Molecular immunohaematology round table discussions at the AABB Annual Meeting, Philadelphia 2014

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Introduction

Use of molecular-based immunohaematology testing is becoming more widespread worldwide in laboratories that are accustomed to the use of blood group serology alone. Molecular immunohaematology issues may be challenging even for some established professionals in the field of blood group serology. At an international meeting, we offered round table discussions on four patient-related and two donor-related topics, which are current and possibly controversial. Six molecular immunohaematology questions were addressed: applications for highly contagious infections, such as Ebola; utility after transfusions in the preceding three months; root cause analysis for unexplained occurrence of anti-D; acceptable turnaround time for red cell genotyping of patients; criteria for donor cohorts to be genotyped; and quality assurance for discrepancies between serological phenotype and licensed red cell genotyping. The opinions polled in this workshop with an international assemblage of more than 100 transfusion medicine specialists were discussed in the light of education and training opportunities and the development of guidance in the field. We provide a summary report of the participants' input to our questions and discuss the topics.

Organisation of the discussion rounds

An international group of transfusion medicine specialists gathered in the 1.5-hour session "Molecular Immunohematology Roundtable" (n. 9131-TC) on Oct 26, 2014 at the AABB Annual Meeting & CTTXPO 2014 in Philadelphia PA, USA. This workshop was offered to any attendee of the conference. A group of participants at a table met with a chaperone to discuss each topic in the form of a question for 10 minutes; the participants remained at the table discussing successive questions while the chaperones changed tables.

Six questions were posed, and opinions and input were polled from the experienced professionals, who gathered with the 12 chaperones. The chaperones, selected prior to the workshop, listened to the participants' viewpoints, clarified questions, took notes regarding the points raised and kept the discussion on track. The six chaperone pairs each consisted of one North American and one international expert in the field. The groups ranged from six to nine participants at each of twelve tables. Before the annual meeting 73 individuals registered for the session; 101 signed up on site and actually attended the session, and 41 returned evaluation forms (41%) after the event (Table I). The format of this workshop and its demographics and evaluations were similar to those in previous years[2,3].

Participants included physicians, medical technologists, and basic scientists from blood donor centres and hospital-based blood banks. Several attendees represented blood banking-related industries. The participants hailed from 14 countries (Table I) and represented a broad range of experience in serology and molecular testing.

Round table discussions

All participants had the opportunity to provide input to the six questions. The six teams of two chaperones each provided the following summaries of their round table discussions, representing only the views of the participants.

Question 1: How can molecular immunohaematology contribute to the care of patients with highly contagious infections, such as Ebola?

Although the question initially appeared nonsensical to several participants, they realised its relevance quickly

Table I - Demographics of the participants.

Parameter and characteristics	Replies (n)	Percentage
Level of experience		
1-5 years	9	22%
6-10 years	2	5%
11-19 years	12	29%
20+ years	18	44%
Total	41	100%
*Position**		
Director/manager	15	36%
Chief/medical director	6	15%
Technologist/technician	5	12%
Scientist/clinical investigator	4	10%
All other replies combined	11	27%
Areas of specialty†		
Patient laboratory testing	16	31%
Clinical practice/patient care	10	20%
Molecular testing	7	14%
All other replies combined	18	35%
Relevance of content		
Excellent	23	59%
Good	16	41%
Other (fair/poor)	0	0%

* Other replies: supervisor/coordinator, lead/specialist (n=3 each); physician, resident/fellow/student (n=2 each); CEO/CFO (n=1).
† Other replies: blood collection, cellular therapy (n=3 each); administration, education/training (n=2 each); communication/PR/marketing, donor product testing, inventory management, quality/compliance, regulatory/legal/ethics, research/development, supplier of products, other (n=1 each).
Multiple replies possible.
Replies may not sum up to 41, because some fields were not answered on all forms.
Recorded countries of origin: USA, Canada, Brazil, Panama; Finland, Italy, Norway, Slovenia, Spain, United Kingdom; Kuwait, Thailand; and Australia, New Zealand.

after discussion of the potential benefits. As in massive transfusion protocols, patients could receive O ccddee red blood cell (RBC) units and AB plasma, while ABO mismatched platelets are routinely transfused based on inventory restrictions. Applying this strategy, any serological testing, including ABO typing can be avoided[4]. Only in cases in which convalescent plasma is transfused might an ABO type be needed. Individuals exposed to Ebola, including health care professionals and USA military personnel deployed to countries with Ebola, are often thoroughly tested for blood groups and antibodies, which can be accessed in the transfusion medicine history. Only especially qualified volunteer staff should handle blood samples known to contain highly contagious infectious agents, and the universal precautions should be enhanced by special protection, equipment and decontamination.

