PROCEDURE

Title: Weak D Testing

Procedure #: 2015BLOODBANK52

Institution: Highlands Regional Medical Center

Address: 3600 Highlands Avenue, Sebring Florida 33870

Prepared by: Anita Smith Date: 6/4/2015

Title: Laboratory Administrative Director

Accepted by: _____ Date: 6-5-15

Title: Laboratory Medical Director

Date Patient Testing Implemented: 8/1/2008

Review of procedure every two years

Reviewed by: _____ Date: _____

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Discontinued testing date: _____

Highlands Regional MEDICAL CENTER

Policy Name: Testing for Weak D	**Department: Blood Bank-Lab**	
Departmental Review:	**Policy #: B1.5**	
INITIATE DATE 08/2008	**DATE REVIEWED/REVISED** 07/2013	**PAGE 1 of 4**

PURPOSE:
The Rh antigen D shows quantitatively variable strength of expression, in some cases requiring an indirect antiglobulin test procedure with a suitable Anti-D reagent for its detection. This procedure is based on the principle of agglutination. Red blood cells that do not agglutinate directly with Anti-D may be further tested for Weak D by the indirect antiglobulin test. This test may be expected to detect both quantitative and qualitative variants of D. It is carried out routinely on all donor bloods appearing to the D negative. Testing of transfusion candidates for Weak D is at the discretion of the laboratory director. During incubation with an IgG Anti-D reagent, human red blood cells possessing the D antigen (whether weakly or normally expressed) become coated with IgG. After being washed to remove unattached IgG, such cells will agglutinate in the presence of antibody directed at human IgG. The presence of agglutination is considered to be a positive test result. Cells not possessing the D antigen do not become coated with IgG during incubation, and accordingly do not agglutinate after being washed and then tested with antibody directed at IgG. The lack of agglutination in the presence of Anti-IgG is considered to be a negative test result and to indicate that no form of D is being detected.

POLICY:
Weak D testing must be performed on the red cells of the infant when determining if an Rh negative mother is a candidate for Rhig.

SPECIMEN:
No special preparation of the patient is required before specimen collection. Blood should be drawn by an approved technique, with or without an anticoagulant. If not tested immediately, the sample should be stored at 2 - 8°C. Storage limits are dependent on the manner of collection and storage conditions.

REAGENTS:
TUBE METHOD:
1. Blood Grouping Reagent Anti-D (Monoclonal-Blend)
2. Anti-Human Globulin Anti-IgG,-C3d; polyspecific or Anti-IgG
3. Coombs Control Cells (EIgG, Strong or EIgG, Weak)
4. Isotonic Saline

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

GEL METHOD
1. MTS Anti-IgG card
2. MTS Diluent 2
3. Blood Grouping Reagent Anti-D (Monoclonal)

QUALITY CONTROL:
To recognize reagent deterioration the reagents must be tested daily with appropriate controls. See under "Daily Quality Control".

PROCEDURE:

TUBE METHOD:

1. Prepare a 3 - 4% suspension of red blood cells in isotonic saline or in their own serum or plasma
2. Place one drop of Anti-D reagent in a clean, suitably labeled test tube.
3. Add one drop of the test cell suspension to the test tube.
4. Mix well and incubate the tube at 37°C for 15 minutes.
5. After incubation, centrifuge the tube.
6. Resuspend the cells by gently shaking the tube and examine macroscopically for agglutination.
7. Record test results. If there is definite macroscopic agglutination, there is no need to proceed to an antiglobulin phase. The test cells are D positive, providing a test for spontaneous agglutination yields a negative result. (Note: A test for spontaneous agglutination could be a parallel ABO test, providing the test cells are not group AB. In the case of a group AB sample, a control test should be carried out with an inert reagent such as Gamma-clone Control Reagent, 3% bovine albumin or the patient's own serum.
8. Wash the cells three times with the tube full of isotonic saline. Decant the saline completely after the last wash.
9. Add one or two drops of Anti-Human Globulin (Anti-IgG or Anti-IgG,-C3d; polyspecific).
10. Mix the contents of the tube and centrifuge.
11. Resuspend the cells by gently shaking the tube, and examine macroscopically for agglutination. Record test results. A hand lens or a concave mirror may be used as an aid in reading.
12. Interpret test results immediately upon completion of the test.
13. To all tests interpreted as negative, add one drop of Coombs Control Cells, centrifuge and examine again for macroscopic agglutination. If agglutination is present, active antiglobulin was present in the tube when the original test was interpreted as negative. If no agglutination occurs, the original test was invalid and must be repeated.

GEL METHOD:

1. Label microtube with appropriate identification.
2. Prepare 0.8% red cell suspension of patient red blood cells to be tested by adding 10µL to one ml of MTS Diluent 2.
3. Add 50 µL 0.8% donor red blood cell suspension to the labeled microtube. Pipette tip should not touch the gel card.
4. Add 25 µL of Anti-D to microtube.
5. Incubate the MTS Anti-IgG card for 15 minutes at 37± 2°C.
6. After incubation, centrifuge the cards in the MTS centrifuge at the preset conditions.
7. 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. Agglutination of the red blood cells at the antiglobulin phase is a positive test result and indicates a weak D antigen (formerly called D^u). The control test cells must have no direct agglutination with Anti-D, even after incubation.

2. No agglutination of the red blood cells is a negative test result and indicates that no D antigen is being detected by the indirect antiglobulin test procedure.

PROCEDURE NOTES:

1. If spontaneous agglutination occurs at Step 6 of the tube method procedure, it is most likely to be caused by the presence of a cold agglutinin or a protein imbalance in the patient's serum/plasma. In most cases, this can be overcome by washing the cells and testing them again.

2. If the red blood cells of a recently delivered woman show mixed-field agglutination in this test procedure, the cause may be the presence of fetal D-positive blood in the maternal circulation. This could indicate the need for a larger-than-normal dose of Rh-Immune Globulin.

3. Red blood cells having a positive direct antiglobulin test cannot be validly tested for the Weak D antigen by means of the indirect antiglobulin test.

4. Red blood cells weakened expression of D may not show uniform reactivity with all Anti-D reagents.

5. Weak D positive donors and newborns are designated D positive. Weak D positive recipients are designated D negative.

REFERENCES:

1. AABB, Technical Manual
2. Reagent Package Inserts

Highlands Regional MEDICAL CENTER

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Departmental Review: **Policy #: B1.5**

INITIATE DATE **DATE REVIEWED/REVISED** **PAGE 4 of 4**
08/2008 **06/2013**

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	5.27.15		
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Initial Implementation Date: _____

Reviewed by: _____ Date: 7/10/13
Department Supervisor

Reviewed by: Angela Lawster Date: 7/8/13
Department Adm. Director

Reviewed by: _____ Date: _____
Department Chief Technologist

Reviewed and Approved by: _____ Date: 7/8/13
Department Medical Director