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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_1 may be detected in blood groups A_2 or weaker A subgroup. Anti-A_1 is often first suspected when an unexpected reaction appears with the A_1 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_1 negative, a tube panel, as described in the *Tube Panel for the Identification of Anti-A_1* 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. **HIS**: The hospital-wide computer system

C. **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.

D. **Selected cell panel**: A panel that is pre-selected based on the antigenic profile of the test RBCs.

E. **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.

F. **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.

G. **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. TWTI, 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 antigram 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 (DAT) Test by Tube Method - Blood Bank.
    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_1

1. A *Tube Panel for Identification of Anti-A_1*, as described in the procedure below, may be indicated if:
    a. the anti-A1 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_1 negative when typed with anti-A_1 lectin, **and**
    d. the reverse A_1 cell is unexpectedly reactive during ABO typing procedures.

    Refer to Transfusion Medicine policy, Resolution of ABO and Rh Discrepancies

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

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_1" 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_1, A_2, 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_1

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-A1* form or the attached *Special Studies Worksheet*.
    a. Three (3) A_1 test RBCs.
    b. Three (3) A_2 test RBCs.
        i. Use three sets of A_1 and A_2 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_1 cells: Use anti-A (Positive Control) and anti-B (Negative Control)
            2. Expired A_2 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_1

1. The antibody investigation may be interpreted as anti-A_1 if all of the following conditions are met:
    a. The patient appears to be blood group A or AB, but the reverse A_1 cell is unexpectedly reactive during the ABO typing procedures, **and**
    b. The patient's RBCs are A_1 negative when typed with anti-A_1 lectin, **and**
    c. In the tube panel for the identification of anti-A_1, the A_1 test cells are reactive while the A_2 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 in accordance with Transfusion Medicine Procedure, SafeTrace (Blood Bank) Application.

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_1 panels for patients at Taylor will be referred to another Corewell 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 Guide (rev 06/27/2024)

Antibody Identification Job Aid (rev. 07/09/2024)

Selected Cell Sticker

Special Studies Worksheet (rev. 07/09/2024)

Approval Signatures

Step Description	Approver	Date
	Ashley Beesley: Mgr, Laboratory	Pending
	Kristen DiCicco: Mgr, Laboratory	7/16/2024
	Fatima Bazzi: Medical Technologist Lead	7/16/2024
	Katherine Persinger: Mgr, Laboratory	7/16/2024
	Karrie Torgerson: Medical Technologist Lead	7/13/2024

Hilary Morey: Medical Technologist Lead	7/12/2024
Suzanne Chahine: Medical Technologist Lead	7/12/2024
Teresa Lovins: Supv, Laboratory	7/12/2024
Kelly Sartor: Mgr, Division Laboratory	7/12/2024
Kelly Sartor: Mgr, Division Laboratory	7/12/2024

Corewell Health

Corewell Health Blood Bank

Antibody Guide

Test Interpretation	Literal	Uses	Antigen NEG Required?***
A-A1	Anti-A1		Y
A-AAC	Auto Anti-C	Autoantibody with C specificity	N
A-AAc	Auto Anti-c (little)	Autoantibody with c (little) specificity	N
A-AAD	Auto Anti-D	Autoantibody with D specificity	N
A-AAE	Auto Anti-E	Autoantibody with E specificity	N
A-AAe	Auto Anti-e (little)	Autoantibody with e (little) specificity	N
A-AAM	Auto Anti-M	Autoantibody with M specificity	N
A-AFa	Auto Anti-Fya	Autoantibody with Fya specificity	N
A-AFb	Auto Anti-Fyb	Autoantibody with Fyb specificity	N
A-AJa	Auto Anti-Jka	Autoantibody with Jka specificity	N
A-AJb	Auto Anti-Jkb	Autoantibody with Jkb specificity	N
A-Bg	Anti-Bg		N
A-C	Anti-C		Y
A-c	Anti-c		Y
A-CD38	Anti-CD38 Monoclonal	Reactivity due to treatment with anti-CD38 monoclonal antibody, such as daratumamab/Darzalex or isatuximab/Sarclisa	N
A-CD47	Anti-CD47 Monoclonal	Reactivity due to treatment with anti-CD47 monoclonal antibody, such as Hu5F9-G4/Magrolimab or ALX148/Evorpacept	N
A-Cob	Anti-Cob		Y
A-Cw	Anti-Cw		Y
A-D	Anti-D		Y
A-Dia	Anti-Dia		Y
A-DIG	Anti-D Passive RhIG	Reactivity due to known RhIG administration	N
A-DNK	Anti-D Unk	Anti D Unknown Origin (unable to confirm Passive vs Allo-D)	N
A-E	Anti-E		Y

