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Forward Typing Determination Of Neonatal ABO and Rh for Patients Less Than Four Months of Age By Tube Method

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document will provide instructions to the Blood Bank staff for determining the ABO group and Rh of neonatal samples by forward typing.

II. SCOPE:

A. This document applies only to neonatal patients (less than 4 months old).

B. For patients greater than 4 months old, refer to Transfusion Medicine policy, Determining the ABO and RhD of Patients Greater Than Four Months Old.

III. PRINCIPLE:

Neonates are immunologically immature and do not produce sufficient levels of ABO antibody to obtain valid graded reverse reactions and valid ABO interpretations using standard ABO typing methods. Therefore, the ABO is determined by forward testing only; reverse typings are not performed.

IV. POLICIES:

A. As with all manual tests, batch testing must be limited to 6 tests per batch. If workload becomes excessive, supervisory staff must be notified immediately.

B. Historical Blood Record Checks

1. Before testing, a technologist must perform a historical record check on each sample. Refer to Transfusion Medicine Policy, Historical Blood Bank Record Check

C. ABO/Rh Discrepancy

1. If an ABO or RhD discrepancy exists, then before entering the interpretations in the Blood Bank computer system the technologist must refer to Transfusion Medicine policy *Resolution of ABO and RhD Discrepancies* and attempt to resolve the discrepancy.

F. ABO/Rh Discrepancy: testing situation when the ABO or RH of the current sample is not in agreement with historical sample; or the graded ABO or RH reactions on the current sample do not yield a valid interpretation.

VI. SPECIMEN COLLECTION AND HANDLING:

Preferred sample is a microtube (heelstick), cord blood or peripheral blood EDTA sample affixed with identifying label.

A. All samples must be labeled in accordance with Transfusion Medicine Policy, Triaging And Identifying Acceptable Samples For Testing.

B. Minimum acceptable volume is 0.5 mL.

VII. REAGENTS:

A. Ortho BioClone Anti-A

B. Ortho BioClone Anti-B

C. Ortho BioClone Anti-D

D. Ortho 7% BSA

E. Immucor Gamma Clone Anti-D

F. Immucor Gamma Clone Control

VIII. EQUIPMENT:

A. Table top centrifuge

B. Lighted agglutination viewing mirror

IX. SUPPLIES:

A. Disposable pipettes

B. Gauze

C. Test Tubes, 10x75 or 12x75mm, plastic or glass

D. Blood Bank Isotonic Saline

X. QUALITY CONTROL (QC):

A. Quality Control of the ABO and Rh Tube reagents is performed daily as described in Transfusion Medicine Policy, *Routine Quality Control of Blood Bank Reagents* and documented in the Blood Bank Computer system or on paper per site procedures.

B. When using the Gamma-clone Anti-D Reagent, the Gamma-clone control must be tested and must be non-reactive in order to interpret the neonate's Rh. Refer to the Interpretation section. This control is documented in the control field of the Blood Bank computer.

XI. BOVINE SERUM ALBUMIN (BSA) CONTROL & DOCUMENTATION IN BLOOD BANK

1. Ortho Bioclone Anti-A for tube "A"
2. Ortho Bioclone Anti-B for tube "B"
3. Ortho Bioclone Anti-D for tube "D"
   Note: Forward typing antisera must be added to tube before patient red cell suspension.

J. Add one (1) drop of the patient's 3-5% saline cell suspension to the test tubes correspondingly labeled as "A", "B", "D"

K. Gently agitate all tube to mix contents. Centrifuge tubes according to the calibrated time of the centrifuge.

L. Observe the supernate in test tube for hemolysis. Gently re-suspend the cell button of each tube. Read, grade, and record the reactions in the Blood Bank computer system using Blood Bank CDM, *Resulting Neonatal Testing on a Worksheet* (if batch testing) or Blood Bank CDM *Single Result Entry* or an appropriate downtime form. Refer to Transfusion Medicine Policy, *Reading, Grading & Recording Test Reactions, .*

M. If the patient's RBCs appear to be AB Positive (reactive with Anti-A, B and D reagents) then test the patient's RBC with the 7%BSA control.

1. Label a test tube with neonates name and [C] for the BSA Control.
2. Add 1 drop of 7% BSA control to the corresponding tube.
3. Add one (1) drop of the patient's 3-5% saline cell suspension to the test tubes.
4. Gently agitate all tube to mix contents. Centrifuge tubes according to the calibrated time of the centrifuge.
5. Observe the supernate in test tube for hemolysis. Gently re-suspend the cell button of each tube. Read, grade and record the reactions in the Blood Bank computer system or on an appropriate downtime form.

N. If the patient's RBC do not appear to be AB Positive, testing with 7% BSA is not indicated. Document the control result field as "NT"

O. Interpret the graded reactions and document this in the blood bank computer system using Blood Bank CDM, *Resulting Neonatal Testing on a Worksheet* (if batch testing) or Blood Bank CDM *Single Result Entry* or on appropriate downtime form. Refer to *Interpretation* section of this document.

P. If the neonate appears to be Rh(D) negative, the technologist should repeat the Rh(D) type using the Gamma Clone Anti D and Gamma-clone control reagents and a new cell suspension before accepting the test results.

1. Prepare a fresh 3-5% saline cell suspension of the neonate's cells in the tube labeled with only the neonate's name. Refer to Transfusion Medicine Policy, Making a Test Red Cell Suspension
2. Label two additional test tubes with patient information and intended use for the tube. For example,
   a. Tube 1 - [Name] Gamma D.
   b. Tube 2 -[Name] Gamma Ctl
3. Add one (1) drop of Gamma-clone Anti-D to tube 1.
4. Add one (1) drop of Gamma-clone Control to tube 2 .
5. Add one (1) drop of neonate 3-5% cell suspension to tube 1 & 2 respectively.

0	0	0	Not indicated	0	0	O Negative
3 - 4+	0	2 - 4+	Not indicated	2 - 4+	0	A Positive
3 - 4+	0	0	Not indicated	0	0	A Negative
0	3 - 4+	2 - 4+	Not indicated	2 - 4+	0	B Positive
0	3 - 4+	0	Not indicated	0	0	B Negative
3 - 4+	3 - 4+	2 - 4+	0	2 - 4+	0	AB Positive
3 - 4+	3 - 4+	0	Not indicated	0	0	AB Negative
+ or 0	+ or 0	+ or 0	+ any strength	+ or 0	+ any strength	INVALID Cannot interpret ABO or Rh; refer to Invalid Graded Reaction section below.

B. Invalid Graded Reactions

1. Reactive Bovine Albumin Control
    a. The 7% BSA must be tested and must be non-reactive in order to interpret the ABO or Rh of a patient who appears to be AB positive (the patient's RBCs react with the Anti-A, Anti-B, and Anti-D reagents). If this control is reactive, then the ABO and Rh cannot be interpreted; refer to Transfusion Medicine Policy, *Resolution of ABO/Rh Discrepancies.*
2. *Reactive Monoclonal Control*
    a. If the Gamma Clone control is reactive, then the results of the Rh(D) type using the Gamma Clone Anti-D reagent may be invalid / falsely positive. Refer to Transfusion Medicine Policy, *Resolution of ABO/Rh Discrepancies.*

C. ABO/Rh Discrepancies

1. An ABO/Rh Discrepancy may occur if:
    a. the ABO or Rh graded reactions are not valid, or
    b. the graded reactions do not yield a valid interpretation, or
    c. the bovine albumin or Gamma Clone control is reactive, or
    d. the current type does not match the historical type
2. If an ABO or Rh(D) discrepancy exists, then before entering the interpretations in the Blood Bank computer the technologist must refer to Transfusion Medicine Policy, *Resolution of ABO/Rh Discrepancies* and attempt to resolve the discrepancy.
    a. If ABO discrepancy can be resolved, the technologist will enter the valid interpretation in the

Step Description	Approver	Date
	Karrie Torgerson: Supv, Laboratory	10/8/2021
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Applicability

Dearborn, Farmington Hills, Grosse Pointe, Royal Oak, Taylor, Trenton, Troy, Wayne