|  |  |
| --- | --- |
| **Policy Statement** | A crossmatch for compatibility must be performed before any red blood cell transfusion except for emergency situations. Saint Agnes Transfusion Services utilizes several methods of crossmatches. These include: immediate spin (tube), gel (full) and AHG (full using tubes and usually enhancement reagents.) Instructions for computer or electronic crossmatches (EXM) are detailed in a separate procedure. |
| **Purpose** | This procedure provides principles as well as instructions for each crossmatch method. |
| **Scope** | This procedure applies to all patients over the age of four months in Saint Agnes who may receive any red blood cells (these would include homologous, autologous and directed donor units.) Please refer to the neonatal transfusion procedure (TRAN 6025 R) for instructions pertaining to patients under the age of four months. |
| **Responsibility** | All Transfusion Services technical staff will follow the procedure as outlined. All technologists are responsible for reading, understanding and following the procedure as written. |
| **Related Documents** | * TRAN 5020 R: Receipt, Inspection and Storage of Blood Products * TRAN 6001 R: Antibody Screen Tube Method * TRAN 6003 R: Serological Testing ABO Rh * TRAN 6006 R: Antibody Identification * TRAN 6008 R: Emergency Release * TRAN 6010 R: Specimen Receipt and Rejection * TRAN 6011 R: 0.8% Red Cell Suspension Preparation * TRAN 6014 Q: ABO Serological Testing Second Specimen for Verification * TRAN 6019 R: Antibody Screen Gel Method * TRAN 6050 R: Quality Control * TRAN 6310 R: ProVue Routine Testing * TRAN 8011 R: Suspected Transfusion Reaction Investigation * AABB Standards for Blood Bank and Transfusion Services, current edition Bethesda, MD. * AABB Technical Manual, current edition, Bethesda, MD. * Harmening, D. Modern Blood Bank and Transfusion Practices, current edition. FA Davis Company, Philadelphia, PA. * Package inserts for reagents and enhancement media |

***Table of Contents***

* **Crossmatch Requirements…………………………………………………...4**
* **Immediate Spin Crossmatch…………………………………………………4**
  + Principle……………………………………………………………..........4
  + Specimen……………………………………………………………........4
  + Materials…………………………………………………………………..4
  + Procedure…………………………………………………………….......5
  + Results…………………………………………………………………….5
  + **Gel Crossmatch…………………………………………………………….......5**
  + Principle……………………………………………………………….......5
  + Specimen………………………………………………………………….6
  + Materials………………………………………………………………......6
  + Procedure…………………………………………………………………6
  + Results…………………………………………………………………….7
  + Limitations………………………………………………………………...8
  + **Tube Method……………………………………………………………………..8**
* Principle…………………………………………………………………...8
* Specimen………………………………………………………………….8
* Materials…………………………………………………………………..8
* Procedure…………………………………………………………………9
* Results…………………………………………………………………...10
* Limitations……………………………………………………………….10
* **Pre-Warm Method……………………………………………………………..11**
* Principle………………………………………………………………….11
* Specimen………………………………………………………………..11
* Materials…………………………………………………………………11
* Procedure………………………………………………………………..11
* Results…………………………………………………………………...13

**Crossmatch Requirements**

Prior to performing a crossmatch, the following requirements must be met:

* 1. Except in cases of emergency release, all necessary pre-transfusion testing must be completed and reviewed if necessary. This includes blood type and Rh, antibody screen, antibody identification for a positive screen and RETYPE specimen for new patients. For emergency releases, refer to TRAN 6008 R.
  2. Verify orders for the number of blood products requested and any special requirements (for example: phenotypically compatible, irradiated, sickle negative and/or CMV negative.)
  3. Verify patient history for any special products or other requirements due to patient’s diagnosis or previous transfusion reactions.
  4. Quality control testing of all equipment and reagents used during the testing process must be performed as indicated. Details can be found in TRAN 6050 R.

1. **Immediate Spin Crossmatch**
   1. Principle: The immediate spin crossmatch is used to determine and confirm ABO compatibility for a recipient by testing a potential recipient’s (patient) plasma with donor red blood cells. It is used on its own for recipients who have a negative antibody screen and no history of clinically significant alloantibodies. It must also be performed as part of a gel crossmatch in order to determine ABO compatibility.
