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Antibody Screening-Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide the Blood Bank staff with instructions for antibody screening using the Micro Typing System (MTS) gel card method or the alternative tube method.

II. INTRODUCTION:

- A. Routine antibody screening is generally performed with automated instrumentation by the gel method. The gel method is the standard reference method at this facility. The following non-exhaustive list describes situations when the manual gel method may be performed:
 - 1. When crossmatched blood or test results are requested urgently,
 - 2. When the automated instrument is unable to test patient samples.
- B. The tube antibody screen is usually performed as part of an antibody identification investigation. For example,
 - 1. If a sample demonstrates non-specific reactivity in gel testing, then perhaps the reactions are due to a factor in the gel system. Removing this factor, by testing with the tube method, may eliminate the non-specific reactions.
 - 2. The reactions of patients with warm autoantibodies may be enhanced in the gel method. Therefore, it may be useful to test for underlying alloantibodies using the 60 minute, no-LISS antibody screen method (60MNL) described in this document.
 - 3. If unexpected reactivity is observed in the reverse typing, see Transfusion Medicine policy, [Antibody Identification](#).

III. DEFINITIONS / ACRONYMS:

- A. **MTS:** Micro Typing System
- B. **AHG:** Anti-human globulin
- C. **CC:** Check Cells

- D. **LISS:** Low-Ionic-Strength Additive Solution
- E. **BBCDM:** Blood Bank Computer Documentation Manual
- F. **L&D:** Labor and Delivery unit/Family Birth Center at each site as applicable
- G. **MD:** Medical Director
- H. **WAA:** Warm Auto Antibody
- I. **Wash by hand:** A process in which contents of a tube are re-suspended in a large volume of saline, centrifuged and the supernatant removed by decanting or pipetting.
- J. **60MNL:** 60 Minute, no-LISS method
- K. **Clinically significant antibody:** An antibody:
 - 1. Is known to cause Hemolytic Disease of the Newborn or shortened survival of antigen positive RBCs.
 - 2. Requires transfusion of antigen negative red cells.
 - 3. Is usually IgG and best detectable with antihuman globulin (AHG).
- L. **Clinically insignificant antibody:** An antibody that:
 - 1. Does not cause shortened red cell survival of antigen positive RBCs.
 - 2. Does not require transfusion of antigen negative red cells.
 - 3. Is usually IgM and reacts best below 37C.

IV. PRINCIPLE:

A. Gel Method (Standard Reference Method)

1. Reagent red blood cells in a hypotonic buffered saline solution are combined with patient plasma to allow antigen/antibody interaction in the upper chamber of the microtube promoting antibody uptake. The detection of antibody occurs when the sensitized red blood cells react with the anti-IgG surrounding the gel beads in the microtube and become trapped by the gel during centrifugation.

B. Tube Method (Alternative Method)

1. The goal of tube antibody screening, like gel antibody screening, is to detect as many clinically significant antibodies as possible, while detecting as few clinically insignificant antibodies as possible. To achieve this goal, the tube antibody screen includes incubation/reading at 37 °C and reading at the antihuman globulin (AHG) phase; it is not read at the immediate-spin phase. Therefore, the tube screen procedure described in this document is not well suited to detect cold-reactive, clinically insignificant antibodies.
2. LISS (low-ionic strength additive solution) is usually used as an enhancement medium in the tube antibody screen. It decreases incubation time by accelerating antibody binding to test RBCs. However, because LISS may enhance autoantibody activity, its use may complicate alloantibody identification in those patients with warm autoantibodies. The section Tube Method Procedure (Alternative Method) in this document may be performed with or without LISS.

V. POLICIES:

A. Reading, Grading, Interpreting Results

1. Gel reactions are read and graded according to the manufacturer's guidelines provided in the

ID-Micro Typing Systems™ Interpretation Guide. Refer also to Transfusion Medicine policy, [Reading, Grading, and Recording Test Reactions](#).

Patient's current antibody screen test results must be compared to previous history. See the Interpretation section and the Comparison of Current Antibody Screen to the Historical Record tables in this document.

B. Historical Record Check

1. Before testing, a technologist must perform a historical record check on each sample. Refer to Transfusion Medicine policy, [Historical Blood Bank Record Checks](#).

