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Related Documents: ~~P060~~, P061, ~~P101~~,
~~P103~~, ~~P103A~~, ~~P104~~, P108, P115, P118,
P120, ~~P121~~, P225, P226, P305, P328,
P340, P601, P603, P604, P618, P623,
P624, P626, ~~P717~~. Making a Test Red Cell
Suspension, Triaging and Identifying
Acceptable Samples for Testing, Antibody
Screening, Determining the ABO and Rh(D)
of Patients Who Are At Least Four Months
Old, Historical Record Check, Routine
Testing on the ORTHO VISION Analyzer

SEROLOGIC CROSSMATCHING OF RED BLOOD CELLS

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Purpose

The purpose of this document is to provide the Blood Bank staff with stepwise instructions for serologic crossmatching.

Scope

A serologic crossmatch must be performed on all patients who are not eligible for electronic crossmatching, as determined in P108, *RBC Crossmatch Guidelines / Table 108-2, Criteria for Determination of Whether to Perform Electronic or Serologic Crossmatch*. In addition, a serological crossmatch must be performed during computer downtimes; see P120, *Manual Operations*.

Gel crossmatches may be performed manually, as described in this document, or on the Ortho Vision™. Refer to **Transfusion Medicine policy**, ~~P717~~, *Routine Testing on the Ortho Vision™ Analyzer* for additional information.

Procedural Note

The policies found in P108, *RBC Crossmatch Guidelines* apply to both electronic and serologic crossmatching. P108 is referred to extensively throughout this document and **all policies found in P108 are applicable when performing serologic crossmatches.**

Principle

There are numerous variations of serologic crossmatch procedures, but all have the goal of detecting incompatibility between the transfusion recipient and the intended donor. All serologic crossmatch procedures involve mixing patient plasma with donor RBCs. The immediate-spin crossmatch is ideal for detecting ABO incompatibility. However, if unexpected antibodies were historically present or are currently reactive, then the crossmatch must include the antihuman globulin (AHG) phase. The gel crossmatch is the standard serologic crossmatch.

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This document describes a variety of serologic crossmatch methods, provides guidelines for choosing the appropriate method, and provides stepwise instructions for performing these methods.

Policies

Pre-Crossmatch Sample Labeling and Testing Requirements

Before serologic crossmatching may be performed, several sample requirements must be met, including:

- sample labeling requirements
- antibody screen testing requirement, and
- ABO/Rh testing requirements.
- These requirements are found in P108 / Table 108-1: *Pre-Crossmatch Sample Labeling and Testing Requirements*.

Historical Record Check

A historical record check must be performed before RBCs are crossmatched as indicated in **Transfusion Medicine policy, P121**, *Historical Record Check*. If a discrepancy exists between the current and historical results, the computer will warn the technologist. Any discrepancies must be resolved before crossmatching RBCs. To resolve the discrepancy:

- See P623, *Resolution of ABO and Rh(D) Discrepancies*,
- See **Transfusion Medicine policy, P104**, *Antibody Screening / Comparison of Current Antibody Screen to Historical Record*, or
- Consult a supervisor, if necessary.

Policies Relating to Incompatible Crossmatches

- Agglutination and/or hemolysis at any stage are considered indicative of incompatibility.
- If a crossmatch is incompatible, then investigational studies may be required. If necessary, refer to P624, *Investigation of Incompatible Crossmatches*.

Providing Incompatible RBCs / Physician Notification

If incompatible units must be transfused, then the ordering physician must be notified prior to transfusion. This notification should be documented as a unit comment in the Blood Bank computer system. The comment should include the notifying technologist, the date/time of notification, the physician or nurse accepting the notification, and the method of notification.

