Chapter 7
PRE-TRANSFUSION LABORATORY TESTING

PRACTICE POINTS

• The laboratory will only accept appropriately labelled forms and samples.
• Blood Group: The lab will determine ABO group by forward and reverse group with comparison to historic records or repeat sample, and perform RhD group.
• Group and Screen: The lab will determine ABO and RhD group and test the patient serum or plasma against red cells of known phenotype (“screen”).
• Cross-match when screen cells are negative, is an “immediate spin” to confirm ABO compatibility.
• Cross-match when screen cells are positive by antibody determination, selection of suitable antigen negative erythrocytes and cross match by antiglobulin testing.
• Finding a compatible unit of blood in a patient with antibodies can result in delay to transfusion.
• Group O erythrocytes are the universal ABO donor cells.
• Group AB plasma is the universal plasma donor.
7.1 LABORATORY SAMPLE LOG AND RECORD KEEPING

The transfusion laboratory will only accept samples which have been appropriately labelled and have an accompanying request (order) form.

All transfusion request forms and samples must have at least two patient identifiers e.g. full name (first and surname) and age or date of birth. If the hospital operates a unique patient identification number system, this number must also be present. The ward or clinic, along with requesting doctor must be identified.

The staff member performing the patient identification and labelling of the sample must sign a declaration (as part of the request form) verifying the sample.

Failure to correctly complete the request forms and label the sample may lead to rejection of the sample.

All blood request forms and accompanying blood samples must be registered in the Laboratory information system or log book. Mandatory information to be recorded in the laboratory is:

- date and time of reception,
- patient first name and surname,
- patient date of birth or age,
- patient specific identification number if available,
- department and ward, and
- name of person who delivered the request.

Request form and blood sample have to be inspected for completeness and integrity before starting the work up.

To avoid any clerical or procedural error –

- If the blood request form is not complete, the ward needs to be contacted to supply the missing information.
- If the blood sample label is not complete and/or the blood sample does not meet the basic requirements of quality and integrity, the ward needs to be contacted to supply a new sample.

- Severe acute haemolytic transfusion reactions are invariably caused by transfusing red cells that are incompatible with the patient’s ABO type. These reactions can be fatal. They most often result from:
  - Errors in labelling the patient’s blood sample.
  - Errors when collecting the unit of blood for transfusion.
  - Failure to carry out the final identity check of the patient and the blood pack before infusing the unit of blood.

7.2 BACKGROUND INFORMATION ON BLOOD GROUPS AND COMPATIBILITY

It is essential that all blood is tested before transfusion in order to:

- ensure that transfused red cells are compatible with antibodies in the recipient’s plasma, and
- avoid stimulating the production of new red cell antibodies in the recipient, particularly anti-D.

All pre-transfusion test procedures should provide the following information about both the units of blood and the patient:

- ABO group,
- RhD type, and
- presence of red cell antibodies that could cause haemolysis in the recipient.

7.2.1 ABO blood group antigens and antibodies

The ABO blood groups are the most important in clinical transfusion practice. There are four red cell types: O, A, B and AB.

All healthy normal adults of group A, group B and group O have antibodies in their plasma against the red cell types (antigens) that they have not inherited:

- Group A individuals have antibody to group B.
- Group B individuals have antibody to group A.
- Group O individuals have antibody to group A and group B.
- Group AB individuals do not have antibody to group A or B.
These antibodies are usually of IgM and may also be IgG class. These are said to be “naturally occurring” as they appear within the first months of life and are present in all people life-long.

7.2.2 ABO incompatibility: haemolytic reactions

Anti-A or anti-B recipient antibodies are almost always capable of causing rapid destruction (haemolysis) of incompatible transfused red cells. Blood which has not been compatibility tested or blood given to the wrong patient may cause life-threatening haemolysis.

Typically, at least one third of unmatched transfusions will be ABO incompatible and may lead to severe or fatal reactions.

7.3 Compatibility and Blood Components

7.3.1 Red Cell Components

In red cell transfusion, there must be ABO and RhD compatibility between the donor’s red cells and the recipient’s plasma.

- Group O individuals can receive blood from group O donors only.
- Group A individuals can receive blood from group A and O donors.
- Group B individuals can receive blood from group B and O donors.
- Group AB individuals can receive blood from AB donors, and also from group A, B and O donors.

Note: Group O erythrocytes can be given to patients with any blood group.

7.3.1.1 RhD red cell antigens and antibodies

Red cells express many antigens. In contrast to the ABO system, individuals rarely make antibodies against these other antigens. Exposure to red cell antigens can occur with previous transfusion or during pregnancy and childbirth. These events can immunise an individual, causing that person to make an antibody.

The most important is RhD. A woman with anti-D due to previous sensitisation and antibody product is at risk of:

- haemolytic disease of the newborn in a subsequent pregnancy, and
- rapid destruction of a later transfusion of RhD positive red cells.

