PURPOSE

The indirect antiglobulin test or antibody screen (ABSC) detects red cell antibodies in the plasma/serum that may reduce the life span of transfused cells (or fetal cells) bearing the offending antigen.

A. Methods of testing of unexpected antibodies to red cell antigens shall be those that demonstrate clinically significant antibodies.
B. They shall include incubation at 37ºC preceding an antiglobulin test using reagent red cells that are not pooled.
C. Hemolysis in the absence of hemolyzed sample or agglutination of any of the red cells in the gel card indicates the presence of an antibody directed against the corresponding antigen, which is present on the screening cells. No agglutination or hemolysis of the screening cells in the gel card is a negative test result and indicates the absence of an antigen/antibody reaction.
D. The actual result of each test observed should be recorded immediately and the final interpretation shall be recorded upon completion of testing.
E. A control system using red cells coated with IgG shall be applied to each antiglobulin test interpreted as negative. When a FDA-Licensed test system is used that does not allow the addition of IgG coated check cells to each antiglobulin test interpreted as negative, controls shall be used according to the manufacturer's written instructions.
F. When clinically significant antibodies are detected, additional testing shall be performed to identify the specificity.

REAGENTS

A. PeG
B. LISS
C. Saline
D. Isotonic saline
E. Antihuman globulin containing anti-IgG
F. Screening cells
G. ID-MTS Gel cards
H. ID-MTS Diluent 2

EQUIPMENT

A. Centrifuge capable of separating cells and plasma/serum
B. VISION
C. 37 ºC heat block
D. Serologic centrifuge
E. Automatic cell washer
F. Agglutination viewer
G. Microscope
H. Transfer pipettes
I. 12 x 75 mm test tubes.

J. ID-MTS Incubator
K. ID-MTS centrifuge
L. ID-MTS dispenser
M. Micropipettes
N. Water bath

SPECIMEN

A. Refer to SFO-WI.0079 'Blood Bank Specimen and Requisition' SOP for specimen expiration date.
B. EDTA /ACD specimen is the specimen of choice.
C. Fully clotted serum specimen can also be used.
D. Requirements for patient identification are the same as those for crossmatch samples. Refer to SFO-WI.0079 'Blood Bank Specimen and Requisition' SOP.

CONTROLS:

A. To confirm the reactivity of reagents, positive controls are tested on each day of use.
B. MTS Diluent 2 must be visually checked to ensure that the liquid is not discolored, turbid or showing any signs of bacterial contamination.
C. If check cells fail to react 2+ - 4+, repeat antibody screen.
D. All equipment and devices are properly maintained and validated/calibrated for use.

PROCEDURE

A. VISION

Vision ABSC is the primary method.

Refer to SFO-WI.1377 'Vision Routine Testing' SOP for operating instructions.

B. MTS Gel Card

Gel is the primary method for **manual** ABSC testing.

Principle:

In this test, the reagent red blood cells in a hypotonic buffered saline solution are combined with patient serum/plasma to allow antigen/antibody interaction in the upper chamber of the microtube. 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 microtube during centrifugation. A completely clotted or EDTA or ACD anticoagulated sample drawn within three days of testing may be used.

1. **Antibody Screen Test Procedure**
   a. Label the MTS Anti-IgG Card™ with the appropriate identification and test information.
   b. Remove the foil seal from the microtubes to be used.
   c. Using an appropriate calibrated pipette, add 50 µL of each of the 0.8% antibody screen cell suspensions at 45^0 angle to its labeled microtube. Do not touch gel card with pipette tip.
   d. Using an appropriate calibrated pipette, add 25 µL of serum or plasma straight down into each of the labeled microtubes.
   e. Incubate at 37±2°C for 15 minutes. Refer to the package insert for comment on extending incubation times.

f. Centrifuge the gel card.
g. Review the centrifuge display is 895±25 RPMs and the timer display counting down from 10 minutes to 00:00 minutes.
h. For manual readings, read the front and the back of each microtube macroscopically and record reactions as described in the interpretation section of the corresponding MTS Gel Card package insert.

