PROCEDURE

Title: ABO & Rh by Tube Method

Procedure #: 2015BLOODBANK54

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: 6/1/2008

Review of procedure every two years

Reviewed by: _____ Date: _____

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Discontinued testing date: _____

Highlands Regional MEDICAL CENTER

Policy Name: ABO/Rh by Tube	Department: Blood Bank-Lab	
Departmental Review:		Policy #:B1.0/B1.1
INITIATE DATE 06/2008	DATE REVIEWED/REVISED 07/2013	PAGE 1 of 4

PURPOSE:
Forward grouping is designed to demonstrate the presence of the ABO and Rh blood group antigens (A,B,and/or D) by testing the red cells with known antisera (Anti-A, Anti-B, and Anti-D). ABO reverse grouping is designed to demonstrate the presence of the expected ABO blood group antibodies (Anti-A or Anti-B) by testing the serum or plasma with known cells (A_1 and B cells). Infants normally do not begin to produce anti-A or anti-B until 3-6 months of age reverse grouping is not required on infants less than 4 months of age.

The presence/absence of A, B and D antigens can be detected by tube or gel microtube method. Agglutination indicates the presence of antigen/antibody reaction while lack of agglutination indicates the absence of antigen/antibody reaction.

SPECIMEN:
No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved techniques.

REAGENTS:
1. Anti-A
2. Anti-B
3. Anti-D
4. Gamma Control
5. Normal Saline
6. A_1 Cells
7. B Cells

Do not use beyond expiration date. Store reagents at 2-8°C. Bring reagents to room temperature (18-25°C) prior to use.

QUALITY CONTROL:
To recognize reagent deterioration, the reagents must be tested daily with appropriate controls.

PROCEDURE:
1. Prepare a 3-5% suspension of patient red blood cells in a clean 12 x 75 test tube.
2. Label six clean tubes with patient identification and respectively A, B, D, Ctrl, A cell, B cell
3. Add one drop of Anti-A, one drop of Anti-B, one drop of Anti-D and one drop of Gamma Control to the appropriately labeled tubes.
4. Add one drop of A_1 cells and one drop of B cells to the appropriately labeled tubes.
5. Add two drops of patient serum or plasma to the tubes containing the A_1 and B cells.
6. Add one drop of the patient's 3-5% cell suspension to the tubes containing Anti-A, Anti-B, Anti-D and Ctrl.
7. Gently mix all tubes and centrifuge in a calibrated centrifuge.
8. For each tube, gently dislodge cell button and inspect for macroscopic agglutination using the optical aid.

9. Record reactions immediately after reading each tube.

REPORTING RESULTS:

1. See Reading and Interpretation of Agglutination Reactions.
2. Agglutination of red cells is a positive result.
3. No agglutination of red cells is a negative test result.
4. The test cannot be interpreted if a positive result occurs in the control tube.
5. Interpretation:

Forward Grouping		Rh Typing		Reverse Grouping		Interpretation
Anti-A	Anti-B	Anti-D	Control	A Cells	B Cells	
+	=	+	=	=	+	A Positive
=	+	+	=	+	=	B Positive
+	+	+	=	=	=	AB Positive
=	=	+	=	+	+	O Positive
+	=	=	=	=	+	A Negative
=	+	=	=	+	=	B Negative
+	+	=	=	=	=	AB Negative
=	=	=	=	+	+	O Negative

PROCEDURE NOTES:

1. Serum grouping tests performed in conjunction with cell grouping should always agree. Discrepancies between reverse and forward grouping should be resolved before interpretation of the blood group.
   a. Repeat testing on same specimen using a saline washed suspension of red cells.
   b. Weak or missing reverse type may be enhanced by incubating at room temperature or at 4°C for 15 to 30 minutes. Include an adult O cell as a control. A positive control indicates the presence of a cold antibody and invalidates the reverse test.
   c. Positive reactions in reverse type may be due to atypical or cold nonspecific antibodies. Warm serum and A_1 and B cells before testing.
   d. Repeat testing on a fresh specimen.
   e. Unexplained positive reactions in reverse grouping may be due to the presence of atypical antibodies or cold nonspecific antibodies. These may include anti-A1, anti-P, anti-M, andit-N, anti-Le(a), and anti-Le(b)
   f. If discrepancy remains refer to reference laboratory.

2. Discrepancies between serum and cell grouping should be resolved prior to transfusion. Group O red blood cells and Group AB fresh frozen plasma are the preferred transfusion alternative for any recipient whose ABO group is in question.

3. Significant variations in red blood cell suspensions may result in false-positive or false negative reactions.

4. Only D negative cord bloods require weak D testing.

REFERENCES:

Blood Grouping Reagent current package inserts
AABB Technical manual 17^{h} edition
CAP Requirement TRM.40100

Highlands Regional MEDICAL CENTER

Policy Name: ABO/Rh by Tube **Department: Blood Bank-Lab**

Departmental Review: **Policy #:B1.0/B1.1**

INITIATE DATE **DATE REVIEWED/REVISED** **PAGE 4 of 4**
06/2008 **05/2013**

Reviewed by	Reviewed Date	Reviewed by	Reviewed Date
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	5-26-15		
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Initial Implementation Date: _____

Taken out of Service: _____

Reason: _____

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