Other important antigens include:

- Rh system: C, c, E, e,
- Kidd,
- Kell,
- Duffy, and
- Lewis.

These antibodies can also cause severe acute or delayed reactions to transfusion.

7.3.2 Plasma and Components Containing Plasma

In plasma transfusion, group AB plasma can be given to a patient of any ABO group because it contains neither anti-A nor anti-B antibody.

- Group AB plasma (no antibodies - can be given to any ABO group patients).
- Group A plasma (anti-B) - can be given to group O and A patients.
- Group B plasma (anti-A) - can be given to group O and B patients.
- Group O plasma (anti-A + anti-B) - can be given to group O patients only.

Safe blood transfusion depends on avoiding incompatibility between the donor red cells and antibodies in the patient plasma.

NOTE: In some disease states, anti-A and anti-B may be difficult to detect in laboratory tests.

7.4 Red Cell Tests

Laboratory quality systems need to be in place to ensure accurate and reproducible test results. The following tests should be available in a transfusion laboratory however several are not available throughout Cambodia yet.

7.4.1 ABO and RhD group

Ideally all samples are tested for ABO group by forward and reverse group. RhD type should be determined on all samples. Comparison to historical records or testing a second sample improve accuracy of testing and reveal any labelling or clerical mistakes. Appropriate controls (positive and negative) are required for all pre-transfusion tests.

Forward group: Testing a dilute sample of patient red cells against standardised anti-A and anti-B (and anti-AB if desired) reagents.

Reverse group: Testing of patient plasma or serum against known A1 and B red cells. This is not required for babies < 4 months age.

RhD group must be determined by direct agglutination using an anti-D reagent.
7.4.2 Screen cells

Testing the patient plasma or serum against 2 or 3 cell suspensions of known antigen phenotype by Indirect Antiglobulin Test (IAT) will detect the presence of any red cell allo-antibodies in the patient. In general, particularly in previously transfused or pregnant patients, a screen cell result is valid for 72 hours before repeat testing is required.

7.4.3 Allo-antibody determination

A positive result with either screen cell indicates the presence of an antibody. Testing patient plasma or serum against a panel of known phenotype red cells may allow determination of antibody specificity. Additional testing, beyond the scope of this document, may be required.

7.4.4 Cross-match

When the screen cells are negative, group compatible erythrocytes are chosen. Patient plasma or serum is tested against a segment of red cells from the unit. Immediate spin will confirm ABO compatibility. Performing an IAT will confirm compatibility with other antigen/antibody systems.

7.5 PRE-TRANSFUSION TESTING

A doctor may request the following combinations of tests:

- Blood group.
- Group and Screen.
- Cross-match.

7.5.1 Blood Group

The laboratory will perform:

- ABO group – forward and reverse group with comparison to historic records or repeat sample.
- RhD group.

These tests will only determine ABO and RhD groups. The serum or plasma will be stored in the refrigerator or freezer for 7 days in case a screen or cross-match is required.

7.5.2 Group and Screen

The laboratory will perform:

- ABO group – forward and reverse group with comparison to historic records or repeat sample.
- RhD group.
- Screen cells – test the patient serum or plasma against red cells of known phenotype.
- Antibody determination if screen cells are positive.

If antibody cannot be determined, the patient serum or plasma will be cross-matched by IAT method to red cells from segments of several units of blood.

7.5.3 Cross-match

The laboratory will perform:

- ABO group – forward and reverse group with comparison to historic records or repeat sample.
- RhD group.
- Screen cells – test the patient serum or plasma against rec cells of known phenotype.
- If screen cells are negative, cross-match (patient serum or plasma against red cells from the segment of chosen ABO/RhD compatible unit) by immediate spin method shall confirm ABO compatibility. Cross-matched erythrocyte or whole blood component may be released.
- If screen cells are positive, antibody determination should occur. Antibody determination and selection of suitable units may take some time leading to potential delay in transfusion. In some cases surgery may need to be delayed.

If the antibody(ies) cannot be determined, the patient serum or plasma will be cross-matched by IAT method to red cells from segments of several units of blood until one unit is found to be compatible.

A patient with a warm auto-immune haemolytic anaemia will have a positive Direct Antiglobulin Test (DAT). These autoantibodies react at 37°C and may prevent a compatible unit being found. If the patient needs a transfusion, ABO/RhD compatible blood which has the lowest reactivity on cross-match testing should be chosen.

7.5.4 Cross-match in laboratories without Screen Cells

All transfusion laboratories must be able to perform ABO and RhD typing; and immediate spin cross-match. These tests will ensure ABO compatibility.

It is preferable that all transfusion laboratories be able to screen for antibodies by screen cells. If that is not possible, ABO/RhD compatible blood needs to be cross-matched using IAT methods with the patient’s serum or plasma.

