PROCEDURE

Title: Antibody Identification

Procedure #: 2015BLOODBANK72

Institution: Highlands Regional Medical Center

Address: 3600 Highlands Avenue, Sebring Florida 33870

Prepared by: Anita Smith Date: 6/12/2015

Title: Laboratory Administrative Director

Accepted by: _____ Date: 6/12/15

Title: Laboratory Medical Director

Date Patient Testing Implemented: 7/8/2013

Review of procedure every two years

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Reviewed by: _____ Date: _____

Discontinued testing date: _____

Highlands Regional MEDICAL CENTER

Policy Name: Antibody Identification	**Department: Blood Bank-Lab**	
Departmental Review:	**Policy #: B2.1**	
INITIATE DATE	**DATE REVIEWED/REVISED 7/8/13, 8/4/14, 5/2015**	**PAGE 1 of 6**

PURPOSE:
Serum thought to contain unexpected antibody(ies) should be tested against at least one panel of commercially prepared type O cells of known antigenic composition to determine antibody specificity. An antibody panel may also be helpful in resolving an ABO discrepancy or incompatible crossmatch

POLICY:
An antibody of IgG class must be antigen typed in each unit of blood prior to being issued. In emergent situations, the potential of adverse effects of transfusing incompatible units should be balanced against the risk of withholding transfusion. Units required for transfusion before antibody identification is complete should be tested with the extended crossmatch and issued as an emergency release.

SPECIMEN:
No special preparation of the patient is required before specimen collection. Specimen is collected in a Pink top tube (EDTA). Clotted whole blood sample (plain Red top) may also be used. Blood should be collected by an approved technique, with or without an anticoagulant. If not tested immediately, the sample should be stored at 2 - 8 C. Antibodies dependent for their detection upon the binding of complement may not be detected if plasma from an anticoagulated sample is used for antibody detection tests. The specimen should not be hemolyzed.

REAGENTS:
TUBE METHOD
1. Commercial panel cells; 2% - 4% cell suspension
2. Anti Human Globulin (Anti-IgG)
3. Enhancement solution
4. Saline

GEL METHOD
1. Commercial panel cells; 0.8% cell suspension
2. MTS Anti-IgG card
3. MTS Diluent 2

Storage Requirements: Panels should be stored in the refrigerator when not in use.

PROCEDURE:
TUBE METHOD:
1. Label a 12 x 75 mm tube for each cell in the panel with the patient's initials and that cell's number and also label a tube for an autocontrol.
2. Prepare a 2% - 4% patient cell suspension for autocontrol.
3. Add 2 drops of the patient's serum to all tubes.
4. Add one drop of the respective panel cell to each appropriately labeled tube.
5. Add one drop of patient's saline suspended cells to the autocontrol tube.
6. Centrifuge each tube. Examinbe for hemolysis and agglutination. Record results.

7. Add two drops of enhancement to all tubes. **Note:** If desired tubes may be incubated at room temperature for 15 to 30 minutes and examined for agglutination prior to the addition of the potentiator at 37^{0}C. This may enhance reactivity.
8. Incubate all tubes at 37°C for at least 10 minutes but no longer than 30 minutes.
9. Spin all tubes and then examine them for hemolysis and agglutination macroscopically. Grade and record results. **Omit this step if PeG is the enhancement and continue to step 8.**
10. Wash all tubes at least 3 times with saline, decanting completely after the last wash.
11. Add 2 drops of Anti-IgG to all tubes and mix.
12. Spin for the calibrated spin time at Coombs phase.
13. Resuspend the cells by gently shaking the tube.
14. Examine the agglutination and record test results.
15. Interpret test results immediately upon completion of the test.
16. To all tests interpreted as negative, add one drop of Coombs Control cells.
17. Centrifuge and examine again for agglutination.

GEL METHOD

1. Label the MTS Anti-IgG card with each screening cell number, autocontrol and appropriate identification
2. Remove the foil seal from the individual microtubes to be used. Foil should be removed immediately before testing or within one hour of testing.
3. Prepare 0.8% patient cell suspension for autocontrol.
4. Add 50 µL 0.8% antibody screen red blood cell suspension and patient cells to the labeled microtube. **Pipette tip should not touch the gel card**
5. Add 25 µL of serum or plasma to each microtube.
6. Incubate the MTS Anti-IgG card for 15 minutes at 37± 2°C.
7. After incubation, centrifuge the cards in the MTS centrifuge at the preset conditions.
8. After centrifugation, remove the cards and observe macroscopically the front and back of each microtube for agglutination and/or hemolysis and record reactions.

REPORTING RESULTS:

1. Once the panel has been completed, interpret the serological results. Identify the antibody(ies) present by examining reaction patterns at each test phase. Once the identification has been determined other antibodies must be ruled out. Although a serum displays a clear reaction pattern, it is important to remember that other antibodies may also be present. When ruling out other antibodies, the following rules should be followed:

    a. When practical, red blood cells carrying a double dose of the relevant antigens are preferred. Rule out using homozygous cells. This is especially important when ruling out anti-M, anti-N, anti-S, anti-Jk(a) and anti-Jk(b) because they are the most likely to show dosage. Anti-K is the exception and can be ruled out with one heterozygous cell.
    b. If the original cell panel doesn't allow for ruling out all antibodies using homozygous cells, then another cell panel or selected cells should be used.

c. If a homozygous cell is not available on a current or expired panel, then negative reactions with two heterozygous cells may be used for ruling out. **Note:** If an expired panel is used QC must be done on the expired panel to determine its sensitivity.

2. It is not always necessary to exclude (rule out) the presence of antibodies to antigens of relatively low incidence such as anti-Cw, anti-V, anti-VS, anti-Kp(a) anti-Js(a) and anti-Lu(a). These antibodies are uncommon, and the corresponding antigens are present on the red blood cells of less than 2% of the random population. If present, these antibodies should be detected when donor rbcs carrying the antigen are crossmatched. These antibodies should be considered when reaction patterns indicate their presence in the patient's serum and when units that should otherwise be crossmatch compatible show up incompatible.

QUALITY CONTROL:
The reactivity of the panel red cells should be confirmed on each day of use and when a new lot of panel cells are first used.

PROCEDURE NOTES:

1. An immediate spin reading or a room-temperature incubation that is read before adding an enhancement medium, or both may enhance the detection of certain antibodies (anti-M, -N, -P1, -I, -Le(a), or –Le(b)) and may help explain reactions detected in other phases. This can be tested with the tube method or with gel using the MTS Buffer card.

2. The autologous control, in which serum and autologous red cells are tested under the same conditions as are serum and reagent red cells, is an important part of antibody identification. The autocontrol is not the same as a DAT

3. Incubation and the presence of enhancement reagents may cause reactivity in the autologous control that is only an in-vitro phenomenon. If the autocontrol is positive in the antiglobulin phase, a DAT should be performed. If the DAT is negative, antibodies to an enhancement medium constituent or autoantibodies that react only in the enhancement medium should be considered. Warm autoantibodies and cold autoantibodies, such as anti-I, -IH, or -Pr, may react by an IAT when certain enhancement media are used; therefore, testing should be repeated in another medium.

4. When the alloantibodies have been identified, the patient's cells must be typed for the antigen(s) corresponding to the antibody(ies) identified following the Antigen Typing Procedure.

5. If the patient types positive for the antigen corresponding to the antibody identified, bring these results to the attention of the supervisor.
   a. An autoantibody may be indicated.
   b. The antigen typing may be invalid due to recently transfused donor cells.
   c. Possible misidentification may have occurred.
   d. Individuals who produce Lewis antibodies should be antigen typed for both Lea and Leb and are usually Le(a) and Le(b) negative. Anti-Le(a) cannot be produced by Le(a-b+)persons.

6. If a complete antibody identification has been done in the previous two weeks, a repeat workup is not necessary if the current sample's results meet the following criteria:
   a. DAT is negative or has not increased in strength
   b. Antibody screen reactions are identical in strength to previous sample's reactivity and reacts with appropriate antigen positive cells

7. For OB/GYN patients who had recently received Rhogam and had a positive antibody screen (anti-D positive due to Rhogam), Resolve Panel A antigen profile is enough to rule out anti-D provided :
   a. that the full set of vials (11 vials) are used
   b. the antigram shows a pattern typical of the anti-D.

8. Sometimes specificity does not fit a clear reaction pattern. If this is the case, the reactions could be caused by the following:
   a. cold autoantibody (can react at 37 degrees and at coombs)
   b. warm autoantibody. If the DAT is positive, then a warm autoantibody may be spilling over into the serum causing positive reactions.
   c. anti-Bg or other white cell antibody
   d. HTLA High Titer Low Avidity

9. If the above reactions do not prohibit ruling out clinically significant antibodies, then no further investigation is necessary. If clinically significant antibodies cannot be ruled out, then further studies must be done.

10. If clinically significant antibodies cannot be ruled out by current in date reagents expired selected panel cells can be used provided that positive and negative controls are run during testing and documented in SoftBank.

11. To aid in the resolution of multiple antibodies, difficult crossmatching problems or to provide specialized services to patients and physicians, which are beyond the scope of the Blood Bank's facilities; specimens are referred to OneBlood, Inc. Reference Laboratory.

12. Full crossmatch on antigen negative units (when appropriate) are compatible.

REFERENCES:

1. AABB Technical Manual
2. Immucor, Inc, Norcross , Ga, Package Inserts:
   a. Anti-Human Globulin Serum, (Anti-IgG-C3d), green
   b. Anti-IgG (Murine Monoclonal)(Green or Uncolored) Gamma-clone
   c. Gamma PeG Polyethylene Glycol Additive for Antibody Detection Tests
3. Ortho-Clinical Diagnostic Inc. Package Inserts:
   a. Reagent Red Blood Cells SURGISCREEN®

b. Anti-Human Globulin Anti-IgG (Rabbit) MTS Anti-IgG Card
c. MTS Diluent 2™Red Blood Cell Diluent

4. CAP checklist, TRM.31250: Reagent expiration dates
5. QSA.05.06.01; Joint Commission Standards, E-Edition effective 01-01-15

HIGHLANDS REGIONAL MEDICAL CENTER
Sebring, FL
Laboratory

DOCUMENT CHANGE RECORD

Document Name: Antibody Identification

Document Section: Blood Bank

Author: Marisabel Ponce-Soto

Please circle one of the following: NEW (REVISION) ARCHIVE

Effective

Description of document, changes, and rationale: Revision based on Joint Commission Recommendation of Running 2 QC on panel cells each day tested
Formal Training of staff required: None Attach email sent to staff about new procedures or changes to procedure if applicable
Method Validation required (attach documents): No
List any changes to the Lab Information system: None

Review and Approval	Signature	Date
Author	Marisabel Ponce-Soto	6/14/15
Chief Technologist		
Admin. Lab Director		
Laboratory Director		

Implementation occurs after signature by Laboratory Director.

Policy Name: Antibody Identification **Department:** Blood Bank-Lab

Departmental Review: **Policy #:** B2.1

INITIATE DATE **DATE REVIEWED/REVISED** 7/8/13, 8/4/14, 5/2015

Reviewed by	Reviewed Date	Reviewed by	Reviewed Date

Initial Implementation Date: _____

Reviewed by: _____Signatures on file_____ Date: _____

Department Supervisor

Reviewed by: _____ Date: _____

Department Adm. Director

Reviewed by: _____ Date: _____

Department Chief Technologist

Reviewed and Approved by: _____ Date: _____

Department Medical Director