Quest Diagnostics

Quest Diagnostics
At
Shady Grove Medical Center and
Washington Adventist Hospital

BLOOD BANK STAFF MEETING

MINUTES

(04/16/2015)

PRESENT: 4.16.2015 @ 0640-0700 (SGMC) STEPHANIE CODINA, HOJAT GOUDARZI, YVONNE NGWA, ANNE RIENKS, NAMRATA SHRESTHA
4.16.2015 @ 1550-1615 (SGMC) STEPHANIE CODINA, SARAH DELINGER, MARIA MORRIS
4.17.2015 @ 0640-0700 (WAH) STEPHANIE CODINA, TSEGAYE NEGASH, VANESSA ROBINSON, SHAKIMAH RODNEY
4.17.2015 @ 1505-1515 (WAH) STEPHANIE CODINA, HABIBA LAKO

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MEETING COMMENCED

Item	Discussion	Action	Follow-up
Minutes			
Tubing Blood	We are officially sending blood products via pneumatic tube at SGMC. That said, we will not tube until the units have the new 2-part form. The units ordered the form, but there was a problem with the way it was printed. They have reordered the form and will begin using it as soon as the replacement arrives. Nursing is very concerned about this process, so we are going to have to ensure them that it is safe. We may also have to push them to use the new process, because it will save time for both nursing and BB staff. Nursing is also concerned that we will not call when we tube blood. They have been told that they need to watch the tube system after they send the request form. It takes approximately 10 minutes to tube the form, issue the blood, and tube the blood. We will call if there is a delay for any reason. We CAN send tubing via pneumatic tube, but we may need to send the tubing in a different carrier than the blood. We WILL NOT send blood products to NICU at this time. We will validate the small products, but we will never send syringes.	None	None
OIC Form	We have officially changed the policy where the T&S is good for the entire hospitalization (inpatients) or 1 year (outpatients) for NON-RBC transfusion. The OIC form has been approved and printed. They will begin the new process as soon as their form arrives.	None	None

Item	Discussion	Action	Follow-up
New Labels	The printers that will print the patient-unit label (instead of the pink form) have arrived and have been programmed. We started the validation and learned that the unit ABO/Rh is mapped to both the patient and unit blood types. Coridian is reprogramming the printers to fix this error. We will resume the validation when remedied.	None	None
Resuspend Cells on Echo	Reminder—you must completely resuspend red cell reagents before putting them on the Echo!! The stirball will keep them resuspended, but it won't resuspend the cells. This is causing testing issues on the Echo.	None	None
CMV Testing	Reminder—we require CMV Ab testing every 2 years on patients who require CMV-seronegative blood products. Please verify the date of testing and order as needed when you see the CMV marker.	None	None
Shift Planner	Please don't forget to log out of shift planner. Please ensure YOU are logged in when requesting vacation. I am getting a lot of feedback from staff that I am approving vacation they did not request. This is creating problems. People who don't want the time off are getting approvals and the people who really want the time off are getting denied, because the other person is already approved for the time off. Also, if you turned off your notifications in shift planner, YOU are responsible for logging in 2-3 times per week to verify your schedule.	None	None
AbID	Scenario: Screen shows "?" results for all E homozygous cells. Panel shows "?" results for all E homozygous cells but one. Testing tech called the Ab "Cannot rule out anti-E." Results were entered as "ASAR." In this situation, you can call the anti-E or say "cannot rule out anti-E." However, in either case, you should give E-negative units until E is ruled out. ASAR was probably not the correct ID to enter in the LIS.	None	None
TRRC Orders	Last meeting, an issue was addressed regarding red cell exchanges at WAH. We (BB) is not receiving the transfuse orders in advance which is delaying the procedures. The front desk is getting the orders. In the near future (sometime in May), they will begin entering transfuse orders for us. They will ONLY enter the product type and number requested. Then, they will give us a copy of the order. We will have to go in and enter the indications, etc. The hospital policy states all blood product orders will get put on the transfusion orders form. We will need to call the doctor's office if we get them on a prescription or in another format and make them fill out the form. The form has the information we need. We will also have to cancel transfuse orders that would not apply such as "cross 4 units for surgery."	None	None
2U XM with Abs	Question: You have a patient with a history of anti-Jka whose screen is currently negative. Do you need to set up 2 units? Answer: YES. We set up 2 units anytime we have a patient with clinically-significant antibodies-----current or historical. Question: Your patient has an ASAR antibody. All clinically-significant antibodies have been ruled out. Do you need to set up 2 units? Answer—No. ASAR is not clinically-significant. Question: Do you need to set up 2 units on a patient who is demonstrating ADRH? Answer: NO. ADRH is not clinically-significant.	None	None

