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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 and interpreting the antibody investigation.

II. CLINICAL SIGNIFICANCE:

A. The goals of an antibody investigation are to identify the antibody(ies) causing unexpected reactivity and to rule-out all common clinically significant antibodies. The initial antibody panel may be interpreted using the exclusion method (aka "cross-out" or "rule-out" method). Antibody specificity may be tentatively excluded when the patient's sample fails to react with test red blood cells (RBCs) known to be positive for the corresponding antigen. Likewise, antibody specificity may be tentatively identified when the patient's sample reacts with test RBCs known to be positive for the corresponding antigen.

B. Confirmatory testing is performed to demonstrate that the tentatively identified antibody(ies) reacts as expected with a sufficient number (three) of test RBCs that express- and lack- the corresponding antigen. Confirmatory testing involves the testing of selected cells to meet these requirements. In addition, when possible the patient's RBCs are antigen typed; they should be negative for the antigen(s) corresponding to any antibody(ies) identified.

III. 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.

IV. PRINCIPLE OF THE TUBE METHOD:

A. The goal of tube antibody identification, like gel antibody identification, 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.

B. 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.

C. 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. Immediate-spin, room temperature incubation, and 4° C incubation may be read to assist in antibody identification in these scenarios.

V. 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. The 60-minute no-LISS method is applied when warm autoantibody reactivity is observed. Additionally, this method is applied for patients currently receiving Daratumumab, and occasionally used for patients with HTLA antibodies.
    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.

VI. DEFINITIONS / ACRONYMS:

A. **MTS**: Micro Typing System

B. **HIS**: The hospital-wide computer system

C. **BBIS**: Blood Bank Information System

D. **ABID**: Antibody Identification Test Code in BBIS

E. **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.

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

G. **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.

H. **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.

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

J. **Confirmatory testing**: Testing of selected cells to prove that a tentatively identified antibody reacts as expected with a sufficient number of test RBCs, generally three (3) test RBCs, that express- and lack- the corresponding antigen.

K. **Exclusion method**: A method of interpretation used in antibody identification investigations in which in which the reactivity of the panel is compared to antigenic profile of the test RBCs.

L. **Homozygous**: Possessing a pair of identical alleles, aka double dose.

M. **Heterozygous**: Possessing different alleles at a given locus, aka single dose.

N. **Dosage**: When an antibody reacts stronger with a test cell demonstrating a homozygous expression of a given antigen than with a test cell demonstrating a heterozygous expression of the antigen.

O. **Clinically significant antibody**: An antibody that:
Is known to cause Hemolytic Disease of the Newborn (HDN) or shortened survival of antigen positive RBCs,
Requires transfusion of antigen negative red blood cells, and
Is usually IgG and best detectable with antihuman globulin (AHG).

P. **Clinically insignificant antibody**: An antibody that:
Does not cause shortened red cell survival of antigen positive RBCs,
Does not require transfusion of antigen negative red blood cells, and
Is usually IgM and reacts best below 37°C.
Antibodies that are usually considered clinically insignificant include the following specificities: Auto-anti-IH, auto-anti-H, auto-anti-I, anti-I, anti-Le^a, anti-Le^b, anti-Lu^a, anti-P_1, anti-M, anti-N, and anti-A_1 reactive below 37°C.

Q. **Passively acquired antibodies**: Antibodies that are transferred from the donor(s) to a recipient

through the transfusion or administration of plasma-containing components (i.e., RhIG, or IVIG administration). Passively acquired antibodies may also be transferred from mother to fetus through pregnancy, and may be present in the neonate after birth.

R. **Alloimmunization**: The process whereby a recipient forms antibodies in an immune response to foreign antigens on donor RBCs.

S. **Designee**: Any Blood Bank technical director, or transfusion medicine fellow.

VII. POLICIES:

A. Standard Panels / Selected Panels / Ficin Treated Panel / Autocontrol

1. In some cases, when non-specific reactivity is originally observed in an antibody screen or panel, the panel will be non-reactive when the sample is re-spun and retested. Whenever repeating an antibody screen or a panel, the sample should be re-spun.
2. 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. weak Unidentified) but no history of an antibody with specificity.
3. 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.
4. Expired panel cells should not be used in a standard panel.
5. 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.
6. 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.
7. It is not always possible to determine the specificity of an unexpected antibody. In complex cases, the use of a ficin treated panel may help to determine the specificity. The reactions of many antibodies may be enhanced with the use of ficin treated cells, most notably antibodies in the Rh, Lewis, Kidd, and P systems as well as cold-reacting antibodies. However, enzyme treatment destroys some red cell antigens (M, N, S, s, Fy^a, Fy^b), thus reducing or eliminating the reactivity of the corresponding antibodies. Refer to the *Ortho 0.8% Resolve Panel C* manufacturer's insert for additional information.
8. An autocontrol must be performed with an antibody identification workup 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. Anti-D QC material (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 or if it fails visual inspection. It should be stored between 2 - 8°C and 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 test cell(s).
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. For tube method antibody invesitgations IgG coated cells must be added to all AHG phase results that are negative.
10. If a test result 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 X.F. *Tube Panel for the Identification of Anti-A_1*, 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 used for patients currently receiving Daratumumab and infrequently indicated for patients with HTLA (high titer low avidity) antibodies.