While no general pathogen inactivation substance is available for blood collection tubes, DNA extraction provides such pathogen inactivation. Most participants knew that whole blood is a much better starting material than buccal swabs for DNA preparation. Working with DNA only, cross-matching would be done electronically based on genotype (dry matching)[5], assuming that RBC units with a red cell genotype are available. This strategy obviates any potential exposure to infectious agents in the regular transfusion medicine laboratory, once the DNA has been prepared with suitable precautions by a biosafety level 3 (BSL-3) laboratory. All manner of reliable antigen testing, not limited to molecular immunohaematology, can be done today on molecular platforms: extract DNA or RNA once and perform all profiles in short order. It was noted that patients with sickle cell disease, typically with African background, should be matched at least for the major Rh and K1 antigens (Chaperones: DAW & SW).

Question 2: In which situation(s) can patients who have been transfused within the past three months be tested for blood groups without red cell genotyping?

No participants felt that anyone requiring antigen testing and transfused within the preceding one month should be tested by standard serological means only, while virtually all participants felt that serological techniques were probably valid at 2-3 months after the most recent transfusion[6-8]. The volume of RBC to which the recipients were exposed would also influence the participants' comfort with employing serological antigen-typing techniques, with larger RBC volume exposure decreasing their reliance on methods other than red cell genotyping[9].

Regarding non-genotyping techniques that could be applied to determine recipient blood group antigen expression in the 3-month window following RBC administration, participants near-universally expressed that they were aware of only two laboratory methods (Table II): RBC reticulocyte harvesting and hypotonic RBC lysis. In the former, reticulocytes - presumably representing the recipient's cells - are harvested via centrifugation or cell gradient separation and tested by serological methods. In the latter, hypotonic saline solutions are used to osmotically lyse RBC, leaving behind those resistant to osmotic lysis and most often representing the recipient's cells, if he or she is a patient with haemoglobin disorders such as sickle cell disease or thalassaemia.

Participants additionally noted that a valid, non-testing option would be to call other hospitals in which the patient had been previously treated to determine whether historical RBC phenotype information was on file (Table II). The strengths and weaknesses of the approaches were summarised by the participants and compared to red cell genotyping (Chaperones: CAT & SLdC).

Question 3: How could patient care be improved if root cause analysis for the unexplained occurrence of anti-D in transfusion recipients became mandatory?

Comments ranged from enthusiastic support for investigation of these unexpected results in all instances to a more selective approach. Many participants thought the information obtained through root cause analysis would help further our understanding of the *RHD* gene and which variants (in both donors and recipients) put patients at risk of alloimmunisation. The question was applied to the two scenarios in which the patient was either Rh positive or Rh negative and developed an anti-D following transfusion (Table III).

Rh-positive patients

Most participants thought an anti-D indicated the presence of a variant *RHD* allele in the patient, and some would not pursue an investigation but rather transfuse Rh-negative blood. However, others suggested an investigation in all patients with other factors increasing the complexity of identifying blood for transfusion, such as in patients with sickle cell disease, chronic transfusion or multiple alloantibodies. It was noted that in Rh positive patients with anti-D, genetic analysis may also uncover variant *RHCE* alleles that may place the patient at risk of additional alloimmunisation in Rh. In such patients, extended red cell genotyping should be performed to guide selection of the safest blood for future transfusions avoiding further alloimmunisation.

The possibility of an anti-D in a Rh-positive patient being an autoantibody should be excluded by further investigation, such as direct antiglobulin tests, autocontrols and adsorption tests. Some participants recognised the importance of excluding an anti-LW, which is rare but often mistaken as anti-D.

