BBGuy Essentials 093:
The Mighty MMA with Sandy Nance
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Sandy: This is Sandy Nance, and this is the Blood Bank Guy Essentials Podcast.

Joe: Hi, everybody! Welcome back to Blood Bank Guy Essentials, the podcast designed to help *anyone and everyone* learn the essentials of Transfusion Medicine. Today on the podcast, I interviewed Sandy Nance, who is the emeritus Senior Director for National Laboratories at the American Red Cross, about a very important test that you may not know much about, the Monocyte Monolayer Assay, or the "MMA." Sandy has stories to tell, and I think you will find the whole thing fascinating and useful.

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OK, let's get back to the MMA. Usually, when a transfusion service or reference laboratory identifies a red cell antibody like anti-K or anti-Fy^a or anti-D, we know exactly what to do next: Find blood that is negative for the antigen target, and transfuse the patient. Occasionally, though, we find antibodies in patients that come with two distinct problems: First, the antigen target is present on just about every blood donor, so it is really hard to find compatible blood, but second, we don't even know if we NEED compatible blood with these antibodies! Some people with antibodies like "anti-Yt^a" or "anti-Vel" will hemolyze transfused blood carrying that antigen, while others just don't! We call these things "antibodies of variable clinical significance," and they lead to the very important question, "Do we search the world for rare units of compatible blood (remember, these are high frequency antigens that are the targets), or can we just transfuse ABO-compatible red cells out of our regular inventory?"

Back in the 1980's, people were using many different tests to try to answer that question. Sandy and a group of other illustrious scientists at the American Red Cross developed the MMA to try to help us with the choice of whether or not a patient with an antibody against a high-frequency antigen can be safely given antigen-positive blood. The MMA is simple in concept, but it's really labor-intensive, so not very many labs do it, and you may or may not have heard of it.

After today, I think you will have a much better understanding of what the test is, and how it can really help in some “no-win” situations.

Let me tell you a bit about my guest today. Sandy Nance, as I mentioned, is the emeritus Senior Director for National Laboratories for the American Red Cross and has provided leadership to multiple programs at the Red Cross and the blood bank world in general, including the American Rare Donor Program and the national American Red Cross SBB Program. She is also an emeritus Adjunct Assistant Professor at the University of Pennsylvania. She has a master’s degree in Pathology from the University of Maryland and received her SBB from the Johns Hopkins Medical Institutions. Sandy developed of the use of polyethylene glycol or PEG for serologic testing as well as today’s topic, the monocyte monolayer assay (MMA).

Sandy’s list of honors and awards is just too long to detail, but they include the John Elliott, President’s, and Sally Frank awards from AABB, the National Blood Foundation Hall of Fame, the ASCP Hall of Fame. She has worked with a veritable “who’s who” of scientists at Johns Hopkins and the American Red Cross (including the late and very, very great George Garratty). She has authored or co-authored over 45 scientific papers and 200 abstracts, and edited 9 books.

I actually interviewed Sandy a number of months ago, and since this interview happened, she has actually retired after a long career spent making a humongous impact! I’m so happy to have managed to talk to her before she left the Red Cross, and I’m also so happy for you to share in that discussion.

So let’s go! Here’s my interview with Sandy Nance on “The Mighty MMA!”

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

Sandy: Hi, Joe, how are you?

Joe: I am doing so great and especially great since we're finally getting the chance to talk about this fabulous test called the "Monocyte Monolayer Assay." I am so excited to do this with you, Sandy. Thank you so much for making the time.

Sandy: It is my pleasure.

Joe: So Sandy, there are people out there that have looked at the title of this podcast, which I cleverly, maybe that's not the right word, called "The Mighty MMA," and they're sitting there going, "MMA, that seems like I should know that." And to a lot of learners, this is something, I think, that is a little bit of a mystery. So why don't you tell us a little bit about, just the high level thumbnail summary, of what the heck this test actually is?

Sandy: Well, for sure, it's not "mixed martial arts." It is, however, technically demanding, and the people who do it may feel like that during the time

they do the assay. But the assay is designed or was developed to really look at what happens to an incompatible red cell when it's transfused to a patient with that antibody. And that is the sole purpose that we use it for today with regard to mostly high-prevalence antigen-negative blood, the utilization of that in a patient that has an antibody to high prevalence antigen, and we don't have the blood available. Things like K-negative blood are relatively easy to find, so we wouldn't probably use it for that, but there are other antibodies of known variable clinical significance for which it has been very useful.

Joe: I am very aware that you played a huge role in the development of this test in the way that it's used today and has been used for quite a while. We won't talk about specific years, Sandy, we don't have to do that...

Sandy: Thank you, Joe.

Joe: You're very welcome. But I am fascinated by the story. I wonder if you would share with us. Can you walk through a little bit how this test was actually developed?

Sandy: I can. As a newbie into George Garratty's research laboratory, that was his most interesting thing that he wanted me to do. And that was my first assignment, to be able to create an assay that could be used to predict the clinical significance of antibodies. That had been his focus and continued to be his focus until he died. And honestly, it was very fascinating to be able to think about developing that test.

And I started by learning how to separate this with his wife at UCLA in her laboratory. And so that was quite interesting as well. And there were a number of researchers at the time that I started that were looking at monocyte assays or mononuclear phagocyte assays, or just that cell line to be able to evaluate things like reticuloendothelial system function like autoimmune hemolytic anemia. And this was a group of people, Moses Schanfield, Branch and Gallagher, Sapanska got into it at that time as well.