   2. Specimen: No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved techniques. A 6ml pink top (K2 EDTA) sample properly labeled (see TRAN 6010 R) drawn within the appropriate time period. The time period is based on the recipient’s status as an inpatient (three days), for pre-admission testing prior to a surgical procedure (up to seven days) or autologous units (valid for the patient’s length of stay or until the unit expires, whichever comes first.)
   3. Materials:
      1. 12 x 75 mm Test Tubes
      2. Rack
      3. Marker
      4. 0.9% Normal Saline solution
      5. Serofuge
      6. Segments of Red Blood Cells units to be crossmatched
      7. Segment devices to obtain blood samples
      8. Disposable pipettes
   4. Procedure:
      1. Select ABO/Rh compatible units to be crossmatched for the patient.
      2. Obtain a segment from each unit to be tested, and placed in an appropriately labeled tube.
      3. Use the segment device to obtain sample as indicated by the manufacturer of the product.
      4. Add enough saline solution to each tube containing red blood cells to obtain a 3 – 5 % red blood cell suspension.
      5. Place two drops of patient’s plasma in appropriately labeled tubes.
      6. Add one drop of 3 – 5 % red blood cell suspension of each unit to be tested to the appropriate tube(s). Mix well.
      7. Perform an immediate spin centrifugation for the time indicated on the serofuge.
      8. Re-suspend contents of tube. With the aid of an agglutination viewer, read macroscopically and concurrently record results.
   5. Results:
2. Absence of agglutination or hemolysis is a negative result and should be interpreted as compatible.
3. If agglutination or hemolysis is observed, the result is positive and therefore the unit would be considered incompatible. Investigation and further testing may be required.
4. Enter the interpretation of the results either in the computer system, on the consult form (TRAN 6006 Fa) and/or on a down time form as applicable.
5. If units are compatible, print cross-match cards.
6. Notify appropriate personnel that blood is ready. Enter their name in the LIS.
7. **Gel Crossmatch**
   1. Principle: The crossmatch compatibility test is used to detect the presence of unexpected antibodies in an intended recipient's plasma directed towards antigens present on donor red blood cells. In this test, the donor red blood cells, suspended in MTS 2 diluent, are combined with patient plasma to allow antigen/antibody interaction in the upper chamber of the micro tube. This results in promoting antibody uptake. The detection of this antibody occurs when the sensitized red blood cells react with the Anti-IgG gel in the micro tube during centrifugation.
   2. Specimen: The same specimen requirements apply as with the immediate spin crossmatch.
   3. Materials:
      1. 12 x 75 mm Test Tubes
      2. Rack
      3. Marker
      4. Serofuge
      5. Segments of Red Blood Cells units to be crossmatched
      6. Segment devices to obtain blood samples
      7. Disposable pipettes
      8. MTS Diluent 2
      9. MTS Anti-IgG gel card
      10. MTS pipettes
      11. MTS disposable pipette tips
      12. MLA pipette
      13. MLA disposable pipette tips
      14. MTS gel card incubator
      15. MTS gel card centrifuge
   4. Procedure:
      1. Select ABO/Rh compatible units to be crossmatched for the patient. The units of blood should be phenotyped for corresponding antigens to the patient’s antibodies if the antibodies are deemed clinically significant. If the units have been obtained from the American Red Cross, verify that the phenotype requested is correctly documented.
      2. Prepare donor unit 0.8% cell suspension, using packed red blood cells from donor unit segments. Refer to procedure TRAN 6011 R for instructions.
      3. Select an MTS gel card and visually inspect the card. Label the ID-MTS Anti-IgG Card with the name of patient, or a foot label from the patient’s label, and unit number from the unit of blood.
      4. Remove the foil seal from the micro tubes to be used.
      5. Using an ID-MTS pipette, add 50µL of each donor unit 0.8% red blood cell suspension to the correct micro tube. Do not touch pipette to gel card.
      6. Using an ID-MTS pipette, add 25µL of patient serum or plasma to the correct micro tube.
      7. Incubate at 37 ± 2oC for 15 minutes.
      8. While the cards are incubating, perform immediate spin crossmatch following the instructions listed above in Section I: Immediate Spin Crossmatch, part d (Procedure.)
      9. Record the results of the immediate spin crossmatch as outlined in part e (Results.)
      10. Centrifuge the gel card at the pre-set conditions of 895 ± 25 RPMs for 10 minutes.
      11. Read the front and the back of each micro tube macroscopically and concurrently record reactions as described in the interpretation section of the corresponding MTS Gel Card package insert.
