

BLOOD CROSSMATCHING: STANDARD OPERATING PROCEDURE

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Department: Blood Banking

Section: Immunohematology

Test: Blood Crossmatching

Specimen: Recipient serum/plasma and donor red blood cells

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DETERMINATION OF BLOOD COMPATIBILITY BY CROSSMATCHING

I. PRINCIPLE

Blood crossmatching is a laboratory procedure performed to determine the compatibility between the donor's red blood cells (RBCs) and the recipient's serum or plasma before transfusion. It helps detect antibodies in the recipient that may react with antigens present on the donor's RBCs and potentially cause a transfusion reaction.

Before crossmatching, the ABO and Rh blood groups of the donor and recipient are confirmed, and the recipient's serum may be screened for unexpected antibodies. The major crossmatch involves testing donor RBCs with recipient serum or plasma, while the minor crossmatch involves testing recipient RBCs with donor serum or plasma.

The major crossmatch may be performed using the Immediate Spin Crossmatch (ISX) or the Antiglobulin Crossmatch. A compatible reaction should show no agglutination or hemolysis.

II. SCOPE

This procedure is applicable to trained laboratory personnel performing blood compatibility testing in the blood banking or immunohematology section. It covers specimen preparation, major crossmatching by Immediate Spin Crossmatch, major crossmatching by the Antiglobulin method, minor crossmatching, interpretation of results, quality control, and reporting.

All procedures must be performed according to the laboratory's approved SOP, validated procedures, and manufacturer's instructions.

III. TEST PARAMETERS

Test Parameter	Description
Test Type	Immunohematology / Compatibility Testing
Specimen	Recipient serum/plasma and donor RBCs
Major Crossmatch	Donor RBCs + recipient serum/plasma
Minor Crossmatch	Recipient RBCs + donor serum/plasma
Immediate Spin Crossmatch	Detects mainly ABO incompatibility and immediate reactions
Antiglobulin Crossmatch	Detects antibodies using the AHG phase
Result	Compatible or incompatible based on observed reaction

IV. SPECIMEN REQUIREMENTS

A. Recipient's Blood

The acceptable specimen is serum or plasma.

1. Specimen type: Serum or plasma
2. Volume: 5–10 mL
3. Serum collection: Red-top tube
4. Plasma collection: Appropriate anticoagulant tube
5. The specimen must be properly identified and suitable for testing.

B. Donor's Blood

1. Specimen: Donor erythrocytes (RBCs)
2. Source: Selected donor blood unit
3. Donor RBCs must be properly identified and appropriately typed.

V. REAGENTS OR MEDIA, SUPPLIES, AND EQUIPMENT

A. Reagents

1. Anti-A serum – used for ABO blood grouping.
2. Anti-B serum – used for ABO blood grouping.
3. Anti-D serum – used for RhD blood grouping and control procedures.
4. Anti-human Globulin (AHG/Coombs' reagent) – used to detect antibodies during the antiglobulin phase.

5. 0.9% Sodium Chloride – used for preparation and washing of RBC suspensions.
6. LISS – used as an enhancement medium for antibody detection when required.
7. Antibody screening cells – used for detection of unexpected antibodies.

B. Supplies and Equipment

1. Test tubes
2. Test tube rack
3. Pipettes and pipettors
4. Centrifuge
5. Microscope
6. Blood bank incubator
7. Blood bank refrigerator
8. Timer or stopwatch
9. Test panels
10. Appropriate personal protective equipment (PPE)

VI. CALCULATION AND/OR CALIBRATION

Crossmatching is primarily a qualitative procedure because the final reaction is evaluated based on the presence, absence, and strength of agglutination or hemolysis.

A. Reagent Calculation for Antiglobulin Crossmatch

Number of drops required:

1 drop × number of patients = total number of drops of Coombs' reagent

Conversion to volume:

Number of drops × amount per drop = total volume of Coombs' reagent

B. Calibration

Laboratory equipment used for crossmatching, including centrifuges, incubators, microscopes, and refrigerators, should be maintained and calibrated according to laboratory policy and manufacturer recommendations.

