BBGuy Essentials 085CE:
What to Do When Everything is Incompatible with Jill Storry
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Jill: Hi. I'm Jill Storry from Lund in Sweden, and this is the Blood Bank Guy Essentials Podcast.

Joe: Hello everyone, and welcome to Blood Bank Guy Essentials, the podcast with one simple goal: To help you learn the essentials of Transfusion Medicine. I am Joe Chaffin, and I'm so glad that you are listening today! You are in for a treat, as I am speaking with Dr. Jill Storry, all the way from Sweden, about a topic that *vexes* blood bankers everywhere (I love that word: "VEXES"), and that topic is, what to do when EVERYTHING is incompatible!

But first, you should know that this *is* in fact a continuing education episode. The free continuing education credit is provided by TransfusionNews.com, and Transfusion News is brought to you by Bio-Rad, who has no editorial input into the podcast. This podcast offers a continuing education activity where you can earn several different types of credit, including: One *AMA PRA Category 1 Credit™*, one contact hour of ASCLS P.A.C.E.® program credit, or one American Board of Pathology Self-Assessment Module (or "SAM") for Continuing Certification. To receive credit for this activity, to review the accreditation information and related disclosures, please visit www.wileyhealthlearning.com/transfusionnews.

You know, in most cases, pre-transfusion workups are simple. We get a sample, we run basic tests on it, like ABO and Rh typing, and we run a screen for non-ABO antibodies (or "unexpected antibodies" as we call them), and everything is just fine. No problem! We issue wonderfully compatible donor blood for patients who need a transfusion.

Every now and then, however, when a transfusion service does that workup, they see that there is incompatibility between the patient's plasma and the cells in the antibody screen, and that suggests that an unexpected antibody is there. And then, in an even smaller proportion of cases, when we try to see what is going on by testing the patient's plasma against a wide variety of other people's red cells (we call that an antibody "panel"), we discover that whatever is in the plasma is incompatible with EVERY other red cell! In other words, NOTHING is compatible!

These situations are a huge challenge, and they end up in time-consuming testing (with funky weird tests like autoadsorption, alloadsorption, and even things like monocyte monolayer assays, for goodness' sake!). And it also leads to delays in blood availability, and confusion and frustration for the providers that ordered the blood. None of that is good, but Jill Storry is going to give you a practical approach to distinguish between the three main causes of what we call the "panagglutinin" pattern, and those three causes are: Warm autoantibodies, antibodies against high-frequency red cell antigens, and multiple alloantibodies. She even throws in a quick discussion of the effect of the multiple myeloma drug daratumumab at the very end of this discussion.

I want you to pay really close attention as she describes the basic ways to look at these three main patterns, the ways that we can categorize them.

Before we start, let me tell you a little about Jill Storry. She is an Associate Professor at the Division of Haematology & Transfusion Medicine at Lund University in Sweden, and she is responsible for the Immunohematology laboratories within the Department of Clinical Immunology and Transfusion Medicine, Laboratory Medicine at Lund. Jill is a world-renowned expert in immunohematology, and she has authored over 80 original papers, reviews, and book chapters on topics related to blood groups. She is passionate about blood groups! She is a section editor for the journal *Vox Sanguinis* and she is on the editorial boards of *Transfusion Medicine Reviews*, *Transfusion*, as well as *Immunohematology*. She's awesome, in other words, and she's got a lot of great things to say to you today!

I'm so excited for you to hear this episode! It is truly one of my favorites. So, let's go! Let's learn what to do when everything is incompatible.

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Joe: Hi, Jill. Welcome to the Blood Bank Guy Essentials Podcast!

Jill: Hi Joe. It's really a privilege to be part of this very exciting series, so thanks for having me here today.

Joe: It's important for us to start off with the basics. So can we just start, Jill, by having you walk us through just a really high level overview of pretransfusion testing when everything goes right. How is this supposed to work when we're getting somebody ready to be transfused?

Jill: What do we do when a patient's sample comes through the door? Well, of course, if there's no blood group already established in the computer system, then we have to start from the blood grouping, antibody screening of course.

And I think it's the regulations in all countries, that we require two samples, two ABO groups and screens to be able to go forward. In some countries, it's required, that then you can just go straight forward and do a crossmatch, if everything's negative. In other countries, you can actually do electronic release, and that's something that we do here.

But that's the dream scenario that you have two ABO and D groups that agree with each other, the records match in the computer system, and the antibody screen is negative.

Joe: Okay. So I've talked a little bit about antibody screens in the past with great and wonderful Sue Johnson, so we don't necessarily have to go into incredible depth on it, but just can you summarize for us, what exactly is an antibody screen or an "antibody detection test," as sometimes people formally insist on calling it.

Jill: Well, it varies certainly from hospital to hospital and country to country, but in general, you test the patient's plasma, by any one of the approved techniques,

whether it's a column agglutination, or microplate, or even tubes, with two to three red cells from different blood donors, and these are often obtained commercially.

Normally then, if it's a two-cell screen, then you'll have two group O, D-positive cells that have been well phenotyped for the other blood group antigens. If you go to three, sometimes it includes an O, RhD-negative sample. You can't do the antibody identification from these, but if one or two of them react positively, then, then you can get a hint occasionally, but you always know that you have to go further and do further investigation.

Joe: If that antibody detection test is positive, how, again, perfect world, should things go from there? What's the normal steps to get to the point where you can crossmatch someone and give them blood?

Jill: Different rules apply to different areas and countries. So sometimes you actually need to ask for a new sample to perform antibody identification. Some areas you can actually go forward and do antibody ID on that existing sample. But either way, you need a sample from the patient that you then test with a panel of somewhere between 8 and 16 different group O cells. Again, many, many places buy these from the different companies, other places prepare their own panel in-house. It all depends on what sort of resources you have.

But in all cases, it's really an extension of the screen. So you're taking another group of well-phenotyped blood donor samples that you can then determine a pattern of reactivity and assign a blood group antibody specificity to.

But in an ideal situation, you would have your antibody screen, do your antibody panel. You'd get a nice clear pattern of reactivity with some of the cells on the panel that you can then see from the antigram that accompanies those panel cells is a clear-cut specificity. Then it's just a matter of walking to your well-managed blood bank, pulling units that are phenotyped for that particular antigen, is antigen-negative, and cross-matching those. That's the smoothest path forward to finding compatible blood for a patient that has antibodies.

Joe: And I'm guessing, Jill, that in your clinical setting, I mean, I can certainly speak for mine, because I work overseeing a immunohematology reference lab, that in hospital transfusion services, that's the way it actually usually goes. Sometimes it feels like the ones that I get are almost always the opposite of that. I end up seeing the ones where things DON'T go that well. Is that your experience, too?

Jill: Oh, absolutely. And actually, I would rather have everything positive or clear cut specificity. The ones that I battle with the most are those that have, yeah, weak 1+ reactions with, I don't know, 7 of the 10 panel cells, and you can't identify it. Those I find very frustrating.

Joe: Thankfully we're not going to talk about those today, Jill.

Jill: Yay!

Joe: Let's…yay! Fantastic. That's good for both of us. But I do want to focus on the scenarios where, as you said, where that, antibody detection test or antibody screen, those cells have positivity, as well as every cell or close to every cell on

the panel. That falls under that category, Jill, that we commonly refer to as "pan-reactive," or "panagglutinins." It's basically where *everything* is incompatible.

So, I wonder if you would just, again, high level, we're going to get into these specific things, but when you see something like that, if you're working in a transfusion service somewhere, or even if you're in a reference lab, and a specimen like that comes in, what are the big picture things that you want to think about?

Jill: Well, quick thing to remember is that you haven't done an autocontrol together with the antibody screen, that's something that comes with your panel. So that's something to look at.

I think the most important thing that you need to do at this point when everything reacts, is find out what the status of the patient is. Because then you know how much time you have to do things. Just see where the patient is, what they've come in for; how long do you have to investigate this antibody? And then it's simply a matter of working through step-by-step to see what you can do. But that would be my main thing: Find out, does the patient need blood soon? How much time do I have? And there's a couple of other things you can check. "Has the patient been recently transfused?", and so on. Those things are important to know before you start your investigation.

Joe: This is something, in my personal opinion, that gets missed all too often. This scenario frustrates me to death: When I hear about a patient that either a hospital transfusion service or a reference lab has been working on their specimen for hours, trying to identify, identify, identify, and then you find out, or you hear from a clinician that is frustrated, that is saying, "My patient is in real trouble here, and nobody seems to be getting that!" So, I think it's so important what you said, to understand that the patient comes first and sometimes you have to make difficult calls. This is my soapbox, Jill. I'm sorry...

Jill: No, no, but I think you're right. In the end, blood transfusion is a medical decision. And I think that, if we're in the lab, we don't have the ultimate responsibility in deciding the patient is transfused or not.

And I think that that's quite a heavy responsibility for the treating clinician. And so, we really need to take into account, the emergency of the situation and understand, is it better to give incompatible blood at this point, or can the clinician really wait to see if his patient can actually cope?

I think we have to be quite humble, really, in our approach to dealing with patients, because even though I think we take quite a great responsibility in any blood bank, it's the doctor at the end that has the greatest responsibility. And I think that's, we will encounter that this panic situation occasionally where people don't know what to do. So I think we're in a position where we can give guidance. We can certainly try and solve the problem. I think in many cases, we do solve the problem. But we can't take away from the fact that that person's wants to step nearer a major decision.

Joe: I think that's so important, and honestly, to the listeners, if that's the one and only thing that people take from this (I hope it's not, but if it is), I would be happy. If we get the message across to people that sometimes you have to make a difficult decision.

And that decision in the end has to be guided by the physician who's taking care of that patient. And sometimes people in my role as a physician may have to jump on the phone or go to the patient's bedside and talk to people about this, because sometimes you have to make a difficult call.

Sometimes you have to make the best call you can based on the information that you have so far to try and keep the patient as safe as possible.

Jill: Absolutely.

Joe: I said during a talk I gave at last year's AABB, Jill, that I would rather deal with a hemolytic transfusion reaction than DEAD, anytime! It's just that simple for me. You got me on my soapbox. I'm going to try and climb down so we can talk a little bit about these possibilities.

So, let's do a case, Jill, let's... It's a case that's partly based on reality, but I've edited some details to protect the innocent (or the guilty in some cases).

Let's start with a young lady who's named "Frieda Johnson." So, here's Frieda: Frieda is a 70-year-old female. She comes into the emergency department. There was a report that she had vomited bright red blood at home. She's a little bit tachycardic, her pulse is 105. Her blood pressure is 100/60, so not terrible, but she's saturating well, 100% oxygen saturation on room air, but she's anxious, and she looks pale and scared.

In the emergency room, they have a point of care instrument that reads for hemoglobin as 6.9 g/dL; okay, 69 g/L to those of you that use the g/L, and she's admitted for evaluation. The emergency docs say, "You know what, it's scary because she vomited bright red blood and she might have esophageal varices, but she's stable enough that we don't have to do an emergency transfusion."

In terms of clinical history that's important: She was pregnant two times, delivered one baby who is currently alive, and she doesn't remember ever being transfused, but her husband says, "Maybe she might've been, but I'm not totally sure."

Given that, her blood is sent to us in the transfusion service. Her ABO testing, she's O positive, no problem. But her antibody screen comes back 4+ positive in all three cells of a column agglutination or gel panel. Uh, so everything's positive. the, antibody ID is done. you know, and at first glance, again, everything is positive. Her autocontrol is positive. The lab decides to do a direct antiglobulin test; it's also positive 4+ with IgG, 1+ with C3.

Okay, Jill, so that's a long story, but what kind of bells is that ringing for you at this point?

Jill: Well, first of all, I'll say it's great that you got so many clinical details from her. I know some of our requisitions come back with like nothing on them, so that's

great. We know her immunizing events, we know she's been pregnant. But then there's a suspicion of a transfusion. That's really good to know. it means that we can't just issue ABO and D compatible blood, which you can with men who don't have a transfusion history that have the same picture, for instance.

So where can we go from here? Well, I think, one, that the DAT is very interesting. The fact that it's 4+ with IgG and 1+ with complement, it's fairly normal for what looks like an autoantibody. The fact that it's positive with C3 is a little bit alarming. It means that maybe there's a bit of an autoantibody that has a cold-reactive phase, possibly, to me.

But in this case, I think that once you've seen those 4+ in all the cells in the panel, there's a couple of ways you can do. I think in a straightforward case like this, I would just go directly to an autoadsorption.

In our lab, we would not perform an eluate. I know that some places do. I know some places that's the rule for some labs, but in my lab, I would simply do an autoadsorption. She doesn't have a current transfusion history. She's vomited red blood, which suggests that it's some sort of GI bleed.

So for me it seems quite straightforward. I would just perform an autoadsorption. I would, do two to three autoadsorptions, checking between them, because with a DAT that was that strong, it's very unlikely you'll pull the antibody out in one adsorption.

Joe: So Jill if I can interrupt you for just a second, let's just make sure that listeners who, some are saying "Autoadsorption. Sure, it makes sense." Some are saying, "Autoadsorption; I think I knew that at one point...." Can you just thumbnail for us: What is an autoadsorption?

Jill: Yeah, sure. Autoadsorption really means you're going to take the patient's red blood cells, you're going to wash them three times so that they're clear of her plasma, which is crazy cause you're gonna add that back. We wash her cells. We've wash and pack her red cells, divide them into, in this case, she's got a hemoglobin of 6.9, so she probably doesn't have too many red cells in that EDTA sample that they've generously given you. So, I would divide that into two aliquots probably.

And then there's a couple of ways you can do autoadsorptions. It's standard practice in my lab, and I think it's done in practice in many labs, is to use the addition of "PEG" [Note: Polyethylene glycol] to do something that we call a "PEG autoadsorption," where you add equal volumes of polyethylene glycol, just the stuff that comes in the bottle, together with equal volumes of plasma. So, we use 1:1:1 in our lab. I know that some people do vary the ratio of red cells. If you have a lot of red cells, you can use a few more in proportion to the plasma that you're adding. Then you incubate that mixture at 37, I think I would say a minimum of 20 minutes. Again, people have different protocols, but 20 minutes for that first adsorption should take a lot of antibody out.

If you adsorb for 20 minutes or so, then spin down the adsorbing mixture, take off the plasma/ PEG mixture that you then have, you can test your screening cells

right away by what is now a PEG antiglobulin test, and see if there's any reactivity left. With this 4+ DAT, you've probably, and 4+ reactivity in the original panel, you've probably got some reactivity left. So then go ahead and do a second adsorption and test again. And again, if you're lucky enough to have enough cells, a third is probably necessary in this particular case.

There are other alternatives. You can strip off the IgG from the patient cells using a couple of commercial kits. One is an EDTA-glycine acid kit, which will remove the IgG. And then you can again, do the same, go through the same performance.

And another, also good way, if you're a lab that has proteases like papain or ficin, is you can actually take the patient cells and treat them with proteases. And then perform a simple adsorption just for the plasma and the patient's protease-treated cells. That works really nicely. But a lot of labs don't have enzymes.

Joe: The logic behind what you're saying is that when you separate the plasma and then remix it, is that the autoantibody is going to be essentially kind of "soaked away." Is that a fair way to put it?

Jill: Yeah, yeah. You're hoping it's going to be, yes, soaked away by the patient's own red cells. And I think sometimes it's, I think it's quite difficult to understand, but there's so much IgG on the patient's cells, is that going to work? I mean, that's a question that I get. Surprisingly, there is room for more in the majority of cases, the patient cells will take up. But again, you cannot say "avoid" that, but you can perhaps make it more efficient by taking the IgG off first. And I know that some labs do use the so-called "EGA kits" EGA to take off IgG.

Can I ask you what you use in your lab actually, in your reference lab?

Joe: We do use EGA in our reference lab. I do know many reference labs in the U.S. that don't, but, for us, we feel like it gives us, well, it avoids as many adsorptions sometimes with strong autoantibodies. That's the logic behind it, anyway.

Jill: Yeah. Yeah. No, it is good logic, and I think that's, it certainly can be recommended.

Joe: To just kind of reset this, we've done our autoadsorption, the idea being that we're "soaking out" that autoantibody, which reacts against the patient's own cells. So what are we looking for in the background? I guess the question is, we've done THAT. So what? How does that help us?

Jill: Yeah. Why do such a fiddly procedure? Yeah, good question. So, the idea is, you know, it's true, you know, it takes time and it, you know, it takes one person away from the bench and so on. The purpose of autoadsorption is, when you have a history like this lady, we know she's been pregnant in her lifetime. She has a husband that thinks she might have had a transfusion. Then she has had the chance to be immunized to antigens that she doesn't have in her own red cells. So, she's had a chance to MAKE antibodies. So, what we're looking for in the autoadsorbed plasma is the presence of ALLOantibodies she might've made many years ago.

And we see them. If you read the literature, approximately 30% of patients who have warm autoantibodies will have an alloantibody in the autoadsorbed plasma. It's a risk we don't want to take.

We know we can't do anything about the autoantibody. We know we're going to have to transfuse against that, but we don't want to really put that patient in a position where they're going to react to something that we should have found, like an anti-E or an anti-K.

Joe: So in other words, we're trying to make sure that that we didn't miss anything in the strong reactions of the autoantibody, we didn't miss an aloe antibody hiding underneath, I guess is what I was going for.