Item	Discussion	Action	Follow-up
Anti-U	WAH currently has a patient with anti-U. Reminder that anti-U is a clinically-significant antibody to a high-frequency antigen. Anti-U reacts with S, s, and U positive cells. We will need to obtain blood products from the rare donor program for this patient.	None	None
Open Forum	Habiba asked the following question: I was going over the primary transfusion reaction investigation procedure. The procedure says to immediately notify the pathologist on call when a septic transfusion reaction is suspected. Do we have to do this every time we see one of the symptoms or is it a combination of symptoms? Answer: You do not have to notify with one of the symptoms, but I would notify if the patient has 2 of the symptoms. A patient can have a fever without other symptoms and it is most likely a febrile reaction. Other symptoms with or without a fever should be called to the pathologist……rigors, vomiting, etc. with a fever should definitely be called. We call the pathologist to get blood cultures on BOTH the patient and the product as part of the investigation. Also, if gram stain is positive or a septic reaction is really suspected, the patient should be placed on antibiotics as soon as possible.	None	None
Meeting adjourned			
Next meeting week of May 4, 2015			

Stephanie Codina
Recorder

Step	Action
7	Ensure that the identification of each tech assisting with the antibody identification is listed on the antigram sheets.
8	Have a second technologist review results **prior** to resulting the antibody in the computer. A. Ask another tech working the same shift to review the antibody workup. If you are working independently **and** the patient **does not** require transfusion, hold the review until change of shift when the next tech comes in. B. If you are working independently **and** the patient **does** require transfusion, fax the workup to another qualified tech at the sister hospital (SGAH or WAH) and ask the tech working in blood bank to review the workup for you. He/she must sign and return the Antibody Identification Form to you.
9	Perform additional testing if indicated. A. If the antibody identified is clinically significant and the patient is pregnant, contact the physician to see if an antibody titer should be performed. This does not apply if patient is being tested at the time of delivery. B. Crossmatch blood products on any patient who has a clinically-significant antibody. a. Crossmatch a minimum of 2 units on each patient with a clinically-significant antibody. b. If the physician ordered more than 2 units to be crossmatched, crossmatch the number of units written in the physician order.

Current Q2 Historical Abs

8.5 Antibody Review (Second Tech Review)

↳ ASAR & ADRH are NOT Clinically Signif.

Step	Action
1	Ensure that all antibody forms contain the following information: A. Patient name B. Patient medical record number C. Testing date D. Identification of each tech involved in the testing process
2	Ensure that the primary panel used is in-date (has not exceeded its expiration date).
3	If the screen and/or panel was tested on the Galileo Echo, verify that the lot number of strips listed on the Echo printout matches the lot number of strips listed on the antigram.

Form revised 10.31.12

One murine monoclonal antibody bound to ^{53}Pro-Pro-Glu-Glu-Glu^{57} of GPA (anti-En^{a}FS), but also reacted with ^{395}Pro-Pro-Glu-Gln^{398} of the cytoskeletal component, protein 4.1 [161]. Monoclonal antibodies directed at different epitopes on GPA have proved extremely valuable in the analysis of the many rare MNS variants described in this chapter.

3.5.4 Pr and Sa antigens and antibodies

The protease-labile Pr antigens [162] were originally named Sp_1 by Marsh and Jenkins [163] and HD by Roelcke [164] (Chapter 25). They are generally detected by cold-active IgM human monoclonal autoantibodies in cold haemagglutinin disease or post-infection [165]. Pr antigens have been subdivided into a number of subspecificities, Pr_1, Pr_2, and Pr_3, distinguished by chemical modification of sialic acid residues with periodate oxidation and carbodiimide treatment (reviewed in [162]). Anti-Sa cold agglutinins are similar to anti-Pr in detecting a sialic acid-dependent antigen, but anti-Sa react, albeit only weakly, with papain-treated cells [166].

