At a glance

Title: Guidance on Blood Type Determination
Sponsoring Committee: Operations and Safety

What is current practice and why address it?

Blood type determination is one of the most crucial components of the process for matching donor organs to transplant candidates. Failure to accurately determine blood type can result in significant adverse events, including graft failure or patient death. The intent of this guidance document is to increase patient safety and create awareness of the importance of addressing all issues that may affect the accuracy of blood type determination.

What's the guidance?

- Reviews **conventional methods** for blood type determination
- Identifies factors impacting blood type **reliability**
- Lists **acceptable** blood type and transfusion **sources**
- Identifies **alternative genetic testing methods** and when they should be used
- Outlines practices to resolve donor **blood type conflicts**

What's the anticipated impact of this document?

- **What it's expected to do**

Help OPOs and transplant programs:

  - Develop procedures when conventional methods of blood testing result in indeterminate or conflicting results
  - Identify triggers for when to use alternative testing methods
  - Identify practices to resolve conflicting blood typing results

- **What it won't do**
  - This guidance document is not a mandate. It provides recommendations for members to consider when resolving conflicting or indeterminate blood typing results.
  - It will not detail procedures for OPOs or transplant programs or take the place of clinical judgment on blood type determination.
  - It does not create or change OPTN policy. There is an accompanying proposal that addresses policy changes.

Themes to consider

- Living donor safety
- Recipient safety
- Organ Procurement Organization (OPO) and transplant program quality improvement

Terms you need to know

- **ABO Blood Type**: The classification of human blood into four groups: A, B, AB, and O
- **Blood Products:** Any therapeutic substance prepared from human blood. This includes: whole blood, blood components, and plasma
- **Conflicting:** Two blood tests from the same donor or candidate that present with different blood typing results
- **Indeterminate:** A blood test that does not provide a clear result
- **Protocol**: a predefined written procedural method
- Click here to search the OPTN glossary
- Click here to view *OPTN Policy 1.2: Definitions*

OPTN

Guidance Document for Public Comment

Guidance on Blood Type Determination

OPTN Operations and Safety Committee

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Contents

Guidance on Blood Type Determination

Sponsoring Committee: *Operations and Safety Committee*
Public Comment Period: *January 22, 2020 – March 24, 2020*

Executive Summary

The OPTN Operations and Safety Committee is charged with ensuring the safety of organ donation and transplantation processes. The Committee periodically reviews transplant and donation-related adverse events and near misses reported to the OPTN by the transplant community. The Committee uses the information to identify potential improvements and policy revisions that may prevent future such occurrences.

Recent reports of events affecting patient safety led to the decision to re-evaluate the requirements for verifying donor blood type. The Committee agreed to re-evaluate the ABO policy and determine the best strategy to address recent concerns. The Committee agreed to develop both a guidance document and policy language.

This guidance document does not create or change OPTN policy. The intent of this guidance is to improve patient safety by providing the transplant community with a resource that offers alternative solutions to address conflicting and indeterminate blood type results. There is an accompanying proposal that addresses policy changes.

This guidance document seeks to:

1. Alert OPOs of triggers and other factors that can cause blood typing discrepancies
2. Identify alternative methods of blood typing in addition to what benefits would be, how widely available they are, and their accuracy
3. Provide best practice processes to address conflicting and indeterminate blood type results

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

There are various factors other than massive transfusions that attribute to indeterminate or conflicting blood type results. The Workgroup discussed the triggers that can contribute to indeterminate or conflicting blood type results. There was also discussion around the inability to determine a timeframe for when the patient's true blood type is measured after having a massive blood transfusion.

The Workgroup discussed in great detail the reliability of historical blood type results. It was agreed that there needs to be a current blood sample and that the protocol per OPTN Policy of drawing two blood samples is standard. Historical information would be important to use as a reference, but clinical decisions should not be based on this information alone.

This guidance document supports the OPTN Strategic Plan of promoting living donor and transplant recipient safety.

