PURPOSE

Specimen submitted for compatibility (pre-transfusion) testing (including for autologous red cell units) shall be ABORh typed and screened for unexpected red cell antibodies. Crossmatch is the final check for compatibility between donor and recipient to ensure proper selection of blood for transfusion. When the recipient has negative antibody screen and no history of antibodies, the antiglobulin crossmatch is omitted and electronic crossmatch or immediate-spin crossmatch is performed to detect ABO incompatibility.

REAGENTS

A. 0.9 % saline
B. PeG/LISS
C. Anti-IgG
D. Check cells
E. MTS IgG gel cards

EQUIPMENT

A. Calibrated centrifuge
B. Test tubes
C. Transfer pipettes
D. LIS for blood bank
E. CIPS computer system
F. Printer
G. Automated Analyzer

SPECIMEN and REQUISITION

Specimens and requisitions must meet the requirements specified in SFOWI-0079 Blood Bank Specimen and Requisition protocol.

CONTROLS

A. If the patient does not have a historical blood type, a Double Check specimen is needed to confirm the ABORh before blood can be dispensed for routine transfusion. Computer/electronic crossmatch can only be performed when there are two concordant ABORh (one must be current & performed locally) in the LIS.
B. ABO/Rh types of all donor units are confirmed and documented appropriately, using a segment from the unit.
C Each unit is visually inspected for acceptability e.g. leakage, discoloration, clots, hemolysis, legible face label, expiration date, before crossmatch.

PROCEDURE

A. Order Processing

1. Check for a local and current Type and Screen.
2. Search previous transfusion records (Millennium and CIPS and/or HealthConnect) for blood type, antibody, special requirements such as Antigen

negative, Irradiated, CMV negative, Hgb S negative blood and comments before compatibility testing.

3. Make sure special needs on requisition/order match the computer system. Call nurse/provider to confirm the special needs and enter into the computer system if patient qualifies.
4. Add-on crossmatch to the current Type and Screen accession number.
5. Select packed RBCs that is ABORh compatible with the patient and which fulfills patient's special needs, e.g. IRR, CMV -, HgbS -, Ag -. Whole blood must be ABO group specific.
6. All identifying information on the order must be matched to the specimen label before commencing serological compatibility testing. Aliases are acceptable if documented in HealthConnect demographics.

B. Blood Selection

1. If the DBCK sample is not received or tested before blood is needed, O Neg pRBCs shall be selected for crossmatch and dispensed as emergency release.
2. If ABO discrepancy cannot be resolved before blood is needed, group O pRBCs shall be selected for crossmatch.
3. Select Autologous first, then Directed (if unit meet special needs) and finally Random pRBC for crossmatch. **NOTE:** Autologous units require crossmatch.
4. In general, Rh Negative or Rh Indeterminate patients shall receive Rh negative Whole Blood or Red Blood Cell components.
5. **SWITCHING from Rh Neg to Rh Pos RBCs**
    a. Rh Neg patients (females over 50 years old and males over 1 year old) can be switched to Rh Pos RBCs in the following situations:
        i. BLOOD SUPPLIER is unable to supply compatible Rh Neg RBCs due to shortage
        ii. Patient's continued transfusion e.g. profuse bleed or MTP, will deplete the **O Neg** pRBCs inventory
        iii. Notify the attending physician immediately and the pathologist of the need to switch and document.
        iv. Follow up with BLOOD SUPPLIER as necessary to get updates as to when the Rh Neg RBCs inventory can be replenished.
6. **Refer to the following SOPs for additional requirements:**
    a. SFOWI-0088 ANTIBODY IDENTIFICATION for patient with antibody
    b. SFOWI-0105 NEONATAL TRANSFUSION
    c. SFOWI-1309 TRANSFUSING PATIENTS POST-HSCT
    d. SFOWI-1316 MANAGEMENT OF PATIENTS ON ANTI-CD38 (DARA)
    e. SFOWI-0096 ANTIGEN TYPING for Sickle Cell, Thalassemia, Severe Congenital Anemias, Autoimmune Hemolytic Anemias and other hematologic diagnoses that require chronic red cell transfusion.

