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<th><strong>INDIRECT ANTIGLOBULIN CROSSMATCH TUBE METHOD</strong></th>
<th><strong>NLBCP-017</strong></th>
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<tr>
<td><strong>Effective Date</strong></td>
<td>2017-06-13</td>
</tr>
<tr>
<td><strong>Version</strong></td>
<td>3.0</td>
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<tr>
<td><strong>Review Due Date</strong></td>
<td>2019-06-13</td>
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Overview

An indirect antiglobulin crossmatch is performed to assure compatibility of red cell units for transfusion in certain patients by incubating recipient’s plasma or serum and donor red cells (obtained from a segment of tubing that was originally attached to the unit to be transfused) then adding anti-human globulin reagent (AHG) to detect any antibody coating of the donor red cells.

Policy

1. Compatibility testing shall be performed before red cells are transfused.

2. When an antibody screen indicates the presence of a clinically significant red cell antibody, or the recipient has a previous history of clinically significant antibodies the red cell donor unit shall be crossmatched using an antiglobulin technique.

3. The red cell donor unit(s) selected for crossmatch shall lack the corresponding antigen(s) to any clinically significant antibodies detected in recipient’s serum or plasma.

4. No delay shall occur in initiating the centrifugation step or reading the reaction after centrifugation is complete. Delay can lead to false-negative results and failure to detect incompatibility.

5. Following transfusion of the first unit of blood, the original recipient blood sample may be used to crossmatch additional units up to 96 hours.

Guidelines

1. Monospecific anti-IgG (which contains only anti-IgG) can also be used in place of AHG.

2. Patient transfusion records shall be reviewed and this review shall be documented. The following information shall be reviewed:

   2.1. Previous ABO and Rh typing;
   2.2. Previous transfusions;
   2.3. Difficulties in blood typing;
   2.4. Previously identified clinically significant red cell antibodies;
   2.5. Adverse reactions to previous transfusions; and
   2.6. Special transfusion requirements.

3. Documentation to link donor unit to recipient shall include:

   3.1. Recipients name;
3.2. Recipient's identification number;
3.3. ABO and Rh groups of the recipient;
3.4. ABO and Rh groups of blood component;
3.5. Name of the component;
3.6. Donor unit number;
3.7. Expiry date of donor unit;
3.8. Compatibility status of the donor unit; and
3.9. Date and time of crossmatch.

Materials
1. Isotonic saline
2. Test tubes (10x75mm or 12x75mm)
3. Transfer pipettes
4. Test tube rack
5. Serological centrifuge
6. Donor unit(s)
7. Patient EDTA whole blood sample or patient serum (Do not use sample drawn into tubes with neutral gel separators)
8. Polyspecific anti-human globulin (AHG) or monospecific anti-IgG
9. IgG sensitized red cells (check cells)
10. 22% albumin or other potentiating solution
11. Cell washer (if available)
12. Waterbath or heating block at 37°C ± 1°C
13. Interval timer
14. Microscope
15. Microscope slides

Procedure
1. Determine specimen suitability.
2. Centrifuge specimen (speed and time as recommended by manufacturer's instructions.)
3. Check specimen for abnormal appearance after centrifuging (e.g. hemolysis.)
4. Create crossmatch worksheet.
5. **Perform patient history check** and check for special requirements (eg. irradiated products).
6. Choose acceptable donor unit(s) from blood bank fridge.
   
   **Note:** If antigen negative red cell units are not available in current inventory, perform antigen typing on the red cells in current inventory or request antigen negative units from blood supplier prior to beginning crossmatch.
7. Record donor unit number(s) on crossmatch worksheet.
8. Label two (2) test tubes for each donor red cell suspension being tested with patient’s serum or plasma.
9. Remove a segment from each donor red cell unit.
10. Return donor unit(s) to fridge.
11. **Wash the donor cells (from donor unit(s) segments) with isotonic saline and prepare a 2-5% red cell suspension(s) in the corresponding labelled tube.**
12. Add the following to each of the second labelled tubes:
   
   12.1. Two (2) drops of patient’s serum or plasma;
   
   12.2. One (1) drops of the donor red cell suspension;
   
   12.3. Two (2) drops of potentiating solution (eg. 22% albumin, LISS)
   
   **NOTE:** Not all facilities use potentiating solution.
13. Mix the contents of the tube(s).
14. Incubate tube(s) at 37 °C for 30 to 60 minutes.
15. Centrifuge tube(s). (Speed and time as recommended by manufacturer's instructions.)
16. Observe macroscopically for hemolysis and agglutination. Grade and record the results.
17. Wash the red cells three to four times with saline completely decant the final wash. Cell washer can also be used.
18. Add two (2) drops of AHG or IgG to the dry red cell button.
19. Centrifuge tube(s).
20. Resuspend the red cell button(s) while examining macroscopically then microscopically for agglutination.

21. Grade and record results on crossmatch worksheet.
   21.1. No agglutination or hemolysis indicates the donor unit(s) is/are compatible.
   21.2. Agglutination or hemolysis indicated the donor unit(s) is/are incompatible.

22. Confirm the validity of negative results by adding IgG-coated red cells (check cells).
   22.1. After check cells are added and centrifuged, agglutination should be present.
   22.2. If agglutination is absent, crossmatch results are invalid. Repeat crossmatch.

**Quality Control**

1. All reagents used shall be controlled as required, before results are released.

2. An ABO and Rh type and antibody screen is performed on the patient sample to be crossmatched. Any discrepancies shall be resolved or antibodies identified before crossmatch is performed.

3. Incompatible results shall be investigated as per facility procedure.

4. IgG sensitized red cells must be added to all negative indirect antiglobulin tests to confirm:
   4.1. AHG or IgG was added; and
   4.2. AHG or IgG added is working.

**Key Words**

Crossmatch, antiglobulin, AHG, IgG
### Supplemental Materials

1. **Is the specimen suitable?**
   - Yes: Centrifuge specimen
   - No: Request new specimen

2. **Is specimen hemolyzed?**
   - Yes: Create crossmatch worksheet
   - No: Perform patient history check

3. **Choose donor unit(s)**
   - Record donor unit number(s) on worksheet
   - Remove segments then return donor unit(s) to fridge
   - Label 2 tubes for each donor unit
   - Wash donor unit segment 3 to 4 times

4. **Prepare 3-5% suspension from washed donor cells in first labelled tube**

5. **To second tube add:**
   - 2 drops patient’s serum/plasma, 1 drop donor suspension, 2 drops of 22% albumin

6. **Mix tube(s)**

7. **Incubate tube(s) @ 37°C for 30-60 minutes**

8. **Centrifuge tube(s)**

9. **Is hemolysis present?**
   - Yes: Further investigation required
   - No: Wash red cells 3-4 times with saline, completely decant the final wash

10. **Add 2 drops of AHG or IgG to dry red cell button**

11. **Centrifuge tube(s)**

12. **Resuspend and examine macroscopically then microscopically**

13. **Agglutination present?**
   - Yes: Further investigation required
   - No: Donor unit(s) are compatible

14. **Further investigation required**

15. **Add 1 drop of IgG coated red cells**

16. **Agglutination present?**
   - Yes: Further investigation required
   - No: **Centrifuge tube(s)**

17. **Further investigation required**
References