Daily calibration should be performed using manufacturer-recommended calibration materials when applicable, and calibration results should be documented in the maintenance log.

VII. QUALITY CONTROL

A. Pre-Analytical Quality Control

1. Properly identify the patient.
2. Verify the request form and specimen identification.
3. Collect the specimen using the appropriate tube and anticoagulant when applicable.
4. Ensure that the specimen is suitable for testing.
5. Keep records of patient information, donor blood products, and crossmatching results.

B. Analytical Quality Control

1. Check that all reagents are properly stored and have not expired.
2. Ensure that laboratory equipment is properly maintained and calibrated.
3. Run appropriate control samples with the patient's specimen.
4. Confirm that control reactions are valid before interpreting the patient's result.

C. Post-Analytical Quality Control

1. Results must be verified by qualified laboratory personnel.
2. Investigate any abnormal or unexpected reaction.
3. Document the crossmatch results together with the donor and recipient information.
4. Document any detected antibodies or other significant findings.

VIII. STEP-BY-STEP INSTRUCTIONS

A. Preparation

1. Verify the patient's identity and transfusion request.
2. Confirm the patient's ABO and Rh blood group.
3. Confirm the ABO and Rh group of the selected donor unit.
4. Review the patient's previous transfusion and antibody history, when available.
5. Perform or review antibody screening when required.
6. Prepare the recipient's serum or plasma.
7. Prepare the donor RBC suspension.
8. Properly label all test tubes.
9. Check the expiration and condition of all reagents before use.
10. Perform appropriate quality-control procedures.

B. Major Crossmatching by Immediate Spin Crossmatch (ISX)

1. If using serum, prepare a 3–5% EDTA-saline suspension of the donor test red cells in appropriately labeled test tubes. If using plasma, prepare a 3–5% saline suspension of the donor test red cells.
2. Prepare the appropriate A1 and B controls in properly labeled test tubes.
3. In labeled 10 × 75 mm or 12 × 75 mm test tubes, add 2–3 drops of recipient serum or plasma.
4. Add 1 drop of each appropriate prepared RBC suspension.
5. Mix the contents of the test tubes thoroughly.

6. Centrifuge according to the laboratory's validated procedure.
7. Gently dislodge the cell button.
8. Examine the reaction macroscopically for agglutination or hemolysis.
9. Grade the reaction.
10. Record the results.

C. Major Crossmatching by Antiglobulin Crossmatch

1. Prepare the recipient's serum, donor RBCs, Anti-D serum, and AHG reagent.
2. Label three test tubes as T (Test), PC (Positive Control), and NC (Negative Control).
3. Add 2 drops of recipient test serum to tube T.
4. Add 1 drop of Anti-D serum to tube PC.
5. Add 1 drop of 5% saline suspension of pooled O Rh-positive cells to each tube.
6. Incubate all test tubes at 37°C for 1 hour.
7. Wash the cells three times with normal saline to remove excess serum and unbound antibodies.
8. Add 2 drops of Coombs' serum/AHG to each tube.
9. Allow the tubes to stand for 5 minutes.
10. Centrifuge at 1,500 rpm for 1 minute.
11. Resuspend the cells gently.
12. Examine the tubes macroscopically and microscopically for agglutination.
13. Grade and record the results.

D. Minor Crossmatching

1. Prepare the recipient's RBCs and donor's plasma or serum.
2. If using serum, prepare a 3–5% EDTA-saline suspension of the test red cells. If using plasma, prepare a 3–5% saline suspension.
3. Prepare the appropriate controls in labeled test tubes.
4. Add 2–3 drops of donor serum or plasma into the labeled test tube.
5. Add 1 drop of the prepared recipient RBC suspension.
6. Mix the contents thoroughly.
7. Incubate at 37°C for approximately 60 minutes.
8. Completely decant the serum or plasma.
9. Wash the cells three times with normal saline.
10. Add 2 drops of Anti-human Globulin (AHG).
11. Mix and allow the tube to stand at room temperature for 5 minutes.
12. Centrifuge at 1,500 rpm for 1 minute.
13. Observe the reaction macroscopically and microscopically for agglutination.
14. Grade and record the result.