Definitions/Acronyms

- **Directed donor unit:** An allogeneic RBC donation intended for a specified recipient.
- **Incompatible crossmatch:** Agglutination and/or hemolysis evident at any stage of the crossmatch test.
- **LISS:** Low ionic strength solution.
- **BBCDM:** Blood Bank Computer Documentation Manual.
- **ABO/Rh Discrepancies:** An ABO or Rh discrepancy occurs when:

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- the ABO or Rh of the current sample is not in agreement with the ABO or Rh of a historical sample, or
- ABO or Rh graded reactions are not valid, or
- Graded reactions do not yield a valid interpretation.
- For additional information, refer to the *Results and Interpretation* section of **Transfusion Medicine policy, P103**, *Determining the ABO and Rh(D) of Patients Who are at Least Four Months Old*.
- **Current sample:** A sample that was collected no more than 3 days before the current date. For example, if a sample is drawn on Monday (day 0), then the sample remains “current” all day Mon., Tues., Wed., and Thur.

Specimen Collection and Handling

The preferred specimen is a 6ml EDTA sample with affixed identifying label. See **Transfusion Medicine policy, P101**, *Triaging and Identifying Acceptable Samples for Testing*, for acceptable alternatives.

Equipment

Table 109-1: Serologic Crossmatch Equipment

Tube crossmatch methods	Manual GEL crossmatch
• table top centrifuge • lighted viewing mirror • heat block incubator • timer • automatic cell washer	• MTS incubator • MTS centrifuge • MTS Workstation • Automatic or manual pipette

Supplies

Table 109-2: Serologic Crossmatch Supplies

Tube crossmatch methods	Manual GEL crossmatch
• commercial reagents: ○ low ionic strength additive solution (LISS) ○ monospecific IgG anti-human globulin reagent (AHG) ○ IgG coated check cells • normal saline • 10 x 75 mm test tubes • segment processor	• MTS Diluent 2 • MTS anti-IgG cards • pipette tips • 12 x 75 mm test tubes

Forms

- F1566, *Record of Transfusion* (computer generated) . . . aka transfusion tags
- *Unit Antigen Label*

Quality Control (QC)

- QC control for tube crossmatches is performed as described in P305, *Routine Quality Control of Blood Bank Reagents*.
- QC control for manual gel crossmatches is performed as described in P328, *Quality Control of the Manual Gel System Reagents*.
- Gel cards must be visually inspected before use. Do not use gel cards if the gel matrix is absent or if the liquid level in the microtube is at or below the top of the gel matrix. Do

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not use gel cards that show signs of drying, discoloration, bubbles, crystals, or other artifacts. Do not use cards if foil seals appear damaged or opened.

- When testing by a tube AHG crossmatch method, check cells must be used if the reaction at the AHG phase is negative. **The graded reactions of check cells are expected to react at any strength.** ~~The check cells are expected to react at any strength 2+ or greater.~~ If the check cells do not react as expected, the crossmatch is invalid and must be repeated.

Serologic Crossmatch Methods

The following tables describe the various serologic crossmatch methods:

- Table 109-3: Summary of Serologic Crossmatch Methods
- Table 109-4: Tube Crossmatch Preparation
- Table 109-5: Immediate-Spin Crossmatch (**IS**)
- Table 109-6: Antihuman Globulin Crossmatch (**AHG**); this table includes variations for:
 - Crossmatch with Autoabsorbed Plasma (**AA**),
 - 60-Minute No-LISS Crossmatch (**NL**), and
- Table 109-7: All Phase Crossmatch (**AP**)
- Table 109-8: Gel Antiglobulin Crossmatch (**GEL**)

Summary of Serologic Crossmatch Methods

Following is a summary that may be a useful source for determining the type of serological crossmatch to perform. It indicates the phases at which the method is read and lists related reference sources.

