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## Antibody Identification - Blood Bank

Document Type: Procedure

### I. PURPOSE AND OBJECTIVE:

This document will provide the Blood Bank staff with directions for performing an antibody identification panel using the gel card or tube method.

### II. PRINCIPLE OF THE GEL CARD METHOD:

- A. In the gel card antibody identification method, reagent red blood cells in a hypotonic buffered saline solution are combined with patient plasma. Antigen/antibody interaction occurs in the upper chamber of a 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.

### III. PRINCIPLE OF THE TUBE METHOD:

- A. In the tube antibody identification method, reagent red blood cells of a known antigenic composition are combined with patient plasma. Comparison of the reaction pattern obtained with the known antigen makeup of the panel aid in determining the antibody's specificity.
- B. When an unexpected antibody affects the ABO grouping of a patient's sample or causes incompatible immediate-spin crossmatches, it should be identified in order to resolve the ABO discrepancy or provide appropriately crossmatched blood. Because these antibodies are most often IgM (cold reacting antibodies), they do not react well in the gel card test system.
- C. The tube method is also useful for demonstrating the presence of antibodies in the ABO system. For example, anti-A<sub>1</sub> may be detected in blood groups A<sub>2</sub> or weaker A subgroup. Anti-A<sub>1</sub> is often first suspected when an unexpected reaction appears with the A<sub>1</sub> reverse cell while ABO typing a patient who otherwise appears to be group A or AB. After typing the patient's red

blood cells (RBCs) to determine whether they are A<sub>1</sub> negative, a tube panel, as described in the *Tube Panel for the Identification of Anti-A<sub>1</sub>* procedure, may be performed.

- D. A tube panel may also be performed to detect anti-A or anti-B in neonatal patients. This will help determine whether a non-group O neonate may receive a non-group O RBC (Medical Director approval required).
- E. The tube method may also be used to reduce the interference by warm autoantibodies in the identification of possible underlying alloantibodies. Warm autoantibody reactivity is often enhanced in the gel card test method and by the addition of LISS (low-ionic strength additive solution). LISS is omitted in the *60-Minute No-LISS Tube Panel* procedure to facilitate the detection and identification of underlying alloantibodies.

## IV. SCOPE:

- A. Routine antibody identification is performed by the gel method, which is the standard reference method at this facility.
  - 1. The gel card method of antibody identification should be utilized for specimens with a positive antibody screen, or an incompatible crossmatch with antigen negative units.
- B. The tube method for antibody identification may be utilized in the following situations:
  - 1. When cold reacting antibodies are suspected. For example:
    - a. Unexpected reverse ABO typing reactions are observed.
    - b. Incompatible immediate-spin crossmatches are observed.
    - c. Mixed-field gel reactions are observed.
  - 2. When warm autoantibody reactivity is observed, the 60-minute no-LISS method is applied.
  - 3. Detecting anti-A or anti-B in neonatal patients to determine whether a non-group O neonate may receive a non-group O RBC.

## V. DEFINITIONS / ACRONYMS:

- A. **MTS:** Micro Typing System
- B. **CDM:** Blood Bank Computer Documentation Manual
- C. **HIS:** The hospital-wide computer system
- D. **Standard panel:** A commercially prepared panel that usually consists of 11 vials of human RBCs. It is usually performed on patients who do not have a historical antibody record.
- E. **Selected cell panel:** A panel that is pre-selected based on the antigenic profile of the test RBCs.
- F. **Unexpected antibodies:** Any RBC antibody (other than naturally occurring anti-A or anti-B that is regularly found in normal serum or plasma) that is currently or was historically present in a sample.
- G. **Wash by hand:** A process in which contents of a tube are resuspended in a large volume of

saline, centrifuged and the supernatant removed by decanting or pipetting.

- H. **Appropriate patient identification:** Sufficient correlating letters or numbers to associate patient sample, requisition, and tubes for testing.

