



**BBGuy Essentials 054:
ABO Discrepancies with Nicole Draper
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Joe: Hi everyone and welcome to the Blood Bank Guy Essentials Podcast, the podcast designed to help you learn the essentials of transfusion medicine. This is Episode 054, and my name is Joe Chaffin.

So, this is an episode that has been called for many times, by many of you, through email and surveys, et cetera. Today we are going to discuss ABO discrepancies, and I'm really excited for you to hear it from my guest Dr. Nicole Draper, from the University of Colorado.

More on that in just a second, but before that you should know that this episode is not eligible for continuing education credit. You can find other episodes where physicians and laboratorians can earn those continuing education credits for absolutely no charge at bbguy.org/podcast. You just look for episodes that end with the letters "CE." You can also visit wileyhealthlearning.com/transfusionnews. The continuing education episodes there are brought to you by transfusionnews.com, and Transfusion News is brought to you by Bio-Rad.

So, ABO discrepancies are probably not my favorite thing in blood banking and transfusion medicine, and that might be an understatement. Those of you that have listened to the podcast I think are very well aware that I love immunohematology. And I've covered many different topics on the podcast, but ABO discrepancies is not necessarily my favorite thing. And from what I've heard from you, in terms of emails that I get through BBGuy.org, as well as site surveys that I've done, one of the more popular requests has been, "When are you going to do something about ABO discrepancies?"

Well, I have resisted that, to be honest, in large part because I was always concerned about doing something so visual in an audio podcast. But I think I've got a solution and I want to share it with you today. I'm really excited to have as my guest today Dr. Nicole Draper, from the University of Colorado School of Medicine, in Denver, where she is an associate professor, as well as the Associate Medical Director of the transfusion service at the University of Colorado Hospital there in Denver.

Nicole LOVES ABO discrepancies! She just totally gets into them, and I'm really glad that someone does, so that she can help teach you this. Nicole is a great teacher. She's a six time winner of the Resident Teaching Award at CU, and she's actually talked about this very topic at AABB Annual Meetings and given terrific presentations.

So, before we get to my interview with Nicole about this, I should let you know there are some ... maybe some prerequisites to this. Not absolutely necessary that you do these, but there are two parts to this. If you go to BBGuy.org/ABO, that's the letters "A, B, and O"; BBGuy.org/ABO, what you'll find there is kind of a primer that I've written, a basic outline of the essentials of ABO testing that will kind of get you ready for this particular podcast. Further, if you go to the show page for this episode, which is BBGuy.org/054, you'll find slides there that will show you the exact examples that Nicole and I will be describing, so that you can actually see the ABO testing and see where the discrepancy is a little more visually.

Now, we're going to try and paint word pictures, so that you can get an idea in your head of what we're talking about. But if you're the kind of person that needs to see it, and print it out, and write something down, totally fine. You can do that again at BBGuy.org/054.

So, I can't wait for you to hear this episode, this interview. With no further ado, I give you my interview with Dr. Nicole Draper on the Essentials of ABO discrepancies.

Joe: Hey, Nicole, welcome to the Blood Bank Guy Essentials Podcast.

Nicole: Thanks for having me.

Joe: It's so great to have you. You and I have known each other for a while. I was trying to think the other day, I think we met somewhere around 2010 or so, is that right?

Nicole: Probably, yeah.

Joe: Yeah. That's when I started working at the blood center in Denver, and you were (and are) colleagues, but back then we worked locally together. So, I just have to tell everyone ... and this cracks me up every time I think about this ... today's topic is ABO discrepancies. What can happen, how we approach, and some great examples of ABO discrepancies. And everyone, Nicole and I, like I said, we've known each for a while, and it just makes me laugh that we have completely, diametrically opposite reactions to ABO discrepancies.

In the past, Nicole, I've described it to you this way. When you get one of these, you go, "Ooh!" When I get one of these I go, "Crap! I hate these things!" So, I just have to ask, why the heck do you like these things so much? Why do they appeal to you so much?

Nicole: I think it's because I like it because it's not straightforward. It requires ... it's a mystery, right, and you've got to ... it's a puzzle. And there's a whole bunch of different options about why you could have this discrepancy and

why you have these weird reactions. And I get to kind of hunt around, and do some investigating, and figure it out, which I think is fun.

Joe: I could accuse you of being a nerd at this point, Nicole, but I won't do that to you.

Nicole: Oh, I'm totally a nerd!

Joe: You have kind of become, on the physician's side certainly, a real go-to expert on this. You've talked at least once, I think several times, at the AABB Annual Meeting about ABO discrepancies. Have you done it more than once, Nicole? I know I've heard you do it once.

Nicole: So, I officially did ABO discrepancies once, and then I included ABO discrepancies in a talk I did about chimerism. So, a couple times.

Joe: Yeah. Well, so folks, Nicole's talks on ABO discrepancies are just full of wonderful examples, and we're going to get some of those today. I tease her about it, but honestly, Nicole, your passion for this comes through. And while I don't necessarily *share* it, I certainly *appreciate* it. How about that, is that fair?

Nicole: That's fair.