Anti-Pr_1, -Pr_2, -Pr_3, and -Sa react with *O*-linked oligosaccharides on sialoglycoproteins [17,167–169]. Most anti-Pr and all anti-Sa recognise immunodominant α2,3-*N*-neuraminic acid groups linked to Gal, but a minority of anti-Pr may recognise α2,6-*N*-neuraminic acid groups [170]. It is probable that anti-Pr_{1-3} detect the predominant form of *O*-glycan, the disialotetrasaccharide shown in Figure 3.2, and that anti-Sa detects incompletely sialylated glycoconjugates (monosialotetrasaccharides) found on the more internal parts of GPA [162]. GPA and GPB express Pr_{1-3} [17,167,168]; GPA is also Sa-active [166,168]. Pr_2 and Sa are also detected on red cell gangliosides [169]. Pr antibodies agglutinate En(a–) cells very weakly and do not agglutinate M^K cells at all [16,106,171]. Unfortunately, no adsorption/elution studies were performed with M^K cells, which would be expected to carry some Pr determinants on other membrane components such as GPC and GPD.

In common with some other autoantibodies directed at determinants on GPA [172], anti-Pr has caused fatal or life-threatening AIHA, which is far more severe than would be predicted from the characteristics of the antibodies [172–176]. Brain *et al.* [176] have proposed a novel mechanism of immune destruction, independent of complement or macrophage classical processes, where antibodies to GPA damage a subpopulation of red cells by increased phosphatidylethanolamine exposure and membrane permeability, and formation of cation-permeable lipid pores.

3.6 U antigen and the GPB-deficient phenotypes S– s– U– and S– s– U+^{var}

3.6.1 U (MNS5) and anti-U

U was the name given by Wiener *et al.* [14,177] in 1953 to a high frequency blood group antigen present on the red cells of 977 of 989 African Americans and all of 1100 white Americans. When, in the following year, Greenwalt *et al.* [12] found a second example of anti-U, it became apparent that U was associated with the MNS system: both U– samples available were also S– s–, a phenotype not previously encountered. Adsorption and elution studies showed that anti-U was not a separable mixture of anti-S and -s [12,178].

U– red cells are almost always S– s–, but S– s– cells are often U+ [83,179–181]. S– s– U+ is often referred to as S– s– U+^{var}. Strength of U antigen expression on S– s– U+^{var} red cells is variable; adsorption/elution tests or sensitive agglutination tests with a particularly potent anti-U may be required for its detection [182]. Alternatively, molecular testing is very effective for distinguishing U– and U+^{var}. Like S– s– U–, the S– s– U+^{var} phenotype is virtually exclusive to people of African origin. About 50% of S– s– red cell samples are U+^{var} [83,181,183]. In this chapter the symbol *u* will represent the gene responsible for U– when it has not been defined by molecular genetic studies.

The precise serological definition of anti-U is unclear, but the term is traditionally used to describe antibodies produced by S– s– individuals to high frequency determinants on GPB. In a study of 17 'anti-U', Storry and Reid [181] found that five failed to react with all S– s– red cells. They called these antibodies anti-U. The other 12, which reacted with S– s– U+^{var} cells, but not S– s– U– cells, they called anti-U/GPB. By these definitions, S– s– U– cells are U–, U/GPB–, whereas S– s– U+^{var} cells are U–, U/GPB+. In this respect, anti-U and -U/GPB could be considered analogous to anti-En^a. S– s– U– cells are totally GPB-deficient, whereas S– s– U+^{var} cells have a variant GPB molecule that expresses neither S nor s. Following transfusion or pregnancy, anti-U may broaden in specificity to become anti-U/GPB and react with S– s– U+^{var} red cells that had previously been non-reactive with serum from the same patient [184,185]. Some individuals with S– s– U+^{var} red cells have made

anti-U or, at least, a U-like antibody [83,186]; one made anti-s [187].

S– s– U– and S– s– U+^{var} cells usually lack the trypsin-resistant 'N' antigen carried on GPB [179,188–190], although weak 'N' activity was detected on isolated sialoglycoprotein from two M+ N– S– s– U+^{var} individuals [191]. Consequently, apart from cells of certain very rare MNS variant phenotypes, M+ N– S– s– red cells are the only cells with no obvious expression of N. Immunised N– U– people are likely to make anti-U and/or potent anti-N, which reacts strongly with the N on both GPA and GPB [188].

He is a low frequency antigen expressed at the N-terminus of a GPB molecule that does not express 'N' (see Section 3.7.4). There is a strong correlation between expression of variant U antigen and He. Of 104 S– s– red cell samples, 51 (49%) reacted with anti-U/GPB, but not anti-U; of these 51 S– s– U+^{var} samples, 36 (71%) were He+ [83]. None of the S– s– U– red cells that were non-reactive with anti-U/GPB was He+.