## VI. POLICIES:

### A. Standard Panels / Selected Panels

1. A standard panel should be performed in the following situations:
  - a. When a patient does not have a historical record of unexpected antibodies.
  - b. When a patient has a historical record of a non-specific antibody (e.g. TWIT, Warm IgG), but no history of an antibody with specificity.
2. A selected panel may be performed in the following situations:
  - a. When a patient has a historical record of unexpected antibodies with specificity. The test cells should be negative for the antigen(s) corresponding to the historical antibody(ies). The selected cell panel should be comprised of antigen positive test cells to rule-out all other antibodies to common red cell antigens.
3. Expired panel cells should not be used in a standard panel.
4. Expired panel cells should only be used in a selected cell panel if in-date cells with the desired antigenic profile are unavailable and appropriate quality control is performed.
  - a. Expired panel cells cannot be used by automated methods.
5. If expired selected cells must be used, observe the donor identification number listed on the manufacturer's antigen to avoid repeat use of the same test cell.
6. An autocontrol must be performed with a panel and should be read at each phase that the panel is read. If the autocontrol is positive, then a direct antiglobulin test (DAT) should be performed. Refer to Transfusion Medicine policy, *Direct Antiglobulin Test (DAT) for Patients Greater than Four Months Old*.
  - a. If an autocontrol is being run alongside testing that includes the use of LISS, the patient's RBCs must be washed prior to preparation of the RBC suspension.

### B. Frequency of Testing

1. The frequency at which a gel panel must be performed is determined by the strength / reactivity and time of the patient's most recent antibody screen. A gel panel is indicated if:
  - a. The last antibody investigation was more than 3 months ago for non-prenatal patients, or 1 month ago for prenatal patients.
  - b. Reactions are inconsistent with expected screening cell reactivity.
  - c. Current test reactivity is stronger than previous results.

## C. Quality Control (QC) Policies

1. Because prolonged exposure of the 0.8% commercially pre-diluted cells to both light and room temperature conditions can cause non-specific reactivity, the Blood Bank will take steps to minimize these conditions. The 0.8% panel cells will be stored in the refrigerator and returned to the refrigerator as soon as possible after use. Panel cells should be brought to room temperature for approximately 15 minutes before use.
2. Panel cells can be used beyond their expiration date. An antigen positive and an antigen negative cell must be run as a control each day of use and must react as expected with the antibody being identified and/or known antisera to prove the cell is viable and reactivity is appropriate. Where possible, the positive control cell chosen should express a single dose of the antigen (heterozygous) to verify that a weak positive reaction can be detected.
  - a. QC of expired cells may be performed by screening an antigen negative and antigen positive cell with known control or antisera to an antigen. Proven reactivity with one antigen is sufficient to prove cell viability. For Example:
    - i. AlbaQ1 (confirmed positive for anti-D) can be tested against D+ and D- expired selected cell.
    - ii. Anti-Fya antisera can be tested against Fya+Fyb+ and Fya-Fyb+ expired selected cell.
  - b. QC of expired cells may be performed by selecting an antigen positive and an antigen negative cell for a corresponding antibody confirmed (with at least 3 positive and 3 negative in date cells) in patient serum. For Example:
    - i. if the patient antibody identified is anti-Fya, and the need is to exclude anti-K, a positive control cell that is K-, Fya + must be chosen, and a negative control cell that is K-, Fya- must be chosen.
    - ii. If the antibodies identified are anti-E and anti-c and the need is to exclude anti-K, 2 positive control cells must be chosen, an E+c- and E-c+ that are negative for the K antigen. If unable to find an E+c- cell on the panel, it is acceptable that the 2 reactive cells chosen are E-c+ and E+c+.
  - c. Documentation of the QC should occur directly on the panel antigram including the manufacturer, lot number and expiration date of the QC vial or antisera used in the testing.
    - i. Royal Oak only: The documentation of expired cell quality control should be done on the *Special Studies Worksheet*.
3. The MTS™ Diluent 2 should not be used beyond the expiration date and should be stored between 2 - 8°C. It should be used at room temperature (18 - 25°C).
4. Test cells must be diluted to 0.8% for use in the gel system; they may be commercially prepared or diluted. Once diluted, test cells should be stored in the refrigerator and have an expiration date/time of the **shorter** of:
  - a. 24 hours from the time of dilution, or

- b. The original expiration date of the panel.
- 5. If diluted test cells are going to be retained after testing, they should be labeled with the following information:
  - a. Test cell number (from the panel)
  - b. Panel manufacturer and lot number
  - c. Original expiration date
  - d. New expiration date and time
  - e. Date of preparation / technologist
  - f. Storage requirements

The *Selected Cell Sticker* may be used for this purpose.