C. Policies Relating to Positive Antibody Screens and Potential Delays

1. If a sample from a patient who is likely to deliver a baby has an antibody history or a positive antibody screen, then it may be necessary to perform additional testing on the cord blood. See site specific Transfusion Medicine policies *Hemolytic Disease of the Newborn Survey/Cord Blood Evaluation*. In this case, the Labor and Delivery (L&D) nurse should be contacted to ensure collection of the cord blood.
2. An external comment should be added to all positive antibody screens in the Blood Bank computer, to notify the patient's caregivers that a potential delay may occur in providing RBCs if a transfusion is required. This comment is placed in the Blood Bank computer system by the medical technologist performing the antibody screen.
3. In some cases, even though a patient's antibody screen is negative, the potential for a delay in transfusion exists. The external comment should also be added in these cases to either the antibody screen or [the patient ABO history result](#).

D. Applicable Policies when using the Tube Method (Alternative Method)

1. Since the MTS Gel Method is the standard reference method, the results of the gel antibody screen (not the tube antibody screen) should be used to decide whether additional studies are required. If necessary, refer to the section Comparison of Current Antibody Screen to the Historical Record tables in this document.
2. When examining for agglutination a magnifying mirror must always be used.
3. If LISS is used, the volume of plasma used in the test must be equal to the volume of LISS.
4. Maximum incubation time must not exceed manufacturer's limits.
5. Because the tube antibody screen is usually performed as part of an antibody investigation, an auto control should also be performed. Refer to the section *Tube Method Procedure (Alternative Method)* in this document.
6. A direct antiglobulin test (DAT) should be performed if the auto control is positive. If necessary, refer to Transfusion Medicine policy *Direct Antiglobulin Test (DAT) for Patients Who Are At Least Four Months Old*.
7. For patients with warm autoantibodies, the tube antibody screen may be performed without LISS. The 3-cell screen (Ortho 3% SURGISCREEN®) is used to perform the 60-minute no-LISS antibody screen for patients with warm autoantibodies.
8. The tube antibody screen described in this document may not detect cold-reactive antibodies; it does not include an immediate-spin phase reading. To detect cold-antibody reactivity, it may be advantageous to perform an immediate-spin, room temperature, and optional 4°C phase reading as described in Transfusion Medicine policy, *Antibody Identification by the Tube Method*.

VI. REAGENTS:

A. Gel Method

1. 0.8% SELECTOGEN[®] Reagent Red Blood Cells
2. 3% SURGISCREEN[®] Reagent Red Blood Cells diluted to 0.8% as described in Transfusion Services Procedure, [Making a Test Red Cell Suspension](#).
3. MTS[™] Diluent 2
4. MTS[™] Anti-IgG Gel cards

B. Tube Method

1. 3% SURGISCREEN[®] Reagent Red Blood Cells
2. Anti-IgG Antihuman Globulin (AHG) Reagent
3. IgG coated check cells (CC)
4. Low-Ionic-Strength Additive Solution (LISS)

VII. EQUIPMENT:

A. Manual Gel Method

1. MTS incubator
2. MTS centrifuge
3. Ortho Workstation
4. Calibrated pipette (electronic or manual)
5. Timer

B. Tube Method

1. Table top centrifuge
2. Lighted agglutination viewer
3. Heat block incubator (37°C)
4. Timer
5. Automatic cell washer or centrifuge

C. Supplies

1. Pipette tips
2. Test tubes, 10 x 75mm or 12 x 75mm, glass or plastic
3. Disposable pipettes
4. Gauze
5. 0.9% Normal Saline

VIII. QUALITY CONTROL:

- A. Quality control (QC) of the reagents used in this policy is performed as described in site specific Transfusion Medicine policies *Quality Control of Blood Bank Reagents*.
- B. All refrigerated reagents should be brought to room temperature before testing.

- C. If the centrifugation phase is interrupted, then all affected specimens must be retested.
- D. If the speed of centrifugation is not at an acceptable level, then all affected specimens must be retested using different equipment if necessary.
- E. Because prolonged exposure of the 0.8% SELECTOGEN[®] reagent RBCs to both light and room temperature conditions can cause non-specific reactivity, the 0.8% SELECTOGEN[®] reagent RBCs will be stored in the original, manufacturer's box or other container, either in the refrigerator or rotated every shift based on site procedures.
- F. IgG coated check cells must be added to all AHG phase results in tube that are negative.