Rh-negative patients

Active immunisation is expected in a Rh-negative patient deliberately transfused with Rh-positive RBC units in the case of a shortage of Rh-negative blood. Transfusion of Rh-positive platelets may be a less common cause of an anti-D[10]. When no apparent cause can be identified, an investigation with root cause analysis was generally considered warranted.

Table II - Available methods and approaches to determine blood group antigens in patients transfused within the preceding three months.

Approach	Strengths	Weaknesses
Historical RBC phenotype	Not affected by recent transfusion. Can be universally applied to all patients. Helpful to avoid problems with evanesced alloantibodies on record at other facilities.	Clerical errors in communication. Unlikely to be available for most patients. If available, records may not reflect antigens of interest.
Reticulocyte harvesting	Can be universally applied to all patients. Rapid turnaround-time (hours). Established technique in some reference laboratories.	Strongly dependent on underlying reticulocyte count. Transfused cells may interfere with test. Complex, labour-intensive test, difficult to perform and interpret. Send out test (1-2 days) for most hospitals.
RBC hypotonic lysis	Rapid turnaround-time (hours). Established technique in some reference laboratories.	Applicable to patients with haemoglobin disorders only. Complex, labour-intensive test, difficult to perform and interpret. Send out test (1-2 days) for most hospitals.
Red cell genotyping	Not affected by recent transfusion. Can be universally applied to all patients. Established technique in some reference laboratories.	Usually acceptable turnaround time (within 1 day). Complex, labour-intensive test, difficult to perform and interpret. Send out test (1-2 days) for most hospitals.

Table III - Haemovigilance for unexplained occurrence of anti-D.

Anti-D observed in:	Differential diagnosis
Rh-positive patient	Variant *RHD* allele
	possibly associated with variant *RHCE* allele
	Auto-anti-D (rare)
	Passively acquired anti-D (rarely free anti-D in recipient's plasma)
Rh-negative patient*	Passively acquired anti-D
	by donor plasma, or
	by immunoglobulin-containing blood products: IVIG, RhIG
	Rh positive RBC unit, mislabelled as Rh negative
	Serologically negative RBC unit with a functional *RHD* allele expressing weak D or DEL phenotypes
	Naturally occurring anti-D (very rare, if ever)

* Complete transfusion history particularly critical. IVIG: intravenous immunoglobulin; RhIG: Rhesus immunoglobulin.

Anti-D may be passively acquired by transfusion of blood components from a Rh-negative donor carrying anti-D. While this information should be on the unit label, it may have been missed or the unit not correctly labelled. It is important to identify these potential errors. Intravenous immunoglobulin[11] and Rh immunoglobulin[12] are other causes. Hence, clinical history is important, and investigation would identify which of these events occurred.

Active immunisation in Rh-negative patients may also occur after transfusion of a serologically Rh-negative RBC unit that carries weak D[13] or DEL[14]. When anti-D is detected despite the seemingly exclusive transfusion of Rh-negative blood units, an investigation was generally considered mandatory to eliminate the possibility of a mistyped unit, or the presence of a Rh variant in the donor. Identification of the cause would prevent future alloimmunisation events. While such haemovigilance cannot help the index patient, molecular typing of the involved donors benefits future transfusion recipients of RBC units from the same donors (Chaperones: EBK & FNP).

Question 4: What are acceptable turnaround times, such as 4 hours, 8 hours, 1 day or longer, for red cell genotyping of patients?

An acceptable turnaround time (TAT) for genotyping in a hospital setting has to be based on the patient's needs. Participants were asked what they believed was an acceptable TAT for four levels of care: (i) urgent, (ii) routine, and (iii) possible/probable clinical need as well as (iv) prophylactic typing in anticipation of future needs. The participants were instructed that their TAT recommendations should be based on what is best for patient care, not what is currently feasible. Moreover, they could assume that testing is available in the hospital transfusion service, so that specimen transportation delays need not be a consideration.

Participants' responses for the median acceptable genotyping TAT were 1 hour for urgent care, 4 hours for routine requests, 8 hours for possible/probable transfusion, and 1 day for prophylactic purposes (Table IV). After the meeting, we compiled currently available TAT for traditional serological compatibility testing: the median TAT for type, screen, and cross-match, as indicated on websites of transfusion services at university and large community medical centres (Supplementary Table S1), was 1 hour for urgent or STAT and 4.5 hours for routine transfusion requests (Table IV).

Some variability in the participants' responses was evident. For example, 25 participants (23%) felt that no genotyping TAT was acceptable for urgent transfusions and gave responses of 0 minutes or no response at all (Table IV). Another 20 participants (19%) thought that TAT for urgent transfusions should be 15 minutes or less, while the most common response (mode) was 60 minutes. For routine transfusion orders, nine participants (9%) felt that genotyping would be impractical and would not speculate on an acceptable TAT. Over three-quarters (76 participants) indicated the TAT should be 6 hours or less and about half (47 participants) considered more specifically 4 to 6 hours to be acceptable. For type-and-screen or type-and-cross-match requests without a definite order to transfuse, i.e. transfusion is possible or probable, a slight majority (60 respondents) thought a TAT in the range of 8 to 24 hours was acceptable. However, a sizable fraction (47 respondents) advocated for a shorter TAT of 6 hours or less out of concern that transfusion could be ordered at any time. This split in opinion was reflected in the large difference between the median (8 hours) and the mode (24 hours, most common reply) of the responses for acceptable TAT (Chaperones: GS and CW).

Question 5: What criteria should be used to select donors for genotyping, when not all donors can be genotyped?

Despite the many platforms currently available[15], the single factor identified by the participants as currently prohibiting all donors from being genotyped was cost. Given this constraint, there was also a universal consensus to select the donors for red cell genotyping by blood group, ethnicity and donation history (Table V).

Table IV - Acceptable turnaround time (TAT) for red cell genotyping.

Clinical need	TAT for red cell genotyping considered acceptable by the participants				Actual TAT for serology (median)†
	Median	*Mean*	*Mode*	*Respondents (n)**	
Urgent	60 min	52.2 min	60 min	81	1 hour
Routine	4 hours	4.3 hours	4 hours	88	4.5 hours
Possible/probable	8 hours	12.1 hours	24 hours	107	n/a
Prophylactic	1 day	1.2 days	1 day	89	n/a

* Participants who did not give a reply were excluded (urgent: n=1; routine: n=10; prophylactic: n=18). Some participants replied "0 minutes" or "not feasible" for urgent (n=25) and routine (n=9) levels of clinical need and were excluded from calculations.
† As currently offered by large medical centres in the USA; random website search for TAT claims of 14 transfusion services (see Supplementary Table S1). n/a: not applicable (no data available).

The preference for the criteria within these three groups varied depending on the needs of the blood centre and country. In the USA, genotyping of African-American or all non-Caucasian donors was widely recommended, primarily to identify antigen-negative blood for patients with sickle cell disease. Some participants from outside the USA suggested typing all donors from non-native ethnic groups in their countries.

Most participants preferred genotyping donors with recent and repeat donation history. However, one participant from an Italian donor centre reported that first time donors, especially if young, were actively selected and rare donors were personally met one-on-one to discuss their results. Participants with red cell genotyping experience reported that many donors who are found to carry distinct rare combinations of antigens in their extended genotype might solidify their role as blood donors if this information was communicated to them (Table VI). Such rare donors identified by red cell genotyping, informed by letter, are provided a special donor card or submitted as donors to the American Rare Donor Program. A few participants mentioned that rare donors were asked to donate whole blood, rather than plasma or platelets, and also to encourage family members to donate and be red cell genotyped. Finally, no participant reported having a policy of communicating to the donor, if red cell genotyping predicted the presence of one or more low incidence antigens.

Few participants tested the genotype of human platelet antigens. Those who did stated that they selected males who were known to be negative for cytomegalovirus and had already been genotyped for HLA antigens. Other participants selected female donors who had not previously been pregnant and males. Some selected donors for extended human platelet antigen genotyping based on their known human platelet antigen-1a/1b status or because they had donated multiple times over the course of a year (Chaperones: MAK & MSL).

Question 6: How should quality assurance handle discrepancies between serological phenotype and genotype, when both methods, serology and red cell genotyping, are licensed by regulatory agencies?

A consistent response was to re-check the paperwork and ensure its accuracy followed by repeat antigen typing using the same techniques, as has been done with any serological discrepancies in the past. If the discrepancy persisted, the serological phenotyping and red cell genotyping should be repeated on the same sample using one or more different techniques available until the discrepancy is resolved (Table VII).

The participants were evenly split as to whether a new sample should be drawn and both serological and molecular tests repeated on a new sample. Many opted for repeat genotyping of the same sample using

Table V - Donor criteria for red cell genotyping.

Parameter	Criteria in order of preference
Blood group	ABO - O with specific Rh type - All O - O and A - All, but AB Rh type - Rh negative - ccddee, CCDee and ccDEE
Ethnicity	African descent Any non-Caucasian Any ethnic group, not native to the country
Donation history	All, but first time donors More than e.g. 2 donations in past year First-time donors as an incentive to donate, when rare genotype is found
Age or gender	Not recommended as criteria for donor selection.

Table VI - Molecular immunohematology as donor motivation, retention and recruitment tool.

Communicate "rare" blood group result* - Inform by letter - Provide special donor card
Discuss "rare" blood group result - Meet donors one-to-one or by phone - Recommend whole blood donation - Offer red cell genotyping for family members who may be encouraged to begin donating blood

* Based on an extended red cell genotype many donors will be "rare" depending on the clinical needs identified in patients.

Table VII - Quality assurance checklist for discrepancies between genotype and serological phenotype.

Initial check (clerical error, technical error or specimen error):
- check paperwork, such as in any discrepancy work-up;
- repeat serology, using same technique as before.
If a discrepancy persists:
- fresh sample may be utilised, if available;
- repeat serology and genotyping, using one or more different reagents and molecular techniques;
- report to quality assurance department, if a test did not perform as expected;
- no need to involve quality assurance department, if all tests performed as expected;
- consider informing the manufacturer of a test that did not perform as expected.
If a true discrepancy between genotype and serological phenotype is confirmed:
- nucleotide sequencing is often the only way to resolve discrepancies caused by rare or novel variants;
- consider including exons, introns, adjacent genomic nucleotide sequences and mRNA analysis;
- an experienced molecular immunohaematology reference laboratory should be involved.

other platforms[15] or nucleotide sequencing or both, if available. Sequencing was suggested particularly for Duffy and Kell discrepancies but the expense of nucleotide sequencing was acknowledged.

A central registry was suggested by some for all such discrepancies. The majority felt, however, that such a discrepancy did not need to be reported to a Quality Assurance department if the tests performed as designed and there was no quality failure. More importantly, many participants felt that the manufacturer should be informed so that trends can be investigated. It was suggested by some that the discrepancy should also be reported to the FDA, because safety is paramount. These considerations apply to the donor and patient setting, but discrepancies observed in patients may be even more informative, when a clinical need prompted the red cell genotyping associated with the underlying discrepancy.

Reporting will depend on the antigen and whether the discrepancy affects a patient or donor. The majority agreed that a patient report should imply antigen-negative transfusion and a report for a blood unit (i.e. donor report) should state "positive" or "indeterminate". (Chaperones: JMM & NS)

Discussion by the authors

The round table groups represented a global cross-section of immunohaematology experience and their input on the six topics is discussed here by the chaperone teams.

Topic 1

Using DNA or RNA as the starting material for testing can obviate any infectious risk from bacteria, virus or prions. While most participants had never considered the fact in the context of molecular immunohaematology, they quickly realised this major advantage of DNA/RNA, relevant not only for highly contagious infections, but also for all kinds of sample handling in the laboratory. There were concerns whether extracted DNA/RNA is actually non-infectious, which can be clarified by education. Dry matching for antigens avoids most transfusion reactions, even if no antibody screen is available[5]; a thorough transfusion medicine history, including previously observed antibodies, is still indispensable and must not be neglected, as for instance O ccddee RBC units would be incompatible for a recipient known to carry an anti-c.

Topic 2

Virtually all participants were aware of the severe limitations of serological methods in patients transfused during the preceding three months. More recent transfusion and larger RBC volume reduce the accuracy further and no reliable threshold for volume or a time frame of less than 3 months has been established[9].