***This column ONLY indicates the RBC antigen requirements of the BBIS. Refer to SOP for additional requirements.

Corewell Health

Test Interpretation	Literal	Uses	Antigen NEG Required?***
A-e	Anti-e		Y
A-f	Anti-f	Need to give c (little) and e (little) antigen negative RBCs	Y
A-Fya	Anti-Fya		Y
A-Fyb	Anti-Fyb		Y
A-Goa	Anti-Goa		Y
A-H	Anti-H		Y
A-I	Anti-I		N
A-Jka	Anti-Jka		Y
A-Jkb	Anti-Jkb		Y
A-Jsa	Anti-Jsa		Y
A-Jsb	Anti-Jsb		Y
A-K	Anti-K		Y
A-k	Anti-k		Y
A-Kpa	Anti-Kpa		Y
A-Kpb	Anti-Kpb		Y
A-Lea	Anti-Lea		N
A-Leb	Anti-Leb		N
A-Lua	Anti-Lua		N
A-Lub	Anti-Lub		Y
A-M	Anti-M		N
MAB	Maternal Antibody	Maternal Antibody reacting in newborn patient	N
A-Mia	Anti-Mia		N
A-Mur	Anti-Mur		N
A-N	Anti-N		N
	NEX	Not Electronic Crossmatch Eligible; used for when an antibody workup is pending	N
A-NSG	Anti-NSG	Nonspecific Gel Reactivity	N
A-NSP	Anti-NSSP	Nonspecific Solid Phase Reactivity	N
A-P1	Anti-P1		N

***This column ONLY indicates the RBC antigen requirements of the BBIS. Refer to SOP for additional requirements.

Corewell Health

Test Interpretation	Literal	Uses	Antigen NEG Required?***
A-S	Anti-S		Y
A-s	Anti-s		Y
A-U	Anti-U	Need to give S and s (little) antigen negative RBCs	Y
A-V	Anti-V		Y
A-VS	Anti-VS		Y
A-Wra	Anti-Wra		Y
A-Xga	Anti-Xga		N
A-Ytb	Anti-Ytb		N
CLD	Cold Antibody	Nonspecific cold antibody or cold autoantibody	N
INV	Invalid	Invalidate the test (prevents inadvertent cancelation of test in LIS)	N/A
WkU	Weak Unidentified	Possible antibody of undetermined specificity	N
WRM	Warm Autoantibody		N

***This column ONLY indicates the RBC antigen requirements of the BBIS. Refer to SOP for additional requirements.

Corewell Health

SPECIAL STUDIES WORKSHEET

Affix label here

ABO/Rh:	**Antibody ID Interpretation:** **Tech:** **Date:**
Gender: M F **DOB:**	**Historical Antibody Record:**

