Tony Casina: Welcome to Ortho Science BYTES. Ortho is proud to sponsor this podcast as a continuing commitment to advanced patient care from donations to patient transfusions. I am Tony Casina and today I am joined by Shane Grimsley. Shane holds a diplomat in pathology from the Royal College of Pathologists in the UK and has been working at the International Blood Group Reference Laboratory, NH Blood and Transplant since 2009, as a laboratory manager from 2015, and senior clinical scientists since 2020. Since 2017, Shane has been the lead scientific advisory to the UK NEQAS Red Cell Genotyping Scientific Advisory Group. He has been an active member of the editorial board for immunohematology as well as a reviewer for sanguineous and transfusion medicine since 2019. Shane is the winner of the 2020 Race and Sanger Award from the British Blood Transfusion Society for Outstanding Contribution to the field of transfusion. Primarily for his work, developing a genotyping platform for accurate prediction of variant phenotypes in patients with sickle cell disorder.

He also is a winner of the 2010 Margaret Ken Wright, Young Scientists of the Year from the British Blood Transfusion Society for his work resolving complex compound heterozygous allele combinations and the identifying of associated antibodies specificities. Shane is an international scientific speaker and a workshop instructor for immuno hematology topics in conference programs such as ISBT. He also has been involved in the identification of new blood group systems, new antigens and novel ills. Shane is helping lead a team of skilled and passionate scientists to resolve the world's most complex immuno hematology cases, contributing to groundbreaking projects that have improved the standards of care for patients. Thank you so much for joining us today, Shane.

Shane Grimsley: Tony, thank you very much for having me. I'm really happy to be here. I'm looking forward to it.

Tony Casina: Okay, let's get started with the first question. What are the key serological clues that indicates you might be working with an antibody to a high prevalence antigen? Are there any watch outs that might mislead you?

Shane Grimsley: So I mean the first clue that you will get when investigating an antibody to a high frequency antigen or an HFA is that all of your panel cells are going to be positive. And that is by far and away the biggest single clue. Beyond that though, there are a few really important factors that I would like people to consider and it is probably obvious to a lot of the listeners. One of the most important things that we want to look at as well as all the panel cells tested, is the autologous control. That's really, really important because if you are dealing with a genuine alloantibody to a high frequency antigen, then of course your autologous control needs to be negative. Another thing that we also advocate, including at this point is the cell of the same ABO group because if the patients group A or B or AB, they could actually just have made an anti HI, which I guess could be defined as an antigen, as a high frequency antigen, but of course it isn't truly because it's very, very weak on group O cells.

So we always advocate running a cell of the same ABO group as your patient and that can really, really help. We do still get some referrals through to our lab that are actually just an anti HI and that the patients are group A and all panel cells are incompatible, but as soon as you put in group A cells you get negative reactions. So those are two things that I would say would be well worth considering.

The other thing that is maybe just worth touching on here is the strength of the reactions because a high frequency antigen can have different strength antigen expression on different cells. So CR 1 or your NOPs, that's maybe a reasonable example of something that does that change in strength of reactions. So I would ask that people consider that because it could be an antibody to a high frequency antigen with variable strength and different cells, or it could in fact be a combination of antibodies that are then reacting at different strengths to different panel cells. So those are the key things that I think people need to look out for and those are the potential red herrings.

Tony Casina: Great, thank you. Now moving forward, what role does ethnicity of the patient play in helping to resolve the identity of the antibody that could be present?

Shane Grimsley: Ethnicity's really important. It's a really significant indicator when you're talking about red cell blood groups and it's something that maybe some people don't fully appreciate, but some antigen negative phenotypes are only found in certain ethnicities. So I think a good example might be anti-Holley within black patients or maybe patients from the Middle East, you might have a gerbich 2 or a YTA, that sort of thing. The other thing to remember I think, not just about high frequency antigens and ethnicity, is also the prevalence of common blood group antigens in different ethnicities. So for example, in Japan, if you needed DIB negative blood, we would almost certainly look in the first instance to Japan, but nearly every donor in Japan is going to be D positive. So if you need a DIB neg, D neg that can get incredibly difficult. So the combination of not just the high but also common blood group phenotypes can be quite critical.

Tony Casina: Thank you for the answer Shane. Now what is generally your first step of identifying an antibody to a high prevalence antigen in your reference laboratory?

Shane Grimsley: Yeah, cheers, Tony. Good question. The most important or the very first step that we would always take would be going through all of the work that the referring lab or referring laboratories have done. It really is a team effort at this point. All of the tests that the lab have done under what conditions and all of the information that they can provide are absolutely critical to helping us know and understand what we are going to do next.

The first piece of wet work in our lab would be to tailor a panel. So the vast majorities of laboratories, when they go about setting up a panel, they'll just take all 10 cells from their panel and they'll test. We don't do it like that. We very purposefully select cells or specific phenotypes and cells based on the

information that we've been given by the referring laboratory. So maybe to give you some context on that, if we had a referral from a man that was papain sensitive, apparent antibody to a high with similar strength reactions in our first panel, we might include to include a gerbich 2 negative cell or something like that. And we are blessed that we have those cells available to us equally. Looking through the information that the referring lab have provided, it can sometimes unlock, it give us really good clues. So we have on occasion had referrals where the common phenotype of the patient is big S neg, little S neg and that gives us a really good clue that the antibody might be anti-U.