Conventional Methods for ABO determination

ABO blood type testing is generally performed using one of three methodologies: tube, gel, or solid phase. Tube methodology is a manual method using separate test tubes for each reaction. Gel column agglutination methodology uses gel or glass beads. Red blood cells and antibodies are combined in microtubes filled with gel matrix, then centrifuged to force the red blood cells through the column. Agglutinated (or clumped) cells remain trapped at the top of the gel column, while non-agglutinated cells travel through to the bottom. In solid phase methodology, A and B antigens or antibodies are adherent to microtiter wells, and red blood cells or serum is added. After washing, indicator red blood cells coated with anti-Immunoglobulin G (IgG) are then added to determine if agglutination occurred. Various platforms have been developed for automation or semi-automation of gel and solid phase methods.

For each of these methodologies, ABO blood group is determined by performing both a forward and reverse blood type. The blood sample is first centrifuged to separate the red blood cells from the plasma or serum. For the forward blood type, red blood cells are combined with reagent anti-A, anti-B, and anti-D antibodies in three reactions to determine the presence of ABO and RhD antigens. The reverse blood type uses the patient's plasma or serum, combined with reagent group A and group B red blood cells, to determine which ABO antibodies are present.

The forward and reverse blood type results should be consistent in order to report the final blood type. If there is a discrepancy between the forward and reverse blood type results, the cause of the discrepancy should be determined prior to reporting a blood type. If the discrepancy cannot be resolved, most transfusion services will treat the patient as blood type O for transfusion purposes until the correct blood type can be determined.

Factors Impacting Blood Typing Reliability

Several clinical situations may result in unreliable serologic blood typing which can lead to mixed field reactions or discordances in the forward and reverse blood typing.

1. **Transfusion:** Patients who receive type O transfusions in emergency situations will often develop a mixed field or discordant typing. Forward typing (patient RBC mixed with commercially available antibody) will be mixed field or non-agglutinated due to the transfused type O red cells, whereas reverse typing (patient plasma mixed with commercially available reagent RBCs) will detect the patient's native anti-A or anti-B antibodies, leading to discordant or indeterminate reports.

   Although case reports have described transfusion impacting a patient's blood type on a temporary basis, there is no information in the literature regarding a time frame post transfusion in which there could be certainty that the blood type results are reliable and no longer impacted by the transfused cells.

2. **ABO Non-identical Stem Cell Transplant:** Patients who have received stem cells from a donor with a different blood type will display a mixed blood type until full engraftment occurs. After engraftment, they will display a different blood type in circulating whole blood from that of the organ allografts.

   Organ donors who have previously received stem cell transplants should be given careful consideration. This information needs to be considered by the OPO medical director and histocompatibility lab. Peripheral blood is typically used for tissue typing on a deceased donor, but in these patients who are prior recipients of stem cells, buccal swabs or lymph nodes need to be utilized to determine both blood type and Human leukocyte antigen (HLA) since the organs and tissues will be incongruent from circulating blood.

3. **Infections and Cancers:** While uncommon, some patients develop an "acquired B" phenomenon as a result of a bacterial infection or malignancy. The underlying infection can cause enzymatic alteration of the group A antigen on cells, and can result in the formation of a "B-like" antigen and discrepant blood type testing. This has been described in patients with specific *Escherichia coli* infections as well as in patients with malignancies of the stomach and intestine.[2] In addition, neonates with Necrotizing Enterocolitis due to *Klesbiella pneumonia* have been inadvertently assigned as blood type B due to the "acquired B" phenomenon.[3] It should also be noted that detection of acquired B is dependent on the anti-B clone used and reagent pH.