IX. BEFORE YOU BEGIN:

- A. Perform the following before starting this procedure:
 1. Verify the patient specimen satisfies all labeling requirements as described in Transfusion Medicine policy [Triaging and Identifying Acceptable Samples for Testing](#)
 2. Centrifuge specimens to obtain clear plasma at the calibrated time and RPM of the centrifuge.
 3. Verify that all QC requirements have been completed as indicated in the *Quality Control* section of this document.
 4. Refer to the Transfusion Medicine policy, [Making a Test Red Cell Suspension](#) or the *ID-Micro Typing Systems™ Interpretation Guide* for additional information as needed while performing this procedure.

X. PROCEDURE:

- A. **Manual Gel Method Procedure (Standard Reference Method)**
 1. Verify the requirements in the *Before You Begin* section of this document have been met.
 2. Visually inspect gel cards before use.
 - a. Gel cards should have a clear liquid layer on top of opaque gel. Do not use if GEL card show signs of damage.
 3. Label a MTS™ Anti-IgG Gel card with patient information and the designated screen cell for each gel card microtube.
 - a. Each antibody screen requires two gel card microtubes.
 4. Remove the foil seal from the gel card, exposing only enough microtubes needed for testing.
 - a. Foil should be removed immediately before testing, not more than one hour before testing.
 5. Pipette 50 µl of well-mixed, 0.8% SELECTOGEN[®] reagent screen cells into the correspondingly labeled microtube.
 - a. Ensure the pipette tip does not touch the gel card.
 6. Add 25 ul of patient plasma into the corresponding microtubes.
 - a. Patient plasma must be added within 15 minutes of the screen cells.
 7. Incubate the MTS™ Anti-IgG Gel card at 37°C for 15 minutes.
 - a. Incubation time may be extended up to but must not exceed 30 minutes.
 8. Centrifuge the MTS™ Anti-IgG Gel card for 10 minutes at the calibrated speed of the gel

centrifuge.

- a. MTS Centrifuge = 895 +/- 25 RPM
 - b. Ortho Workstation = 1032 +/- 10 RPM
9. Read both the front and back of the MTS™ Anti-IgG Gel card for agglutination. Grade the reactions in the microtubes.
 10. Record and interpret the graded antibody screen reactions in the Blood Bank computer system or on an appropriate downtime form.
 - a. Add the result delay comment (**ABHX**) to the antibody screen test results for all patients with positive antibody screens.
 - b. Add the **NEX** antibody code to the patient profile to flag the patient as Ineligible for Electronic Crossmatch while antibody identification is pending. Note: The NEX code can be inactivated once the antibody identification is complete and results are on file.
 11. Compare the current antibody screen results to the results of the previous antibody screen to determine what additional actions are required.

Comparison of Current Antibody Screen to Historical Record		
Current Gel Screen	Most Recent, Previous Gel Screen (including those performed at any Corewell Lab Site)	Additional Actions
Negative	Not Applicable (Patient has no previous record)	None
	Negative	None
	Positive and antibody ID was performed within last 3 months.	Repeat current antibody screen <u>if performed using a manual method</u> to verify test accuracy. Consider possibility of WBIT
	Positive and the most recent antibody ID was performed more than 3 months ago	None. No further action is required (report out the negative antibody screen result).
Positive	Not Applicable (Patient has no previous record)	Perform antibody identification studies. Refer to Transfusion Medicine policy <i>Antibody Identification</i>
	Positive	Consider repeat antibody screen before releasing a positive result <u>if reaction strength is weak and there is potential for false positive result.</u>
	History of a warm autoantibody (WAA) or an Anti-CD38.	Refer to Transfusion Medicine policy <u>Warm Autoantibody Investigation.</u>
	Positive and one (1) of the following three (3) conditions exist: 1. Last antibody	Perform an antibody identification. Refer to Transfusion Medicine policy <u>Antibody Identification</u>

