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BLOOD GROUPING SERUM ANTI-D,ANTI-Rh₀ (Monoclonal/Polyclonal Blend) A Latex Test

SUMMARY AND EXPLANATION

The Rh₀(D) antigen was first recognized in 1939¹. Since the initial recognition of the D antigen, over 40 different antigens are now known to be part of the Rh system. Most Rh blood group antibodies are immune, produced in response to stimulation by pregnancy or transfusion. The D antigen is highly immunogenic and has been reported to stimulate the production of anti-D in 50-85% of D negative individuals who are exposed to D positive blood². Anti-D is of considerable importance since this antibody can cause severe Rh Hemolytic Disease of the Newborn (HDN) and hemolytic transfusion reactions. The D antigen and its weakened form D^u are therefore, commonly considered in the routine selection of blood for transfusion^{3,4}. Optimal detection of D^u cells by anti-D requires the application of an indirect antiglobulin test procedure. The commonly used terms Rh positive and Rh negative, refer specifically to the presence or absence of the D antigen. The frequency of Rh positive persons in the general Caucasian population is 85%. A number of theories that explain the genetic inheritance of Rh antigens have been proposed and three commonly used systems of Rh terminology have been developed based on different genetic models. More detailed information on the Rh system, its inheritance and nomenclature may be obtained from the references cited^{2,3,4,5}.

BIOLOGICAL PRINCIPLE OF THE TEST

The test used with this blood grouping reagent is based on the principle of hemagglutination. Incubation of test red cells with CORTEZ DIAGNOSTICS, INC. Anti-D Blood Grouping Serum will result in a specific antigen-antibody reaction if the corresponding D antigen is present on the tested red cells. Visible detection of this reaction is demonstrated by agglutination of the cells. No agglutination indicates a negative test result and within the limitations of the test procedure, indicates the absence of the corresponding D antigen.

PRODUCT DESCRIPTION

CORTEZ DIAGNOSTICS, INC. Anti-D Blood Grouping Serum for slide and rapid tube test is prepared from human monoclonal Anti-D and human serum containing Anti-D obtained from immunized donors. CORTEZ DIAGNOSTICS, INC. Anti-D is designed for use in slide or rapid tube tests, and provides a specific, qualitative test for the detection of the corresponding D antigen on human red blood cells. The diluent used for this reagent contains sodium chloride, 6.0 to 8.0% bovine serum albumin, and phosphate buffer. Sodium azide at a final concentration of 0.1% is added as a preservative.

CAUTION 1: Sodium azide may react with copper and lead plumbing to form explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

Do not use if marked turbidity or other observable indications of product alteration occur. These signs may indicate bacterial contamination and/or product deterioration. Do not use beyond expiration date.

CAUTION 2: Source material from which this product was derived has been tested in accordance with approved procedures and found non-reactive for HBsAg and antibody to HIV. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, this product should be considered potentially hazardous and handled with appropriate caution.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient/donor is required prior to specimen collection. Blood samples should be collected by approved medical procedure. Blood collected with or without anticoagulant is acceptable, if testing is performed without delay. If a delay in testing is unavoidable, red cells from clotted samples, EDTA or heparinized anticoagulated samples should be separated from the serum/plasma, washed and stored in a red cell preservative solution (such as CORTEZ DIAGNOSTICS, INC. PHYSIOSOL) at 2-8°C for no longer than 35 days.

ACD, CPD and CPDA-1 anticoagulated blood samples may be tested up to their expiration date if storage is maintained at 2-8°C. Prolonged storage of red cells prior to testing may result in deterioration of red cell antigens and resultant weaker than expected test reactions.

MATERIALS AND EQUIPMENT NOT SUPPLIED

1.SLIDE TEST: Glass slides; pasteur pipettes; centrifuge; applicator sticks; isotonic saline; illuminated Rh viewbox with surface temperature of 40-50°C.

2.RAPID TUBE TEST: Glass test tubes (10x75 mm or 12x75 mm); pasteur pipettes; centrifuge; isotonic saline.

3.D^u TEST: Polyspecific Anti-Human Globulin or Anti-IgG; IgG sensitized reagent control cells (Coombs Control Cells); 37°C incubator; optical aid.

4.OTHER RECOMMENDED MATERIAL NOT SUPPLIED: Control cells of known Rh phenotype.

TEST PROCEDURE

A. SLIDE TEST METHOD

1. Prepare a 40-50% suspension of test red cells. Red cell suspensions may be prepared in autologous or group compatible serum or plasma.
2. Place one drop of CORTEZ DIAGNOSTICS, INC. Anti-D (for slide and rapid tube test) on one end of a labelled, prewarmed slide on an illuminated Rh viewbox.
3. Add two drops of the prepared 40-50% suspension of test red cells to each drop of the CORTEZ DIAGNOSTICS, INC. Anti-D.
4. Using separate, clean applicator sticks, thoroughly mix each red cell suspension over an oval area of approximately 20 x 40 mm.
5. Slowly tilt the Rh viewbox back and forth and examine for macroscopic hemagglutination. Agglutination may be apparent within a few seconds; however, observation should not continue beyond 2 minutes.

B. TUBE TEST METHOD

1. Prepare a 3-5% suspension of test red cells. Red cell suspensions may be prepared in autologous or group compatible serum or plasma, or in isotonic saline.
2. Dispense one drop of CORTEZ DIAGNOSTICS, INC. Anti-D (for slide and rapid tube test) into an appropriately labelled test tube.
3. Add one drop of the prepared 3-5% suspension of test red cells to the test tube.
4. Mix the contents of the test tube thoroughly.
5. Centrifuge for:
 - a) 30 seconds at 3200-3400 rpm (900-1000 rcf)
 - b) or centrifugation of equivalent force.

NOTE: The centrifugal force applied should be the minimum required to produce a clear supernatant and a clearly delineated red cell button that can be easily resuspended. No single centrifugation speed or time can be recommended for all types of available centrifuges or test applications. Centrifuges should be calibrated individually to determine the optimal time and speed required to achieve the desired results.

6. Gently resuspend red cell button and examine macroscopically for agglutination. Do not examine microscopically.
7. Grade and record results.

C. D^u Test Method:

1. Prepare a 3-5% suspension of test red cells. Red cell suspensions may be prepared in autologous or group compatible serum or plasma, or in isotonic saline.
2. Dispense one drop of CORTEZ DIAGNOSTICS, INC. Anti-D (for slide and rapid tube test) into an appropriately labelled test tube.

3. Dispense one drop of isotonic saline or 6.0 to 8.0% bovine albumin solution into a second, appropriately labelled test tube to serve as a control.
4. Add one drop of the prepared 3-5% suspension of test red cells to each tube.
5. Mix contents of both tubes thoroughly and incubate at 37°C for 15 minutes.
6. Wash the cells in each tube three or four times with isotonic saline. (Decant supernatant saline completely following each wash and ensure thorough resuspension and mixing of red cells with each new addition of saline for subsequent washes).
7. Completely decant supernatant saline following last wash and blot tube edge on a clean absorbent tissue to ensure the removal of all residual saline and a resultant "dry" red cell button.
8. Add two drops of Polyspecific Anti-Human Globulin or Anti-IgG to the "dry" button of cells in each tube (Refer to appropriate manufacturer's Directions for Use for Anti-Human Globulin).
9. Mix gently but thoroughly to resuspend red cell buttons.
10. Centrifuge for:
 - a) 15 seconds at 3200-3400 rpm (900-1000 rcf)
 - b) or centrifugation of equivalent force.

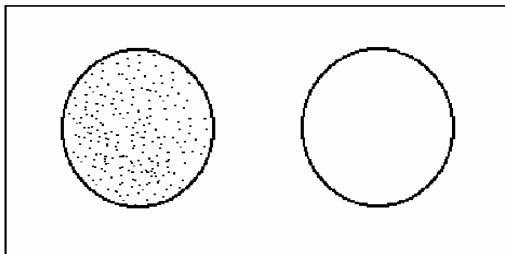
- NOTE:** The centrifugal force applied should be the minimum required to produce a clear supernatant and a clearly delineated red cell button that can be easily resuspended. No single centrifugation speed or time can be recommended for all types of available centrifuges or test applications. Centrifuges should be calibrated individually to determine the optimal time and speed required to achieve the desired results.
11. Gently resuspend red cell buttons and examine macroscopically for agglutination. Do not examine microscopically.
 12. Grade and record results.

