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Weak D Testing

Document type: Procedure

I. PURPOSE AND OBJECTIVE:

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

II. SCOPE:

A. Indications for Weak D Testing

1. **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.
2. **Autologous Donors**: The ABO and Rh of an autologous blood donor and the intended recipient are expected to be identical. However, in some cases a Rh(D) discrepancy arises: the pre-transfusion sample is tested as Rh(D) negative, but the autologous unit is labeled as Rh(D) 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(D) 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.
3. Weak D testing may be performed to help resolve a Rh(D) discrepancy; to help differentiate between a wrong blood in tube (WBIT) event or a mistyped sample.
4. Weak D testing is indicated on neonatal samples for purposes of determining maternal RhIG candidacy.

B. 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. Note that some partial D patients are capable of developing anti-D.

III. PRINCIPLE:

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

B. 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.

IV. NOTES:

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

1. The current sample types as Rh(D) positive on the Vision™ and the current or historical sample typed as Rh(D) negative in the tube method at the immediate-spin phase.
    a. One possible explanation is that the patient is weak D / partial D positive. The Anti-D reagent in the gel card is capable of detecting the patient's D antigen, while the Anti-D clone in the Ortho BioClone tube Anti-D reagent is not.
    b. Other possible explanations are a WBIT event or mistyped sample involving the current or historical sample.
2. The current sample types as follows: Rh(D) positive on the Vision™, Rh(D) negative in the tube method at the immediate-spin phase, and weak D /partial D positive when tested as described in this document. These are typical results for a weak D / partial D positive patient; the possibilities of a WBIT event or mistyped sample have been ruled out. The patient's Rh(D) type is edited to weak D positive in the Blood Bank computer. Refer to Transfusion Medicine Policy Resolution of Rh(D) Discrepancies for additional information.

V. 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.

A. If a sample shows visible hemolysis, lipemia, or icterus then the sample must be adequately washed before weak D testing.

B. If the patient has a positive Direct Antiglobulin Test (DAT) the weak D test may be falsely positive. Note that the control should be positive in this situation; refer to the policy *Appropriate Anti-D Reagents and Controls* and to the *Interpretation* section of this document.

C. Note that mixed-field reactivity should be observed in Rh(D) testing and weak D testing if the patient was recently transfused with Rh(D) dissimilar RBCs. The weak D test should not be interpreted if mixed-field reactivity is observed.

VI. REAGENTS:

A. Ortho™ BioClone Anti-D reagent

B. Ortho™ 7% BSA

C. Immucor™ Gamma™ Clone Anti-D reagent

D. Immucor™ Gamma™ Clone control

E. Monospecific Anti-IgG reagent

F. Ortho™ Coombs Control Cells

G. Blood Bank Isotonic saline

VII. EQUIPMENT AND SUPPLIES:

A. table top centrifuge

B. lighted viewing mirror

C. disposable pipettes

D. Test tubes, 10 x 75mm or 12 x 75mm, plastic or glass

VIII. QUALITY CONTROL:

A. 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.

B. A negative control is required for interpretation of the weak D test. See the policy *Appropriate Anti-D Reagents and Controls* and the *Interpretation* section of this document.

C. 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.

IX. PROCEDURE:

A. 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.

1. If a sample shows visible hemolysis, lipemia, or icterus then the sample must be adequately washed before weak D testing.

B. Label four test tubes to identify the patient and the following:

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

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

C. Add one drop of reagent to each correspondingly labeled test tube.

D. 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.

E. 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*.

F. Incubate the tubes for 15 minutes at 37°C±1°C.

1. Weak D testing is not read at 37°C.
2. Incubation may be extended for up to 30 minutes, if desired.
3. Incubation for the upper end of this time range may enhance reactivity.

G. Wash the tubes at least three times either manually or by using the cell washer

1. 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.

H. Add two drops of monospecific Anti-IgG. Mix well and centrifuge.

I. 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*.

J. Add one drop of Ortho™ Coombs Control Cells to each test tube in which the graded reaction with Anti-IgG is negative.

K. Agitate tubes to mix, centrifuge and then read, grade and record the results for the Coombs control cells.

1. The reaction after the addition of the Coombs control cells must be positive or the test is not valid and must be repeated.

X. INTERPRETATION:

The weak D test is interpreted as follows:

A. 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.

B. 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.

C. The weak D test should not be interpreted if mixed-field reactivity is observed with the Anti-D reagent(s).

D. The weak D test should not be interpreted if the 7% BSA or the Gamma® Clone control is positive.

Gel Anti-D Microtube	Gel Control Microtube	Tube Ortho BioClone Anti-D reagent	Tube 7% BSA Control	Tube Immucor Gamma Anti-D reagent	Tube Immucor Gamma Control	Interpretation
4+	0	2+ - 4+	0	2+ - 4+	0	Rh positive
0	0	0	0	0	0	Rh negative

w+ - 3+	0	w+ - 1+	0	w+ - 1+	0	Weak D positive

E. **Canned Messages for Patients who are Weak D Positive**

The technologist should add the appropriate blood bank comment to the patient's ABO/Rh test, depending on the patient's age / sex / description, and whether the patient is weak D positive or whether the patient has an unresolved Rh(D) discrepancy

Patient Demographics (age, sex, description)	Comment Code	Message Description
Females within childbearing years (< 50 years old)	WK+CB	The patient may be weak D or partial D positive. Rh negative RBCs will be used if transfusion is necessary. Patient may be a RhIG candidate. Consult the Blood Bank with any questions.
Males < 18 years old	WK+YM	The patient may be weak D or partial D positive. If transfusion is necessary, the Blood Bank will attempt to provide Rh negative RBCs.
Females > 50 years old Males > 18 years old	WK+OL	The patient may be weak D or partial D positive. The Rh of any RBCs that may be required for transfusion will be based on Blood Bank Standard Operating Procedures.
Neonates delivered at Beaumont Health to an Rh negative or Weak D+ mother.	WK+B	This neonate may be weak D or partial D positive. If a transfusion is necessary, the Blood Bank will provide Rh negative RBCs. The patient who gave birth to the weak D / partial D neonate may be a RhIG candidate. Consult the Blood Bank with any questions.

XI. REFERENCES:

1. American Association of Blood Banks, *Technical Manual*, current edition.
2. American Association of Blood Banks, *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.

Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
	Muhammad Arshad: Chief, Pathology	1/12/2022
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Applicability

Dearborn, Farmington Hills, Grosse Pointe, Royal Oak, Taylor, Trenton, Troy, Wayne