Perform and Initial	Complete and Initial Each Task or NA= not applicable
	Delay comment added to ABSCN
	Add 2 RBCs to order if banded
	Check / Add NEX
	Troy Only: Positive ABSCN called to provider Name: Date / Time:
	Recent transfusion/pregnancy history: include where, # of products, type of product, date, (G#,P#).**Source** of transfusion/RHIG Hx: **Rhig date:**
	Yellow card created, documented, reviewed if site applicable
	Check / Add EPIC flag
	Attach instrument printout
	Document patient **Care Everywhere**:
	Is patient having surgery / C-section? Y N OR Date:
	Patient requirements / messages: ____ Irradiation, ____ HGBSN ____ Washed, ___Jehovah's Witness ____ IgA Deficient, ____Caucasian ____ Stem Cell Transplant, _____CMVN Other: _____
	# RBC added for OR : (Double the MSBOS/matrix requirement):
	ALL phases, enhancements, incubation time, reaction strengths, and check cells documented
	Panels performed are initialed and dated
	Ab Screen correlates with ABID
	Antibody interpretation documented on top of antibody card and on **Antigram Conclusion.**

Perform and Initial	Complete and Initial Each Task or NA=not applicable
	Auto control performed if **positive: order and perform DAT**
	All "rule outs" completed (1 homozygous or 3 heterozygous) or per SOP (D-passive, WAA....).
	Answer ABID as indicated; previous antibody added to ABID comment. Appropriate QC rack selected.
	Units on order from reference lab? Y N
	Cord Blood Requested? Y N Baby RBC unit typed? Y N Add cord request comment to ABSCN and on order comment.
	Titer Needed? Y N
	Eluate indicated? Y N Eluate Results:
	Antigen Typing performed unless DAT pos (cannot test AHG phase) or if transfused <90d.
	PINFO patient profile note with transfusion history, antibody History, current findings
	Crossmatches performed: _____IS (EI, GND,RND, Computer Down.....) _____GEL (alloantibody, Cold Antibody.....) _____60minNoLIss (WAA reacting, HTLA, CD38) _____ All phase (Anti-A1) _____ Combination required (gel and IS.....)
	Incompatible crossmatches investigated
	Needs genotype send-out: Y N
	For WAA add special crossmatch requirements to patient profile notes.
	Day-Shift Review
	Verify patient and unit antigen typing's, genotypes, etc. have been billed
	Transfusion Reaction Evaluation If all 4 conditions of a delayed transfusion reaction are met, consult MD. Name of MD: Delayed Transfusion Reaction approved? Y or N
	Additional work:
	CABID ordered
	CABID forms scanned and emailed to MD
	ABID reviewed acceptable. Date:

Store in 1°-6°C **SELECTED CELL#**________
MANUF__________LOT#________
EXP DATE__________
PREP. DATE__________
EXP DATE__________TIME________
PREPARED BY __________

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PREPARED BY __________

Corewell Health

Special Studies Worksheet

Name:
MRN:
DOB:

Test Date:
ABO/Rh:
Patient Location:
Known Antibodies:

Antibody Screening

Method: Circle One Manual or Vision™	Mfg. and Lot#	Results I :	Results II :

Patient Antigen Typing

Anti-sera/specificity														
Method (tube or gel)														
Anti-sera: Mfg., lot#, exp date														
Controls: Panel mfg, lot#, exp. date														
Controls: test cell #	Pos:			Neg:				Pos:			Neg:			
Pre-txn sample date														
Positive control	IS	RT	AG	CC	Gel	A/P	INT	IS	RT	AG	CC	Gel	A/P	INT
Negative control	IS	RT	AG	CC	Gel	A/P	INT	IS	RT	AG	CC	Gel	A/P	INT
Patient	IS	RT	AG	CC	Gel	A/P	INT	IS	RT	AG	CC	Gel	A/P	INT
Inert control														
Tech / Test date:														

Expired Cell Quality Control

Anti-sera / specificity										
Method (tube or gel)										
Anti-sera: Mfg., lot#, exp date										
Controls: Panel mfg , lot#, exp. date										
Controls: test cell #	Pos:			Neg:		Pos:			Neg:	
Positive control	IS	RT	Gel	A/P	INT	IS	RT	Gel	A/P	INT
Negative control	IS	RT	Gel	A/P	INT	IS	RT	Gel	A/P	INT
Tech / Test date:										

