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: 03-05-2023	Number: PC-0038.032

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
- Complete history check regarding prior Rh D typing and transfusion history must be performed in LIS, blood supplier and other hospitals prior to resolution of discrepancy
- 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.

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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	• 12 x 75 glass tubes	• Calibrated Serologic Centrifuge • Calibrated Cell washer

• Anti-IgG • IgG coated control cells	• Blood bank transfer pipettes	• 37 ºC Heat block • Agglutination viewer

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

INSTRUCTIONS:

STEP	ACTION

1	Label tube(s) per SOP *Labeling for Manual Testing*
	If initial ABO/Rh test tubes are / **Then**
	Available: Use the D tube and patient cell suspension from ABO/Rh typing to continue testing
	• Label one tube for control
	• Go to next step
	Not available:
	• 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

2	Add 1 drop of the ABO/Rh Control reagent to the control tube
3	Add 1 drop of the 3-4% patient cell suspension to the control tube
4	Mix and incubate the anti-D and control tubes at 37°C ± 1 for 15-30 minutes
5	Wash tubes 3 times with blood bank saline
6	Add 2 drops of anti-IgG to each tube
7	Mix and centrifuge
8	Resuspend the cell buttons gently, and examine for agglutination
9	Read, grade, and record the reactions
10	Add 1 drop of IgG Coated Control Cells to all negative tests
11	Mix and centrifuge
12	Read, grade, and record the results
13	Go to section" Interpreting & Reporting Results"

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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
Note: %DU Weak D will be automatically ordered in LIS on patients ≤ 3 days old when Rh status is reported as negative. This can be cancelled if not needed for neonatal specimens per policy</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><th>Field</th><th>DAHG</th><th>DUCC</th><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><th>Field</th><th>DAHG</th><th>DUCC</th><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~~
• Non Prenatal</td><td>• Rh Negative
• Add a PB comment: WKDP = Patient is weak D positive
• Case ~~Workup~~ will be reviewed by TSL MD on call for determination o~~f~~ additional workup, Rh D genotyping and finalization of Rh D status~~e is indicated~~</td></tr>
<tr><td>Positive</td><td>• Prenatal</td><td>• Rh Negative
• Add a PB comment: WKDP = Patient</td></tr>
</table></td></tr>
</table>

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Step	Action		
			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. • 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. ○ 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.~~ ~~○ Document the following in SQ BOP as a BBCS comment:~~ ~~▪ Date and time of notification~~ ~~▪ Name of TSL MD notified~~ ▪

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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:

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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.
3/1/2023: Updated to clarify weak D policy for non prenatal patients, %DU added as reflex on Rh negative neonatal samples ≤ 3 days old