- 6. If diluted test cells are going to be discarded immediately after testing, they should be labeled with the panel lot number and test cell number (from the panel).
- 7. If the centrifugation phase is interrupted, then all affected tests must be repeated.
- 8. If the speed of the centrifugation is not at an acceptable level, then all affected tests must be repeated using different equipment, if necessary.
- 9. IgG coated cells must be added to all AHG phase results that are negative (tube).
- 10. If a test with IgG coated cells is negative, then the test must be repeated.

## D. Tube Panel for the Identification of Anti-A<sub>1</sub>

- 1. A *Tube Panel for Identification of Anti-A<sub>1</sub>*, as described in the procedure below, may be indicated if:
  - a. the anti-A<sub>1</sub> has not been previously identified, **and**
  - b. the patient appears to be blood group A or AB, **and**
  - c. the patient's RBCs are A<sub>1</sub> negative when typed with anti-A<sub>1</sub> lectin, **and**
  - d. the reverse A<sub>1</sub> cell is unexpectedly reactive during ABO typing procedures.

Refer to Transfusion Medicine policy, *Resolution of ABO Discrepancies for A Subgroups and Patients with Anti-A<sub>1</sub>*.

## E. 60-Minute No-LISS Tube Testing

- 1. To reduce the interference of warm autoantibodies and to facilitate the identification of underlying alloantibodies, the tube antibody panel may be performed without LISS in patients with a history of warm autoantibody reactivity or when directed by the Medical Director or designee.
  - a. Note that this testing is also infrequently indicated for patients with HTLA (high titer low avidity) antibodies.

Refer to Transfusion Medicine policy, *Warm Autoantibody Investigations* and Transfusion Medicine policy, *HTLA/Anti-Bga Antibody Investigations*

## F. Immediate Spin (IS) or Room Temperature (RT) Phase Reading for Suspected Cold Reacting Antibodies

1. It may be helpful to perform an IS phase reading to aid in the detection or identification of a suspected cold reacting antibody. If the IS phase reading is non-reactive, then the reactivity of the suspected cold reacting antibody may be enhanced by incubating the tubes at RT for 15 minutes and reading at the RT phase.
  - a. Refer to the *Notes* section near the end of this document; in some cases 4°C incubation may also be used.

## G. Detection of Anti-A or Anti-B in Non-Group O Neonates Receiving Non-Group O RBCs

1. On rare occasions, a non-group O neonate may receive a non-group O RBC. This may occur if a directed donation or rare unit is requested for the neonate.
2. A tube panel must be performed using neonatal plasma to detect anti-A or anti-B, according to the *LISS Tube Antibody Identification* procedure below, with the following specifications:
  - a. An autocontrol is not required.
  - b. Use only two (2) test cells, from a set of commercial "A<sub>1</sub>" and "B" reverse typing cells.
  - c. The panel should be performed with LISS and will be read at the immediate spin, 37°C, and antihuman globulin phases.

## H. Reading / Grading Reactions and Interpretation of Panels

1. Reactions are read and graded according to Transfusion Medicine policy, [Reading, Grading, and Recording Test Reactions](#).
2. Panels shall be interpreted according to Transfusion Medicine policy, *Interpretation of Antibody Investigations*.
3. If numerous non-specific reactions are present:
  - a. A warm autoantibody may be present.
  - b. The non-specific reactions may be due to a preservative in the 0.8% reagent red cells. It may be necessary to test these samples using either the gel card method (using 3% test cells that are diluted to 0.8%) or by the tube method.

## VII. SPECIMEN COLLECTION AND HANDLING:

- A. The preferred specimen is a 6mL EDTA sample with affixed identifying label. Refer to

Transfusion Medicine policy, Triaging and Identifying Acceptable Samples for Testing - Blood Bank.

- B. Plasma from a lavender top tube (CBC) may be used for additional studies provided it meets all labeling requirements.

## VIII. REAGENTS / EQUIPMENT / SUPPLIES:

### A. Gel method

1. Calibrated pipette (electronic or manual)
2. 10 x 75 mm or 12 x 75 mm test tubes
3. MTS™ Diluent 2, a hypotonic buffer saline solution
4. MTS™ Anti-IgG Card, Anti-IgG (Rabbit) suspended in gel
5. Reagent test RBCs that are commercially pre-diluted or diluted to 0.8%

### B. Tube method

1. 10 x 75 mm or 12 x 75 mm test tubes
2. Lighted viewing mirror
3. Disposable pipettes
4. Saline
5. Coombs control cells (IgG-coated check cells)
6. LISS
7. Anti-IgG AHG
8. Table top centrifuge
9. 37°C heat block
10. Antibody identification cells (may be standard panel, selected cell panel, or commercial A<sub>1</sub>, A<sub>2</sub>, and B cells).

