# BBGuy Essentials 090CE:
# *RHD* Genotyping; We Can do Better! with Sue Johnson
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**Sue**: Hi! I'm Sue Johnson from Versiti / Blood Center of Wisconsin, and this is the Blood Bank Guy Essentials Podcast.

**Joe**: Hi everyone, and welcome or welcome *back* to Blood Bank Guy Essentials, the podcast that is designed to help you learn the essentials of Transfusion Medicine. This is episode 090CE, and my name is Joe Chaffin. I'm thrilled, and mean *thrilled*, to welcome my friend Sue Johnson back to the podcast! I'm going to tell you what we are going to discuss in just a moment.

But first, some housekeeping: This *is* a continuing education episode. The free continuing education credit is provided by TransfusionNews.com, and Transfusion News is brought to you by Bio-Rad, who has no editorial input into the podcast. This podcast offers a continuing education activity where you can earn 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 (we are aware that whose are being discontinued at some point). To receive credit for this activity, to review the accreditation information and related disclosures, you just need to visit www.wileyhealthlearning.com/transfusionnews. Please note that continuing education credit is no longer available two years after the date this episode was released; in other words, the CE for this episode will expire in May of 2023.

A short time ago, I had the amazing opportunity to interview one of my favorite people, and that is Sue Johnson. Were together at a virtual conference that was put on jointly by the California Blood Bank Society and LifeStream Blood Bank in Southern California. Sue and I talked at length about what to do with those who test weakly for the D antigen the RhD antigen, and Sue summarized some new recommendations and struggles we have seen with the full implementation of what we call *RHD* genotyping for those who do test weakly for D by serologic methods. Because of the generosity of both CBBS and LifeStream, I am going to share that interview with you today, recorded "live" before a big group of people on Zoom! I'm just so excited to tell you that you can also WATCH this episode if you prefer (you probably want to see Sue, I don't think you want to see me), but you can watch this episode by going to the show page for this episode, BBGuy.org/090, or my YouTube page (YouTube.com/bloodbankguy).

If you are unfamiliar with Sue Johnson, well, shame on you! Sue is the Director of Clinical Education at Versiti Blood Center of Wisconsin. She is also the director of the Specialist in Blood Banking Program at Versiti and

the Transfusion Medicine Program at Marquette University. Sue is Associate Director of the Indian Immunohematology Initiative, which is a program designed to improve general immunohematology knowledge in South Asia. She is a sought-after speaker and world-class immunohematology expert. To add to all of that, I don't know if this is her BIGGEST accomplishment, but episodes of Blood Bank Guy Essentials featuring Sue have been downloaded well over 25,000 times, making her the most popular guest in the history of this podcast! That's pretty cool, right? I think so…

Anyway, I'm sharing this interview with you with virtually no edits whatsoever, just so you can experience it how it was "live." I hope you enjoy it! *RHD* Genotyping, how we can do better!

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**Joe:** Sue, welcome back to the Blood Bank Guy Essentials Podcast.

(Group Chatter and Applause)

**Joe**: That's awesome. I am so happy right now! Thank you, CBBS, that's just terrific! Sue, welcome.

**Sue**: Thank you [LAUGHS]. Oh, that was awesome.

**Joe**: That was great.

**Sue**: Thank you very much.

**Joe**: That was great. Well, Sue, I am delighted to have you back on the podcast, and I'm extra delighted to have all the wonderful attendees at the CBBS/LifeStream Virtual Transfusion Medicine Conference today with us. We are recording this live. Sue, you doing okay? How's it going?

**Sue**: I'm doing great. I'm doing great.

**Joe**: Well, Sue, today we are going to have hopefully a great conversation about something that I know is really near and dear to your heart, something that you've been involved in for a long time, and a couple of papers specifically and projects that you played a big role in, along with a very august group of blood bankers.

Everyone, I just want you to be aware, I had a conversation, whew, boy, four or five years ago with Connie Westhoff on this podcast. You can find that conversation at BBGuy.org/005, and it was about the recommendation, Sue, that you and the other folks in the working group came up with. It was published in Transfusion 2015. Jerry Sandler was the lead author. The name of it was "It's Time to Phase in *RHD* Genotyping for Patients with a Serologic Weak D Phenotype." A really groundbreaking piece of information and a groundbreaking set of recommendations.

Sue, first, can you tell us just a little bit how you got involved in this project?

**Sue**: Sure. When you look at everybody that was involved, it was like, "Whoa." It was very humbling. I got involved, one, from being the immunohematologist in the group, like the hands-on, the "tube shaker," looking for the reactions, right? Also, I was there as the AABB representative on this working group, because it was the working group that came together from CAP and AABB, along with experts, scientific experts, and the Armed Forces and ACOG, American College of Obstetrics and Gynecology, so it was a really diverse group.

**Joe**: There were some very important recommendations that were made. Again, everybody, you can refer to the article in the previous podcast. But before we get to those, because I want to review those, Sue, before we go to what you guys are saying now. But let's talk a little bit about D variants in general. Let's step back a little bit and set the stage, because that article title obviously talked about serologic weak D. Obviously that's not the only D variant out there, so let's step back and talk first about just D inheritance in general. How are D antigens inherited? How does the *RHD* genetics work?