BB History Form (Tech / Date)

Sent to RN (RN#)	To:
Record updated	

ARC Form (Tech / Date)

Written on form	
Record updated	

RhIG Information

Called BRL or RN (Emp / D / T / Tech):	
RhIG date provided by:	
Date RhIG Rec'd:	

Suspected Transfusion Reaction

Transfused in the last 3 weeks? (Y/N)
Positive AC or DAT? (Y/N)
New unexpected antibody identified? (Y/N)

Tube DAT Results

Reagent	IS	RT	CC	
Poly				Tech/ Date:
IgG		NI		
C3				
Saline control (tube DAT):				
Gel DAT:				

Tech / Supervisory Review Checklist

Task	Tech	Date	SR (Tech)
ABSC matches ABID			
ABID field / interpret			
Patient Notes			
Antibody Card			
Manual Billing			
CABID required			
ABHX (delay comment)			
NEX antibody			

Antigen Typing

No TXNS in 90 days			
Results in computer			
Appropriate test cells			
Inert Control			

WAAs

Patient "CR" Note			
Genotype / QC Rev			
RBCs XM'd correctly			
Adsorption, if required			

Titer

Appropriate test cell			
TITER resulted			
Sample frozen			

Eluate

QC documented			
ELUATE ordered			
Interpretation			

Antibodies Identified:	Antibodies Not Ruled out:	Supervisory Review and Moved from SR Rack by: (Tech / Date)

Refer to Transfusion Medicine policy, *Interpretation of Antibody Investigations*

Corewell Health

Special Studies Worksheet

Name:
MRN:
DOB:

Test Date:
ABO/Rh:
Patient Location:
Known Antibodies:

Titration for HTLA/Bga Antibody

Test Cells (mfg. lot#, cell#, exp. Date)	Gel Results 1:10	1:20	1:40
Tech / Test Date:			

Eluate Panel

Eluate manufacturer:	
Lot #:	
Exp. Date:	

LW Neutralization (Optional) QC

Sal: LW:

LW ABSC (Required) QC

ABSC Mfg/Lot#/Exp:	SCR	Gel	AG	CC
	I			
	II			

LW vs, Reverse Cells (if required)

Reverse Cells Mfg/Lot# /Exp:	Cell	Gel	AG	CC
	a			
	b			

Eluate Interpretation:

Tech / Test Date:

Allohemagglutinin Titer

Patient's blood type	
ABO antibody(ies) for which titer is performed	
A_1 test cell: mfg, lot #, and exp.	
A_2 test cell: mfg, lot #, and exp.	
B test cell: mfg, lot #, and exp.	
Sample date:	

☐ Gel ☐ Tube Dilution	A_1 Titer RT	A_1 Titer AHG	A_1 Titer CC	A_2 Titer RT	A_2 Titer AHG	A_2 Titer CC	B Titer RT	B Titer AHG	B Titer CC
1:1									
1:2									
1:4									
1:8									
1:16									
1:32									
1:64									
1:128									
1:256									
1:512									
1:1024									
1:2048									

Titer Result	RT	AHG
A_1		
A_2		
B		

Tech / Test Date:

Antibody Titer

Antibody	
Test cell: phenotype mfg, lot#, cell #, exp. date	
Control sample date	
Current sample date	
Current aliquot frozen (√)	
Submit copy to BBMD (√)	

Dilution	Control Sample AG	Control Sample CC	Current Sample AG	Current Sample CC
1:1				
1:2				
1:4				
1:8				
1:16				
1:32				
1:64				
1:128				
1:256				
1:512				
>512				

Control Sample Titer:	
Current Sample Titer:	
Tech / Test Date:	

Anti-A_1 Tube Panel

Cell	Manufacturer	Lot # / Cell #	Exp. Date	IS	RT	4°C	37°C	AHG	CC
A_1									
A_1									
A_1									
A_2									
A_2									
A_2									
O									
O									
O									
A/C									

Tech / Test Date: