Guidance for ABO Subtyping Organ Donors for Blood Types A and AB

Contents

Summary

The “Guidance for ABO Subtyping Organ Donors for Blood Groups A and AB” was originally developed in 2011. This revised guidance contains updates to OPTN Policy references and additional resources. It provides amended information about special considerations such as neonates. The guidance document is one of several resources to assist OPTN members with subtyping questions and practices. The goal is to continue expanding organ access for candidates through transplanting non-A_1 donors.

Key Points

- Persons who are in primary blood groups (also known as blood type) A and AB can be further tested to determine a more specific subgroup (also known as a subtype).
- Subtypes for blood type A include A_1, A_2, A_x, A_{int} and others, but the most common is A_1.
- If the donor is **not** subtype A_1, it means they have less A antigen on their red blood cells (RBCs) and organs, which allows them to donate to recipients outside of their primary blood type.
- OPTN Policies refer to all subtypes that are not A_1 as non-A_1. Therefore, a donor who is primary blood type A and subtyping results show that the donor does not have the A_1 subtype is referred to as having blood type A, non-A_1.
- When reporting to the OPTN Contractor, A_2 is used as shorthand for any blood type A subtype other than A_1 (i.e. non-A_1, negative for A_1). A_2B is used as shorthand for any blood type AB subtype other than A_1B (i.e. non-A_1B, negative for A_1B).
- Perform subtyping before the donor receives any RBC transfusions.
- ***Any*** blood transfusion can affect the accuracy of subtyping results despite the donor’s hemodilution status.
- OPOs and transplant hospitals should consult with their blood banks to consider special issues that might impact results (such as RBC transfusion and neonates) and follow their recommendations.
- It is never acceptable to use two out of three results for a subtype determination. If there are any discrepant results, then *only* primary type can be used for allocation.
- There are no standards for how laboratories should report ABO subtypes. The International Society for Blood Transfusion Committee on Terminology for Red Cell Surface Antigens has created a standardized numerical format for reporting red cell subtypes, but this is not suitable for everyday communication. Popular terminology often uses terms: A_1, A_2, A_1B, and A_2B. OPTN Policies use the term non-A_1 for any subtype that is not A_1.
- When reviewing results, be cautious not to confuse the Rh factor result with the ABO subtype result.
- Routine testing determines the presence or absence of the A_1 antigen only. It does not determine the actual sub-type. Typing is either positive for A_1 or negative for A_1. This is why the term A, non-A_1 is used for a donor that does not have the A_1 antigen.

- The absence of A_1 does not necessarily equal A_2 due to the existence of multiple subtypes.
- **Subtyping results must not be reported to the OPTN Contractor or used for organ allocation when:**
    - The results do not match or indicate the same result. It is never acceptable to use two out of three results. If one result is different, then only primary type must be reported and used for allocation.
    - Pre-RBC transfusion specimens are not available for initial and/or confirmatory testing of subtyping.

What is required by OPTN Policy?

The OPTN has several policies that allow the transplantation of donors who have a non-A_1 subtype into candidates of other blood types. These policies include:

- *Policy 9.7.B: Points Assigned by Blood Type*
- *Policy 8.5.D: Allocation of Kidneys by Blood Type*
- *Policy 13.7: OPTN KPD Screening Criteria*

Requirements for Blood Type Determination

Clinical policies and information about how to perform transplants that are primary blood type incompatible but are done with certain subtyping results are determined by the transplant program. When these types of transplants are planned, the OPTN has policies about how to determine a subtype for a deceased donor (*Policy 2.6.B: Deceased Donor Blood Subtype Determination*) or a living donor (*Policy 14.5.B: Living Donor Blood Subtype Determination*). OPTN policies mandate that all deceased and living donors, as well as candidates, be blood typed on two separate occasions, and these rules apply to subtyping as well. By definition, this means the blood must:

1. Be drawn on two separate occasions
2. Have different collection times
3. Be submitted as separate samples
4. Have results indicating the same blood type

Requirements for Blood Subtype Determination

If testing determines that a deceased donor's primary blood type is A, then you must also subtype that donor. The only exception to this rule is when no blood samples are available before the donor is given red blood cell (RBC) products. Subtyping is optional for living donors and blood type AB deceased donors. If the donor is found to be blood type A, non-A_1, a second subtype must be drawn (different draw time, different draw occasion) for confirmation. It is important to note that:

All subtyping for deceased or living donors must be completed before the donor receives any red blood cell transfusions.

What is a Subtype?

We are all familiar with the blood types A, B, O and AB.