## IX. PROCEDURE:

### A. Documentation

1. The attached *Antibody Identification Job Aid* (required for Troy Blood Bank) is available to assist with work flow and documentation.
2. The attached *Special Studies Worksheet* will be required to be completed at Royal Oak.
3. If using either the *Job Aid* or *Special Studies Worksheet*, document it with the following information:
  - a. Computer generated patient label (which includes the patient's name, MRN, and birthdate).
    - i. If a computer generated patient label is not available, manually document at a minimum the full patient name and Medical Record Number (MRN).



- b. Blood type, if available
  - c. Date studied
  - d. Antibody screen results and lot number
4. Manually write patient information or place a computer generated patient label on a copy of the antigram that will be used for testing. If a selected cell panel is performed, then place the patient labels on each selected cell printout of the antigram / test cells used. These copies must be initialed and dated by the technologist performing the testing.

## B. Manual Gel Method

Gel panels can be run manually as per the procedure below or will be performed on the ORTHO VISION analyzer.

1. Label MTS™ Anti-IgG Cards with the appropriate patient identification and lot numbers. Label each microtube with the panel cell numbers. The last microtube may be labeled as "AC" for the autocontrol.
  - a. A standard panel will require 2 MTS™ Anti-IgG Cards (11 cells + AC).
2. Prepare a 0.8% autocontrol. Refer to Transfusion Medicine policy, [Making a Test Red Cell Suspension](#).
3. Remove the foil seal from the gel cards.
  - a. Foil should be removed immediately before testing, not more than 1 hour before testing.
4. Ensure each vial of the panel is well mixed prior to testing.
5. Using an appropriate calibrated pipette, add 50 µl of each antibody panel red blood cell (0.8%) to the correct microtube, and 50 µl of the autocontrol (0.8%) cell suspension to the "AC" microtube.
  - a. Pipette tip should not touch the gel card.
6. Using an appropriate pipette, add 25 µl of serum or plasma to the microtube(s) that contain red blood cells from the previous step.
  - a. Plasma must be added within 15 minutes of panel cells.
7. Incubate at 37°C for 15 - 30 minutes. Incubation may not exceed 30 minutes.
8. Centrifuge the gel card at the calibrated speed of the gel centrifuge for 10 minutes.
  - a. MTS centrifuge: 895 ± 25 RPM
  - b. Ortho Workstation: 1032 ± 10 RPM
9. Read and grade the front and the back of each microtube macroscopically.
10. Record the graded reactions on the antigram copy or the selected cell panel copy.
11. Interpret the results in accordance with Transfusion Medicine policy, *Interpretation of Antibody Investigations*.



## C. Automated Gel Method

1. Verify appropriate maintenance and quality control has been performed.
2. Load red cell panels onto the analyzer.
  - a. Touch RESOURCES.
  - b. Touch REAGENTS.
  - c. Touch quadrant 1, 2, or 3 for reagent red cells.
  - d. Touch LOAD/UNLOAD.
  - e. Insert reagent rack with well mixed reagent cells.
  - f. Close the LOAD STATION DOOR.
3. Load the barcoded patient sample.
  - a. Touch the SAMPLES MENU.
  - b. Select the quadrant you wish to load.
  - c. Touch LOAD / UNLOAD.
  - d. Place the samples onto the load station, using the provided handles on the rack.
  - e. If you have more than one rack to load touch the other quadrants and load your racks.
  - f. Close the LOAD STATION DOOR when you are done.
4. After the samples that require manual ordering are loaded, they will turn orange because there is no order assigned to the sample yet. Create the order.
  - a. Click the orange sample (not the sample rack "wedge").
  - b. Touch CREATE ORDER.
  - c. Verify the correct sample bar code is in the field 1st Sample ID.
  - d. Verify the field 1st Sample liquid type field says CENTRBLOOD.
  - e. Touch ASSIGNED PROFILES (will be highlighted in red) and select the appropriate panel profile.
    - i. To run selected cell panels, choose the panel profile with selected cells to be tested.
    - ii. Touch Disable Assays.
    - iii. Touch Panel Cells that are NOT being tested, Panel cells that are being tested should be in white.
  - f. Enter patient identification on the sample if not already displayed
    - i. Touch Patient ID field and enter patient Medical Record number in the field.
    - ii. Touch Last Name and enter patient last name.
    - iii. Touch First Name and enter patient first name.