7.5.5 Urgent blood for transfusion

Pre-transfusion testing, selection of unit(s) and
cross-match may take an hour or more. Urgent clinical need for blood can be provided:

- **Immediate requirement** – provide O RhD negative erythrocytes or whole blood for women of child bearing potential. Males may receive O RhD positive erythrocytes or whole blood.
- **Urgent (< 15 minutes)** – ABO/RhD group should be determined and ABO compatible erythrocytes or whole blood can be supplied.
- **Urgent (< 45 minutes)** – ABO/RhD and screen cells should be known. Either ABO compatible or cross-matched erythrocytes or whole blood can be supplied.

### 7.5.6 An IAT cross-match is always necessary in case of:

- Newborn (until the age of 4 months); always cross-match with serum of the mother.
- Patients with congenital or acquired abnormality in the erythropoiesis for which frequent erythrocytes have to be given [e.g. thalassaemia major and (congenital) hypoplastic anaemia].
- Patients in whom previously erythrocyte allo-antibodies have been detected.

### 7.6 SELECTION OF BLOOD COMPONENTS

All erythrocyte and whole blood transfusions require ABO/RhD typing, antibody screen and cross-match performed.

The specific unit of erythrocytes or whole blood should be selected according to age of the units. Generally, good inventory management is to use the oldest units first. The main exceptions to this rule are intra-uterine, neonatal and young children transfusions. These patients should receive youngest blood to reduce the risk of storage lesion problems e.g. high potassium.

All plasma products and platelets should be released oldest units first.

A compatibility label should be attached firmly to each unit of blood by the laboratory, showing the information seen in Table 7.1 (below). The label should be attached in such a manner that the compatibility label and the blood product cannot become separated.

### 7.7 BLOOD COMPONENT STORAGE AND DELIVERY IN THE HOSPITAL

Refrigerators and freezers in which blood products are stored, should fulfill the requirements of Good Manufacturing Practice (GMP). This means that they must be provided with a temperature registration and an acoustic alarm. The course of the temperature must be documented. Refrigerators and freezers should be periodically validated (test on high and low alarm) and regularly defrosted and cleaned.

Transportation should always follow the principles of “cold chain” and “direct routing”. If blood needs to travel significant distance or may not be transfused within 4 hours, then the blood should be transported and stored short term in a validated insulated container called a “shipper”.

Tubes containing blood, reagents, food, etc shall not be stored in a blood storage cabinet. So called domestic refrigerators and freezers are not suitable to store blood products for transfusion.

### 7.7.1 Erythrocytes and Whole Blood

To keep the quality and to prevent bacterial growth they shall be stored at a temperature of 2–6°C (see Table 7.2). Erythrocytes warmed up to > 10°C

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**Table 7.1 The information necessary for a compatibility label.**

<table>
<thead>
<tr>
<th>THIS BLOOD IS COMPATIBLE WITH:</th>
<th>UNIT NR:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient’s name:</td>
<td></td>
</tr>
<tr>
<td>Patient’s hospital reference number or date of birth:</td>
<td></td>
</tr>
<tr>
<td>Patient’s ward:</td>
<td></td>
</tr>
<tr>
<td>Patient’s ABO and RhD group:</td>
<td></td>
</tr>
<tr>
<td>Expiry date:</td>
<td></td>
</tr>
<tr>
<td>Date of compatibility test:</td>
<td></td>
</tr>
<tr>
<td>Blood group of blood unit:</td>
<td></td>
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</tbody>
</table>
may not be restored and shall be transfused within 24 hours. Erythrocytes shall not be stored for more than half an hour outside the refrigerator for transport to the ward or performing a compatibility test etc.

7.7.2 Platelets

These shall be transfused as soon as possible after reception in the hospital blood bank or ultimately within 6 hours after delivery by the blood bank. They must be stored until transfusion at room temperature (20–24°C) (not in the refrigerator!) and must be gently rocked or agitated while stored. In a conditioned platelet storage cabinet platelets have a shelf life of maximal 5–7 days (see Table 7.2).

7.7.3 Fresh Plasma and Cryoprecipitate

To preserve the activity of the coagulation factors, FFP must be stored at a temperature of –25°C (1 year) or < –30°C (2 years) (see Table 7.2).

Thawing may take place in a water bath at 30-37°C (refresh, clean/disinfect daily) packed in an extra plastic bag or by means of any other validated method (e.g. specially developed microwave).

After thawing the product (fresh plasma) must be transfused as soon as possible to preserve the coagulation factors.

Table 7.2 Storage conditions of blood products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Storage temperature</th>
<th>Shelf life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (EC) in additive solution</td>
<td>2–6°C</td>
<td>35 days</td>
</tr>
<tr>
<td>Platelet concentrate (PC)</td>
<td>20–24°C</td>
<td>5–7 days</td>
</tr>
<tr>
<td>Frozen Fresh Plasma (FFP)</td>
<td>&lt; 25°C</td>
<td>1 year</td>
</tr>
</tbody>
</table>