U is generally resistant to denaturation by sialidase, trypsin, chymotrypsin, papain, and ficin. Unusual examples of anti-U, however, do not react with papain-treated cells and an antibody component to a papain-sensitive determinant (UPS) was identified in about 50% of sera containing anti-U [192]. U-like alloantibodies in two S– s– U+^{var} and two S– s– U– individuals were non-reactive with ficin-, pronase-, α-chymotrypsin-treated red cells, non- or weakly reactive with papain-treated cells, and reactive with trypsin-treated cells [186], resembling in this way some U-like autoantibodies [193].

S– s– U– red cells do not show most of the unusual serological reactions associated with reduced sialic acid that are characteristic of red cells deficient in GPA (Section 3.5.1.1), though *Glycine soja* lectin may agglutinate U-deficient cells [194].

Other rare phenotypes in which the red cells may be S– s– U– are the Rh-deficiency phenotypes (Section 5.16.5) and phenotypes arising from homozygosity for hybrid genes encoding the rare SAT and St^a antigens (Sections 3.10.3 and 3.14.2).

Further details of anti-U, including clinical significance and autoanti-U, can be found in Section 3.18.10. Anti-U^Z and anti-U^X are described in Section 3.18.11.

3.6.2 Biochemistry

S– s– U– red cells are deficient in GPB. This has been demonstrated by failure to inhibit anti-S, -s, or -U with SGPs isolated from S– s– U– cells, by SDS PAGE of red cell membranes or isolated SGPs, and by immunoblotting with antibodies and lectins directed at determinants on GPB [23,24,189–191,195–198,]. Red cells of individuals heterozygous for *u* have roughly half of the normal quantity of GPB [189,190,195]. Small quantities of GPB, about 2–3% of normal, were detected on S– s– U+^{var} cells [191].

GPB normally carries about 11 *O*-glycans and S– s– U– and S– s– U+^{var} red cells demonstrate a reduction in sialic acid by about 15% compared with normal cells [195,199]. Cells of individuals heterozygous for *u* have about a 9% sialic acid reduction [195]. Unlike the GPA-deficiency phenotypes, S– s– U– is not associated with any apparent alteration of band 3 [195].

U appears to be a labile structure requiring lipid for full expression [200]. In this respect it resembles En^{a}FR, which is located close to the membrane on GPA (Section 3.5.3). From the results of anti-U haemagglutination-inhibition tests with GPB extracts, in the presence of lipids, amino acid residues 33–39 of GPB appeared to be essential for U antigen expression [200], but U expression also appears to be dependent on an interaction between GPB and RhAG (Section 5.20). Unlike S and s, U, as defined by most anti-U, escapes denaturation by α-chymotrypsin treatment of intact cells, because the cleavage site for chymotrypsin is between residues 32 and 33. Some U-like antibodies, however, are non-reactive with α-chymotrypsin-treated U+ red cells, suggesting that their determinants are closely related to S and s [186].

3.6.3 Molecular genetics

The S– s– U– phenotype results from homozygosity for a deletion of *GYPB* (*GYPB*Null*) encompassing exons B2–B6 of *GYPB* and also including exon E1 of *GYPE* [53,57,83,197,201] (Figure 3.7). The deletion includes the whole of the sequence of *GYPB* encoding the mature protein.

At least four genes are responsible for S– s– U+^{var}, all of which are responsible for alternative splicing of all or part of exon B5 of *GYPB* and all of which have the S sequence encoding Met48 [83]. The most common (83% of samples) has g>t at position +5 of the donor splice site of intron 5, which causes skipping of exon B5 and loss of the region that usually constitutes the membrane-spanning domain of GPB (Figure 3.8). The reading frameshift abolishes the translation stop codon close to the 5′ end of exon B6 so that the C-terminus of the glycoprotein is elongated by a novel sequence of 41 amino acids. The most common form of this gene, *GYPHe(P2)* (*GYPB*03N.03*), has a *GYPA* insert within exon 2 responsible for He expression, whereas the less common form, *GYPB(P2)* (*GYPB*03N.04*), has the

