolysis at 37C, and when they read it at the anti-human globulin phase, the IAT, it's completely negative. They do a DAT and it's positive only for C3. So that was a lot of introduction to kind of get you to what at that point you would be thinking of, but what would you be thinking of at that point, Sue?

Sue: Oh, at that point I would be thinking about a cold autoantibody of some sort. It could have some specificity, you know, oftentimes they are auto-anti-I specificity, but it classically would react best at immediate-spin in a test tube method. And because it's IgM, it likes that phase the best, which ultimately then gives you a little bit of that weak variable reaction when you do a gel test or a solid-phase test, for example (but mostly gel is where it'll give you that trouble). And, then we can usually work around these antibodies pretty easily in a test tube method by avoiding the immediate-spin phase of testing and going right to a 37C antiglobulin test.

Joe: Well again, there's a lot of places that we could go from there, but I do want to get your take on something, Sue, in regard to this. What I will see oftentimes in people discussing cases like this is, they will they will use the words (and I'm not directly quoting but I'm paraphrasing), but people will say, "Well, let's just pre-warm it away"...

Sue: [SCOFFS]

Joe: Wow, that got a reaction right away!

Sue: I knew that's what you were going to say! [LAUGHS]

Joe: Well, I want to give you the chance to talk about that, because I think there is a perception out there that, you know, applying 37C can make those ugly things that we don't want to deal with go away. And personally, I'll state right from the beginning: I think that's dangerous. Am I overreacting?

Sue: No! Not at all. And I totally agree with you. It's interesting, because I know in my career working in an IRL that there was a point when we would say, "Well, try prewarm before you send it." Because, you know, for that reason. However, since, oh, I can't remember the exact year the paper came out [NOTE: It was 1994. Reference: **Immunohematology** 1994;10(3): 83-86], but it was a paper published by Jill Storry and Delores Mallory on an anti-Vel, which is an antibody to a high-frequency antigen that was not identified initially. The lab that had the sample prewarmed the antibody, so the antibody was non-reactive in a prewarm test. They went ahead and gave incompatible units, and the patient had a fatal hemolytic transfusion reaction. That actually caused, I mean it was like this great paper (not a great situation), but a paper that really caused people to step back and say, "Hey, what are we doing here with the prewarm?" And I'm aware now of numerous examples in our own experiences, techs (especially it always seems to be off-shifts and such because you're trying really hard to get blood for the patient), they'll prewarm, and then it turns out that it's an anti-Jk^{b}, for example, or other real antibodies. And there's been a number of good studies done that have shown that **you can prewarm clinically significant antibodies**. Well, the anti-Vel was one, but anti-Jk^{b}, -Fy^{a}...any weak reactive antibody, you can prewarm it away, meaning it won't be reactive. So, yeah, I totally agree. So, our caveat and anything that we teach in our procedures here we say, "You must identify the antibody if you're going to do the prewarm test." So, you can DO the prewarm, but you should know what you're prewarming...

Joe: Right! Oh, I love that!

Sue: ...so that you have a good handle on it. Yeah. Totally makes me nervous now!

Joe: Me too. And I see it all too often. But I've got to move on because I'm getting annoyed just thinking about it.

Sue: We didn't even mean to go there, but I couldn't help it!

Joe: I know. It's perfect. So, **here's the last one**, Sue, and I know you'll recognize this scenario, and I hope everyone listening will as well. But I want to make sure that it's clear. So, let's say we have a patient who comes into the hospital. This is not his first hospital; he has gone to different places. Apparently, he was in another facility a couple of weeks ago. By report he was transfused there are a couple of weeks ago with no problems, but we don't have any more history than that. We don't have any records or anything. We do an antibody screen on him. It is positive, it's not a real discernible pattern on the antibody screen, but on the panel, it turns out to be an anti Jk^{a}, pretty well-defined. The DAT is done; it is positive with the polyspecific reagent, but it's kind of weird. When we're looking at it on the gel DAT, and

you've got some cells that are up at the top that look positive, but some cells that are down at the bottom that look negative. And the same thing is true with the anti-IgG. So, you've got the "mixed-field" appearance for the DAT. What should we be thinking about at that point?

Sue: This is a CLASSIC example of a patient that's presenting with a delayed transfusion reaction. No antibody a few weeks ago, and this is that case of evanescence where they had no trouble at the previous hospital because they didn't detect that antibody, it was low level. And now it's come back because they were transfused with Jk^a-positive units, and now the antibody is back, the IgG response, the antibody is present, coating the transfused red cells (which would explain the mixed-field in the DAT). So, the DAT is showing us some cells are at the top of the column, and some cells are at the bottom. So, the cells at the top are the agglutinated transfused red cells, probably a smaller portion than the patient's own cells, which are Jk^a-negative, which would be at the bottom of the column. So, classic scenario, and I purposely said, "delayed transfusion reaction" versus a "hemolytic" or "serologic," because, at this point this is where we would need people like Dr. Joe here to find out a little bit more clinical information before we can say is it a hemolytic reaction or not.

Joe: That's so important. And Sue, I have to jump on that, because I see a lot of confusion about that as well: The difference between a delayed serologic reaction and delayed hemolytic reaction is just purely, is the patient hemolyzing? That's it. If in that scenario that you just described, you call me and I investigate, and there's no evidence of hemolysis, that's a "delayed serologic." If there is evidence of hemolysis, that's a "delayed hemolytic." Pretty simple, right?

Sue: Yeah.

Joe: Shouldn't be that hard, but people do get it confused a LOT. And really that case, Sue, is just kind of the case that I did in number three with the negative antibody screen, the compatible computer crossmatch, but later hemolysis, that's just catching that case later on. And quite frankly, that's kind of doing you a favor because you can jump on this, you can talk to the clinicians about, "Gee, we've got to be watching this patient, because this patient really could (if he's not already) hemolyzing, he really could." As opposed to the other scenario, where you get caught by surprise.

Sue: Right. Absolutely. I mean, the fact that you're seeing transfused red cells in that DAT is telling you that there's still red cells there, and there could be some hemolysis coming.

Joe: There could be. Absolutely. OK. Well Sue thank you so very much for being with me. I can't even tell you once again how much I appreciate what you've done with me here. I know this is going to be really, really helpful for people, so thank you so much.

Sue: You're very welcome. My pleasure.

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Joe: I always have a great time talking to Sue. She is incredibly knowledgable, and it's really fun to speak with her. I hope that our discussion was helpful for you, too.

Stay tuned for more episodes of the Blood Bank Guy Essentials Podcast, because I have a lot of fun things coming your way. The next episode will be a discussion with Dr. Aryeh Shander, one of the true leaders in Patient Blood Management. Aryeh and I will discuss the one critical part of Patient Blood Management that is completely ignored by way too many hospitals! And it puts you behind the eight ball from the very beginning. That's coming soon, so don't miss it!

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!