2. No agglutination or hemolysis of the screening cells in the gel card is a negative test result and indicates the absence of an antigen/antibody reaction. Hemolysis in the absence of a hemolyzed sample or agglutination of any of the red cells in the gel card indicates the presence of an antibody directed against the corresponding antigen, which is present on the screening cells.
3. Use prediluted 0.8% screening cells or prepare 0.8%-screening cells.
4. To prepare 0.8% from 3% screening cells,
   a. Label three test tubes I, II, and III; include lot number, date and time of preparation and initial.
   b. With an appropriate pipette, dispense 0.5 ml of each antibody screen cell sample into its appropriately labeled tube and centrifuge one (1) minute to pack the red blood cells.
   c. Remove the supernatant
   d. Add 1.5 ml of MTS Diluent 2™ to each tube. Mix gently. The final cell suspension should be approximately 0.8% and is stable for 24 hours. For best results, the suspension should not be less than 0.6% or exceed 1.0%.
5. **Limitations**
   a. Anomalous results may be caused by fibrin, which can be avoided by re-spinning the specimen and repeating the test.
   b. Interpretation of mix field reaction should be cautious with consideration of patient's clinical history.

C. PeG

Principle:

PeG creates a low-ionic test environment that increases the rate of antibody uptake during incubation. PeG antibody screen is used when MTS Gel gives an invalid result after repeating with re-spun specimen.

1. **Procedure**
   a. Place 2 drops of plasma, serum or eluate to be tested in a small, properly labeled test tube.
   b. Add 1 drop of 3% screening cells.
   c. Add 2 drops of PeG and mix well.
   d. Incubate for 15 minutes at 37°C. The incubation time may be extended to 30 minutes if desired.
   e. Examine for hemolysis. **Do not centrifuge.**
   f. Wash cells 3-4 times without interruption.
   g. Add 2 drops of anti-IgG immediately.
   h. Mix well and centrifuge for a time appropriate to the calibration of the centrifuge at HIGH speed.
   i. Resuspend the cells by gentle agitation.

j. Examine for agglutination macroscopically and microscopically. Record results.

k. Confirm the validity of all negative tests by adding 1 drop of IgG coated check cells.

NOTE: Weak reactions can be enhanced by using 3 drops PeG, 3 drops plasma and extending incubation time up to 30 minutes.

2. **Limitations**

a. Polyethylene glycol has a tendency to precipitate serum globulins. When testing samples containing elevated globulin levels, three washes may not be sufficient to remove unbound protein. If precipitated globulin remains enmeshed in the red cell button, it may neutralize the anti-IgG and cause a false negative result.

b. Precipitation of fibrinogen may be observed when testing plasma samples. It may be necessary to wash the cells more than 3 times to remove all unbound human protein.

D. LISS

Principle:

Incubation of patient plasma/serum and test cells (reagent red blood cells or donor cells) at 37^{o}C is carried out in a low ionic strength milieu through the addition of a LISS (low ionic strength salt solution) reagent. The low ionic environment enhances the first stage of hemagglutination (sensitization) and allows the shorter incubation time to provide for adequate uptake of antibody by the test cells. LISS antibody screen is used when MTS Gel or PeG result is invalid due to warm/cold antibody or Gel/PeG agglutinins and when sample contains elevated globulin.

1. **Procedure**

a. Place 2 drops of plasma or serum to be tested into properly labeled test tubes.

b. Add 1 drop of 3% screening cells.

c. Add 2 drops of LISS to each test tube.

d. Mix the content of each tube thoroughly.

e. Incubate at 36-38 ^{o}C for 15 minutes. Incubation at 37^{o}C may be extended up to 30 minutes. **CAUTION:** Weaker reactions may be obtained if tests are incubated **less** than 10 minutes or **more** than 30 minutes.

f. Centrifuge each tube at high speed for an appropriate calibrated time.

g. Examine the supernatant fluids for hemolysis. Resuspend the cells by gentle agitation and examine for agglutination (LISS 37 C PHASE). Record results.

h. Wash the red cells 3-4 times with saline.

i. Add 2 drops anti-IgG to each tube and mix thoroughly.

j. Centrifuge at high speed for an appropriate calibrated time. Gently resuspend each cell button and examine macroscopically for agglutination (ANTIGLOBULIN PHASE). **NOTE:** Microscopic examination is discouraged when using LISS Ortho Antibody Enhancement Solution. Record results.

k. Confirm the validity of all negative reactions by adding 1 drop of IgG

coated check cells.

