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Document Contact	Kelly Sartor: Mgr, Division Laboratory
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Interpretation of Antibody Investigations

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide the Blood Bank staff with policies and procedures for interpreting antibody investigations.

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. DEFINITIONS AND ACRONYMS:

A. **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.

B. **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.

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

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

E. **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.

F. **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.

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

H. **Unexpected antibodies**: Any antibody (other than the 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 patient's sample.

I. **Clinically significant antibody**: An antibody that:

    1. Is known to cause Hemolytic Disease of the Newborn (HDN) or shortened survival of antigen positive RBCs,
    2. Requires transfusion of antigen negative red blood cells, and
    3. Is usually IgG and best detectable with antihuman globulin (AHG).

J. **Clinically insignificant antibody**: An antibody that:

    1. Does not cause shortened red cell survival of antigen positive RBCs,
    2. Does not require transfusion of antigen negative red blood cells, and
    3. Is usually IgM and reacts best below 37°C.
    4. Antibodies that are usually considered clinically insignificant include the following specificities:

        a. Auto-anti-IH, auto-anti-H, auto-anti-I, anti-I, anti-Le^a, anti-Le^b, anti-P_1, anti-M, anti-N, and anti-A_1 reactive below 37°C.

K. **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.

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

M. **HIS**: The hospital information system.

N. **BRL**: Beaumont Reference Laboratory.

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

IV. POLICIES

A. 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%.

B. 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.

C. Antibody Exclusions when a Warm Autoantibody (WAA)

1. When a WAA is currently reactive in a patient's sample, then antibody exclusions may be performed using the 60 minute no-LISS tube method in some cases. Refer to Transfusion Medicine policy, *Warm Autoantibody Investigations*, for additional information.

D. 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. 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 the Comment Text of the Blood Bank computer.
    a. This may also be documented on the *Special Studies Worksheet* in the space provided for "Antibodies Not Ruled Out".

E. 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.
    b. 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.
    c. For those antibodies that cannot be excluded, the antibody field of the patient's computer record should be updated to include this specificity (under Patient / Edit / Antibodies). 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 the Comment Text 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 corresponding antibody and updating the Comment Text.

F. 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, it is not necessary to confirm the presence of that antibody in subsequent investigations.
    b. A technologist may use the Ortho 0.8% Resolve Panel C or extend the incubation at 37°C for 30 minutes to attempt to enhance reactivity.
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., "too weak to identify" (TWTI) or Warm IgG Antibody, Non-specific.
    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.

G. Confirming the Presence of Multiple Antibodies

1. 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.

H. 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.

I. 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. 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 comment shall be added to the Blood Bank computer record indicating the sample collection date, and whether the antibody to the low incidence antigen is reactive.
        i. This may also be documented on an Antibody Card/Folder.

J. Confirmation of an Antibody to a High Frequency Antigen

1. 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:

a. 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)

2. 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.

K. Policies Specific to Patients with Anti-U

1. The U antigen is a high incidence antigen. Patients who develop anti-U are usually African American and the patient's own RBCs type as S-s-. The plasma may appear to be pan-reactive, but will likely be non-reactive with S-s- test RBCs. For additional information on antigen typing the patient and donor, and transfusion of patients with anti-U, refer to Transfusion Medicine policy, *Policies Specific to Patients with Anti-U*.

L. 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 clinically insignificant.
4. A complete phenotype shall be performed on 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., antibodies that are too weak to identify (TWTI).

M. Direct Antiglobulin (DAT) Testing

1. An autocontrol is performed with all panels. If the autocontrol is positive, then a DAT must be performed.

N. Repeat Testing

1. Whenever repeating an antibody screen or a panel, the sample should be re-spun. In some cases, when non-specific reactivity is originally observed in a panel, the panel will be non-reactive when the sample is re-spun and retested.

O. The Use of Expired Test Cells

1. Expired panel cells should not be used in a standard panel.
2. Expired panel cells should not be used in a selected cell panel unless in-date cells with the desired antigenic profile are unavailable and appropriate quality control has been performed.

P. The Use of a Ficin Treated Panel

1. 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.

Q. Obstetrical Patients with Apparent Anti-C and Anti-D

1. 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.

R. Antibody Titer Requirements for Obstetrical Patients with Clinically Significant Unexpected Antibodies

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 also be performed for patients with antibodies of varying clinical significance, i.e., anti-M and anti-N.

S. Determination of whether Anti-D Specificity is related to Passive Anti-D due to RhIG Administration, or Alloimmunization

1. 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. An antibody titration may be helpful in making this determination. A titer may be performed and the investigation will be interpreted, depending on the elapsed time between the date of the RhIG injection and the collection date of the sample in which the passive anti-D is detected. Refer to Transfusion

Medicine policy, Policies Specific to Patients with Passive Anti-D (Due to Recent RH Immune Globulin Administration).

T. 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 in Transfusion Medicine policy, Antibody Identification.
2. It is not necessary to perform repeat tube panels to confirm the presence of anti-A_1 for patients with a history 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.*

U. Non-Specific Reactivity in Antibody Investigations

1. The following policies apply when a sample exhibits non-specificity in the gel panel:
    a. If the strength of the strongest reacting cell is 2+ or stronger, then the antibody shall be classified as a **Warm IgG** Non-Specific antibody.
    b. If the strength of the strongest reacting cell is 1+ or weaker, then the antibody shall be classified as a **TWTI** (Too Weak to Identify).
    c. 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). 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 Possible Reactions to Preservative in Test Media (**PRESV**), which typically has a mixed-field appearance.
        ii. The patient's antibody field will be updated as "Not Eligible for Electronic Crossmatch."

V. 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 Blood Bank computer system by the medical technologist performing the antibody screen, if an

antibody investigation is not required, or performing the antibody investigation, if an investigation is required.

a. **ADELY**: Possible delay if blood products are requested due to positive antibody screen or historical antibody record.

b. **ADELX**: Due to the patient's antibody, there will be an extensive delay before RBCs are available. Please contact the Blood Bank immediately if emergency-issue / uncrossmatched blood is needed. The Blood Bank has notified the patient's nurse or other caregivers of this delay, and has asked the nurse or other caregivers to notify the patient's physician, if applicable, based on the patient's clinical status.

    i. This canned message also includes prompts for documentation of the RN/caregiver notified, date/time or notification, and Blood Bank technologist.

W. Requesting a Cord Blood Sample

1. If a sample is received for an obstetric patient who has a history of unexpected antibodies, then a cord blood should be requested. This sample will be used to perform the testing described in Transfusion Medicine policies, *Hemolytic Disease of the Newborn (HDN) Survey* and *Cord Blood Evaluation*. This request will be documented in the Blood Bank computer as a comment to the order of the maternal Type & Screen.

X. 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.

Y. 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.*

Z. 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. WAA (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 or WinRho. 4. A warm IgG antibody 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, PRES (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 DUNK (unknown whether anti-D specificity is due to alloimmunization or RhIG). CABID order is not required but paperwork should be submitted for review. 4. Anti-A_1 5. TWTI (strength 1+ or less) 6. 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 Blood Bank computer 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.

V. PROCEDURE:

A. Perform an antibody panel. Results should be recorded on:
    1. A copy of the antigram that was used, or

2. RO only: A copy of the selected cell antigram from the Antigen Plus program.

B. All antigrams should be initialed and dated, and documented with indelible ink. If running panels on the VISION, panel antigram lot numbers should be reconciled against the vision printout before recording reactions onto the antigram.

C. **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.

D. **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.

E. **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.

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

G. If applicable, antigen type the patient's RBCs for the antigen(s) corresponding to the antibody(ies) identified.

H. After completing an antibody investigation, the technologist will place all of the paperwork for the investigation in the designated area for Antibody Review.

VI. NOTES:

A. 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.

B. 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.

C. 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.

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

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

VII. 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.

Attachments

Antibody Screens, Panels, and Crossmatch Job Aid

Approval Signatures

Step Description	Approver	Date
	Vaishali Pansare: Chief, Pathology	9/21/2023
	Ann Marie Blenc: System Med Dir, Hematopath	9/20/2023
	Muhammad Arshad: Chief, Pathology	9/15/2023

	Ryan Johnson: OUWB Clinical Faculty	9/8/2023
	Kristina Davis: Staff Physician	9/7/2023
	Jeremy Powers: Chief, Pathology	9/6/2023
	John Pui: Chief, Pathology	9/6/2023
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Mgr, Division Laboratory	9/6/2023
	Abigail Swaney: Medical Technologist Lead	9/6/2023
	Fatima Bazzi: Medical Technologist Lead	9/5/2023
	Ashley Beesley: Mgr, Laboratory	9/1/2023
	Kristen DiCicco: Mgr, Laboratory	8/31/2023
	Katherine Persinger: Mgr, Laboratory	8/31/2023
	Michele Ferla: Medical Technologist Lead	8/31/2023
	Teresa Lovins: Supv, Laboratory	8/31/2023
	Karrie Torgerson: Supv, Laboratory	8/31/2023
	Hilary Morey: Medical Technologist Lead	8/31/2023
	Kelly Sartor: Mgr, Division Laboratory	8/31/2023
	Kelly Sartor: Mgr, Division Laboratory	8/31/2023