But our focus was completely ... In the early years, we looked at autoimmune hemolytic anemia, because George was very interested in that, but we quickly honed in on this prediction of the clinical significance of alloantibodies. And that started, I think the first publication was two years after I joined. And then collecting data with the use of other assays, like Chromium 51, looking at IgG sub-classing, really trying to have clinical correlates to be able to say whether the assay was in fact predicting what happened when the patient was transfused incompatible units.

Joe: Were you fresh out of school, Sandy? When you started with George, was this your first ... You walk into the lab and George says, "Here's what I want you to do?"

Sandy: That is what happened, but I had started, I had been six years in Johns Hopkins and was the lead technologist there before I wanted to move to California. There's a romantic story here, that I'm still married to the man I moved for, but we won't go into that today, Joe.

Joe: Okay, fair enough.

Sandy: So when I walked in the lab in California, he said, "I have an assay I want you to develop." Bingo. That second week I was in Eileen Garratty's laboratory in UCLA.

Joe: So again, I want to make sure that people understand that this is not just shaking a few tubes. This is an assay that's very, very labor intensive, right? You were spending, I'm guessing, lots and lots of time and lots and lots of thought in figuring out how to do this well.

Sandy: I did, and I used a lot of my own blood, because it was readily available, and I didn't have to ask permission. So definitely in the first three years, if you look at the lab books, Sandra Nance, those were the monocyte sources. And I learned a lot about storing monocytes and the age of a sample in transportation. We'll get into it probably later, but why do we not use patient monocytes? Why is that not used? Well, the reason behind that was the timed studies of drawing my own blood and then evaluating it the next day at these timed intervals and showing that the blood that was drawn yesterday was of almost no value. The monocytes were dead.

So once you take them out of the body, they start to die. So it is technically demanding in that you need to be quick, and you need to make sure that you have them in the right media. Those experiments of finding the right media, of isolating the cells, of finding the right media to put the monocytes onto, occupied much of the first year.

Joe: We will get into the specific details of how this works and all the stuff that you have to do in order to prepare it. But I wonder if you would just give us the high-level look at what the basic concept of the test is from the perspective of, how are the monocytes supposed to interact with red cells to give you answers?

Sandy: Sure. And the concept behind it would be that if I isolate monocytes from myself, from anyone, and it would be good to have a qualified stable of, I'll call them "volunteers," but they actually are employees, that we know how their monocyte activity is, to qualify them before use. Because of course, there's going to be people who might have not as reactive monocytes and maybe hyper-reactive monocytes. The controls becomes very important to make sure that you have consistent reactivity.

And what we do today is take our stable, and we'll draw two of them. And we wash up all the cells so that we remove all the ABO antibodies. And at

the end, we'll combine them, and that gives us one, a more stable mixture. It also helps us to have more volume, so we can do more tests.

So the concept really would be much like PCH. If you've heard of intravascular phagocytosis, we're looking for that monocyte on my tissue culture chamber slide to be slightly activated and to recognize that there is a sensitized red cell that it would like to eat, essentially.

So they migrate towards these red cells that ... We'll go through the assay, of course, but they actually can move towards these red cells. And then they either attach, so they're adherent, or they actually get to a stage for phagocytosis. Early researchers and some still using assays used CO2 to drive the monocyte to phagocytosis. But in fact, any adherent red cell means that there is a positive reaction, because the monocytes inherently will not ingest or attach to cells that are not coated.

So the first assays, the first reading of the assay, we were very particular about the stain that we used, so we could really see the cells inside the monocytes. And the first assay, of course, these were not COVID times, so everybody gathered around this one microscope, and we all were looking down the scope, tremendously excited that the assay quote, had worked.

Joe: I wish there were pictures that we could share of that scene.

Sandy: Oh my goodness. Well, if you've ever seen George Garratty do a happy dance, that was it.

Joe: Okay. That's fantastic. Sadly, I never got that privilege, but that would have been a sight, I'm sure.

Sandy: Oh, yes.

Joe: I think what I'm understanding from you is that if a red cell is coated with an antibody, then what we're looking for in the test is for an activated monocyte to basically either grab onto that red cell and have it stick to the outside, or actually ingest the monocyte to phagocytize it. Is that accurate?

Sandy: Absolutely. That is accurate.

Joe: Okay. And monocytes don't do that to uncoated red cells?

Sandy: There's a normal range that we have established over the years, and it was 0 to 3% of reactive monocytes in a counting base of 600 monocytes. And the reason we chose 600 was because if you count 200 monocytes and you have greater than 20% reactivity, your CV is 7%. So that's good. But when you start to have less active monocytes, reactive monocytes positive, then your CV goes crazy. So then we counted 600 cells to bring the CV to 7%. But you're right that there is some amount of adherent red

cells that could happen in a normal situation, unsensitized cells or sensitized cells in a patient who now then is determined to have a negative MMA.

So we used a 0 to 3% cutoff, but George Garratty's group, with Pat Arndt did some elegant studies looking at the actual laboratory values and the clinical impact of what I would call "gray zone reactivity, "so between 3 and 5%. How important is that? And what they found was that the patient may not have had normal cell survival, but certainly the cells weren't destroyed right away. So in a clinical situation, that information could be very useful in a patient who's having significant low hemoglobins and symptoms because of their anemia.

So we use a 0 to 3%, because we did not do those studies, but George Garratty's laboratory uses 0 to 5%. And if we have an assay that comes in at 4 or 5%, we will quote that paper so that the clinicians can make an informed decision.