- g. Touch SAVE and START.
5. When completed, any gel cards with positive or questionable reactions will be automatically be placed in the manual review rack. Retrieve the cards by going to RESOURCES > MANUAL LOAD REVIEW > LOAD/ UNLOAD.
6. Use the manual review options to modify and accept the results.
7. Print the ORDER REPORT and record the graded reactions on the antigram sheet of the corresponding panel. Retain the original instrument printout with the antigram sheet for review.
8. Interpret results.

## D. LISS Tube Antibody Identification

1. Label a test tube with appropriate patient identification; prepare a 2 - 4% cell suspension of the patient's own previously washed RBCs for the autocontrol.
2. Label a set of test tubes with appropriate patient identification. Number these tubes consecutively, corresponding to the panel cells. Label one tube "AC" for the autocontrol.
3. Combine test cells and patient plasma in the following order:
  - a. Add 2 drops of patient plasma to each of the test tubes.
  - b. Add one (1) drop of the panel cells to the correspondingly labeled tubes.
  - c. Add one (1) drop of the patient's RBCs to the "AC" tube.
  - d. The order in which patient plasma, cells and LISS are added is important. If LISS is added before the plasma, then the test cells may hemolyze. LISS will be added in step 6.
4. Agitate tubes to mix.
5. Determine whether an IS or RT phase reading will be performed. See VI.F *Immediate Spin (IS) or Room Temperature (RT) Phase Reading for Suspected Cold Reacting Antibodies*.
  - a. If not indicated, proceed to step 6.
  - b. If indicated:
    - i. Centrifuge tubes according to calibrated time.
    - ii. Observe the supernate for hemolysis and resuspend the cell buttons.
    - iii. Read, grade, and record results under the "IS" column on the copy of the antigram.
    - iv. If a RT phase reading is indicated, then incubate for 15 minutes at RT, repeat i-iii (above), and record results under the "RT" column.
6. Add 2 drops of LISS to each tube.
7. Incubate the tubes at 37°C for 15 minutes.
  - a. Incubation may not exceed thirty (30) minutes.
8. Remove tubes from incubator and centrifuge tubes according to calibrated time.

9. Observe the supernate in the tubes for hemolysis. Gently resuspend the cell button. Read, grade, and record test results under the "37°C" column on the copy of the antigram.
10. Wash tubes in an automatic cell washer for four (4) cycles.
  - a. Alternatively, wash by hand four (4) times.
11. Add two (2) drops of Anti-IgG AHG. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
12. Gently resuspend the cell button. Read, grade, and record test results under the "AHG" column of the antigram.
13. Add IgG-coated check cells to all AHG phase results that are negative. Agitate tubes to mix. Centrifuge according to calibrated time.
14. Gently resuspend the cell button. Read, grade, and record coated cell test results under the "CC" column on the copy of the antigram.
  - a. Coated cells must be reactive (any strength) otherwise the test must be repeated.
15. Interpret results.

## **E. 60-Minute No-LISS Tube Panel**

1. Label a test tube with appropriate patient identification; prepare a 2 - 4% cell suspension of the patient's own previously washed RBCs for the autocontrol.
2. Label a set of test tubes with appropriate patient identification. Number these tubes consecutively, corresponding to the panel cells. Label one tube "AC" for the autocontrol.
3. Combine test cells and patient plasma as follows:
  - a. Add 3 drops of patient plasma to each of the test tubes.
  - b. Add one (1) drop of the panel cells to the correspondingly labeled tubes.
  - c. Add one (1) drop of the patient's RBCs to the "AC" tube.
  - d. Do not add LISS.
4. Agitate tubes to mix.
5. Incubate the tubes at 37°C for 60 minutes.
  - a. Incubation may not exceed 60 minutes.
6. Remove tubes from incubator and centrifuge tubes according to calibrated time.
7. Observe the supernate in the tubes for hemolysis. Gently resuspend the cell button. Read, grade, and record test results under the "37°C" column on the copy of the antigram.
8. Wash tubes in an automatic cell washer for four (4) cycles.
  - a. Alternatively, wash by hand four (4) times.
9. Add two (2) drops of Anti-IgG AHG. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
10. Gently resuspend the cell button. Read, grade, and record test results under the "AHG" column

- of the antigram.
11. Add IgG-coated check cells to all AHG phase results that are negative. Agitate tubes to mix. Centrifuge according to calibrated time.
  12. Gently resuspend the cell button. Read, grade, and record coated cell test results under the "CC" column on the copy of the antigram.
    - a. Coated cells must be reactive (any strength) otherwise the test must be repeated.
  13. Interpret results.

## F. Tube Panel for the Identification of Anti-A<sub>1</sub>

1. Label a test tube with appropriate patient identification; prepare a 2 - 4% cell suspension of the patient's own previously washed RBCs for the autocontrol.
2. Gather the following 9 test cells (if possible); document the test cells identification on the Resolution of ABO Discrepancies for A Subgroups and Patients with Anti-A<sub>1</sub> Form or the attached *Special Studies Worksheet*.
  - a. Three (3) A<sub>1</sub> test RBCs.
  - b. Three (3) A<sub>2</sub> test RBCs.
    - i. Use three sets of A<sub>1</sub> and A<sub>2</sub> cells from different lot numbers. If in-date cells are unavailable, then an expired cell may be used as long as appropriate quality control is performed.
      1. Expired A<sub>1</sub> cells: Use anti-A (Positive Control) and anti-B (Negative Control)
      2. Expired A<sub>2</sub> cells: Use anti-A (Positive Control) and anti-B (Negative Control)
  - c. Three (3) type O test RBCs.
    - i. Surgiscreen cells are preferred.
3. Label a set of 10 test tubes with appropriate patient identification. Number the tubes consecutively 1 - 9 and "AC" for the autocontrol.
4. Combine test cells and patient plasma in the following order:
  - a. Add 2 drops of patient plasma to each of the test tubes.
  - b. Add one (1) drop of the panel cells to the correspondingly labeled tubes.
  - c. Add one (1) drop of the patient's RBCs to the "AC" tube.
  - d. The order in which patient plasma, cells and LISS are added is important. If LISS is added before the plasma, then the test cells may hemolyze. LISS will be added in step 11.
5. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
6. Gently resuspend the cell button. Read, grade, and record test results under the "IS" column of the antigram.
7. Incubate the tubes at room temperature for 15 minutes.

8. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
9. Gently resuspend the cell button. Read, grade, and record test results under the "RT" column of the antigram.
10. If the IS and RT phases are non-reactive, it may be helpful to also include a 4°C phase as described in the *Notes* section near the end of this document.
  - a. To complete a 4°C phase:
    - i. Incubate the tubes at 4°C for 15 minutes.
    - ii. Agitate tubes to mix.
    - iii. Centrifuge tubes according to calibrated time.
    - iv. Gently resuspend the cell button. Read, grade, and record test results under the "4°C" column of the antigram.
11. Add 2 drops of LISS to each tube.
12. Incubate the tubes at 37°C for 15 minutes.
  - a. Incubation may not exceed thirty (30) minutes.
13. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
14. Observe the supernate in the tubes for hemolysis. Gently resuspend the cell button. Read, grade, and record test results under the "37°C" column on the copy of the antigram.
15. Wash tubes in an automatic cell washer for four (4) cycles.
  - a. Alternatively, wash by hand four (4) times.
16. Add two (2) drops of Anti-IgG AHG. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
17. Gently resuspend the cell button. Read, grade, and record test results under the "AHG" column of the antigram.
18. Add IgG-coated check cells to all AHG phase results that are negative. Agitate tubes to mix. Centrifuge according to calibrated time.
19. Gently resuspend the cell button. Read, grade, and record coated cell test results under the "CC" column on the copy of the antigram.
20. Coated cells must be reactive (any strength) otherwise the test must be repeated.
21. Interpret results as described in the *Results / Interpretations* section at the end of this document.

## **X. RESULTS / INTERPRETATIONS:**

- A. Hemolysis or agglutination of any of the panel cells is a positive test result and indicates the presence of an antibody directed against the corresponding antigen that is present on the panel cells.
- B. No agglutination or absence hemolysis of the panel cells is a negative test result and indicates the absence of an antigen / antibody reaction.

- C. Interpretation of mixed-field reactions must be done with caution. The presence of fibrin, clots or particulates may result in some cells layering at the top of the gel. IgM antibodies may give mixed-field reactions.
- D. If a tube panel was performed according to the policy *Detection of Anti-A or Anti-B in Non-Group O Neonates Receiving Non-Group O RBCs*, then the results may not correlate with the neonatal blood type. Because the neonate's immune system is immature, antibodies present in the neonatal circulation are usually of maternal origin. Therefore, the results obtained in this panel will likely correlate with the maternal blood type.
- E. Identification of anti-A<sub>1</sub>
  - 1. The antibody investigation may be interpreted as anti-A<sub>1</sub> if all of the following conditions are met:
    - a. The patient appears to be blood group A or AB, but the reverse A<sub>1</sub> cell is unexpectedly reactive during the ABO typing procedures, **and**
    - b. The patient's RBCs are A<sub>1</sub> negative when typed with anti-A<sub>1</sub> lectin, **and**
    - c. In the tube panel for the identification of anti-A<sub>1</sub>, the A<sub>1</sub> test cells are reactive while the A<sub>2</sub> and group O test cells are non-reactive.
- F. Results are interpreted as described in Transfusion Medicine policy, *Interpretation of Antibody Investigations* and entered in the LIS using established antibody result codes using Blood Bank CDM - ABID Resulting.

## XI. LIMITATIONS:

- A. Antibodies with levels below the threshold level of detection may not be detected by these tests.
- B. An antibody showing dosage may fail to react with heterozygous cells.
- C. False-positive results may occur if antibodies to components of the preservative solution are present in the serum tested.
- D. Significant variations in red blood cell suspensions may result in false-positive or false-negative reactions.
- E. Anomalous results may be caused by fresh serum, fibrin or particulate matter in serum or plasma, or red blood cells that stick to the sides of the microtube. If this occurs, it may be helpful to run a wooden stick through the plasma, re-centrifuge the sample, and repeat the antibody screen. The use of EDTA plasma may also minimize these anomalous results.
- F. Strict adherence to the test procedure is critical to test performance.

## XII. NOTES:

- A. IgM antibodies may fail to react sufficiently at immediate spin or the room temperature phase. A 4°C incubation for 15 minutes may enhance reactivity. This incubation, followed by centrifugation and reading for agglutination, may be included in the procedure. These incubations should be performed after the immediate spin or room temperature reading, and prior to 37°C incubation. Note, however, that in some cases a 4°C incubation may result in spontaneous agglutination.

- B. Reactivity of warm autoantibodies is enhanced in the presence of LISS. The focus of an antibody identification when a warm autoantibody is present should be avoiding reactivity of the warm autoantibody in order to identify any clinically significant underlying alloantibody.
- C. Anti-A<sub>1</sub> panels for patients at Taylor will be referred to another Beaumont Blood Bank for follow up and resolution.

## XIII. REFERENCES:

1. AABB, *Technical Manual*, current edition.
2. AABB, *Standards for Blood Banks and Transfusion Services*, current edition.
3. College of American Pathologists, *Transfusion Medicine Checklist*, current edition.
4. ID-Micro Typing Systems™ Interpretation Guide.

## Attachments

[Antibody Identification Job Aid](#)

[Selected Cell Sticker](#)

[Special Studies Worksheet](#)

## Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	5/31/2022
	Muhammad Arshad: Physician	5/27/2022
	Ryan Johnson: OUWB Clinical Faculty	5/25/2022
	Jeremy Powers: Chief, Pathology	5/23/2022
	John Pui: Chief, Pathology	5/19/2022
	Vaishali Pansare: Chief, Pathology	5/19/2022
Policy and Forms Steering Committee (if needed)	Kelly Sartor: Supv, Laboratory	5/19/2022



Policy and Forms Steering Committee (if needed)	Gail Juleff: Project Mgr Policy	5/18/2022
	Craig Fletcher: System Med Dir, Blood Bank	5/18/2022
	Michael Rasmussen: Supv, Laboratory	5/13/2022
	Rebecca Thompson: Medical Technologist Lead	5/13/2022
	Anji Miri: Supv, Laboratory	5/12/2022
	Teresa Lovins: Supv, Laboratory	5/11/2022
	Karrie Torgerson: Supv, Laboratory	5/11/2022
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	Kelly Sartor: Supv, Laboratory	5/10/2022

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