Compatibility Table for Packed RBCs

Recipient Blood Type	O POS	A POS	B POS	AB POS	IND POS
Donor blood type	O POS	A POS	B POS	AB POS	O POS
	O NEG	A NEG	B NEG	AB NEG	O NEG
		O POS	O POS	A POS	
		O NEG	O NEG	A NEG	
				B POS	
				B NEG	
				O POS	
				O NEG	
Recipient Blood Type	**O NEG**	**A NEG**	**B NEG**	**AB NEG**	**IND NEG**
Donor blood type	O NEG	A NEG	B NEG	AB NEG	O NEG
		O NEG	O NEG	A NEG	
				B NEG	
				O NEG	

Compatibility Table for Whole blood (donor must be ABO group specific with recipient)

Recipient Blood Type	O POS	A POS	B POS	AB POS	IND POS
Donor blood type	O POS	A POS	B POS	AB POS	Do Not Give Whole Blood
	O NEG	A NEG	B NEG	AB NEG	
Recipient Blood Type	**O NEG**	**A NEG**	**B NEG**	**AB NEG**	**IND NEG**
Donor blood type	O NEG	A NEG	B NEG	AB NEG	Do Not Give Whole Blood

C. Reading and Interpreting Serologic Compatibility Testing

1. Read and record results concurrently. Record final interpretation at completion of testing.
2. Agglutination between donor cells and patient's plasma/serum at any phase is interpreted as incompatible.
3. If hemolysis is observed when using patient's **serum**, the result is interpreted as incompatible.
4. No agglutination between donor cells and patient's plasma/serum is interpreted as compatible.

RESULT OBSERVATION for All Serological Crossmatch Methods

If	Then	Crossmatch Interpretation
Agglutination or hemolysis (serum) at any phase and any method	Indicates **incompatibility** due to the presence of antibody directed against the corresponding antigen on the donor cells.	Incompatible
No agglutination or no hemolysis	Indicates compatibility between recipient and donor.	Compatible

D. NEGATIVE Antibody Screen <u>And</u> NO History of Clinically Significant Antibody(s)
Perform computer/electronic crossmatch if patient qualifies (see table below). Otherwise perform serological immediate spin crossmatch by Tube or Gel (using buffer gel card) methodology.

1. **Electronic Crossmatch**
   a. **Note:** CLS **<u>must</u>** qualify patient for Electronic Crossmatch based on the eligibility requirements below. Do Not qualify patient based only on Millennium's Eligibility icon.
   b. Refer to Computer SOP for instructions on performing Electronic Crossmatch (EXM).

Electronic Crossmatch Eligibility Tables

Eligible for Computer Crossmatch
Current T/S performed at SFO. Two concordant blood types. Current ABSC negative. No history of clinically significant antibody(ies).
pRBC only (including Directed and Autologous).
No Blood Bank comments to disallow Electronic Crossmatch.

NOT Eligible for Computer Crossmatch
Only one blood type (No Double Check or No historical blood type upload).
Current antibody screen is positive.
History of clinically significant antibody(s).
Unexplained Typing Discrepancies on the current sample.
Neonates - see SFOWI-0105 Neonatal Transfusion SOP for details.
Whole blood - has not been validated for EXM.
Granulocytes and bloody platelets - have not been validated for EXM.
Laboratory Computer System is not available.

2. **Immediate Spin Crossmatch**
   a. **Serological Immediate Spin Crossmatch** (IS) should be performed:
      i. When the laboratory computer system is not available.
      ii. When crossmatching type specific whole blood (Rh POS patient

can receive Rh NEG whole blood). **NOTE: All recipients must receive ABO group specific whole blood.**

iii. When the historical ABORh has not been uploaded to the current computer system.

iv. When AHG crossmatch is perform, IS crossmatch must be performed to detect ABO incompatibility. LISS Ortho Antibody Enhancement solution detects ABO incompatibility, making IS crossmatch unnecessary.

b. **TUBE Immediate Spin Crossmatch Procedure**

Steps	Action
1	Add 1 drop of a 2-5% donor cell suspension to 2 drops of patient's plasma in a properly labeled test tube.
2	Centrifuge at high speed for calibrated time and read immediately and macroscopically.
3	Results must be entered in the computer as they are read.
4	If serum is used and hemolysis is observed, the crossmatch must be interpreted as incompatible.

c. **Buffer GEL Immediate Spin Crossmatch Procedure**

<table>
<tr><th>Steps</th><th>Action</th></tr>
<tr><td>1</td><td>Prepare 0.8% cell suspension from a segment of the donor unit.
<table>
<tr><th>Steps</th><th>Action</th></tr>
<tr><td>a</td><td>Label a 12 x 75 mm test tube for each donor to be tested.</td></tr>
<tr><td>b</td><td>Dispense 1.0 ml of MTS Diluent 2 into the labeled tube(s).</td></tr>
<tr><td>c</td><td>Add 10 ul of the donor packed red blood cells.</td></tr>
<tr><td>d</td><td>Mix gently.</td></tr>
</table>
0.8% final cell suspension are stable for 24 hours.</td></tr>
<tr><td>2</td><td>Buffer Gel Compatibility Test procedure
<table>
<tr><th>Steps</th><th>Actions</th></tr>
<tr><td>a</td><td>Label the MTS Buffer Gel Card with the appropriate identification and test.</td></tr>
<tr><td>b</td><td>Remove foil of the wells that will be used for testing from card.</td></tr>
<tr><td>c</td><td>Add 50uL of 0.8% donor cells into microtube well at 45^0 angle. Do not touch pipette to gel card.</td></tr>
<tr><td>d</td><td>Add 50uL of patient’s plasma at 90^0 angle into the same well.</td></tr>
<tr><td>e</td><td>Centrifuge the gel card at 895±25 RPM for 10 minutes.</td></tr>
<tr><td>f</td><td>Read the front and the back of each microtube macroscopically. Record results</td></tr>
<tr><td>g</td><td>Cover microtube(s) with tape for review later.</td></tr>
</table></td></tr>
<tr><td>3</td><td>Results
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Hemolysis (if serum is used) or agglutination of the red cells in a microtube of the gel card</td><td>Indicates incompatibility due to the presence of an antibody directed against the corresponding antigen that is present on the donor cells</td></tr>
<tr><td>No agglutination or no hemolysis of the red cells and a complete sedimentation of all red cells at the bottom of the microtube.</td><td>Indicates negative results and the absence of an antigen/antibody reaction. The unit is compatible.</td></tr>
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</table>

d. If the **immediate spin crossmatch is incompatible** (current and historical antibody screen negative), one or more of the following steps should be performed to resolve problem:

i. Wash donor cells and repeat crossmatch.
ii. Retype the recipient and donor.
iii. Perform DAT on the donor cells.
iv. Saline replacement technique may be helpful to distinguish between rouleaux and agglutination.
v. If repeat crossmatch is still incompatible, perform an antibody screen and antibody panel at immediate spin and room temperature.