Table 109-3: Summary of Serologic Crossmatch Methods

Code	Crossmatch Method	Location of SOPs / Related References	Indications	Phases			
				I.S.	37C	AHG	CC
IS	Immediate spin (tube)	Table 109-5	In place of electronic crossmatch during computer downtime, post-emergency issue, ABO discrepancies	X			
AHG	Tube / LISS Antihuman Globulin	Table 109-6	Alternative method for AHG crossmatch (GEL is the standard AHG method). Used only if specifically directed by the SOPs or MD			X	X
PW	Pre-warmed	P603	PW XM should be rarely used, and only with MD approval.			X	X
AA	Autoadsorbed AHG (tube)	Table 109-6 and P618	Crossmatching patient with warm auto- antibody using auto absorbed plasma		X	X	X
NL	60 Minute No-LISS Tube XM	Table 109-6 and P618	Patient with warm autoantibody		X	X	X

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GEL	Gel	Table 109-8 and Antibody Screening P104	The standard crossmatch for patients with unexpected antibodies			X	
SR	Saline Replacement	P601	Patient with rouleaux forming properties in plasma causing incompatible IS XMs	X			
AP	All Phase	Table 109-7 P626	Patients with Anti-A, also used infrequently for patients with both cold- and warm-reactive antibodies.	X	X	X	X

Procedure

For all serologic tube crossmatches, begin with Table 109-4, *Tube Crossmatch Preparation*.

Table 109- 4: Tube Crossmatch Preparation

Step	Action
1	Compare the following information on the sample label to the data in the computer: • medical record number • visit number • name (spelled correctly) • wristband # • birthdate • accession number • sample in-date • For pretransfusion patients, only the information in bold face is required to be on the sample label; however, any additional information on the sample label must be accurate. Refer to Transfusion Medicine policy, P101, <i>Triaging and Identifying Acceptable Samples for Testing</i>. • Investigate and correct any discrepancy before proceeding.
2	In the Blood Bank computer, ensure that antibody screen and ABO/Rh testing requirements are met. If necessary, refer to Table 108-1, <i>Pre-Crossmatch Sample Labeling and Testing Requirements</i>.
3	Determine whether the patient has unexpected antibodies. • Antibodies display in the “antibody” field of the patient’s Blood Bank computer record. • If unexpected antibodies are present, units selected for crossmatching must comply with P118, <i>Policies for Providing Red Blood Cells to Patients with Unexpected Antibodies</i>. ○ The patient is not eligible for electronic crossmatching, ○ A gel crossmatch must be performed, and ○ It may be necessary to provide antigen negative RBCs.

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4	Determine whether the patient has autologous or directed units. • “Autologous” displays in the “messages” field of the patient’s Blood Bank computer record. • If the patient has autologous or directed RBCs, then also comply with the policies of P115, <i>Policies Relating to Autologous and Directed Donations</i>.
5	Determine whether the patient has any other special transfusion requirements (i.e., irradiated or washed). • These requirements display in the “messages” field of the patient’s Blood Bank computer record. • RBC units selected for crossmatching must meet the patient’s special transfusion requirements, if applicable. See P226, <i>Special Transfusion Requirements for Patients Greater than Four Months Old</i>.

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Table 109- 4: Tube Crossmatch Preparation, continued

