PROCEDURE

Title: Antibody Screen-Gamma PeG

Procedure #: 2015BLOODBANK67

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: 4/1/2015

Review of procedure every two years

Reviewed by: _____ Date: _____

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Reviewed by: _____ Date: _____

Discontinued testing date: _____

Highlands Regional MEDICAL CENTER

Policy Name: Antibody Screen – Gamma PeG **Department: Blood Bank-Lab**

Departmental Review: **Policy #: B1.6**

INITIATE DATE **DATE REVIEWED/REVISED 04/2015** **PAGE 1 of 4**

PURPOSE:
The detection of unexpected antibodies in the serum or plasma of a patient or donor is accomplished by testing against selected red blood cells possessing between them the common inherited blood group antigens (C, c, D, E, d, M, N, S, s, P1, K, k, Lea, Leb, Fya, Fyb, Jka, Jkb). The addition of polyethylene glycol/low-ionic-strength solution both enhances the sensitivity of the test and enables the incubation time to be reduced to ten minutes. The anti body detection procedure is based on the principle of agglutination.

SPECIMEN:
No special preparation of the patient is required before specimen collection. Blood should be collected by an approved technique, with or without an anticoagulant. IF not tested immediately, the sample should be stored at 2° to 8°C. Antibodies dependent for their detection upon the binding of complement may not be detected if plasma from an anticouagulated sample is used for antibody detection tests.

REAGENTS:
1. Reagent Red Blood Cells for detection of unexpected antibodies
2. Gamma PeG™ - Polyethylene Glycol Additive for Antibody Detection Tests
3. Anti-Human Globulin Anti-IgG, -C3d, poly specific or Anti-IgG
4. Coombs Control Cells

Store all reagents at 2° to 8°C. May be left at room temperature (up to 30°C) while in use. Do not use beyond expiration date.

QUALITY CONTROL:
Reagent Red Blood Cells must be examined daily for hemolysis or color change and are tested daily with appropriate controls.

PROCEDURE:
1. Label an appropriate number of test tubes for each reagent cell. An autologous control is not required in antibody screening, but can be set up optionally.
2. Deliver two drops of serum or plasma to each tube.
3. Thoroughly mix each vial of antibody screening cells in turn, and add one drop of the suspened cells to each appropriate tube in sequence.
4. To all tubes add two drops of Gamma PeG.
5. Mix all tubes thoroughly and incubate at 37°C for 10 to 15 minutes. (Incubation may be extended to 30 Minutes)
6. Examine for hemolysis without centrifugation. Hemolysis may be an indication of a positive reaction. Antibodies of the Lewis, P, Kidd or Vel Systems may cause hemolysis of incompatible cells when the serum being tested is freshly drawn. Hemolysis will probably not be seen if plasma is being tested.
7. Wash cells in all tubes at least three times.

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

8. In washing the cells, the tubes must be filled with saline, the saline must be carefully decanted after each wash, and the cells must be thoroughly re-suspended when adding saline for the next wash. Decant the saline completely after last wash.
9. Add one or two drops of Anti-Human Globulin in accordance with manufacturer's directions. The kind of Anti-Human Globulin used is at the discretion of the using institution. It may be of rabbit or murine monoclonal origin, but should be Anti-IgG unless a particular lot of Anti-IgG,-C3d, poly specific has been demonstrated to yield specific reactions when testing is carried out by the polyethylene glycol test procedure.
10. Mix well and centrifuge.
11. Resuspend the cells by gently shaking the tube.
12. Examine the agglutination and record test results.
13. Interpret test results immediately upon completion of the test.
14. To all tests interpreted as negative, add one drop of Coombs Control cells.
15. Centrifuge and examine again for agglutination.

REPORTING RESULTS:

1. No agglutination or hemolysis on any screening cell suspension is a **negative** test result and indicates that no antibody directed at an antigen present on the cells is being detected. **NOTE:** Antibody identification panel must be performed to confirm a positive antibody screen. SoftBank will automatically change immediate spin cross match to AHG crossmatch.
2. Hemolysis or agglutination of one or more individual screening cell suspensions is a **positive** test result and indicates the presence of an antibody directed against an antigen present on the cells.
3. Notify nursing of positive screen and delay in testing for compatible units. Notification must be documented in SoftBank (added in the comment box).

PROCEDURE NOTES:

1. Agglutination occurring after the addition of Coombs Control Cells to a test interpreted as negative assures that active Anti-Human Globulin is present in the test mixture, and that the negative test result is valid.

2. No agglutination after the addition of Coombs Control Cells to a test interpreted as negative indicates the Anti-Human Globulin was either inactivated or omitted. The negative test result is invalid and the test must be repeated.

3. Red blood cells must not be suspended in this low-ionic strength additive solution because polyethylene glycol tends to cause aggregation of the red blood cells, the usual examination of the test mixture for **direct agglutination cannot be undertaken, either after an immediate spin or following incubation at 37°C.** This may cause antibodies that are wholly IgM to be missed. However, the fact that the sensitivity of the anti-globulin test is enhanced means that most IgG antibodies are detected more reliably than in other test systems.

4. The identity of an unexpected antibody detected during screening may be determined by testing the serum or plasma against a panel of red blood cells and matching the reaction pattern of the serum or plasma against the antigen profiles of the panel.

5. Complement-binding antibodies may not be detected if plasma is used for the test, or if anti-IgG is used instead of anti-IgG,-C3d; polyspecific.

6. Polyethylene glycol has a tendency to cause large-molecule protein to precipitate. In patients having an increased gamma globulin level, or when testing plasma, precipitated protein may be visible to the naked eye in the test mixture. This does not interfere with the sensitivity of the test, but extra care is needed to assure that unattached immunoglobulin is completely removed during the washing phase of the antiglobulin test. The precipitated globulin may become enmeshed in the deposited cell button; hence, and additional wash may be called for, and it is especially important to assure that the cells are resuspended thoroughly at the start of each wash. Not all automated cell-washing centrifuges achieve this with uniform efficiency.

7. Antibodies may be present in a serum or plasma but not be detected if their level of potency is below the threshold of detectability by the test procedure.

8. An Autologous control or DAT is not required or recommended as part of routine pretransfusion testing. An Autologous control, however, is of value when performing antibody identification. Autologous control is not the same as DAT.

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

HIGHLANDS REGIONAL MEDICAL CENTER
Sebring, FL
Laboratory

DOCUMENT CHANGE RECORD

Document Name: Antibody Screen - Gamma Reg

Document Section: Blood Bank

Author: Marishel Prophet

Please circle one of the following: NEW REVISION ARCHIVE

Effective

Description of document, changes, and rationale: Procedural note added (#8)	
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): None	
List any changes to the Lab Information system: None	

Review and Approval	Signature	Date
Author	Marishel Prophet	4-30-2015
Chief Technologist		
Admin. Lab Director		
Laboratory Director		

Implementation occurs after signature by Laboratory Director.