Guidance for ABO Subtyping Organ Donors For Blood Groups A and AB

Purpose

The ABO Subtyping Work Group of the Operations and Safety Committee has developed this document to assist transplant professionals in understanding ABO subtyping practices and terminology.

Introduction

This document addresses the laboratory testing, interpretation, and reporting of ABO blood subtype status (A_1 and A_2) in blood group A or group AB organ donors. It is common practice in many transplant centers, donation service areas (DSA) and organ procurement organizations (OPO) to subtype donors as A_1, A_1B, A_2 or A_2B. It is important to know that the technically accurate term for A_2 and A_2B donors is "A_1-negative" or "A, non-A_1" because A_2 is not directly tested for and many other rare subtypes exist (e.g. A_3, A_{int}, etc.). Blood group "A, non-A_1" organs are transplanted in many centers into blood group O or B candidates, and blood group "AB, non-A_1B" organs into blood group B candidates. The Organ Procurement and Transplantation Network (OPTN) has had, since 2002, a voluntary variance that allows the transplantation of blood group "A, non-A_1" kidneys into B candidates. As a part of its liver allocation system, the OPTN allows for transplantation of livers from donors that are blood group "A, non-A_1" into O candidates. Clinical policies and practices as to whether, when, and how to perform these transplants are determined by the transplant center, DSA, OPO, and their physicians, and are not intended to be in the scope of this document. This blood group A subtype terminology (A_1 and non-A_1) should not be confused with the Class I histocompatibility antigens HLA-A1 and HLA-A2.

ABO Blood Group: Background

We are all familiar with the blood groups O, A, B, and AB. The OPTN mandates that all deceased and living donors as well as candidates be ABO typed on two separate occasions. Blood types are determined by enzymes that add sugars to form either the group A or the group B antigens. Individuals who are blood type O lack the enzyme to add those sugars and have an H precursor substance that gives the individuals their O blood type. Blood group antigens are found on many cells, including red blood cells (RBCs), and cells inside blood vessels of all vascular organs that are routinely transplanted. The reason these blood group antigens are clinically important in transplantation and blood transfusion is because individuals have naturally occurring antibodies to blood group antigens they do not have. Those antibodies are termed isoagglutinins. 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. To explain, when an incompatible transplantation takes place, for instance a blood type B organ transplanted into a blood type O individual, that organ would likely be rejected immediately due to B isoagglutinins in the blood type O patient reacting with the B antigens on the vessels of the transplanted organ. Isoagglutinins are antibodies that can react with the blood group antigens on the cells of the organ being transplanted.

Eighty percent of blood group A and AB persons are subtype A_1 and A_1B, respectively. The other 20% of these blood groups are subtype "non-A_1," most often A_2 (or A_2B), but occasionally a more rare subtype (e.g. A_3, A_{int}, etc.). Blood group "A, non-A_1" individuals express only about 20% of the normal level of group A antigen on their RBCs and organs. A_1 phenotyping 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 group "A, non-A_1", 25% of group "AB, non-A_1B" persons) (American Association of Blood Banks (AABB) Technical Manual, 2008).

Testing for the A1 Phenotype

Determination of a donor's A_1 RBC phenotype 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 group 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 blood group A donor does not test positive for A_1 it is called a non-A_1.*

Other Group A Variants

One group 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 group A African-Americans (5-8%). All of the other group A variants, such as A_3, A_{end}, and A_x, are rarely seen (<1:1000 group A persons) and are much weaker in expression overall than group non-A_1, and therefore presumably would be equivalent to A_2 for organ-transplant purposes. Laboratories using anti-A_1 lectin should follow the manufacturer's directions carefully. From the perspective of transplant safety for the use of non-A_1 organs, any RBC reaction with anti-A_1 lectin, as performed according to the manufacturer's directions, should be regarded as A_1-reactive by the transplant center, unless proven otherwise.

Inaccurate A1 Typing in Transfused Patients and in Infants

Recent RBC transfusions may cause inaccurate A_1 phenotyping, and therefore pre-RBC-transfusion specimens obtained before RBC transfusions are given should be used for A subtyping. (Plasma and platelet transfusions would not affect RBC typing results.) Some organ donors may have been given emergency group O RBCs before a blood bank specimen was collected. Experiments with *in-vitro* mixtures of group O and A_1 RBCs suggest that A_1 typing could become falsely negative if more than 75% of the RBCs are group O (Unpublished, Ramsey et al., Transfusion 50(2S):168A, 2010). However, it is difficult to estimate precisely how many units of group O RBCs this might represent, depending on the patient's size, amount and rate of blood loss, timing of the transfusions, and intravascular volume status.

Neonates and infants do not fully express their ABO antigens, and all manufacturers of anti-A_1 lectin caution against inaccurate false-negative results in these patients.

A_1 and "non-A_1" Terminology

Blood banks and manufacturers use many different and even confusing terminologies for results of A subtyping. This can cause confusion when transplant centers, DSAs, or OPOs need to identify the accurate subtyping for transplant compatibility. As discussed above, the actual subtype test looks for whether a blood group A or AB donor's RBCs react with anti-A_1 lectin. The following columns can be regarded as synonyms:

Blood Group A Subtype Determination	
A_1-reactive group A, synonymous with:	**A_1-nonreactive group A, synonymous with:**
A_1-positive	A_1-negative*, or non-A_1
A_1	A_2

* "Negative" does not refer to Rh type

Blood Group AB Subtype Determination	
A_1-reactive group AB, synonymous with:	**A_1-nonreactive group AB, synonymous with:**
Blood Group AB, A_1-positive	Blood Group AB, A_1-negative*, or non-A_1B
A_1B	A_2B

*"Negative" does not refer to Rh type

The term "A_2-negative" should not be used. In case of any ambiguity in a donor's A_1 typing report, the transplant center should obtain clarification from the donor facility and the testing laboratory.

Frequently asked Questions regarding ABO subtyping

What is the difference between the term subgrouping and subtyping?

In the laboratory, "subgroup" properly refers to the classification of ABO phenotypes (e.g., A_1, A_2, A_{int}, etc.), and "subtype" refers to the serological testing process that is performed to identify ABO phenotypes (e.g., typing for the blood group). However, these two terms have become interchangeable in common usage. AABB publications use the term A_2 subgroup.

Are there issues with testing samples that are hemodiluted?

Blood bankers use the term pre-transfusion to mean "before any transfusions of red blood cells or red cell containing products." They do not use the term "non-hemodiluted specimen" because it implies that a little transfusion may be okay, but that is not the case. If the patient has received even just one unit of red blood cells, then the subtyping results could be affected.

What are the most frequent sources of errors in ABO subtype testing and reporting?

Factors affecting subtyping testing results include RBC transfusion and the possibility of specimen mixup or mislabeling. In the laboratory, Bryan, et al, noted an increased testing error rate for A_2 subtyping compared to regular ABO typing, and suggested that genetic variations and differences in reagents might contribute to variability in test results (Transplantation 82:733, 2006). After testing is completed, laboratories which do blood type A or AB subtyping infrequently may not have established a uniform format in their standard operating procedures (e.g., a "free-text" report versus computerized report terms). In addition, false-negative subtyping results may be seen when testing neonates and infants.

What do I do if I cannot obtain a pre-transfusion sample(s) for testing?

When pre-transfusion specimens are not available for initial and confirmatory testing of subtyping, the safest approach to placing organs from donors that have received blood

transfusions is to allocate the organs based on the donor's primary blood type without consideration of subtyping.

Is there a time between RBC transfusions that could be considered as "safe" to subtype donors or candidates and receive an accurate subtype result?
There is currently no data identifying how many group 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 up to 120 days. If group A RBC transfusions have taken place and the subsequent subtype is A_1, the safest thing to do is to allocate donor organs as an A. Conversely, if a group A_1 donor has received multiple emergency group O RBCs, there is danger of a false-negative A_1 typing; again, if no pre-transfusion specimen is available, the safest thing to do is to allocate donor organs as group A.

What does negative for A_2 mean?
Strictly speaking, there is no test for the A_2 antigen—only a test for whether the A_1 antigen is present or not. The terms "negative for A_2" or "A_2-negative" should not be used.

Are there standards for laboratory reporting of ABO subtyping?
There are no standards for laboratory reporting of 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 phenotypes, but this is not suitable for everyday communication. The recommendation for "popular" terminology is A_1, A_2, A_1B, and A_2B. AABB is currently considering standardizing the nomenclature for future use.

If I am unsure about the interpretation of a laboratory report on ABO subtyping, what should I do?
The laboratory may be able to clarify a specific result and/or address its reporting format for future subtyping results. When there are questions about how to interpret subtyping results or whether testing performed is accurate, the safest approach is to allocate the organs based on the donor's primary blood type without consideration of subtyping.

Should there be consideration of molecular testing for subtyping when pre-transfusion specimens are not available?
An A_1/A_2 distinction post transfusion may be impossible by serology but is possible by molecular techniques. However, molecular typing is not routinely available except in reference laboratories. Few centers have ready access to this type of testing due to time constraints of the organ allocation process.