Joe: Okay. So, I would like to ... before we start in with specific examples, I would love to hear from you from the perspective of a physician. Now, a lot of the folks listening to this podcast are physicians that are early in training, people that are going to potentially be involved in overseeing hospital transfusion services. So, I would like to know from your perspective, from the physician's perspective, when you hear from your staff that, they come in and they say, we have an ABO discrepancy, after you get over your rubbing your hands in glee, how do you approach these things? What are kind of the general philosophies that physicians should be aware of in terms of the resolution of ABO discrepancies?

Nicole: So, usually ... our techs are fairly used to ABO discrepancies here because we do a lot of transplants, and so we get a fair amount. But when they first come to me, usually what they're asking me for is they've done all the basic testing and they want to know if there's more they should do, what's the true explanation of this? And so, what I mostly provide is I go through the chart. Because in ABO discrepancies, it's so important to look at the history because different sets of reactions, there are so many potential explanations that you often can't figure out just from doing the testing. And that history is so important to determine what it truly is, and how much of a problem it is, if you're going to have to get them special units, if you're going to have to use the ... give them universal blood type until you can figure it out.

So, largely my part is sort of looking at the history, and then determining where we go beyond the basic reactions or the basic testing.

Joe: And do you get involved, heavily, in the ... well, I mean, we're going to show some examples of how these reactions can occur. Do they bring things to you and say, "I'm stuck, help me, Dr. Draper?"

Nicole: Oh yeah, often. I mean, they'll say, "I found this, I think this is the answer, but I'm not sure," right? So, they're ... "it might be a subgroup, it might be an interfering antibody, it might be this, or this, or this, or this, and we really need the history to determine what that is." And that's mostly my role, is should we do anything super-crazy and special, should we send it to the reference lab, or that kind of stuff.

Joe: Got it, okay. Well, so I think that there's a lot of important stuff in what you just said, but I really think either from the laboratory perspective, or from the physician's perspective, it is hugely important what Dr. Draper just said, it's hugely important that we understand the context, clinically, of when these things are occurring.

It can make, as you said, Nicole, all the difference in the world. And it can make what appears to be a complex serologic problem become a fairly simple problem as soon as you know what the history is. So, agree with you completely there that we've got to be involved in helping to figure that out. That's super-important.

Before we get into the specifics of these cases, I want to share two things with you. First, ABO, kind of by definition, is a super-visual blood group system. And Nicole and I really thought long and hard about how we were going to do this, because this is an audio podcast, obviously, and so we're going to have to paint some word pictures so that you can kind of visualize what we're talking about. However, if you'll go ... this is one episode where actually going to the Blood Bank Guy website will really, really help you a lot.

First, the show page for this episode is BBGuy.org/054, that's BBGuy.org/054. If you go there, what you'll see is when Nicole is describing a particular scenario, there will be a slide on the site there, on the show page, that you can pull up and you can see visually exactly what she's describing. We'll do our best to make sure that if you're driving in your car, you can understand what we're talking about. But again, if you prefer the visual, that's where it will be.

The other thing is that if you'll just go to the show page again, I will have a link to a brand new blog post that I've done, a basic description of the ABO system. If you find yourself in the position of not completely understanding the background of ABO, how the system works, how the basic testing normally works, I've done a blog post there so that you can kind of get that

background [NOTE: BBGuy.org/ABO]. You might want to start there before you listen to this podcast, if that's your scenario.

So, all that being said, Nicole (sorry for that long intro), I would love to get started with this and take off on your very first case scenario. So, why don't you take it away?

Nicole: Okay. For our first scenario, number one, we have a standard ABO/Rh type. And when we test the patient's red cells, we get a strong reaction with the anti-A. We also get a very strong reaction with the B cells on the back type, so consistent with someone who's blood type A. But we have this 1+/weak reaction with A1 cells. So, we have a discrepancy.

Our front type technically looks like blood type A and our back type looks like an O. So, this needs to be resolved. We use automated testing initially. And where we are, which is a good thing to do, is always to repeat your testing, potentially repeat it by a different method. So, we go to tube testing, to check and see if we can resolve the issues that way. And we still get the same aberrant reactions. So, tube testing didn't necessarily solve our problem.

So, in this case, typically rules that I use are: "**Weak reactions are usually the discrepant reactions.**" So, I've got a 1+ reaction that's very weak, and I think this is probably my discrepancy. The second rule I use is, "**Antibody problems are much more common than antigen problems.**" So, you're much more likely to lose or gain an antibody than you are to lose or gain, and have a problem with an antigen, typically.

So, in this case, I've got a weak reaction that is an antibody problem. And so I think, "Oh, this is probably an antibody issue that we need to try to explain." Of course, we know that with the blood group A, we have quite ... subgroups are pretty common. Our A2 subgroup is about 20 percent of the population. And so, one thing that typically we check for right up front is, is this person an A subgroup and do they maybe have an abnormal reaction because they are a subgroup?

So, in this scenario, when they did their tube testing, they did the anti-A1 lectin and it did come up with no reactivity. So, this person looks like they are probably an A2 subgroup. And so, this very well might be a true anti-A1 antibody that this person has made because they are a subgroup.

Joe: So, Nicole, let me interject for just a second and ask you this question (and we won't get into every detail like this simply because there's too much to cover), but I get a lot of questions, especially from physicians and certainly from laboratorians that are just starting off, about this whole "lectin" thing. Do you mind taking just a real quick side light to describe what you mean when you talk about a lectin reaction?

Nicole: So, a lectin is something that is not an antibody, but it does have a very specific reaction like an antibody. They come from plants. And if you basically make kind of a "tea" out of the plant at just the right concentration, it will react very specifically with certain antigens and not with others.

So, A1 lectin reacts with A1 and not any of the subgroups.

Joe: And the fancy name for that lectin in this case?

Nicole: ...is "*Dolichos biflorus*."

Joe: Oh yeah, Boards question. I like it.

Nicole: Yes.

Joe: Okay. Sorry for that side light, but I think that's important to just make sure that everybody's on the same page. So, you were saying that in this case we tested this patient's red cells with our anti-A1 lectin and it showed no reaction. And tell us again, how does that help you?

Nicole: So, that says to us that this person is an A subgroup. And A subgroups are known to be able to make an anti-A1 antibody. So, they'll make an antibody against the most common A group, which is A1. So, the techs kind of thought this is probably our explanation, but there are lots of ... not everyone who's an A2 makes an anti-A1. Actually, a minority of people do. So, we don't have ... you can't say this is your guaranteed explanation unless you look at their history.

Joe: Yay, history! I like it.

Nicole: Yay history, which is my favorite part. Going through the chart and figuring them out.

Joe: Nerd.

Nicole: I know. I wear it proudly, a total nerd.

So, in this case, you might have some other interfering antibody. We probably want to know what their antibody screen looks like, do they have some other red cell antibody that might be causing some interference here? Rouleaux potentially can cause some interference, if they have a lot of protein, all sorts of different things.

So, I go and look up the history. And this happens to be a 25-year-old woman, she just came in for a prenatal type and screen. She doesn't have any significant history, but she was in a motor vehicle accident 10 years ago, she did get transfused. And so, she probably got some A1 red cells. She saw them as foreign, and she made an anti-A1.

Joe: Nice. So, in a scenario like this, Nicole, what does this mean if you ... well first, do we have enough information at this point to say that she's a subgroup, or is there more work that we have to do?

Nicole: Nope. We have enough information to say she's a subgroup. We don't have any evidence of any other antibody to explain this, it's consistent with an anti-A1. So, she looks like she's a subgroup with an anti-A1.

Joe: Okay. What does this mean from the transfusion perspective?

Nicole: So, anti-A1s are usually not significant antibodies. They're IgM, cold-reacting antibodies. They don't typically act like other ABO antibodies. So, people have varying theories about what they want to do about this. So, if you're very conservative, which we are, we actually avoid giving any A1 antigen. Some people will do testing to see if the antibody reacts at body temperature or not, and then decide if they want to avoid the A antigen or not. And some people will say, "Oh, this hardly ever reacts, it's not significant, you can have A red cells." So, there's no defined way to deal with it. You kind of get to decide, depending on how liberal or conservative you are.

Joe: Okay. So, more a local medical director decision.

Nicole: Yup.

Joe: Okay. All right, fair enough. So, we have a case of where we gained an extra antibody in our reverse grouping or our "back typing," whatever you want to say. And in this case, it resolved to be an A subgroup, in particular an A2 making an anti-A1. Cool.

Okay. Well, let's move on to the next one, Nicole, unless there's something else you want to cover with that scenario.

Nicole: Nope.

Joe: Okay, good to go. Rock and roll, the next one.

Nicole: So, here's just another one very similar, but this person their front type looks like they're a blood type B, they have a strong reaction with anti-B. And then, on their back type, they have 2+ reactions with A cells and a 1+ reaction with a B cell. So, we're a little unsure exactly. They seem to be a B, according to their front type, but technically they're typing as an O.

And, again, if we sort of follow our general rule that the weakest reaction is usually the discrepant one and antibody problems are more common, we're going to assume that that 1+ reaction with the B cells is the discrepant reaction, the one that doesn't belong that we need to explain. And then, in this case, there are B subgroups, right, but we don't really have good ways or easy ways to test for them in standard laboratory

testing. So, we're not going to get as easy of an answer or basically find a B subgroup like we did on the last case. And it's less likely that that's going to be our answer.

So, we repeat tube testing. We basically get the same problem, we still have reactivity with A cells and B cells on our back type. And so, now we need to try to figure out why. And so, again, go to the history, kind of figure out which road to go down.

And in this case, our antibody screen is very informative. So, basically, when I got the page for the antibody screen, the techs had put no answer, they had put asterisks instead of reactions and they had put a note at the bottom next to their asterisks that said, "We couldn't read this. The red cells were all stuck to the side of the tube and they wouldn't come off."

Joe: Uh-oh.

Nicole: And they gave me a picture. So, I was like, "Great, okay." So, that makes me think I have something that's horribly sticking my red cells together, right? And this is probably another problem with there being something in the patient's plasma that just sticks all the red cells together.

So, I go looking through the patient's history. And I look at their lab results first, and there's a whole bunch of comments on their B12 testing, their folate testing, their hemoglobin testing that they can't give answers because they're getting interference from an antibody. Then it turns out that this patient has an IgM level that is at 5,000 and the upper limit of our test is 280. So, really, really high. He's diagnosed with Waldenstroms macroglobulinemia and that is probably our interfering antibody. Huge amount of protein sticking all the red cells together.

So then, this one's a little hard to resolve, right? Like how do you get rid of all this IgM that's just in his plasma? So, we might not be able to truly resolve this, or you might have to go to some special techniques. You might just say we believe the front type and we think he's a B, or if you can find some history. So, this was a little harder because there's not a good way to get rid of all that IgM, at least not in our standard lab, so.

Joe: But the history in this case obviously really points you towards ... I mean, the obvious conclusion would be that something's going on there. Were you able to ... was it so interfering that you couldn't do ... as you said, your staff couldn't really do antibody screens, you couldn't really work him up very well. I mean, I guess the question is, what are the options, how are you going to handle this guy from the transfusion perspective?

Nicole: Yeah. So, basically right, if you can't rely on your antibody work up, then you have to try to phenotype someone, just figure out what their phenotype is and try to match them that way. We did phenotyping, to try to get him cells. And then, you could potentially get rid of your IgM if you treat

with DTT. And so, you could try to resolve your antibody testing that way, but we don't ... that would be a reference test for us. We don't do that locally, so you would have to send it out.

And actually, the techs found that after his sample had sat in the fridge for two days, at a really cold temperature, most of the IgM attached to his own red cells and they were able to get clean reactions on his plasma.

Joe: Nice, nice. So, for the learners out there, what Nicole just said at the DTT getting rid of the IgM ... I can't help myself, Nicole.

Nicole: That's okay.

Joe: So many people listening to this podcast are really just getting going; just thumbnail that for us, if you don't mind. Why would DTT, or dithiothreitol, why would that help you with eliminating some of the activity of IgM antibodies?

Nicole: So, IgM is basically a pentamer of five of our sort of basic antibody structures attached together, and they're attached by disulfide bonds. And DTT cuts disulfide bonds. So, if you treat with DTT, it cuts the disulfide bonds and removes the IgM reactivity. But because IgGs, which is what we usually care much more about, they're more clinically significant because they are just single units, they don't rely on the disulfide bonds, and so it doesn't harm the IgG.

Joe: Super important. Okay. That's somewhat of an odd case. I don't think that happens super often, but really interesting.

I wonder if before we move on from the antibody gain cases, we've talked about a scenario with an ABO subgroup, we've talked about a situation of massive hypergammaglobulinemia, are there other things that we might see when you have one of those antibody gains?

Nicole: Oh, yeah. You could definitely get just cold-reacting antibodies. Very common things like anti-M, warm autoantibodies are pretty common. In this day and age, right, we have all of these drugs that are antibodies. You have Daratumumab and there's an anti-CD47, so IVIG, right? There's a whole bunch of drug options that are antibodies that can interfere, lots of more common possibilities.

Joe: Right, right.

Nicole: But I like the crazy cases... right?

Joe: But given your... I was going to say, given your previously established "nerd status," you went for the Waldenstroms. I like it. That's fantastic. All right. So, two really illustrative and interesting cases, even for someone

like me. I found both of those interesting. So, let's move on to another scenario, Nicole.

So again, everyone, we've covered the antibody gain scenarios. Nicole's going to take us in a different direction now. Nicole, take it away.

Nicole: Okay. So, for our next case, in this one we have reactivity with anti-A and anti-B reagents. So, the patient looks like blood type AB on our front type. But we only have reactivity with B cells on the back type, which looks like blood type A. And so, here again, if we take our first rule, the weak reaction is usually the questionable one, that would be on the front type that we have this inappropriate B reactivity. So, that's the one we're probably going to try to investigate first, that we seemed to have gained an antigen.

And so, we can try to look up the patient's history, different possibilities we might consider of how you can gain an antigen, which isn't all that common. So, classically people talk about acquired B, right? So, I know you have a very nice presentation on acquired B, which I have watched. But here you kind of get the ... you might have the classic history of an older person that had colon cancer, some kind of colon issue, they end of gram negative bacteremia or gram negative sepsis. But that doesn't happen very often, just given our reagents now. They don't really find acquired B very well, but techs like to ask about it still.

So, it might be some rare AB phenotype, which is when you get the A transferase, but also isn't very specific and it will pick up a few just plain galactoses, instead of just the N-Acetylgalactosamine. And it will make a little bit of the antigen, along with predominantly A, but that's also pretty rare.

Where I am, because I'm at a transplant center that has like nine transplant doctors, we see this thing a lot from our stem cell transplant patients. So, that happens to be what this case is, is this is actually a woman who was originally blood type A and she got a stem cell transplant from someone who was blood type B. And so, she's in the process of kind of converting to become a blood type B.

Joe: In that case, the clinical history answers the problem for you.

Nicole: And especially because these gain of antigens are so uncommon, you really need to rely on the history to figure out what's happening here. And, of course, this could potentially be transfusion, but again it would be an incompatible transfusion, right? The best explanation for this would be someone who's blood type A who got B red cells, which is possible, but hopefully that doesn't happen very often.

Joe: That's generally frowned upon, right? Okay. So, I can't help myself before we leave that, because you mentioned acquired B and for those who are

just thumbnailing this, Nicole, what would be the classic clinical history for someone with acquired B?

Nicole: So, the classic clinical history is someone who has a colon issue, especially colon cancer, something that's breaking down the wall of their colon and allowing those GI tract bacteria, those gram negative bacteria, to get into their bloodstream. And then, the gram negative bacteria have a deacylating enzyme that cuts the acyl part off of our N-Acetylglucosamine on our A and makes it kind of look like a B, but only with ... usually there's really one clone of monoclonal reagent that reacts with it the best, and that clone doesn't get used very often anymore. And so, we don't really see ... people might have acquired B, but we actually don't pick it up very well anymore, so. And it's not clinically significant usually anyway, so it's okay that we don't see it.