	<p>investigation was more than 3 months ago for non-prenatal patients, or 1 month ago for prenatal patients.</p> <p>2. Reactions are consistent with expected screening cell reactivity. See the supplement below.</p> <p>3. Current test reactivity is stronger than previous results.</p>	
	<p>Positive, and all of the following three (3) conditions exist:</p> <p>1. Last antibody investigation was less than 3 months ago for non- prenatal patients, or 1 month ago for prenatal patients.</p> <p>2. Reactions are consistent with expected screening cell reactivity. See the supplement below.</p> <p>3. Current test reactivity is the same or weaker than previous results.</p>	<p>None (report out positive antibody screen). The ABID test that reflexes may be canceled.</p>
<p>Note: When determining whether reactions are "consistent" or "inconsistent" with expected screening cell reactivity, consider antigenic profile of the screening cells used for historical and current antibody screen. This can be accomplished by reviewing the antigram of the previous screening cell Lot number. Another Corewell site may be contacted to obtain this information if necessary.</p>		

12. If no additional actions are required, ensure the sample is capped and stored as directed in Transfusion Medicine policy, *Storing and Disposing of Patient Samples*.

B. Tube Method Procedure (Alternative Method)

1. Verify the requirements in the *Before You Begin* section of this document have been met.
2. Label five test tubes with the patient name and the intended use of the tube, including the corresponding screen cell, the auto control (AC), or the patient's 3% red cell suspension. See the example below:
 - a. Tube 1 – [Name] "SC 1"
 - b. Tube 2 – [Name] "SC 2"
 - c. Tube 3 – [Name] "SC 3"
 - d. Tube 4 – [Name] "AC"
 - e. Tube 5 – [Name] "3%"

3. Prepare a 2 – 4% red cell suspension in the tube labeled "3%" using the patient's own RBCs.
 - a. Refer to Transfusion Medicine policy, [Making a Test Red Cell Suspension](#) for additional information.
4. Combine the 3% SURGISCREEN[®] reagent, patient plasma, patient red cell suspension, and LISS (if applicable) in the corresponding tubes in the order described below:
 - a. Add patient plasma to the four tubes labeled SC 1, SC 2, SC 3, and AC.
 - i. If LISS will be used, add 2 drops of patient plasma per tube.
 - ii. If LISS will not be used (60MNL), add 3 drops of patient plasma per tube.
 - b. Add 1 drop of each 3% SURGISCREEN[®] into each of the corresponding tubes labeled SC 1, SC 2, and SC 3.
 - c. Add 1 drop of the patient 2 – 4% red cell suspension into the tube labeled AC.
 - d. If applicable, add 2 drops of LISS into the tubes labeled SC 1, SC 2, SC 3, and AC.
 - i. The order in which patient plasma, red cells, and LISS are added is important to prevent hemolysis of the red cells.
 - ii. For patients with warm autoantibodies (WAA), LISS should be omitted. Perform a 60-minute no-LISS screen using the 3% SURGISCREEN[®] screen cells.
5. Gently agitate the test tubes to mix the contents.
6. Incubate the antibody screen and auto control test tubes for the time indicated below:
 - a. If LISS was added, incubate the test tubes at 37°C for 15 minutes. Incubation times may be extended up to but must not exceed 30 minutes.
 - b. If LISS was not added (60MNL), incubate the test tubes at 37°C for 60 minutes. Incubation times may not be extended.
7. Remove the test tubes from the incubator and centrifuge them according to the calibrated time of the centrifuge.
8. Observe the supernate in the test tubes for hemolysis. Gently resuspend the cell button of each tube. Read, grade, and record the reactions in the Blood Bank computer system or on an appropriate downtime form for the 37°C phase.
9. Wash the test tubes in an automatic cell washer for 4 cycles.
 - a. Alternatively, the test tubes can be manually washed by hand 4 times, decanting completely after each wash.
10. Add 2 drops of Anti-IgG AHG to the washed test tubes and gently agitate to resuspend the cell button.
11. Centrifuge the test tubes according to the calibrated time of the centrifuge.
12. Observe the supernate in the test tubes for hemolysis. Gently resuspend the cell button of each tube. Read, grade, and record the reactions in the Blood Bank computer system or on an appropriate downtime form for the AHG phase.
13. Add IgG coated check cells to any of the test tubes that are negative.
14. Centrifuge the test tubes according to the calibrated time of the centrifuge.
15. Gently resuspend the cell button of each tube. Read, grade, and record the reactions in the Blood Bank computer system or on an appropriate downtime form for the check cell phase

(CC).

16. Interpret the results from the 37°C, AHG, and CC phases and document this in the Blood Bank computer system or on an appropriate downtime form.
 - a. Refer to the *Interpretation* section of this document.
17. If testing is complete and no additional actions are required, ensure the sample is capped and stored as directed in Transfusion Medicine policy *Storing and Disposing of Patient Samples*.