   5. Results:
      1. No agglutination or hemolysis of the red cells is a negative test result and indicates the absence of an antigen/antibody reaction. This indicates that the unit is compatible.
      2. Hemolysis or agglutination of any of the red cells in a micro tube of the gel card indicates the presence of an antibody directed against the corresponding antigen that is present on the donor cells. The unit is considered incompatible for the recipient. Further testing and investigation might be necessary.
      3. Enter the interpretation of the results in both the computer system and on the consult form (TRAN 6006 Fa) and/or on a down time form as applicable.
      4. When entering results in Meditech, enter gel results in the AHG field with a comment that it was performed in gel.
8. If units are compatible, print cross-match cards.
   * 1. Notify appropriate personnel that blood is ready. Enter their name in the LIS.
9. Limitations:
   * 1. Antibodies below threshold level may not be detected by this test.
     2. False-positive results may occur if antibodies to components of the preservative solution are present in the plasma tested.
     3. Significant variations in red blood cell suspensions (<0.6 or >1.0%) may result in false-positive or false-negative reactions.
10. **Tube Method**
    1. Principle: The crossmatch compatibility test is used to detect the presence of unexpected antibodies in an intended recipient's plasma directed towards antigens present on donor red blood cells. In this test, the donor red blood cells, suspended in buffered saline solution, are combined with patient plasma and an enhancement reagent to allow antigen/antibody interaction through various phases of testing. The detection of this antibody can occur at any phase. This method can be utilized in several different scenarios:
       1. Patients whose plasma may react with components in gel
       2. Patients whose antibodies were detected using the tube method of antibody screening
       3. In the event that gel cards or associated reagents may not be available (shortage, recall, QC issues, etc.)
    2. Specimen: The same specimen requirements apply as with the immediate spin crossmatch.
    3. Materials:
       1. 12 x 75 mm Test Tubes
       2. Rack
       3. Marker
       4. Serofuge
       5. Segments of Red Blood Cells units to be crossmatched
       6. Segment devices to obtain blood samples
       7. Disposable pipettes
       8. Enhancement reagents (PEG, AES/LISS) if applicable
    4. Procedure:
       1. Select ABO/Rh compatible units to be crossmatched for the patient. The units of blood should be phenotyped for corresponding antigens to the patient’s antibodies if the antibodies are deemed clinically significant. Make sure that all the units have been tested according to the antiserum manufacturer package insert. If the units have been obtained from the American Red Cross, verify that the phenotype requested is correctly documented.
       2. Obtain a segment from each unit to be tested, and placed on an appropriately labeled tube.
       3. Use the segment device to obtain sample as indicated by the manufacturer of the product.
       4. Add enough saline solution to each tube containing red blood cells to obtain a 3 – 5 % red blood cell suspension.
       5. Place two drops of patient’s plasma in appropriately labeled tubes.
       6. Add one drop of 3 – 5 % red blood cell suspension of each unit to be tested to the appropriate tube(s).
          1. If using PEG, centrifuge for one minute and examine for hemolysis; record concurrently if present. Go to the next step (vii.)
       7. Add two drops of the selected enhancement media used during the testing process. If using saline method, no additional enhancement media should be added at this time.
       8. Incubate the tubes for 15 to 30 minutes in a heat block. The incubation for 30 minutes must be used if no enhancement media has been added.
       9. Remove the tubes from the heat block.
          1. If using PEG, tubes may be examined before hemolysis. Do **not** centrifuge, go immediately to step xi.
          2. If using AES or saline only, go to the next step.
       10. Centrifuge the specimen. Re-suspend the contents of the tube. Read and record the results concurrently with the aid of an agglutination viewer.
       11. Wash the tubes 3-4 times.
       12. Remove the tubes from the cell washer and add 2 drops of AHG-IgG reagent to each tube.
       13. Centrifuge the tubes for the time indicated on the centrifuge.
       14. Re-suspend the contents of the tube. Read and record the results with the aid of an agglutination viewer.
       15. Add Coombs Control (Check Cells) reagent red cells to the tubes when a negative reaction has been obtained.
       16. Centrifuge the tubes for the time indicated on the centrifuge.
       17. Re-suspend, read and concurrently record results. Agglutination must be seen to consider the test valid.
       18. Enter the interpretation of the results in both the computer system and on the consult form (TRAN 6006 Fa) and/or on a down time form as applicable.
