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-0038.02
TITLE: **Weak D Manual Tube Testing**		

PURPOSE:
To provide instructions for performing Weak D testing using a manual tube technique

PRINCIPLE & CLINICAL SIGNIFICANCE:
Principle
Red blood cells from patients are tested with commercial anti-D reagent to determine if the D antigen is present on the patient's red blood cells. In the presence of D antigen, Anti-D reagents bind to the antigen on sample red blood cells and cause an antigen-antibody reaction visible as red blood cell agglutination. RBCs with weakened D antigen may require incubation with the anti-D reagent or testing through the antiglobulin phase for detection.

Clinical Significance
In some recipients determined to be weak D, the possibility exists that pregnancy or transfusion with D+ RBC containing components could result in the production of an allo anti-D.

POLICY
- Weak D testing is performed on the following specimens when the initial Rh result is negative:
    - Cord blood specimens for determination of maternal Rh immune globulin eligibility of a Rh-negative mother
    - Specimens from donors or potential donors of blood or stem cell components (does not include red blood cell component type confirmation testing)
- Weak D testing may also be utilized for ABO/Rh type discrepancy resolution
- For newly identified weak D positive perinatal females:
    - Send the sample to reference lab for weak D type 1,2,3, genotype evaluation
    - Notify the TSL MD on-call 8 am to 5 pm to determine the need for Rh immune globulin.

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 and Order Receipt*

REAGENTS/SUPPLIES/EQUIPMENT:
Reagents:	Supplies:	Equipment:
• Anti-D • ABO + Rh Control • Blood Bank Saline • Anti-IgG • IgG coated control cells	• 12 x 75 glass tubes • Blood bank transfer pipettes	• Calibrated Serologic Centrifuge • Calibrated Cell washer • 37 ºC Heat block • Agglutination viewer

QUALITY CONTROL:
Quality Control is performed each day of use.

INSTRUCTIONS:

<table>
<tr><th>STEP</th><th>ACTION</th></tr>
<tr><td>1</td><td>Label tube(s) per SOP Labeling for Manual Testing
<table>
<tr><th>If initial ABO/Rh test tubes are</th><th>Then</th></tr>
<tr><td>Available</td><td>Use the D tube and patient cell suspension from ABO/Rh typing to continue testing
• Label one tube for control
• Go to next step</td></tr>
<tr><td>Not available</td><td>• Label 3 tubes: one for patient red cell suspension, one for control and one for anti-D
• Prepare an approximate 3-4% patient cell suspension
• Add the following to the anti-D tube
○ 1 drop of Anti-D
○ Add 1 drop of the patient’s cell suspension</td></tr>
</table></td></tr>
<tr><td>2</td><td>Add 1 drop of the ABO/Rh Control reagent to the control tube</td></tr>
<tr><td>3</td><td>Add 1 drop of the 3-4% patient cell suspension to the control tube</td></tr>
<tr><td>4</td><td>Mix and incubate the anti-D and control tubes at 37°C ± 1 for 15-30 minutes</td></tr>
<tr><td>5</td><td>Wash tubes 3 times with blood bank saline</td></tr>
<tr><td>6</td><td>Add 2 drops of anti-IgG to each tube</td></tr>
<tr><td>7</td><td>Mix and centrifuge</td></tr>
<tr><td>8</td><td>Resuspend the cell buttons gently, and examine for agglutination</td></tr>
<tr><td>9</td><td>Read, grade, and record the reactions</td></tr>
<tr><td>10</td><td>Add 1 drop of IgG Coated Control Cells to all negative tests</td></tr>
<tr><td>11</td><td>Mix and centrifuge</td></tr>
<tr><td>12</td><td>Read, grade, and record the results</td></tr>
<tr><td>13</td><td>Go to section” Interpreting & Reporting Results”</td></tr>
</table>

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

Results Reporting in Sunquest

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Select the appropriate patient specimen in “Blood Order Processing” (BOP)</td></tr>
<tr><td>2</td><td>Add; DU (Weak D test) in the Add Spec. Test window, if not previously ordered</td></tr>
<tr><td>3</td><td>Enter the results and interpretation
<table>
<tr><th colspan="3">If</th><th>Interpretation</th><th>Sunquest Key</th></tr>
<tr><td>Field</td><td>DAHG</td><td>DUCC</td><td rowspan="3">Negative</td><td rowspan="3">N</td></tr>
<tr><td>PT</td><td>0</td><td>+</td></tr>
<tr><td>C</td><td>0</td><td>+</td></tr>
<tr><td>Field</td><td>DAHG</td><td>DUCC</td><td rowspan="3">Positive</td><td rowspan="3">P</td></tr>
<tr><td>PT</td><td>+</td><td>ND</td></tr>
<tr><td>C</td><td>0</td><td>+</td></tr>
</table></td></tr>
<tr><td>4</td><td>Add the Antigen/Antibody code corresponding to Du test interpretation to flag the patient’s historical record:

Enter ;PB (Patient Problem Info) in the Add Spec. Test window
Enter the code below:

<table>
<tr><th>If</th><th>Enter</th></tr>
<tr><td>Weak D Positive</td><td>;WKDP</td></tr>
<tr><td>Weak D Negative</td><td>;WKDN</td></tr>
</table></td></tr>
<tr><td>5</td><td>Select the initial ABO/Rh test and interpret the Rh as follows:
<table>
<tr><th>Weak D Interpretation is</th><th>And patient is</th><th>Rh Interpretation</th></tr>
<tr><td>Negative</td><td></td><td>Rh Negative</td></tr>
<tr><td>Positive</td><td>Female >50 years old
Male non-SCCA</td><td>Rh Positive</td></tr>
<tr><td>Positive</td><td>SCCA patients
Female ≤ 50 years old who are not pregnant</td><td>Rh Negative
Add a PB comment:
WKDP = Patient is weak D positive</td></tr>
<tr><td>Positive</td><td>Prenatal</td><td>Rh Negative
Add a PB comment: WKDP = Patient is weak D positive
Add a BBC comment:
RHREC = Further D antigen characterization should be done through Rh D genotyping to determine the need for Rhogam. If genotyping is not done or not available, patient is a candidate for Rh Immune globulin.</td></tr>
</table></td></tr>
</table>

Step	Action			
				• Send the sample to reference lab for weak D type 1,2,3, genotype evaluation • Notify the TSL MD on-call 8 am to 5 pm (to determine the need for Rh immune globulin. o Document the following in SQ BOP as a BBCS comment: ▪ Date and time of notification ▪ Name of TSL MD notified
		Positive	Cord Blood - male	• Rh Positive
		Positive	Cord Blood - female	• Rh Positive • Add a PB comment indicating the following “Consider Rh negative for transfusion purposes • Notify the TSL MD on-call 8 am to 5 pm (to determine the need for Rh immune globulin. o Document the following in SQ BOP as a BBCS comment: ▪ Date and time of notification ▪ Name of TSL MD notified

CALIBRATION:
NA

PROCEDURE NOTES AND LIMITATIONS:

- Calls from patient providers regarding Rh genotyping should be referred to the TSL MD on-call.
- Resulting the Rh type as positive in a weak D positive patient may result in a QA failure that can be overridden using BBR along with a free text comment indicating the “Patient is weak D positive”.
- Mixed-field agglutination should be investigated for possible cause – refer to SOP *ABO/Rh Discrepancy Resolution*
- 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
  - By adding one drop of packed RBCs to approximately 1-1.5 mL of blood bank saline
- Results are considered invalid and must be repeated if negative reactions do not produce agglutination following the addition of IgG Coated Control Cells

REFERENCES:

- Technical Manual. Bethesda, MD: AABB Press, current edition
- Standards for Blood Banks and Transfusion Services. Bethesda, MD: AABB Press, current edition

RELATED DOCUMENTS:

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

APPENDICES:

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:
07/21/2021: Clarification when to notify the TSL MD for consideration of genotyping, Rhogam eligibility, and adding “consider genotyping” comment to test results. Updated related SOPs.