Background

Blood type determination is one of the most crucial components of the process for matching donor organs to transplant candidates. Failure to accurately identify blood type can have catastrophic consequences for organ transplant recipients receiving organs from a donor whose blood type has been determined or reported inaccurately. Thus, steps should be taken by members of the organ donation and transplantation community to educate themselves on the processes for blood type determination, the strengths and weaknesses of blood type testing methods, factors that can impact the reliability of blood typing, and steps that can be taken to evaluate and address those factors that impact ABO typing reliability to mitigate risks to transplant candidates awaiting lifesaving gifts.

OPTN policy requires that host OPOs ensure that two donor blood samples are used to determine blood type. Host OPOs are also required to develop and comply with written protocols to resolve any conflicts with primary blood type results and to verify key information, including donor blood type and subtype, prior to organ recovery. The policy does not address testing methods or additional factors that can affect blood typing results. This guidance document serves as a resource on blood type determination and includes additional methodologies and factors that should be considered when addressing indeterminate or conflicting blood typing results. It was developed in consultation with relevant subject matter experts, and stakeholders.

In 2014, the Operations and Safety Committee performed a Failure Modes and Effects Analysis (FMEA)[1], where all stages of ABO testing were extensively reviewed. Based on this analysis, there were ABO policy changes that were implemented. At the time, when there was no pre-transfusion specimen available for testing, the Committee's response was to create a policy requirement for Organ Procurement Organizations (OPOs) to have their own protocol. Recent reports of events affecting patient safety led to the decision to review the requirements for verifying deceased donor blood type. One of the events that led to the development of this guidance document centered on deceased donor blood typing results that were affected by massive blood transfusions.

The Committee agreed to take a holistic approach to consider all factors that might influence blood typing results. The Committee also believed that developing a comprehensive guidance document would be appropriate to help educate the community on additional methodologies and testing that could be considered when presented with indeterminate or conflicting blood typing results.

The Committee formed a joint Workgroup with representation from the following OPTN Committees: Operations and Safety, Membership and Professional Standards, Organ Procurement Organization, and Histocompatibility. The Workgroup also included blood bank experts.

The Workgroup discussed various topics to better understand the factors that can lead to indeterminate or conflicting blood typing results, and the current practices performed to resolve them.
The Workgroup first established goals that would be addressed in the guidance document. The Workgroup agreed that the guidance document should create awareness of the various factors that can contribute to indeterminate or conflicting results and the alternative methodologies that are available and should be considered to resolve these cases.

[1] November 12, 2014, OPTN Operations and Safety Committee Report to the Board of Directors. Available at https://optn.transplant.hrsa.gov

been drawn from the correct patient to prevent conflict that may have occurred due to possible sample labeling error.

Some OPOs have employed policies to re-draw donor blood samples after an interval of time has passed and have the samples re-tested for blood type. While this may resolve some conflicts it may not always be a reliable means since no criteria is known for determination of when a donor would revert to their natural blood type. Re-testing may result in further conflict or such a practice may result in blood type results that are no longer in conflict and enable more confidence in the original result.

The utilization of alternative (new) testing methods for determination of blood type DNA-based determination of blood type as described above could be an adjunct in efforts to resolve conflicting, discrepant or indeterminate blood type results.

As a last resort, when donor blood typing results remain in conflict and unable to be resolved, the safest course of action is to consider the donor to be blood type AB to ensure that only AB blood type candidates, as universally ABO compatible recipients, would be considered to receive the organs from that donor. This does however carry the consequence that urgently ill candidates in need of a lifesaving transplant may be excluded from consideration of the organs in such a scenario.

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Appendix

DNA-Based Determination of ABO

When the ABO gene was cloned in 1990, it was found that the genes for A the B glycotransferase enzymes differ by four single nucleotide polymorphisms (SNPs) in exon 7, designated according to the cDNA sequence as c.562C/G (p.176Arg/Gly), c.703G/A (p.235Gly/Ser), c.796C/A (p.266Leu/Met), and c.803G/C (p.268Gly/Ala). Group O, representing loss of transferase activity, most often resulted from a nucleotide deletion in exon 6, c.261delG (p.Thr88Profs*31), although a number of other genetic backgrounds have been reported.[24] To date, several hundred different ABO allele sequences have been catalogued by the International Society of Blood Transfusions (ISBT) Red Cell Immunogenetics and Blood Group Terminology working party, however this is not a comprehensive list and new alleles are still being discovered primarily associated with weaker than expected antigen expression (i.e. A and B subgroups) that can cause serologic typing discrepancies between forward and reverse ABO typing .[25] The ABO subtypes (e.g. A_2, A_{weak}, A_x, B_3, B_{weak}) are associated with genetic changes elsewhere in the coding, or less often regulatory, region of the ABO gene. Importantly, although numerous A and B alleles have been defined, the original four SNPs are the essential differences that distinguish the A and B phenotypes. Group O is most often associated with homozygosity for the nucleotide deletion in exon 6, c.261delG, although to date, at least 15 other genetic changes have been found to cause an O phenotype.[26] Methodologies for ABO genotyping target the A and B exon 7 SNPs along with one or more of the known O genetic changes. Some of the assays also include the more common A_2 subtype.