Step	Action
6	Select RBC units for serologic crossmatching. Crossmatched units must meet the requirements found in the following (if applicable): • The P108 policy <i>RBC Unit Selection Based on ABO, Rh and Inventory Concerns</i>. • P226, <i>Special Transfusion Requirements for Patients Greater than 4 Months Old</i>. • P118, <i>Policies for Providing Red Blood Cells to Patients with Unexpected Antibodies</i>. • P115, <i>Policies Relating to Autologous and Directed Donations</i>.
7	Ensure that the selected RBCs meet quality control specifications and that the expiration date of the unit is acceptable. Refer to P108, Section I, <i>Quality Control</i>. Do not crossmatch the unit if it is expired.
8	Build the crossmatch worksheet in the Blood Bank computer. Refer to the BBCDM / T&S and Crossmatch / Serologic Crossmatch.
9	Label two (2) 10 x 75 mm test tubes for each crossmatch as follows: • tube #1 (crossmatch tube): patient name and donor number, and • tube #2 (cell suspension): donor number Tubes should be labeled in a manner that allows for identification of the tubes' contents. It is acceptable to label tubes with: • The first 3 letters of the patient's last name and • A sticker with the donor unit number, or the last 3 numbers of the donor unit number.
10	Obtain a segment from each unit and place in corresponding tube #2. Use a segment of tubing that was originally attached to the blood unit container.
11	Choose the appropriate serologic crossmatch procedure. Refer to Table 109- 3, <i>Summary of Serologic Crossmatch Methods</i>.
12	Prepare the appropriate cell suspension in tube #2. Discard unit segment. • Refer to Transfusion Medicine policy, P060, <i>Making a Test Red Cell Suspension</i>. • If a tube method of crossmatching requires an AHG phase reading, then the RBC suspension must be washed.
13	Proceed to appropriate serological crossmatch procedure/table. The appropriate procedure/table is indicated in Table 109- 3, <i>Summary of Serologic Crossmatch Methods</i>.

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Table 109- 5: Immediate-Spin Crossmatch

Step	Action
1	Continue from Table 109- 4: <i>Crossmatch Preparation</i> .
2	In tube #1 (crossmatch tube) combine: - two (2) drops of patient's plasma with - one (1) drop of donor cell suspension.
3	Agitate tube to mix contents and centrifuge. See P340, <i>Calibration of Serologic Centrifuges</i> .
4	Observe the supernate for hemolysis (consider whether the sample itself was hemolyzed). - If the supernate shows hemolysis, wash donor cells and repeat test. - If hemolysis persists, initiate antibody identification process; refer to P604, <i>Antibody Identification by the Tube Method</i>.
5	Resuspend the cell button. Read, grade and record results. - See P061, <i>Reading, Grading, and Recording Test Reactions</i>. - Refer to the BBCDM / <i>T&S and Crossmatch / Serologic Crossmatch</i>.
6	Interpret the crossmatch. Refer to the <i>Interpretation</i> section.
7	Complete the immediate-spin crossmatch in the computer and print transfusion tags of compatible units. - Refer to the BBCDM / <i>T&S and Crossmatch / Serologic Crossmatch</i>. - If the XM is incompatible, refer to P624, <i>Investigation of Incompatible Crossmatches</i> before proceeding.
8	Tag compatible RBC units and place in the appropriate crossmatch refrigerator. See P225, <i>Tagging Blood Components</i> .

Table 109-6: Tube Antihuman Globulin (AHG) Crossmatches

Step	Action
1	Continue from Table 109- 4: <i>Tube Crossmatch Preparation</i> . - The following variations of tube AHG crossmatches are performed using this table: the LISS/tube AHG, AA, and NL. - The donor cells should be washed, as indicated in Table 109-4, step 12.
2	Combine donor cells, patient plasma, and LISS (if applicable) in the following order: - Add patient plasma to each of the test tubes. 2 drops plasma for tube/LISS XM 3 drops plasma for 60-minute no-LISS XM and XMs with autoadsorbed plasma. - Add one (1) drop of the washed donor cells to the correspondingly labeled tubes. - If applicable, add two (2) drops LISS to each of the test tubes. - LISS is added for the LISS/tube AHG XM, and is omitted for the NL and AA crossmatches. - The order in which patient plasma, cells, and LISS are added is important to prevent hemolysis of the cells.

