BBGuy Essentials 099:
Rh Immune Globulin (Teaching Episode)
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Joe: Hi, this is Joe Chaffin and this is The Blood Bank Guy Essentials Podcast.

Well, hello, everybody. Welcome back to Blood Bank Guy Essentials, the podcast that's just designed to teach you the essentials of transfusion medicine. My name is Joe Chaffin, and I am your host. I'm so glad to be back with you. Some of you have noticed and I've received a lot of kind emails asking if I'm okay. I am! Life has a way of hopping up on you and things happen, but this is the first Blood Bank Guy Essentials Podcast episode in a little while. It's the first one of 2023. And I have some really great changes coming for Blood Bank Guy Essentials, some big plans for 2023. And I'm excited to share those plans with you.

But first, you should know that this particular episode is not a continuing education episode. You can find episodes where you can get free continuing education hours at bbguy.org/podcast. They're all labeled with the letters "CE." You can also find those podcasts at wileyhealthlearning.com/transfusionnews, as well as, of course, any podcast outlet like Apple Podcast, Google Podcast, Spotify, et cetera. Those CE episodes are courtesy of transfusionnews.com, and Transfusion News is brought to you by Bio-Rad, who has no editorial input into this podcast.

Well, some of you are longtime listeners to this podcast; I'm really grateful for you. Some of you have just found it recently; I'm grateful for you as well. This podcast started all the way back in 2016, and I really had one simple goal, just to share the essentials primarily through interviews with experts in blood banking and transfusion medicine. And I'm still going to do that.

In fact, all the continuing education episodes that are coming up are going to be "interview style," where I'm talking with an expert about something that they are sharing with you so that you can learn over the span of about 45 to 50 minutes or so, somewhere in that ballpark. But I'm also going to do episodes like today's, where I'm actually teaching a particular topic. And in this case today, you actually get to be a "fly on the wall" while I'm going over something with a single student or sometimes, a group of students.

Most of these episodes are going to be shorter than those CE episodes, but they're still going to be done with the eye towards fulfilling my goal for this podcast, which is, again, to just help you learn the basics. One thing I'm super proud of is my affiliation with transfusionnews.com and Wiley Health Learning, as you've already heard about. And one of my favorite aspects of transfusionnews.com is the Transfusion Medicine Question of the Day.

I should tell you, if you're not subscribed to that, to the Transfusion Medicine Question of the Day, you really should consider it. If you're a learner, that there is just a treasure trove of information there. It's completely free. They'll actually send you three new questions every week. Typically, the questions during a week follow a theme, but not always. But at any rate, they're wonderfully written and they're really, really super

helpful. So just go to transfusionnews.com and click the question of the day link at the top right, and you'll get there.

The reason I bring that up is actually, because this episode is one where I'm actually discussing one of the questions on transfusionnews.com. It was a transfusion medicine question of the day from December of 2021. The question itself was actually written ... Actually, it's two questions that were written by Lorraine Blagg, who's from the Johns Hopkins Hospital, I should say. The question was on basically how we dose and how we do calculations surrounding the dosage of Rh immune globulin to prevent anti-D formation in Rh negative pregnant women.

It's such an important topic. I've actually done a video on this before a number of years ago, but I find that learners really still struggle with this. And in fact, not just learners, there's actually good data showing that a lot of people have trouble with this. I really think that, in many ways, it's made too complicated. And especially if you read, no offense to the Tech Manual, I love the Tech Manual, it's a wonderful text. I'm on record as saying that, but I think it's too complicated there. I'm going to try and simplify it for you a little bit.

Today, you're going to get to listen to me in a teaching session with a resident of mine named Jessica Hudson. Jessica is a third-year pathology resident at Loma Linda University Medical Center where I do my primary teaching. Jess is also an assistant editor at bbguy.org. And from the first time I met Jess, she's always told me about her interest in transfusion medicine. You're going to hear about something that's a little disappointing, but I still like her okay, anyway. All right. No more messing around. Let us go. Here's a Blood Bank Guy teaching session on Rh immune globulin dosage.

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Joe: Jessica Hudson, you are on the Blood Bank Guy Essentials Podcast. How cool is that?

Jessica: It's pretty darn cool, actually, Dr. Chaffin. Happy to be here.

Joe: I'm really excited to have you. But first, I have heard a horrible rumor about you. And I'm really, really, really upset because as long as I've known you, which is, I guess, about three years now, I think I've known you about three years, it has always been my complete understanding that Jessica Hudson was going to be a transfusion medicine physician. And I hear ugly, ugly rumors, ugly rumors, Jess. I mean, it couldn't be worse that you're considering... Micro? Oh my gosh, what's going on?

Jessica: I know. I know. I'm so sorry about that. I didn't betray you, I promise. I still plan to pursue transfusion fellowship. You know it's my one true love. I think the combination-

Joe: Argh.

Jessica: ... of the two, as you well know, Dr. Chaffin, someone's got to love micro as there's a great deal of overlap. Someone's got to do it. I'm...

Joe: No truer statement has been made, that somebody has to love Micro!

Jessica: That's right, and I do.

Joe: It certainly isn't me.

Jessica: Yes, that's right. We all know that. We know that. Yes, indeed.

Joe: Right, fair enough. I think I have made that point on more than one occasion, but let's go from there…

Jessica: You have.

Joe: Jess, I'm so excited that you agreed to do this. We are going to have a little teaching session. We're going to talk about how we dose Rh Immune Globulin, in particular, to prevent Hemolytic Disease of the Fetus/Newborn from anti-D. I've talked to everybody about what's going on and where these questions came from. What I didn't mention is that interestingly enough, and it's interesting to me anyway, these questions were listed as "easy" on the Transfusion Medicine Question of the Day site, both of them.