ABO genotyping by exon specific amplification and Sanger sequencing allows for unbiased evaluation of the ABO gene, enabling detection of rare and novel ABO genetic changes, although Sanger sequencing is unable to define the cis/trans haplotype phase of heterozygous changes. This can be overcome by using primers specific to A, B, or O alleles to amplify the target or in the sequencing reaction. For routine clinical sequencing, Sanger sequencing is performed for ABO exons 6 and 7, and when serologic reactivity suggests the presence of a subgroup phenotype as the basis for a discrepant forward and reverse type, the remainder of the gene is sequenced including promoter regions located upstream of the ABO gene within intron 1 associated with weakly expressed ABO subtypes.[27,28] Sanger sequencing is not scalable for testing large number of samples and the results require interpretation by subject matter experts.

One of the first ABO genotyping assays was based on polymerase chain reaction (PCR) amplification of ABO exons 6 and 7 followed by restriction fragment length polymorphism analysis (PCR-RFLP).[29] Since this PCR-RFLP assay can distinguish between A, B, and the two most common O genetic backgrounds it is still used by reference labs as an initial assay in ABO genotyping workups (only two American Association of Blood Banks (AABB) accredited reference laboratories in the United States do ABO genotyping) as RUO LDT testing. The PCR-RFLP assay requires subject matter expert interpretation of the restriction enzyme digestion patterns.

ABO genotyping methods targeting multiple SNPs have proven to be scalable, and reliable. For example, allele specific PCR using sequence-specific primers (PCR-SSP) have been developed to determine ABO genotype using by targeting the key ABO genetic changes.[28] These PCR based methods have also been extended to use real-time quantitative PCR to simplify detection and allow for automated software based interpretation.[30] One benefit of PCR based methods is that allele specific phasing reactions can be incorporated into them to define the cis/trans haplotype of important genetic positions. Recently the

use of a high density SNP array have also been reported for a scalable ABO genotyping method in large population level datasets capable of genotyping thousands of samples per batch.[31]

Several groups have recently published the use of both short and long read next generation sequencing (NGS) for ABO genotyping from whole genome sequencing, whole exome sequencing, and targeted NGS,[32–40] including the use of automated interpretive software.[32,35,36] One of the major advantages of NGS is that it allows for evolution of the entire ABO gene including novel genetic changes. In addition, in most cases short read NGS can fully phase the most import genetic changes, which when combined with long read NGS can fully phase the entire ABO gene. In addition, by running hundreds of samples per batch targeted NGS can reduce the per sample cost of ABO genotyping. However, current NGS methodologies still require several days for library preparation and sequencing.

Although, transfusion of red blood cells can interfere with serologic ABO typing, blood group genotyping, including ABO, has been shown to not be influenced by transfusion.[41–44] This is because blood group genotyping, like HLA molecular typing, is performed using genomic DNA isolated from recipient white blood cells which are generally not affected by red blood cell transfusion. However, in situation of granulocyte transfusion or stem cell transplant, ABO genotyping results need to be interpreted based on the clinical context.

ABO genotyping has proven to be highly accurate across methodologies, including some studies of deceased donors. Targeted NGS of just ABO exon 6 and 7 with automated software interpretation was 99.6% concordant to serologic ABO testing in 453 samples, with two discordances likely due to false negative serologic testing from weak expression.[34] NGS based whole exome sequencing with automated software interpretation of ABO exons and nearby intronic regions was 100% concordant with ABO serologic testing.[40] NGS based whole genome sequencing and automated software based evaluation of the entire ABO gene in 200 samples was 100% concordant with serologic ABO typing.[36] Targeted NGS of the entire ABO gene has also been applied to a set of 40 discordant serologic cases, in which it was able to explain the majority of discordances by identifying ABO alleles encoding ABO subtypes, weak ABO variants, hybrid ABO enzymatic activity, and novel genetic changes.[38,45] Most recently, targeted NGS of ABO exons 2 to 7 with automated software interpretation of 100 deceased donors was 100% concordant with serologic ABO typing.[46] Similarly, ABO genotyping with PCR-SSP and real-time PCR in 500 deceased donors was 100% concordant with ABO serologic typing and clarified discordant forward and reverse reactions, mixed field serology, and weak anti-A_1 lectin results.[47]

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