The majority of participants appreciated that RBC reticulocyte harvesting and hypotonic lysis of RBC are complex and laborious techniques that may only be available in some laboratories, applicable to small groups of patients and, ultimately, may still yield inaccurate information regarding blood group antigens (Table II). Despite these well-recognised limitations of serological methods, many participants reported having seldom or never used red cell genotyping for blood group typing after a recent transfusion. The participants communicated current restrictive access to genotyping as the reason for continuing to rely on serological methods.

Responses from this group indicated that in order for blood group genotyping to be more practical for assessing recently transfused patients, techniques need to be: (i) faster, so that the TAT meets clinical needs, (ii) less expensive or billable as a medical laboratory test to recoup costs associated with the potentially expensive molecular testing, and (iii) more widely available, i.e. not simply offered at reference centres and laboratories. We concur with a recently published conclusion[16] that there is no good excuse for continuing to rely on old techniques known to yield misleading results frequently, even if they are rapidly available and cheap. Time constraints did not permit discussion of why a much improved technology has to be less expensive before it should replace inexpensive techniques that are known to be inferior[16] and incapable of resolving the clinical question.

Topic 3

Although deliberate transfusion of Rh-positive RBC units to Rh-negative recipients is universally applied in the case of shortage of Rh-negative units, such decisions are not reportable or liable to follow-up and hardly any aggregate data are gathered[11]. Hence, the causes of newly encountered anti-D in patients and donors are not routinely researched and the question as to whether the occurrence can be explained is not even raised. However, the participants were well aware of the various differential diagnoses that could be explored in a root cause analysis (Table III), improving transfusion safety and appropriate utilisation of Rh immunoglobulin in women of childbearing age[12]. Although the clinical utility was readily recognised, root cause analysis is rarely performed[13,17,18] and not mandatory in any health care system. With a few rare exceptions, root cause analysis of unexplained anti-D requires molecular evaluation of the patient or donor or both.

Topic 4

For most hospitals red cell genotyping is a send-out test with TAT ranging from 1 to 7 days. Faster TAT and more widespread use of red cell genotyping have the potential to further improve transfusion safety[19]. For example, there are times when the optimal RBC product is not transfused because clinical urgency necessitates transfusion before genotyping results are available. Shortened TAT, achieved either by hospital transfusion services establishing their own in-house genotyping capability or by improved service from reference laboratories, could reduce such quality failures.

At our 2013 round tables, TAT was not considered a limitation by participants from the industry (Discussion 4)[3] but those from hospitals regarded TAT as a limitation to red cell genotyping (Discussion 6)[3]. The key question, and the one posed to the round table discussion participants, was: How short do genotyping turnaround times have to be for optimal patient care? The participants' responses proved to be nearly identical to the current turnaround-times of traditional serological compatibility testing for urgent and routine transfusions (Table 4), based on comparative data from a medical centre website survey (Supplementary Table S1). Prolonging TAT beyond currently acceptable timeframes was considered undesirable by the participants.

A gap analysis revealed that genotyping TAT available today already meet the urgency needs of many possible/probable transfusions (i.e. 8 hours), if genotyping is done in-house, and of prophylactic genotyping requests (i.e. 1 day), if it is done either in-house or sent out. Currently achievable genotyping TAT approach, but generally still exceed, the TAT advocated by the participants for routine transfusion (i.e. 4 hours).

The TAT goal of 1 hour for urgent transfusions is beyond current capabilities. Nevertheless, it should be noted that advances in molecular diagnostic testing in microbiology have led to the development of FDA-approved multiplex polymerase chain reaction testing platforms that can, for example, detect and identify 20 bacterial and viral respiratory pathogens at a time with a total TAT of about 1 hour[20]. Red cell genotyping with a 1-hour TAT does, therefore, seem to be within the realm of possibility, once transfusion medicine provides a market for such products. When a TAT of 1 hour becomes technically feasible, red cell genotyping may not only outperform the TAT of routine serology; red cell genotyping may also improve the quality and timeliness of patients' care beyond the capabilities of the current serological testing.