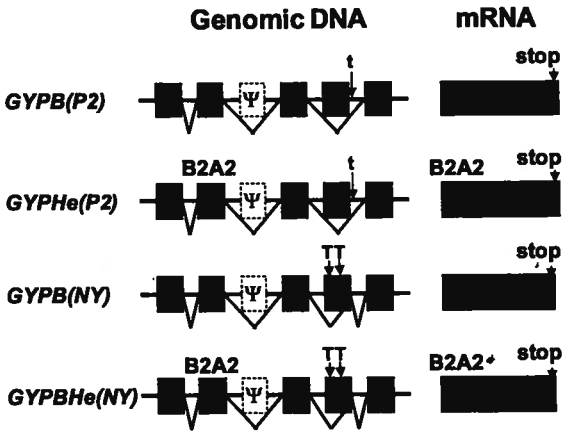

Figure 3.8 Four genes responsible for S– s– U+^{var} phenotype, showing genomic DNA with B2A2 hybrid exon 2 in genes encoding He and mis-splicing of exon 5 and resultant aberrant mRNA. There is no evidence that mis-spliced mRNA is represented as a protein in the red cell membrane [83].

normal 'N' sequence in exon B2 [83,202]. The abnormally spliced transcript encodes a variant protein of 81 amino acids, but this was not detected at the red cell surface [83].

Two other U+^{var} genes, *GYPHe(NY)* (*GYPB*03N.02*) and *GYPB(NY)* (*GYPB*03N.01*), have 208G>T and 230C>T changes in exon B5 that result in activation of a cryptic splice site at 251G causing partial skipping of exon B5 (Figure 3.8). *GYPHe(NY)* and *GYPB(NY)* have the He and 'N' sequences in exon 2, respectively. The coding sequence predicts a 43-amino acid protein, with no hydrophobic membrane-spanning domain, and which has not been detected by immunoblotting analysis [83].

The abnormal splice site sequences associated with the genes responsible for S– s– U+^{var} phenotypes suggest that skipping of exon B5 would not be absolute so that some normally spliced transcripts and low levels of normal GPB.He or GPB would be produced. This would explain the weak U and He expression detected by haemagglutination and the detection of weak bands representing a 24 kDa protein, the size of GPB, by immunoblotting with monoclonal anti-He or anti-GPA+GPB [83,183]. The low levels of GPB.He or GPB could result in conformational changes that are responsible for the absence of S and for the production of anti-U in a few individuals with S– s– U+^{var} red cells [83].

3.6.4 Frequency studies

Results of screening donors with anti-U are unreliable, because they vary according to the proportion of S– s– U+^{var} samples that give positive or negative results with the antibody reagent used, though the frequency in U– African Americans varies from 0.2 to 1.4% [188]. Table 3.2 shows M, N, S, and s phenotype frequencies in African Americans, together with genotypes in which the S– s– phenotype is assumed to have resulted from homozygosity for *u* at the *Ss* locus. The *MN* and *Ss* haplotype frequencies derived from studies of African American and African populations shown in Table 3.7 reflect a similar approach. Of 126 Pygmies from Congo, 35% were U– [203]. No S– s– U– individual was found among 1000 Bantu-speaking people of Natal [204], whereas three were found among 1000 black antenatal patients from the Eastern Cape [205].

PCR with allele-specific primers revealed that 94% of African Americans with the S– s– U+^{var} phenotype have an *He* allele of *GYPB*; the remainder have an 'N' allele of *GYPB* [83]. Analysis of an *Eco*R1 site that is ablated by the intron 5 mutation in *GYPB(P2)* and *GYPHe(P2)* showed an allele frequency of 2.5% in African Americans [83]. From a molecular analysis of 267 African Americans, eight were heterozygous for *GYPB(P2)* or *GYPHe(P2)*, one was homozygous for *GYPB(NY)* or *GYPHe(NY)*, and in four *GYPB* was deleted [89].

Although extremely rare, the U– phenotype has been identified in people of non-African descent. S– s– U– members were found in a white family from France [196] and in a family originating from India [206]. Six of 324 Finnish Lapps [84] and two of 63 Central American Indians from Honduras [85] were S– s–.

3.7 M and N variants representing amino acid substitutions within the N-terminal region of GPA and GPB

M and N antigens are determined by the sequence and glycosylation of the N-terminal five amino acids of GPA and GPB (Table 3.5). Amino acid substitutions within this pentapeptide may affect expression of M or N and may create a new antigen. Three such variants are described in this section: M^{s} and M^{c} on GPA; He on GPB.

3.7.1 M^{s} (MNS11)

M^{s}, a very rare antigen first described in 1958 [207], is encoded by a gene that produces virtually no M or N antigen. Undetected, an *M^{s}* (*GYPA*Mg* or *GYPA*11*) allele in a family could result in apparent exclusion of parentage as an M+ N– (*M/M^{s}*) parent can have an M– N+ (*N/M^{s}*) child.