Detection and Identification of alloantibodies to Red Cell Antigens
Antibody Screen Test

Iranian Blood Transfusion Organization

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Detection and Identification of unexpected Antibodies to Red Cell Antigens

Requirements of IBTO (AABB) Standards for a Blood Transfusion Request

1. Positive identification of the recipient and the recipient blood samples.

2. ABO group and Rh typing of the recipient's blood.

3. Red cell antibody detection tests for clinically significant antibodies using the recipient’s serum/plasma (Antibody Screening).

4. Comparison of current findings on the recipient’s sample with records of previous results.
Detection and Identification of unexpected Antibodies to Red Cell Antigens
جستجو و شناسایی آنتی بادیهای غیرمنتظره عليه آنتی زندهی گلوبول قرمز خون

5. Confirmation of the ABO group of red cell components.

6. Confirmation of the Rh type of Rh negative red cell components

7. Selection of components of ABO group and Rh type appropriate for the recipient

8. Performance of a serologic or computer cross match

9. Labeling of products with the recipient’s identifying information
Detection and Identification of unexpected Antibodies to Red Cell Antigens

- Clinically significant alloantibodies
  - cause a transfusion reaction
  - decrease survival of RBCs
  - are usually IgG antibodies
  - react at 37°C or in the antihuman globulin (AHG) phase
Detection and Identification of unexpected Antibodies to Red Cell Antigens

Antibody Screen

Definition: Testing the patient’s serum/plasma with group “O” reagent red cells in an effort to detect atypical antibodies

<table>
<thead>
<tr>
<th>Small percentage of general population has detectable RBC antibodies</th>
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<tbody>
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<td>0.2 - 2 Percent</td>
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- IBTO (AABB) requirement states that method used to detect clinically significant antibodies must include
- Incubation at 37°C
- Use of an antiglobulin test
Detection and Identification of unexpected Antibodies to Red Cell Antigens

Groups that an antibody screen test is required

1. As part of pre transfusion testing on the recipient.
2. Routine prenatal testing for obstetric patients
   evaluate HDN
   Candidacy for RhiG
3. Donors of allogenic blood and blood products stem/progenitor cells
Procedures
1-Tube Technique

- An indirect antiglobulin test performed in a test tube 12×75 mm or 10×75mm
- Patient's Serum/Plasma + RBCs with known Antigen
  - Immediate spin phase
  - to detect antibodies reacting at room temp
  - may lead to the detection of clinically insignificant cold Abs.

- 2 drops patient plasma + 1 drop reagent red cells

Centrifuge and observe for hemolysis and agglutination
37°C incubation phase
- immunoglobulin G (IgG) molecules sensitize antigen - carrying RBCs
- enhancement media may be added
- depending on the enhancement media, tubes might be centrifuged and observed for hemolysis or agglutination
- the degree of reactivity is graded as 0 to W+ to 4+
- the degree of agglutination should be judged only after all of the cells have been dislodged from the bottom of the test tube

Add enhancement media, Incubate at 37°C, Red cells sensitized with antibody

Centrifuge and observe for hemolysis and agglutination
3+

SEVERAL LARGE AGGLUTINATES-CLEAR BACKGROUND

ONE SOLID AGGLUTINATE

NOTE:
Partial or Complete Hemolysis is a Positive Reaction

4+

NEGATIVE: NO AGGREGATES

NEGATIVE: NO AGGREGATES (Microscopic)

PSEUDOAGGLUTINATION OR STRONG ROULEAUX (2+)

ROULEAUX: Microscopic (original magnification x10; enlarged 240%) NOTE: The "stack of coins" appearance of the agglutinates
AHG or Coombs phase

Wash with 0.9% Saline 3-4 times to remove unbound antibody

Add 2 drops AHG reagent

Centrifuge and observe for hemolysis and agglutination

If negative, add Coombs control cells
AHG or Coombs phase
- the antibody in the AHG reagent will create a bridge between sensitized RBCs
- no antibodies → no sensitized RBCs → no agglutination
- hemolysis may appear as a loss of cell button mass
- depending on the enhancement media, reactions may be observed macroscopically only, or macro-and microscopically

To confirm the negative test, Coombs control cells (check cells) will be added to the tube
RBC Reagents

- the RBC reagent used in the antibody screen come from group O individual Anti-A & Anti-B not interfere
- typed and known for the most common and the most significant RBC antigens
- cells suspended at a concentration between 2-5% in a preservative diluent
- packaged in sets of 2 or 3 cells
- at least one cell positive for each of the following antigens D, C, c, E, e, K, k, Fya, Fyb, JKa, JKb, Lea, Leb, P1, M, N, S and s
- each set is accompanied by a lot- specific antigen profile sheet.
- Ideally, to detect antibodies that show dosage or low concentration there will be homozygous expression of some antigens (double dose)

<table>
<thead>
<tr>
<th>Some blood group systems that exhibit dosage</th>
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<tbody>
<tr>
<td>Rh (except D)</td>
</tr>
<tr>
<td>Kidd  Jk(a-b+)Homozygous Jk (a+b+)Heterozygous</td>
</tr>
<tr>
<td>Duffy Fy(a+b-) Homozygous Fy (a+b+)Heterozygous</td>
</tr>
<tr>
<td>MNSs</td>
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<tr>
<td>Lutheran</td>
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- testing blood donors, use of a pooled screening reagent is acceptable from two different individual
Antibody Screening Cells
Produced by IBTO
Recipient antibody Screening

Dosage exhibition
- Rh (except D)
- Kidd
- Duffy
- MNSs
- Lutheran
<table>
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<th>Use of antibody screening cells to detect antibodies vs. cross-match</th>
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<tr>
<td>• The screen cells will test for most clinically significant antigens</td>
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<tr>
<td>• The cross-matched unit of blood will possess some of the antigens</td>
</tr>
<tr>
<td>• The screen cells will have cells with homozygous expression of many antigens, more reliable in the detection of weakly reacting antibodies</td>
</tr>
<tr>
<td>• Donor units may or may not have homozygous antigen expressions</td>
</tr>
<tr>
<td>• The RBCs age and antigen expressions begins to weaken, prepared screen cell sets are diluted in a preservative to maintain antigen integrity</td>
</tr>
<tr>
<td>• Expiration date on donor unit may extend up to 42 days unites used for cross-match some near expression date, may miss antibody due to weaken antigen expression</td>
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</table>
**Enhancement Reagents**

to increase the sensitivity of the test system
may allow for a shortened incubation time

**Various enhancement reagents may be added to the cell/plasma mixture before 37°C incubation**

**22% Albumin**

Albumin works by reducing the zeta potential, dispersing the charges, thus allowing the RBCs to approach each other.

**Low Ionic Strength Solution (LISS)**

Contains glycine in an albumin solution. In addition to lowering the zeta potential, LISS increases the uptake of antibody on the RBC during sensitization phase.

**Polyethylene Glycol (PeG)**

PeG in a LISS solution removes water from the test system, concentrating any antibodies present. This increases the degree of RBC sensitization. Generally, PeG test system, are more sensitive than LISS, albumin, or saline system.
Electrical charges surrounding Red Blood Cells prior to the addition of enhancement media.

Electrical charges surrounding Red Blood Cells with the addition of an enhancement medium.
AHG Reagents

It is required that the reagent contain anti-IgG when used for antibody detection and pre-transfusion compatibility. Polyspecific AHG reagent or broad spectrum coombs’ serum contains antibodies to both IgG and complement components, either C3 and C4 or C3b and C3d. Antibodies for C3d is more desirable in the reagent, since there are more on the RBC surface during complement activation.

The presence of complement in the AHG may lead to the detection of clinically insignificant antibodies, to avoid, you can choose to use monospecific AHG regent containing anti IgG only.
Coombs control cells

any test that is negative following the addition of the AHG reagent should be control by the addition of coombs control cells.

Rh- positive cells that have been coated with anti – D Proves

- there was adequate washing performed
- AHG reagent was added
- the reagent was working properly
Tube technique

Advantages
- flexibility
- commonly available laboratory equipment
- Inexpensive

Disadvantages
- instability of the reactions
- subjective nature of grading by technologist
- amount of free time
- failure of the washing phase
Automated instruments have helped to lessen the tedious workload of ABO/Rh and antibody screens on donors.
Automation

- Methods
  - Tube technique
  - Solid phase technique
- Gel technique
Interpretation of antibody screening

- Agglutination or homeolysis at any stage of testing is a positive test result.
- Need for antibody identification
- In what phase did the reactions occur?
- Is the autologous control negative or positive?
- Did more than one screening cell sample react?
- Is homolysis or mixed-field agglutination present?
- Truly agglutination or is rouleaux present?
using a-- three cell screen, a negative result with all 3 cells gives, 95% confidence that no clinically significant antibodies are present

26% of antibodies become undetectable over time, median time of 7 months

**Influencing factors**

- sensitivity of antibody screening are influenced by:
  - Cell to serum ration
  - Prozone  →  false negative
  - Postzone  → false negative
  - Temperature
  - Length of incubation
  - pH 6.8-7.2
Basic Antibody Identification Techniques

- Interpreting results
  - Positive and Negative
  - Exclusion or “crossing out” and rule out

Antibodies to High-Incidence Antigens
anti-H, I, P, LW, Sda, Vel, Ch, Rg, U, Js

Antibodies to low-Incidence Antigens anti-Wr
Selecting Blood for Transfusion

For a patient with clinically significant Antibodies (37°C and IAT)
- Red cell should be tested and be negative for the appropriate antigen
- Even if Ab. Is no longer detectable to prevent a secondary immune response
- An antiglobulin cross-match is required
- The absence of Ag should be confirmed with a potent commercial antisera
- IBTO requires use of licensed (commercial) reagents