Topic 5

As a follow up to the 2012 round table discussion (Theses 3 and 4)[2], we explored factors determining eligibility of donors for red cell genotyping, because currently no participant reported universal red cell genotyping of donors at any blood centre. Cost constraints restricted genotyping to certain donors and, while the subsets of donors varied to satisfy the specific needs of the donor centre and country, the basic rationales for donor criteria were clearly outlined and widely shared (Table V). Cost is becoming less of a concern[15], as groups continue to show similar or lower cost for genotyping than for serology in various donor settings[21-24]. Blood group, ethnicity and donation history determined the selection, and no participants thought it was prudent to select donors by age or gender. Distinct rare combinations of antigens are often found when testing for extended red cell genotypes and sought after to facilitate dry matching. Rare red cell genotypes may be used as a novel donor management tool (Table VI)[25].

Topic 6

Reporting to the Quality Assurance department was not considered necessary, if all tests performed as designed. The work-up of discrepancies was of much concern for practitioners and had been raised in each round table discussion before (Theses 3 in 2012[2] and 2013[3]); this year's participants suggested pragmatic handling of the inevitable discrepancies (Table VII), which will occur when any new technology is introduced: Re-checking the paperwork and sample draw as well as repeat serological and molecular testing were considered standard praxis for all discrepancies[26,27]. The need for repeat testing of a new sample was less clear and more problematic

because many patients may have been discharged or donors would not return for a redraw. Although ideally fresh samples should be used for repeat testing, most participants would consider the original sample acceptable if a fresh sample was not available. It was noted that even nucleotide sequencing of the involved blood group gene may still not resolve the discrepancy, because some laboratories only perform nucleotide sequencing of the exon with the single nucleotide polymorphism that encodes the blood group antigen. Nucleotide sequencing of the entire full length of the gene may be more informative in such cases. In rare cases, even full length nucleotide sequencing may still not resolve the discrepancy if other modulating genes or post-transcriptional or post-translational processing is causing the discrepancy.

Conclusions

This international forum on six current molecular immunohaematology topics documented the knowledge, acceptance and concerns among an international group of transfusion medicine specialists. Collating these data may further this developing field, because the perception of experienced specialists will shape the adoption of new technologies for molecular immunohaematology. Such technologies are known to benefit patients at a level that cannot be achieved by any serological approach.

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Supporting information

Additional Supporting Information may be found in the online version of this article (Supplementary Table S1: Website survey of turnaround times for RBC compatibility testing by serological methods).

Authorship contributions

WAF and GAD designed the topic statements and organised the session. The six teams of two chaperones, who volunteered to participate, each wrote the summaries of their discussion rounds. GAD moderated the session; and WAF compiled and wrote the report.

Disclosure of conflict of interest

CAT holds a research grant from Terumo BCT. GAD is the inventor of European patents on red cell genotyping owned by the Canadian Blood Services. GS receives research support from Immucor. JMM is an employee of Grifols, Inc. MAK is on the speakers' bureaus of Agena Bioscience and Immucor. WAF is inventor of patents owned by the German Red Cross Blood Service Baden-Württemberg-Hessen and holds intellectual property rights for RHD *genotyping applications. The remaining authors do not have a conflict of interest relevant to this article.*

Statement of disclaimer

The views expressed do not necessarily represent the view of the National Institutes of Health, the Department of Health and Human Services, or the U.S. Federal Government.

Approval of the first molecular immunohaematology assay for blood group genotyping by the US Food and Drug Administration (FDA) was announced on May 23, 2014, while several *Conformité Européenne* (CE)-labelled test kits have been available for more than 10 years[1]; the CE label certifies that a test kit may be used for in vitro diagnostic purposes in the European Union. If laboratory-developed tests are used for the care of patients in the USA, such tests will come under the authority of the Clinical Laboratory Improvement Amendments (CLIA) categorised as tests of either "high" or "moderate" complexity.