Joe: Have you seen a real, live case of acquired B, Nicole?

Nicole: I have not.

Joe: I mean, I can count on I think two fingers the number I've seen and it was very early in my career. Like you said, that reagent is not used very often anymore. So, it's found on exams, I think, more than it is in real life.

Nicole: Exactly, yup.

Joe: All right, good deal. So, that is an example of antigen gain, as Nicole said. A pretty uncommon scenario. The classics that you should be aware of, obviously acquired B, as she mentioned transplantation a huge one, patients with hematopoietic stem cell transplantation, some weird genetic stuff like B(A) or A(B) phenotypes, and other oddities. But again, the classics, probably acquired B and transplantation, that you should really, really focus in on.

Anything more on gain of antigen, Nicole?

Nicole: Nope. But you could always have interfering things like rouleaux, you might get an anti-reagent antibody, something that interferes. But those usually will show up ... those are potentials to cause multiple types of ABO discrepancies, so.

Joe: Got it, got it. Okay. So, we've been through gain of antibody, and we've been through gain of antigen. I'll let you guys guess what's coming next, but I'm going to give it to Nicole. Nicole, fire away.

Nicole: All right.

Joe: What's your next scenario?

Nicole: So, our next scenario is someone who has a very strong reactivity on the front type, 4+ with anti-A, looks like blood type A. On the back type, we get nothing, no reactivity. So, technically looks like blood type AB. So, here we don't really have a weak reaction, right, so we can't use our first rule. We either have a really strong reaction or we have nothing. But we can use our second rule, which is antibody problems are more common.

And if we go to tube testing, we actually do get some weak reactivity with B cells. So, that actually resolves our discrepancy right there. Now our front type is consistent with A and our back type is consistent with A. So, we basically resolved that discrepancy really easily by just doing tube testing, but we might want to ask ourselves why is their antibody reactivity so weak? And because we usually do automated testing, it might happen again, and again, and again. And so the techs don't have to keep trying to resolve this over and over again, and do extra testing, if we can figure out the reason and just put an explanation in their history, we don't have to work it up every time.

So, in this case, losing antibodies are pretty common, right? There are lots of reasons to be immunosuppressed, to lose antibodies. Diseases, immunosuppressive drugs, all sorts of things. So, this guy happened to be a 74-year-old man who had multiple myeloma. He had also recently gotten some steroids. And when we looked at his protein electrophoresis, it showed us right there that his IgM level was below the detectable limits.

So, we have an immediate explanation of why he has a weak antibody reactivity. We can put that note in his chart and the techs don't have to do a full work up every time.

Joe: Right. So, are we talking about like a treatment effect, Nicole? I mean, most people when they think about myeloma, they think about having too much antibody. What's the mechanism there?

Nicole: Yeah. I mean, it kind of depends. Sometimes you're right, if you have myeloma, depending on what immunoglobulin type it is, it can suppress the other immunoglobulins. So, in this case, this man's IgG was really high and it was actually somewhat suppressing his IgM. And probably his recent steroid treatment was also suppressing his antibodies.

Also, sometimes we lose antibody reactivity when we get older, right? So, he's 74, his antibody reactivity inherently probably isn't as strong as it was when he was 20. So, multiple reasons.

Joe: That makes sense. Okay. And so, Nicole, you've mentioned that in this particular case that the ... you didn't see anything on the automated testing with the reverse type, with the B cells, but you saw weak reactions with the B cells on the tube typing. Is that happening that way fairly common? I mean, are you talking ... when you're saying tube testing, are you talking

doing anything extra with the tube testing or just standard, routine tube testing?

Nicole: Just standard, routine tube testing. Sometimes we'll just get a little bit stronger reactions. The techs like to say that the machine "shakes too hard," and sometimes it shakes away weak reactions that when they don't shake so hard, they see the reaction, so.

Joe: That's awesome. Everyone, just in case you didn't catch that, the machines don't "shake tubes."

All right, Nicole, let's move on to the next case. But before we do, I'm wondering, could you go with me on just a little bit of a detour. People sometimes write to me and they ask me why we don't routinely use the anti-A,B reagent on the front typing in tube tests anymore. I usually tell them that's not very many cases where that would help, but I just wondered if I could get your perspective on that. How do you feel about that?

Nicole: I mean, I think that our reagents with the monoclonal antibodies now are so good, I mean they're so strong, that they're very reliable. And spending the extra money and doing the extra work to do an anti-A,B routinely just doesn't seem like it's worth it to me.

Joe: I would agree with that. Occasionally, in reference lab world, we'll see cases where as part of ... as just one of the tools in your toolbox it might be helpful to kind of pick up something when the reactions are not showing anything. But I agree with you completely, routine use not necessarily valuable.

Sorry for that detour, I appreciate that.

Nicole: That's okay because it's actually going to show up on the next case, so perfect intro!

All right. So, Scenario 3-B has some anti-A,B. So, fairly similar, right? So here we have no reactivity on our front type, looks like blood type O. And we have a question mark, we hit a lot of question marks, I hate question marks, but the machine doesn't want to commit, right? So, we have a question mark with A cells. So, when we do it by tube testing, we get no reactivity whatsoever, which is why the techs then pulled out the anti-A,B to see if maybe it would help them find some reactivity on the front type, and it didn't. So, we have nothing. So, we're going to go with our rule of antibody problems are more common and just kind of try to assume that our antibodies are weak for some reason and this person is really blood type O. And also, look up the history, right?

So, this is a 30-year-old pregnant woman, this is a prenatal type and screen. And looking through her history, she doesn't have autoimmune

disease, she doesn't have anything that could explain to me why she has no antibodies. But hunting back further in her history, I found out nine years before she had clearly typed as O positive, with a four plus and a two plus reaction on her back type. So, her antibodies disappeared somewhere in the last nine years.

And so, the techs went through some effort, right, of trying to cool things down, adding extra drops of plasma, multiple things to try to bring up her back type. And finally, after incubating at 4 degrees C for 30 minutes, they did get a 2+ reaction with A cells and only a microscopic reaction with B cells. So, then we're trying to figure out why did her back type disappear.

So, transfusion would be a pretty common thing, right, if for some reason she got a whole bunch of AB plasma, but she didn't have that history. No immunosuppressant drugs, none of that. We finally figured out that she has combined variable immune deficiency, which is an inherited immune deficiency where you pretty much can lose all of your antibodies. And we did testing on her, and she had undetectable IgA, IgG, and IgM, so.

Joe: So, wait. Did you say that she had previously tested normally? With this being inherited, you would think that that wouldn't have been the case.

Nicole: It usually shows up later. So, most people it shows up later in life and they sort of lose antibody production in their 20s or 30s.

Joe: Okay. So, that's a weird case. But, Nicole, do you mind taking us through ... we've obviously talked about in these two scenarios about loss of antibody, and you've given us a case of common variable immunodeficiency, you've given us a case of multiple myeloma. So, what else might you think of in those loss of antibody cases?

Nicole: So, you just think about multiple reasons that someone might be immunosuppressed. So, if they have autoimmune diseases, they're on immunosuppressant drugs, people with cancer who are getting chemotherapy, people who are congenitally immunodeficient in some way. Sometimes the ABO subgroups can look like they're a loss of antibody. And then, when you do some further work up you sort of figure out, oh no, actually it's kind of weak antigen instead of antibody.

And, of course, transfusion. So, transfusion a pretty common reason to have loss of antibody, right? If someone gets emergently transfused with a whole bunch of AB plasma, then they can look like they have temporarily lost their antibody. And then, of course, transplantation can cause anything, stem cell transplants.

Joe: Yeah. That's the fun part, right-

Nicole: Right.

Joe: ... that you get to see all the time in your practice, the transplantation stuff.

So, okay everyone, here's where we are now. We have gone over three different categories of different situations with ABO discrepancies. We have talked about the antibody gain scenarios first, and then we did the antigen gain, and now we have done antibody loss. I'm guessing everybody kind of knows where we're headed with this, but, Nicole, hit us with scenario number four.

Nicole: All right. So, number four is a patient who has no reactivity on their front type, and then they have 4+ reactivity on their back type with A. And so, we're wondering where in the world did their front type go, or why do they only have anti-A in their back type if they're really a blood type O. So, here again we don't really have a weak reaction, we have nothing or we have strong reactions.

And so, in tube testing we get no change basically, same reactivity. And so, we're trying to figure out what happened here. So, we go and look in the patient's history. This is where you might also want to pull out your anti-A,B, see if you can bring up some front type reactivity. And this guy has pancreatic cancer, that's his most significant history, and he's getting some chemotherapy. But chemotherapy usually causes us a back type problem and he has a nice, strong back type. So, we don't really think of this as an immunosuppressant issue.

But there are some cancers, so primary pancreatic, ovary, and biliary tract that are known to sort of get revved up and secrete too much soluble ABO antigen. So, if you've got a whole bunch of soluble antigen sort of floating around in the plasma, then it can suck up a whole bunch of your antibody, and enough of it doesn't bind to the red cells to actually get them to agglutinate. So, the way we solve that problem is we wash.

So, we wash the patient's red cells and we retest. And there is another slide for scenario four that shows what happens after we washed. And clearly you can now see that his red cells are blood type B. Ta-dah! So, we got rid of all that neutralizing stuff that was in his plasma.

Joe: So, Nicole, correct me if I'm on the wrong track here, but the way I always described scenarios like this to trainees is, imagine you're a reagent antibody and you've got the choice of reacting against these big, honking red cells that have all kinds of weird electric potential and stuff, or these lovely soluble antigens that are just kind of floating free. Our reagent red cells love those soluble antigens. And like you said, they can get used up, soaked up, neutralized, et cetera, and you basically don't have any left to react against the red cells.

Is that a fair way to put it or am I making it too simple?

Nicole: No, you described that perfectly.

Joe: I like simple things, I'm a simple man. So, that's all good. Okay, awesome. So, that's a loss of antigen associated with pancreatic cancer. I'm so glad that you did that case.

Nicole: Yeah. So, it's sort of an "apparent" loss of antigen, right? Like the antigen's still there, but you can't see it initially unless you do a little work. I have seen cases of true loss of antigen, which the cases that I've seen are in AML. So, because our red cell, right, precursors are from the stem cell, the myeloid stem cell, as the myeloid leukemia is coming from, you can actually get sort of stem cell abnormalities that are not only leading to the myeloid leukemia, but they can also lead to abnormalities in your red cells. And I have seen people with really strong AML, with a whole lot of blasts in their system, that basically start losing their ABO expression on their red cells. They truly have a loss of antigen.

Joe: Okay. Yeah, that's a great point. This is apparent loss, but it's really there. But in cases like that, it is a true loss. Okay. I'm so glad you did the pancreatic cancer one though because that ... I have always had a difficult time helping people understand that visually and conceptually, and I think you explained that perfect. So, nice work there.

So, loss of antigen. Anything else with loss of antigen? I'm guessing transplantation would also be in [there], like it is with everything...

Nicole: Oh, yeah, always.

Probably ... I mean, the most common reason people usually see loss of antigen is because of massive transfusion or just chronic transfusions. So, if you get massively transfused with O red cells and you're really a blood type A or B or something else. And we also, our sickle cell patients who are getting chronic exchange transfusions, usually this ... the special red cells that get set aside for them and are phenotypically matched are O. And so, they just ... every four to six weeks they get exchange transfused with O red cells. And so, they pretty much always just look like they're blood type O.

Joe: Got it. So, outstanding. So, we've covered the four main categories; the antigen and antibody gains and the antigen and antibody losses. Nicole, I think you have some fun, weird stuff. We're descending deeply into nerd territory now, everyone. Just a warning, Nicole's going to take us deep. Let's move on to your last couple of cases that you want to share with us to illustrate some different stuff. Hit us.

Nicole: Okay. So, these are all cases of "mixed fields." So, sort of technically not a discrepancy, but it tells you ... a mixed field usually suggests to you that there are two red cell populations, and one of those populations might be discrepant with the type that you're seeing on your plasma type or your antibody type.

Joe: Let me interject for just one second. Again, for the learners, when you say "mixed field," can you describe what that means or how we would see that if we were shaking a tube or looking on, say, a gel column or something?

Nicole: Of course. So, like I said, mixed field usually, classically tells you you have two different red cell populations. And so, for example, if you add anti-A, if you have a population of A cells, they're going to react. So, in a tube they're going to agglutinate, or on a gel card they're going to get stuck up at the top of the gel. And then, if you have a population of cells that are something else, they're B, they are not going to react. So, you're either going to see them free floating in a tube or you're going to see them move their way down to the bottom of the gel card. And so, you're going to see this mixed reaction of some cells that are reacting very clearly and some cells that are not reacting very clearly, which is why it's called mixed field.

Joe: Perfect.

Nicole: All right. So, I live in Nerdville, right, and sometimes I get excited about the crazy stuff. It's good to explain the basics. So, here we had a patient that we only have a front type here, we don't have a back type, and that's because this is a neonate. So, we routinely don't do the back type on neonates because it often has mom's antibodies in there and we know we're going to get strange reactivity. And it's not trustworthy because it's mom's antibodies, not baby's. So, we just do a front type on our babies.

And here, when we our regular testing, our immediate spin testing, we get a 2+ mixed field with the anti-A. And then, they washed it multiple times. And so, what this is, this is a cord specimen. And in cord specimens, they're often kind of junky. They can get a whole bunch of Wharton's jelly and stuff in them that makes them not specifically stick together. So, the techs thought, "Well, maybe this is contaminated. We'll try to wash away the Wharton's jelly or wash away this interfering stuff." But it didn't work. They washed it 12 times, still got the same reactions.

Joe: One thing about blood bankers, we are persistent, are we not?

Nicole: Yes, yes. I will make it work, right? No.

So, then we thought ... what you think of here is it might be the Wharton's jelly, but washing didn't get rid of that, so it probably isn't. Another thing is the cord can get mom's blood into the system, right? So, you might have a mixture of baby's blood and mom's blood and truly you have two different blood types. So, it would help us to know what mom's blood type is. So, her blood type is A positive.

So, we're getting reactivity with A here, might be mom's blood with baby having a different blood type. But when we do anti-A,B ... there it comes again, anti-A,B ... we don't get mixed field. So, if we truly ... if the baby were truly like blood type O, we should get mixed field with the anti-A,B

and we don't. So, this seems like these are really baby's red cells. They just ... some of them react better with an anti-A reagent than others. This baby's only 30 weeks. He had to get delivered early because his mom had horrible preeclampsia. So, baby's have weak ABO expression. It's partly protective for them.

So, on the next slide we actually kind of have a picture that depicts what the difference is between a baby's ABO blood group and someone who's older. And basically, babies, protectively, have like these single ABO antigens, that's just sort of one little tail sticking up there. So, it's really close down to the red cell membrane and there's a whole lot less expression. Then, when we get older and we create these very branched, big antigens, that we have 10 times more ABO expressions, and it's a big thing that sticks way out from the red cell membrane, and it reacts more easily with antibodies.

Joe: So, Nicole, when you say it's "protective," help us understand that. What do you mean that it's protective for a baby?

Nicole: So, mom and baby are necessarily going to be ABO compatible. So, especially if mom is blood type O, she has the anti-A,B antibody that is an IgG, it can cross the placenta, and it can react with baby's red cells. And so, if the ... in the United States, blood type O is the most common. So, we have a whole lot of O moms that are having babies that are blood type A or blood type B. And so, if they have less ABO expression on their red cells, they are much less likely to hemolyze from mom's antibody.