Joe: Everyone, I will put the link to not only that paper that Sandy just mentioned, which I believe, if I'm quoting the right one, that Pat and George published in September 2004 "Transfusion." I will put the link to that on the show page for this episode, everyone. You can find that as well as multiple things that, Sandy, you've published on the MMA on the show page at BBGuy.org on the Blood Bank Guy website for this episode. So be sure to check out the references for this, everyone. There's lots of good stuff there.

Okay. So again, I just want to make sure that everybody's really clear on this. So you've got a test where the basic principle is, the more the monocytes in this test either grab onto, the more red cells are adherent to those monocytes or the more red cells are phagocytized, the higher percentage is suggestive, in vitro, that the antibody that's involved in coding those red cells might be clinically significant. Is that a fair way to put it?

Sandy: I'll just change one thing that you said-

Joe: Please do.

Sandy: ... in that we count the reactive monocytes, not the number of red cells that are either attached or phagocytized. So it is the percent reactive monocytes in our assay, being if you count 200 monocytes, what percentage of them are reactive essentially?

Joe: Got it. That's better. Sandy, given that, I would suggest, from my perspective, that the MMA is probably the best known in vitro test over the years. And certainly we'll talk a little bit later about some of the data that you guys have to try to support its use. But are there other alternatives? Are there other things that people have done with this same kind of

interaction with the monocytes to show in a lab setting whether or not an antibody might be significant?

Sandy: Absolutely. In the early days, I think people were clamoring, and still are clamoring, to be able to get something that will predict, well, the first thing would be whether a patient will form an antibody. That would be really helpful. But whether the antibody the patient forms is clinically significant. And so we worked with researchers. We actually published a paper with a group at the NHS in the UK, when they were looking at chemiluminescence, and their assay was actually better for predicting fetal maternal hemolytic disease. And ours was better at predicting the survival of transfused red cells. So it was very curious, interestingly enough.

And the other assay that has been used, I just thought it was going to be a fabulous approach, which it's called "ADCC, or antibody-dependent cell-mediated cytotoxicity. And I love that because it had a way that you could look at the adherent cells and then you could also quantitate the phagocytosis cells, because first you would burst the red cells and then you would burst the monocytes. So it just seemed perfect. But unfortunately, the data didn't really come down to being the best predictor for our purpose, for the prediction of transfused red cell survival in the incompatible realm of testing. So I just love the concept. So in the same time that we were developing the monocyte assay, there was the monocyte assay that we were doing, the antibody-dependent cell-mediated cytotoxicity, chemiluminescence and then the mononuclear phagocyte assay that was being developed by Branch and Gallagher.

Joe: I think it's fair to say that at this point anyway, the MMA, it's clearly the one that is most familiar to people. And I assume is the one... Is it your perspective that it's the one that's being done the most?

Sandy: That's a very good question because I think that there are other assays that are being used in other arenas, but for this purpose, I would say yes. And I think that the advantages for why this probably, "Won," or why it's used a lot is that one, it was our laboratory and then I moved to Philadelphia where I took over the American Rare Donor Program and we needed it. We needed it yesterday.

So the advantages to the MMA really focus on, it's technically demanding, but it doesn't require a lot of expensive or dangerous equipment. So we don't really need to have a Geiger counter. We don't need to have a gamma counter. We don't need to have any funny chemicals. We really can just do it with blood and the patient's sample. It's pretty amazing actually that we are able to do that. And then it is read microscopically, and there have been some studies looking at putting it through a flow cytometer. And that piece still holds some attraction for me since I'm also very interested and have done a lot of work on flow cytometers. But this seems to be the one that was the most predictive. So we stuck with that.

And the techs who are performing this assay, who might be listening to the podcast, will say, "This is definitely a technically difficult and demanding test," because you can't stop. When you're spinning of course, you could go do something. But once you've got rolling on the test, there's no stopping. You need to just keep moving and add the cells quickly because as soon as you take them out of the body, they start to die.

Joe: Before I get to talk about the specifics of how this is done and the complexity of it, I would be remiss if I didn't address something that I'm guessing that most people that have read about how to manage, well scenarios like you were mentioning with a high frequency, an antibody against a high frequency antigen, you'll read in textbooks people talk about doing transfusion and doing Chromium 51 survival studies, which frankly, Sandy, I've read about my whole career and I've never actually done. But I know people still talk about it. And other sources that talk about doing an, "In-vivo crossmatch." Obviously, those are different things than what we're talking about here with MMA. But do you have any thoughts on those? And have you experienced people doing in particular the straight Chromium 51 survival studies?

Sandy: Yes. And they were the comparative assays that we used to prove the monocyte test validities. And I have been involved when I was at John's Hopkins. We did do Chromium 51 survival studies. Because they had a laboratory that also used chromium to do blood volume studies, they were able to just adapt it to use incompatible red cells. It's a fascinating experience honestly, because you can tell immediately whether the cells are being destroyed. The interesting part for me, and we also had partners when I was in Los Angeles at numerous hospitals that were doing it at the time, the teaching hospitals, mostly. But I guess I would say that the attraction to me was it actually happened in the body. The non-attraction to me was we didn't transfuse a whole unit. We transfused just a small aliquot of cells. George's thought was that potentially, the antibodies would have much more opportunity to destroy those little bits of red cells than if you had transfused a whole unit. But nonetheless, it was the gold standard to be able to measure cell survival and used by Mollison in his publications and in his studies for years. So that was our gold standard.

The in-vivo crossmatch, interesting you say that. We just had a case where we didn't quite know how to assess whether a patient would reform an antibody. So there was a trial that was done using, it was a Parabombay patient that had a history of a very strongly reactive anti-H and anti-A. And the thought was what will happen if we transfuse a unit? We didn't know, right? And the patient didn't have the antibody at the time. So this, "in-vivo crossmatch," the concept was used to see whether the patient would reform the antibody.

And I know that in a pinch, in times when you can't do the studies and the patient is moribund, I had a great medical director who said, "Just

transfuse something red. We need to treat the patient and we'll do the studies if they need to be done as they can be done, but let's treat the patient." That has stuck with me forever. And when people say, "We'll wait for the monocyte assay, or we'll wait for this," no. You need to be really looking at the patient and figure out if the patient needs to have something.

Joe: You are speaking my language there, Sandy. As a medical director for an immunohematology reference lab here in Southern California, I can't tell you how many times I've had that conversation with people. It usually revolves around people with warm autoantibodies, for example, where someone's freaked out about transfusing them. And I say to my staff and I say to people all the time, "You cannot wait. If the patient is in need of transfusion, you transfuse and deal with it later, figure it out later." You can't let a patient suffer while you're trying to cross every T and dot every I with your workup. You just can't. I'm right there with you. Hallelujah.

Sandy: Yeah. It's so important for people to think that way. Sometimes, I cringe when we know that blood is usually available the minute you need it in a hospital setting. And when you say there's an antibody in the person, the physician or the physician's assistant doesn't quite understand that this is not going to be available right away. It's not understood that it's not going to be available for days.

Joe: All right, Sandy. So I think that we are in solid agreement on that. Let's leave that point and let's get back to our friendly MMA, our "mighty" MMA that we're talking about. I think that it's important for people to understand, as you mentioned, the complexity of doing this. So if someone says to you, "Hey, Sandy, I'd really like to implement the MMA in my own hospital transfusion service," or even in a local AABB-accredited immunohematology reference lab. What do you think about something like that? Is this something that everyone should be thinking about doing?

Sandy: Well we certainly have laboratories besides mine that are performing the MMA in their IRL, immunohematology reference lab. And I certainly think it's doable. It does take a dedicated staff. And I have this rule of three in that it's a three minimum staff members have to be trained in a unique assay because you have to have it available. So as long as you can dedicate that time to the training of those three staff, and it does take the best part of a day and a half to perform the assay and then read the slides. You have to be able to have that bandwidth. And I know today, it's very difficult to get staffing and to be able to guarantee that you could do the assay in a given timeframe could be difficult.

So if you have that all worked out and you have the interest and you can get the training. We offer, every year, we do some international training of people, mostly from laboratories that are involved with the ISBT working party on rare donors and have trained 11 countries in how to do the assay.

And many have implemented the assay. We interestingly enough just completed our assessment of the New Zealand implementation of the MMA. So I'd say it can be done. It definitely takes enough staffing and enough awareness of how much time it actually takes.

The other piece to that is I think it would be difficult if you wanted to have this, "Stable of volunteer MMA donors." So the donors of the monocytes, you want to have had them pre-qualified and you want to be able to utilize their monocytes for the assay. So I would say it's technically demanding and my techs would say, "You bet it's definitely demanding." But I think it's something that if there's an interest, I definitely think that it's useful. You won't get a lot of them in our laboratory, probably see the most, but it's really up to I think the directors to be able to source the monocyte, source the people who can be trained.

Joe: You've said that a couple times, Sandy, forgive me for pounding in something to everyone that you've already said. But I think a lot of people have the misconception that the MMA is done using the patient's own monocytes.

Sandy: We thought early on that that was going to be ideal as well. And we did about three years of study of trying to obtain the patient's monocytes. And it can work if you have the patient in your city and they could either be ferried over very quickly and within an hour of draw. But to have them shipped, we get samples from all over the country and sometimes internationally. And so for that to work would be impossible. So to those who use it, and have the patients in their hospitals and can do that, I think that's great. But we needed to have an assay where we could do it on all patients.

Joe: Makes total sense. All right, well the time has come. All you immunohematology nerds out there, the time has come for Sandy to give us some details on how this test is done. So Sandy, we don't have 20 minutes for you to go through it, but if you would just give us the high level, what are the steps that are taken just so people can get a clear mental picture of how this is done?

Sandy: So I won't take you through the six hours that it takes us to do what I'm about to say...

Joe: Good.

Sandy: So first, we want to have the fresh monocytes from the two donors. So we'll take heparinized samples from two people, probably two tubes, three tubes, depending on how many tests we need to run, very early in the morning. And we will separate the white cells and the mononuclear layer. So that will be the lymphocytes and the monocytes.

So in our prep, we will wash those cells, those mononuclear cells that has lymphocytes and monocytes three times. And we'll be washing them in a solution that is Hanks' buffered saline or RPMI to be able to keep the monocytes alive. When we are finished washing, we have washed away all the ABO antibodies, we're ready to combine them. And so we will combine them, make sure that they're very mixed up and there's no clumps. And then we will put them onto a tissue culture chamber slide. And it's eight wells and so we use this, it's still in production today, amazingly enough after 35 years, amazingly enough. But it was designed for tissue culture. So it's great because it has the wells that's covered. And what happens in the application of this mononuclear layer is that the monocytes will stick, and as I said before, become slightly activated on the glass slide. But the lymphocytes don't stick at all.