Jessica: Is that so?

Joe: Yeah, they were listed as easy, both of them. Only 50% of people that answered it got it right. That's-

Jessica: Can't be easy.

Joe: It can't be that easy, huh? And I will admit, these questions are asked a little differently than they're normally asked, and we'll talk about that a little bit towards the end. But why don't we start with something? I'm going to give you something right at the beginning that I want you to remember for forever, for forever.

Jessica: It's like a mini mental status exam...

Joe: It is, and it's the ... Yeah, that's right. Exactly. As you know, because we've had a conversation similar to this previously when you were on my rotation, I look at how we do these calculations maybe a little bit differently than the textbooks do. We'll talk about the fact that when you look at the text and you read the text, I should say, you're going to see some stuff that has a lot of zeros and a lot of points and a lot of 0's. And to me, that's really hard to remember. I came up with something. Actually, one of my residents a very long time ago came up with something that I have used ever since, and I want you to keep this in mind as we go through. Here's your memory tool for the day. You ready?

Jessica: I'm ready.

Joe: Okay, here we go. It's "five over three for RhIG," five over three for RhIG.

Jessica: And it rhymes. How could you forget it?

Joe: It shockingly rhymes. Five over three for RhIG. Everybody listening, keep that in mind. Five over three for RhIG. When you hear that and when we go over exactly how to do these problems, that will make a whole lot more sense to you. But for now, what is it, Jess?

Jessica: Five over three for RhIG.

Joe: Thank you so much. That's outstanding. All right. We're going to come back to that. Let's talk first a little bit about Hemolytic Disease of the Fetus/Newborn because that really is important for us to have the context behind the problem that I'm going to read to you guys in just a few minutes. For HDFN, Jess, let's just see what you know about it. In terms of Hemolytic Disease of the Fetus/Newborn, I mean, what comes to your mind when you think about it? How exactly does Hemolytic Disease of the Fetus/Newborn happen in a nutshell?

Jessica: Sure. Broad brushstrokes, thousand foot view. It means when there's maternal antibodies that are able to cross the placenta and attack the developing baby or fetus, it's usually associated with particular antibodies because we know some antibodies can cross the placenta and other ones inherently cannot. As you've taught me, it's actually a active process, not a passive process. We worry about particular antibodies that can cross, and so we try to prevent those antibodies from forming so that they don't cross. But if they do, then they develop hemolytic disease of the fetus and new newborn.

Joe: They certainly have that potential. Tell me what characteristic feature of the antibodies is necessary for them to be able to be moved across the placenta? What kind of subclass do they have to be, in other words?

Jessica: I believe they need to be small. I think the IgMs are too large, as far as I remember. But the IgG, which are small-

Joe: There you go.

Jessica: ... they make a little Y shape can cross, can cross the placenta...

Joe: Everybody listening to this, we're chuckling because I just made a dopey little hand signal with my hands…

Jessica: a Y!

Joe: ...to show tiny little arms of IgG. Yeah, that's exactly right. They need to be IgG. And you said it correctly, it's not just a passive process where IgG diffuses across the placenta. It actually is an active process where the placenta actually transports IgG across from mom circulation into baby circulation. And that actually forms the basis of baby's immunity once the baby is born. IgM, though, is not able to be transported. The antibody has to be IgG.

And that leads me a little bit to the way I look at HDFN when we're trying to decide whether or not an antibody is capable of causing HDFN. The first thing I look at is, are the antibodies in that blood group system typically IgG or IgM. Pretty simple, right? As you know, if you look, for example, at a blood group like the Lewis blood group system, well, Lewis is related to ABO. The antibodies against either Lea or Leb are typically IgM. Guess what? They don't cross the placenta, and that is generally the case. You'll see rare examples.

But generally, the case that they don't cross the placenta. Same thing within the MNS system. Anti-M is typically IgM. Even though, again, you'll find rare case reports,

generally speaking, IgM antibodies don't cross. Anti-M and anti-N wouldn't cross. On the other hand, the biggest blood group that we worry about, that we spend so much time that we're going to talk about today, were the antibodies typically are IgG and can cross the placenta would be what? What's the classic?

Jessica: The Rhesus, the D.

Joe: Absolutely. The Rh blood group system and in particular, the D antigen, antibodies against the D antigen. Now there are other, obviously, Rh antibodies and in fact, the antibody against a "little c" can do a similar thing to anti-D. A lot of the other ones can cause HDFN, but it's typically more mild. And we'll talk about what mild versus severe HDFN is in just a sec. But the other one that I want to make sure that everybody remembers, so everybody listening to this podcast, if you went to the Bahamas for a second, come back to me for just a second because this is so important.

We spend a ton of time worrying about HDFN and trying to prevent HDFN due to anti-D, and that's what the rest of this podcast is going to be about. However, there is an antibody in another blood group system, not the Rh blood group system that actually can cause more severe "HDFN" than anti-D. And that antibody, I say quote and unquote because there's not really a lot of hemolysis, it's much more of a severe suppression of neonatal or fetal, I should say, erythropoiesis. Jess, do you remember what that antibody is? What's that famous one?

Jessica: Oh, right. The OBs always worry about "Kell kills!"

Joe: Kell kills, there you go. That's exactly right. Anti-K [Note: We say that as "anti-big K"] is famous for causing a very severe suppression of fetal erythropoiesis that we call HDFN, but it's not really hemolytic. Again, that's a topic for another day. I have a whole video on that, on the blood bank guy website if you're interested in more.

But when we talk about HDFN, I mentioned severe versus non-severe. Let's just talk, again, very high level overview, two different things in particular that can happen to the baby, prior to the baby being born or being manifested after the baby is born.

The first of those is erythroblastosis fetalis, the old fashioned name for hemolytic disease of the fetus/newborn. And it basically describes a scenario where hemolysis is happening and the baby is cranking out immature red cells, erythroblastosis, because so they can be nucleated and erythroid precursors circulating around for the baby. But the other end of that spectrum is hydrops fetalis. And, Jess, what's your picture of hydrops? What's going on with hydrops?

Jessica: The babies are very edematous, swollen. They look like the Michelin man. They're very bloated. And as far as I know, the red cells are being attacked. They're being lysed. I think the baby's hemoglobin is extremely low.

Joe: Often, yeah.

Jessica: And I'm not sure if it's due to the rupture of the red cells that causes fluid accumulation, but I know the babies are quite rotund.

Joe: They are. And in fact, there's a really good reason for it. And the reason is the phenomenon known as "extramedullary hematopoiesis," so EMH. When the baby's bone marrow is trying to keep up with all this hemolysis and it can't keep up with all this hemolysis, baby starts trying to make red cells wherever it can. And one of the places where the baby makes red cells is the liver. The liver gets taken over with islands of extramedullary hematopoiesis. And suddenly, oops! The liver can't make some of the proteins that it normally makes, including?

Jessica: Albumin.

Joe: Albumin. And suddenly, you've got a scenario where the baby doesn't have the proteins intravascularly to keep the water in, and the water just scoots out and you get a baby that is severely hydropic. In the old days, that used to be fatal most of the time, by the way, Jess. But a number of babies that might have been born with fatal hydrops in the past are able to be saved nowadays with early and aggressive management.

Of course, the other thing that we worry about with HDFN when it gets severe is what happens to the baby after the baby is born, in particular with the bilirubin for the baby, the indirect bilirubin for the baby. And you know, of course, when red cells are hemolyzed, the hemoglobin goes out, the hemoglobin is metabolized. And generally speaking, it's changed from indirect bilirubin to direct bilirubin and it's cleared from the circulation. Well, it's super important to understand why that becomes a problem for the baby more after the baby's born. How come babies don't get born with severe hyperbilirubinemia, generally? [NOTE: Yep, I said "don't get born"; shakes head at self sadly...]

Jessica: Because mom's circulation takes over.

Joe: Exactly, yeah. Mom takes care of that indirect bilirubin before the baby is born. After birth, obviously, the baby loses that connection.

Jessica: Physically.

Joe: Yeah. Again, all those things being super important to have as a background. Over the years, we have focused enormously on HDFN, as I mentioned, caused by anti-D. D is, of course, the main Rh antigen. Those of you that aren't blood bankers, it's the one that when we say someone's "Rh positive" or "Rh negative," we really mean that they're "D positive" or "D negative." It's just the name for a particular antigen, the main antigen in the Rh blood group system, it's hugely important. It's the most immunogenic antigen that we have.

In other words, if you are negative, there is no other blood group antigen that's more likely to cause an antibody if you see that antigen. The risk is really, really high, comparatively speaking, to most blood group antigens. In fact, just from transfusion, the risk is around 22% to 25% or so, but that's still way more than any other blood group antigen in terms of the risk. Again, let's focus in on anti-D HDFN. In the old days, before the 1960s when Rh immune globulin was invented, if an Rh negative mom delivered an Rh positive baby, she had about a 14% to 16% risk of making anti-D.

And that caused problems, typically not with that pregnancy, but typically with the second pregnancy. In most cases, the immunizing event, and this is really important to understand, the immunizing event, the event that causes a D negative mom to form anti-D is typically delivery. It doesn't usually happen in the first, second, or third trimester prior to delivery, unless something else is going on. Unless there's some sort of trauma or a procedure that's done, an amniocentesis or an intrauterine and transfusion, things like that, that raises the risk, obviously.

But just in routine pregnancies, typically, delivery is the time when mom gets immunized to the D antigen. Again, typically, no involvement in the first pregnancy, but then involvement in the second pregnancy. When we do these interventions, it knocks that 14% to 16% risk down to about 0.1%. That's a huge decrease. That's a massive decrease. And the risk is really, really tiny. But I always want to point out to people the fact that we could do absolutely everything right.

We could do absolutely everything by the book and still 1 out of 1,000, that's 0.1%, 1 out of 1000 D negative moms delivering a positive baby could still make anti-D. It is not impossible to see anti-D. If you see it, it doesn't necessarily mean that somebody screwed up. It could just mean that for whatever reason, either mom had a bigger fetal maternal hemorrhage than we realized or somebody didn't do the calculation quite right. Oh, we can't have that. That's why we're doing this podcast or whatever. Again, even if everything's done completely properly, there is still a risk of that mom making anti-D.

Jessica: I have a quick question related to this. We're focusing on D negative mom, right? D negative mom, D positive baby.

Joe: Correct.

Jessica: Do you know in the studies or any literature, if the ABO status of the mother means anything, it's like, oh, are they more or less likely if they're A negative or if they're O negative or B negative? Does that matter or it's literally doesn't matter across the board if you're D negative, the risk is the same?

Joe: That's a great question, and it's a hugely important question too. And there is a relationship. If there is ABO incompatibility between mom and baby, for example, if mom is group O and baby is group A, those cells, when they go into mom's circulation, are going to be hemolyzed so fast. There's a much smaller likelihood, and there's probably a number out there, and anybody listening, if you know the number, I don't know it off the top of my head, please feel free to put it in the comments for this episode.

But, yes, the ABO incompatibility reduces the risk of RhD alloimmunization, just as a result of the fact that the red cells get cleared, they get hemolyzed so fast. Again, I don't remember the exact number, but I'm sure many of my immunohematology friends will be happy to supply me with that.

Jessica: I'm sure. You have so many friends.

Joe: Well, I'm fortunate that way.

Jessica: You are.

Joe: Let us walk through how a pregnancy works for an RhD negative mom. And everyone, I just want you to realize, what we're talking about is here in the United States, there are some variations depending on what country you're in. Different countries have different doses of Rh immune globulin, for example. Different countries have different ways of monitoring moms and different ways, different standard times at which they administer RhIG. I'm going to stick to what we do here in the United States.

Please check your local listings for something that works in your country if you are not in the United States. Jess, somebody's Rh negative and she discovers she's pregnant and she's cruising along and she has her first visit. Oh, I don't know, let's just say she comes in at 8 to 10 weeks or so for her initial visit, her initial labs, et cetera. Is there any action or any injection or anything that we do in the first trimester?

Jessica: Well, I would like to confirm that she is D negative.

Joe: Good. I agree.

Jessica: An antibody screen probably wouldn't be a bad idea. Ask about her transfusion history and other things, just more of a risk assessment. But at this point-

Joe: Sure.

Jessica: ... RhIG is not technically given at this interval.

Joe: You are correct. That is absolutely true. And the American College of OB/GYN has some very specific guidelines on exactly when the initial ABO and Rh should be done. And I don't have those at the tip of my fingers, but you're right. If you know somebody's Rh negative or if you can test them to find out that they're Rh negative, you can take some steps to preliminarily get ready for what's going to happen in the future. Because the key visit for an Rh negative mom, the most important visit is the 28-week visit or around 28 weeks gestation.

That's the point at which that mom is going to get her initial dose, typically speaking, if no pregnancy complications have happened before this. That's the point at which she's going to get her first dose of Rh immune globulin. That's a really important thing to understand that 28-week visit, we're going to check an antibody screen because we want to make sure she doesn't already have anti-D. Why is that important, Jess? Why do we care whether or not she has anti-D?

Jessica: Well, if she has anti-D, unfortunately, there's not a whole lot that we can do as far as reduce the risk in a safe manner, as far as I'm aware.

Joe: Yeah, yeah. Cat's out the bag already if she's made her own. But that's such an important point. The caveat, if she has made her own, the number one question anytime anybody in blood bank world sees an Rh negative mom who has anti-D, the number one question should be, has this person received Rh immune globulin somewhere else? It is so easy to get caught into that trap and say, oh, she's already made it, boom. We don't need to give her more. And that may not be true. Careful questioning of the patient is really, really important in that setting.

Back to the RhIG for just a second, you've noticed, I'm sure, because you know this is one of my pet peeves, that I've called it Rh immune globulin over and over and over in this episode. And I'm sure some people are sitting there going, "does he mean RhoGAM? Is that what he's talking about? Does he mean RhoGAM?"

Jessica: I used to say RhoGAM, yeah.

Joe: Oh my goodness, gracious.

Jessica: Oftentimes.

Joe: I remember the first time you said it. I think my hair caught on fire.

Jessica: Oh, you corrected me hard, yeah!

Joe: Gosh. Well, I hope it was semi-gently. But anyway, so here's the point, and you've heard me say this enough that you probably could recite this word for word. But basically, RhoGAM is a trade name. RhoGAM is a type of RhIG from one particular manufacturer. And I will say, they've done an excellent job at marketing because when people think of RhIG, they think of RhoGAM. Again, fine, that's fine. But it's like calling all facial tissue "Kleenex."

Jessica: Kleenex, yeah.

Joe: All facial tissue is not Kleenex. Some of it's Puffs, some of it comes from Walmart, some of it comes from Costco. You get the message. I will say RhIG all the time. Those of you that want to call it RhoGAM, fine. We know what you mean, but we're calling it RhIG in this episode.

Jessica: Dr. Chaffin, and doesn't RhoGAM have particular ... There's particular amounts in ... It's actually more specific, right? There's a particular dose of it, all the different things. It's like when we're talking to our clinicians, we may not have RhoGAM at our institution or-

Joe: Might not.

Jessica: ... it's not a direct ... Not all brands are exactly identical, right?

Joe: They aren't. You're exactly right. And some of them have heparin and some of them don't have heparin. Some of them are IM only, intramuscular injection only. Some of them are okay with either IM or IV, and some are IV only. Again, there's a lot of variation, and that's way beyond the scope of what we're talking about today. But you're absolutely right. And if you're working somewhere and you're involved in this, I would suggest that you grab a copy of the package insert and take a look and see.

In the United States, a so-called standard vial of RhIG or a standard single dose of RhIG has 300 micrograms of anti-D. Again, it's concentrated high titer anti-D, comes from a D negative donor who's been immunized with D positive red cells and the antibodies been harvested, and here you go. It's purified, it's treated, all that stuff. It's good. 300 microgram is with the way it's traditionally been described. Internationally, even more, it's described in IUs, and that's 1500 IU corresponds to the 300 microgram dose.

The most important conversion that I want you to be sure to remember is that that 300 microgram/1500 IU dose covers 30 mL of D positive blood or 15 mL of D positive red blood cells. 30 mL of D positive whole blood and 15 mL of D positive red cells. Most RhIG used in the United States has that particular dose. There is another version of RhIG that's not used very often. It used to be called the mini dose. It used to be a 50 microgram or 250 IU dose to a 50 international unit. It only covered basically 5 mL of D positive blood.