    Refer to the applicable Transfusion Medicine policy for more information on these situations, Warm Autoantibody Investigations, DTT Treatment and Testing - Blood Bank, and HTLA/Anti-Bga Antibody Investigations

F. All-Phase Tube Testing 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 Transfusion Medicine Policy, Investigation of Cold Reacting Antibodies - Blood Bank, for more information.
2. Furthermore, 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.

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 the guidelines below X. I. Results/Interpretations.
3. If numerous non-specific reactions are present:
    a. 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.
    b. An antibody to a high incidence antigen may be present. Refer to Section VII. V. *Confirmation of an Antibody to a High Frequency Antigen*
    c. A warm autoantibody, HTLA antibody, or interference with the drug Daratumumab may be present.

I. General Antibody Exclusion Requirements

1. Antibody specificity may be conclusively excluded when the patient's plasma, tested by the gel method, is non-reactive with:
    a. At least one (1) in-date test RBC demonstrating homozygous expression of the corresponding antigen. This is the preferred method of exclusion.
    b. At least three (3) different test RBCs demonstrating heterozygous expression of the corresponding antigen. This is the alternative method of exclusion, and should **only** be used when it is not possible to exclude an antibody with at least one (1) homozygous, in-date test RBC.
2. The above antibody exclusion requirements apply to all antibody investigations, with the following noted exceptions:
    a. **Alloanti-D**: Alloanti-D may be excluded when the patient's plasma, tested by the gel method, is non-reactive with at least 3 Rh(D) positive test cells.
    b. **Passive anti-D**: If passive anti-D due to recent RhIG administration is detected in a patient sample, then other antibody specificities may be conclusively excluded when the patient's plasma, tested by the gel method, is non-reactive with at least one (1) test RBC demonstrating heterozygous expression of the corresponding antigen.
    c. **Kidd antibodies**: Anti-Jk^a or anti-Jk^b may be excluded when the patient's plasma, tested by the gel method, is non-reactive with at least one (1) test RBC demonstrating homozygous expression of the corresponding antigen. Anti-Jk^a and anti-Jk^b should not be excluded with heterozygous test RBCs. If in-date, homozygous test cells are unavailable, then a Kidd antibody should be excluded with three heterozygous in-date test RBCs and one (1) out-of-date homozygous cell.

i. Kidd antibodies are dangerous as they may cause severe acute hemolytic transfusion reactions (HTRs) or delayed HTRs. Kidd antibodies may show dosage and have the tendency to drop below detectable levels. For these reasons, the Blood Bank should err on the side of caution when excluding Kidd antibodies. Antigen typing the patient's own pre-transfusion RBCs will help determine whether the patient has developed anti-Jk^a; the prevalence of the Jk^a antigen in the general population is approximately 77%.

J. Antibody Exclusions Based on Patient's Phenotype

1. As an alternative to excluding antibodies based on the general requirements stated above, antibodies may be excluded based on the patient's phenotype. In order for alloimmunization to occur, a patient's RBCs are generally negative for the antigen corresponding to the particular antibody. For example:
    a. Testing the RBCs of a patient yields the following results:
    D- C- E+ c+ e+ K- Fya+ Fyb+ Jka+ Jkb+ S- and s+
    Many antibody specificities may be excluded based on these results including anti-E, anti-c, anti-e, anti-Fy^a, anti-Fy^b, anti-Jk^a, anti-Jk^b, and anti-s. Based on these results, this patient is generally capable of making only anti-D, anti-C, anti-Kell, and anti-S.
2. The use of the patient's phenotype to exclude antibodies should be done with caution, as some antibody specificities have been demonstrated in the patient's serum even though the patient's RBCs are positive for the corresponding antigen. Most notorious are examples of auto-anti-M and warm autoantibodies with e-like specificity. Also, variant and silent genes may affect antigen density on a certain red cell, causing the cell to react differently than other cells of the same apparent phenotype. Therefore, the preferable method of exclusion is the panel, not the phenotype. The genotype may be used as a method of exclusion.

K. Exclusion of Antibodies to Low Incidence Antigens

1. For most investigations it is not always possible, and therefore not required, to exclude antibodies corresponding to low incidence antigens.
2. If the following specificities are not excluded and there is reasonable suspicion of the presence of anti-C^w, anti-V, anti-Kp^a, anti-Js^a, or anti-Lu^a, document this in a Patient Profile Note in the BBIS. Gel-AHG-Crossmatched units should be provided for the patient, refer to Thansfusion Medicine policy, Policies for Providing Red Blood Cells to Patients with Unexpected Antibodies.
    a. This may also be documented on the *Special Studies Worksheet* in the space provided for "Antibodies Not Ruled Out".
    b. Refer to section VII. N. *Confirmation of an Antibody to a Low Incidence Antigen* for more information.

