Status **Pending** PolicyStat ID **15958842**

Beaumont

Origination	8/9/2021
Last Approved	N/A
Effective	7/21/2024
Last Revised	7/5/2024
Next Review	2 years after approval

Document Contact	Kelly Sartor: Mgr, Division Laboratory
Area	Laboratory-Blood Bank
Applicability	All Beaumont Hospitals

Weak D Testing

Document type: Procedure

A. PURPOSE AND OBJECTIVE:

The purpose of this document is to provide instructions for performing the weak D test.

B. INTRODUCTION:

A. Many different genetic mutations are capable of causing the weak D expression. Weak D is a *quantitative* variant of the Rh antigen; a reduced expression of the Rh antigen is displayed on the intracellular RBC membrane. Most weak D patients do not develop anti-D.

B. Partial D is a *qualitative* variant of the Rh antigen; some epitopes of the Rh antigen are lacking on the extracellular RBC membrane. Partial D patients can develop anti-D.

C. Different clones of anti-D reagent can give different reactions with patients with weaker expressions of D antigen. This can result in different reactions with different anti-D reagents and also cause variations in reactivity between different methods of testing - tube, solid phase and gel testing.

D. The weak D/partial D status of a patient is typically discovered when discrepant Rh results are obtained from the gel and tube testing.

E. The weak D test is used to detect forms of the D antigen that are not agglutinated at the immediate-spin phase of testing but that require an indirect antiglobulin phase for detection. The RBCs of weak D and partial D patients may display variable reactivity with different anti-D reagents at both the immediate-spin and antiglobulin phases. The weak D test does not differentiate between weak D and partial D; molecular testing by a reference laboratory would be required.

C. SCOPE:

Indications for Weak D Testing

1. Weak D testing is generally performed to help resolve a Rh(D) discrepancy; to help differentiate between a wrong blood in tube (WBIT) event or a mistyped sample.
2. Weak D testing is indicated on neonatal samples for purposes of determining maternal RhIG candidacy.
3. **Zygosity testing**: weak D testing may be performed when zygosity testing is specifically ordered by an obstetrician on the male partner of a pregnant woman who has developed anti-D. This testing will be used to calculate the probability that the fetus is Rh(D) positive.
4. **Autologous Donors**: The ABO and Rh of an autologous blood donor and the intended recipient are expected to be identical. However, in some cases an Rh discrepancy arises: the pre-transfusion sample is tested as Rh negative, but the autologous unit is labeled as Rh positive. This discrepancy may arise because weak D testing is not performed on pre-transfusion samples at this facility, but donor centers are required to use a test method designed to detect weak D. If this Rh discrepancy is encountered, weak D testing as described in this document should be performed on the pre-transfusion sample to rule out the possibility of a collection or labeling error involving the pre-transfusion sample or the autologous unit.

Contraindications for Weak D Testing

1. Weak D testing is not indicated for routine pre-transfusion testing.
2. Weak D testing is not indicated for routine obstetrical testing.
3. Weak D testing is not indicated for patients who have developed Anti-D.

D. SPECIMEN COLLECTION AND HANDLING:

The preferred specimen is a 6 ml EDTA sample with affixed identifying label. Refer to Transfusion Services policy, Triaging and Identifying Acceptable Samples for acceptable alternatives.

1. If a sample shows visible hemolysis, lipemia, or icterus then the sample must be adequately washed before weak D testing.
2. If the patient has a positive Direct Antiglobulin Test (DAT) the weak D test may be falsely positive and can not be performed.
3. Do not perform weak D testing on patients recently transfused or on patients receiving Rh immune globulin.

E. REAGENTS:

1. Ortho™ BioClone Anti-D reagent
2. Ortho™ 7% BSA
3. Immucor™ Gamma™ Clone Anti-D reagent

4. Immucor™ Gamma™ Clone control
5. Monospecific Anti-IgG reagent
6. Ortho™ Coombs Control Cells
7. Blood Bank Isotonic saline

F. EQUIPMENT AND SUPPLIES:

1. table top centrifuge
2. lighted viewing mirror
3. disposable pipettes
4. Test tubes, 10 x 75mm or 12 x 75mm, plastic or glass

G. QUALITY CONTROL:

1. Quality Control is performed once per day on all lot numbers of reagents and cells currently used for testing. Refer to site specific reagent quality control procedures.
2. A negative control is required for interpretation of the weak D test.
3. Ortho™ Coombs Control Cells must be added to all weak D test results that are negative at the AHG phase. If a test result with IgG coated cells does not produce a positive result, then the test must be repeated.