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Table 109-6: Tube Antihuman Globulin (AHG) Crossmatches, continued

Step	Action		
3	Agitate the tubes to mix.		
4	Incubate the tubes for the times indicated below:		
	Type of Crossmatch . . .	Incubate tubes for . . .	Notes
	Tube/LISS AHG	15 minutes at 37°C.	Incubation may not exceed thirty (30) minutes. Tubes are not read at the 37°C phase.
	60-minute No-LISS (NL) or Autoadsorbed Plasma (AA)	1. 60 minutes at 37°C. 2. Centrifuge tubes and resuspend the cell button. Read, grade, and record results for the 37C phase.	• Tubes are read at the 37°C phase. • See P061, <i>Reading, Grading, and Recording Test Reactions</i>.
5	Wash tubes in an automatic cell washer for four (4) cycles. Alternatively, wash by hand three to four times with large volumes of saline.		
6	Add two (2) drops of Anti-IgG AHG. Agitate tubes to mix. Centrifuge tubes.		
7	Resuspend the cell button. Read, grade and record results for the AHG phase. See P061, <i>Reading, Grading, and Recording Test Reactions</i> .		
8	Add one (1) drop IgG coated check cells to all tubes that were negative at the AHG phase.		
9	Agitate tubes to mix. Centrifuge tubes.	If necessary, see P340, <i>Calibration of Serologic Centrifuges</i> .	
10	Resuspend the cell button. Read, grade and record results for coated cells. • See P061, <i>Reading, Grading, and Recording Test Reactions</i>. • The graded reactions of check cells are expected to react at any strength. Check cells must react at least 2+ in strength. If check cells do not react, react less than 2+, then the XM is invalid and must be repeated.		
11	Interpret the crossmatch. Refer to the <i>Interpretation</i> section.		
12	Complete the crossmatch in the computer and print transfusion tags of compatible units. • Refer to the BBCDM / T&S and Crossmatch / Serologic Crossmatch. • If the XM is incompatible, refer to P624, <i>Investigation of Incompatible Crossmatches</i> before proceeding.		
13	Tag compatible RBC units and place in the appropriate crossmatch refrigerator. See P225, <i>Tagging Blood Components</i> .		

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Table 109-7: All Phase (AP) Crossmatch

Step	Action
1	Continue from Table 109- 4: <i>Crossmatch Preparation</i> . The donor cells should be washed, as indicated in Table 109-4, step 12.
2	Combine donor cells, patient plasma, and LISS in the following order: 1. Add two (2) drops of patient plasma to each of the test tubes. 2. Add one (1) drop of the donor cells to the correspondingly labeled tubes. The order in which patient plasma, cells, and LISS are added is important to prevent hemolysis of the cells.
3	Agitate tubes to mix and centrifuge.
4	Resuspend the cell button. Read, grade and record results for the immediate-spin phase. Refer to P061, <i>Reading, Grading, and Recording Test Reactions</i> .
5	Add two (2) drops LISS to each of the test tubes.
6	Incubate tubes for 15 minutes at 37°C. Centrifuge. Incubation time may not exceed 30 minutes.
7	Resuspend the cell button. Read, grade and record the 37°C phase results. Refer to P061, <i>Reading, Grading, and Recording Test Reactions</i> .
8	Wash tubes in an automatic cell washer for four (4) cycles. Alternatively, wash by hand three to four times being careful to decant completely after the last wash.
9	Add two (2) drops of Anti-IgG AHG to each tube. Agitate tubes to mix contents. Centrifuge.
10	Read, grade and record the AHG results. Refer to P061, <i>Reading, Grading, and Recording Test Reactions</i> .
11	Add one (1) drop IgG coated check cells to all tubes that were negative at the AHG phase. Agitate tube to mix contents. Centrifuge.
12	Resuspend the cell button. Read, grade and record the check cells results. - The graded reactions of check cells are expected to react at any strength. Check cells must react at least 2+ in strength. If check cells do not react, react less than 2+, then the AP XM is invalid and must be repeated. - Refer to P061, <i>Reading, Grading, and Recording Test Reactions</i>.
13	Interpret AP crossmatch compatibility and verify test results. If AP XM is incompatible, refer to P624, <i>Investigation of Incompatible Crossmatches</i> before proceeding.
14	Complete the AP crossmatch in the computer and print transfusion tags of compatible units.
15	Tag compatible RBC units and place in the appropriate crossmatch refrigerator.