IX. REPORTING RESULTS

A. Major Crossmatch by Immediate Spin Crossmatch (ISX)

Nonreactive donor RBCs + valid controls: The donor unit is considered compatible based on the immediate-spin phase.

Reactive donor RBCs + valid controls: A cold-reactive autoantibody or alloantibody may be suspected. Further testing, such as an antiglobulin crossmatch, should be performed according to the laboratory procedure.

Nonreactive donor RBCs + invalid controls: The test is invalid. Confirm the ABO group of both donor and recipient and repeat the necessary testing.

Reactive donor RBCs + invalid controls: Do not release the blood unit. Investigate the discrepancy and follow the laboratory's approved procedure.

B. Major Crossmatch by Antiglobulin Crossmatch

No agglutination: Compatible based on the test.

Agglutination: Incompatible; the blood unit should not be transfused until the incompatibility is investigated.

C. Minor Crossmatch

No agglutination: Compatible.

Agglutination: Incompatible.

X. LIMITATIONS OF METHODS

A. Possible Unwanted Positive Reactions

1. Autoantibodies
2. Alloantibodies
3. Contaminated samples
4. Polyagglutination
5. Polycarboxyl-dependent antibodies
6. Passive antibodies

B. Possible Unwanted Negative Reactions

1. Omission of red cells or test sample
2. Contaminated sample
3. Weakly reactive Anti-A and/or Anti-B
4. Newborn sample
5. Weakly expressed A and/or B antigens on donor red cells

XI. TROUBLESHOOTING

Problem	Possible Cause	Solution
False-positive crossmatch	Unexpected antibody or testing error	Repeat the crossmatch and investigate the reaction
False-negative crossmatch	Testing or specimen error	Repeat the crossmatch
Inconsistent or weak agglutination	Variation in one of the test components	Recheck the test using another donor unit when appropriate
Unexpected reaction	Possible antibody, specimen, or testing problem	Review ABO/Rh typing, antibody history, controls, and specimen
Invalid control	Incorrect control or testing problem	Repeat the test and verify ABO grouping and control reactions

XII. BIOSAFETY

1. Treat all blood specimens and blood components as potentially infectious.
2. Wear appropriate personal protective equipment (PPE).
3. Perform proper hand hygiene before and after handling specimens.
4. Avoid direct contact with blood and blood products.
5. Handle needles, lancets, and other sharps carefully.
6. Dispose of contaminated materials in appropriate biohazard containers.
7. Dispose of sharps in approved sharps containers.
8. Clean and disinfect the work area according to laboratory policy.
9. Follow standard precautions when handling all human specimens.

XIII. WASTE DISPOSAL

1. Dispose of used blood specimens and contaminated materials according to institutional biohazard-waste procedures.
2. Dispose of used test tubes and disposable materials in the appropriate biohazard container.
3. Dispose of sharps immediately in an approved sharps container.
4. Clean and disinfect the work area after testing.
5. Perform proper hand hygiene after completing the procedure.

XIV. TEST PERFORMANCE SUMMARY

Characteristic	Blood Crossmatching
Test Type	Immunohematology / Compatibility Testing
Specimen	Recipient serum/plasma and donor RBCs
Major Crossmatch	Donor RBCs + recipient serum/plasma
Minor Crossmatch	Recipient RBCs + donor serum/plasma
Immediate Spin Crossmatch	Primarily detects immediate/ABO incompatibility
Antiglobulin Crossmatch	Detects antibodies through the AHG phase
Incubation for Antiglobulin Method	37°C for 1 hour
Cell Washing	3 times with normal saline
AHG Centrifugation	1,500 rpm for 1 minute
Compatible Result	No agglutination or hemolysis
Incompatible Result	Agglutination and/or hemolysis

XV. REFERENCES

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