4. **Elevated Globulin Levels:** Patients with multiple myeloma, amyloidosis, hyperfibrinogenemia, Waldenstrom macroglobulinemia, plasma cell disorders or those who receive plasma expanders, such as dextran, may display a protein to plasma abnormality. This can lead to rouleaux formation and false appearance of agglutination on forward typing that may be inconsistent with reverse typing.[4]

5. **A_{weak}, B_{weak}, and Blood Type Subgroups:** Antigen expression can become so weak that it is not detected by forward typing, with no natural antibodies present on the reverse reaction. In addition, some subgroups may not express some forms of the blood type red blood cell (RBC) antigens, which can cause discordant forward and reverse patterns. For example, patients with type A_2 may possess anti-A_1 antibody (estimated in 1-8% of A_2 individuals and 22-35% of AB individuals) [5], which would render the reverse typing discordant from forward typing. Such patients would display forward type of A, but reverse type of O in the event that antibodies to A_1 are present in the type A_2 patient.[6]

6. **Age:** Patients that are very young or elderly may have weakly reacting antibodies, or missing antibodies that renders the blood typing incongruent.

   It is well described that while newborns express A and B blood type antigens, which would be detectable on forward typing, they do not produce antibody to blood types until 3-6 months of age. Until this age, the blood type antibodies present are maternal from placental transfer.[7] Newborns

should only be typed using forward typing, as reverse typing may result in discordant or unreliable results. If a newborn has received any type O transfusion, extreme caution should be exercised with regard to organ donation, as the newborn can be incorrectly typed as O using forward typing only.

Similarly, elderly patients may not possess enough antibody for reliable reverse typing, resulting in discordance. In one study, a 66 year old otherwise healthy patient was deemed to be type O on forward typing, but did not show anti-A or anti-B on reverse typing until the amount of serum utilized in the testing was doubled.[8] This would result in a discordant forward type O and reverse type A, B, or AB.

7. **Immunosuppression:** Patients severely immunocompromised, due to disease, therapy, or depressed immunoglobulin levels may not mount an appropriate amount of antibody to reliably perform reverse typing, for the same physiologic reasons mentioned above.[9]

Acceptable Blood Type and Transfusion Sources

OPOs rely on a number of potential sources for donor blood type testing. Commonly these may be the donor hospital blood bank, the OPOs contracted infectious disease laboratory and/or the OPOs tissue typing laboratory. OPTN Policy requires at least two sources of ABO type from donor samples drawn at separate times and ideally these samples would be obtained prior to transfusions which may impact blood typing results. If a potential donor was treated at another hospital prior to transfer to the donor hospital or recovery center that originating hospital may have pre-transfusion samples available for testing.

All known and available blood type results of the donor should be reviewed to ensure there are no conflicting results. To have, for example, two recent blood typing results that are in conflict with a historic blood type from a previous hospital admission should call into question the reliability of blood typing results and action must be taken to resolve this conflict.

Though historical blood typing results may be available from past hospitalizations these results may be used only as a means of confirming blood typing performed during the donor's current admission course rather than as a primary source of the donor's blood type. The best source of ABO typing by blood sample is ideally a sample obtained prior to the donor receiving blood transfusions.

As referred above in the section titled "*Conventional Methods for Blood Type Determination*", donor blood typing determination performed by hospital blood banks considers the perspective of the patient as a blood product recipient. Thus, if there are discrepant forward and reverse blood typing results the blood bank may err on the side of assigning the result as blood type O to ensure the patient would receive blood type compatible O blood transfusions. This of course creates a concern if that patient then becomes an organ donor and the reliability of donor blood type may be in question.

When considering the reliability of blood type results transfusion history must be considered as it can impact the reliability of such testing. It is important for OPOs to consider what blood products the donor may have received in all phases of the admission course, including pre-hospital or any other hospitals where the patient may have been treated prior to a transfer to the donor hospital or recovery center.

Alternative (new) Testing Methods for Determination of Blood Type: DNA-based Determination of Blood Type

Since the early 1900s, blood typing has been performed by serological methodology.[10] This has consisted of a forward and reverse typing which together are evaluated and must agree to give a valid blood type phenotype. However, when patients have been transfused out of their own blood type, or discrepancies between the forward and reverse typing or mixed field typing is seen, DNA based testing may be considered.

