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: 06-20-2019	Number: PC-0020.02

TITLE: ABO/Rh Manual Tube Method

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

PRINCIPLE & CLINICAL SIGNIFICANCE:
Principle
Plasma and cells from patients are tested with commercial reagents to determine if the A, B and/or D antigens are present on the red cells and if the corresponding A and B antibodies are present in the plasma

Clinical Significance
Transfusion of ABO/Rh-incompatible blood can be associated with acute intravascular hemolysis, 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:
- Weak D testing (one time) is required when initial typing with Anti-D is
    - Nonreactive for the following patient categories:
        - Cord blood and neonate specimens (for maternal Rh immune globulin eligibility)
        - Samples from donors or potential donors of blood or stem cell components (this does not include red blood cell component type confirmation testing)
    - Discrepant or discrepancies exists between the current and historical ABO/Rh. These discrepancies must be resolved before issuing type specific blood components (refer to SOP *ABO and Rh Discrepancy*)
- Plasma components and platelets may be released after one ABO/Rh performed by the University of Washington Transfusion Service Laboratory (UWTSL) is on record
- Historical ABO/Rh testing from Bloodworks Northwest (BWNW) shall not be used for compatibility testing or allocating any blood components to patients

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

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 • 2 reverse type • 1 patient cell suspension
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* • 3 forward type • 1 patient cell suspension
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: ABORh Result Interpretation

ABORh 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	+	0	+	+	O POS	O,P
0	0	0	0	+	+	O NEG	O,N
+	0	+	0	0	+	A POS	A,P
+	0	0	0	0	+	A NEG	A,N
0	+	+	0	+	0	B POS	B,P
0	+	0	0	+	0	B NEG	B,N
+	+	+	0	0	0	AB POS	L,P
+	+	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 a cord blood or neonate samples
- Negative on a donor sample

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

Results Reporting in Sunquest

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td>1</td><td>Select the test in “Blood Order Processing” (BOP)</td></tr>
<tr><td>2</td><td>Enter the reactions in the ABO/Rh grid</td></tr>
<tr><td>3</td><td>Complete the ABO/Rh interpretation except for the following circumstances:
<table>
<tr><th>If the specimen is from a(n)</th><th>And</th><th>Then</th></tr>
<tr><td>Any patient</td><td>Reactions do NOT match the ABO/Rh Interpretation Tables and/or are discrepant from previous results</td><td>• Click <Accept>
• Resolve discrepancy before entering the ABORH interpretation
(See SOP ABO/Rh Discrepancy)</td></tr>
<tr><td>Cord Blood specimens</td><td>Rh D negative</td><td>• Click <Accept>
Perform Weak D testing before entering interpretation (refer to SOP Weak D Testing)</td></tr>
<tr><td>All SCCA & all female patients < 50 y.o.</td><td>1+ Rh D Reactivity</td><td>• Report as Rh negative
• Add a PB comment: WKDP = Weak D positive</td></tr>
<tr><td>Prenatal specimens (PREN)</td><td>1+ Rh D Reactivity</td><td>• Report as Rh negative
• Add a PB comment: WKDP = Weak D positive
• For new weak D positive results on prenatal samples, add a BBC comment: RHREC = Patient is a candidate for Rh Immune globulin. Further D antigen characterization can be obtained with Rh D genotyping.</td></tr>
</table>
NOTE: If patient has a previous history of Weak D testing documented in the LIS, repeat weak D testing is not required.</td></tr>
</table>

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.
- Mixed-field agglutination should be investigated for possible cause prior to interpreting results.
- 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*
SOP *Quality Control of Manual Bench Reagents*
SOP *Labeling for Manual Testing*
SOP *Grading Reactions*
SOP *ABO/Rh Discrepancy*
SOP *Weak D Manual Tube Testing*

APPENDICES:

NA

UWMC SOP Approval:

UWMC CLIA Medical Director	Mark H. Wener, MD	Date
Transfusion Service Manager	Nina Sen	Date
Compliance Analyst	Christine Clark	Date
Transfusion Service Medical Director	Monica Pagano, MD	Date

UWMC Biennial Review:

Date

Date

06/20/19 – 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**.**