Tony Casina: Very interesting. And how would your approach on this be if you were in a laboratory that did not have as many resources as a reference laboratory?

Shane Grimsley: Yeah, it's a good question and a question we're asked quite regularly. And I do try to remind myself and other people in the team that we really are blessed in the resources that we've got, but there is actually quite a lot that you can do without those resources. So even with a standard panel of cells, you can use those antibody antigen characteristics. Again, we are maybe blessed or we certainly are blessed because we are exposed to these antibodies really quite regularly, so we feel like we get to know them. Whereas for an awful lot of other labs, it genuinely is a rarity to encounter them. So knowing and understanding those characteristics can be quite difficult, but it certainly is one of the first steps that we engage in. In the absence of rare cells or in the absence of that experience, if you are confident that an antibody to a high frequency antigen is present and you do those things, I said all cells are reactive, including a cell O, the same ABO group and your auto controls neg. A really important step is to do a full phenotype or genotype.

Again, I sort of mentioned in the previous answer if you get a result like Big S neg, little S neg or Duffy neg or B neg, that can be a big clue as to what your antibody specificity might be. So you can use your common blood group phenotypes to help inform next steps or decisions with regard to blood to provide. One thing we would also advocate would be to do an absorption with a cell that is matched for the common blood group phenotypes of your patient. And the reason we would advocate doing that is what you can do is you can pull out the antibody to the high frequency antigen and then using that absorbed plasma you can test to see if there are any antibodies to underlying common blood group phenotypes. And this can be really, really useful because if then you are pushed into an urgent situation where blood is required, at least you know what if any underlying antibodies are present and you can avoid those antigens.

If you don't have cells of the same red cell phenotype as your patient, then a method that is practiced certainly in the UK is to use differential absorption. That's where you have a pair of cells. One might be JKA pos B neg, and the other would be JKA neg B pos. By absorbing with those cells, you can then test both sets, of its own plasma and arrive at the same conclusion. Identifying or excluding underlying antibodies. And this is really actually really helpful then

when you make that referral to a tertiary reference lab like ours because we will then know if any antibodies are underlying and what those specificities are, which will really speed up our investigation as well. Of course that in and of itself won't resolve the specificity of the antibody to the high, but it can be really useful as I say, if you then need to transfuse that patient in extremists.

Tony Casina: Thank you. Can you also please describe what other serological techniques would you use from a stepwise approach to evaluating a sample?

Shane Grimsley: Yeah, absolutely. There's loads of different techniques that we can employ. So one of the ones that we would turn to reasonably early in our investigation is what we call enzymes or enzyme studies. So what we would do here is we again, we would take a cell with the same common blood group phenotype as the patient and we would treat an aliquot of that red cell with a particular enzyme. And then a second aliquot with a different enzyme. So in much the same way in your routine panel testing, it's very normal to have untreated cells and papain treated cells. And by doing that, that can help you tease out what antibody specificity are present.

We use all sorts of different end zones. So we do use papain in our initial panels, but when we are doing enzyme studies, we use papain trypsin, climate trypsin, we'll also use chemicals so AET as a reducing agent. And these enzymes in chemicals, they have a different effect on different antigens. And what you can then do is you can look at your pattern of positive and negative results that you get with your different enzyme and chemical treated cells and that can really help narrow down the search for what blood group system or for potentially which specific antigen you are looking for. And that can be a really, really useful, useful technique.

The other thing we would turn to quite easily, which again would require an antigen match cell would be to make eluate. So when we do this, what we are doing is we are isolating that antibody to a high frequency antigen. So if you imagine you a patients plasma, which contains at least one antibody, but it could contain many more antibodies and it could also depending on the ABO group, contain anti A, anti B and or anti A, B so.

So by making the eluate from a group O cell that is antigen matched, that eluate then should hopfully contain only the antibody to the high frequency antigen. Because you'll leave any anti A and anti B behind because that can't be absorbed onto that cell. And you'll leave a potentially underlying anti JKA as an example also behind because that can't be absorbed onto your phenotype match cell. So isolating the antibody to a high frequency antigen to via an eluate is something that we do really quite readily and it can then help us match cells because in the absence of doing that, you don't know what if any other antibodies are present.

And when we do test that eluate, again, we are very blessed. So we have various indicator cells that we would include. So LUA neg, B neg is a good

example of an indicator cell because different antigens and different blood group systems are suppressed in that phenotype, not just Lutheran. And so that then can indicate that the antibody specificity, if it's weaker on [inaudible 00:15:42], it might be one of those specificities and we can go away and we can investigate that more earnestly. And other indicator cells like cord cells, RHnull cells, they're also available and really, really powerful. So yeah, lots of different techniques that we can turn to serologically, but that can all be used to help [inaudible 00:16:02] what the different specificities are present in the patient's plasma.

Tony Casina: And following that question, are there other more recent techniques, reagents or methods that you have found useful for these cases?

Shane Grimsley: Yeah, it's something now in the fullness of time that I'm trying to get myself exposed to increasingly really is the clinical care of these patients and what we can truly do for them. I think the biggest piece of advice I would give for people working in blood banks is the blood transfusion is really complicated and you are an expert in what you are doing. So your opinion and your input as to how to help care for this patient, I think really will be valued and listened to.

So of course you probably won't be that patient's doctor and that provision of blood will be the responsibility of someone else. But a doctor, having someone in their corner that knows and understands transfusion and can offer them expert advice, really is helpful to that medic. So don't be shy, I would suggest in discussing these cases with your medics that may be in charge or may be affiliated with your blood banks and learn from one another because you'll probably have lots of theoretical knowledge about some of the nuances of different blood group systems. But they'll have lots of clinical and practical knowledge that you two can learn from and can bounce off one another. So yeah, I think without getting too specific, it would be to be confident in what and to use that information to help that clinician in the provision of blood.

Tony Casina: Thank you. Shane, working with the hematologist or physicians is key in treating patients exhibiting these cases. Do you have any recommendations on that area to guide the treatment or further follow ups?

Shane Grimsley: Yeah, there's two things I think I'd like to share on that one. I think I'll go with at firstly, I'll say that there are lots of different approaches to resolve in these cases. That's partly why I'm so passionate about what we do is that there are loads of different ways of unpick these puzzles. It's almost a logic problem and there are different keys that can help unpick different locks and sometimes you could open the same lock with different keys, you can use different tools to arrive at the same answer. And I really like that. So it's just a case of knowing and understanding the tools that are available, knowing and understanding their limitations, but also their strengths and applying them in a logical and coherent manner. And what I really like, we can sit down and have conversations like this and three different people might come up with three

slightly different approaches based on the same problem put in front of them and all of them will have merit.

And I really enjoy that and I really enjoy in our lab every morning we get together, we have what we call a huddle where we sit around and people discuss their cases, discuss the results they got the day before, and they talk about what their next steps might be. And it's really interesting to see how other staff members come in and say, "Oh, I had something like this and I did that", and it unpicked it really quick.. And I really, really enjoy that aspect of it. So no one understands the tools, I think it's my first piece of advice, and apply them in as logical a manner as you can. And you will unpick a lot of these problems. And my last piece of advice would be to enjoy it. It can be infuriating. I think also, particularly if you then need to send that sample off to a reference lab. But rest assured that all of the work that you've done preceding is an important part of the puzzle and it is an important part of getting to that answer. So enjoy the process, do what you can to try and resolve it.

Tony Casina: And to close this fascinating conversation for all of our listeners, can you share any words of wisdom about antibodies to high prevalence antigens? Any advice?

Shane Grimsley: Yeah. So one of the recent-ish developments, which we've certainly found useful of the soluble recombinant blood group proteins. And part of their power is that they often express a protein that is the entire blood group system. So when I talk about this, I often use anti YTA as an example, and the soluble blood group protein that was available was sort of marketed as inhibiting anti YTA, which of course it does. But we were able to pinpoint specificities to other YT related antigens because of this soluble recombinant protein.

So we actually identified YT 3, YT 4 and YT 5 because we had antibodies that we could show were not anti YTA, but that were inhibited by the YT blood group protein. So it was a high frequency antigen to a different epitope within that protein, and we found that a really, really powerful tool. So yeah, the soluble recombinant proteins have been a very welcome addition to our toolbox. And of course genotyping, genotyping is coming on and it offers a really useful, not even add-on, but a testing that can be done in parallel with the serology. And of course there are strengths and there are limitations with phenotype in versus genotyping, and that debate will rage on probably forever. But from my perspective, it's a really nice tool to use alongside and actually the serology and the genotype and they do compliment one another quite nicely.

Tony Casina: I really want to thank you for taking the time with us today, Shane, and giving us your experiences and insights on this fascinating topic. It's been a pleasure to talk with you and again, thank you so much for your time today on this podcast.

Shane Grimsley: Thank you, Tony. Thank you for the invite. Absolutely enjoyed it. Thank you very much and I hope your listeners have enjoyed it too.

Tony Casina: I hope you all enjoyed this podcast episode about resolving antibodies to high prevalence antigens. There are complexities and tools to identify them. Make sure to review the sessions within the podcast description for any reading materials that we've suggested. Based on today's podcast, I'll leave you with our pop quiz. What are some examples of rare blood types? You can go back and listen again. Thank you for listening. Please subscribe to Ortho Science BYTES, our monthly podcast where there will be discussions on more complex questions we face every day in our labs. Brought to you by Ortho Clinical Diagnostics, Pioneering advances and Diagnostics for 80 years because every test is a life. Take care, stay healthy and safe.