So after a 37 degree incubation, we'll take off the supernatant. And what we're left with is a monolayer of monocytes on the tissue culture chamber slide. And we've been incubating our red cells at the same time, no additive solutions, just red cells in a saline media and then in RPMI with the patient's antibody. So of course, we're going to want to have antigen positive, antigen negative autologous cells if we can with and without fresh complement for all antibodies except RH. And that would be so that we have provided a fresh source of complement. If there's any indication that the patient has a low complement level, we have corrected for it.

So once we remove the supernatant from our first incubation, we're going to put the sensitized cells on. Then we'll put them back into this dry air incubator for another 60 minutes. That's when you can go to lunch, you can do anything you want. But after that, you are back in the lab.

So now, we've incubated for 60 minutes. And what has happened during that time is that the red cells have by gravity just come down on top of the monocyte monolayer. And the monocytes have an opportunity then to interact or not interact as they want to, right? At the end, we'll take off this supernatant. And then we have this monolayer of monocytes with red cells on top, either ingested, attached or not, and then we will just rinse the slide and stain it. And that's the beauty of it. Once you have everything done and you rinse the slide, you can actually evaluate it immediately, but we usually want to put a cover slip on it and observe it under oil so we're able to preserve it.

Once the slide is made, then you would read it. And as I said, we would be reading the controls, we would be reading the patient, and then we would also have had a positive control that we would need to have reactivity within the range we've established. So, making sure that the monocytes actually were reactive and the assay "worked." So we have positive and negative controls, along with the patient antigen positive, antigen negative, with and without complement, and then we'll just test the cells alone. We

have never had a cell come up by itself, but I guess you would never know. So we want to have those controls tested as well.

And then, we're going to be reading under oil, as I said, and we'll count 200 monocytes. If the reactivity is greater than 20%, we'll quit, because our CV is at an appropriate range, and if it's less, we'll count more. And 600 monocytes is when it gets technically demanding, and not difficult, but demanding, is to count up to 600 cells and then do the calculations to see whether it's within the normal range of 0 to 3% or greater than 3%. So that's it in a nutshell, that's six hours.

Joe: Okay. So everyone, Sandy has some great, great pictures that she's agreed to let us share, to let us see, of this process. I'm going to put a couple pictures of this process and what this looks like on the show page for this episode. Again, go to BBGuy.org and find this episode and you will see some fabulous pictures of just exactly what Sandy is describing. So, that's awesome.

Sandy, you mentioned, and you've already covered this, but I just want to make sure it's clear that there is a slight difference in your cutoffs in your lab as opposed to George's lab. And I know that in things that you've published in the past, you make the statement and I'm paraphrasing that each lab that's doing this kind of needs to set up their own thresholds and cutoffs, but the 3% versus 5% thing, would you like to elaborate on that a little bit more?

Sandy: Sure, I can. So in the studies that we did in George's lab and in the assay that was set up in the National Reference Laboratory for Blood Group Serology, when it was in DC, before it moved to Philadelphia, did their own parallel studies for the cutoffs. They also validated it against different patient groups and antibodies. So, that piece becomes really important. And even more important if there's any modification that's done to the test, because, picture this, I mean, if you're enhancing the reactivity in any way or decreasing the reactivity in any way that would impact whether it then correlated with the clinical course of a patient receiving an incompatible blood transfusion. So those studies have to be done to "prove" that your assay does predict relevance. That part's pretty important.

And speaking to the cutoffs, the original cutoffs in George's lab in Delores Mallory's laboratory in DC were 0 to 3%. And those were arrived at independently. But then when George and Pat Arndt looked back at their work and were able to look at the laboratory and clinical evaluation of some of the patients that they studied, they found that the 5% cutoff led to an outcome where the patient survived, the red cells may or may not have survived normally like their 120 days, but there was clearly no clinical reaction and the laboratory values may have been off a bit, but they didn't result in a significant impact to the patient. And that's important, because if the patient really is very anemic and needing transfusion, the clinicians

could decide that this is worth taking this risk of a small decrease in the transfused red cells survival rather than not transfusing the patient. It's so important just, as we said before, to give something red. And if you can give something that's incompatible and follow it with something that's compatible, like we have done that in the past too, in the effort to try to get blood, maybe we had to import it internationally, the patient has had to get incompatible blood and then followed by a compatible blood transfusion.

Joe: So Sandy, you've talked about the 3% in your lab, 5% in George's lab threshold, if it's above that, if you see reactivity above that, that that's obviously highly indicative of the potential for clinical significance. Has anybody looked at a higher threshold, like a magic number that says, "Wow, if it's above THIS, then it is DEFINITELY going to be a problem"? Is there anything like that out there?

Sandy: I'm glad you mentioned that, because in the paper that George and Pat published, they were looking at, where should they draw the line on whether it was clinically relevant. And they did look at cutoffs that were beyond the 5%, and showed that at 20% there was definitely signs of a clinical reaction, not only laboratory, but clinical. These signs could be jaundice, fever, chills, that sort of really clinically-evident sort of reaction, but I'm not aware of anyone who has published studies similar to those. So, wouldn't it have been great if we could have used 20%, there could have been a whole lot more patients who we could have predicted and been happy with transfusing them incompatible blood, but didn't quite work out that way.

Joe: That actually brings me, Sandy, to something that I think is really important. I have come across people that when they have an MMA result in front of them, it feels like they're using it as like a get out of jail free card or a guarantee that, "My gosh, this number was 2.5%, so this patient is definitely going to be safe." So, I want to give you the opportunity to add some context from your perspective on how people should use these results.

Sandy: I don't think there's *any* guarantee, Joe, but I would say that it gives you a whole lot more background in a patient that might be clinically compromised to be able to go ahead and transfuse, and maybe a slight reaction would not be a good thing for the patient. So I think it has to be really taken with a clinical situation on the patient.

I also think that when you're talking about a patient who is going to be transfused again and again, and again, just to be conscious that that every time the patient is transfused, you would need to reevaluate the situation and do another MMA, because you could have changes. We've had several patients who have exhibited changes in their MMA and have come around to destroy the blood.

One of our patients was not very compliant with appearing at the transfusion date, and actually delayed two weeks in coming in. And in the early days, we would have thought that was okay, but the patient was chronically transfused. And the patient's antibody changed within that time, because we had done an MMA two days before the intended transfusion. And by the time the two weeks rolled around, the antibody had changed and completely destroyed the whole unit. So I am very aware that there could be changes in time.

And I also just think we are looking at a test and the test has to be interpreted by physicians who are near the patient and understand what the ramifications could be. We have used the MMA to encourage people to go ahead and transfuse incompatible or status unknown blood and have not had repercussions other than this one Lu^{b} case that I just talked about the patient.

Joe: You published in an abstract form a couple of years ago, I believe at the Boston AABB if I remember right, an update of what you guys have seen in your lab regarding the use of the MMA. I wonder if you would just take us through a little bit, over those years, what have you seen in terms of the most common high-incidence antibodies that you've evaluated?

Sandy: For sure, it's the ones who have been reported in literature to be variable. So, that would include Gerbich system antibodies, Yt^{a}, for sure is our highest number of evaluations. And I think that it's also driven by how many red cells are available on these cases. So we have evaluated ones that are quite rare, Lan, for example, because the blood is really difficult to find. And for us in importing blood from other countries, the FDA requires that we would have done work to prove that the blood is not available in the US. So that would include sibling testing, evaluating for autologous use, and performing the MMA. So we proved that we don't have any blood in the country that is suitable for this patient that's why we have to import under an IND.

So, we definitely have numbers. I mean, I think that we have done over 200 anti-Yt^{a}s. And it's pretty interesting that it's not half and half, but it certainly it's a big number, probably three-quarters of them are positive, but then there's this percentage that is negative, which is joyful because we can have the transfusion happen today, right? You don't have to get blood in from somewhere else or frozen units, and it just becomes incredible.

We had a patient, I don't know if you want to hear a story, Joe...

Joe: Sure, please.

Sandy: We had a patient, there were two of them, actually, at the same time that had anti-Vel and they overlapped, requiring over 150 units, and we

imported from abroad. We also used the MMA to say, "Could we use the Vel-positive blood?" And in fact, one of the patients, we could have, but the patient was slated for transplant, and the transplant was going to be a Vel-positive donor. In keeping with what the patient's treatment plan was, we didn't want to restimulate that Vel to a higher level and then have the transplant be impacted. So in that case, it was a negative MMA, but we did not transfuse Vel-pos. So there's different ways to approach the results that you get based on the patient's clinical situation.

Joe: That is so key, what you just said, Sandy. And I think that is something, unfortunately, that sometimes does get missed. It's not just a number and, "Whoo-hoo, we have a good number, we can plow ahead," it has to be put into context. I love the way you put that.

You mentioned something there that I want to expand on just a little bit before we talk about maybe a couple of real world style examples anyway. I know you play a very large role in the American Rare Donor Program, how does the MMA fit in with requests that you get for blood through the American Rare Donor Program?

Sandy: Well, I'll start maybe at the easiest part, which is we get the request, there's blood in the bank somewhere and it's shipped and no problem. That's the majority of what the American Rare Donor Program, the outcome is. However, there's probably 10% of the cases for which there is not blood available immediately. So in our situation, we will try to evaluate how soon we would have the blood, let the requesting institution know that, one, there's no active units in the system, two, we're recruiting, but it could take some time, do you want to import? Do we want to try the MMA? I mean, we use it as a tool in our fight against trying to put blood in the hands of the people who need it. So, I think it's an option. I think it's our tool to get to a place where the patient can get blood.

And if the patient is going to have surgery, then we'll, of course, evaluate autologous, we will see if there's siblings. If it's an emergent need, we may decide to do the MMA right away and also do simultaneous importing of blood. So it depends, again, I hate to go back to this, but it depends on what is the situation of the patient and how fast do they need the blood? And we can use the MMA. It's not a quick test as you know. So, we would need to have it shipped, it arrives one day, we test the next day, we get the result the next day. So, it is a three-day delay, and so at that same time we could be importing blood.

Joe: I think that gives us some really good context, Sandy. And I wonder if you would be willing to just give your thoughts on these scenarios that I'm going to fire at you.

Sandy: Absolutely. Just keep in mind that I would not be the physician in charge of the patient.

Joe: Completely understand, completely understand. Okay, so let's talk about a fairly straightforward example, but I think this is probably illustrative of a lot of the requests that you get for use of the MMA. So we have a 64-year-old female who was admitted for a total hip replacement. She has an antibody screen and her panel that are positive at AHG against all test red cells. They can't figure it out, they send it to the local reference lab and the local reference lab does their work as AABB accredited Immunohematology Reference Labs do, and they identify an anti-Yt^{a}. And you already mentioned, that Yt^{a} is the most frequent antibody that you guys evaluate on the MMA. So, the reference lab is saying, "By gosh, we need Yt^{a}-negative blood, and we're wanting to see what you in your lab would tell us about that."