L. Unable to Exclude Clinically Significant Antibodies

1. If unable to exclude any clinically significant antibodies, then RBCs shall be provided for

transfusion that are negative for the antigen(s) corresponding to any antibody(ies) that were not excluded serologically.

a. If the patient's own red cells are antigen positive then it is not necessary to provide RBCs for transfusion that is/are negative for the antigen(s) corresponding to any antibody(ies) that were not excluded serologically. Note the following exception for the C and E antigens:

    a. The RBCs of patients who appear to be C or E positive may actually have a variant expression of C or E. Patients with these variant expressions may be capable of developing the corresponding antibody. Therefore, if unable to exclude anti-C or anti-E serologically and the patients RBCs appear to be C or E positive, then RBCs for transfusion must be C or E negative.

b. For those antibodies that cannot be excluded, the Special Requirements tab of the patient's record in the BBIS should be updated to include this requirement. Example: "E negative RBCs". The computer logic will then prevent the issuance of red cells that are positive for the applicable antigen. In addition, a notation should be made in a Patient Profile Note in the BBIS e.g., "Unable to R/O anti-C".
Note: If future studies provide information to rule-out the suspected antibody, consult the Medical Director or designee prior to removing the antigen negative Special Requirement and adding a new Patient Profile Note to document the decision.

M. General Confirmatory Testing Requirements

1. An antibody specificity may be confirmed when the patient's plasma tested by the gel method is reactive with at least three (3) antigen positive test cells, and is non-reactive with at least three (3) antigen-negative test cells. The screening cells **may be counted** to meet these requirements. If an insufficient number of test cells exist in the initial panel and antibody screen to meet these confirmatory testing requirements, then the technologist should test additional selected cells to meet these requirements.

    a. Note that if a patient has a previously identified antibody (either at a Corewell Health Facility or non-Corewell Health Facility), it is not necessary to confirm the presence of that antibody in subsequent investigations.

    b. To attempt to enhance reactivity a technologist may use the Ortho 0.8% Resolve Panel C (untreated and ficin treated) or extend the incubation at 37°C for 30 minutes.

2. Exception to the general confirmatory testing requirements: Passive anti-D due to recent Rh Immune Globulin (RhIG).

    a. Passive anti-D due to recent RhIG is confirmed when the patient's plasma tested by the gel method is reactive with at least three (3) Rh(D) positive test cells on a standard panel, and is non-reactive with at least (3) Rh(D) negative test cells. The screening cells may **not** be counted to meet these requirements. A technologist may test the ficin treated Ortho 0.8% Resolve Panel C to attempt to enhance the Passive Anti-D due to recent RhIG. If fewer than three (3) Rh(D) positive test cells on the standard panel or ficin treated panel are reactive, then another specification must be

made; i.e., WkU (Unidentified Antibody).

b. Note: If the reaction strengths are greater than 2+, you may bring the results to the Medical Director or designee for further discussion of antibody interpretation.

3. When multiple antibodies are present, the following should be demonstrated:
    a. For each antibody identified, the patient's plasma tested by the gel method should be reactive with at least three (3) test cells that are positive for the corresponding antigen. These three (3) test cells should be negative for all of the other antigens corresponding to the other antibodies identified.
    b. In addition, the patient's plasma tested by the gel method should be non-reactive with at least three (3) test cells that are negative for each of the antibodies identified.

N. Confirmation of an Antibody to a Low Incidence Antigen

1. A low incidence antigen is defined as an antigen with a prevalence of ≤ 1% in the general population. In some cases it may be helpful to confirm the presence of an antibody to a low incidence antigen. However, the resources spent on this endeavor should be minimal, because the low prevalence of the antigen in the donor population should make it easy to find compatible gel crossmatches. An antibody to a low incidence antigen may be suspected if the investigation is otherwise resolved, but:
    a. One and only one extraneous reaction is observed in a panel, or
    b. One and only one incompatible crossmatch is observed, and the unit is negative for all antigens corresponding to the patient's known antibodies.
2. It may be helpful to test a selected cell panel consisting of several cells that are positive for low incidence antigens represented on in-date panel cells; for instance, the more common ones like C^w, V, Kp^a, Js^a, and Lu^a. If any of these cells are reactive, then additional cells that are positive for that low incidence antigen may be tested, if available, to confirm the presence of the antibody to the low incidence antigen.
3. Refer to Transfusion Medicine policy, Policies for Providing Red Blood Cells to Patients with Unexpected Antibodies. As indicated in this policy, it may be necessary to demonstrate whether the antibody to the low incidence antigen is currently reactive.

O. Determining whether an Antibody to a Low Incidence Antigen is Reactive

1. If an antibody to a low incidence antigen has been identified and if antisera is unavailable for typing the RBC units, it may be necessary to determine whether the antibody is currently reactive before red blood cells are crossmatched for clinically significant antibodies. Proceed as follows to determine whether an antibody to a low incidence antigen is currently reactive:
    a. Test the patient's sample against three 3 test cells that are positive for the antigen corresponding to the antibody. Consult the Medical Director for further direction if 3

test cells are unavailable or if there is variable reactivity amongst the 3 test cells.

b. This testing shall be performed every 90 days.

c. A patient profile Note shall be added in the BBIS indicating the sample collection date, and whether the antibody to the low incidence antigen is reactive.

    a. This may also be documented on an Antibody Card/Folder.

