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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.

D. Requirement for Two Separate ABO/Rh Typings

1. All patients must have two (2) complete, separate sets of ABO/Rh results in the Blood Bank computer before RBCs are crossmatched. The source of these two separate typings may be:
    a. Two manual typings of the current sample, performed by two different technologists, or
    b. Testing of both the current sample and a history of a historical sample of any method, or
    c. Testing of the current sample and testing of separate sample with different collection time.

E. Testing by the Tube Method

1. All manual neonatal forward typings shall be performed by the tube method as described in this document; not by the gel method.

F. Appropriate Blood Bank Computer Test Code, Anti-D Reagent, and Control

1. NNPR: This test code is used by the first technologist who types the sample.
    a. The results for the NNPR code do not interface to the HIS.
    b. The appropriate Anti-D reagent for this test code is the Ortho BioClone Anti-D reagent.
    c. The 7% BSA control must be tested if the neonate appears to be AB positive.
2. NTYPE: This code is used by the second technologist who types the sample or when testing a second collection.
    a. The results for the NTYPE code do interface to the HIS.
    b. The appropriate Anti-D reagent for this test code is the Gamma Clone Anti-D reagent.
    c. The Gamma Clone control must be tested with the Gamma Clone Anti-D reagent.

G. Rh Negative Results for Patients with No Previous Record (NPR)

1. If the neonate appears to be Rh(D) negative, the technologist should repeat the Rh(D) type using a new cell suspension and the Gamma Anti-D and Control reagents before accepting the test results.
2. This repeat testing is documented in Blood Bank Test system using the Repeat Rh

test code (RRHT) or utilizing an internal comment on NNPR test code, for example "Repeat Rh negative with Gamma-clone reagents".

H. Weak D Testing

1. Weak D Testing will not be performed on neonatal samples, except if to determine the RhIG candidacy of the neonate's mother. Refer to Transfusion Medicine Policies, *Weak D*, *Cord Blood Evaluation / HDN Investigation* or *Rh Typing of Cord Blood Samples*.

V. DEFINITIONS:

A. CDM: Computer Documentation Manual

B. Neonate: Patient from birth to four months of age

C. HIS: Hospital Wide Computer System

D. Interface: the computer process by which test results are sent from the Blood Bank computer system to the HIS.

E. NNPR: Neonatal No Previous Record; the computer test code that is ordered when a neonatal or pediatric patient does not have a previous ABO/Rh test result in the Blood Bank computer.

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. Note: Cord Blood Samples from multiple births are not acceptable for any testing. Samples obtained direct from infants of multiple birth are required to complete any blood bank 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 COMPUTER:

A. In order to interpret the ABO or RhD of a patient who appears to be AB positive (RBCs react with the Anti-A, Anti-B, and Anti-D reagents), a Bovine Serum albumin control must be tested and must be non-reactive.

B. The 7% BSA should be visually inspected prior to use. Product should be clear, slightly yellow-yellowish brown in color, and free from particulates.

   1. Unopened and refrigerated (2-8°C), the stability is until expiration date.
   2. Opened and refrigerated (2-8°C), the stability is ≤ 28 days when tightly sealed.

C. The purpose of the control described below is to prevent potential false positive results with the Anti-A, Anti-B, and Anti-D reagents.

   1. If the patient's RBCs appear to be AB positive (RBCs are reactive with the Anti-A, Anti-B, and Anti-D reagents) then potential false positive results are a concern. Testing with the 7% BSA shall be performed.

      a. If testing with this control is reactive, the ABO and RhD cannot be interpreted; refer to Transfusion Medicine policy Resolution of ABO/Rh Discrepancies.

XII. BEFORE YOU BEGIN:

A. Verify the patient specimen satisfies all labeling requirements as described in Transfusion Medicine policy, Triaging And Identifying Acceptable Samples For Testing. Verify all patient information from the specimen match the information in the Blood Bank computer system.

B. Verify that all QC requirements have been completed as indicated in the Quality Control section of this document.

XIII. PROCEDURE:

A. Verify the requirements of the BEFORE YOU BEGIN Section have been completed.

B. Label four test tubes with the patient information and the intended use of the tube, including the corresponding reagents or the patient's 3% red cell suspension. See the example below:

C. Tube 1 - [Name] "A"

D. Tube 2 - [Name] "B"

E. Tube 3 - [Name] "D"

F. Tube 4 - [Name]
Tube 4 will be used to make the neonate's cell suspension.

G. If testing more than one patient sample in a batch, build a worksheet in the Blood Bank computer by barcode scanning the order number(s) from the specimen label(s). Scan the samples in the same order as they are organized in the rack. Refer to Blood Bank CDM, *Resulting Neonatal Testing on a Worksheet*.

H. Make a 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.

I. Add one (1) drop of typing sera to the test tubes correspondingly labeled as "A", "B", "D" .

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.
6. Gently agitate all tube to mix contents. Centrifuge tubes according to the calibrated time of centrifuge.
7. 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.
   Note: If testing with Gamma-clone reagents and the gamma control is reactive, the ABO and RhD cannot be interpreted; refer to Transfusion Medicine policy Resolution of ABO/Rh Discrepancies.
8. Document this repeat testing in Blood Bank Test system using the Repeat Rh test code (RRHT) or utilizing an internal comment on NNPR test code, for example "Repeat Rh negative with Gammaclone reagents".
   Reminder: Weak D testing is not required unless typing is performed as part of Rhogam assessment for neonates's mother.

Q. If testing is complete and no additional actions are required, ensure the sample is capped and stored as directed in site specific Transfusion Medicine Policies, Storing and Disposing of Patient Samples.

XIV. INTERPRETATIONS:

A. Valid Graded ABO and RhD Reactions in Test Tubes

1. Valid graded ABO and RhD reactions in test tubes are defined in the following table:

If the test is	then the graded result must be
Forward ABO grouping	negative or 3-4+
Rh (D) typing	negative or 2-4+
7%BSA or Gamma Clone Control	negative

a. Negative Result – No agglutination and no hemolysis of the red blood cells is a negative test result, indicated by a smooth cell suspension after resuspension of the cell button.

b. Positive Result – Agglutination and/or hemolysis of the red blood cells is a positive test result. Agglutination must be of the strength listed in the table above to be considered a valid graded reaction. Refer to Invalid Graded reactions below, if applicable.

c. The test can not be interpreted if BSA control is reactive.

d. A mixed-field reaction is considered an invalid graded reaction and must be investigated. Refer to Invalid Graded Reactions, below.

ABO and Rh(D) Interpretation

ABO		Rh(D)				
Anti-A	Anti-B	NNPR Test Code (First Type)		NTYPE Test Code (Second Type)		
		Ortho BioClone Anti- D	7%BSA	Gamma Clone Anti-D	Gamma Clone Control	Interpretation
0	0	2 - 4+	Not indicated	2 - 4+	0	O Positive
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 Blood Bank Computer system.
    b. If an ABO discrepancy remains unresolved, the technologist will enter the interpretation GND (Group not Determined).
    c. If an Rh discrepancy remains unresolved, the technologist will enter the interpretation RND (No Rh Determined).

XV. REFERENCES:

1. American Association of Blood Banks, Technical Manual, current edition.
2. American Association of Blood Banks, Standards for Blood Banks and Transfusion Services, current edition.
3. Blood Grouping Reagents: Anti-A, Anti-B, Anti-A,B BioClone, e631200442, revision date January 2017
4. Blood Grouping Reagent: Anti-D BioClone, e631200462, revision date January 2017
5. Blood Grouping Reagent: Gamma-clone Anti-D (Series 4), 336-9, revision date 02/2013
6. Blood Grouping Reagent: Gamma-clone Control, 3022-3, revision date 06/2017

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Applicability

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