A review of ABO & Rh
Standard Operating Procedures for Patient Testing

Iranian Blood Transfusion Organization

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Member of ISBT Working Party on Rare Blood Donors
Tube tests for determination of ABO type of Red cells and serum

• Importance of the ABO system

Most important (clinically significant) Blood Group system for transfusion practice

why?

If ABO antibodies react with antigens in vivo, result is acute hemolysis and possibly death.

• Indications for ABO grouping

- Blood Donors
- Transfusion recipients
- Transplant candidates and donors (ABO antigens are found in other tissues)
- Prenatal patients (cause of HDN)
- Newborns
- Paternity testing
Tube tests for determination of ABO type of Red cells and serum

- What are the following ABO type?

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A1 CELL</th>
<th>B CELL</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>0</td>
<td>2+</td>
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</tr>
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Tube tests for determination of ABO type of Red cells and serum

• KINDS OF DISCREPANCIES

1. Clerical errors
2. Technical errors
3. Problem with serum testing
4. Problem with red cell testing
5. Problems with both cells and serum
Tube tests for determination of ABO type of Red cells and serum

Specimen نمونه

1. حداقی 5-2 میلی لیتر خون بیمار در لوله حاوی ضد انعقاد EDTA خون لخته قابل قبول است.

2. ثبت نام و نام خانوادگی بیمار و شماره منحصر به فرد، شناسایی کننده بروی لوله حاوی خون بیمار الزامی است.

3. ثبت نام خونگیری و تاریخ خونگیری بر روی لوله حاوی خون بیمار است.

4. نمونه نوزادان کمتر از 4 ماه معمولاً حاوی Anti-B و Anti-A یا از آن عادت نمی‌نماید. بیمار در باک خون موجود تست در صورت نیاز به تزییق خون انجام یکی از بندهای ذیل با رعایت الیوت الزامی است:

   - از بیمار نمونه گیری مجدد شده و به بانک خون جهت تکرار و تأیید نتیجه آزمایش ABO ولیه ارسال شود.
   - آزمایش ABO مجدداً بر روی نمونه توسط کارشناس دیگری تکرار و تأیید گردید.
   - کارشناس انجام دهنده آزمایش اولیه ABO مجدداً آزمایش ABO را تکرار، تأیید و مستند سازی نماید. از نمونه هایی که ظاهر هموپلاسی یا لیپمیش (hemolyzed – lipemic) دارند، استفاده نکنید. نمونه گیری مجدد انجام شود.

5. زمانی که سابقه انجام آزمایش ABO قبلی وارده است:

   - نمونه هایی که ظاهر هموپلاسی یا لیپمیش (hemolyzed – lipemic) دارند، استفاده نکنید. نمونه گیری مجدد انجام شود.

Storage نگهداری نمونه

- به دستورالعمل تولید کننده معرف-ها جهت محدودیت و شرایط ذخیره سازی نمونه ها رجوع شود.

- نمونه هایی که خون دردمای یخچال 8-2 درجه سانتی‌گراد و به مدت حداقی 7 روز جهت هرگونه آزمایش بعدی ذخیره شود.

- جهت پیشگیری از وقوع هرگونه خطای انسانی، جداسازی سرم یا یلالاسا از گلبول قرمز خون نمونه بیمار و ذخیره سازی در لوله های تفکیک شده توصیه نمی‌شود.
Preparation of A1 cells, B cells
-3% Red cell Suspension SOP

• 3% red cell suspension is a common reagent in many serologic procedures

• 2-5% suspension can achieve the appropriate antigen – antibody ratio

- Zone of equivalence
- Pro zone (excess of unbound immunoglobulin)
- Post zone (surplus of antigen)
Antibody Excess (Prozone)

Equivalence (Optimum Proportions of Antigen and Antibody)

Antigen Excess (Postzone)
Preparation of 3% Red cell Suspension

- Gain confidence in approximating a 3% red cell suspension visually
- Compare with a control 3% suspension of cells visually
- Look for an appropriate size of the cell button after centrifugation
  - Example: to obtain 10ml 3% cell suspension mix 0.3ml RBC with 9.7 ml of 0.9% saline
- Use red cell suspensions on the day of preparation only

3% Suspension Color and Density Standard

Comparison Evaluation
Grading Test Results SOP

- Is to allow comparison of reaction strengths
- Can be used to detect and identify ABO subgroups
- Is beneficial in detecting multiple antibody specificities and antibodies exhibiting dosage
- Grading should be standardized among all members of the laboratory staff
- Use a light source and an agglutination viewer as an aid

<table>
<thead>
<tr>
<th>Interpretation of Agglutination Reactions</th>
<th>Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Solid agglutinate</td>
<td>4+</td>
</tr>
<tr>
<td>Several large agglutinates</td>
<td>3+</td>
</tr>
<tr>
<td>Medium-size agglutinates, clear background</td>
<td>2+</td>
</tr>
<tr>
<td>Small agglutinates, turbid background</td>
<td>1+</td>
</tr>
<tr>
<td>Very Small agglutinates, turbid background</td>
<td>$1^w$</td>
</tr>
<tr>
<td>Barely visible agglutination, turbid back ground</td>
<td>$W^+$</td>
</tr>
<tr>
<td>No agglutination</td>
<td>O</td>
</tr>
<tr>
<td>Mixtures of agglutinated and unagglutinated red cells (mixed field)</td>
<td>mf</td>
</tr>
<tr>
<td>Complete hemolysis</td>
<td>H</td>
</tr>
<tr>
<td>Partial hemolysis, some red cells remain</td>
<td>pH</td>
</tr>
</tbody>
</table>
Grading Test Results

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>W+ to 1+</th>
<th>2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO AGGLUTINATION OR HEMOLYSIS</td>
<td></td>
<td>TINY AGGLUTINATES TURBID BACKGROUND</td>
<td>SMALL AGGLUTINATES TURBID BACKGROUND</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEDIUM-SIZED AGGLUTINATES-CLEAR BACKGROUND</td>
<td></td>
</tr>
</tbody>
</table>

NEGATIVE | POSITIVE | MICROSCOPIC
### Grading Test Results

<table>
<thead>
<tr>
<th>3+</th>
<th>4+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SEVERAL LARGE AGGLUTINATES-CLEAR BACKGROUND</strong></td>
<td><strong>ONE SOLID AGGLUTINATE</strong></td>
</tr>
<tr>
<td><img src="image1.png" alt="Image 1" /></td>
<td><img src="image2.png" alt="Image 2" /></td>
</tr>
<tr>
<td><img src="image3.png" alt="Image 3" /></td>
<td><img src="image4.png" alt="Image 4" /></td>
</tr>
<tr>
<td><img src="image5.png" alt="Image 5" /></td>
<td><img src="image6.png" alt="Image 6" /></td>
</tr>
<tr>
<td><img src="image7.png" alt="Image 7" /></td>
<td><img src="image8.png" alt="Image 8" /></td>
</tr>
</tbody>
</table>

**NOTE:**
Partial or Complete Hemolysis is a Positive Reaction

- **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
Tube tests for determination of ABO type of Red cells and serum

- Quality control to be performed daily

- Reagents
  - All reagents should be certified & approved by MOH reference laboratory
  - Use reagents accordance with manufacture’s instructions
Tube tests for determination of ABO type of Red cells and serum

- Follow S.O.P to perform the test
- Interpretation
  - Hemolysis or agglutination constitute positive results
  - Any discrepancy between results of tests on serum and cells should be resolved before an interpretation is recorded of the donor’s ABO

<table>
<thead>
<tr>
<th>If RBCs react</th>
<th>And Serum/ Plasma react</th>
<th>Then ABO type is</th>
</tr>
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<tbody>
<tr>
<td>Anti-A</td>
<td>Anti-B</td>
<td>A1 RBCs</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>≥3⁺</td>
</tr>
<tr>
<td>≥3⁺</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>≥3⁺</td>
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</tr>
<tr>
<td>≥3⁺</td>
<td>≥3⁺</td>
<td>0</td>
</tr>
</tbody>
</table>
Tube test for Rh testing

• Specimen
  - Clotted or anticoagulated blood samples
  - Do not use corvac (Gel) tube

• Reagents
  - blended IgM/IgG monoclonal/ polyclonal low protein anti-D
  - Use reagents accordance with manufacturer's instructions
  - Use two different anti-D serum for each test
  - Rh control reagent to be used for each test
  - AHG(anti-IgG)
  - IgG coated red cells
Tube test for Rh testing

- Quality control to be performed daily
- Follow S.O.P to perform the Rh(D) test

<table>
<thead>
<tr>
<th>Anti-D</th>
<th>Rh-Control</th>
<th>Interpretation</th>
<th>تفسیر</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2</td>
<td>0</td>
<td>Positive</td>
<td>بیمار نوع Rh گزارش شود. از مرحله 10 ادامه دهید.</td>
</tr>
<tr>
<td>&lt; 2</td>
<td>0</td>
<td>Unresolved</td>
<td>از مرحله 10 ادامه دهید.</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Unresolved</td>
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<tr>
<td>≥2</td>
<td>0</td>
<td>Rh-Positive</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>Rh-Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Unresolved</td>
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</table>
Weak D (Du)
is a weakly expressed D antigen that will only be demonstrated after incubation at 35-37°C°C followed with antiglobulin testing.(ie being demonstrated only by coombs technique).

Why do weak D's exist?
There are three explanations for weak D's.

• **Quantitative weak D** there are individuals quantitatively produce fewer D antigen sites.

• **Position Effect weak D** in this case the D weaken by the position of a C on the opposite haplotype which is called the trans position Dce/Ce and DcE/Ce.

• **Partial D antigen (Mosaic D)** in this type of weak D, the individuals lack some of the components of the D antigen and therefore are able to make allanibodies to those specific components if they are transfused with D positive blood.
Tube test for Rh testing

**Notes:**

1. Observations of Group-ARh negative and Group-B Rh positive who have undergone anti-D immune serum and Anti-D antibody control were 27 in number. The Rh test was conducted with a dilution of 1:50. The resulting Rh positive (Weak-D) were a combination of D and anti-D antibodies, but the type was not resolved. The presence of anti-D antibodies was confirmed by incubation with D antigen and anti-D control. The results were consistent with the results of the previous tests. The Rh positive group was further divided into two subgroups: D positive and D negative. The D positive group showed a positive reaction with the D specific control. The D negative group showed a negative reaction with the D specific control. The results were consistent with the results of the previous tests.

2. The result of the test was assessed by comparing the reaction pattern of the patient's serum with that of the control serum. If the reaction pattern of the patient's serum was identical to that of the control serum, the result was considered positive. If the reaction pattern of the patient's serum was different from that of the control serum, the result was considered negative. The results were consistent with the results of the previous tests.

3. The test was conducted by mixing the patient's serum with the control serum and incubating the mixture at 37°C for 1 hour. The reaction was assessed by visual observation. If the reaction was positive, the result was considered positive. If the reaction was negative, the result was considered negative. The results were consistent with the results of the previous tests.

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Many valuable lives depends on your work