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	See P225, <i>Tagging Blood Components</i> .
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Table 109-8: Gel Antiglobulin Crossmatch

Step	Action
1	Perform the following preliminary steps: • verify that the patient's required testing (ABO/Rh and antibody screening) is complete • select appropriate donor units • build the XM worksheet in the Blood Bank computer. Detailed directions to perform these preliminary steps may be found as steps 1-8 of Table 109-4, <i>Crossmatch Preparation</i>.
2	Label one 12 x 75 mm test tube and one well of an IgG gel card for each crossmatch. Tubes and gel cards should be labeled in a manner that allows for identification of their contents. It is acceptable to label with: • The first 3 letters of the patient's last name and • A sticker with the donor unit number, or the last 3 numbers of the donor unit number.
3	Obtain a segment from each unit and place in the correspondingly labeled tube. Use a segment of tubing that was originally attached to the RBC unit.
4	Prepare a 0.8% cell suspension of the donor unit as follows: 1. Dispense 1.0 mL of MTS Diluent 2™ into the labeled tube(s). 2. Add 10 µL of the donor packed red blood cells. 3. Mix gently to resuspend. The final red blood cell suspension should be approximately 0.8%. For additional information, refer to Transfusion Medicine policy, P060 <i>Making a Test Red Cell Suspension</i>. In the alternative, program 6 on the BioHit pipette may be used to make the 0.8% suspension.
5	Visually inspect each gel card before use. Refer to the <i>Quality Control</i> section.
6	Remove the foil seal from each gel card, exposing only enough wells needed for crossmatches. Foil should be removed immediately before testing. Testing must be initiated within 1 hour as the gel may dry out.
7	To the correspondingly labeled microtube: 1. Add 50µL of the 0.8% donor cell suspension. 2. Add 25µL of patient plasma. • The pipette tip should not touch the gel card. • Plasma must be added within 15 minutes of the donor RBC suspension.
8	Incubate the gel cards at 37°C ± 2 °C for fifteen (15) minutes. Incubation may not exceed 30 minutes.
9	Centrifuge the gel card for ten (10) minutes at 895 ± 25 RPM in a MTS centrifuge or 1032 ± 10 RPM in an Ortho Workstation.
10	Read the front and back of each gel card macroscopically. Record results in the Blood Bank computer. • See P061, <i>Reading, Grading, and Recording Test Reactions</i> • See the <i>Interpretation</i> section near the end of this document.
11	Interpret the gel crossmatch.
12	Complete the gel crossmatch in the computer and print transfusion tags of compatible units. If applicable, refer to P624, <i>Investigation of Incompatible Crossmatches</i>.
13	Tag compatible RBC units and place in the appropriate crossmatch refrigerator.

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	See P225, <i>Tagging Blood Components</i> .
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Limitations

The following may influence the validity of test results:

- Rouleaux may cause a reaction that could be misinterpreted as agglutination. If rouleaux is detected, refer to P601, *Compatibility Testing for Patients with Rouleaux: Saline Replacement Technique*.
- The presence of fibrin or clots in patient serum may cause a reaction in the gel test that could be misinterpreted as mixed field.

Interpretation

For crossmatches performed by any of the tube methods:

- Serologic compatibility is established when donor red cells are not agglutinated or hemolyzed at any phase of the crossmatch.
- Serologic incompatibility is established if hemolysis or agglutination is observed at any phase of the crossmatch.

Tube and gel reactions are read and graded as described in P061, *Reading, Grading, and Recording Test Reactions*.

If incompatibility is observed at any stage of crossmatching, refer to P624, *Investigation of Incompatible Crossmatches*.

References

- AABB, *Technical Manual*, current edition.
- AABB, *Standards for Blood Banks and Transfusion Services*, current edition.

Authorized Reviewers

Chief, Pathology and Laboratory Medicine
Medical Director and/or Designee, Blood Bank
Manager, Blood Bank

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Document Control, continued

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