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Joe Chaffin: Alright! Hi everyone, I'm delighted today to introduce Dr. Connie Westhoff! Welcome to the Blood Bank Guy Essentials Podcast, Connie.

Connie Westhoff: Thank you, Joe. I'm pleased to be here!

Joe: Thank you. So great to have you! I want to tell everybody about you, a little bit. Dr. Connie Westhoff directs the Laboratory for Immunohematology and Genomics, at the New York Blood Center. She's also an adjunct Assistant Professor in the Department of Transfusion Medicine at the University of Pennsylvania. She lectures all over the place, nationally/internationally, and she's published, oh—roughly, about a bazillion—ok it's more than 80 scientific papers, and she's also written numerous book chapters. Connie is an Associate Editor for the Immunohematology and Genomics Section of Transfusion and she's also an editor of the AABB Technical Manual. And if I sound a little nervous today, it's because I am talking to a true, honest to goodness Hall of Famer! I say that for real! Connie is actually an original inductee to the National Blood Foundation Hall of Fame. I just saw that the other day, Connie, and again, I wasn't intimidated before, but now I am! (laughs)

Connie: Well, you shouldn't be!

Joe: Ok I admit, I'm not *that* scared because I know you're an incredibly nice person! So we've got that going for us...

Connie: Well, Joe, you're a Hall of Famer as far as your education skills you do, and I think that this is a wonderful addition to our education that you've taken on.

Joe: Thank you so much! Well, I do always like to start off a particular way in a podcast, and there's so many people that are just starting off in their career and their training, and I always really like to know from people like yourself, beyond your bio, can you just tell us a little bit about what got you interested in blood banking to start with? What led you there and what keeps you going there now?

Connie: Well, I've been in blood bank forever—the early 80's actually! But in high school and college I always kind of gravitated towards the sciences and biology, because I felt like there were so many new things to learn, and I really like the laboratory environment. I had a friend who was a med tech, and it sounded like a really perfect profession, where you got to be involved with patient care—rarely on the laboratory diagnosis side. And I also liked blood bank when I did become a med tech because of my interested in genetics. So after working for a number of years as a SBB I did go back to do a research PhD in Immunology and Molecular Genetics, because that was the time we were starting to clone the genes and I realized that, “Gee, these blood group antigens are encoded by genes and wouldn't it be cool to be involved in the cloning of the genes?”

Joe: And obviously, I know that a lot of your early work and your PhD work, involved work with the Rh Blood Group System, correct?

Connie: That's right. So Rh was—well, I was very naive, I thought, “How can there be, you know, 50 different Rh antigens? There can't be 50 different genes in populations!” Well, I was wrong in that, there were even more than 50 different genes in Rh populations, there are about 200. But I thought you could simplify Rh if we knew about the genes. So, it has helped us explain what's going on, but it certainly hasn't simplified things.

Joe: Well that is for sure! But, we're going to try to make things as simple as possible today. Today, you and I are going to talk a little bit about testing for RhD. And further, a little bit about some of the new information, or at least a new approach, to variant RhD antigens that you have done—you along with a group of experts has done—really some impressive work in terms of guiding us down the right pathway. But before we get to that, I really just want to start from the beginning, and let's just talk basics. So, let's just say you're in a blood bank, you're a technologist working in a blood bank, and someone sends in a sample to you for pre-transfusion testing. What are the basic tests that a blood bank transfusion service has to do for someone with pre-transfusion testing?

Connie: Sure. For Rh typing, certainly, on the face of it, it's a simple test. You use an anti-D antibody reagent and you add it to the patient's red cells and you spin the cells to bring them close together, to allow those anti-D antibodies to cross-link the D antigen between different red cells, if the antigen is present. And we read agglutination, you know, that's what blood banks are based on is agglutination, when the D antigen is present, the red cells will agglutinate. But there is some variation...

So if you're doing a tube test, though, I just wanted to mention, one has an option there of adding other step to the test and taking that negative or weak reaction onto the indirect antiglobulin test, that's called a "Weak D Test" where you actually incubate the cells and the antisera and you wash them after incubating it 37 degrees and then you add the antiglobulin sera to detect bound anti-D, so you have an option there if you're doing a tube test to go on and do what we call a Weak D Test.