NOTE: Weak reactions can be enhanced by using 3 drops LISS, 3 drops plasma and extending incubation time up to 30 minutes.

2. **Limitations**
   a. The ionic strength of the test system is dependent on the amount of serum used. Alteration of the ionic strength of a LISS procedure by the addition of excess human serum will increase the ionic strength and decrease the sensitivity of the test system.
   b. The order in which cells, serum/plasma and LISS are added in the test is important. Addition of LISS to red cells prior to the addition of serum/plasma may lead to slight hemolysis of the red cells.

E. Saline

Principle:

The patient's plasma/serum is incubated with saline suspended cells. No enhancement solution is used so the incubation period is longer than required and increase cell and serum/plasma ratio. The advantage of saline technique is that no enhancement may minimize or eliminate interference cause by clinically insignificant cold agglutinins and use of enhancement solution will aggravate the problem of abnormal proteins, which may cause cells to agglutinate (e.g. multiple myeloma).

1. Procedure
   a. Place 3-4 drops of patient's serum into a properly labeled test tube.
   b. Add one drop of 3% screening cells to the tube.
   c. Mix and incubate at 37 ^{o}C for 30-60 minutes.
   d. Centrifuge for an appropriate calibrated time at high speed.
   e. Observe for the presence of hemolysis.
   f. Resuspend the cells by gentle agitation and examine macroscopically for agglutination. Record results.
   g. Wash cells 3-4 times without interruption.
   h. Add 2 drops of anti-IgG immediately.
   i. Mix well and centrifuge for an appropriate calibrated time at high speed.
   j. Resuspend the cells by gentle agitation.
   k. Examine for agglutination macroscopically and microscopically. Record results.
   l. Confirm the validity of all negative tests by adding 1 drop of IgG coated check cells.

F. Prewarm

Principle:

Prewarming may be useful in the detection and identification of red cell antibodies that bind to antigen only at 37^{0}C. The test is particularly useful for testing sera of patients with potent cold-reactive autoantibody that may mask the presence of clinically significant alloantibodies.

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.

1. **Procedure**
   a. Prewarm a bottle of saline to 37°C.
   b. Add 1 drop of 3% saline-suspended screening cells to each properly labeled test tube.
   c. Place the tubes containing the screening cells and a tube containing a small volume of the patient's plasma and a pipette at 37 °C; incubate for 5-10 minutes.
   d. Using the prewarmed pipette, transfer 3-4 drops of prewarmed plasma to each tube containing prewarmed screening cells. Mix the tubes without removing them from the incubator.
   e. Incubate for 45-60 minutes.
   f. Without removing the tubes from the incubator, fill each tube with prewarmed 37°C saline. Centrifuge and wash 3-4 times with prewarmed saline.
   g. Add 2 drops of anti-IgG. Mix well. Centrifuge for an appropriate calibrated time at high speed.
   h. Resuspend the cells by gentle agitation. Read macroscopically and microscopically for agglutination. Record results.
   i. Confirm the validity of all negative tests by adding 1 drop of IgG coated check cells.

G. Cold ABSC at IS, RT and 4^0C

Refer to 'Cold-reactive antibodies' section in SFOWI-0088 'Antibody Identification' SOP.

H. Results and Documentation

1. Reactions must be recorded as they are read.
2. Results should be entered directly into the LIS.
3. The use of Supplemental Worksheet is recommended when multiple ABSC methods are employed.
4. Only one ABSC method and interpretation should be verified in the LIS. All other results should be entered as Result Notes attached to the Interpretation.

PROCEDURE NOTES

A. To result manual ABSC, i.e. Gel, PeG, LISS, Saline, etc instead of the Vision result, cancel the Vision ABSC in ORV and order ABSC Man in DOE or alternatively, add the method i.e. 'Gel' or ‘SF_ABSC’ for PeG/LISS/Saline as Result Note at the Interpretation field.
B. Result Entry by accession number and use Antibody Screen as the test group.
C. Refer to manufacturer's insert for more information on limitations and troubleshooting.

REFERENCE

A. AABB Technical Manual, current edition, Bethesda, MD.
B. AABB, Standards for Blood Banks and Transfusion Services, current edition, Bethesda, MD.