WEAK D TESTING

I. **Principle:**

The D antigen is highly immunogenic. It is necessary to detect weakened expression of the D antigen in certain populations in order to prevent the formation of anti-D in Rh negative individuals. These populations include blood and organ/tissue donors, and infants of Rh negative mothers. Other patient populations may be tested as per local protocol.

II. **Purpose:**

The Weak D test is performed in order to detect weak expression of the D antigen on red cells, when the cells being tested have failed to react with anti-D at the immediate spin phase. The red cells in question are incubated at 37°C with anti-D and then tested with anti-human globulin (AHG) reagent. If the D antigen is present on the cells, the anti-D will sensitize the cells, resulting in agglutination at either the 37°C phase or the AHG phase. Lack of agglutination at both phases indicates the D antigen is not present on the red cells.

III. **Specimen**

The specimen of choice is red blood cells (RBCs) in EDTA anticoagulant. Other acceptable anticoagulants include ACD, CPD, CP2D, CPDA-1, and heparin. A specimen drawn without anticoagulant is acceptable provided there is no neutral gel separator in the tube. [False positive results may occur in specimens drawn using gel separator tubes.] Specimens should be tested as soon as possible, or stored at 1-10°C to limit false reactions due to contamination of the specimen.

IV. **Equipment and Reagents:**

Chemically modified anti-D typing serum
12 x 75 test tubes
Isotonic saline
Dispo pipettes
Serofuge
37°C incubator
IgG specific anti-human globulin
IgG sensitized reagent red cells
Agglutination mirror
Rh-hr control
6% albumin
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V. Controls:

1. See the D typing procedure for D controls.

2. If the cells being tested are positive at the AHG phase, the cells may be tested for *in vivo* antibody sensitization using the Direct Antiglobulin Test (DAT). A positive DAT will invalidate the weak D test result.

3. Alternatively, a control such as Rh-hr control or 6% albumin may be tested in parallel. Any agglutination in the control tube invalidates the weak D test.

4. All tests that are negative at the AHG phase should be tested against Coombs Control Cells (check cells) to verify proper technique has been followed. A negative result with Coombs Control Cells invalidates the weak D test results.

VI. Procedure:

1. Place one drop of low protein anti-D into an appropriately labeled test tube. (If running a control in parallel, label a second tube and add 1 drop of control reagent to that tube).

2. Prepare a 2-5% suspension of washed red cells to be tested. Place one drop in the tube containing the anti-D (and one drop in the control tube). Mix and incubate at 37°C for 15-30 minutes.

3. Centrifuge and read macroscopically. If definite agglutination is observed with anti-D (and no agglutination is observed in the control), it is unnecessary to continue. Cells are D positive. If negative, continue with the test.

4. Wash a minimum of three times with saline. After the final wash, the tube should be blotted dry to ensure that all residual saline has been removed to prevent the antiglobulin reagent from being diluted.

5. Add 2 drops of IgG specific antiglobulin reagent, mix, centrifuge and read both macroscopically and microscopically.

6. If positive, perform a DAT on the patient’s cells. If a control was tested in parallel, and the control is negative at the AHG phase, the DAT is unnecessary.

7. If negative, add 1 drop of IgG coated cells (Coombs Control Cells). Centrifuge. Read macroscopically for agglutination.
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VII. Reporting Results:

1. If the weak D test is negative, report the patient as Rh Negative.

2. If the weak D test is positive, and the control (DAT or control reagent) is negative, report the patient as Rh Positive.

3. If the weak D test is positive, and the control is positive, the test is invalid.

VIII. Additional Notes:

1. Weak D testing is performed on neonatal specimens if the infant’s mother is Rh negative. Donor red cells that initially type as Rh Negative will have weak D testing performed. It is not necessary to determine whether a recipient’s red cells are of the Weak D phenotype because no harm results from giving these individuals Rh negative red cells.

2. The weak D test will be positive on patients who have a positive DAT. If the patient is to receive a blood transfusion, the recipient should be considered Rh negative and must receive Rh negative blood products.

IX. References:


Manufacturer’s directions.