University of Washington Medical Center 1959 NE Pacific Street. Seattle, WA, 98195 Transfusion Services Laboratory Policies and Procedures Manual	Original Effective Date: 02-11-2016 Revision Effective Date: 05-23-2022	Number: PC-0020.03
TITLE: **ABO/Rh Manual Tube Method**		

PURPOSE:
Provide instructions for determining the ABO and Rh type of patients using a manual tube technique

PRINCIPLE & CLINICAL SIGNIFICANCE:
Principle
Patients' red blood cells and plasma are tested with commercial reagents to determine if the A, B and/or D antigens are present on the red cells (front type) and if the reciprocal A and B antibodies are present in the plasma (back type).

Commercially available Anti-A, Anti-B and Anti-D reagents bind to the corresponding antigens on red blood cells and cause an antigen-antibody reaction visible as red blood cell agglutination. The four ABO blood groups are determined by the absence or presence of A and B antigen on the red blood cells.

Plasma is tested for the presence of corresponding antibodies using reagent red cells expressing A and B antigens. The corresponding antibody present in the serum or plasma of the patient binds the reagent red blood cells and cause a visible antigen-antibody agglutination reaction.

Following the Landsteiner's rules of ABO typing, patients with red blood cells expressing A, or B, or O or AB, will demonstrated anti-B, anti-A, anti-A,-B and no antibodies in their plasma, respectively.

When a discrepancy exists between the ABO antigen red blood cell expression and the expected ABO antibodies in the plasma, resolution of the discrepancy is necessary prior to providing type specific blood components for transfusion.

Rh type is serologically determined by the absence or presence of D antigen on the red blood cells.

Clinical Significance
Transfusion of ABO/Rh-incompatible blood can be associated with acute intravascular hemolysis, disseminated intravascular coagulation, renal failure, and death. Likewise, transplantation of ABO-incompatible organs is associated with acute humoral rejection. Because of clinical consequences associated with ABO incompatibilities, ABO/Rh typing is the foundation of pretransfusion testing and important before transplantation

POLICY:
- Only ABO/Rh test results performed by UWMC TSL may be used for compatibility testing and allocation of blood components for transfusion. Historical ABO/Rh results from Bloodworks Northwest (BWNW) or other facilities may not be used.

- This procedure applies to testing of both peripheral and cord blood specimen. Reverse typing is not performed on cord blood specimens.
- Refer to SOP ***ABO/Rh Discrepancy Resolution***
    - Discrepancies between forward types and back type reactions
    - Weak or discrepant Rh typing reactions
    - Between current and historical results
    - Discrepancy between test methods (ie: manual tube vs Ortho Vision)
- Discrepancies must be resolved before issuing type specific blood components
- Mixed field agglutination discrepancies must be resolved for all specimens including cord blood and neonatal specimens – refer to SOP ***ABO/Rh Discrepancy Resolution*** for resolution. Cord blood and neonatal specimens may be contaminated, and a new specimen required.
- Weak D testing should be performed for initial Rh D negative reactions prior to interpretation of ABO/Rh test results for the following – refer to SOP *Weak D Manual Tube Testing*:
    - Neonate of a Rh-negative mother for determination of maternal Rh immune globulin eligibility (cord blood or neonatal specimens)
    - Rh negative donors or potential donors of blood or stem cell components (does not apply to routine type confirmation of red blood cell components)

SPECIMEN REQUIREMENTS:

EDTA is preferred and if not tested soon after collection, should be stored at 1-6°C
Red top clotted blood specimens are also acceptable
See SOP *Specimen Acceptability and Order Receipt*

REAGENTS/SUPPLIES/EQUIPMENT:

Reagents:	Supplies:	Equipment:
• anti-A • anti-B • anti-D • A_1 cells • B cells • ABO + Rh Control • Blood Bank Saline	• 12 × 75 glass tubes • Blood bank transfer pipettes	• Calibrated serologic centrifuge • Calibrated cell washer • Agglutination viewer

QUALITY CONTROL:

Quality Control is performed daily

INSTRUCTIONS:

TABLE OF CONTENTS:

Adult ABO/Rh Manual Testing

STEP	ACTION
1	Label 7 test tubes per SOP *Labeling for Manual Testing* • 4 forward type tubes • 2 reverse type tubes • 1 patient cell suspension tube
2	Add 1 drop of reagent anti-A, anti-B, anti-D and ABO/Rh control to the respectively labeled tubes for forward type
3	Add 2 drops of patient plasma/serum to tubes labeled for reverse type
4	Prepare an approximate 3-4% cell suspension using patient red cells in the respectively labeled tube
5	Add 1 drop of the 3-4% patient's red cell suspension to tubes labeled for the forward type
6	Add 1 drop of reagent A_1 cells and reagent B cells to the respectively labeled tubes for the reverse type
7	Mix gently and centrifuge for time posted
8	Resuspend the cell button and examine for agglutination and/or evidence of hemolysis
9	Read, grade, and record the reactions (refer to SOP *Grading Reactions*)
10	Go to section *Interpreting & Reporting Results*