Advances in technology allow for blood type genotyping using molecular methods. These include:

- Sanger sequencing
- Polymerase chain reaction (PCR) with restriction fragment length polymorphism analysis (PCR-RFLP)[11]
- PCR using sequence-specific primers (PCR-SSP)[12]
- Real-time quantitative PCR[13]
- High density DNA arrays[14]
- Next generation sequencing (NGS)[15-23]

All of the above genotyping methods originated under research protocols as research use only (RUO) and have been implemented for clinical testing in a small number of large reference labs as laboratory in house developed assays (LDT) evaluated in concert with serologic reactivity of the sample. This has limited the number of labs capable of performing ABO genotyping. However, recent vendor supplied kits have been developed for blood type genotyping using PCR-SSP, real-time PCR, and targeted NGS. Importantly, all of these vendor supplied kits use techniques and instruments already employed by most tissue typing labs. As such, the PCR-SSP and real-time PCR methods are of particular importance for deceased donor testing, since they can be done within the required time constraints. Of these, real-time PCR is the most attractive method for deceased donors since it has a streamlined assay setup and the PCR products are detected by the instrumentation allowing for automated interpretation by vendor supplied software. Real-time blood type genotyping could be routinely performed alongside existing histocompatibility typing lab workflows on deceased donors to resolve serologic forward and reverse blood typing discordances, help interpret mixed-field reactions, and when evaluated with serologic blood typing by subject matter experts can resolve the inherited blood type, especially in situations of massive red blood cell transfusion.

Further information can be found in the appendix.

Triggers for When to Use Alternative Methods

In those circumstances where blood typing results may be in question OPOs should perform a thorough review of all results, including the specific forward and reverse typing results, to ensure there are no discrepancies or unreliable results.

Certainly in situations where there are conflicting donor blood typing results OPOs are required to have written protocols in place to attempt to resolve the conflicting results.

More importantly, in the circumstances where a donor has received blood products prior to the availability of required samples for donor blood typing, the potential impact on post-transfusion results should be considered.

In any circumstances where there are blood typing results received by the OPO that are "Indeterminate" due to conflicting forward and reverse blood typing, all results should be reviewed. These results should be viewed in conjunction with transfusion history, donor medical history and admission course for factors that may have led to an indeterminate result which then may call into question other results received.

Practices to Resolve Donor Blood Type Conflicts

There are a variety of practices employed by OPOs to resolve conflicting or indeterminate donor blood typing results.

Resolution of indeterminate results may be achieved with a review of donor transfusion history and review of blood type forward and reverse typing results. In some scenarios a donor may express blood type O by forward typing and a different blood type by reverse typing when the donor has received un-crossmatched blood type O transfusions which can convolute the forward typing result.

For example, if a donor receives massive transfusions of blood type O packed red blood cells (PRBCs), then blood type forward-typing indicates blood type O with reverse-typing indicating blood type A, it is likely the blood type O blood transfusions have affected the forward typing by reflecting the blood type of the transfused PRBCs. In such a scenario, the safest course of action is to conclude the donor is blood type A. Concluding that the donor is blood type O in error would potentially expose transplant candidates to organs that are incompatible for transplant. By concluding the donor is blood type A in this scenario (subtyping in this scenario would not be an option) then all candidates matched to the donor would be ABO type A or AB (or Platelet Transfusion Refractory (PTRs) listed as accepting organs of incompatible blood type as allowed by policy).

It is best in such scenarios to consult with blood banking physicians and scientist experts to review the entirety of the circumstances, donor medical history, transfusion history and blood type results to ensure the safest course is followed when the final determination of donor blood type is made. If there is doubt about the conclusions of donor blood typing, extreme caution should be exercised to avoid the possibility of exposing candidates to such risk.

Conflicting blood typing results are certainly the more concerning scenarios OPOs may face. In the event the donor blood typing by one lab or blood draw time is conclusive but conflicting with the conclusive results of another lab result or result on a donor blood sample drawn at a different time, the OPO should review of donor transfusion history and review of all forward and reverse blood type results obtained to determine the source of the conflict. The reliability of the blood sample source must also be called into question in such a scenario. OPO Medical Directors and Blood Bank Experts should be consulted to investigate the source of the potential error.

OPTN policy requires that blood type be determined using two blood samples drawn at separate times. The purpose of this requirement is to confirm blood type determination and ensure that samples have

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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