Status **Active** PolicyStat ID **14316248**

Beaumont

Origination	12/1/2023
Last Approved	11/1/2023
Effective	12/1/2023
Last Revised	11/1/2023
Next Review	10/31/2025

Document Contact	Kelly Sartor: Mgr, Division Laboratory
Area	Laboratory-Blood Bank
Applicability	All Beaumont Hospitals

Antigen Typing - Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide policies and procedures relating to antigen typing of patient and donor units.

II. SCOPE:

A. Antigen testing is performed on patient's red blood cells (RBCs) to aid in the confirmation of antibody(ies) identified in the patient's plasma. With rare exception, when an alloantibody is present in a patient's plasma, the corresponding antigen is absent from the patient's RBCs.

B. RBC donor units are typed for the antigen(s) corresponding to the patient's clinically significant antibody(ies) prior to crossmatch, to help ensure recipient-donor compatibility. For these patients, donor units must be negative for the antigen(s) corresponding to the patient's clinically significant antibody(ies).

III. DEFINITIONS:

A. **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).

B. **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. Anti-IH, auto anti-H, anti-I, anti-Le^a, anti-Le^b, anti-P_1, anti-M, anti-N, and anti-A_1.

C. **Rare antisera**: Antisera that are not readily available commercially due to factors such as extreme cost or technological production difficulties.

IV. POLICIES

A. Antigen Typing Methods

1. Testing methodology can differ from one site to another based on different testing methodologies from one reagent vendor to another as well as approved test validations. For example gel testing has been validated at some sites for Rh antigen testing as well as for some reagents that require indirect antiglobulin phase.
2. The technologist will determine the appropriate reagent and method by which to perform antigen typing based on available anti-sera, and site test validations. Refer to the attachment, *Antigen Typing Job Aid*.

B. Requirements to Follow the Manufacturer's Directions

1. When antigen typing, the technologist must review and follow the applicable manufacturer's insert.
    a. If the manufacturer's insert indicates the choice of using 1 or 2 drops of anti-sera, then use 2 drops.
    b. If the manufacturer's insert recommends incubation range, ie. 5 -15 minutes, the longer of the incubation times will be used for all negative tests or positive tests with a reaction grade less than 2+. Refer to policy, *Grading of Reaction for Patient and Donor Samples* below.
2. These inserts are maintained in the Manufacturers' Inserts Binder located in each blood bank department.

C. Antigen Typing Policies Relating to the Patient's Transfusion History

1. Before antigen typing a patient's RBCs, the technologist should obtain a patient history as described in Transfusion Medicine policy, Obtaining Patient Histories. Antigen typing should not be documented to the patient's record unless a history has been obtained.

2. Samples of patients who have been transfused with RBCs within the preceding 90 days cannot be accurately antigen typed because the RBCs circulating in their body may be a combination of their own cells and transfused cells.

D. Antigen Typing Policies Relating to a Positive Direct Antiglobulin Test (DAT) and/or Positive Autocontrol

1. Antigen testing cannot be performed with antisera that require an IAT (indirect antiglobulin test) if the tube DAT and/or the autocontrol is positive. Saline reacting antisera that do not require IAT can be used if available.
2. If the patient's autocontrol is weakly positive with gel testing but the Direct Coombs is negative with the tube method, the antihuman globulin (AHG) tube method should be employed to determine a patient's antigen status.

E. Inert Control Requirements

1. Due to the potential for false positive reactions, most manufacturers' inserts recommend the use of an inert control when positive antigen results are obtained. This inert control is expected to be non-reactive. For example, a monoclonal control may be tested when using the Rh individual or phenotype cards, or a DAT may be performed as a control when antigen typing by the indirect antiglobulin tube method, etc.
    a. For patient samples with negative antigen results the inert control is not required (false positive results are not a concern in this case).
    b. For **patient** samples with positive antigen results, the inert control must be tested if indicated by the manufacturer's insert. If the patient antigen result is positive, and the inert control is positive or the inert control is not tested, then the positive antigen result is invalid.
    c. For **donor** units with positive antigen results, this inert control will not be tested. Any antigen positive units will be considered and labeled as preliminary positive.

    Refer to page 2 of the attachment, *Antigen Typing Job Aid* for more details.

F. Patients with Unexpected Antibodies

1. Once an unexpected antibody has been identified in a patient's plasma, the patient's RBCs should be tested for the corresponding antigen, if possible.
2. If a clinically significant antibody is present in a patient's plasma, then the RBC donor units selected for transfusion should be negative for the antigen corresponding to the antibody.

    Refer to Transfusion Medicine policy, Policies for Providing Red Blood Cells to Patients with Unexpected Antibodies.

G. Clinical Indications for Extended Phenotype/ Molecular Genotyping

1. A complete phenotype or RBC genotype will be performed if:
    a. The patient has 3 or more identifiable antibodies (not including non-specific antibodies).
    b. The patient has a warm autoantibody (WAA).
    c. The patient has sickle cell disease or thalassemia.
    d. The patient has a newly identified passive CD38 antibody or blood bank receives notification that the patient will begin treatment with the CD38 drug.
2. This phenotype should include Rh(D) testing and testing for the following 11 antigens: C E c e K Fy^a Fy^b Jk^a Jk^b S and s
3. The phenotype may be performed at a Corewell Health Blood Bank, but may be beneficial or required to send the patient sample out to a reference laboratory instead.

H. Indications for Submitting a Sample to a Reference Laboratory for Molecular Genotyping

1. It may be beneficial to send a sample to a reference laboratory for molecular genotyping instead of performing a phenotype at a Corewell Health Blood Bank. Examples of these benefits include:
    a. Molecular genotyping extracts and tests genetic material from the patient's white blood cells, not the red blood cells. This allows the determination of the patient's genotype even if they have been recently transfused, or that have a positive DAT and/or autocontrol.
    b. Molecular genotypes provide more antigen results than if the phenotype was performed at a Corewell Health Blood Bank due to the limited antisera availability.
    c. Molecular genotypes have the ability to detect antigen variants that may not be detectable during serologic testing.
2. Do not submit a sample to a reference laboratory if the antigen typing is only being performed as part of a routine antibody investigation. For example:
    a. Anti-E is identified in a routine antibody investigation on a patient who has been recently transfused. Do not submit a sample for E typing. Instead, add the message "Unable to Antigen Type" to the patient's Blood Bank computer record.
3. Refer to Transfusion Medicine Policy, Submitting Samples to External Reference Laboratories.

I. Documentation of Antigen Results from Blood Suppliers and Reference Laboratories

1. Antigen types on donor units that are confirmed by a blood supplier (testing was performed on current donation) do not need to be tested at Corewell Health and will be documented as "official" results (not preliminary) under *Inventory / Edit / Antigen.* A *Unit Antigen Label* will also be documented and affixed to the unit.

   Note: Blood supplier/ reference lab may use unlicensed antisera to confirm antigen results if the antisera is rare. This result is entered as confirmed in the Blood Bank computer.

2. Antigen types on donor that are historical or unconfirmed by the blood supplier (testing was not performed on current donation or obtained from Versiti Antigen Query) will be considered preliminary and may not be crossmatched for a patient who has the corresponding clinically significant antibody unless / until the unit has been tested and found to be antigen negative at Corewell Health. Note: Unconfirmed units may be emergency issued if necessary.
3. All unconfirmed antigen types that are not tested at Corewell Health will be documented as a preliminary result in the computer. The units will have the *Unit Antigen Label* attached with a notation that the preliminary results were provided by a reference lab.
4. Patient antigen results (including molecular genotyping) are documented in the blood bank computer system upon receipt. Verify that the results agree with previously documented results. If a molecular report is received add a comment to the patient's record indicating that we have a molecular report and save a copy of the molecular report in the designated reference laboratory file / folder in the department.

J. Preliminary Antigen Results of Donor Units Tested at Corewell Health

1. The antigen results (positive and negative) of units screened without licensed antisera (retained patient serum) at Corewell Health are documented as preliminary in the computer and will be labeled as preliminary with a *Unit Antigen Label*. These units may not be crossmatched for a patient who has the corresponding clinically significant antibody unless / until the unit has been tested and found to be antigen negative. Refer to Transfusion Medicine Policy, *Preliminary Antigen Screening*.

K. Labeling Donor Units with Antigen Results

1. The results of all units tested at Corewell Health will be documented in the Blood Bank computer and the *Unit Antigen Label* will be affixed to the unit.
2. The green side is used for all confirmed antigen negative results (results that are not preliminary). The negative antigen result is circled and the donor unit number is documented using a sticker from the unit if possible. This green side is initialed and dated by the technologist who antigen tests and tags the unit. The Cosign section will be initialed by the technologist who confirms that the antigen testing is recorded in the computer at time of selection/issue. (If possible this should not be the same technologist when additional staff is

available). The Billed section of the tag should be completed when the AGTYP action code has been applied to the patient for whom the first screening occurred.

3. The yellow side is used for preliminary antigen results. For example, for historical or unconfirmed antigen results from the reference lab or in situations where we preliminary screen with retained patient serum. The preliminary typing is recorded in Blood Bank computer accordingly and the preliminary antigen result is circled and the donor unit number is documented using a sticker from the unit if possible. This yellow side is initialed and dated by the technologist who antigen tests and tags the unit. It is not necessary to cosign preliminary antigen results.

L. Appropriate Test Cell for Antigen Positive Control

When applicable, these are should be heterozygous for antigen of interest.

Antisera / Gel card	Appropriate Test Cell for the Antigen Positive Control
anti-C	R1r (C+c+)
anti-E	R2r (E+e+)
anti-c	R1r (C+c+)
anti-e	R2r (E+e+)
anti-K	Kk
anti-Fy^a or anti-Fy^b	Fy(a+b+)
anti-Jk^a or anti-Jk^b	Jk(a+b+)
anti-S or anti-s	Ss
anti-M or anti-N	MN
anti-Le^a	Le(a+b-)
anti-Le^b	Le(a-b+)
anti-P_1	P_1 +weak (if available)
anti-C^w	C^w+
anti-A_1	A_1 cell for positive control; A_2 or B cell for negative control

M. Graded Reactions of Patient or Donor Samples

1. Valid Graded Reactions
    a. To interpret the antigen typing result of a patient or donor sample as negative, the test must be non-reactive.
    b. To interpret the antigen typing result of a **patient** sample as positive, the reaction strength must be 2+ or greater, and the inert control (if indicated) must be non-reactive. Weak positive or 1+ reactions on patient samples are considered invalid and should be repeated or reviewed with Medical Director.
    c. To interpret the antigen typing result of a **donor** sample as positive, any reactivity

shall be interpreted as positive. However, if the reaction strength is less than 2+ (is weak+ or 1+) repeat testing should occur.

N. Requirements to Consider an Antigen's Prevalence when Antigen Typing Units

1. When antigen typing donor units, the technologist should consider the antigen's prevalence in the general population, which may be obtained from the AABB *Technical Manual*, for the following purposes:
    a. To estimate the number of units that should be tested to find the desired number of antigen negative units. For example:
        i. The prevalence of the Jk^a antigen in the general population is approximately 77%. Therefore, the percentage of Jk^a negative donors is approximately 23%. The technologist types 10 units and should expect to find 2 Jk^a negative units.
    b. To verify that the antigen's prevalence observed in the units tested roughly corresponds to the antigen's prevalence in the general population. For example:
        i. A technologist types 20 units for Jk^a and observes that 17 of them are Jk^a negative and 3 are Jk^a positive. The technologist should repeat this testing, because the antigen's prevalence that is observed in the tested units (15%) does not roughly correspond to the antigen's prevalence in the general population (77%).
2. If an antibody to a low incidence antigen has been identified and if antiserum 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. Refer to Transfusion Medicine Policy, Interpretation of Antibody Investigations: Determining whether an Antibody to a Low Incidence Antigen is Reactive

O. Policies Relating to Antigen Typing by the IgG Gel Card Method Using Bottled Reagent Antisera Derived from a Human Source that Requires an IAT

1. The technologist must review the manufacturer's insert for the reagent being used before antigen typing by the IgG gel card method to confirm that the reagent is derived from a human source that requires an IAT.
2. The technologist must review the Job Aid to verify that gel procedure was validated for the antiserum at the testing site.

P. Policies Relating to Antigen Typing by the Tube Method

1. Before antigen typing by the tube method, the technologist must review the applicable manufacturer's insert. The technologist must follow the policies, directions, inert control requirements, etc. in the manufacturer's insert. The procedure is intended to supplement the procedures found in the manufacturer's inserts.
2. If the manufacturer's insert indicates that an indirect antiglobulin phase is required, then check cells must be used in all tubes in which the graded reaction is negative. The check cells must be positive (any strength). If the check cells reaction is not positive, then the results for that tube are considered invalid and must be repeated.
3. All patient and donor cells must be washed and resuspended to 2% - 4% before testing, even if the manufacturer's insert has a statement such as "samples *may* be washed and resuspended prior to testing".

Q. Policy to Document the "Open Date" on Each Vial of Reagent Antisera

1. The open date and technologist's initials will be written on each vial when it is opened (when it is used for the first time).

V. SPECIMEN COLLECTION AND HANDLING:

A. The preferred patient specimen is a 6 mL EDTA sample with affixed identifying label. For acceptable alternatives, refer to Transfusion Medicine policy, Triaging and Identifying Acceptable Samples for Testing.

B. For testing donor units, a segment from the unit in a test tube labeled with the unit number will be used for the antigen testing.

C. See the manufacturer's insert corresponding to the specific antisera being used for any special specimen requirements.

VI. REAGENTS / EQUIPMENT / SUPPLIES:

A. When using the MTS Monoclonal Rh Gel Cards™:

1. MTS Individual (Monoclonal) Cards™
2. MTS Monoclonal Rh Phenotype Cards™
3. MTS Monoclonal Control Card™
4. MTS Diluent 2 PLUS™
5. 12 x 75 mm test tubes
6. MTS Ortho Workstation or MTS Centrifuge
7. Commercially prepared test cells for use as antigen positive and antigen negative

controls, 4% ± 1% suspension

8. Pipette capable of delivering 25 µL
9. Pipette capable of delivering 10 µL or 12.5 µL

B. When using the IgG Gel Card Method with Reagent Antisera Derived from a Human Source that Requires an IAT:

1. MTS Diluent 2™
2. 12 x 75 mm test tubes
3. Pipette capable of delivering 25 µL
4. Pipette capable of delivering 50 µL
5. MTS Ortho Workstation or MTS Centrifuge/Heat Block
6. MTS Anti-IgG Gel Cards™
7. Reagent antisera (verify with the manufacturer's insert that the reagent requires an IAT phase and that the reagent is derived from a human source)
8. Commercially prepared test cells for use as antigen positive and antigen negative controls, 0.8% suspension

C. When using the Tube Method:

1. Reagent antisera
2. Commercially prepared test cells for use as antigen positive and antigen negative controls, 4% ± 1% suspension
3. IgG-coated cells (check cells)
4. Antihuman globulin (IgG)
5. Buffered saline
6. 10 x 75 mm or 12 x 75 mm test tubes
7. Disposable pipettes
8. Timer
9. Heat block
10. Centrifuge
11. Automated cell washer (if applicable)

VII. REAGENT HANDLING

A. All reagents, anti-sera, and diluents must be visually inspected prior to use to ensure that the liquid is not discolored, turbid, or showing any signs of bacterial contamination.

B. Each well of the gel card should have a clear liquid layer on top of the opaque gel. Do not use the gel card if:

1. The gel matrix is absent.
2. The liquid level in the microtube is at or below the top of the gel matrix.

3. It shows signs of drying, discoloration, bubbles, crystals, or other artifacts.
4. Foil seals appear damaged or opened.

C. The Rh individual and phenotype gel cards should be stored upright at 1°C to 8°C, and brought to room temperature before use.

D. IgG cards should be stored upright at 2°C to 25°C and brought to room temperature before use.

E. All reagents, antisera, diluents, and gel cards must be used within the manufacturer's expiration date. Any exceptions must be approved by the Blood Bank Medical Director or designee, and may be used only if the positive and negative controls are tested and work as expected.

VIII. QUALITY CONTROL (QC):

A. Quality Control (QC) is performed once per day of use for each antiserum/methodology used, and is documented in the computer as described in the Blood Bank CDM - *Resulting the QC Rack* or on paper per site specific QC policies. QC results are documented only for phases tested.

B. Appropriate positive and negative controls for antigen typings must be tested once per day of use for each antiserum/ methodology used.

1. The reaction strength of the positive control must be 2+ or greater. If the reaction strength of the positive control is not 2+ or greater, then the quality control is considered to be failing.
2. The negative control must be non-reactive. If the negative control is reactive (any strength) then the QC is considered to be failing.

C. Failing QC

1. If the QC fails, then all of the patient and donor samples and the positive and negative controls for that batch must be repeated with the same lot numbers (if possible). If the QC fails after this repeat testing then:
    a. Place the applicable antisera or Rh gel cards in quarantine; order additional antisera or gel cards, if necessary.
    b. If possible, repeat all testing with a new lot number.
    c. Patient and donor antigen typing results may not be released unless quality control is valid.
    d. Notify the supervisor by documenting all failed QC in a variance (even if the QC passes upon repeat).

IX. PROCEDURE:

A. Antigen Typing by the Rh Gel Card™ Method using the MTS Individual (Monoclonal) Cards™ or the MTS

Monoclonal Rh Phenotype Cards™

1. Visually inspect all reagents, diluents, and gel card(s) before use.
2. Label a 12 x 75 mm test tube to identify each patient or donor test cell being tested. For example:
    a. For each donor unit, label one test tube with the donor unit number.
    b. For each patient sample, label one test tube with the patient's last name.
3. In the tube(s) that were labeled in the step above, prepare a 4% ± 1% suspension of each patient or donor test cell as follows:
    a. Manual pipette:
        i. Dispense 0.5 mL of MTS Diluent 2 PLUS™ into the test tube.
        ii. Add 25 µL of packed red blood cells.
        iii. Mix gently to re suspend.
    b. Biohit or Sartorius Electronic pipette:
        i. Program the pipette to program #7.
        ii. Aspirate 200 µL of MTS Diluent 2 PLUS™.
        iii. Aspirate 15 µL of air into the pipette tip.
        iv. Aspirate 10 µL packed RBCs and wipe the outside of the tip.
        v. Purge all contents from the tip into the 12 x 75 mm test tube and mix.
4. Label one well of the MTS C, E, e, or e Individual (Monoclonal) Gel Card to identify each of the following:
    a. Patient last name or donor unit number.
    b. Positive control and negative control (commercially prepared).
    c. An MTS Monoclonal Rh Phenotype Card™ may be used instead of individual cards.
5. If indicated, also label one well of the MTS Monoclonal Control Card for each patient or donor sample. This well is used to test the inert control, if indicated (and if not already tested as part of the patient's ABO/Rh type or Rh Phenotype card).
6. Remove the foil seal from the gel card(s); expose only enough wells needed for testing. Testing must occur within one hour of removing the gel card foil.
7. Add 10 - 12.5 µL of the 4% ± 1% patient test cell, donor test cell, or control cell to the correspondingly labeled wells of the gel card(s).
    a. The volume of the red cell suspension depends on which setting is available on the pipette.
    b. The pipette should not touch the card.
8. Centrifuge the gel card in the MTS centrifuge for 10 minutes at 895 ± 25 RPM or in the Ortho Workstation for 10 minutes at 1032 ± 10 RPM.

9. Observe the front and the back of each well macroscopically.
10. Read, grade, and record all test results and interpret the graded reactions.

B. Antigen Typing by the IgG Gel Card Method using Bottled Reagent Antisera Derived from a Human Source that Requires an IAT

1. Visually inspect all reagents, diluents, and gel card(s) before use.
2. Refer to the manufacturer's insert and verify that the reagent was derived from a human source and requires an IAT phase.
3. Label a 12 x 75 mm test tube to identify each patient or donor test cell being tested. For example:
    a. For each donor unit, label one test tube with the donor unit number.
    b. For each patient sample, label one test tube with the patient's last name.
4. In the tube(s) that were labeled in the step above, prepare a 0.8% cell suspension of each donor or patient test cell as follows:
    a. Manual pipette:
        i. Dispense 1 mL of MTS Diluent 2™ into the test tube.
        ii. Add 10 µL of packed red blood cells.
        iii. Mix gently to resuspend.
    b. Biohit or Sartorius Electronic pipette:
        i. Dispense 1 mL of MTS Diluent 2™ into the test tube.
        ii. Program the pipette to program #6.
        iii. Aspirate 100 µL of MTS Diluent 2™.
        iv. Aspirate 15 µL of air into the pipette tip.
        v. Aspirate 10 µL packed RBCs and wipe the outside of the tip.
        vi. Purge all contents from the tip into the 12 x 75 mm test tube and mix.
5. Label an MTS Anti-IgG Gel Card to identify the antiserum specificity. Also label one well of the gel card to identify each of the following:
    a. Patient last name or donor unit number.
    b. Positive control and negative controls (commerically prepared).
6. Remove the foil seal from the gel card(s); expose only enough wells needed for testing. Testing must occur within one hour of removing the gel card foil.
7. Add 50 µL of the 0.8% patient or donor test cell (prepared in step 4) and 50 µL of the 0.8% control cells to the correspondingly labeled wells of the gel card(s). The pipette should not touch the card.

8. Add 25 µL of the antiserum to the correspondingly labeled well(s).
9. Incubate the gel card(s) fo 15 minutes at 37°C ± 2°C.
10. Centrifuge the gel card(s) in the MTS centrifuge for 10 minutes at 895 ± 25 RPM or in the Ortho Workstation for 10 minutes at 1032 ± 10 RPM.
11. Observe the front and the back of each well macroscopically.
12. Read, grade, and record all test results and interpret the graded reactions.

C. Antigen Typing by the Tube Method

1. Obtain and review the manufacturer's insert for the applicable antisera being used.
2. Visually inspect all reagents before use.
3. Label 12 x 75 mm test tubes to identify the patient test cells, donor test cells, and positive and negative control cells as follows:
    a. Patient test cells
        i. Label 2 test tubes as follows:
            i. Tube 1: Patient's last name (for cell suspension).
            ii. Tube 2: Patient's last name and antisera specificity (for antigen testing).
    b. Donor test cells
        a. Label 2 test tubes as follows:
            i. Tube 1: Donor unit number, place a segment in this tube (for cell suspension).
            ii. Tube 2: Donor unit number and antiserum specificity (for antigen testing).
        b. It is preferable to use the stickers with the donor unit number as opposed to handwriting for labeling the test tubes.
    c. Positive control
        a. Label to identify as the positive control and with the antiserum specificity.
    d. Negative control
        a. Label to identify as the negative control and with the antiserum specificity.
4. If indicated by the manufacturer's insert, label tubes and perform an appropriate inert control.
5. In the correspondingly labeled tubes, prepare a 2% - 4% washed cell suspension of each patient or donor test cell. Note that in some cases the manufacturer's insert indicates that the positive and negative control test cells must also be washed and resuspended.
6. Following the directions in the manufacturer's insert, combine the appropriate number of drops of antiserum and the patient, donor, or control test cells to the correspondingly labeled tubes. If the insert indicates the choice of using 1 or 2 drops of antiserum, then use 2 drops.
7. If indicated by the manufacturer's insert, incubate the test tubes for the specified time and at

the specified temperature.

8. Read, grade, and record all test results and interpret the graded reactions.
    a. If the manufacturer's insert recommends incubation range, ie. 5 -15 minutes, extend the incubation time to the longer of the incubation times for all negative tests or positive tests with a reaction grade less than 2+.
    Refer to policy, *Grading of Reaction for Patient and Donor Samples* below.

X. RECORDING TEST RESULTS:

A. All unit antigen results are ordered and resulted in the computer as described in the Blood Bank CDM - *Unit Antigen Typing*.

B. All patient antigen results are ordered and entered into the computer as described in Blood Bank CDM - *Single Result Entry*.

C. Donor A_1 subgroups are specifically ordered by a physician for transplantation purposes. This testing must be done on duplicate on separate samples. These antigen results are ordered and resulted in the computer as described in the Blood Bank CDM - *Documentation of Donor A_1 Subgroups and Other Antigen Results Specifically Ordered by a Physician*.

D. During computer downtime, the *Antigen Typing Downtime Worksheet* will be used for recording test and QC results.

XI. INTERPRETATION:

A. Gel Method

1. Negative result - No agglutination and no hemolysis of the red blood cells is a negative test result.
2. Positive result - Agglutination and/or hemolysis of red blood cells is a positive test result. Red blood cells may remain suspended at the top of the gel or are dispersed throughout the gel in varying degrees. A few red blood cells may form a button in the bottom of the microtube in some positive reactions.
3. Documentation of a negative inert control is required before a positive antigen test may be interpreted.
4. A very weak reaction on one or both sides of the microtube is not an expected result. Further investigation should be performed before interpretation.
5. Interpretation of mixed-field reactions must be done with caution. The presence of fibrin, clots, or particulates may result in some red blood cells layering at the top of the gel. Mixed-field reactions are generally only observed in tests containing a dual population of red blood cells, such as a transfused patient, bone marrow recipient, or when a pooled red blood cell sample is used for testing. However, not all mixed red blood cell situations have a sufficient minor population to be detected.

B. Positive Inert Monoclonal Control

1. If the MTS monoclonal control is tested and is reactive, testing should be repeated with further investigation; red blood cells washed with warm saline prior to testing may be used.

C. Tube Method

1. Negative result - No agglutination and no hemolysis of the red blood cells is a negative test result.
2. Positive result - Agglutination and/or hemolysis of the red blood cells is a positive test result.
3. Documentation of a negative inert control is required before a positive antigen test may be interpreted.

XII. NOTES:

A. If a patient has anti-Le^a or anti-Le^b, then the patient's RBCs should be typed for both Le^a and Le^b. Generally, the patient's RBCs should be negative for both antigens.

B. If anti-N is identified, then the patient's RBCs should be typed for S and s as well as N.

   a. If the patient types as N-S-s-, then the anti-N identified is considered clinically significant. In this situation N antigen negative units should be provided to the patient. Antibody titers are also indicated for obstetric patients.

   b. If the patient types as N negative and S or s positive, then the anti-N identified is usually be clinically insignificant; antigen negative units are not required but should be crossmatch compatible.

XIII. REFERENCES:

1. AABB, *Technical Manual*, current edition.
2. College of American Pathologists, *Transfusion Medicine Checklist*, current edition.
3. Micro Typing Systems™ Procedures: *Rh Phenotyping Using Individual Gel Cards (Monoclonal Anti-D, Anti-c, Anti-E, and Anti-e) and Rh Phenotype Using Monoclonal Rh Phenotype Gel Cards*.

Attachments

Antigen Typing Downtime Form

Antigen Typing Job Aid

Approval Signatures

Step Description	Approver	Date
	Jeremy Powers: Chief, Pathology	11/1/2023

	Muhammad Arshad: Chief, Pathology	10/31/2023
	Kristina Davis: Staff Physician	10/26/2023
	Ann Marie Blenc: System Med Dir, Hematopath	10/26/2023
	Ryan Johnson: OUWB Clinical Faculty	10/24/2023
	Vaishali Pansare: Chief, Pathology	10/20/2023
	John Pui: Chief, Pathology	10/20/2023
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Mgr, Division Laboratory	10/20/2023
	Abigail Swaney: Medical Technologist Lead	10/20/2023
	Michele Ferla: Medical Technologist Lead	10/18/2023
	Fatima Bazzi: Medical Technologist Lead	10/18/2023
	Kristen DiCicco: Mgr, Laboratory	10/17/2023
	Hilary Morey: Medical Technologist Lead	10/17/2023
	Katherine Persinger: Mgr, Laboratory	10/17/2023
	Karrie Torgerson: Supv, Laboratory	10/16/2023
	Teresa Lovins: Supv, Laboratory	10/13/2023
	Ashley Beesley: Mgr, Laboratory [KG]	10/13/2023
	Kelly Sartor: Mgr, Division Laboratory	10/12/2023
	Kelly Sartor: Mgr, Division Laboratory	10/12/2023