E. POSITIVE Antibody Screen Or History of Clinically SIGNIFICANT Antibody(s)

1. **Antiglobulin (AHG) Crossmatch Methods/Enhancements**
   a. Automated or Manual Gel IAT, PeG, LISS, Saline or Prewarmed.
   NOTE: Only use Prewarmed when instructed by Supervisor or recommended by Reference Lab.
2. **AHG Crossmatch Method/Enhancement Selection**
   a. In general, use the same crossmatch method/enhancement as the one used to identify the antibody.
3. **ABO Incompatibility Detection**
   a. Include immediate spin tube test or immediate spin using buffer gel card except when using LISS Ortho Antibody Enhancement Solution.
   b. LISS Ortho Antibody Enhancement Solution detects ABO incompatibility, making immediate spin crossmatch unnecessary.
4. **Current or History of Clinically Significant Antibody**
   a. RBC units must be confirmed phenotype negative for the antigen which corresponds to the patient's Clinically **Significant** Antibody, unless commercial antiserum for phenotyping is unavailable.
   b. Refer to SFOWI-0088 Antibody Identification for additional crossmatch requirements.
   c. Consult with Reference Lab if needed.
5. **Current Positive Antibody Screen due to Clinically Insignificant Antibody**
   a. Examples of Clinically Insignificant Antibodies with optimal reactive temperatures below 37^0C are anti-P1, anti-Le(a), anti-Le(b), anti-M, anti-N, anti-Lu(a) and anti-A1.
   b. When Clinically **Insignificant** Antibodies show reactivity at IS or RT, perform AHG crossmatch using LISS Ortho Antibody Enhancement Solution.
   c. When Clinically **Insignificant** Antibody reacts at 37^oC under strict warm condition, it may be necessary to crossmatch antigen negative units at AHG phase unless commercial antisera are unavailable.
   d. Refer to SFOWI-0088 Antibody Identification for additional crossmatch requirements.

VISION

Step	Actions
1	Label a 10 x 75 mm test tube for each donor to be tested with barcoded unit#.
2	Dispense the entire content of one or two donor segments into each test tube.
3	Load Anti-IgG Gel card(s).
4	Load patient sample.
5	Program the sample for Crossmatch-IAT. **NOTE:** VISION cannot perform Crossmatch-IS. Manual tube XMIS must be performed.
6	Load donor sample(s).
7	Vision will interpret the IAT compatibility results.
8	When tests are completed, print results and enter into LIS. Initials on printout.
9	Second CLS reviews the manual result entry accuracy and initials on the printout also.

IgG GEL

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<tr><th>Steps</th><th>Action</th></tr>
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<tr><td>c</td><td>Add 50uL of 0.8% donor cells into microtube well at 45^0 angle. Do not touch pipette to gel card.</td></tr>
<tr><td>d</td><td>Add 25uL of patient’s plasma at 90^0 angle into the same well.</td></tr>
<tr><td>e</td><td>Incubate at 37ºC for 15 minutes.</td></tr>
<tr><td>f</td><td>Centrifuge the gel card at 895±25 RPM for 10 minutes.</td></tr>
<tr><td>g</td><td>Read the front and the back of each microtube macroscopically. Record results</td></tr>
<tr><td>h</td><td>Cover microtube(s) with tape for review later.</td></tr>
<tr><td>i</td><td>Perform IS crossmatch by tube or Buffer Gel card to detect ABO incompatibility.</td></tr>
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</table>

PEG

Step	Actions
1	Add 2 drops of patient's plasma/serum in a properly labeled test tube.
2	Add 1 drop of 2-5% donor cells.
3	Centrifuge at high speed for calibrated time.
4	Examine for agglutination (IS phase to detect ABO incompatibility). Record results.
5	Add 2 drops of PeG to each test tube
6	Mix and incubate at 37^{o}C for 15 minutes.
7	Wash 3-4 times.
8	Add 2 drops anti-IgG.
9	Centrifuge at high speed for calibrated time.
10	Examine for agglutination (AHG phase) macroscopically and microscopically. Record results.
11	Confirm all negative tests by adding 1 drop of check cells. Record results.

LISS (Ortho Antibody Enhancement Solution)

Steps	Actions
1	Add 2 drops of plasma in a properly labeled test tube.
2	Add 1 drop of 2-5% donor cells.
3	Add 2 drops of LISS to each tube.
4	Mix and incubate at 37^{o}C for 15 minutes.
5	Centrifuge.
6	Resuspend the cells and examine for agglutination (37^{o}C phase). Record results.
7	Wash 3-4 times.
8	Add 2 drops anti-IgG.
9	Mix and centrifuge for calibrated time.
10	Resuspend and examine for agglutination (AHG phase) macroscopically. Record results.
11	Confirm negative reactions with 1 drop of check cells**.** Record results.
12	LISS Ortho Antibody Enhancement Solution detects ABO incompatibility, making immediate spin crossmatch unnecessary.