XI. INTERPRETATION:

Gel Antibody Screen Results Interpretation

Reaction of Screen Cell I	Reaction of Screen Cell II	Antibody Screen Interpretation
0	0	Negative
+	0	Positive
0	+	Positive
+	+	Positive

"+" indicates the presence of agglutination or hemolysis (any strength, including mixed-field)

"0" indicates the absence of agglutination or hemolysis

Tube Antibody Screen Results Interpretation

Reaction of Screen Cell I		Reaction of Screen Cell II		Reaction of Screen Cell III		Antibody Screen Interpretation
37°C / AHG	CC	37°C / AHG	CC	37°C / AHG	CC	
0	+	0	+	0	+	Negative
0	+	0	+	+	NT	Positive
0	+	+	NT	+	NT	Positive
+	NT	0	+	0	+	Positive
+	NT	+	NT	0	+	Positive
0	+	+	NT	0	+	Positive
+	NT	0	+	+	NT	Positive
+	NT	+	NT	+	NT	Positive

Auto Control Reactions:

37°C / AHG	CC	Interpretation	Additional Actions
0	+	Negative	None
+	NT	Positive	Perform a DAT

"+" indicates the presence of agglutination or hemolysis (any strength, including mixed-field)

"0" indicates the absence of agglutination or hemolysis

"NT" indicates that the test phase was not tested.

NOTE: Interpretation of mixed field reactions must be reviewed and can not be interpreted as positive with out

further investigation. The presence of fibrin, clots of particulates may result in some cells layering at the top of the gel. Reactions which are mixed fields in appearance may also be due to cold antibodies reacting with corresponding antigens in the upper portion of the microtube. These will then be trapped in the top portion of the gel at the time of centrifugation resulting in a positive reaction. These reactions must be confirmed with repeat testing or antibody investigations.

Note: When determining whether reactions are "consistent" or "inconsistent" with expected screening cell reactivity, consider antigenic profile of the screening cells used for historical and current antibody screen. This can be accomplished by reviewing the antigram of the previous screening cell Lot number. Another Corewell site may be contacted to obtain this information if necessary.

XII. LIMITATIONS:

- A. Antibodies specific for low incidence antigens not represented on the test cells will not be detected.
- B. Negative antibody screens may occur on patients that were previously positive if the antibody titer has dropped below detectable levels.
- C. The presence of fibrin, clots or particulates may result in some cells layering at the top of the gel giving the appearance of a mixed field reaction. If this occurs, it may be helpful to run a wooden stick through the plasma, re-centrifuge the sample, and re-run the antibody screen.
- D. False positive results may occur if a card that shows signs of drying is used in testing.
- E. False positive results may occur as a result of components in the gel system. It may be necessary to test these samples using:
 1. By gel testing, using 3% test cells that are diluted to the 0.8%. This is described in Transfusion Services procedure, [Making a Test Red Cell Suspension](#)
 2. The tube method (alternative method), described in this document.
- F. If the screening cells are not mixed before use or are pipetted improperly, then false positive or false negative reactions may occur.
- G. Plasma expanders such as Hydroxyethyl Starch (HES) may contribute to non-specific reactivity in the MTS system.
- H. Tube antibody screen results should not be compared with gel antibody screen results when deciding whether antibody identification studies are required. Refer to the *Comparison of Current Antibody Screen to Historical Record* tables in this document.
- I. Erroneous test results may be obtained for multiple reasons, such as technical errors, sample mix-ups, and wrong blood in tube (WBIT) events. If erroneous test results are obtained due to any of these reasons, a variance should be submitted. If any erroneous results were resulted in the Blood Bank computer system, a corrected report should be performed and/or the results should be invalidated.

XIII. REFERENCES:

1. AABB, *Technical Manual*, current edition.
2. AABB, *Standards for Blood Banks and Transfusion Services*, current edition.
3. *ID-Micro Typing Systems™ Interpretation Guide*.
4. Malyska H., Weiland, D. *The Gel Test*, "Laboratory Medicine" 1994;81-5.

Attachments

[Antibody Screens, Panels, and Crossmatch Job Aid \(rev. 05/23/2024\)](#)

Approval Signatures

Step Description	Approver	Date
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