Neonatal ABO/Rh Manual Testing

STEP	ACTION
1	Label 5 test tube per SOP *Labeling for Manual Testing* • 4 forward type tube • 1 patient cell suspension tube
2	Add 1 drop of reagent anti-A, anti-B, anti-D and ABO/Rh control to the respectively labeled tubes for forward type.
3	Prepare an approximate 3-4% cell suspension of patient cells in the respectively labeled tube.
4	Add 1 drop of the 3-4% patient's red cell suspension to tubes labeled for forward type.
5	Mix gently and centrifuge
6	Shake gently to resuspend the cell buttons and examine for agglutination and/or evidence of hemolysis.
7	Read, grade, and record the reactions (refer to SOP *Grading Reactions*)
8	Go to section *Interpreting & Reporting Results*

CALCULATIONS/INTERPRETATIONS/RESULTS REPORTING/NORMAL VALUES/CRITICAL VALUES

Interpretation

Table 1: ABO/Rh Result Interpretation

ABO/Rh Result interpretation							
Forward grouping		**Rh D Typing**		**Reverse grouping****		**Resulting in SQ**	
Anti-A	Anti-B	Anti-D*****	Control	A_1 cells	B cells	**Interp.**	**Sunquest Entry**
0	0	≥2+	0	+	+	O POS	O, P
0	0	0	0	+	+	O NEG	O, N
≥2	0	≥2+	0	0	+	A POS	A, P
≥2+	0	0	0	0	+	A NEG	A, N
0	≥2+	≥2+	0	+	0	B POS	B, P
0	≥2+	0	0	+	0	B NEG	B, N
≥2+	≥2+	≥2+	0	0	0	AB POS	L, P
≥2+	≥2+	0	0	0	0	AB NEG	L, N

ND = not done

***** Perform Weak D testing prior to reporting ABO/Rh result if reaction with anti-D is:

- Negative on cord blood specimens for maternal Rh immunoglobulin evaluation
- Negative on a donor specimen

****** Reverse grouping is not performed on neonatal specimens or patients < 4 months old and is resulted as INT (infant not tested) in the testing grid

Results Reporting in Sunquest

STEP	ACTION
1	Select the test in “Blood Order Processing” (BOP)
2	Enter reactions in the ABO/Rh grid
3	Enter test interpretation except in the following circumstances:

If	Then
Reaction pattern does NOT match a reaction pattern listed in **Table 1: ABO/Rh Result Interpretation** or are discrepant with historical results	• Click <Accept> • Resolve discrepancy before entering the interpretation – refer to SOP ***ABO/Rh Discrepancy Resolution***
Unexpected mixed field agglutination in any specimen including cord blood and neonatal specimens	• Click <Accept> • Resolve discrepancy before entering the interpretation – refer to SOP ***ABO/Rh Discrepancy Resolution***
Rh D negative reaction for the following:	Perform Weak D testing before entering interpretation - refer to SOP *Weak D*

STEP	ACTION	
	• Cord blood specimens for maternal Rh immunoglobulin evaluation • Donors or potential donors of blood or stem cell components	*Manual Tube Testing Procedure* **NOTE:** If patient has a previous history of Weak D testing documented in the LIS, repeat weak D testing is not required
	1+ Rh D reactivity	• Refer to SOP *Weak D Manual Tube Testing Procedure* for interpretation instructions

NOTES AND LIMITATIONS:

- 3-4% suspension of RBCs may be prepared by one of the following:
  - Using volume estimation with comparison to the reagent red cells for visual verification
  - Adding one drop of packed RBCs to approximately 1-1.5 mL of blood bank saline
- Positive reactions characteristically show 3+ to 4+ agglutination by reagent ABO antibodies; reactions between test serum and reagent red cells are often weaker. Any discrepancy between the results of the tests with serum or plasma and red cells must be resolved before an interpretation is recorded for the patient's or donor's ABO group.
- Positive ABO/Rh control invalidates test. Refer to SOP *ABO/Rh Discrepancy*

REFERENCES:

- Technical Manual. Bethesda, MD: AABB Press, current edition
- Standards for Blood Banks and Transfusion Services. Bethesda, MD: AABB Press, current edition
- Reagent Manufacturer's Insert; current version

RELATED DOCUMENTS:

SOP *Specimen Acceptability and Order Receipt*
SOP *Quality Control of Manual Bench Reagents*
SOP *Labeling Tubes and Gel Cards for Testing*
SOP *Grading Reactions*
SOP *ABO/Rh Discrepancy Resolution*
SOP *Weak D Manual Tube Testing*

APPENDICES:

NA

UWMC SOP Approval:			
UWMC CLIA Medical Director	Andrew Bryan, MD	Date	
Transfusion Service Manager	Nina Sen	Date	
QA Manager	Tayler Reeves	Date	
Transfusion Service Medical Director	Monica Pagano, MD	Date	
UWMC Biennial Review:			
		Date	
		Date	

REVISIONS:
06/20/2019: Revised as part of Ortho Gel Method implementation to add a control tube to all ABO/Rh to be standard with the gel method and result entry in the LIS**.**

07/21/2020: Clarifying decision map for weak D testing and discrepancy resolution for neonatal mixed field reactions. Updated related SOPs. Increased interpretation strength minimum reaction strength to 2+.