**Weak D**

**PRINCIPLE:** Red blood cells that appear to be Rh negative by direct test methods may be further tested for Weak D. The Weak D test detects variants or weak forms of the D antigen. Testing of transfusion candidates for Weak D is at the discretion of the laboratory.

**SPECIMEN COLLECTION:** No special preparation of the patient is required prior to specimen collection. Blood should be collected by approved techniques. The sample should be stored at 2 to 8C. EDTA or clotted samples should be tested within 10 days.

**REAGENT:** Seraclone Anti-D (RH1) Blend Rapid Tube. Anti-Human Globulin Anti-IgG, Coombs Control Reagent Red Blood Cells sensitized with IgG antibody. Do not use beyond expiration date. Store at 2 to 8C. May be at room temperature (20 to 30C) while in use. (Refer to product insert for additional information)

**QUALITY CONTROL:** To recognize reagent deterioration the reagents must be tested daily with appropriate controls. See QUALITY CONTROL procedure.

**\*\*\*RH CONTROL\*\*\***

• If a DAT has been performed and is negative, an Rh control is not required.

• If a DAT has not been performed, a commercial Rh control, an Rh control of 6% albumin, or saline can be

tested concurrently with the Anti-D.

**PROCEDURE: (All reagents must be at room temperature before testing)**

**NOTE:** Steps 2 and 3 may be interchanged, but do it one way or the other. Be consistent.

|  |  |
| --- | --- |
| **Step** | **Action** |
| 1 | Prepare a 3-5% suspension of red blood cells to be tested in isotonic saline.  (Washed or unwashed cells may be used) |
| 2 | Add one drop of Anti-D reagent to appropriately labeled tube. |
| 3 | Add one drop of Rh Control if appropriate. (See **\*\*\*Rh Control\*\*\*** above) |
| 4 | Add one drop of rbc suspension into the tube(s). |
| 5 | Mix and incubate tube(s) at 36-38 C for 15-30 minutes |
| 6 | After incubation: Mix tubes gently and then centrifuge\*. Gently resuspend the red cell button and examine macroscopically for agglutination.  • If the reaction is negative continue with #7.  • If anti-D reaction is positive, change the immediate spin reaction to positive. Add a comment:: “Anti-D positive after 37 C incubation”. |
| 7 | Wash the cells in the tube(s) three times with tubes full of isotonic saline. Decant completely after the last washing. |
| 8 | To the tube(s), add two drops of Anti-IgG Anti-Human Globulin. |
| 9 | Mix the contents of the tubes gently and centrifuge\*. Resuspend the red cell button by gentle agitation and examine macroscopically for agglutination. |
| 10 | To all negative antiglobulin tests results, add 1 drop of Coombs control cells (e.g., check cells). Record results. |

**\*Note: Centrifuge spin and wash time are noted on each centrifuge.**

**RESULTS:**

• No agglutination of the red blood cells is a negative test result and indicates the D antigen is not

demonstrable by the Weak D test method.

• Agglutination of the red blood cells is a positive test result and indicates the presence of the D antigen by

the Weak D test method. For a positive weak D (Du): Report Rh as positive and comment “weak positive”.

• **Positive Weak D (Du):**

a. Positive Weak D test results are valid only if it can be shown that the red cells exhibit a negative direct

antiglobulin test (DAT) or Rh control. A negative Weak D test requires no further confirmation.

b. Red blood cells that agglutinate by the Weak D phase of testing and demonstrate a positive direct

antiglobulin test or Rh control cannot be validly typed for the D antigen. Rh negative blood should be

transfused until the discrepancy is resolved.

**COMMENTS:**

• CAUTION: Mixed-field agglutination in the Weak D test on a woman who recently delivered may indicate

an admixture of maternal Rh neg. and fetal Rh positive blood.

• NOTE: Once a Weak D test has been performed and found negative on 2 separate samples, further Weak

D testing is unnecessary. A comment “Du Confirmed \_\_\_\_(date)” should be entered into patient blood

bank history.

**LIMITATIONS:**

• Red blood cells demonstrating a positive direct antiglobulin test cannot be accurately tested for the Weak

D antigen.

• Reactions with red cells exhibiting weakened expressions of the D antigen may show varied reactivity as

compared to those obtained with other anti-D reagents.

• Refer to product inserts for additional limitations.

**REFERENCES:** AABB Technical Manual. 17th ED. 2011

**PACKAGE INSERT:**

• Bio test: Blood Grouping Reagent Anti-D (RH1) Blend Seraclone® Human Monoclonal Blend. Driesch,

Germany.

• ORTHO® Anti-Human Globulin Anti-IgG (Rabbit). Raritan, NJ: Ortho Diagnostic Systems Inc.

Bio test: Coombs Control Cells. Driesch, Germany

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