So, you can take this from either the perspective of your lab receiving an MMA in this situation or them directly requesting Yt^{a}-negative blood from the ARDP without doing an MMA. How would you approach a scenario like that?

Sandy: Well, from the perspective of the ARDP, so the American Rare Donor Program, we would put this through as a search for Yt^{a}-negative blood. And, we would fill the request if the blood was available straight out. If there was not blood available, then we have to evaluate, can it can be recruited? Now Yt^{a} is generally available, but I can tell you that ABO plays a role, additional alloantibodies play a role. So, in your scenario, if it's a straightforward single Yt^{a}, and it's not an O negative patient, then I think we can probably provide the blood without even thinking about an MMA.

But, if it was an O negative with anti-, list whatever you want, Jk^{b}, Fy^{a}, whatever and the blood is not immediately available, then we should evaluate whether the Yt^{a} is clinically relevant. For that we would recommend an MMA.

Joe: Some of our listeners may not be super familiar with Yt^{a}. I think it's fair to summarize it as saying that it's... You said this before, it's variable. The clinical significance of anti-Yt^{a} can be quite variable. So, given that, it's interesting to me that you don't choose to use MMA more as a "gatekeeper" with the ARDP.

Sandy: Yeah. I think that it would always depend on how we can obtain the blood. There's a recent publication in Transfusion that suggests that you should just go ahead and transfuse unknown status blood. And, then if the patient has a reaction, think about Yt^{a}-negative or MMA. So, I think that because we've been so able to fill the orders, this is an infrequent occurrence. But as I said, when there's complications, that's when we would probably need to evaluate. And, I think it's reasonable to evaluate the patient's antibody by MMA, in advance of trying to import blood from another source, like international source. I mean, that makes sense to me.

Joe: Fair enough. Let's do a second case and I'm going to base it on that case actually. So, that 64-year-old female got her total hip replacement. She got an MMA. It was below the threshold, below the cutoff. So she received a couple of units of Yt^a untested, we presume it's probably Yt^a positive. She got a couple of units of red cells during her surgery. No problem, no complications, no issues, but she's back. It's three months later, she still has the anti-Yt^a. It appears she has something else. The immunohematology reference lab works it up, identifies the antibody. And, the question at that point becomes, "Well, wait a minute. So we've got an MMA from three months ago that was totally fine. So we don't have to worry about it. We don't have to send another MMA to evaluate that Yt^a." Someone says that to you, Sandy, how do you respond to that?

Sandy: This is such an important question. I'm so glad you ask it, because it's so important. And, it's not something that is logical. The patient is a responder of course, because they have an anti-Yt^a. We have that it's clinically irrelevant, but they formed another antibody and we don't know what happened to whether the anti-Yt^a changed its status, its reactivity. And, we have a couple examples in the literature where such a thing has happened. And we have certainly numerous examples that are unpublished. But, the MMA has to be performed again as close to the time of the next transfusion as possible, because it could have changed its reactivity characteristics, and now it could be clinically relevant. Just like I said, with that Lutheran case, never say never. So, you need to really repeat the MMA. And, I think that becomes okay in this case, because it's a hip, it's not a chronic transfusion case. The chronic transfusion cases, I think become more difficult because that means that you'd have to do it each time. And, sometimes that's required if we can't get the blood.

Joe: I wonder if you would comment on this question: If the MMA is below the threshold and it suggests you can transfuse without an abundant risk anyway of hemolysis, then obviously what you just said, repeating as close as possible to the next transfusion is important, especially if the person is exposed. But, what if it's positive, Sandy? Is it a "once positive, always positive" thing for the MMA?

Sandy: Oh, I love that you asked this question, because in the olden days I would have said, "once positive, always positive." But we've actually had an example of a case where we had to rethink that. And that was a bone marrow transplant where the antibody characteristics changed completely. It's a different immune system. And so once positive, always positive doesn't count there. So, the patient's antibody was persistent, so it stayed for a period of time. And the question was, the patient had an anti-E and (it wasn't an exotic antibody), but -E and -e, so was the --e still clinically relevant because they got a e-positive transplant. They had a positive DAT. Did we need to continue to give e-negative blood and E-negative blood?

So, that question could be answered by the MMA. So that, so no, once positive, always positive, let's look at the clinical picture and see what happened with the patient.

Joe: But, short of having something like that example, generally speaking, it would be a once positive always positive?

Sandy: It is. And, the other scenario that we have seen is that we'll see a patient's antibody disappear. It's not serologically detected. So, what do you do then? If the patient went into another hospital, nobody would know, but we know. And, so we could prevent a delayed hemolytic transfusion reaction. So, I would say, yes once positive, always positive.

Joe: My little nerd antenna are just going crazy. I'm so happy right now! This is beautiful. Okay, so a few more cases. We're starting to run a little short on time so I'll do this quickly. This is an example of something that I have definitely seen the MMA used for, but I wanted to get your take on it.

So, we've got a fairly young man, 27 year old guy, has a motorcycle accident. He has some sort of a transfusion history from his wild youth, but when they go to transfuse him, all red cells are incompatible. He's got "something" in the background that is broadly reactive and they send it to the local immunohematology reference lab. They can't figure it out either. This guy needs to get transfused. And granted, I know there are timing issues here, but I guess the question is, is there a role in the "cry for help" scenario? When you can't identify something that's high frequency, can the MMA play a role in determining whether or not a patient can be safely transfused?

Sandy: We have definitely seen this scenario and we have definitely used the exact red cells that could be used for the transfusion in the MMA to see whether those red cells could be transfused. And, we have done that. Yes. So, that's definitely true.

The other piece to this, is it's a different scenario, but in our situation where we're looking for matching red cells at the DNA level, we have different tiers of that matching, much like HLA. So, we either have the complete match. We have a partial match or we have no match, but it's close to what the person's alleles look like. And, it's really important, we have used the test often in looking at a tier three match, which is not really a match to any of the alleles the patient carries, but it looks more closely like it than say a normal wild type.

And, we have used that to differentiate whether we can transfuse a certain allele tier. So, looking at a tier two, which is a match of at least one of the patient's alleles, tier three, close match, not really a match. And, we've been able to help in that area that mostly it's valuable to think about when

a patient has a novel allele and you are not going to find a match in the population.

That's been really interesting for us to evaluate how we can use the MMA to do that. In that case, we can either use cells that are clearly molecularly characterized, or we can use the actual units that are going to potentially be used for transfusion.

Joe: I have three other examples and I'm not going to give a whole case scenario just in the interest of time, Sandy. But, I think that these are important and you've kind of covered this before, but I want to make sure we comment on them. The first example would be in a scenario where you receive a sample from someone who's clearly looking to tell whether or not an antibody, let's just pick an antibody that may cause HDFN, like anti-M. OBs are, as you know, are very familiar with the fact that a few anti-Ms can cause hemolytic disease of the fetus and newborn. What if you get a sample in your lab for MMA to evaluate for the possibility of HDFN?

Sandy: Yeah. We're not going to do it. Because, the MMA has not been validated to be used as predictor for the baby's outcome in cases of HDFN, potential HDFN. But, we could use it for the mom and we have had cases where they do need... It's a high prevalence antigen; not M, but, it is one that is of unknown clinical significance. So, not for HDFN for the baby, but for the mom, yes.

Joe: Okay. Got it. Okay. What about the scenario, the next case, where we've got someone who has a very strong warm auto antibody that doesn't appear to be clinically causing hemolysis at the moment, but the heme-onc is very concerned that this autoantibody might start causing hemolysis in his patient. How do you react when you receive a sample to evaluate for the possibility of hemolysis in an autoantibody?

Sandy: It sounds to me like you want me to do it "least incompatible" by MMA?

Joe: Oh God.

Sandy: We don't do that.

Joe: You said the phrase! You said the phrase! I'm breaking out in hives.

Sandy: The phrase that shall not be said.

Joe: Yeah, I'm breaking out in hives.

Sandy: Exactly. So, no, we wouldn't be able to use it for the prediction of the clinical relevance of transfused cells. But, there have been authors who have used the MMA to evaluate the reticuloendothelial system of the patient. So, I think that the MMA could have an application in that area, but not for the prediction of transfused red cell survival.

Joe: One last one. And again, just quickly. Someone who has an antibody of whatever type that appears to be IgM in nature. Say it reacts only at immediate spin and I don't know, DTT treatment takes away the activity. So, it's pretty obviously an IgM type. Does the MMA have any role in IgM antibodies?

Sandy: This is another excellent point to make and I'm glad you asked the question in that the Monocyte essay, as we have developed it, cannot be used to assess IgM antibodies. So no, especially important if there's a mixture of both IgM and IgG, we wouldn't want to predict based on what we get in the MMA.

Joe: Okay. Okay. That makes total sense. All right. Well, Sandy, we've covered a lot of ground in an hour and this is really just a fascinating story to me. And I think that we've, I hope anyway, that we've left our listeners in a much better place to understand this fabulous test that you played such a huge role in developing.

We can call you "the developer of the MMA" for this particular use. And, that's really exciting to me to be able to talk to you about it. So thank you so much for taking the time.

Sandy: It has been my pleasure to talk to you today, Joe.

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Joe: Hey everybody, it's Joe. As I mentioned, you can go to BBGuy.org/093 for images and links to some of the articles that Sandy and I mentioned in this interview. 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 did want to re-emphasize one point Sandy made repeatedly in this interview. The American Red Cross National Laboratory uses 3% as their cutoff for a “positive MMA,” as Sandy explained. Other labs, and I’ve seen this frequently, use 5%. Don’t get freaked out about that. You should look at the report you receive from the lab that does your MMA, and just understand their reference range and why they chose it. No big deal.

As always, I hope you will consider going to Apple Podcasts to give this podcast a rating and subscribe. Since the last episode, someone with the screen name “Uyyenie” did just that, and said, “Love this podcast! I’m learning tons from this, also their website is helpful too when it comes to basic learning.” Thanks, Uyyenie, for the kind review (and by the way, there is no “their website” at this point! It’s just *me*, and I appreciate the kind words). Reviews help others discover the podcast, so I’d really appreciate any of you giving one, and you could hear your review read on the next episode!

My next episode is another interview I did a while ago, with Dr. Sujit Sheth from Cornell. Sujit is a hematologist who is an expert on thalassemia, and that's something that people in our world need to understand FAR better than we do! I can't wait to share that interview with you soon.

But until that time, 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. I'll catch you next time on the Blood Bank Guy Essentials Podcast.