H. PROCEDURE:

1. Label a test tube to identify the patient cells. Prepare a 3 – 5 % saline cell suspension of the patient's cells in the labeled tube using Transfusion Services Policy, Making a Test Red Cell Suspension.
    a. If a sample shows visible hemolysis, lipemia, or icterus then the sample must be adequately washed before weak D testing.
2. Label four test tubes to identify the patient and the following:
    a. The Ortho™ BioClone Anti-D reagent
    b. 7% BSA
    c. The Gamma Clone Anti-D reagent
    d. The Gamma Clone control

    Royal Oak and Troy: Rh testing with the two antisera is not performed similutaneously. Repeat Rh testing with Gamma reagents is performed and documented by a second technologist in the blood bank system.

3. Add one drop of reagent to each correspondingly labeled test tube.
4. Add one drop of the patient's 3 – 5 % cell suspension (prepared in step A) to each correspondingly labeled test tube. Mix well and centrifuge.

5. Re-suspend the RBCs by gently shaking. Read, grade, and record the reactions at the Immediate Spin (I.S.) phase in accordance with Transfusion Medicine Policy, *Reading, Grading, and Recording Test Reactions*.
    a. If the 7% BSA or the Gamma® Clone control is positive. Repeat Rh Control with the following:
        i. Place one drop of the red cell suspension in a tube.
        ii. Wash three times with Blood Bank Saline
        iii. Add one drop of Blood Bank Saline to the tube.
        iv. Mix to re-suspend the cells
        v. Centrifuge for speed and time optimal (written on centrifuge) for the serofuge used.
        vi. Check for agglutination.
    b. If the red cells still show agglutination, repeat steps 1a-1f using warm blood bank saline.
    c. If the red cells still show agglutination after warm saline wash then the Rh typing is invalid. Assume the patient is Rho D negative and transfuse accordingly.
6. Incubate the tubes for 15 minutes at 37°C ±1°C.
    a. Weak D testing is not read at 37°C.
    b. Incubation may be extended for up to 30 minutes, if desired.
    c. Incubation for the upper end of this time range may enhance reactivity.
7. Wash the tubes at least three times either manually or by using the cell washer
    a. The washing phase of the weak D test must be carried out without interruption, and the reactions should be graded immediately after addition of the anti-IgG reagent.
8. Add two drops of monospecific Anti-IgG. Mix well and centrifuge.
9. Re-suspend the RBCs by gently shaking. Read, grade, and record the reactions of the weak D test in accordance with Transfusion Medicine Policy, *Reading, Grading, and Recording Test Reactions*.
10. Add one drop of Ortho™ Coombs Control Cells to each test tube in which the graded reaction with Anti-IgG is negative.
    a. Agitate tubes to mix, centrifuge and then read, grade and record the results for the Coombs control cells.
        i. The reaction after the addition of the Coombs control cells must be positive or the test is not valid and must be repeated.
11. Interpret the graded reactions for the Weak D test and document this in the Blood Bank computer system or on an appropriate downtime form. Refer to the *Results & Interpretation* section of this document.
    a. All Weak Positive patients should have the appropriate result comment added to the result as per the chart below.

I. RESULTS & INTERPRETATION:

A. **The weak D test is interpreted as follows:**

1. Weak D / partial D positive: the weak D test is reactive with either the Ortho® BioClone Anti-D reagent or the Gamma-clone® Anti-D reagent, and the controls are negative. Result the weak D test in the Blood Bank computer system as positive.
2. Weak D / partial D negative: the weak D test is non-reactive with both the Ortho® BioClone Anti-D reagent and the Gamma-Clone® Anti-D reagent, and the controls are negative. Result the weak D test in the Blood Bank computer system as negative
3. The weak D test should not be interpreted if mixed-field reactivity is observed with the Anti-D reagent(s).
4. The weak D test should not be interpreted if the patient is known to be DAT positive.
5. The weak D test should not be interpreted if the 7% BSA or the Gamma® Clone control is positive.