**Sue**: Okay. It's pretty complex, but from the perspective of just the basics, on chromosome 1 is the gene for *RHD*, and *RHD* is 10 exons of coding information. Actually, the genetic material actually faces the *RHCE* gene and... Oh, perfect. We have these 10 exons. At the really simple level, if we inherit the *RHD* gene, then what happens is that actually codes for the expression of the *RHD* protein, and the *RHD* protein is a protein that it's called transmembrane, so it goes in and out of the red cell membrane many times. We always look at it in this one dimensional image, but it is probably a protein that looks more like, I always visualize it as like a cone, like an ice cream cone sort of.

**Joe**: Nice. Nice. Okay. So *RHD* inherited separately from *RHCE*. We don't have a whole lot of time to go over that, but that relationship is important, right? The fact that those two loci are right next to each other, is an important thing?

**Sue**: Absolutely. Those two being so close together, you can imagine how if they're on the gene facing each other, there's a lot of different genetic mutations that can occur. There can be information from, for example, the *RHCE* gene that ends up in the *RHD* gene, and a whole big conglomeration of things can occur. Then that protein that looks so nice and beautiful in a one dimensional image can get kind of messed up, right? The interesting thing, if you think about, there's this protein, and it's a pretty big protein, it is 30... So the RhD protein is very similar to the RhCE protein, and there are about 34 to 37 amino acid differences between RhD and RhCE. Interestingly, then you wonder if you're looking at this protein, where is D? What exactly is D?

**Joe**: Yeah.

**Sue**: Well, we don't really exactly know, which is part of our challenge. We're trying to detect something on that protein that will consider us as being RhD positive.

**Joe**: Yeah, I mean, as you look it and you see the differences in the amino acids, it's hard to believe that there's such a dramatic difference in the immunogenicity of D versus the other Rh antigens, right?

**Sue**: Right.

**Joe**: I mean, it's vastly different.

**Sue**: Vastly different.

**Joe**: From just not that many… Go ahead…

**Sue**: Right. But the interesting thing is, when you are RhD negative or Rh negative, you in most cases lack the protein.

So then if you're lacking the protein, then that means if you were transfused to somebody that doesn't have it and you have it, now that's 34 to 37 amino acids, at least, that are going to look foreign.

Right? Which does help explain why it's so immunogenic from that perspective.

**Joe**: That makes sense. That makes sense. Okay. You mentioned that there are things that can go wrong. Can you talk us through just a few, just from the big picture scale, yeah, of what kind of things can happen.

**Sue**: Right. We know that there's all sorts of genetic mutations or variations that can occur between these two genes. So there can be just simply an amino acid or a base pair change backing up, right? A base pair change that from, for example, a G to a C, that will change the amino acid, that maybe that amino acid is important in how we detect the protein or how the body sees the protein. It could be that base pair change could cause nonsense, so there's no information or frameshift, something doesn't get expressed. It can be mutations that occur in the introns, the splice site mutation, so lots of different things that can happen.

Then the other thing that can happen is that you have these gene conversion events, where in proportions of the CE gene, for example, will end up in the D gene. I kind of like looking at this one, because I can imagine now, what does the protein look like? The protein looks like, well, there's some D and then there's some CE protein, and then there's some D protein, right? You might still call them "Rh positive," like D positive, depending on where the antibody's reacting.

The other one that's really interesting is that a piece of the *RHD* gene can end up in the *RHCE*. That one's really interesting, because depending on, I mean, really, if you didn't have a D gene normally and all you have is that one exon from D in that CE protein, actually some of those will type as RhD positive, which is crazy, and they shouldn't be, right? Those should be D negative.

**Joe**: Yes, that should. By all rights, they should be.

**Sue**: Right.

**Joe**: Ah. Okay. Well, so again, there's a whole lot more that could be said about that. I could tell just looking at your face during that you were ready to just roll into 30 minutes on this stuff, but we don't have time for that, Sue, for crying out loud.

**Sue**: Absolutely.

**Joe**: So let's slow down a little bit, and let's talk a little bit about what this actually means in terms of D, in terms of the D expression, I guess, is what I'm trying to say. With all those different genes and with all those different alleles, I should say, and all the different possibilities, what are the results of that? When we talk about D variants, are there kind of different groups or different types of D variants that we can see?

**Sue**: Yeah, absolutely. So that's our challenge, right? There are now greater than 500 *RHD* alleles that have been reported, and that number grows all the time. I look at Transfusion every month, and there's new ones being reported. The tricky part for us is at the practical level, is what are these alleles showing up as, right? So does an individual have mostly an RhD protein that maybe is altered in expression but has normal epitopes, and those people we could call RhD positive. Then some others might have pieces like that one we just showed. They might have pieces of D or pieces of CE, and they are actually what we would consider "partial Ds." Those individuals would have those partial Ds, right? Those are the ones that we worry about for future transfusions or in pregnancy, because those individuals, if they get exposed to a normal RhD protein, they'll see that as foreign, and then they'll make what looks like anti-D to the piece that they're missing.