Joe: And that's important, that's something I think a lot of beginners miss that the option to do a Weak D Test is not necessarily—well, in fact it's not available as a part, anyway, of some of the more, I guess we could call them more "modern platforms" available for RhD testing, correct?

Connie: That's right. So the gel and the solid phase don't have that second step. But when we were all doing tube tests, it was very common to do an indirect antiglobulin test as part of Rh typing. But when that change was in the 80's, when we got the monoclonal reagents which were very good at detecting weaker than normal D antigens on red cells, at the initial spin, so a lot of us dropped the indirect antiglobulin test on patients samples at that time.

Joe: I see. So that's again, an interesting and important point, that the "Weak D Test", as you referred to it, the indirect antiglobulin test is done—is it fair to say that it's kind of a "just to make sure kind of test"? Someone who tests as D negative, or weaker than expected D, that you use that indirect antiglobulin test to prove that they really are or are not D positive?

Connie: That's right. It's more sensitive, so it will detect weaker, lower levels of D antigen. But some of us have dropped it also, because then the DAT on the patient's own cells, if there's any antiglobulin on the patient's own cells, like if they have a positive DAT that could interfere with the test and make it confusing to interpret. So that's kind of a second reason why it's been dropped because there's no harm in treating a patient as Rh negative. So, on the patient side, the indirect anti-globulin test is optional, but of course, as you've mentioned previously, it's not part of the gel.

Joe: Right, okay. So that's the testing for D obviously that our hypothetical person in the blood bank would do, just your basic ABO grouping, do your testing for D, and then an antibody screen. We're not going to talk about those other tests today, we're going to stick with testing for D. Okay, here's a dumb question. It's a dumb question because I'm asking it, but I do get asked this sometimes! People get a little bit confused, what's the—and I know, trust me on this, this is a silly question. People get a little bit confused when we talk about "D," and then sometimes we call it "RhD," and then we've got a gene that's

capital "RHD", just clear up the mystery there for us, Connie, I know you can do it quickly!

Connie: Well, "D" all by itself always means the antigen. "RhD" can mean the antigen or the protein but we try and use it when we're talking about the protein, and then capital "RHD" means the gene, so there is a pecking order to the terminology, but if you're talking about the antigen and you just use big D that's all you really need to use.

Joe: That's fair! So when we talk about D testing and you've already kind of mentioned this and alluded to it, there are some potential complications to it, I mean in many cases the testing is simple and straightforward but in some cases, as you've already said, things aren't quite so straightforward. So what makes it different? Why is D different from typing someone for K or for Jk^a, those seem pretty simple? "Yes it's there, no it's not there." Why are there these variations? I guess, what's different about the D antigen?

Connie: Yeah, that's a really good question. I call it the problem with the D antigen is that the variability in expression from on different patient red blood cells, for example, when your typing for the Kidd antigen or the K antigen, that protein is almost always expressed in high levels on the red cells and there's not very many variations in populations between different individuals. The genes are sequences are the same, but at the Rh locus there's a whole bunch of mutations going on and so, as many as 1% to 4% of the patients you type, will have some variations in their gene. And that variation in gene translates to different levels of the antigen on the red cells. Though, some of them will be harder to detect than others and so, that's not true with Kidd or Kell usually, except in rare instances. There's not a variation in the level of antigen on the red cells like there is for RhD.

Joe: I see. So there are variations in the antigen strength, obviously because of gene issues. Are there any other differences that make things weird with D testing, like from one lab to another or perhaps one reagent to another?

Connie: Yes. So, that's another good point. With the K or k or Jk^a or Jk^b, you're detecting a single difference in the protein. It's a single epitope. But when you're doing D typing, you're detecting the presence or absence of this whole protein. The whole protein, the whole 409 amino acids are either there or not. Now that should be simple, either have it or you don't have it. The ones that are causes problems are when it is there, and they are in a lower amount of protein than normal or in a different epitope pattern. So, for example, if you're an Rh positive, you have multiple epitopes of D on your cell surface, where if you're Jk^a or Jk^b, that's a single epitope difference that the reagents are detecting. This

variation then in the both antigen strength and the epitopes are what cause a problem.

Joe: So you used a word there that I just want to make sure our audience is very clear on. You talked about how with Rh D you have multiple epitopes. Let's make sure that everyone understands. Can you just give us a thumbnail on epitope? What does that mean?

Connie: So epitope is the part of the antigen that binds to the antibody. So when you respond to D, if you're Rh negative and you see the D antigen, you're seeing lots of bumps on the cell surface, lots of epitopes that are different from your D antigen. You don't have the D antigen. So you make an antibody to all of those foreign bumps that are—I'm sorry for using non-scientific terminology!

Joe: (laughs) That's okay!

Connie: But I look at the Rh protein as “the ship on the sea” of the red cell membrane and it has different bumps and bruises that the antibody is directed to. When we had anti-D reagents that were directed to all kinds of epitopes of the D protein, they were called polyclonal antibodies. Typing was a little bit easier, now with the monoclonal antibodies, they react with one epitope on D and if that's altered, then the reaction is not as strong as you would expect it to be.

Joe: That's so important, again, I think students miss that. The trade-off with going with monoclonal, which obviously you would assume anyway, is a potent and powerful antibody, but the tradeoff is that you're only dealing, by definition, with monoclonal with one particular epitope, right?

Connie: That's right and then what happens is, these monoclonal reagents out there, in different manufacturer's reagents are different. So for example, we have 11 different reagents to choose from when you're typing someone for D, and those 11 different reagents react with a different epitope of the D antigen. So that alone is responsible for some of the variability we see when we're typing patients. We may get a positive reaction with one reagent and a negative reaction with the other, because of the epitope that it's directed to. But all that really tells you is, not that you've made a mistake, though you can always go back and make sure you haven't made a mistake. It tells you though, that there's something different about that person's D antigen.

Joe: Right. And “Different things about the D antigen” is a topic that students have struggled with for a long time. In fact, I will be honest and tell you that many people that aren't just students, sometimes struggle with the concept. I

did a podcast, a couple of years ago on D variants that I can't even believe the questions I've gotten as a result of it. So it's clear that people really get messed up with this whole D variant thing. So if you could, can you talk about the main variations, or at least the main categories of, I don't want to say, the "abnormal" types of D, but I guess they are, the non-standard forms of D and what do they mean? The weak D and partial D in particular, people get very confused about. So what are the differences and why should we care?

Connie: (laughs)

Joe: That was heresy, wasn't it? "Why should we care?" How dare I say that to you! I apologize!

Connie: It isn't a simple topic, but it is one is probably worth trying to kind of sort out in your mind. So we've learned a lot from the genetics, and I want to make clear that whenever you see a reaction that is not the strength you expected it to be, it's not 3-4+ like what we usually see, or it's different from one reagent or another, it's telling you that there's variation in the gene in that patient. And not like I said, depending upon the people you're typing it could be 1% or 4%, so all of you in your career are going to come across that. The problem is though, some of these genetic changes doesn't put the patient at risk to make an anti-D, whereas some of the genetic changes do put you at risk for anti-D. And now that we're finally looking at the genes, we're being able to associate who's at-risk and who isn't at-risk.

And so we have two terms we use. We use a term "weak D" because these people usually have lower levels of expression of D, but they don't have a change in their epitopes. The bumps on the surface of the ocean don't really differ from normal. So these people are not prone to make anti-D. Now I never say never, but 99.9% of them won't make anti-D. They're just what we call a "weak D." Then we use this other term, "partial D," meaning you're *missing* some of those epitopes and those bumps on the surface. So, normal D is going to look foreign to you and you're at-risk to make an antibody to the bump you're missing, the epitope you're missing and those we call "partial." This is terminology that's been around for a number of years, and when we started looking at genes there were some arguments that we should just put them all in one pot, but we've really realized that we can use weak D, especially weak D we call Types 1, 2, and 3, which are the most common ones that don't have an alteration that's going to be at-risk for anti-D in one camp, and they're not at-risk and those with partial D are at-risk. Now we use to use "D^u" terminology; D^u is an obsolete term. We really should no longer be using that. **We now use "weak D" to indicate folks that are generally not at-risk for anti D, they just have low expression levels and "partial D," meaning they have different epitopes, they**

are at-risk for anti-D. Can't tell this from the serology, though. These are going to react similar or variable, so any time you see a reaction that's not what you expect, not the strength you're expecting, it could be either a "partial category" or a "weak category." So you can't tell from that serologic reactivity, whether that patient is at-risk for anti-D or not.

Joe: That's a really important point. Two things on that: #1) The fact that you can't tell these apart serologically. There's been somewhat conflicting advice regarding that historically, but I feel the same way you do. And you're very clear on that, that serology is not definitive between weak D and partial D, Connie, is that fair?

Connie: That's right. And you know, there have been 2 camps previously, when you saw this reactivity and you didn't have the genetic analysis to back you up. People would be conservative and then treat these people as Rh negative, that was appropriate. Or people would err on the other side and would risk exposing them to anti-D and to call them positive. So some people were calling them negative and some people called them positive and we still have that very much in practice out there, some call them positive, some call them negative so that they're not at-risk for anti-D.

Joe: So that's point number one—actually I may have 3 points in this! Point #2) Is the "reaction weaker than expected." And I wrote that down when you said it because I think we just need to make sure that people understand that. When you say reaction weaker than expected, what should we expect with anti-D testing? What is normal?

Connie: What is normal is 3 to 4 +, certainly often, 4+, 3+ or 4+. So we don't like to be prescriptive, that's why I always say it's "weaker than you expect in your hands and your reagents and your method," because it's all so varied depending upon, like I said the reagent, it will depend upon whether it's a tube test or a gel card. It will depend upon which reagent, which clone you're using, so, it's always best to put it relative to your experience in your lab and what's normal in your lab. People usually often say 2+ or weaker.

Joe: Okay. And is that specified in the package insert for the reagent or does that vary?

Connie: And again to add to the confusion every reagent manufacturer says something different. Some say agglutination less than 1+, you should do an indirect antiglobulin test, some say a reaction less than 2+, you should evaluate it, maybe it's a false positive. So manufacturer's instructions cautions vary and

adding to the confusion. But like I said, the best advice I give people is, if it's weaker than you expect in your lab, with your reagents, and with your typing.

Joe: And point #3 is just to summarize just what you said, because I think it's a essential for our audience to understand this. So you described the two main D variants: "weak D" and "partial D" and I want to make sure that everyone is very clear on the consequences of both of these groups of patients receiving D positive blood. For a "weak D" patient, who has a quantitative issue, lower than expected D but normal epitopes, especially if they're Types 1, 2 and 3, is it fair to say they are not prone to make an anti-D in that situation, receiving D positive blood?

Connie: Exactly, they're not at-risk for anti-D and women are not candidates for Rh immune globulin.

Joe: Ah! Excellent point! We will come back to that. The second one is the "partial D" where they have a qualitative issue. They're missing epitopes and so as a result of that, if they see a normal D antigen from a regular D positive donor they are at risk potentially of making anti-D, correct?

Connie: That's right. They are at-risk and if they're a woman, you know, she probably should be given Rh negative blood. Sorry guys! We're not discriminating here, but we are trying to do best practice and save the Rh negative blood for the people that will have long term consequences.

Joe: Excellent! So, everything you've told us we haven't really broken into anything that's incredibly new. Most of this has been known for some time and described for some time. Certainly we're discovering more and more and more mutations and significantly due to the work and your lab are doing. But since we've known about this for a while, do we have any evidence that we have an issue in laboratories? Shouldn't everyone be handling this pretty much the same?

Connie: Well that would be nice, but with the practice of medicine, you know, there is a lot of variations, but at the same time we're trying to work towards some standardization across the industry. I'm sure most of our audience have maybe been exposed to a situation where someone thinks they're negative but they're really typing positive. They are typing positive but thought to be negative. And the CAP survey, a number of years ago, just showed how variable practice was across the whole spectrum of the profession. If people saw a weaker than expected reaction, if it was a woman, someone would call them negative. But if someone would always call them positive so that they wouldn't waste Rh negative blood. All this variability in practice makes us not

very scientific about what we're doing. So I think most people feel that it is a drawback to all this variability.