SALINE

Steps	Actions
1	Add 2-4 drops of patient's plasma into a properly labeled test tube.
2	Add 1 drop of 2-5% donor cells to the tube.
3	Mix and centrifuge at high speed for calibrated time.
4	Examine for agglutination (IS phase to detect ABO incompatibility). Record results.
5	Incubate at 37ºC for 30-60 minutes.
6	Centrifuge at high speed for calibrated time.
7	Observe for hemolysis.
8	Resuspend and examine macroscopically for agglutination (37ºC phase). Record results.
9	Wash 3-4 times
10	Add 2 drops anti-IgG.
11	Mix well and centrifuge at high speed for calibrated time.
12	Resuspend and examine for agglutination (AHG phase). Record results.
13	Confirm negative result by adding 1 drop of check cells. Record results.

PREWARMED

Caution: Prewarmed technique has been shown to result in decreased reactivity of some potentially significant antibodies and caused weak antibodies to be missed. Do not use this technique to eliminate unidentified reactivity. Only use Prewarmed method when instructed by Supervisor or recommended by the Reference Lab.

PREWARMED TUBE

Steps	Actions
1	Prewarm a bottle of saline to 37ºC.
2	Add 1 drop of 2-5% donor cells to properly labeled tube.
3	Place a small volume of the patient's plasma in another labeled test tube.
4	Incubate the donor cells and patient’s plasma separately at 37ºC for 5-10 minutes**.**
5	Add 2-4 drops of prewarmed plasma to each tube containing prewarmed RBCs. Mix the tubes without removing them from the heat block.
6	Incubate for 30-60 minutes.
7	Wash at least 3 times with prewarmed saline.
8	Add 2 drops anti-IgG.
9	Mix well and centrifuge.
10	Resuspend and examine for agglutination. Record results.
11	Confirm negative result by adding 1 drop of check cells. Record results.
12	Perform IS crossmatch by Tube or Buffer Gel card to detect ABO incompatibility.

PREWARMED GEL

Steps	Actions
1	Label the MTS Anti-IgG Card with the appropriate identification and test information.
2	Remove the foil from the microtubes to be used.
3	Place a small aliquot of the 0.8% of the donor cells in a test tube in the MTS incubator for 5-10 minutes.
4	Place the card and an aliquot of the plasma in the MTS incubator for 5 to 10 minutes.
5	Add 50uL of 0.8% donor cells into microtube well at 45^0 angle. Do not touch pipette to gel card.
6	While keeping the card in the incubator, add 25uL of prewarmed plasma at 90^0 angle into the same well.
7	Incubate at 37±2ºC for 15 minutes. Refer to package insert for comment on extending incubation times.
8	Centrifuge the gel card at 895±25 RPMs for 10 minutes.
9	Read front and back of the card. Record results.
10	Cover microtube(s) with tape for review later.
12	Perform IS crossmatch by Tube or Buffer Gel card to detect ABO incompatibility.

SUMMARY TABLE FOR CROSSMATCH METHODS

NOTE: Include immediate spin crossmatch by Tube or Buffer Gel card when performing IAT crossmatch, except when using LISS Ortho Antibody Enhancement Solution, to demonstrate ABO incompatibility.

Methods	Donor Cells	Patient's Plasma	Incubation	Reading Phase
IMMEDIATE SPIN (TUBE)	1 drop	2 drops	None	IS
IMMEDIATE SPIN (GEL)	50uL 0.8%	50uL	None	IS
GEL IAT	50uL 0.8%	25uL	15 min, 37ºC	AHG
PEG (the number of drops should equal the drops of plasma)	1 drop	2-4 drops	15 min, 37ºC	AHG
LISS (the number of drops should equal the drops of plasma)	1 drop	2 drops	15 min, 37ºC	37ºC and AHG
SALINE	1 drop	2-4 drops	30-60 min, 37ºC	37ºC and AHG
PREWARMED	1 drop prewarmed cells	2-4 drops prewarmed plasma	30-60 min, 37ºC	Wash with prewarmed saline, AHG
PREWARMED GEL	50uL prewarmed 0.8% cells in gel card	25uL prewarmed plasma	15 min, 37ºC	AHG

F. Incompatible Crossmatch Due to Autoantibody

1. For patients with **warm autoantibody,** crossmatch will be incompatible and least incompatible units should be given if underlying alloantibody(s) has been ruled out.
2. **If underlying alloantibodies cannot be ruled out**, blood that is phenotypically matched with the patient can be crossmatched.
3. Notify the patient's physician as soon as possible. Physician's approval is required for release of incompatible crossmatched RBC.
   a. The physician must sign the 'PHYSICIAN'S APPROVAL FOR TRANSFUSION OF INCOMPATIBLE CROSSMATCHED RBCs' form which is valid for the duration of the current T/S.
   b. I f the physician only wants to approve individual RBC unit, he/she can sign on each product chart copy stamped/written with the following:

      Provider # _____ Date _____
      Approve to transfuse the least incompatible RBC by
      MD _____

G. Transfusion Service Crossmatch Report/Tag

1. After the crossmatch results is entered and verified in the computer system a Transfusion Service Crossmatch Report/Product Chart Copy with an adhesive label will be generated.
2. The Crossmatch Report and Crossmatch label must have the following information:
   a. Patient’s/Recipient’s full name.
   b. Patient’s/Recipient’s medical record number
   c. Patient’s Group and Rh
   d. Donor Unit Number
   e. Donor Unit Group and Rh
   f. Donor Unit Expiration Date
   g. Technologist initials.
   h. Indication that the unit is compatible, incompatible, etc.
   i. Donor unit blood attributes if applicable.
3. Affix the Transfusion Service Crossmatch label to the back of each unit before issuance. The label shall remain attached on the unit until completion of the transfusion.
4. Affix one Prepare RBC accession# aliquot label to the top right quadrant of each yellow copy of the Transfusion Report. Discard any extra aliquot label.
5. Paper clip the Transfusion Report/Product Chart Copy with the requisition and file.

H. Storage of Crossmatched RBC Units

1. For non-Surgery patients, place crossmatched RBC units on designated shelves labeled with ABORh that matches the donor’s ABORh in 1-6^{0}C blood storage refrigerators.
2. For Surgery patients, place crossmatched RBC units in a bucket and place bucket

on the top shelf of 1-6^{0}C blood storage refrigerators according to the last 2 digits of the patient's MRN.

PROCEDURAL NOTES

A. **Blood Products Exempted from Compatibility Testing**
   1. Plasma, platelets (that contains less than 2 mL RBC) or cryoprecipitate do not require crossmatch.

B. **Apheresis Granulocytes and Platelets that contain > 2 mL RBCs**
   1. Perform **IS crossmatch** of **Apheresis Granulocytes and bloody Platelets** with the recipient's plasma unless patient has atypical antibody which requires AHG crossmatch instead.
   2. The donor blood cells for crossmatch may be obtained from a sample collected at the time of donation, otherwise a segment from the unit can be used.

C. **Compatibility Testing Detection and Limitation**
   1. It is rare for the antiglobulin phase of the crossmatch to detect a clinically significant antibody if the patient's antibody screen test is negative.
   2. An antibody to a low incidence antigen may cause incompatible crossmatch if the antigen is present on the donor cells.
   3. Even with the advances in blood group serology, compatibility testing will not detect all unexpected antibodies, nor will it guarantee normal survival of transfused cells.

D. **Compatibility Testing during Massive Transfusion Protocol**
   1. Electronic Crossmatch Dispense can be used when the patient is qualified.
   2. Refer to SFOWI-0110 Massive Transfusion for additional information.

REFERENCE(S)

A. AABB, Technical Manual, current edition, Bethesda, MD.
B. AABB, Standards for Blood Banks & Transfusion Services, current edition, Bethesda, MD.
C. Manufacturer's Inserts, current revision.