Enzymes that add sugars to form either the type A or the type B antigens determine the blood type. Individuals who are blood type O lack the enzyme to add those sugars and have an H precursor substance that gives them their O blood type. You can find blood type antigens on many cells, including RBCs and cells inside blood vessels of all vascular organs that are routinely transplanted. The reason these blood type antigens are clinically important in transplantation and blood transfusion is that individuals have naturally occurring antibodies to blood type antigens they do not have. Those antibodies are termed isoagglutinins. Isoagglutinins are antibodies that can react with the blood type antigens on the cells of the organ being transplanted. For instance, blood type O individuals have A and B isoagglutinins, blood type B individuals have A, blood type A individuals have B, and blood type AB individuals have no isoagglutinins.

When an incompatible transplantation takes place, such as transplanting a blood type B organ into a blood type O individual, that organ would likely be rejected immediately. The rejection occurs because the B isoagglutinins in the blood type O recipient react with the B antigens on the vessels of the transplanted organ.

Eighty percent of blood type A and AB persons are subtype A_1 and A_1B, respectively. The other 20% of these blood types are subtype non-A_1. Most often the subtype is A_2 (or A_2B), but occasionally it may be a more rare subtype like A_3, A_{int}, etc. Blood type A, non-A_1 individuals express only about 20% of the

normal level of type A antigen on their RBCs and organs. A_1 subtyping is not routinely performed in compatibility testing; however, some patients and donors may be identified as A, non-A_1 or AB, non-A_1B in the course of routine blood bank typing because they have anti-A_1 antibody in their plasma (1-8% of type "A, non-A_1" and 25% of type "AB, non-A_1B" persons[1]).

Why does it matter?

An ABO subtype (A_1 vs. A, non-A_1) allows organs to be allocated to additional candidates for both deceased and living donor transplants. A person who is primary blood type A normally could not donate their organ to a candidate who is blood type B. If the person who is blood type A also has a non-A_1 subtype, then they could possibly donate a kidney to a person who is primary blood type B (or O), depending on other factors.

When should we use and not use subtyping results for allocation?

Subtyping results can only be used when both samples were obtained before any RBC transfusions, and subtype testing results (both initial and confirmatory) are clear, valid, and match each other. You must not use subtype testing if you question the validity or interrelation of the ABO subtype testing results or if pre-transfusion specimens are not available for both initial and confirmatory subtyping testing. In these situations, the safest approach is to allocate the organs based on the donor's primary blood type only. It is inconsistent with OPTN Policy to use two out of three results if even one of the results does not indicate the same subtype. Conflicting results include those from transplant hospital or another lab if reported to the OPO prior to or during allocation.

What can interfere with test results?

If a donor recently received an RBC transfusion, the A_1 subtyping result may be inaccurate and therefore you should obtain all subtyping samples before RBC transfusions occur. Plasma and platelet transfusions do not affect RBC typing results.

For example, if you gave an organ donor an emergency blood type O RBC transfusion before you collected the subtyping specimen, then the A_1 typing could be inaccurate. Experiments with *in-vitro* mixtures of blood type O and A_1 RBCs suggest that A_1 typing could become falsely negative if more than 75% of the RBCs are type O [2]. Since it is difficult to estimate precisely how many units of blood type O RBCs need to be given to affect the efficacy of the test (as this depends on the patient's size, amount and rate of blood loss, timing of the transfusions and intravascular volume status) you must obtain all samples before RBC transfusion.

In the event that the potential donor received a RBC transfusion in the past (as opposed to the current hospitalization), then OPOs and transplant hospitals must determine the time, if any, since transfusion that they consider safe to perform subtype testing. Currently no data identifies how many blood type A RBC transfusions it may take to change the subtyping result from non-A_1 (A_1 negative) to A_1. Transfused RBCs have a half-life of 30 days and the "youngest" RBCs in the blood bag would circulate for up to 120 days.

Infants and Neonates

Neonates and infants do not fully express their ABO antigens. Manufacturers of anti-A_1 lectin also have varying warnings in their package inserts such as "results should be interpreted with caution in infants less than one year of age"[3] and "cord blood and specimens from infants cannot be accurately typed with anti-A1 lectin since the A1 antigen is not fully developed on red blood cells until the age of six months"[4].

[1] John Roback et al., eds., AABB Technical Manual 17th edition (Bethesda, MD: AABB, 2008).
[2] Glenn Ramsey et al., "Abstract Presentations from the AABB Annual Meeting and CTTXPO, Baltimore, MD, October 9-12, 2010," Transfusion 50 (2010): 168A.
[3] Anti-A1 Lectin Package Insert, Core Diagnostics LTD.
[4] IFY H241Dolichos-A1 Lectin Anti-A1 Ver 1.0 2015, Hemo Bioscience

Umbilical cord blood is another consideration for neonates and it is generally recommended that you not use cord blood cells to determine primary ABO or subtype. OPO and transplant programs should consult with their blood banks to consider this issue and adjust practices accordingly.

What do the results say?

The wide range of terminologies used by blood banks and manufacturers to describe subtyping results is confusing. It is particularly confusing when transplant programs or OPOs need to identify the accurate subtyping for transplant compatibility. As mentioned earlier, the actual subtype test looks for whether a blood type A or AB donor's RBCs react with anti-A_1 lectin. The following tables provide subtype terminology used by the OPTN, along with synonymous terms that you may also see on typing results.

Blood Type A Subtype Reporting Terminology:

OPTN	**A_1**	**A, non-A_1**
Other terms used	A_1 positive	A_1 negative
	A_1 reactive	Non A_1
	-	A_2

Blood Type AB Subtype Reporting Terminology:

OPTN	**A_1B**	**AB, non-A_1B**
Other terms used	AB, A_1 positive	AB, A_1 negative
	AB, A_1 reactive	AB, A_1 non-reactive
		A_2B

When reporting to the OPTN Contractor, A_2 is used as shorthand for any blood type A subtype other than A_1 (i.e. non-A_1, negative for A_1). A_2B is used as shorthand for any blood type AB subtype other than A_1B (i.e. non-A_1B, negative for A_1B).

Common issues in subtype reporting:

Some of the most common issues found in reviewing and reporting subtype results include:

- Unclear subtyping results
- Uncertainty whether the term negative or positive refers to Rh factor or subtype
- Discordant results (results that do not seem to indicate the same subtype)
- Not knowing the time of the last transfusion
- Not knowing if the donor had a transfusion at a prior health care facility or in transit

What should I do when I am not sure how to report results?

A patient's age, transfusion status, and testing methods of the laboratory can all affect the efficacy of the test. If you have questions about how to interpret subtyping results or whether testing was performed accurately, your safest approach is to report and allocate the organs based on the donor's primary blood type and not to consider subtyping. For all blood type A donors, the host OPO must document either that subtyping was completed or the reason it could not be completed.

More technical information about subtyping

Determination of a donor's A_1 RBC subtype is performed with anti-A_1 lectin, an FDA-approved test reagent. Lectins are non-antibody proteins, which bind with high specificity to a particular carbohydrate structure. Anti-A_1 lectin is extracted from the lentil-like seeds of the plant Dolichus biflorus (horse gram). Anti-A_1 lectin binds to the A_1 carbohydrate and agglutinates A_1 or A_1B RBCs in a suspension. When type A or AB RBCs are not agglutinated by anti-A_1 lectin, the RBCs are negative for A_1.

Strictly speaking, there is no (non-DNA) test for the A_2 antigen— only a test for whether the A_1 antigen is present or not. Therefore, when a blood type A donor does not test positive for A_1 the result is called an A, non-A_1. Other type A variants exist. One type A variant called A_{int} (intermediate) is partway between A_1 and A_2 in strength and can give weak reactions in A_1 typing. A_{int} is found most often in blood type A African-Americans (5-8%). All of the other type A variants, such as A_3, A_{end}, and A_x, are rarely seen (<1:1000 type A persons) and are much weaker in expression overall than type A, non-A_1, and therefore presumably would be equivalent to non-A_1 for organ-transplant purposes. Laboratories using anti-A_1 lectin testing should follow the manufacturer's directions carefully.

From the perspective of transplant safety, **any** reaction with anti-A_1 lectin, when performed according to the manufacturer's directions, should be regarded as positive or reactive for A_1. This would not be considered safe for potential use as a non-A_1 donor by the transplant program, unless proven otherwise. OPO and transplant programs should consult with their blood banks if needed regarding guidance or additional questions.

Who can help?

Your local blood bank is a great resource. OPOs and transplant hospitals should establish a relationship with them as they can advise on protocol development and answer questions. Other national groups such as the American Association of Blood Banks (AABB) (http://www.aabb.org/Pages/default.aspx) have additional resources.

Resources to learn more

- UNOS Connect, the location for all your transplant education materials, is available at:

  https://unosconnect.unos.org/

Two courses are available on UNOS Connect that relate to subtyping:

- ABO Subtyping (SFT 116)
- ABO Typing and Subtyping (SYS104)

- OPTN/UNOS "Guidance for Transplant Program Participation in the Transplantation of Non-A1/Non-A1B (A2/A2B) Donor Kidneys into Blood Group B Candidates" is available at: https://optn.transplant.hrsa.gov/resources/guidance/
- You can also find resources organized by specific organ types:

  Kidney and pancreas
  Liver and intestine
  Heart and lung
  Vascularized composite allograft