Joe: Understand. Well, so that brings us to some of the work the you and the team of experts that you worked with have published over the last year or so, with some recommendations from AABB. So I want to just talk through those. In particular, so that I make sure that I'm setting the stage properly, I think that your recommendations, your team's recommendations revolve around the management of pregnant ladies who test with variable D patterns and blood recipients that test with variable D patterns. Is that correct? Is that the 2 areas you're focusing on primarily?

Connie: That's right. So the variability in practice leads to a patient being treated as positive in one hospital and negative in another hospital, and so, to try and begin to address that, there were 2 issues here. One is, who needs Rh immune globulin? Let's get that right! Who's at-risk? We want to limit Rh immune globulin use to those women who are at-risk. And the second thing is our Rh negative blood supplies. We want to conserve those for the people who need them and traumas and the neonates, etc. The work group was formed to address some kind of standardization of testing and to begin to use genotyping in these certain situations to decide what to transfuse or to give Rh immune globulin or not. So that was the work group challenge.

Joe: Got it. Okay, let's start with the pregnant ladies scenario. However you want to do it, whether you want to just summarize the recommendation or maybe take us through an example case or something? How would you prefer to outline how this works?

Connie: So maybe I can just summarize what the focus was. This Rh work group was formed already in 2013, and it had representatives from CAP (the College of American Pathologists), AABB, ABC, Red Cross, ACOG (who are the OB/GYN folks), and from the armed services. And we were charged to develop some kind of recommendation to get people to start using Rh genotyping. If you have this kind of situation when you have a D typing discrepancy, something's positive, another reagent's negative, or you see a serologic weak D type in an OB woman. So the first population we wanted to address with Rh genotyping, is those OB women. Who should get Rh negative blood and who should get Rh immune globulin? And start to phase in the use of Rh genotyping by applying it to this very critical group of patients—the pregnant woman. And at the same time, avoid unnecessary Rh immune globulin and unnecessary transfusion of Rh negative red cells. So the first issue was, why do we care about excess Rh immune globulin? Joe, do you care about excess Rh immune globulin? (laughs)

Joe: (laughs) I'm sad to admit, Connie, I believe it was back in 2012, the podcast that I mentioned earlier where I talked about D variants, I am on record, I'm on the internet saying, "Who cares?!? What's the big deal? So we give a little unnecessary RhIG! It's worth the trade off to be safe." But in my defense, I don't think at that time, anyway, well certainly your task force hadn't been formed, and there wasn't easy availability to RhD genotyping. So I claim some innocence, but truthfully, I may have made too strong of a statement back then anyway. Sorry...go ahead.

Connie: Well, I would have agreed with you 100% back then, to be conservative, etc. and give her the Rh immune globulin, because we really didn't have any good alternative, did we? And certainly this is a very safe product. It has a great track record, but it's a human blood product. It's manufactured from pooled plasma from paid donors, so they must be actively immunized. Although there are no reports of transmission of hepatitis in the U.S. or virus or HIV, nothing like that was in this product, there's always a potential for new emerging agents to get into a blood product that we don't know about. Certainly I was around when HIV came around and so, that makes it kind of an ethical issue if you give a biological product that you don't really need to give. Women who have gotten this product say, "It hurts!" It's a large volume, intramuscular usually, and we have women that seek us out because they don't want to get this blood product. When we looked at it, though, the work group, with weak D Type 1, 2, and 3 in mind, there are about 25,000 or more doses of Rh immune globulin that are being given unnecessarily just in the U.S.

Joe: How many???

Say that one more time?!?

Connie: 25,000? That doesn't sound like a lot.

Joe: Well, annually though? 25,000 annually.

Connie: 25,000 annually are being given that don't need to be given. And certainly, the idea here is if a woman has this kind of—what I call a "funky Rh typing" and she's sent off for genotyping, that can become part of the results, can become part of her medical record, and she may have another pregnancy and the genotyping doesn't have to be repeated that second pregnancy. It's part of her medical record and so, it would save.

Joe: And to be clear, what your work group recommended was not to do RhD genotyping for all Rh negative moms, but just for ones with the discrepant or the serologic "weak D's" basically, is that correct?