P. Confirmation of an Antibody to a High Frequency Antigen

A. A high frequency antigen is defined as an antigen that is prevalent in 98- 99% of the general population. An antibody to a high frequency antigen may be suspected if all test cells are reactive but the autocontrol is non-reactive. When this is suspected, a selected cell panel may be tested using test cells that are negative for the high frequency antigens listed below, if they are available:

    1. k (Cellano), U (use a S-s- test cell), Kp^b, Js^b, Lu^b and Fy^3 (use a Fy(a-b-) test cell)

B. Knowing the patient's race may also lend a clue; for instance, anti-U or anti-Fy^3 is found almost exclusively in African Americans. In most cases, it will be necessary to use the services of an Immunohematology Reference Laboratory to confirm the presence of an antibody to a high frequency antigen.

C. Refer to Transfusion Medicine policy, *Handling of Patients with Anti-U*, for additional information on antigen typing the patient and donor, and transfusion of patients with anti-U.

Q. Patient Antigen Typing Requirements

1. The patient's RBCs should be antigen typed and should be negative for the antigen(s) corresponding to any antibody(ies) identified. However, patient antigen typing may not be indicated for those patients who have been recently transfused or who have a positive direct antiglobulin test (DAT).

2. If anti-Le^a or anti-Le^b is identified, then the patient's RBCs should be typed for both the Le^a and Le^b antigens. The patient's RBCs should be negative for both antigens.

3. If anti-N is identified, then the patient's RBCs should be typed for S and s as well as N. If the patient types as N-S-s-, then the anti-N identified is considered clinically significant. If the patient types as N negative and S or s positive, then the anti-N identified may be considered clinically insignificant.

4. A complete phenotype or genotype shall be performed for patients with 3 or more identifiable antibodies and patients with a detectable warm autoantibody. This will be done on the day shift or, if time allows, on off shifts. A phenotype is not required if the antibody cannot be identified; i.e., Unidentified Antibody (WkU). Refer to Transfusion Medicine Policy, Antigen Typing - Blood Bank: Clinical Indications for Extended Phenotype/ Molecular Genotyping.

R. Policies specific for Obstetrical Patients

1. An antibody titer should be performed on all obstetrical patients with clinically significant unexpected antibodies once per month throughout the pregnancy, as indicated in Transfusion Medicine policy, *Antibody Titration*. A titer should only be performed for patients with antibodies of varying clinical significance if specifically requested by the caregiver, i.e., anti-M and anti-N.
2. If both anti-C and anti-D appear to be present in the sample of an obstetrical patient, then the Blood Bank should determine whether anti-D, anti-C, and/or anti-G is present. This distinction is important because some patients who appear to have both anti-C and anti-D may not actually have anti-D; they may actually have anti-G. In these cases in which anti-D is not present, Rh Immune Globulin may be indicated. This determination is made as described in Transfusion Medicine policy, *Differentiation of Anti-D, Anti-G, and Anti-C*, or by sending a sample to the Versiti Reference Laboratory. The Medical Director should be consulted on all cases in which both anti-C and anti-D appear to be present in the sample of an obstetrical patient.
3. In some cases, it may be difficult to determine whether anti-D specificity is related to passive anti-D due to RhIG administration, or to alloimmunization. Refer to Transfusion Medicine policy, Policies Specific to Patients with Passive Anti-D (Due to Recent RH Immune Globulin Administration).

S. Unexpected Antibody Reactivity that Interferes with ABO/Rh Typing

1. Unexpected antibody reactivity that is detected in the ABO/Rh typing may be due to a cold reacting antibody. Perform the panel by the tube method, as described below in X.D. *LISS Tube Antibody Identification*.
2. For patients with a history of Anti-A_1 it is not necessary to perform repeat tube panels to confirm the presence of anti-A_1 unless reverse typing with A_2 cells does not resolve the discrepancy.
3. ABO/Rh typing should be performed / resolved as described in Transfusion Medicine policy, Resolution of ABO and Rh Discrepancies - Blood Bank.

T. Non-Specific Reactivity in Antibody Investigations

1. The following policies apply when a sample exhibits non-specificity in the gel panel:
    a. If all clinically significant antibodies have been ruled out, then the antibody shall be classified as a **WkU** (Unidentified Antibody).
    b. Non-specific reactions may be due to a preservative or component in the pre-diluted test cells supplied by the manufacturer. In this case, all or most of the test cells will be reactive and the autocontrol is usually non-reactive (no preservative in the auto control). These reactions may have a mixed-field appearance. It may be helpful to repeat the gel screen or panel using 3% test cells that are diluted by the Blood Bank

to 0.8%; see Transfusion Medicine policy, Making a Test Red Cell Suspension. The 3-cell Ortho Surgiscreen is useful for this purpose. If all of the panel or Surgiscreen test cells are non-reactive when diluted in this manner, then:

i. The investigation may be interpreted as **NSG** (Nonspecific Gel Reactivity).

ii. If on a future visit, the patient's antibody screen is non-reactive, the **NSG** antibody can be deactivated and the patient will be eligible for electronic crossmatch (providing they do not any other antibody history).