It was used typically when someone had an elective or therapeutic abortion during the first trimester. First trimester stuff, abdominal trauma during the first trimester, when the baby has such a small blood volume, that's really all you needed to cover. I don't see a whole lot of many doses around anymore. I think some places still use it, but I don't think the majority of places still use it. When we talk about giving a certain number of vials of RhIG in the United States, we mean a certain number of vials of 300 microgram/1500 international units. That's okay if you remember that. But what is important to remember is that one vial per 30 mL of D positive blood. That conversion is hugely important. You got to have that one burned into your brain.

Jessica: I think I got a question wrong about this, actually, because the question was saying about packed red cells-

Joe: Ah, yes.

Jessica: ... and I said 30, but it's not.

Joe: You said 30 when it's supposed to be 15, yeah.

Jessica: Yes. And so-

Joe: If you're on an examination and they specify packed red cells specifically, you cut that number in half and it's 15 mL of D positive packed red cells. I hate it that they ask that because that is not used very often. I think it does still show up on exams here and there. Okay, so a couple of other things real fast. We're still talking about the 28-week visit when we're basically giving mom one standard vial, one standard dose, one vial of Rh immune globulin at 28 weeks, at or around 28 weeks, if she has not already made anti-D. And by the way, if you're in doubt, give her the RhIG. If you don't know, if you can't tell for sure, just give her the RhIG. Really, I would rather-

Jessica: One dose?

Joe: ... err on that side of things. One vial. If you're not sure, if she's got an anti-D floating around and it's low titer and you're not sure if she's made it, maybe she's not a great historian, I would rather give her one vial of RhIG at that point and figure it out later than not do it and have a problem later on in her pregnancy or at the time of delivery. Important to remember, Jess, why do we give that dose at 28 weeks? What's so magical about 28 weeks? Well, there's nothing really magical about it. I think you're aware of the half-life of RhIG. In other words, when you give somebody really any antibody and to the anti-D and RhIG, in particular, for our purposes, when you give them that a dose of that, how long does it hang around? What's the half-life?

Jessica: 25 days.

Joe: It's 25 days, exactly right. Yay! If you do the math, I love how happy you are that you knew that one. That's outstanding.

Jessica: With doubt still.

Joe: Yes. If you give someone a dose at 28 weeks and the half-life is about 3.5 weeks, basically, you can do the math and you're going to see that that anti-D should hang around to protect mom from D positive red cells for until about ... I mean, there should be about 10% left at the time of delivery. Again, the idea is that that 28-week dose is to protect her from these somewhat unlikely event that she's going to have a feto-maternal hemorrhage during that last trimester. Again, it doesn't happen all that often, but it absolutely can happen.

And that's the point of doing that 28-week dose. We need to actually talk about one thing before we move on, and this is important. How the heck does RhIG work, Jess? What do we know about that? Why does this thing work? Giving somebody anti-D when we're trying to protect them, how does that ... I mean, what's the mechanism behind that?

Jessica: I think we don't actually really know.

Joe: Trick question alert, trick question alert.

Jessica: Oh, did I get it right? Okay, good.

Joe: You're right. We don't actually know. I mean, historically, we've thought that we knew. In other words, the obvious thing, and what I learned when I was a medical student was, well, okay, so the anti-D is floating around in mom's circulation and D positive red cells come in and the antibody grabs onto that D positive red cells and magically makes them removed, and so that mom doesn't see them and mom doesn't make the antibodies. Well, maybe there's some component of that.

There probably is some component of that, but I think it's probably more likely that there's some other component that we don't understand, just the way that we don't really totally understand how IVIG works. That, to me, frankly, is voodoo. I don't get how IVIG works. You give somebody a whole bunch of antibodies and they stop making their own, okay, all right, fair enough. But I think there's some component of that. But the reality is we don't know, for sure, how anti-D in the Rh immune globulin works. Again, it's definitely going to bind to those red cells, but how does it mask it from mom's immune system? It's hard to make that mental picture and imagine that, but it does. And, yay, cool that it does. And so I guess we should just be happy that it does work, right?

Jessica: Yes, absolutely.

Joe: Okay, so let's go from where we were. We talked about the first trimester. We're basically not doing a whole lot. 28 weeks is the big important point in an Rh negative pregnant woman's life. We give her the RhIG, we give her one vial, typically. And then we come to after she delivers the baby, the postpartum time. And so a couple things

need to happen postpartum. For an Rh negative mom, we need to first see if she's delivered an Rh positive baby. That is hugely important, and that gets missed sometimes. I want to make sure that people are very well aware of that.

If you're not getting a cord blood sample to check baby's Rh type, that's a problem. That's a hole in your system, if you're not routinely getting cord blood samples for Rh negative moms, D negative moms. Because we need to see whether or not we need to give her RhIG postpartum. And it's a hugely important question. If the baby really is D negative, great. Hooray! We're done. Nothing more to do. But if the baby is D positive, we have more work to do.

And that's the stuff that you know is coming, the stuff that we ... Where we're basically trying to ask the question when a D negative mom delivers a D positive baby, we have one main super, super, super, super important question that we have to ask right off the top. It's going to determine everything else that we do. What's the most important question that we're trying to ask for that Rh negative mom who's delivered an Rh positive baby? What's the most important question?

Jessica: How much RhIG do we give?

Joe: Exactly, right. That is 100% correct. We know we're going to give her at least one. What we're trying to decide is, are we going to give her more than one? And that's why we embark on a series of tests that are pretty much routine in blood banks all across the United States and really, in blood banks all around the world. Some places do it differently. But generally speaking, the first test that we're going to do is a feto-maternal hemorrhage screen. It's typically called the "Rosette test." Rosette test, most people are familiar with it.

Basically, you're adding anti-D to mom's red cells or a sample of mom's blood, I should say. Anti-D would gather around and attack while binds to D positive baby red cells. And then we add indicator red cells, which in some formulations are sheep red cells, and they form rosettes. Basically, we look at mom's blood under the microscope, we count the rosettes, we look at the package insert. And the package insert says, if you have more than X number of rosettes, that's a positive. It's a yes/no test.

If it's below that number, whatever the package insert says, then great, we're done. We're giving one vial of RhIG. If it's above, then we have to figure out just how many more than one vial of RhIG we're going to give. The entire purpose of the Rosette test or any Joe: feto-maternal hemorrhage screen is just to answer the question, is one vial of RhIG enough for this person?

Jessica: Got it.

Joe: A positive Rosette test says it might not be enough.

Jessica: So…

Joe: We've got to go on and do other tests. Go ahead.

Jessica: Just to confirm, so if you have a negative fetal blood screen, no indication that there's significant exchange of red cells, you still give one vial anyway?

Joe: You always give one vial.

Jessica: Okay, sounds good.

Joe: An Rh negative mom delivering an Rh positive baby will always get at least one vial of RhIG.

Jessica: Perfect.

Joe: If the feto-maternal hemorrhage screen is positive, the Rosette test in most laboratories, then she might need more than one. We don't know for sure. We need to go on and do a quantitative test. The Rosette test or the Fetal Bleed Screen as it's also called feto-maternal hemorrhage screen, whatever, is a qualitative test. It's a yes/no test.

Jessica: Yes/no.

Joe: One vial enough, one vial maybe not enough. If one vial is maybe not enough, we go on and we do the quantitative tests, which are the Kleihauer-Betke and flow cytometry, one of the two. In most labs in the United States, it's the Kleihauer-Betke test. The Kleihauer-Betke, clunky, awkward, and awful as it is, my apologies to anybody who's related to either Kleihauer or Betke, frankly, but it's not a great test. It's a challenging test to interpret. Have you ever actually seen one of these, Jess, in your residency so far?

Jessica: I have not seen one in the flesh, but I have seen what looks like test questions that have pictures associated with it. But, no, I haven't seen one.

Joe: At some point, next time you're on your transfusion medicine rotation, be sure to go to the transfusion service and actually take a look at one. They are challenging to interpret, they're not easy. Basically, the principle, as you know, is that we make a blood smear of mom's blood, flood that slide with citric acid. And basically, it operates on the principle that citric acid elutes out hemoglobin A, but leaves hemoglobin F, which is, theoretically, in the baby's red cells, or should be in the baby's red cells behind.

And so you get ghost cells in the background, those are moms, and you count the bright red ones, those are babies. You typically count 2,000 red cells and basically take the number of bright red cells and divide that by the 2,000 cells that you've counted, and that gives you a percentage. That's the Kleihauer-Betke percentage. Does that make sense? You looked like you wanted to ask me something.

Jessica: Yeah. Yes. How do we count 2,000 cells? I feel like I would need a cell counter.

Joe: You do need a cell counter. Yeah, you do need a cell counter. That's the easiest way to do it.

Jessica: Got it.

Joe: You have a cell counter and you just pop, pop, pop, pop, pop, pop, pop. Just like when you're doing peripheral smears.

Jessica: Perfect, okay.

Joe: Yup. That's exactly right. That is frankly a fairly crude and awkward way to do it. But it's technically fairly simple to do, and most laboratories don't do flow cytometry. Flow cytometry, unquestionably, is far more accurate and in a perfect world, is better. Unfortunately, flow, it requires different reagents, it requires different QCs, and maybe the volume is not high enough. I didn't mention this before, but I think it's 98%, 99% of people that get a Rosette Test, a feto-maternal hemorrhage screen, it's negative.

You don't get very many positives that you have to do either a Kleihauer-Betke or a flow cytometry test. Most hospital laboratories don't have the volume of these that they would want to do flow on them. Again, I'm not going to talk about flow, simply because we just don't see it all that often. But if you did have flow, you would get a percentage from flow as well. And that percentage is what becomes really important when we talk about how to do these problems. Let's look at this first question.

Actually, both of these questions were written by Lorraine Blagg from Johns Hopkins. The first question is, and again, everybody, you can find this on the website at bbguy.org/099 or on the transfusion medicine question of the day site at Transfusion News. A postpartum blood sample from a D negative woman with an estimated blood volume of 5,000 mL who delivered a D positive baby today is received in the laboratory for a Rosette test, which is positive, the Kleihauer-Betke test, and again, that makes sense. You do the Rosette test.

If it's positive, then you go on to do the quantitative test. The Kleihauer-Betke test identifies 16 fetal red cells out of 2,000 RBCs counted. What is the estimated feto-maternal hemorrhage? Now, I will tell you, and there are four choices, by the way, 0.8 mL, 16 mL, 25 mL, 40 mL, or 54 mL. I will tell you that this question is written a little bit differently than the way I typically see these questions. Most commonly, you'll see the intro to that question.

In other words, they'll take you all the way through the Kleihauer, and then they'll say, "Okay, how many vials of Rh immune globulin would this patient need are indicated?" This asks an intermediate step. In other words, it's asking the estimated fetal-maternal hemorrhage, which you can get really simply by taking that 16 fetal red cells out of 2,000 RBCs and just divide it. And that gives you a percentage. I think it's like 0.8%. 16 divided by 2,000 is 0.008.

And then you take that 0.008 and multiply it by mom's blood volume, which they told you earlier in the question, was 5,000 mL. And that gives you the answer to this question, which is 40 mL. Again, this is not the way that you typically see these questions. Normally, you're able to skip to the end and you're able to use my handy dandy little formula that I said. Oh, by the way, Jess, what is that formula?

Jessica: Five over three for RhIG.

Joe: Yes, exactly right.

Jessica: Nailed it!

Joe: In most circumstances, nailed it, you're able to figure out that percentage, which is again, 0.8%. Take that, multiply it by five, divided by three, and you'll get the vials of

RhIG. We're going to come back to that. But again, in this particular case, Lorraine asked everybody to figure out the intermediate step, which is to determine the amount of feto-maternal hemorrhage. As I mentioned, only 50% of people who answered this question, and it's out of a lot of people at the time I printed this, it was about 550 people that had taken it, and only half had gotten that right.

Again, it's not super hard, but it is something that people do struggle with. And again, it's a reason potentially why you see that maybe RhIG does not get dosed as well as it should. All right. That's the first part of the question. The second part of the question is, and this was on the next day that the second part came out, a postpartum ... Or maybe this was from the same day. Actually, this was from the same day. I'm sorry about that. This is part two.

A postpartum blood sample from a D negative female who delivered a D positive baby today has an estimated feto-maternal hemorrhage of 40 mL. That's just summarizing what we found in the first part by the Kleihauer-Betke test. How many vials of 300 microgram RhIG should be given. And the answer choices are one, two, three, and four, basically. Again, my perspective on this, Jess, is that, by far, the easiest way to do this is once you know the Kleihauer-Betke percentage, once you have that, again, we were given, it was 16 red cells out of 2,000 red cells that we counted. We divide 16 divided by 2000, that's 0.008 or 0.8%. If you take that raw number, that 0.8%, 0.8, and you use our magical formula, which is what?

Jessica: Five over three.

Joe: Five over three. 0.8 times 5, divided by 3. 0.8 times 5 is 4, 4 divided by 3 is 1.33. And so you get the answer to this question, which is 1.3 vials of RhIG. What was that? Oh, oh, I'm sorry.

Jessica: No, it's not.

Joe: My producer is telling me that's not one of the choices. Oh, no.

Jessica: Dang it, yes.

Joe: That's not one of the choices. Wait a minute, what's going on? Oh, well. We have some more work to do, don't we? Well, okay, Jess, I'm going to let you show off here for a second. How do we decide when we have a number that's something point something, how do we decide, actually, how many vials to give the patient?

Jessica: Yes, absolutely. Usually, we don't get a nice round number when we calculate these numbers. In this case, it's 1.3. We're going to use that 0.3 as our game changer number. If it's 0.5 or up, we're going to round up, so it'd be 1.5, we round it up to 2. And then we always add one vial, so it'll be three.

Joe: Ah, okay, okay.

Jessica: Now in this case, we have 1.3, so it's not 0.5. We're going to leave it at one, round down to one, and then always add one vial, so it should be two vials. Did I get it?

Joe: Correct. And that's ... You did. You got it 100% right. That is exactly the way that you would do this. And we always add a vial, simply because we know that the Kleihauer-Betke test isn't the greatest test. We always want to give ourselves a cushion, and that cushion is super important. That is the standard way that we do questions like this. In this particular case, the way that Lorraine asked this question, it's still simple. I mean, again, we know that it's 40 mL. 40 mL is the amount of fetal maternal hemorrhage, and we know that one vial covers 30 mL of baby blood.

So 40 divided by 30 also gives you 1.3. I should be really clear on this, Jess, because I don't want you to just remember five three for RhIG. I do want you to remember that, but I don't want you to just remember it. The five part comes from mom's blood volume. In most of these questions on exams, they won't tell you either mom's blood volume or mom's weight. And so as a result, you have to assume that her blood volume is a "standard" five liters. That's where the five comes from. The three on the bottom comes from that 30 ml calculation.

And you can sit down and you can do all the math and you can cross off the zeros and you can see how I ended up with five over three. But I hope you'll trust me when I say that the numbers work out so that if you use the five for the blood volume and the three to represent that amount of baby blood, even though we know it's 30, but we're using mathematically three, that will give you that right answer every time. You never want to mess with that bottom number. You're always going to do the Kleihauer-Betke times something over three.

And if they don't give you mom's blood volume, you're going to use five. You're going to assume mom's blood volume is five. Now, if they do give you mom's blood volume, let's just say they said her blood volume is 6,000 mL or 6 liters. Well, then you just change that five to a six. Or if they tell you that she weighs 100 kilos, so if she weighs 100 kilograms, then you would take that 100 kilograms and use the standard conversion, which is typically 70 mL per kilo. 100 times 70 would be 7,000 liters blood volume, 7,000 milliliters, excuse me, blood volume.

Then you would take that seven liters and put the seven in instead of five. And the same would be true if you found her blood volume was 5.5, 4.2, 3.1, whatever. You use her blood volume in liters on the top of that five three, and that will get you that right answer every time. It's really not all that hard, and I hope that those of you who are listening that we're painting enough word pictures that you're seeing that that five over three is your friend, it makes things so much simpler, as opposed to looking at percentages and wondering, well, in our example, do I use 0.008 or do I use 0.8?

Just remember, you use the percentage. If the percentage is 0.8%, don't screw around with moving decimal points. Just keep it as it is, 0.8 times 5, divided by 3, we get 1.3, and we round up. The other thing I want to say, Jess, about the rounding rules ... I'm on a roll. The other thing I was going to say about the rounding rules is that you're 100% right. If the number to the right of the decimal when you do your calculation is five or more, you round up, then you round up again. I find it easier to think about how I learned how to round back in elementary school and high school.

Well, the rounding rules are pretty simple, right? If you see a 1.3 and I ask you to round that to the nearest whole number, you would round it to 1. If I saw a number that was 1.5 and I said, round it to the nearest whole number, I would round it up to 2. Basically, all you're doing is just doing the same thing that you learned how to do when you were a kid and you're rounding to the nearest whole number, then adding one for that cushion. Just as you said, we're always going to-

Jessica: Always one.

Joe: ... add one for that cushion. Always add one for the extra cushion. Okay, so we have gone, man, longer than I thought we were going to go. But this was fun. Do you have any other questions for me, Jess?

Jessica: Yes. Are there any contraindications for RhIG?

Joe: Really, the main one is if someone's already made their own anti-D. There are others potentially, such as if someone has severe allergic reactions to RhIG injections. You said earlier that there's not just anti-D and not just IgG in Rh immune globulin. In some formulations, there's IgA in there. If someone's IgA deficient, then there's the potential risk of anaphylaxis. And though I'm personally not super familiar with examples of this, if someone has heparin induced thrombocytopenia, some forms of RhIG do have heparin in them.

You would want to check that before you gave that to somebody. The other thing that people ask me about, by the way, is whether IV injection or IM injection is most useful. All I would say is it 100% depends on what formulation you have. If you've got the IV version, I don't really have any heartache with doing that. Typically, the IV version gets used in other clinical situations like an Rh positive person with ITP. That's another potential indication for Rh immune globulin. But either way, either way work. I do want to leave you with one last thing, Jess, unless you had another question for me.

Jessica: No, bring it on.

Joe: Okay, so I did some math the other day. You've probably noticed I'm a bit of a nerd when it comes to math and when I was-

Jessica: What?

Joe: Yeah, I know. Shocking, right? I did some math the other day and I said, okay, at what point should I be worried that my Kleihauer result is inaccurate? Because as we talked about, all it's looking for is hemoglobin F. And if mom has hemoglobin F, if mom has either a hemoglobinopathy where she has increased hemoglobin F or if she has hereditary persistence of hemoglobin F, then realistically you could have a number when you do your Kleihauer, that's super high. What doesn't make sense?

If you look at a standard three average ... I think average baby weight in the United States is 3.2 kilograms. I looked this up because I'm just that much of a nerd. And so if you look at a 3.2 kilogram baby and baby blood volume is found by multiplying 100 mL per kilo, times the baby's weight in kilograms, that would give you ... Let's just say, it gives you 320 mL or 300 mL or so of baby blood. That's the baby's entire blood volume.

And you try and dilute that entire baby blood volume into a maternal blood volume of five liters. That's in the range of 6% or so.

If you see a Kleihauer-Betke come back that's 9%, 10%, 15%, there is no way that's right. There is no way that's accurate. That would be more than the baby's entire blood volume. And if the baby's alive, then that doesn't make any sense. Just keep that in mind. It's just a red flag that tells you, hey, maybe before I go jump and giving a dozen vials of RhIG for this mom, I should make sure that this is real. And it may require different testing. It may require a send out for flow cytometry to quantify her feto-maternal hemorrhage.

Jessica: Dr. Chaffin-

Joe: That's just a little tidbit. Yes?

Jessica: I have a question. In that same vein, is there false positive Fetal Bleed Screen that we should be aware of some causes that can cause that where we shouldn't trust it?

Joe: Yeah. The indicator red cells will react to any red cells that are coated with antibody, basically. If mom has a positive DAT, let's just say mom has a maternal ... She has a red cell autoantibody, for example, then that's going to give you a falsely elevated fetal bleed screen. That is definitely a thing to keep in mind. That's in the package insert, I believe, for the Rosette test. Those indicator red cells don't really care whether it's D positive red cells or not. Well, they do to an extent, but they will still falsely agglutinate, falsely form rosettes, I should say, around red cells that are coded with antibody of any kind. Positive DATs can definitely throw it off.

Jessica: Cool. Thanks.

Joe: Okay. Everyone listening, I hope this has been helpful for you. This was a trial run. It's something I'm going to do a whole lot more often in the future. And, Jess, thank you for being the guinea pig. I appreciate it.

Jessica: Oh, always a good time to be a guinea pig with you, Dr. Chaffin.

Joe: All right. Thank you so much, Jess. Take care. We'll talk to you later.

Jessica: Thank you so much, Dr. Chaffin.

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Joe: Hi, everybody. This is Joe with just a couple of quick closing thoughts. As I said before, this podcast really has just one goal, to teach you the essentials of transfusion medicine. I love doing that and I hope that comes through when you hear me teach. That enthusiasm that you hear is just ... That's who I am. It's what I absolutely love to do. And so from now on, it's my commitment to you to do that as often as I can, the best way I can in ways that'll hopefully help you as much as possible. I want you to know that I do read every single comment that's made on the website.

I also read every single comment that's made in Apple Podcasts, as well as look at reviews, et cetera. And again, positive or negative, I try to learn from things that I can

certainly do better. And if you say that I talk too fast, I would say, you know what? You're right, sometimes I do. That's for sure. But to that end, I would love it if you would go to Apple Podcasts in particular to rate and subscribe and comment about this podcast. It really does help other people who are in your situation, as learners, help to find the podcast and hopefully, it'll benefit them as well.

As I mentioned before, I'm looking forward to 2023 with my next episode coming up soon, the 100th episode, which will be a continuing education episode, followed by tons of great interviews with great teachers and leaders in our field, alternating with teaching sessions and question of the day discussions. That's really what I plan to do, is alternate the continuing education episodes with basic teaching sessions that might not be quite as long. We'll see. We'll feel it out as we go along. But hopefully, you're going to be hearing from me a whole lot more in 2023 and beyond.

And I can't wait to do that. But until then, until we see each other again, my friends, I hope that you smile, have fun, tell the ones that you love just how much you do. And above all, never ever stop learning. Thanks so much for listening. Take care. I'll see you next time on The Blood Bank Guy Essentials Podcast.