Connie: That's right. That's always focused on the serologic "weak D's." And then on the transfusion side, if you see a young woman or a female patient with this kind of typing, some people give Rh negative blood. But if you're going to have a patient that is chronically transfused or going to get a lot of units, it behooves folks to actually genotype these patients, so that we really manage responsibly our Rh negative blood supplies. The Rh negative blood needs have been increasing throughout the years.

Joe: And I wanted to ask you about that, because that's—I think in virtually every newspaper around the country over the last couple years, all we've heard about is the declining amount of blood that's being used. That "patient blood management is working, by God, and we're decreasing the amount of blood that's being used." So again, I guess the obvious question is, if that's the case, why are we so concerned about a little "unnecessary Rh negative blood use?"

Connie: Well, you're certainly right that blood use has been declining and that's really been a phenomenal decrease in blood usage. But what we've seen at New York Blood Center, and certainly other centers have really seen this, is the **actual Rh negative needs are increasing**. So it used to be in 2000, about 7% of our inventory had to be Rh negative to serve our Rh negative customers needs. Now it's 10% or more and some ABC Centers are saying over 13% or more. And if any of our audience are an Rh negative blood donor they know that they're being called and bugged continuously. And so where's that going and that need going? Well we certainly know that trauma takes Rh negative blood which is suspicious and we think the patients with sickle cell disease, a lot of hospitals maybe want C, E, Kell negative blood and they know that also happens to be your Rh negative blood supply is also C, E, Kell negative used. So some of the use is happening there, we like to really, really discourage that. Because we want to keep it for the female children, women of child-bearing age, and for traumas. The work group actually tried to look at this and model it and determined that about 50,000 units a year in the U.S., 50,000 Rh negative units are given unnecessarily to this patient population that has a weak D Type 1, 2 and 3 that really don't need the Rh negative blood.

Joe: Wow. In light of the many millions of transfusions we do every year, that seems a small number, but speaking as a blood center medical director, and dealing with Rh negative shortages, seemingly everyday, that has potential to help.

Connie: It certainly isn't going to hurt to have those extra 50,000 around for the trauma and the babies, right?

Joe: Right! Absolutely. And again, just to make it clear, we're not talking about all Rh negative recipients being genotyped. We're talking about those with these variant types.

Connie: Exactly. And we're talking not necessarily, your 80 year old grandfather, although we all love our 80 year old grandfathers....and he's not going to get more than one unit or so, most people will give him a positive unit. Not everybody makes anti-D, especially hospitalized patients, but we're focusing this whole campaign on female, children, women of child-bearing age. That is our first line of patient who needs the Rh negative blood.

Joe: Got it. So one thing that I wanted to actually meant to ask this earlier, but in the work that you've done so far, in terms of those with these unusual D types, when you genotype them, what proportion of them, or do you have a rough idea of what proportion of them are coming out to be "weak D" Types 1, 2, or 3 vs. anything else where you would treat them more conservatively?

Connie: That's a really good question. We have a very diverse population and about 75% of them would have unnecessarily gotten Rh immune globulin, 75% of them are weak D Type 1, 2, and 3 and about 25% of them are partial D and really need the Rh immune globulin. It will differ on your population that your actually typing, but we've seen that hold up in different areas of the U.S., also.

Joe: Those are pretty good odds! I'm sorry, Connie, I interrupted you...

Connie: About 75% of them don't need the Rh immune globulin. But no one wants to really play that numbers game—getting the genotype. It does break up a lot of ethnic groups, you know your Hispanic, your African Americans are more likely to be "partials", your Caucasians are more likely to be weak D type 1 and 2, but there are some partial in the Caucasians also, like DVI that are fatal HDN. You never want to play this game based on ethnic group.

Joe: Right! (laughs) So I will tell you, since the recommendations came out last year, I've had many questions from hospitals that my blood center serves, and I will tell you that the biggest thing that they have said to me, well first, the biggest thing they've said is trying to understand exactly what was being recommended. The reason I kept harping on the "not all are Rh negative people are getting genotyping" thing, that's one of the things people ask me a lot. But aside from that, the second thing they ask about is cost and turn around time. Obviously, you and I both know that for turn around time you can't have this back in a day, but how do you respond to those questions and concerns and what practical things can people do while they're waiting, for example, for genotyping to come back?