Jill: Exactly.

Joe: We could talk about this for a month, I think, Jill, because I'm fascinated by your take on all this, but in the interest of time, let's say that we've done that and…Oh no, no! Jill, hold on! The ER is calling and saying that she started throwing up again and now they, definitely need blood. What do we do now, Jill? What are we going to do?

Jill: Yeah, it happens, doesn't it? Yeah. I think what I would do, and again, not all labs have the same thing. I would test her red cells with monoclonal reagents to Rh antigens and K. I would give out blood that's based on her Rh and K phenotype, ABO compatible, Rh and K, and say, "This is all we can do right now."

If you crossmatch, of course they're all going to be 4+ incompatible. So you're not giving anybody any information they don't already have. Again, she hasn't been transfused recently, so at the very worst, you can give out ABO and D-compatible blood in this sort of emergency. I don't think you're going to do her more harm than not transfusing her.

We have a policy here and again, Europe is slightly different to America in terms of how much we phenotype, but when we have some of these patients, we just do an automated Rh phenotype and a K phenotype and give out blood on that. I don't know if that happens much more in the U.S. I know people are phenotyping and genotyping their blood so much better, so there's so much more on the shelf.

Joe: So, there was actually a BEST Collaborative study that was done on this not too long ago. And I'll put that reference in the show notes. And what we're seeing in the U.S., Jill is, to answer your question of what we're doing, it's very, very common in people with autoantibodies without underlying allos to match for Rh and K. That happens almost... it's not universal, but it happens very commonly. And sometimes, people, if they have an autoantibody with an underlying allo of any kind, well, a significant one anyway, they'll do pretty close to, as much as possible, full phenotype match. So that's becoming a whole lot more common in the U.S. Is that similar to what you guys are doing in Sweden?

Jill: Yeah, it is. And yeah, we want, in a perfect world, you know, you would match all the common antigens that we think are clinically important, but it's not particularly practical. But yeah, we do try to match it as much as possible. We still don't have

any very good *evidence* it makes a difference, but there's certainly enough in the sickle cell world to suggest that in matching as much as we can does help in preventing antibody production.

Joe: So let's go back for just a second to our case. Let's just imagine that little, that little panicked phone call didn't happen, and we've had the time to work through and we've had the time to get to the place where we can say with confidence, as much confidence as possible, anyway, this appears to be a warm autoantibody that's reacting in the way that they often do it. It appears very strong. There are no underlying alloantibodies.

What kind of conversations, Jill, do you think that people should be having with clinicians in these cases? Because, as you know, sometimes clinicians may not be as well versed in blood banking and transfusion medicine things as we would like them to be, and sometimes they say "transfuse with an incompatible cross-match? Can I do that? How is that possible?" How do you have those conversations?

Jill: I pass them straight over to my medical colleague [Laughs]

Joe: Thank you so much! [Laughs]

Jill: No, but it's a difficult conversation to have. I think, quite often, these are actually hematology patients, so those medical colleagues are much more in the know than somebody that comes in to the ER, like this lady. I mean, you just have to explain that we've done everything we can do, this is autoantibody that we really can't match for. Trying to get around the "least incompatible" recommendation that many of us, including us, still stick on our reports.

But that is a bit of a red flag, I think when we've done the work we've done, we should just be able to give out those ABO and D-compatible units. But, just to say to the clinician, "this is what we can provide you with." It's putting ourselves in the shoes of that clinician that then has got to put incompatible blood into a patient. It's not something they've done very much of. It can't be a very comfortable position to be in.

Joe: I think that you're 100% right that we have to try and explain to them as best we can. I will tell you a couple of things that that bother me. There are some places in the United States, and I don't know if this happens in Sweden or not, I would hope not, but there are still places in the United States that force their clinicians to sign what people call a "waiver," and if you read the language on some of these things, it's just horrifying.

We're asking clinicians to sign things that basically say, "I accept 100% of the responsibility if something horrible happens to this patient," and they're going, "WHAT?! This is crazy!" Have you encountered any of that in your career, Jill?

Jill: In my career, yes, but not in the last 17 years that I've been in Sweden.

Joe: Thank God! Good, good. Well, again, there is a take home message for listeners here. If you and your hospital have something where you're forcing your clinicians to sign something when they get blood with an incompatible crossmatch, that's

not necessarily a bad thing, for an acknowledgement. But, please take a look at what's on that form and make it logical. Don't make it draconian, because, quite frankly, it doesn't matter what they're signing. Nobody is waiving responsibility. We in the transfusion still have responsibility. It's not all on the clinician, so be careful with that language, everyone. Sorry, Jill. That's again, another little soapbox.

Jill: I've gotta agree with you.

Joe: One other thing I'll say before we leave warm autoantibodies, I got in trouble, well, two years ago at the AABB meeting when I was on the platform and I said something to the effect of (and I have a tendency to speak too strongly sometimes, I admit it), but I said to a large group of people in the audience that I thought that “least incompatible” was the dumbest phrase that we use in Transfusion Medicine. And people came up to me afterwards and said, "Joe, that's mean. How could you say things like that?" Well, so, before we leave this, again, least incompatible, as you said, I completely agree, it's a pretty meaningless phrase. How can we banish this, Jill? How can we get rid of this silly phrase?

Jill: Yeah. It's a very good question. What do we mean? If all cells react 4+, there's no “least” or “most” incompatible, they're just incompatible. And again, if you have an autoantibody that's reacting somewhere between 1 and 3+, if you then crossmatch and find units that are 1+, are they really any better than those that are 3+? I don't know. I think we just have to say to the clinicians, "We've done all we can, we'll provide you with the safest blood we can, given our workup. This is where we are.”

Yeah, we've gone back and forth on language and what we should write. And it's difficult, but I think "least incompatible" is meaningless.

Joe: All right. So, Jill, we've talked about a scenario where everything appears incompatible, however, we have a positive autocontrol and a positive DAT, and we've worked through that, saying that we're trying to deal with the fact that this could be and, in fact was, in our case, a warm autoantibody.

So, let's go back to our friend Frieda Johnson, and Frieda, like many comic book characters, exists in many different planes. So, let's just imagine an alternate reality, where Frieda comes into the hospital with the same bleeding esophageal varices that we talked about before, the same initially fairly benign vital signs, and the same ABO and Rh results.

But this time, while the antibody and the panel look pretty close to the same, let's just say they're not quite as strongly reactive, but they're still pan-reactive. The reactions are fairly strong. They're fairly uniform, but this time the autocontrol is negative, as is the DAT. What pops into your mind right away, Jill? And how would you approach a case like this?

Jill: Okay. if all the reactions were 4+, I'm probably looking at an antibody to a high prevalence, high frequency antigen. But what I can't exclude is, has she got these really strong antibodies to more than one antigen? So, am I looking at multiples?

These are my favorite cases. I have to say. I just, I mean, not in an emergency situation, they're not, but certainly from a reference lab perspective, I think these are the most exciting.

So, what do you do? Well, again, you need to find out how much time you have. I think I've said that before, but it's really important. How much time do I have to investigate? And in fact, these are a little bit more scary than an autoantibody situation where you know ultimately you can give out blood that's ABO and D compatible and you're not going to do much harm.

Here you have an antibody that is clearly an alloantibody, since the auto and the DAT are negative, so you really sort of need to get to the bottom of it before, you know, can you transfuse blood that is not compatible or do you have to scramble to find blood that's compatible? So, it's a bit of a different situation.

I would start sort of in "two legs" as it were. On the one hand, I would phenotype the patient's red cells for all common blood group antigens; Fy, Jk, S, s, that sort of thing. I want to know, what CAN she make of the common antibodies?

And then once I've got her phenotype, then I will select two to three test RBCs that are the identical phenotype, as much as I can; Rh-matched, K-matched, and so on, and test my plasma. Does it react 4+ with those cells, or is it negative with some and positive with others? And the idea being that if the plasma does not react with two to three samples that are the same phenotype as hers, then we're looking at multiple antibodies, strong multiple antibodies. If they do react with cells that are phenotypically similar, then we're looking at an antibody to a high-prevalence antigen. So that gives you a start.

Joe: You're establishing the phenotype, the basic phenotype for the patient, certainly with the common antigens, you're finding cells that are as identical as possible to the patient, and you're reacting the patient's serum/plasma against those cells. If they're negative, then perhaps you have multiple common alloantibodies. It it's positive, though, then that leads you more in the direction of high frequency. Did I summarize that properly?

Jill: Exactly. I think any reference lab would say that the money spent phenotyping the patient (because sometimes that's brought up as an unnecessary cost), the money spent phenotyping the patients red cells is money well-spent, because that really is the basis for your investigation.

Joe: Great, great tip. Okay, let's imagine that we did that, and that the reactions came up positive, that for those phenosimilar cells that you were testing against, the reactions come up positive. Where do you go from there?

Jill: Yeah. Well, I think where I would go first is, I would test the plasma with ficin- or papain-treated cells. You don't have to test the whole panel. And I would also DTT treat them at the same time.

I think DTT has become more popular because of patients being treated with anti-CD38. I think many of us have DTT-treated cells in our labs now. So that's not as foreign a thought as it would have been perhaps, four or five years ago. So, I

would look to see how does this antibody react with protease-treated cells and with DTT-treated cells. And DTT is a reducing agent, so specific for some blood group systems.

Joe: Before we go further, I think one thing that I should have asked you earlier, and just again to kind of get this in people's minds, and I know that if you, if I were to ask you to list all the high frequency antigens, that would be insane. But in your practice, just generally speaking, is there kind of a group of high-frequency antigens that you see more commonly than others? Are there some examples I guess I should say, of what kind of specificities we might end up with in a case like this?

Jill: Yeah, it really depends on where you're living. And I say that because you can't generalize about a population so easy as you can maybe in Sweden be thinking, “Oh, the Swedes are quite uniform,” perhaps. So maybe we'd be looking at, anti-k, for instance. Anti-Vel is another antibody that we see more often here than you see in the general population. And anti-Kp^{b}, we see occasionally, anti-Lu^{b}. Depending on the age of the patient, that sounds crazy, but we can maybe look for anti-JMH, which is something that's found in older people, for some reason. Anti-Yt^{a} is not that unusual.

So, there's a couple, but again, if get little “fingerprints” with those protease and DTT treated cells, you can find a direction. And again, too, if you're in Southern California, then I would probably look for anti-Di^{b}…

Joe: Sure. “Diego B.”

Jill: …thinking that would be more common. In an area that has a lot of African Americans, or African descent, Js^{b} is something that's quite a common specificity. Anti-U. The test cell manufacturers know that. And any reference, not that makes his own panel also normally has a pretty representative spread. So they'll probably have cells that are, you know, are Js^{b}-negative in an area that has more people of African descent. I think that the manufacturers have been pretty clever about some things.

Joe: So, Jill, I interrupted you when you were talking about how you're hitting these things with a panel of different things, like different enzymes and DTT and things like that. I'm assuming that when you do that, there are patterns of reactivity that sort of point you in different directions. Is that accurate?

Jill: Yeah. I think, I really think this is why I love these cases so much, because, with papain and ficin and DTT, you can truly, truly narrow down the specificity to, you know, selected blood groups. Using those two, can we say, "chemicals," you can eliminate an enormous amount of different blood group systems.

We know, for instance, if it's reactive with papain and nonreactive with DTT, you're looking at an antibody in the Kell blood group system or the Lutheran blood group system, and you can focus your investigation. You can thaw cells that are negative for the "null cells." So you can narrow down.