U. Policies Relating to Positive Antibody Screens and Potential Delays in Providing Components

1. A comment should be added to all antibody screens that are positive or if any test results or the patient's history have the potential to delay the Blood Bank's ability to provide compatible RBCs. This comment should be added to warn the caregivers of the potential delay. This includes both inpatient samples and outpatient samples. The comment is placed in the BBIS by the medical technologist resulting the antibody screen.
    a. **ABHX**: Patient has a history of an antibody. There will be a delay in obtaining compatible Red Blood Cells.

V. Serologic Indications for Possible Delayed Transfusion Reaction

1. The technologist should consider initiating a Suspected Transfusion Reaction Evaluation (STRE) if:
    a. The patient has a positive antibody screen on the current sample in which new, unexpected antibody reactivity is detected, **and**
    b. An antibody screen performed within last four (4) weeks was negative, or did not demonstrate the same unexpected reactivity present in the current sample, **and**
    c. The patient received a RBC transfusion in the last four (4) weeks, **and**
    d. Either transfused RBC is antigen positive (via testing of RBC segments, or statistic probability of antigen exposure if >6 RBC units transfused over 4 weeks) or the new antibody is detectable in the patient eluate.
2. The technologist shall consult with the Medical Director (MD) to determine whether to initiate a STRE if the above conditions are met. If the MD is consulted, then this shall be documented on the *Special Studies Worksheet / Job Aid,* if applicable. In addition to performing the steps found in Transfusion Medicine policy, *Laboratory Investigation of a Suspected Transfusion Reaction*, it is essential to obtain a patient history, as described in Transfusion Medicine policy, Obtaining Patient Histories.

W. Review of Antibody Investigations

1. All antibody investigations will be reviewed for completeness and accuracy. This review is documented on the *Special Studies Worksheet / Job Aid* or on the panel sheets provided.

   Refer to site specific Transfusion Medicine policies, *Review of Antibody Investigations.*

X. Medical Director Review of Antibody Investigations

1. The Medical Director(MD) will perform a consult for antibody identification (CABID) for investigations which fit the criteria described in the table below.

Cases Reviewed by the Medical Director (CABID)	Cases not Reviewed by the Medical Director (No CABID)
1. An antibody specificity can be determined, the first time the antibody is identified; e.g., Anti-K, Anti-Fya, Anti-M, etc. Include any associated titer, eluate studies with the documentation. 2. WRM (Warm autoantibodies) that are detected for the first time. 3. Anti-CD38 or any passively acquired antibody detected except passive Anti-D due to RhIG. 4. A WkU non-specific is detected with strength 2+ or greater. 5. HTLA	1. Investigations which do not result in the determination of antibody specificity; e.g; CRAUS, NSG (antibody to a preservative in the gel media), etc. 2. Investigations of previously identified antibodies in which the rule-out panel is negative. 3. Passive anti-D due to RhIG 4. DNK (unknown whether anti-D specificity is due to alloimmunization or RhIG). CABID order is not required but paperwork should be submitted for review. 5. Anti-A_1 6. WkU (strength 1+ or less) 7. If an HDN Survey is performed then do not order a CABID test (unless otherwise indicated), but provide a copy of the paperwork to the MD.

2. The technologist responsible for the supervisory review will order the CABID test code in the HIS if it is determined that the investigation meets the criteria for Medical Director review and forward all paperwork to the Medical Director as directed in site specific policies, *Review of Antibody Investigations.*
3. MD will result the CABID test code.

VIII. 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.

IX. 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).

X. PROCEDURE:

A. Documentation

1. The attached *Antibody Identification Job Aid* (required for Troy Blood Bank) and *Special Studies Worksheet* (required for Royal Oak) are available to assist with work flow and documentation.
2. 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

3. 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 and documented with indelible ink 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. Verify appropriate quality control has been performed.
2. 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.
3. 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.
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 6.
5. Agitate tubes to mix.
6. Determine whether an IS or RT phase reading will be performed. See VII.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 a column labeled "IS" 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 a column labeled "RT" on the copy of the antigram.

v. If a 4° C phase reading is indicated, then incubate for 15 minutes at 4° C, repeat i-iii (above), and record results under a column labeled "4°" on the copy of the antigram. (Read Notes section below regarding 4° incubation)

7. Add 2 drops of LISS to each tube.
8. Incubate the tubes at 37°C for 15 minutes.
    a. Incubation may not exceed thirty (30) minutes.
9. Remove tubes from incubator and centrifuge tubes according to calibrated time.
10. Observe the supernate in the tubes for hemolysis. Gently resuspend the cell button. Read, grade, and record test results under a column labeled "37°C" on the copy of the antigram.
11. Wash tubes in an automatic cell washer for four (4) cycles.
    a. Alternatively, wash by hand four (4) times.
12. Add two (2) drops of Anti-IgG AHG. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
13. Gently resuspend the cell button. Read, grade, and record test results under a column labeled "AHG" on the copy of the antigram.
14. Add IgG-coated check cells to all AHG phase results that are negative. Agitate tubes to mix. Centrifuge according to calibrated time.
15. 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.
16. Interpret results.

E. 60-Minute No-LISS Tube Panel

1. Verify appropriate quality control has been performed.
2. 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.
3. 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.
4. 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.
5. Agitate tubes to mix.
6. Incubate the tubes at 37°C for 60 minutes.
    a. Incubation may not exceed 60 minutes.

7. Remove tubes from incubator and centrifuge tubes according to calibrated time.
8. 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.
9. Wash tubes in an automatic cell washer for four (4) cycles.
    a. Alternatively, wash by hand four (4) times.
10. Add two (2) drops of Anti-IgG AHG. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
11. Gently resuspend the cell button. Read, grade, and record test results under the "AHG" column of the antigram.
12. Add IgG-coated check cells to all AHG phase results that are negative. Agitate tubes to mix. Centrifuge according to calibrated time.
13. 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.
14. Interpret results.

F. Tube Panel for the Identification of Anti-A_1

1. Verify appropriate quality control has been performed.
2. 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.
3. 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.
4. Label a set of 10 test tubes with appropriate patient identification. Number the tubes consecutively 1 - 9 and "AC" for the autocontrol.
5. 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.

6. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
7. Gently resuspend the cell button. Read, grade, and record test results under the "IS" column of the antigram.
8. Incubate the tubes at room temperature for 15 minutes.
9. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
10. Gently resuspend the cell button. Read, grade, and record test results under the "RT" column of the antigram.
11. 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.
12. Add 2 drops of LISS to each tube.
13. Incubate the tubes at 37°C for 15 minutes.
    a. Incubation may not exceed thirty (30) minutes.
14. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
15. 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.
16. Wash tubes in an automatic cell washer for four (4) cycles.
    a. Alternatively, wash by hand four (4) times.
17. Add two (2) drops of Anti-IgG AHG. Agitate tubes to mix. Centrifuge tubes according to calibrated time.
18. Gently resuspend the cell button. Read, grade, and record test results under the "AHG" column of the antigram.
19. Add IgG-coated check cells to all AHG phase results that are negative. Agitate tubes to mix. Centrifuge according to calibrated time.
20. Gently resuspend the cell button. Read, grade, and record coated cell test results under the "CC" column on the copy of the antigram.

21. Coated cells must be reactive (any strength) otherwise the test must be repeated.
22. Interpret results as described in the *Results / Interpretations* section at the end of this document.

XI. 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. Additionally, IgM antibodies may give mixed-field reactions.

D. **Tentative Antibody Identification**- Examine the positive/reactive test cells in an attempt to make a tentative identification. Look for a pattern among the group of antibodies that have not yet been conclusively excluded between the reactivity of the patient's sample and the antigens on the reactive test cells. Consider the possibilities of multiple antibodies or of dosage.

E. **Tentative Antibody Exclusions**- Examine the negative/non-reactive test cells (one at a time) to begin tentatively excluding antibody specificities. If the non-reactive test cell displays a:

    1. Homozygous expression of an antigen, then cross off the antibody on the antigram. The antibody is conclusively excluded.
    2. Heterozygous expression of an antigen, then place a mark on the antigram, above the name of the antibody.

F. **Confirmatory Testing** - Perform confirmatory testing as the reaction pattern is observed.

    1. If the pattern appears to indicate a **single** antibody specificity:
        a. Confirm the presence of the antibody by verifying that the patient's plasma is reactive with at least three (3) antigen positive test cells, and is non-reactive with at least three (3) antigen-negative test cells. The screening cells may be counted to meet these requirements.
        b. Confirm that all clinically significant antibodies are excluded with at least one homozygous test cell (preferred), or at least three (3) heterozygous test cells (alternative).
        c. If necessary, test additional selected cells to meet above requirements.
    2. If the pattern appears to indicate **multiple** antibody specificities:
        a. Confirm the presence of each antibody by ensuring that for **each** antibody identified, the patient's plasma tested by the gel method should be reactive with at least three (3) test cells that are positive for the corresponding antigen. These three (3) test cells should be negative for all of the other antigens corresponding to the other antibodies identified.
        b. The patient's plasma is non-reactive with at least three (3) test cells which

lack the antigens corresponding to all the identified antibodies.

c. Verify that all other clinically significant antibodies are excluded with at least one homozygous test cell (preferred), or at least three (3) heterozygous test cells (alternative).

d. If necessary, test additional selected cells to meet above requirements.

G. Verify that the identified antibody(ies) correlate(s) with the antibody screen reactivity. If the results do not correlate, then further investigation is required.

H. If applicable, antigen type the patient's RBCs for the antigen(s) corresponding to the antibody(ies) identified.
After completing an antibody investigation, the technologist will place all of the paperwork for the investigation in the designated area for Antibody Review.

I. 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.

J. 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.

K. ABID results are entered in the BBIS using established antibody result codes in accordance with Transfusion Medicine Policy, SafeTrace (Blood Bank) Application.

1. Historical and new antibody(ies) should be selected and resulted in the ABID Interpretation.
2. Add result comments as described below:

ABID	Comment ID	Literal Text/Free Text
WkU	WKUID	Possible antibody of undetermined specificity present.
WkU plus additional Antibody	WKUNABRO	Previously identified _____. Possible antibody of undetermined specificity present." Free text to edit and clarify as necessary.
A-NSG		Free text: Antibody reactivity may be due to preservative in test media.
A-DIG	POS RHIG	Positive antibody screen suspected due to Rh immune globulin given on ____. All common

		clinically significant antibodies other than anti-D have been ruled out at this time.
A-DNK		Free text: Unknown whether anti-D specificity is due to alloimmunization or RhIG
Historical, no new antibodies identified	ABRO	Previously identified anti -___. Other common, clinically significant alloantibodies ruled out.
Historical with new antibody(ies) identified	ABHIS	Newly identified anti-___, previously identified anti-___ reactivity present. Other common, clinically significant alloantibodies ruled-out.
WRM	WRMRO	Warm autoantibody reactivity present. Common, clinically significant alloantibodies ruled out by the manual, alternative method of testing.
CLD	CABID	Cold reactive antibody identified using manual, alternative method. (Add "Auto" if applicable)
A-CD38	DARALISS -OR- DARAHX -OR- DARA	Positive antibody screen suspected due to daratumamab treatment. All other common clinically significant antibodies ruled-out by manual, alternative method. - OR - Unable to rule out all common clinically significant alloantibodies due to history of daratumamab treatment. Phenotypically matched RBCs will be given for antibodies not ruled out. -OR- Sent to Versiti on _____. All underlying common clinically significant alloantibodies ruled-out with DTT-treated cells, except anti-K. Give K negative PRBCs if patient is antigen negative for K. (Remove "Sent to Versiti" if not applicable)

L. If there are any antibodies that can not be ruled out or confirmed add a Special Requirement in the BBIS for the applicable antigen, ie. "K negative RBCs".

M. Add a Patient Profile Note in the BBIS briefly summarizing the antibody investigation workup. Examples:

1. 60MNL=pan reactive, AC=1+, neg DAT, DTT=neg
2. Gel R/O = neg, AC = neg (R/O = Rule Out, AC = Auto Control)

XII. 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.

XIII. NOTES:

A. 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.

B. Anti-A_1 panels for patients at Taylor will be referred to another Corewell Blood Bank for follow up and resolution.

C. Panel interpretations should logically agree with reactivity seen in the antibody screen. If all antibody screen activity is not accounted for by the identified antibody(ies), further investigation should be performed; see a supervisor or lead technologist if necessary.

D. When a tube panel is performed, care should be taken to observe the various phases of reactivity which may suggest the presence of more than one antibody.

E. If a patient has previously identified antibody(ies), then a selected cell panel to eliminate other clinically significant antibodies may be substituted for a standard panel.

F. Weak antibody reactivity in the gel may be enhanced by longer 37°C incubation (up to 30 minutes).

G. All testing profiles may not be validated and/or in use at every Corewell Health location for all methods. Only testing and methods that have been implemented and properly quality controlled in each individual Corewell Health Blood Bank shall be performed at that location.

XIV. 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)

Antibody Screens, Panels, and Crossmatch Job Aid Revised 10/1/24

Selected Cell Sticker

Special Studies Worksheet (Rev 092424)

Approval Signatures

Step Description	Approver	Date
	Jeremy Powers: Chief, Pathology	10/23/2024
	Muhammad Arshad: Chief, Pathology	10/22/2024
	Kristina Davis: Staff Physician	10/17/2024
	Masood Siddiqui: Staff Pathologist	10/14/2024
	Ann Marie Blenc: System Med Dir, Hematopath	10/11/2024
	Hassan Kanaan: OUWB Clinical Faculty	10/11/2024
	Ryan Johnson: OUWB Clinical Faculty	10/11/2024
	John Pui: Chief, Pathology	10/11/2024
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Mgr, Division Laboratory	10/11/2024
	Hilary Morey: Medical Technologist Lead	10/11/2024
	Fatima Bazzi: Supv, Laboratory	10/10/2024
	Kristen DiCicco: Mgr, Laboratory	10/10/2024
	Suzanne Chahine: Medical Technologist Lead	10/10/2024
	Katherine Persinger: Mgr, Laboratory	10/10/2024
	Ashley Beesley: Mgr, Laboratory	10/9/2024

Teresa Lovins: Supv, Laboratory [KS]	10/9/2024
Karrie Torgerson: Medical Technologist Lead [KS]	10/9/2024
Kelly Sartor: Mgr, Division Laboratory	10/9/2024
Kelly Sartor: Mgr, Division Laboratory	10/9/2024

Applicability

Dearborn, Farmington Hills, Grosse Pointe, Royal Oak, Taylor, Trenton, Troy, Wayne

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.

ANTIBODY SCREEN, CROSSMATCH, AND PANEL JOB AID

Code	Ab screen/ panel / XM method	Indications	Phases						Screen / panel / XM Composition	Incubation time	Notes
			IS	RT	4°C	37°C	AHG	CC			
IS	Immediate Spin (test tube)	In place of electronic crossmatch during computer downtime, post-emergency issue and/or ABO discrepancies	X						2 drops plasma and 1 drop donor cells (Washed not required)	No incubation Spin and Read	
AHG XM	LISS/ Antihuman Globulin (test tube)	Alternative method for GEL testing. (GEL is the standard AHG method). ***Used only if directed by SOP or MD***					X	X	1 drop washed donor cells, 2 drops plasma, 2 drops LISS	15 minutes at 37°C	Tubes ***are not read*** at the 37°C phase
AHG *PANEL*	LISS/ Antihuman Globulin (test tube)	Alternative method for GEL testing. (GEL is the standard AHG method). ***Used only if directed by SOP or MD***				X	X	X	1 drop cells, 2 drops plasma, 2 drops LISS	15 minutes at 37°C	Tubes ***are read*** at 37°C
PW	Pre-warmed (test tube)	PW testing should be *rarely used* and ONLY with MD approval					X	X	1 drop screen / panel cells and 3 drops plasma	60 minutes at 37°C	Tubes ***are not read*** at the 37°C phase
AA	Autoadsorbed AHG (test tube)	Patient with warm autoantibody (WAA) using auto absorbed plasma				X	X	X	1 drop washed cells and 3 drops auto-absorbed plasma	60 minutes at 37°C	
NL	60 minute No LISS XM (test tube)	Patients with warm autoantibody (WAA)				X	X	X	1 drop washed cells and 3 drops plasma	60 minutes at 37°C	Tubes ***are read*** at 37°C
GEL	Gel Anti-IgG card	The standard method for patients with unexpected antibodies					X		50 µL of 0.8% cell suspension and 25 µL of patient plasma	15 minutes at 37°C	Centrifuge gel cards for 10 minutes
SR	Saline Replacement	Patients with rouleaux forming properties in plasma	X						2 drops plasma and 1 drop screen / panel cells – see procedure for details	No incubation Spin and read	
AP *XM*	All phase	Patients with Anti-A1 and infrequently used for patients with ***both*** cold and warm reacting antibodies	X			X	X	X	1 drop washed donor cells, 2 drops plasma. After IS add 2 drops LISS	After IS, incubate 15 minutes at 37°C	Tubes are read at all phases
AP *PANEL for AntiA1*	All phase	Patients with Anti-A1	X	X		X	X	X	1 drop screen/panel cells, 2 drops plasma. After RT add 2 drops LISS	After IS, incubate 15 minutes at 37°C	Tubes are read at all phases
AP *PANEL for Cold Ab*	All phase	Patients with cold reacting antibodies	X	*	*	X	X	X	1 drop screen / panel cells, 2 drops plasma. After IS add 2 drops LISS	After IS, incubate 15 minutes at 37°C	* Tubes may be read at all phases.

Note: Donor cells that will be carried through Coombs must be washed.

Note: Not all test codes are performed at all Beaumont Health hospitals.

Revised 10/01/2024

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 :	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:	Written on form	
Record updated		Record updated	

ARC Form (Tech / Date)

RhIG Information

Called Dr. office or RN (Emp / D / T / Tech):		Transfused in the last 4 weeks? (Y/N)	
RhIG date provided by:		Positive AC or DAT? (Y/N) Hemolysis Check? (Y/N)	
Date RhIG Rec'd:		New unexpected antibody identified? (Y/N)	

Suspected Transfusion Reaction

Tube DAT Results

Reagent	IS	RT	CC	Tech/ Date:
Poly				
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 cmt)			
NEX antibody (remove if applicable)			
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, *Antibody Investigation and Interpretation*

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	
A1 test cell: mfg, lot #, and exp.	
A2 test cell: mfg, lot #, and exp.	
B test cell: mfg, lot #, and exp.	
Sample date:	

☐ Gel ☐ Tube Dilution	A_1 Titer RT	AHG	CC	A_2 Titer RT	AHG	CC	B Titer RT	AHG	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	CC	Current Sample AG	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:

Store in 1°-6°C **SELECTED CELL#**________
MANUF__________LOT#________
EXP DATE__________________
PREP. DATE________________
EXP DATE__________TIME________
PREPARED BY ______________

Store in 1°-6°C **SELECTED CELL#**________
MANUF__________LOT#________
EXP DATE__________________
PREP. DATE________________
EXP DATE__________TIME________
PREPARED BY ______________

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MANUF__________LOT#________
EXP DATE__________________
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EXP DATE__________TIME________
PREPARED BY ______________

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**:
	Antibody 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: