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Antigen Typing - Dearborn 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. The technologist will determine the appropriate reagent and method by which to perform antigen typing and will consider the gel autocontrol, DAT, and transfusion history.

C. 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).

D. Patients with sickle cell disease and thalassemia will be transfused with RBCs that are partially matched to their own phenotype. This is done in attempt to prevent alloimmunization to the highly antigenic C, E, and K RBC antigens in this frequently transfused population. Refer to Transfusion Medicine policy, Policies Specific to Patients with Sickle Cell Disease and Thalassemia.

E. Neonatal RBCs may be antigen typed to assess the risk of Hemolytic Disease of the Newborn (HDN) when the mother has unexpected antibodies. Refer to Transfusion Medicine policy, Cord

Blood Evaluation, for additional information.

F. A complete phenotype may be performed on patients with 3 or more identifiable antibodies and patients with a detectable warm autoantibody. A phenotype is not required if the antibody cannot be identified; i.e., antibodies that are too weak to identify (TWTI).

G. 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.

H. If anti-N is detected, then the patient's RBCs should be typed for N, S, and s. Refer to Transfusion Medicine policy, Interpretation of Antibody Investigations: Patient Antigen Typing Requirements for additional information.

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

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

IV. POLICIES

A. 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. The RBCs circulating in their body and being test may be a combination of their own cells and transfused cells.

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

1. In some cases, antigen testing cannot be performed if the DAT and/or the autocontrol is positive; e.g., if the reagent requires an IAT (indirect antiglobulin test). Refer to the *Inert Control Requirements for Positive Antigen Results* section of this document.

C. Antigen Typing Methods

1. C, E, c, and e:
    a. The preferred method is by the Rh gel card method.
    b. The alternate method is by the tube method.
2. Fy^a, Fy^b, S:
    a. The preferred method is by the IgG gel card method. Reagents for these antigens that are derived from a human source and that require an IAT phase have been validated.
    b. The tube method may be used for reagents that are not derived from a human source or that do not require an IAT phase.
3. All other antigens (besides C, E, c, e, Fy^a, Fy^b, S):
    a. The required method is the tube method.

D. Recording Test Results

1. All unit antigen results are ordered and resulted in the computer as described in the Blood Bank CDM - *Unit Antigen Typing*. Quality Control (QC), which is performed once per day of use for each antisera / methodology used, and is documented in the computer as described in the Blood Bank CDM - *Resulting the QC Rack*; QC results are documented only for phases tested. During computer downtime, the *Antigen Typing Downtime Worksheet* will be used for recording test and QC results.
2. All patient antigen results are entered into the computer as described in Blood Bank CDM - *Single Result Entry*. QC, which is performed once per day of use for each antisera / methodology used will be documented in the Blood Bank computer. QC results are documented only for phases tested. During computer downtime, the *Antigen Typing Downtime Worksheet* will be used for recording test and QC results. Note that patient antigen tests are not typically ordered by physicians and when recorded in this manner, they do not interface to the Hospital Information System (HIS) with the exception of donor A_1 subgroups and antigen results specifically ordered by the physician.
3. Donor A_1 subgroups are specifically ordered by a physician for transplantation purposes. In addition, the patient's physician will occasionally order antigen results: e.g., on the partner of an obstetrical patient with antibodies to help assess the risk of HDN. 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*. Note that

when resulted in this manner, the results will interface to the HIS.

E. 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.
3. The Blood Bank may not stock the anti-sera corresponding to a patient's antibody to a low incidence antigen. Refer to Transfusion Medicine policy, Policies for Providing Red Blood Cells to Patients with Unexpected Antibodies.

F. Phenotype for Patients with 3 or More Identifiable Unexpected Antibodies or Detectable Warm Autoantibodies

1. A complete phenotype or RBC genotype will be performed on patients with 3 or more identifiable, unexpected antibodies or a detectable warm autoantibody.
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 Beaumont Blood Bank, but may be beneficial to send the patient sample out to a reference laboratory instead.

G. 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 Beaumont 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 Beaumont 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. Indications for submitting a sample to a reference laboratory for molecular genotyping include:
    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.

3. 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.

H. Documentation of Antigen Results from Blood Suppliers, Reference Laboratories, or Antigen Screening at Beaumont Health

1. Generally, antigen types that are confirmed by the supplier will be documented as "official" results (not preliminary) under Inventory / Edit / Antigen, and antigen types that are unconfirmed / historical will be documented as preliminary, as described in the following table:

Antigen Result	Appropriate Actions
Reference Lab confirmed donor units (testing was performed on current donation)	The antigen type does not need to be tested at Beaumont. Record the confirmed results in the Blood Bank computer. Confirmed antigen results may print on the reference lab unit face label or be labeled with an antigen tag that is affixed to the unit. If the antigen results are printed on the face label, a Beaumont *Unit Antigen Label* will also be documented and affixed to the unit. A reference lab may use unlicensed antisera to confirm antigen results if the antisera is rare.
Reference Lab unconfirmed donor units (testing was not performed on current donation)	If a patient requires antigen negative RBCs, these unconfirmed results must be tested and document at Beaumont. All unconfirmed antigen types that are not tested at Beaumont will be documented as a preliminary result in the computer. If a preliminary reference lab antigen label is not affixed to the unit, the unconfirmed results will be documented as preliminary results in the Blood Bank computer system and the units will have a Beaumont *Unit Antigen Label* attached; include a notation that the preliminary results were provided by a reference lab.
Reference Lab patient antigen results	Document the reference lab results in the Blood

(including molecular)	Bank computer. It is not necessary to wait for the Final Reference Lab Report in order to document the antigen results. When the reference laboratory report is received, verify that the results agree with the previously documented results. The reference lab may send a preliminary report by fax or may telephone antigen results. 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.
Antigen Screening at Beaumont	The antigen results (positive and negative) of units screened without licensed antisera (retained patient serum) at Beaumont are documented as preliminary in the computer and will be labeled as preliminary with a Beaumont *Unit Antigen Label*.

I. Preliminary Antigen Results of Donor Units Tested at Beaumont Health

1. A unit with a preliminary negative antigen result 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. Antigen results of donor units tested at Beaumont are considered preliminary in the following cases:
    a. For antigen positive donor units, if the inert control was not tested when indicated.
    b. For antigen results that are obtained by screening at Royal Oak using retained patient antisera.

J. Labeling Units

1. The results of all units tested at Beaumont will be documented in the Blood Bank computer and the *Unit Antigen Label* shall be affixed to the unit.

K. Inert Control Requirements for Positive Antigen Results

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. For example, a monoclonal control may be tested when using the Rh individual or phenotype cards, a DAT may be performed as a control when antigen typing by the indirect antiglobulin tube method, etc. This

inert control is expected to be non-reactive. Note that these controls are not required when the antigen results are negative (false positive results are not a concern in this case). Due to this potential for false positive results, the following policies apply:

a. 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.

b. For **donor** units with positive antigen results, this inert control will be tested if required by manufacturer insert when testing was performed by the AHG method or if donor unit is AB positive *and* antigen testing is done by monoclonal Rh method. Note: It is also acceptable to label an antigen positive units as preliminary if the inert control is not tested.

L. Potential False Positivity if All Patient Results are Positive

1. If all of a patient's antigen types are positive by a given method, then the chance that the results are falsely positive is increased. This is especially true for AB positive patients, and if the DAT is positive. Therefore, if the patient's DAT is positive and all of the patient's antigen types are positive by a given method, do not interpret the results. Bring the case to a Medical Director for consultation.

M. Appropriate Inert Controls

Codes for the Inert Control	
Code	**Description**
CALB	7% BSA control
CDATG	Gel DAT or gel autocontrol non-reactive
CDATT	Tube DAT non-reactive
CGABO	Inert gel monoclonal control well non-reactive; see ABO/Rh results
CGLOT	Inert gel monoclonal control well non-reactive; tested with antigen type.
CAAN	Another antigen(from same mfg insert) was negative
CAANG	Another antigen was negative by gel method
CNTDP	Control not tested; donor unit documented as preliminary
CNMFG	Control not indicated by manufacturer's insert

Antigen	Method	Appropriate Inert Controls (for positive patient antigen results)	Code
C E c e	Preferred method is antigen typing by the Rh Gel Card Method.	The MTS Monoclonal Control - typically tested on the VISION as part of the patient's ABO/Rh typing.	CGABO
		The MTS Monoclonal Control may be	CGLOT

		tested in parallel with the antigen testing.	
		If the sample is found to be negative for any of the Rh antigens tested by this method.	CAAN
	Alternative method is antigen typing by the Tube Method.	Negative 7% BSA control.	CALB
		If the sample is found to be negative for *any* of the Rh antigens tested by this method.	CAAN
Fy^a Fy^b S	Preferred method is antigen typing by the IgG Gel Card Method - using bottle reagent antisera derived from a human source that requires an IAT.	Negative gel autocontrol (tested as part of an antibody panel) or negative gel DAT (neonates)	CDATG
		If the sample is found to be negative for Fy^a, Fy^b, S when tested by the gel method.	CAANG
Fy^a Fy^b S	Alternative method is antigen typing by the Tube Method.	Negative tube DAT.	CDATT
		Negative gel autocontrol - tested as part of an antibody panel.	CDATG
Jk^a Jk^b s Kell	Required method is antigen typing by the Tube Method.	Negative 7% BSA control.	CALB
		If the sample is found to be negative for either Jk^a or Jk^b when tested by the tube method.	CAAN
All other antigens	Required method is antigen typing by the Tube Method.	Refer to the manufacturer's insert. Most inserts indicate that a negative DAT may be used to guard against false positive antigen results.	Varies
		If the sample is found to be negative for another antigen on the same manufacturer's insert.	CAAN
		This comment may be used in cases where the manufacturer's insert does not require an inert control.	CNMFG

N. Reagents / Antisera / Diluents / Gel Cards

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

c. It shows signs of drying, discoloration, bubbles, crystals, or other artifacts.

d. Foil seals appear damaged or opened.

3. The Rh individual and phenotype gel cards should be stored upright at 1°C to 8°C, and brought to room temperature before use.
4. IgG cards should be stored upright at 2°C to 25°C and brought to room temperature before use.

O. Expiration Date of Reagents

1. 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.

P. Positive and Negative Controls / Failing QC

1. Appropriate positive and negative controls for antigen typings must be tested once per day of use for each antisera / methodology used.
    a. 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.
    b. The negative control must be non-reactive. If the negative control is reactive (any strength) then the QC is considered to be failing.
2. Failing QC
    a. 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:
        i. Place the applicable antisera or Rh gel cards in quarantine; order additional antisera or gel cards, if necessary.
        ii. If possible, repeat all testing with a new lot number.
        iii. Patient and donor antigen typing results may not be released unless quality control is valid.
        iv. Notify the supervisor by documenting all failed QC in a variance (even if the QC passes upon repeat).

Q. Appropriate Test Cell for Antigen Positive Control

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 or 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 cell for negative control

R. 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 must be non-reactive.
    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+), a variance shall be completed and submitted.
2. Invalid Graded Reactions
    a. Weak+ or 1+ reactions on patient samples are considered invalid.
    b. All weak+ or 1+ reactions observed on patient or donor samples shall be documented in a variance. The Medical Director or designee will then determine whether additional investigation is needed.

S. 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%).

T. 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 for Fy^a, Fy^b, or S by the IgG gel card method. The procedure has been validated, and may therefore be used, only if typing for Fy^a, Fy^b, or S with a reagent that is derived from a human source and that requires an IAT.

U. 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 tube 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".

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

1. A "Received / Date" sticker will be affixed to each vial of reagent antisera upon receipt. In addition, 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 Cards™
4. MTS Diluent 2 PLUS™
5. 12 x 75 mm test tubes
6. MTS Ortho Workstation
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
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. 12 x 75 mm test tubes
7. Disposable pipettes
8. Timer
9. Heat block
10. Centrifuge
11. Automated cell washer (if applicable)

VII. QUALITY CONTROL (QC):

A. QC is performed once per day of use for each antisera / methodology used.

B. It is documented in the computer as described in the Blood Bank CDM - *Resulting the QC Rack.*

C. QC results are documented only for phases tested.

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

VIII. 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 resuspend.
    b. 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 MTS Monoclonal Control Card™ to be used to the test the inert control for units and patient (if not already tested as part of the ABORh type).
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. 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 contests from the tip into the 12 x 75 mm test tube and mix.

5. Label an MTS Anti-IgG Gel Card to identify the antisera 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. Also label one well of MTS™ IgG Card for each patient or donor sample. This well is used to test the inert control, if indicated.
7. 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.
8. 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.
9. Add 25 µL of the antisera to the correspondingly labeled well(s).
10. Incubate the gel card(s) fo 15 minutes at 37°C ± 2°C.
11. 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.
12. Observe the front and the back of each well macroscopically.
13. 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 antisera 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 antisera specificity.

d. Negative control
    a. Label to identify as the negative control and with the antisera 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 antisera 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 antisera, 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. If the manufacturer's insert specifies an incubation range, incubate for the longest time specified in the range. Note: Further incubation is not necessary if a valid positive result is obtained with immediate spin.
8. Continue following the directions in the manufacturer's insert to determine the antigen typing results of the patient or donor sample and of the positive and negative controls.
9. Read, grade, and record all test results and interpret the graded reactions.

IX. RECORDING TEST RESULTS:

A. QC ,which is performed once per day of use for each antisera / methodology used will be documented in the Blood Bank computer.

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

C. All patient antigen results are entered into the computer as described in Blood Bank CDM - *Single Result Entry*. Note that patient antigen tests are not typically ordered by physicians and when recorded in this manner, they do not interface to the Hospital Information System (HIS) with the exception of antigen results specifically ordered by the physician.

D. Patient's physician will occasionally order antigen phenotyping on the partner of an obstetrical patient with antibodies to help assess the risk of HDN and Donor A_1 subgroups specifically ordered by a physician for transplantation purposes. These antigen tests using specific test codes 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. When ordered/resulted in this manner, the results will interface to the HIS.

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

X. 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.

XI. NOTES:

A. If a patient with antibodies needs blood quickly, it may save time to begin crossmatching the patient with several units that have not yet been antigen tested. If any of the units are compatible by this "screening" method, these units may then be antigen tested.

B. When antigen typing a unit for a patient, bill the patient in the Blood Bank computer.

XII. 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

Approval Signatures

Step Description	Approver	Date
	Jeremy Powers: Chief, Pathology	Pending
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Supv, Laboratory	6/27/2022
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy	6/27/2022
	Kimberly Geck: Dir, Lab Operations B	6/26/2022
	Kelly Sartor: Supv, Laboratory	6/24/2022
	Kelly Sartor: Supv, Laboratory	6/24/2022

Beaumont HEALTH

Antigen Typing Downtime Worksheet

Date and Tech	Patient/ Donor Number	Antigen	Method (Tube or Gel/IgG or Gel/Rh)	Anti-sera or Rh Gel Card (Mfg / lot# / exp)	* Controls													Donor R		
					Panel (Mfg /lot# / exp)	Pos Cell #	Neg Cell #	Pos Ctrl Result					Neg Ctrl Result								
								IS	RT	AG	CC	GEL	IS	RT	AG	CC	GEL	IS	RT	AG

* Results are documented only for phases tested.

Applicable Policy: *Antigen Typing*
Additional copies of this form located in the downtime bin.