Then there's some people that have such weak D antigen expression, called DEL, and there's over 40 alterations that have been known for that. There's also some non-functional *RHD* genes. They're just turned off. They just won't go. But our biggest challenge is trying to differentiate the ones that we can consider the weak Ds. Some of them but not all of them we can consider Rh positive, and others we should treat as being Rh negative, RhD negative.

**Joe**: I guess that kind of gets to the fundamental question, which is that when you see a patient, a patient or a donor, obviously it could be either way,

with a D variant, what's the fundamental question that you have to answer?

**Sue**: Right. Well, the fundamental question is, should we treat them as RhD positive or RhD negative, right?

**Joe**: Yeah.

**Sue**: That is it.

**Joe**: Ugh. That's the challenge, and that's what I know that you guys were attempting to address in that 2015 paper. But before we go further, Sue, there's something that, well, it's weighing a little heavily on my heart, I will admit it. It's simply this. When we talk about... Hang on. Let me stop the screen share, because I got to see your face when we talk about this. Over the years, I've been doing this a long time, I have seen a lot of variation in how people say or write things in terms of the terminology in immunohematology. The Rh system is no exception to that.

Just for example, you and I were talking a little while ago, and I was telling you that I was about to write something. I was about to write "D positive," and in my head I was going, "Well, wait, is it 'RhD positive,' or is it just 'D positive?' Ugh, I don't know which way to go with this!" So I want to give you just a second. I'm wondering, it would be so nice, it would be so wonderful, so amazing, if there were a place where, I don't know, say a very interested blood bank person could go, to find how do we do this? What's proper terminology, not just in the Rh system, but in all of immunohematology? Is there such a place, Sue?

**Sue**: There absolutely is!

**Joe**: Yay!

**Sue**: I had the great honor to actually be an associate editor for the 20th edition of the Technical Manual. One of the things that we worked really hard to do was to standardize our terminology. Yeah. The terminology, I mean, we had emails and phone calls and all sorts of things around how we should refer to things. Yeah, in the Rh system, for example, if we're talking about the protein, it's the big R, little h, big D or capital D, RhD protein. If we're talking about the gene, it's all caps, big R, big H, big D. If we are talking about an antigen, it can be a big D (we still use "little d" as a designation of no D antigen but not generally in writing). If you're talking about it in terms of somebody doesn't really know, you really should include that big R, little h, D, like RhD. Out of context, if you just talk about D, most people, unless you're with blood bankers, don't know what that is.

**Joe**: Right. Right. Okay. So what you're telling me is, not just for the RH system, but if I want to know, for example, if I'm going to write the Kidd A antigen, if I'm going to write "Jka," and, oops, I don't have a superscript.

Do I put a little up caret? Do I do a parenthesis? How do I write it? I could find that in the technical manual?

**Sue**: Absolutely. Absolutely. In fact, we worked hard at that as well. The titles of the other blood group systems are, and specifically we used ISBT terminology, the abbreviation, to also get people away from saying, "Oh, it's an anti-Duffy A."

It's not "Duffy," right? It's the "FY" blood group system, and the antigen is "FYA." Now you got me going.

**Joe**: I know. It's a big soapbox for you, I know.

**Sue**: It is.

**Joe**: That's okay. That's okay. Well, thank you for that. Everyone, please keep that in mind, because if you write something or say something wrong, Sue will find you. No matter where you are, she will find... I'm kidding. She's a nice person. She wouldn't do that.

With all that being said, so we've kind of set the stage for D variants, and in particular, the things that we're going to talk about most today are the weak D, partial D distinction.

As you said, in particular, and that's probably not the most fair way to put it, but most importantly, in a D variant, does that D variant have the potential when exposed to make an anti-D or not? That's a fairly simple way to look at it. Is that fair?

**Sue**: Yep.

**Joe**: Okay, good.

**Sue**: That's perfect.

**Joe**: So in 2015, that paper that we discussed talked about ways to kind of make that distinction. If you wouldn't mind, can you just walk us through, again, we've talked about it before in BBGuy.org/005, with Connie Westhoff, can you talk through what the basic recommendations were?

**Sue**: Sure. One of the things that we talked about right up front was just coming to an agreement on what we would term a weak D, "serologic weak D." So a serologic weak D by definition is any type that we have that on initial typing is less than or equal to 2+, whether... It doesn't matter. Any method. Then the other piece that we included in that is also we would consider a serologic weak D phenotype as anybody that had a discordant result. So you have a record that they're D positive, and now you're typing them as RhD negative, right? So those would also be included in that group.

Once you knew that you have... So we had that definition. It actually took us a long time to agree on that, but once we had that definition, then the

recommendation was that we strongly encouraged that in women, in prenatal women and women of childbearing, that when we identified these discrepant D typings or weak expressions, that those should be *RHD* genotyped. That was number one. Then, of course, we talked about transfusion recipients but didn't make that recommendation.

Then also included in those recommendations is that when we had done *RHD* genotyping and we determined that it was a weak D Type 1, Type 2 or Type 3, those individuals were very, very, very unlikely to make an anti-D and that we should call them "RhD positive" and then treat them as Rh positive, meaning that they would not need to get Rh immune globulin, or if they needed a transfusion, they could be transfused with Rh positive blood.

**Joe**: Just one thing to check on there, Sue, the types 1, 2, and 3, are those common?

**Sue**: Yeah, extremely common. Well, in the general population and especially the original work was done in Germany and here in the US as well I could tell you in our laboratory, 80 to 90% of the weak D types that we see are weak D Type 1, 2, or 3.

**Joe**: Okay.

**Sue**: The interesting thing, why we feel pretty confident with... well, very confident with these... is that their mutations are base pair changes which result in an amino acid change. Those amino acid changes are either intra-cellularly, so inside the red cell, or transmembrane, so they occur within the membrane itself.

Right? What you can imagine then is that the amino acids that are exposed that are on the outside of the red cell membrane are all the same. There's no changes there so that you won't see a positive unit or a positive baby or Rh positive baby as being foreign.

**Joe**: Gotcha. That's so important. So for those of you that are with us on the CBBS webinar, you can see the slide that I have up right now. If you're listening to this on the regular podcast, you might want to check the website for this, because this is a really important image, Sue. Talk us through, so what you were saying is that those changes are either... On this image, this is the outside, this is the inside, right?

**Sue**: That's the inside, correct.

**Joe**: So the changes are not on the outside, right?

**Sue**: Right.

**Joe**: That's what's important.

**Sue**: Exactly. So you could see the Type 1 and 2 are very common, and those two are smack in the middle, right? Probably. I'm sure there's a little fluctuation in a red cell membrane, but they look like they're in the red cell membrane itself and that bilipid layer of the lipids that come together in the red cell membrane.

Then the third one, the Type 3, is actually internal, right in the beginning of the protein, and doesn't... Interestingly, it causes weakened expression of the antigen. So in most cases, these will type serologically, immediate spin, just mixing anti-D with the red cells, almost always they'll be negative, and it takes that weak D test, that antiglobulin test, to detect the antigen. So it does impact expression, but everything on the outside is the same, right? It doesn't change.

**Joe**: Gotcha. That makes total sense for why you wouldn't think that if someone saw this... Sorry, let me back up. It makes total sense for why they wouldn't see a normal form of D as being foreign. It just looks similar to what they have, right?

**Sue**: Right. Right. Exactly.

**Joe**: That's cool. All right. That's really important. So Types 1, 2 and 3 are 80 to 90% of the weak D types, as you said. Again, functionally, just so I'm clear, the position that was taken on those is that when you have a serologic weak D and identified as Type 1, 2 or 3 on the molecular test, that you should consider those as D positive in pregnancy and transfusion settings? Is that accurate?

**Sue**: Yes. Yes.

**Joe**: Did you guys make any calculations for what the benefit of that might be?

**Sue**: Yeah, absolutely. The calculations, I believe, that we would save about 14,000 doses of Rh immune globulin in the US that wouldn't need to be given a year, which would be significant, right? Across the world, I know there's a shortage at times of Rh immune globulin, so just even thinking about that, it would open up more for people. RhIG, it's a pharmaceutical product. We haven't had any issues, knock on wood, but still if you don't have to get it...

**Joe**: Those that say it's no big deal are the people that haven't gotten it, I think.

**Sue**: Right. Right, yes.

**Joe**: I don't imagine it's a real fun thing, especially when you get to those multi, multi injections, right? It's more than just one.

**Sue**: Oh yeah, definitely.

**Joe**: Of course. I obviously haven't been pregnant. I haven't received it, but I actually have talked to people that have received RhIG, and they've said it's not the most fun thing in the world.

**Sue**: Yeah. I believe it. I am an AB positive, so I am totally positive. I have no trouble.

**Joe**: We've had this discussion before. You're AB and I'm O neg, so we're a pair.

**Sue**: That's right, the trauma pack.

**Joe**: Okay. So that all makes sense. Consider those as like if they're Rh positive, but anything other than Type 1, 2 or 3 in that initial paper should be treated as Rh negative? Was that accurate?

**Sue**: Yes. I believe we did mention Type 4, weak D type 4.1 but weren't ready to make a recommendation at that point.

**Joe**: Got it, okay. With that being said, and obviously, as I said, Connie and I talked about that, all those recommendations at length in the past. What can you tell us? What can you update us on what's happened since then?

**Sue**: Sure.

**Joe**: What were you guys seeing first? I guess the first and most important question is were people starting to follow this?

**Sue**: They were. What we've seen is definitely in all the individuals that were authors on the paper, Connie's lab, Sunitha's lab, they started to see more orders for *RHD* genotyping, and we absolutely did as well. In most cases, it was in pregnancy. It definitely was an uptick in the orders across the country, all the labs that do the testing.

So that was a great thing. That's a really great thing.

**Joe**: I have a feeling there's "but" there. I say that simply because the paper that you guys followed up with in 2020 that will... I'll give you the name of it in just a second. Just by reading that paper you can see that there were challenges. Can you talk us through what some of the big challenges... Okay, so on the one side, good, people started using it.

**Sue**: Right.

**Joe**: On the bad side maybe, or the more challenging side, there were some issues. So what were the issues that you guys saw?

**Sue**: Sure. This is an issue that we see always just from being in an immunohematology reference lab world, is we'll provide a result, but then there's an interpretation that actually will then be put into the hospital system, right? That's cool. I mean, I understand it.

**Joe**: We get it. What's the problem?

**Sue**: Yeah, exactly. I know, and I've heard this from many people, that they're challenged by their hospital LIS system just to even use the right term for a weak D testing, right? I still hear some of the systems actually have "Du" (that's the only time I'm saying it).

**Joe**: I didn't hear it. No, no, no, no, no, no.

**Sue**: No, no, no, yeah. So I know that that's a challenge. Then also the other challenge was the reports, right? Even if you're trying to be very clear and to call this Rh positive and interpret this result if it's a weak D Type 1, 2 or 3 as Rh positive, then from that setting I think what was happening was that we lost the translation, and it's that call these people Rh positive.

So if there's any doubt, and we've all been taught this, if there's any doubt you call them Rh negative. So the people that are experts in this are confident, but when you're giving it to people that don't have that knowledge, it got to be all jumbled up, right? So that was a big thing. So these patients are still being treated as RhD negative.

**Joe**: Ugh. Yeah. So good intentions and everything done right up to the last step, it's just the implementation of the results that you were seeing challenges with?

**Sue**: Right. Right. Again, that was like the group that came back together again, yeah.

**Joe**: Before we go to what you guys recommended, there is one specific group that I wish to call out, not to embarrass them, but I think it's important, because when you were talking before about who was involved in the initial process, you mentioned the American College of OB-GYN, that they were involved in making these first recommendations. But it doesn't seem like necessarily that the message got through to all aspects of their organization. Can you tell us a little bit about the challenges with what ACOG has come out with and their recommendations?

**Sue**: Sure, I would love to. Thank you, because I was waiting. I didn't want to call them out, so...

**Joe**: They're great people, but-

**Sue**: They are.

**Joe**: …there were some challenges here.

**Sue**: Absolutely. So interesting, right? So just like the AABB... I'll say that, right? They have a group of experts that will come together, and they work on their practice bulletins. They had a practice bulletin back in 1999. That was the original. I don't know if it was the original, but that was the one that everybody was working off of for years and years and years. Dr. Queenan

was part of our group and we thought everything was good. But then when they got their committee together to now update their practice bulletin, which got published in August of 2017, they talked about... I mean, it was great. They called out the working group, said now *RHD* genotyping is good, we should consider that.

Then they did a couple of things. One, they said there was a lack of comprehension of the cost benefit analysis, and we actually had done that. There's been some papers about that, and it's actually pretty equivalent in price to serology, so that wasn't the issue at all.

But the other thing they did was, which was really what blew us away, was that they said... I have to read it because it still blows me away. It says, "Clinicians are advised to administer RhD immune globulin to patients with weak D blood type in appropriate clinical situations." Okay. Then the part that shocked us all was, "By the same rationale as that for RhD typing blood donors until further scientific and economic studies are available," and we're like, "What? Blood donors? That's even going backwards."

**Joe**: Oh boy.

**Sue**: Yeah. Yeah. Then I think it was, that's when I believe was Dr. Bill Flegel, who everybody knows, and Dr. Denomme, Greg Denomme, they said, "Let's get the group back together again."

**Joe**: "There's a minor disconnect here we've got to fix."

**Sue**: Right. Right. Yeah, yeah, exactly.

**Joe**: So you guys did that, you guys got back together, and the result of that is the updates to the 2015 paper.

**Sue**: Correct.

**Joe**: Those of you that are listening to this, you can find the reference to that on the show page for this episode at BBGuy.org, but the paper was, the lead author was Dr. Bill Flegel, as you mentioned. The paper is "It's Time to Phase out 'Serologic Weak D Phenotype' and Resolve D Types with *RHD* Genotyping Including Weak D Type 4." That was in Transfusion in 2020. Again, you can find the reference on the show page.

I already know, you've already said what you set out to do with that. Why don't you just talk through, if you would, your main recommendations in that paper?

**Sue**: Sure. So getting everybody back together again, so this was now... Just to clarify, we weren't working as the working group coming together, CAP and AABB. This was just the gang, the authors of it, bringing us back together again to say, "Hey, we need to readdress this," right? So all these awesome people, back together. The title to me of the paper said do away with serologic weak D or whatever, however that terminology is, but we

don't mean to do away with it. What we mean to say is serologic weak D phenotype is still... still use that terminology, use that definition. But when you can do *RHD* genotyping, that is the direction you should be going, and then those results should be reported. The final results should be reported to the clinicians, right?

For example, we should not defer the interpretation of those results to practitioners, because they don't understand it, and that ACOG bulletin really was our evidence, so we did talk about it in the article that came out, because it really was the evidence that they don't understand all the science that's gone into this.

**Joe**: In other words, am I interpreting what you're saying right, you wouldn't want to put on a report "serologic weak D phenotype" and stop. It would be if the person presented that way with, as you described before, a D typing that's either discordant from the past or weaker than expected, that you wouldn't just say it's a serologic weak D, but that you would resolve it with the genotyping and THEN say, "This is what this means" on the report.

**Sue**: Right. Exactly, exactly, yep.

**Joe**: That makes excellent sense. I guess what I'm a little confused about, Sue, and I admit nowadays I spend a lot of my time in reference labs after most of my career in hospitals, but in reference lab world, putting out a report without saying what to do with it, that doesn't make a lot of sense. Is that different in transfusion service world? Is it more of a challenge there?

**Sue**: Yeah. It is because it's, again, it's those LIS systems, hospital systems, that they have. They're not built that way, right? I know you can have impact into the logic tables and all of that, but that's going to take time. I understand that, right?

But if we don't start to do that and start to demand it, it's not going to happen. You can have more of a voice, I think, than a lot of blood bankers realize when they are setting up their systems or they're implementing a new blood bank software.

**Joe**: Yes, and I know that people that are listening to us right now are the ones that are parts of transfusion services in big hospital networks that are going, "I have no say in how my lab is set up," especially the LIS. So everyone listening, and those of you that are on the CBBS webinar, we get it. It can be a real challenge, and we understand that. I hope that those in those big networks are going to listen to this, because it really is important. This recommendation, I think, is huge, because otherwise you lose all the benefits. Well, maybe not all, but significant portions of the benefits of what you're doing with molecular.

**Sue**: Right. Yeah, absolutely, right. We have this available now.

**Joe**: Exactly.

I didn't cover this before, but I mentioned it with Connie. I think it's important just to reemphasize this. I know you can't give a particular number to this, but cost-wise is doing *RHD* genotype over the roof expensive?

**Sue**: No. No, not at all. In fact, when there was the one cost analysis done comparing it to the workup you would do to evaluate if you're going to give Rh immune globulin or not, almost equivalent in price.

**Joe**: Nice. Nice.

**Sue**: Yeah, almost equivalent. Then plus you have the extra cost of giving RhIG.

**Joe**: Right. Absolutely, and not to say nothing of the potential risks of giving someone an injection.

**Sue**: Right.

**Joe**: In theory anyway, these results are something that can go with somebody forever?

**Sue**: Right.

**Joe**: It's not like you ever have to be RH genotyped again...

**Sue**: One time, right, yeah. It's the one time.

**Joe**: Really important. To summarize so far, we've said that the new recommendation was to make sure that there's an interpretation given, a "What do I do with this result" to the clinicians. Don't leave it to the clinicians to try and interpret it themselves. But I know there was something else that you made a recommendation on, and it's something that we briefly touched on before, and that's weak D types 4.0 and 4.1. Could we talk about those just a little bit?

**Sue**: Sure. Sure. Those two have been hotly debated. I think the weak D type 4.1 we're feeling much better about. There again, the amino acid changes. There's four base pair changes in the DNA, but in the protein itself there's three amino acid changes. Again, they are all in the membrane, transmembrane, or they're intracellularly, so they're inside the red cell membrane. The one might be right on the edge of the interface. Who knows exactly. But this one we felt confident in saying we can call it weak D 4.1, Type 4.1 Rh positive. So that was added to the recommendations.

Now, weak D type 4.0 is similar but different. There's a few different amino acids. Again, there's only actually two amino acid changes that are shown that are again are intra, probably transmembrane, in the red cell membrane itself. There's a couple of other base pair changes. This

particular one, though, is a little bit more controversial from the perspective of this particular weak D type 4.0 is found more often in a diverse population.

For example, again, New York Blood Center sees a lot of different, a very diverse population, and they've been able to put together, and they have seen patients that have weak D type 4.0 that have had anti-D. This one, from their perspective in a diverse population, probably want to be more cautious with. But there's other studies that have been done, beautiful studies from Tunisia, which is in North Africa. They have found many weak D type 4.0s, and they have only had, I believe, one auto anti-D. Then there was one anti-D that was reported in the French hemovigilance system.

So the debate when we have these patients with weak D types that make anti-D is always, is it truly an alloantibody, or is it an autoantibody? That's always the debate, and the challenge to differentiate that in most cases, especially when you're getting a sample referred to your reference laboratory, you don't get a lot, and you might get enough... Well, you can do your genotyping, but the serology is putzy and complicated to do the absorptions to prove whether it's auto or allo.

In the North Africa/French population, I know a weak D type 4.0, they're very confident to call them Rh positives. In those individuals that are in more ethnically diverse population, there's more hesitation. We recommended weak D type 4.0. You could call Rh positive, but in an abundance of caution, especially in pregnancy, you may want to consider them as being Rh negative at this point.

**Joe**: Okay. Interesting, okay. That one obviously comes with a little bit of shall we say a little bit of an asterisk, I guess, and that's kind of what you were showing on your slide.

**Sue**: It was. It was. The other interesting thing... I just have to mention this because it's super-cool.

**Joe**: Please.

**Sue**: We were talking about before there was just like early view Transfusion is this amazing article on 3D modeling of the Rh protein-

**Joe**: Nerd alert, everyone.

**Sue**: Nerd alert. It's super cool, by Flock, et al, and it is... I've got the paper right here. Anyway, they talk about interactions of this protein, and you see a one-dimensional picture of it, right? It looks like this curvy thing in the red cell membrane, but you know it's more of a channel. What's going on inside the red cell membrane that's causing this protein expression to be altered, right?

So they actually with modeling, computer modeling, said actually for 4.0, that there could be 14 or 15 different interactions with the membrane. Yeah, but whereas a weak D Type 2 and 3 have one. It's like, "Oh, there's more going on," right?

**Joe**: Everyone is sitting there going, "I don't know what Sue just said, but man, she really means it."

**Sue**: I do.

**Joe**: That's fantastic. No, I'm teasing you. You know that. It is fascinating, and I think the point of course is that we have to understand we show these as two-dimensional things on paper, but they are not. There's so much complexity to it that we are still trying to figure out and still trying to understand. So I'll give you your nerd moment.

**Sue**: Thank you. Thank you.

**Joe**: No problem. Okay. So you've said that we should, that the new recommendations include not sending out results without interpretations whenever possible, that the paper said to change 4.0 and 4.1 from weak to partial D. I'm sorry, to weak D from partial D, so treat them as D positive with the caveat that there's some concern about 4.0. Was there anything else? Did you talk anymore about other stuff that you had said previously or-

**Sue**: Yeah. We did also mention that it would not be a bad idea, no recommendations, but it wouldn't be a bad idea to identify those serologic weak D phenotypes, right? So maybe consider using two different serologic methods or two different anti-D reagents. We know at least one of the manufacturers automatically runs two anti-D on their automation. I know our friends in Europe have done that for a long time. That's a way to identify. Not a requirement but would be something to think about, right?

**Joe**: I admit I'm a little puzzled by that, because I think you've talked about before, and we may... I can't even remember. We may even have a slide about it. I thought that there was a big challenge to identify weak D serologically.

**Sue**: Yeah, there is, so that's the other thing, I guess. But we wanted to mention it also, just to get people's awareness there. There are actually different, 20 different anti-D reagents now that are available. I mean, some have similar clones, but they've, depending on what manufacturer, they have different diluents, secret ingredients, in the reagent that will make it, give you in most cases a nice, strong, positive result.

The other thing that the manufacturers have is the challenges up until now the FDA... I shouldn't even say up until now, but the FDA hasn't really required them to define how the anti-D reagents should react with these different weak D and partial D types. They will give you a little information

in the package insert, but it varies greatly. In large part, it's because the manufacturers haven't had a library of molecularly characterized weak D, partial D types. I think that will be coming as well, so that eventually we'll be able to do a better job at detecting these.

**Joe**: Okay. Well, so I think we've summarized the new recommendations, and I hope that everyone listening has a really clear picture.

Everyone, you should get ahold of this paper. Again, you can find it on the BBGuy site. Just as a reminder, it's in Transfusion 2020, and again, lots of great information in there. But we have just a couple minutes before we finish, so I want to just take a second and ask you a couple other questions that have come through the chat function.

**Sue**: Sure.

**Joe**: Yay!

**Sue**: Can I add one thing?

**Joe**: Yes, please. Yeah, go ahead.

**Sue**: I was just going to say, the last thing that I didn't mention is we are encouraging the standard setting committee, the Standards for Transfusion Services of Blood Banks and the IRLs to take a stronger stand on the genotyping.

**Joe**: Nice. Thank you.

**Sue**: I needed to add that.

**Joe**: You did, and I forgot to ask you, so thank you. You're 100% right. Okay. There've been a couple of questions that have come through, so I want to just in the few minutes that we have, let me get them to you. Let's see. Virginia asks, "Are weak D 1, 2 and 3 weaker in expression because there are fewer sites on the RBC membrane?"

**Sue**: That's a great question. Yes, there actually have been quantified, and yeah, we see Type 1, 2 and 3 all have less. Our normal D phenotypes would have probably more like 14 to 20,000 antigen sites that have been calculated, whereas the weak D type 3, for example, I think, is like 500 antigen sites.

**Joe**: Wow.

**Sue**: Yeah. So there's huge, huge decrease anywhere from 10,000 down to that hundreds.

**Joe**: Gloria asked an important question, and I think this is, it's valid to ask this. I know what part of your answer will be, but she asks, "Is part of the problem due to maybe the delay and expense of the molecular testing?" I

know how you feel about expense. We've already talked about that, but what about the delay?

**Sue**: That's a great question. I think genotyping labs are trying to do it more expeditiously, giving a good turnaround time. I think that now there's some molecular platforms that I think, especially the larger transfusion services that are looking at, that you can purchase a chip that will be able to help you differentiate, and the prices are coming down on that as well. But yeah, that's definitely... I'm sure it's a factor, especially if you have to send the sample out.

**Joe**: Right. I agree. That's been to me, just personal aside, in my personal practice that's been the biggest challenge. I want an answer right now. I don't want to wait a week or a few days or whatever it is.

**Sue**: Yeah. Yeah. For prenatal patients, usually you have the time, but if we move the recommendation to actually say, "Let's think about this for transfusion now as well." That's going to be the biggest issue there, I think.

**Joe**: Agree completely. All right. Let's see. I'm trying to get through as many of these as we can. Erin has a question. "Any recommendations for the difference of strength of reactivity between two different anti-D reagents in order to recommend the genotyping?"

**Sue**: Oh, that's a great question. I wish I could give you one that I was confident in. The reason that I say that is I just had a SBB student named Crystal Theiler who worked with, we had a multi-group, and we looked at every single anti-D reagent with molecularly characterized samples, and we'll be getting it published hopefully in the next six months or so. It was great variation. We even saw a weak D type 1, I think. We tested a bunch, and there was one that was 3+ at immediate spin. So yeah, I'm not confident.

**Joe**: So the answer, unfortunately, Erin, is no, we don't have a great recommendation.

**Sue**: No. I mean, there might be ones that will give you a little bit better, but... yeah.

**Joe**: Lori asks another question. She says, "We have seen completely negative reactions on our solid phase ECHO analyzer with two anti-D reagents, but then we do tube testing, and it's 2+. What do you do with that?"

**Sue**: Oh, that's a great question. Yeah, so that's a challenge, because when you test on an analyzer, it is programmed to shake, for example, four ways this way, four ways horizontal, six ways vertical, right? It's programmed, so if you have weak antigen expression, the machine just doesn't know that, whereas as a human, when they shake a test tube, they're being hopefully gentle, and they're also looking for it too at that point. So it's probably still weaker expression of the antigen. I do know, though, that some people have told me where they've sent out a bunch of them for genotyping, and

they come back as normal *RHD*. But I don't know. It's a hard one. That is a tough one.

**Joe**: That is a tough one.

**Sue**: Yeah, I guess you almost have to do a little validation.

**Joe**: Everybody's liking THAT!

**Sue**: Yeah.

**Joe**: I only have time for one more. I'm sorry, there's a couple left, but this one's not fair. Sunitha is asking a question. That's not fair. She knows more than I do about this. It's not fair. Okay, but I'll ask it anyway. "Amazing job," which is of course due to Sue. "Would you reconsider how to classify RhD 4.0 by that recent paper that you were mentioning showing the protein modeling?"

**Sue**: Yeah. You know, I actually have been thinking about that, Sunitha. That's a great question. I actually, the outcome of the paper showed... I love that they said "you cannot use one method to always be a hundred percent confident." They said you have to use serology with the protein modeling, with the genotyping and the molecular characterization. We're still learning. Yeah, we might have to get the gang together again.

**Joe**: Maybe so. Well, so that's all the time we have for questions. I'm sorry, everybody, there's one or two more, but we got to run. Sue, anything before we go?

**Sue**: Yeah, and I just wanted to acknowledge the gang. Of course we mentioned Dr. Flegel, Greg Denomme, Dr. Queenan, Margaret Keller, Connie Westhoff, Dr. Sandler is amazing, and Dr. Katz, Meghan Delaney, Ralph Vassallo, Clayton Simon, and I already mentioned Crystal. Yeah, I just had to say thank you to all of them. They'll all part of this.

**Joe**: Well, this has been a blast. I want to thank all the attendees, the CCBS/LifeStream virtual transfusion medicine conference for joining us and for everyone listening, thank you so much, and Sue, for you, my friend, thank you very much for being here.

**Sue**: Thank you very much. This has been great.

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**Joe**: Hi everyone. Before you go, don't forget to check the show page for this episode at BBGuy.org/090 for direct links to those articles that Sue and I talked about in this episode. And also, again, if you want to watch this on video, you can watch it there. As you heard, we answered some questions at the end of this interview, but if you have something else that came up that's burning in your brain as you listened to this interview today, please

just go right there to the show page, put in the question, and I'll make sure that Sue gets a look at it.

Remember, if you are a physician or a laboratorian, be sure to go to wileyhealthlearning.com/transfusionnews to get your hour of totally free continuing education credit. My thanks for the continuing education sponsorship to Transfusion News, to Bio-Rad who brings you Transfusion News, as well as of course to Wiley Health Learning.

I've mentioned before that I really appreciate it if you would go to Apple podcasts and subscribe and give this podcast a rating and review. That really just helps other people find the podcast. I really do read every single review that's on there and some of you are just too kind, others of you, ehhhh… [laughs]

The next episode, which is coming very soon, will be a discussion with Dr. Ruchika Goel on platelet transfusion in situations where we have always thought we shouldn't transfuse platelets, like TTP, ITP, and Heparin-induced Thrombocytopenia or "HIT." I plan to follow that episode with some more great stuff like discussions of the monocyte monolayer assay test, transfusion in thalassemia, and lots of other great topics coming throughout the summer of 2021!

But until then, my friends, I hope that you smile, and have fun, and above all, never, EVER stop learning. Thank you so much for listening. I'll catch you next time on the Blood Bank Guy Essentials Podcast.