Connie: Right. Cost is a major driver of what we do, right? And it should be, you know, the cost-benefit ratio has to be there to make it feasible. One of the things the work group did was ask the Kacker group et al*, to look at this with us and see if this makes sense. Is there a cost savings if you're doing Rh genotyping or not, or is this an added cost to the system? In that analysis, we ask specifically how much should Rh genotyping cost to be a wash or to be a cost savings? And the assumptions made here is that this testing would be done on pregnant females with serologic weak D phenotypes, so weak D. And we compared it to managing that woman as if she was Rh negative, giving her the shot, giving her Rh immune globulin. Under the assumption that this would be done during her first pregnancy, when her Rh typing was done. Now we all know that a lot of times it doesn't happen, you don't get a type on this woman until she's delivering. Then it becomes a part of her medical record for her next pregnancy so it doesn't help you necessarily on your first pregnancy because you got that turn around time of a few days. But then look at the direct medical cost assessed over 10 to 20 year periods for all the population in the U.S.. The number they came up with that genotyping needs to cost about \$256—in the range of \$256 to \$300. I know those of us that do typing have targeted that as the cost that this service must break even at. And then it needs to be sent back and become part of the medical record now. We've tried real hard here, I'm not going to be an advertisement for the New York Blood Center to make that a 5 day turn around or less, and we've been pretty successful at that and I think most labs can turn it around. But as you know, that doesn't help you when the patient is wanting to get her shot or not and go home. The idea is that hopefully this also filters into the OB/GYN offices. That's why ACOG was involved in these discussions, so that she gets genotyped and typed at her office visit. Many of you are doing the initial OB typings from the doctor's offices and this is where you can intervene.

**Kacker S et al, "Financial implications of RHD genotyping of pregnant women with a serologic weak D phenotype," Transfusion 2015;55:2095–2103*

Joe: Right. That's such an important point. It's not a stat test, obviously...

Connie: That's right.

Joe: ...and it's better done when you're planning ahead. What I've been telling hospitals when they're in the situation where they have someone in-house, and they're having to make the decisions, that based on the data that you guys have published, I would treat them conservatively while they're waiting for the genotyping to come back. Is that fair?

Connie: That's right, and that's a medical decision, that you as the Medical Director would make and others might make it differently, but that's certainly is a medical decision at that point. But hopefully this becomes prevalent enough that this becomes part...my vision is certainly that it becomes a natural part of an OB workup at the OB's office. Women are being offered lots of different genetic tests all the time at the OB/GYN level, and a \$250 test to get your Rh status determined genetically so you have it right and for best practice and best care, I think is a very reasonable thing for OBs.

Joe: And the fact that it's a one-time cost, you get it done once and that's all you have to do, obviously has some benefits as well.

Connie: That's right. It requires a laboratory, though, that specializes in blood group genetics, and that knows the serology side of it, because this is a research test, an RUO test, it's not an FDA-licensed test, and we don't think many genetic tests will ever be truly FDA-licensed. You might say, "Why not? Why isn't a genotype FDA-licensed?" Well, the patient can have any one of hundreds of different mutations in their D gene, and so until we can do full gene sequencing, will we always be 100% accurate on what the D gene status of that patient is? But right now, we can very accurately test if they are weak D type 1, 2, or 3, or all of the known partials. Like I said, it won't be an FDA-licensed test but it is an RUO test that's used in conjunction with the serologic typing for decision-making.

Joe: Excellent! Well, Connie, this has been really informative and really enlightening and I can't thank you enough for taking the time to go through this with us and for being willing to answer my silly questions and just be as basic as we can. I want to ask you, are you willing to allow me to put a couple of your slides to illustrate some of your points on the Blood Bank Guy website?

Connie: Sure, that would be great! It's been fun, and I know this isn't a simple topic, and it's one that I deal with every day so it can be complex though for people to just jump right into it. But I applaud your efforts to educate us all and get the dialogue going.

Joe: It's my pleasure, and you made it simple, so thank you so very much! Connie, thank you for being on the Blood Bank Guy podcast!

Connie: Thank you!