B. **The Rh Type is interpreted as follows:**

Rh Typing Interpretation					
Patient Age/ Sex/ Description	**Anti-D Graded Reactions by Tube Method**	**Control**	**Rh Interpretation**	**Result Comment Code**	**Message Description**
Neonate (performed for RhIG purposes)	Weak + or 1+	0	Rh Negative	**WKDPOS**	Weak D result is positive. Infant's mother should receive a post-partum dose of RhIG
Females ≤ 50 years old	Weak + or 1+	0	Rh Negative	**DVAR**	
Males ≤ 15 years old	Weak + or 1+	0	Rh Negative	**CDDVAR**	The Rh results suggest a possible D variant. Without genotyping of the RHD gene for additional information, for purposes of transfusion, the patient will be treated as Rh negative.
Females > 50 and Males > 15 years old	Weak + or 1+	0	Rh Positive	**DVARP**	The Rh results suggest a possible D Variant, the patient will be treated as **Rh positive.**
Any	Any strength	Reactive (any strength)	Rh Negative*	**RND**	Unable to determine the Rh(D), patient will be treated as Rh negative

J. D Variant Testing for Pregnant Females

A. D Variant Analysis will be performed on all pregnant females that are determined to be possible D variants.

B. This testing will be referred to Versiti Wisconsin for testing. Sample will be submitted for both 3040 - Weak D and 3240 - Partial Rh Analysis testing profiles. Refer to Transfusion Medicine policy, Submitting Samples to a Reference Laboratory.

C. Once D variant testing results are available:

1. If no weak/partial D is detected:
    a. Correct the most recent blood type to D positive with the result comment **DTCHNG:** D typed changed to positive based on results from D Variant testing.
    b. Add note to patient profile: **DVARNEG -** Weak and/or partial D analysis performed by Versiti Wisconsin. No weak D detected. No partial D detected. Patient can be treated as D positive.
2. If weak D/partial D is detected and results indicate the variant can be treated as D positive:
    a. Correct the most recent blood type to D positive with the result comment **DTCHNG:** D typed changed to positive based on results from D Variant testing.
    b. Add note to patient profile: **DVARPOSP -** Weak and/or partial D analysis performed by Versiti Wisconsin. Analysis detected: _____. Patient can be treated as D positive.
3. If weak D/partial D is detected and results indicate the variant can be treated as D negative:
    a. No change to prior blood type is indicated. Continue to honor the D variant result beyond the established age ranges, unless otherwise indicted by the TM/BB medical director.
    b. Add note to patient profile: **DVARPOSN** - Weak and/or partial D analysis performed by Versiti Wisconsin. Analysis detected: _____ . For clinical purposes this patient should be considered as Rh D negative and is a candidate for antenatal/postnatal Rh immune globulin prophylaxis. This patient should receive RhD negative blood if red cells transfusions are necessary.

REFERENCES:

1. AABB, *Technical Manual*, current edition.
2. AABB, *Standards for Blood Banks and Transfusion Services*, current edition.
3. Immucor™ / Gamma Anti-D (Monoclonal Blend) Gamma-clone®, Manufacturer's Insert, 10/2007.

4. Immucor™ / Gamma Gamma-clone® Control, Manufacturer's Insert, 10/2007.
5. Ortho™ Blood Grouping Reagent Anti-D (BioClone®), Manufacturer's Insert, March 2012.

Approval Signatures

Step Description	Approver	Date
	Ann Marie Blenc: System Med Dir, Hematopath	Pending
	Karrie Torgerson: Medical Technologist Lead	7/5/2024
	Kristina Davis: Staff Physician	6/14/2024
	Muhammad Arshad: Chief, Pathology	6/11/2024
	John Pui: Chief, Pathology	6/10/2024
	Jeremy Powers: Chief, Pathology	6/7/2024
	Hassan Kanaan: OUWB Clinical Faculty	6/6/2024
	Masood Siddiqui: Staff Pathologist	6/5/2024
	Ryan Johnson: OUWB Clinical Faculty	6/5/2024
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Mgr, Division Laboratory	6/5/2024
	Kristen DiCicco: Mgr, Laboratory	6/5/2024
	Fatima Bazzi: Medical Technologist Lead	6/5/2024
	Katherine Persinger: Mgr, Laboratory	6/4/2024
	Suzanne Chahine: Medical Technologist Lead	6/4/2024
	Ashley Beesley: Mgr, Laboratory	6/4/2024

Hilary Morey: Medical Technologist Lead	6/4/2024
Teresa Lovins: Supv, Laboratory	6/4/2024
Kelly Sartor: Mgr, Division Laboratory	6/4/2024
Kelly Sartor: Mgr, Division Laboratory	6/4/2024