    5. Results:
11. No agglutination or hemolysis of the red cells is a negative test result and indicates the absence of an antigen/antibody reaction. This indicates that the unit is compatible.
12. Hemolysis or agglutination of any of the red cells indicates the presence of an antibody directed against the corresponding antigen that is present on the donor cells. The unit is considered incompatible for the recipient. Further testing and investigation might be necessary.
13. Enter the interpretation of the results in both the computer system and on the consult form (TRAN 6006 Fa) and/or on a down time form as applicable.
14. If units are compatible, print cross-match cards.
15. Notify appropriate personnel that blood is ready. Enter their name in the LIS.
    1. Limitations:
       1. False-positive results may occur if antibodies to enhancement solutions are present in the plasma tested.
       2. Significant variations in red blood cell suspensions (<2% or >5%) may result in false-positive or false-negative reactions
16. **Pre-Warm Method**
    1. Principle: Prewarm screens are used to determine the possibility of antibodies that could be masked when the patient has a strong cold antibody. This test will be done in conjunction with other methods to ensure that no alloantibodies are missed.
    2. Specimen: The same specimen requirements apply as with the immediate spin crossmatch.
    3. Materials:
       1. 12 x 75 mm Test Tubes
       2. Rack
       3. Marker
       4. Segments of Red Blood Cells units to be crossmatched
       5. Segment devices to obtain blood samples
       6. Disposable pipettes
       7. Serofuge
       8. Heating Block 37° C.
       9. Mono-specific antihuman globulin (IgG)
       10. Coombs Control red cell reagent (Check Cells)
       11. Isotonic Buffered 0.9% Saline
    4. Procedure:
       1. Select ABO/Rh compatible units to be crossmatched for the patient. The units of blood should be phenotyped for corresponding antigens to the patient’s antibodies particularly if the antibodies are deemed clinically significant. Make sure that all the units have been tested according to the antiserum manufacturer package insert. If the units have been obtained from the American Red Cross, verify that the phenotype requested is correctly documented.
       2. Obtain a segment from each unit to be tested, and placed on an appropriately labeled tube.
       3. Use the segment device to obtain sample as indicated by the manufacturer of the product.
       4. Add enough saline solution to each tube containing red blood cells to obtain a 3 – 5 % red blood cell suspension.
       5. Add one drop of 3 – 5 % red blood cell suspension of each unit to be tested to the appropriate tube(s).
       6. Label an additional tube with the patient’s last name and first initial. To that tube, add a sufficient amount of drops of the patient’s plasma to be able to crossmatch each unit.
       7. Place all tubes in the 37° C incubator, incubating the plasma and cells separately for 10 to 15 minutes.
       8. Put a bottle of saline in a plastic bag and place in the 37° C water bath. (Make sure that water does not get into the saline bottle.)
       9. After the 10 to 15 minutes incubation, using a pipette remove some pre-warm plasma and add two drops to tubes that contain the donor red blood cells.
       10. Incubate for another 30-60 minutes.
       11. After the incubation period, add pre-warmed saline to each tube.
       12. Centrifuge for at least one minute.
       13. Decant the saline.
       14. Wash at least two more times using the warmed saline.
       15. After the last wash and decanting, add 2 drops if anti-IgG.
       16. Centrifuge the tubes for the time indicated on the centrifuge.
       17. Re-suspend the contents of the tube. Read and record the results concurrently with the aid of an agglutination viewer. For questionable results a microscope can be used with caution.
       18. Add Coombs Control (Check Cells) reagent red cells to the tubes when a negative reaction has been obtained.
       19. Centrifuge the tubes for the time indicated on the centrifuge.
       20. Re-suspend, read and concurrently record results. Agglutination must be seen to consider the test valid.
       21. Enter the interpretation of the results in both the computer system and on the consult form (TRAN 6006 Fa) and/or on a down time form as applicable.
    5. Results:
       1. No agglutination or hemolysis of the red cells is a negative test result and indicates the absence of an antigen/antibody reaction. This indicates that the unit is compatible.
       2. Hemolysis or agglutination of any of the red cells indicates the presence of an antibody directed against the corresponding antigen that is present on the donor cells. The unit is considered incompatible for the recipient. Further testing and investigation might be necessary.
       3. Enter the interpretation of the results in both the computer system and on the consult form (TRAN 6006 Fa) and/or on a down time form as applicable.
       4. If units are compatible, print cross-match cards.
       5. Notify appropriate personnel that blood is ready. Enter their name in the LIS.