If they react equally strongly with papain-treated cells and DTT-treated cells, even though you don't feel like you've got an answer, you've actually excluded all those blood group systems that *are* affected by one or the other of those reagents. And again, you can focus, and that list is actually quite small when you even said, even if it includes maybe 14 different blood group systems, when you look at the high incidence antigens in those systems, you can eliminate quite a few directly.

So it helps you, it doesn't answer the question, but it helps you.

Joe: I know you love this and we could talk for a lot longer on this, but in the interest of time, let's say that we identify a particular antibody. How do we decide whether this antibody is important? How do we decide if we have to get blood that's antigen-negative in those cases? What do we do?

Jill: That's a really good question. And I'm not sure that I have a great answer. Many of them are pretty obvious. If you have an anti-Di^{b}, you don't really want to go against an anti-Di^{b}, but the antibodies that are difficult are things like anti-Yt^{a}, anti-Ge3 (I would say anti-Ge2 isn't particularly significant, but anti-Ge3 is significant). Some of the Cromer antibodies, you don't know whether they're going to be difficult for your patient or not.

We had a very interesting case this year from one of our labs here that turned out to be a case of anti-Gy^{a}, which is in the Dombrock blood group system, and it was a 2+. So we looked around for what the recommendations were and took those from the English blood service who say that, “if it's 2+ or less, then we just give incompatible blood. if it's stronger than 2+, we try and get blood that's negative for that antigen.”

So there's a lot of practicalities involved in patients that have antibodies to high prevalence antigens, because, if it's not something that's common in your area, then you've got to call the rare donor registry and try and get units in. And this is no acute situation. This is something that takes a lot of time. So if you have somebody calling from the ER as you did for the previous case and saying, "We need blood!", this is a really tricky call for any clinician. You probably have more experience than I do, in this area, but I must say, I think I would stand there sort of panicked because the 4+ antibody and say, "I don't know."

You'd have to try, I guess. You'd have to try.

Joe: Well, in the end, you're exactly right. And I think that the conversations that I have with clinicians in these situations, and thankfully they're not all that common, thank goodness, but the times that you do have to make that "transfuse or not" decision, what I always tell clinicians is that ANY transfusion should be a risk-benefit…weighing the risks versus the benefits. If the clinician is telling me that, "My patient is going to die without a transfusion," then I have to do the best I can with what I have available at that moment.

It’s not satisfying for anybody. Nobody’s happy with that. Everybody wants a perfectly compatible, antigen-negative unit. Yay! Hooray! But that's not reality sometimes.

Jill: Yeah, and again, in this case where if Frieda is still continuing to vomit the bright red blood or she has coming out the other end, then yeah, then any blood is going to be better than no blood. And presumably, if it's coming out that fast, her immune system's not going to do too much with it…we can hope.

Joe: Yes. Well, so you've talked us through a little bit that I think is super important and one of the things that we look for in those settings is obviously what have other people reported about this antibody? I know that people talk about things like biological crossmatches, and in the old days we used to talk about chromium 51. I personally haven't seen chromium 51 used for years, so I don't want to talk about that. But do you have any thoughts on biological cross-matching, Jill?

Jill: Yeah, we recommend them occasionally here. And I, I mean, I said to my colleagues, "What is a biological cross-match?" And they said, "Oh, you know, you give a little blood, and then you draw from the other arm, and you look for hemolysis." Actually I could think that in a case like this with a strong antibody, that would be an appropriate test.

We moved away from biological cross-match recommendations, and now write on our reports that, "Extra observation of the transfusion is recommended," because we don't want the nurse or the doctor walk away from the patient while they're being transfused.

We want them to, you know, check the vitals as that blood's going in. So, that's something. So I think, biological crossmatch seems a little unscientific to me, but, people do keep an eye on things.

Joe: One of the reasons that I've kind of taken it out of my vernacular is the last thing I want someone to do is walk into a patient's room and say to the patient or their family, "Hey, we're just going to try this and see what happens." It sounds like a great idea, right?

Jill: Yeah. No, it's true. I've completely skipped over tests like the "Monocyte Monolayer Assay," which are very useful. I mean, there is now a lot of collected data on the MMA over the last 20, 30 years that can be extremely useful in determining whether your antibody is going to cause a problem.

I think we can stand by those data really.

Joe: For those that aren't clear on what that is, Jill, let's talk real quickly about the MMA, the monocyte monolayer assay, and what it is.

Jill: Okay. So this is an *in-vitro* test, and you're really looking to see, how rapidly or how readily the macrophages, normal peripheral monocytes, are going to "take up" sensitized red cells.

So you take the patient's plasma and mix it with cells that are incompatible, that carry the antigen, the target antigen. Let them incubate for awhile. Then you wash them. Then you "feed them" to macrophages that you've put onto a very special microscope slide that has chambers. You allow those to incubate with the macrophages for a set time. Then you wash away all the unbound red cells, antibody, and so on. And then you stain slide with a normal hematological stain

like Giemsa, and just have a look under the microscope and see how many red cells are adhering to macrophages, how many red cells have been engulfed and eaten by macrophages. It's a very visual test.

You count the percentage of red cells that are either adhering or have been eaten by macrophages. And, there's a scale. So if it's less than 5% red cells bound or eaten, then it's considered that that antibody is not particularly clinically significant. And then there's a mid range, and then there's a range where you would expect there to be hemolysis.

Joe: I have used that test and recommended that test quite often in my career. In the U.S., there's only actually a couple of labs that do it. Do you do that on site?

Jill: Actually, we don't yet. We send those sort of samples to our Stockholm colleagues, actually. It's not technically difficult, but it It is a test that requires some practice and interpretation practice. And I think anybody that's spoken to Sandy Nance about this, she'll say the same thing. If you don't have many of these cases, it's better to send them to a lab that does the test more regularly, I would say.

Joe: I would agree with that. So, last but not least with this case of antibody against a high frequency antigen, you mentioned rare donor registries. And that's, again, kind of perfect world scenario: If we identify what the antibody is, we decide through whatever mechanism that this antibody has either has the potential to be or has a history of being clinically significant, meaning it can cause hemolytic transfusion reactions, of course…The rare donor registries are something that hospital transfusion medicine folks and learners don't necessarily understand. So what does that mean when we say we're calling the rare donor registry? What does that functionally mean to us and to our patient?

Jill: Yeah, so the rare donor registry is a database of blood donors from around the U.S. (and I'll talk about the American Rare Donor Registry right now), where different blood centers inform the rare donor registry that they have units that have a rare phenotype, and that these are blood donors that can be used for patients in different areas. And if you have an antibody to something that's clinically significant, this is something, this is a request that normally your reference lab will put in for you. And, the rare donor registry will then check its database to see where appropriate units can be found.

And they will, as far as I remember, they will arrange shipping of those units to your hospital for your patient. If you're sending a request directly from a hospital, often they will ask that you submit a sample of the patient's blood so they can just confirm the antibody. And that might seem a bit controlling, but it is a good thing.

There's some anti-HI's that are being identified; antibodies that are not significant. it's a very, very good system. And there are similar rare donor registries in many, many different countries around the world.

Joe: And I guess the take home point is that while they are wonderful tools, you're not going to get a unit rolling into your hospital the day of, or oftentimes the next day. Right? It takes a while.

Jill: Yeah, it takes a while. But it's an amazing resource and I'm actually part of the International Society of Blood Transfusions rare donor party, which is actually chaired by Christina, Ms. Francis, in New York, and we tried to…It's a very international effort to try and get information from the whole of the world, who's using what? What the phenotypes that are most needed? Where all the repositories of those units that people to go to? And so on. So that work's ongoing.

Joe: Well, that's fantastic and obviously super important, but I think that we need to move on to talk about one more thing, at least, before I let you go, Jill.

And so let's go back to our friend Frieda. And we've kind of alluded to, I think, in the second case, a little bit where we're going with this third one, but let's just, give a slightly different presentation for Frieda.

In her third reality, she comes in again with the same basic clinical history, but her family reports that about a year ago, she had a similar episode where she got transfused 15 units of red cells or so, a lot of units that she received about a year ago. Again, her antibody screen and her panel are positive pretty much across the board, but this time there's a decent amount of variation. There's some 1+, there's some 4+. There's some things in between. And the autocontrol and DAT are negative. Is that ringing any bells for you, or what should people think when they see something like that?

Jill: Yeah, it's ringing a lot of bells. It's also a case that I would enjoy to work on. And I think the key thing about that is that she hasn't been transfused *recently*, so you can do so much more with a patient sample when they haven't been transfused recently.

Here again, I would absolutely phenotype her. What can she possibly have made with those 15 units that she was transfused a year ago? Once you have a picture of what she can make, you can look at the panel, look at the panel that you have. You see those variable reactions. Can you make out any patterns? Very often an Rh antibody will react more strongly than the rest of the panel, for instance. Can you deduce anything from there?

And then taking the knowledge that you have from her phenotype, I would select cells as best I could, that represent, one at a time, if you can, phenotypically matched except for K positive, phenotypically matched except for Fy^a positive, and so on, and try and set up a little mini-panel where you can exclude and include different antibodies. And if you have panel that's reacting, or a plasma that's reacting with all the panel cells variably, you're probably looking at two to three antibody specificities.

So, it's definitely puzzle work, but here, all that you're actually limited by is the number of different. antibody investigation cells that you have in your fridge as to how you can deduce it.

And I would also say, don't be afraid to use the donor blood you have in your fridge. If you have a well-phenotyped donor base, then take a little segment bit from one of the units if you need to build up a panel, so that you don't have to rely

all the time on reagent panels. Use the resources you have actually sitting on your shelves.

Joe: Jill, with this multiple alloantibody workup, that's something that, again, there's a lot of detail that we could go into, but generally speaking, I think that we get the overview. I do want to just go back to these three examples and just, if I can get your thoughts on just at a quick glance, just a quick, if you looked at these panels, what is the big thing that points you in the direction of first, the warm autoantibody, second, the antibody against a high frequency antigen, and third, against multiple alloantibodies? What are the key, if you can put it in a thumbnail, what are the key tips when you see these panels that point you in one of those three different directions?

Jill: Okay, let's take Frieda with her positive autocontrol. There, the tip for me was the even reactivity and the strong autocontrol/DAT.

In the second case, where she has even, it's the even reactivity with the panel cells and a negative autocontrol that says, okay, I'm looking at a high most likely.

The third case, there we have uneven reactivity, and a negative autocontrol. Then you're most likely looking at multiples, but don't forget that some blood group antigens show tremendous variability on red cells. So high incidence antigens like JRA, LAN, even Vel, can vary a lot in their expressions. So it doesn't always answer the question, but you'd be most likely looking at multiples if you've got variable reactivity like that.

Joe: Fantastic. And that's really what I wanted to make sure that we got across, that while cases can vary, in terms of just the classic stereotypical patterns, those are kind of the three general patterns that you're looking for. So that's perfect.

I do want to cover a couple more things before I let you go, Jill. And part of this is just me being selfish because I have you on the phone and it's fantastic for me to have these conversations with you. One of the things that we didn't do with our cases, with our wonderful friend Frieda, is that we did not give her recent transfusions and... You've... yes, because we're trying to be kind, but that's not always reality. So again, without going into massive depth on this, how does that change things, if Frieda has been transfused recently? And what are the things that you might want to look for in those settings?

Jill: This is really a nightmare situation, I think, because you know, as you will have noticed going through, I rely so much on the patient's phenotype to help me out, and if patient's been transfused in the last three months, you don't have that. If you have time, and of course, you're in a bigger lab, what we do in a lot of these cases is actually go directly to genotyping. A lot of labs are using these really goods kits to do genotyping and to try and get our phenotype from those.

But if you don't have that luxury, then it's so tricky. In terms of what appears to be an autoantibody in case number one, then you can do parallel alloadsorptions with donor cells. This is perhaps a little more detailed than we can have the time to go into, but you can choose, for instance, an R1R1, Jk^a-neg, and an R2R2, Jk^b-neg, for instance, and treat those red cells with ficin, and you can use those to

perform the adsorptions to remove the patient's alloantibodies...sorry, AUTOantibodies to look for alloantibodies.

In the case of a patient that looks like they have an antibody to a high frequency antigen, but they've been transfused, again, then you have to just work on that second leg. See what you can do with testing the patient's plasma with papain-treated and DTT-treated cells. Can you get a little bit of a hint and then, you just have to thaw cells and see what you can do in terms of narrowing it down. So it's more work. I think that's basically it.

And again, with the last case, then you're really looking at pulling a lot more cells and seeing, can you puzzle, can you work out the puzzle by, you know, you select more cells, see if you get a negative reaction, and so on. But in all those cases, genotyping, if you have the time, will help you. But I know that it's not available to everybody. But it helps.

Joe: Before I let you go, let's talk about one other one that you alluded to earlier that I think is really important for us to mention. And that's the scenario where, again, let's go back to Frieda. I love Frieda, so we're going to do Frieda one more time.

Frieda comes into the ED and she's got essentially the same presentation. But in this reality, her family says, "Oh, by the way, she has this disease. Um, it's, ah, what is it? Oh, 'Multiple myeloma.' That's it. She's got multiple myeloma, and they just started on this weird new drug." Uh-oh, I know you see where I'm going,

Jill: Yeah, yeah.

Joe: …for those that are listening, how does that history impact the potential of what you might see?

Jill: Well, patients with multiple myeloma, these days, are being treated pretty much directly with an antibody called, well it's an antibody called "anti-CD38." . And it's marketed in different names, but it's, called "daratumumab," that's its generic name. And this antibody will attach to the patient's red cells and also attach to all the panel cells, and give you a picture of an antibody that can look like an autoantibody, because sometimes the patient cells are DAT-positive, or it can look like a fairly weak antibody to a high-incidence antigen. And, certainly before we realized what was going on, we did a lot of work on these patients, because we just had no idea what was happening.

It's rare that you get a 4+ reaction with patients on DARA. It does happen. But then again, it's helpful to know the patient's history and it's worth double-checking with the clinicians to find out, is there anything that you should know?

From the laboratory point of view, we have, I think many of us gone to testing DTT-treated cells, dithiothreitol, I realized I hadn't said what “DTT” was. And this is a reducing agent. And what it does is it breaks disulfide bonds and proteins and it straightens them out. And if an antibody recognizes an antigen that's sensitive to DTT, then it won't react. This is the case with CD38. So, if you treat red cells with DTT, then these DARA antibodies don't react.

That's been the sort of standard recently. And then, again, certainly in Sweden, and I think in most places, we also phenotype these patients and give out blood that is at least Rh and K-matched. Some people go a bit further, but that seems to be standard of care.

I think the fun thing, just as a last point, is that now we're starting to... I've seen one report so far, but now we're starting to be able to look back at these patients and see, do they actually make antibodies, because they're transfused quite a lot. and it looks as though they are not really making antibodies, so that's great news.

Joe: All right, Jill, before I let you go, I want to give you the chance to tell me is there, anything else that we may have missed as we were talking, any other practical tips that we should be aware of?

Jill: Well, actually, I was thinking, when I was so enthusiastic about describing investigating antibodies due to high frequency antigens that sometimes we go gung-ho down the wrong track, and we must also remember that sometimes we're looking at an anti-IH in a group A patient. Those can be pretty strong. And, I myself spent a couple of days investigating what turned out to be a not a very significant antibody. Just keep those in mind. Think of the, what is it? Think of the horses and not of the zebras. I think that's something that's a...

Joe: I love that. Absolutely. OK, that's awesome. Do you have any closing thoughts for us, Jill, before I let you go?

Jill: Well, there's something that always comes to my mind when I'm presented with these sorts of cases, and it's a quote that I heard Ed Snyder from Yale give one time at a BBTS meeting many years ago. And he ended his talk, I thought it was brilliant, with a quote, well, I'm quoting him that said, "Providing blood for a corpse is not a therapeutic triumph." And I think that's such a great saying. And I think it really brings us back to the clinician perspective. We can't afford to be late with blood. So that stuck with me all these years.

Joe: That is fantastic. I love that. Well, Jill, this has been such an honor and such a pleasure. I cannot possibly thank you enough for hanging out with me. Thank you so much for doing this.

Jill: Thanks, Joe. It's been really fun, and even with the time difference, it's great!

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Joe: Hey everyone, it's Joe with just a couple of quick closing thoughts. I just can't tell you how honored I was to have Dr. Storry on for this episode. I want to thank Dr. Meghan Delaney for introducing me to Jill. What a great time I had talking to her. My thanks to Dr. Daniela Hermelin, who helped enormously with the continuing education materials.

Speaking of that, I want to mention one more time that this is a continuing education activity. So if you're a physician or a laboratorian, don't forget to visit wileyhealthlearning.com/transfusionnews to get your hour of totally free continuing education credit. My thanks for that, as always, to Transfusion News, to Bio-Rad who brings you Transfusion News, as well as, of course, to Wiley Health Learning.

If you have a minute, please go to Apple Podcasts to give this podcast a rating, a review, and to subscribe. You know, I read every one of those reviews (even the non-complimentary ones!), and I really appreciate all of you that have done that already.

I hope you'll join me again for upcoming episodes on donor infectious disease testing, transfusion in thalassemia, and some more details on working with autoantibodies.

But until then, my friends, I hope that you smile, and have fun, tell the ones that you love that you do (especially nowadays), and above all, never, EVER stop learning. Thank you very much for listening. I'll catch you next time on the Blood Bank Guy Essentials Podcast.