Joe: Got it. That's excellent. So this one, in this case, it was just a function of the baby being so young, and not at all anything anybody did wrong, and just the way the baby is.

Nicole: Yup. And you just randomly get a few of those red cells that have enough A expression to react and a few of them that don't, so.

Joe: Got it. Okay, awesome. Well, I think you have one more to share with us, and on the slides it's Scenario 5-B. So, why don't you hit us with that one.

Nicole: So, this is sort of my pet thing that I love. I love chimeras, all different kinds of chimeras, I think they're so cool. But I haven't seen an ABO chimera personally. The one chimera I've dealt with personally was identified by discrepancies in HLA typing. But this is a really good example of what you see if they are identified by discrepancies in ABO typing.

And so, here we have a man who donated blood, and he was found to have these abnormal reactions on the gel card. And so, he has a mixed field reaction with anti-B. And then, his back type only reacts with A. So, he truly looks like he's blood type B, but he has a mixed field.

So, the question is, why does this perfectly healthy donor who showed up to donate, has never had a transfusion, why does he have a mixed field? They investigated further, did some flow cytometry testing, and found that he had 5 percent of his blood cells were B positive. So, he actually had a mixed field in his D type also, and 95 percent of his cells were O negative.

And so, this is a situation that can happen very commonly in twins. It's called "hematopoietic chimerism." And basically what happens is if the twins have a placenta that overlaps each other and they have some shared circulation through the placenta, they can share stem cells from their bone marrow with each other. And then, they can permanently have mixed blood types. This is probably the most common form of natural chimerism that you'll find in people, is in these twins who've shared their stem cells in utero.

Joe: And this actually brings up an interesting point in terms of the formation of ABO antibodies. If this person is 95 percent O negative, someone who is just kind of naively looking at this might say, well, why the heck didn't this person make anti-B? And I think it's a pretty easy answer, why don't you tell us?

Nicole: Well, because they're ... they've got 5 percent B cells. And so, they do have that B antigen. Even though it's a really small amount, it's still enough that your body doesn't see it as foreign. So, it says this is me, even if it is in a really small percentage and you don't make an antibody.

Joe: I'm sorry, Nicole, I'm descending into Nerdtown with you, but I love that because I think people have a misperception that the "naturally occurring" ABO antibodies just happen out of the blue. I mean, yes, you don't have to have a transfusion, but it's still ... the immune system is still the immune system. It sees something that isn't recognized as self and it's making an antibody. It's just not necessarily from seeing red cells, right?

Nicole: Right, yup. So, it's probably ... you know, in our case when we make our antibodies to ABO, it's from getting exposed to plant material, to bacteria in our gut, multiple things that look very similar.

Joe: Well, Nicole, I have to tell you, you have almost ... and I'm saying "almost" ... made me love these things, but I'm still me, so I probably won't love them. But honestly, this has been a really great look at how to approach these, some great classic examples.

Again, folks, you can find the slides for every one of these scenarios at BBGuy.org/054. Please go there and check these out. Again, we've tried to paint the word pictures as best we can. So, if you're running, or riding in your car, or whatever, you can understand it, but you'll probably get the most benefit from seeing this also on the website. There will be links there as well. And please, go ahead and comment. I'll try and get Nicole to

answer any of your wacky questions that come up because god forbid I should answer ABO discrepancy questions, Nicole, know what I'm saying?

Nicole: You could do it, I have faith in you.

Joe: Oh man, I'll struggle with it. But, Nicole, thank you so very much for being with me, this has really been fun. I greatly appreciate it.

Nicole: Thank you so much for having me. It's been a pleasure to share this thing that I love and find so interesting.

Joe: Well, I'm not sure that Nicole completely convinced me to fall *totally* in love with ABO discrepancies as a result of that conversation, but I really do think that she gave you a super-useful framework; kind of a way to approach ABO discrepancies. I think that if you follow the rules that she gave you, I think you're going to have no trouble with the vast majority of these cases. Certainly, the more advanced, the more difficult ones, those would be more of a challenge, but this was intended to be a primer, the basics, the essentials, if you will.

So, again, let's talk about the three things that she mentioned. **First, get the history.** The clinical history helps you so much in the evaluation of ABO discrepancies. It can make the entire difference in evaluating the case. And the second and third rules involve how you actually look at the reactions themselves, both on the forward grouping and the reverse grouping, the front type and the back type, the cell grouping, the serum grouping, whatever you choose to call those things.

The first of those two rules is that the **weakest reactions are usually the discrepant reactions.** So, look for the weak ones. Remember ABO reactions are usually very strong. A weak one may show you where the problem is. The second rule, in terms of evaluating the reactions, is that **antibody problems are more common than antigen problems.** Again, keep those in mind and I think you'll have no trouble with the vast majority of these cases, especially on examinations for those of you that are in those situations.

So, the next episode IS a continuing education episode where I'll be covering the critical developments of late 2016 and 2017 with Drs. Claudia Cohn and Melissa Cushing. This is based on an amazingly great summary that they did in the April 2018 edition of the journal *Transfusion*. You're going to love this. There's lots of really, really great stuff in that. So, I can't wait for you to hear it, it is coming soon.

So, until then, as always, I hope that you smile, and have fun, and above all, never *ever* stop learning. Thanks a lot. We'll catch you next time on the Blood Bank Guy Essentials Podcast.