References

1) Flegel WA. Blood group genotyping in Germany. Transfusion 2007; **47**: 47S-53S.
2) Flegel WA, Johnson ST, Keller MA, et al. Molecular immunohematology round table discussions at the AABB Annual Meeting, Boston 2012. Blood Transfus 2014; **12**: 280-6.
3) Flegel WA, Castilho L, Delaney M, et al. Molecular immunohaematology round table discussions at the AABB Annual Meeting, Denver 2013. Blood Transfus 2015: **13**: 514-20.
4) Katz LM, Tobian AA. Ebola virus disease, transmission risk to laboratory personnel, and pretransfusion testing. Transfusion 2014; **54**: 3247-51.
5) Denomme GA, Flegel WA. Applying molecular immunohematology discoveries to standards of practice in blood banks: now is the time. Transfusion 2008; **48**: 2461-75.
6) Eshleman JR, Shakin-Eshleman SH, Church A, et al. DNA typing of the human MN and Ss blood group antigens in amniotic fluid and following massive transfusion. Am J Clin Pathol 1995; **103**: 353-7.
7) Brauner P. DNA typing and blood transfusion. J Forensic Sci 1996; **41**: 895-7.
8) Wenk RE, Chiafari PA. DNA typing of recipient blood after massive transfusion. Transfusion 1997; **37**: 1108-10.
9) Gonsalkorale K, Vanhecke C, Badami KG. Red blood cell phenotyping after transfusion: an in vitro model. Immunohematology 2013; **29**: 93-6.
10) Cid J, Lozano M, Ziman A, et al, Biomedical Excellence for Safer Transfusion. Low frequency of anti-D alloimmunization

following D+ platelet transfusion: the Anti-D Alloimmunization after D-incompatible Platelet Transfusions (ADAPT) study. Br J Haematol 2015; **168**: 598-603.
11) Flegel WA. Pathogenesis and mechanisms of antibody-mediated hemolysis. Transfusion 2015; **55**: S47 - S58.
12) Sandler SG, Flegel WA, Westhoff CM, et al. It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. Transfusion 2015; **55**: 680-9.
13) Flegel WA, Khull S, Wagner FF. Primary anti-D immunization by weak D type 2 RBC. Transfusion 2000; **40**: 428-34.
14) Kim KH, Kim KE, Woo KS, et al. Primary anti-D immunization by DEL red blood cells. Korean J Lab Med 2009; **29**: 361-5.
15) St-Louis M. Molecular blood grouping of donors. Transfus Apher Sci 2014; **50**: 175-82.
16) Wagner FF. Why do we use serological blood group phenotype determination in chronically transfused patients? Blood Transfus 2014; **12**: 1-2.
17) Elhence P, Veena S, Sharma RK, Chaudhary RK. Root cause analysis of transfusion error: identifying causes to implement changes. Transfusion 2010; **50**: 2772-7.
18) Karim F, Moiz B, Shamsuddin N, et al. Root cause analysis of non-infectious transfusion complications and the lessons learnt. Transfus Apher Sci 2014; **50**: 111-7.
19) Tsongalis GJ, Coleman WB. Clinical genotyping: the need for interrogation of single nucleotide polymorphisms and mutations in the clinical laboratory. Clin Chim Acta 2006; **363**: 127-37.
20) Rand KH, Delano JP. Direct identification of bacteria in positive blood cultures: comparison of two rapid methods, FilmArray and mass spectrometry. Diagn Microbiol Infect Dis 2014; **79**: 293-7.
21) Wagner FF, Bittner R, Petershofen EK, et al. Cost-efficient sequence-specific priming-polymerase chain reaction screening for blood donors with rare phenotypes. Transfusion 2008; **48**: 1169-73.
22) Jungbauer C, Hobel CM, Schwartz DW, Mayr WR. High-throughput multiplex PCR genotyping for 35 red blood cell antigens in blood donors. Vox Sang 2012; **102**: 234-42.
23) Gassner C, Meyer S, Frey BM, Vollmert C. Matrix-assisted laser desorption/ionisation, time-of-flight mass spectrometry-based blood group genotyping--the alternative approach. Transfus Med Rev 2013; **27**: 2-9.
24) Latini FR, Gazito D, Arnoni CP, et al. A new strategy to identify rare blood donors: single polymerase chain reaction multiplex SNaPshot reaction for detection of 16 blood group alleles. Blood Transfus 2014; **12** (Suppl 1): s256-63.
25) Politou M, Gialeraki A, Valsami S, et al. Integration in groups of donors may modify attitudes towards blood donation. Blood Transfus 2015; **13**: 336-7.
26) Myhre BA, McRuer D. Human error - a significant cause of transfusion mortality. Transfusion 2000; **40**: 879-85.
27) Dzik WS, Beckman N, Selleng K, et al. Errors in patient specimen collection: application of statistical process control. Transfusion 2008; **48**: 2143-51.

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