QUALITY CONTROL

Appropriate control tests are essential for all laboratory test procedures.

1. If a patient control is run simultaneously with the test and shows agglutination no valid conclusion concerning the test result can be reached.
2. The application of IgG sensitized reagent control cells (such as CORTEZ DIAGNOSTICS, INC. Coombs Control Cells) is considered an essential control procedure to confirm the validity of weak or negative antiglobulin tests.
3. It is strongly recommended that the reactivity of Blood Grouping Sera be confirmed each day of use by control tests with known antigen positive and negative red cells. Positive control cells should be selected to represent weak expression of the specific antigen and where applicable, appropriate cells may be selected from heterozygous donors whose red cells express a single dose of the respective antigen.

INTERPRETATION OF TEST RESULTS



Positive

Negative

POSITIVE (slide and rapid tube test): Agglutination of test red cells with CORTEZ DIAGNOSTICS, INC. Anti-D indicates the presence of the corresponding D antigen (within the accepted limitations of the test procedure).

POSITIVE (D^u Test): Agglutination of test red cells with CORTEZ DIAGNOSTICS, INC. Anti-D by D^u test procedure only (indirect antiglobulin test), indicates that the red cells are of the D^u variety.

NEGATIVE: No agglutination of test red cells with CORTEZ DIAGNOSTICS, INC. Anti-D indicates the absence of the corresponding Rh antigen within the accepted limitations of the test procedure.

LIMITATIONS OF THE TEST PROCEDURE

1. Red cells that have a positive direct antiglobulin test (DAT) may on rare occasion agglutinate "non-specifically" in tests with Anti-D Blood Grouping Sera (for slide and rapid tube test). Rarely, examples of test cells may spontaneously agglutinate in low protein media⁶. Such false agglutination may not always be detected by control tests using saline media⁶.
2. The use of unwashed test red cells, suspended in plasma or serum may promote false positive reactions associated with rouleaux formation, autoantibodies, or more rarely, antibodies to constituents of CORTEZ DIAGNOSTICS, INC. Anti-D diluent. The routine use of well washed red cells for rapid tube tests may reduce the incidence of such false positive reactions.
3. Red cells that have a positive direct antiglobulin test cannot be accurately tested for D^u.
4. Some red cells may express variant Rh antigens and may therefore demonstrate weaker than expected reactions with Anti-D.
5. Delays in reading antiglobulin tests, overvigorous resuspension of red cell buttons and other technique variables associated with antiglobulin test performance may result in weaker than expected or false negative D^u test results.
6. Rare, false positive reactions may occur, associated with the presence of unsuspected antibodies to low incidence antigens. Such antibodies may infrequently and unavoidably occur as contaminants of human blood grouping reagents.
7. False negative or unexpectedly weak reactions may occur with red cells that have been subjected to prolonged storage and/or inappropriate storage conditions.
8. Other variables such as improper technique, inappropriate centrifugation or incubation, improperly cleaned glassware and/or contaminated materials may cause false negative or false positive results.

SPECIFIC PERFORMANCE CHARACTERISTIC

CORTEZ DIAGNOSTICS, INC. Anti-D (for slide and rapid tube test) has been tested and found to specifically agglutinate human red cells if the corresponding D antigen is present, when used according to the Recommended Directions for Use. Each lot of CORTEZ DIAGNOSTICS, INC. Anti-D has been tested to ensure potency and specificity according to methods recommended by U.S.-F.D.A. standards.

Specificity tests for CORTEZ DIAGNOSTICS, INC. Anti-D (slide and rapid tube test) are performed using extensive panels of Rh negative donor cells that include cells positive for antigens that occur with a frequency of 1% or greater in the North American population. In addition, each lot of CORTEZ DIAGNOSTICS, INC. Anti-D is tested and shown to be non-reactive in serological tests with selected Bg (a⁺) donor cells when used according to the Recommended Directions for Use.

The absence of antibodies to the low frequency antigens, M^e and W^r is not always confirmed; however, when test cells are available, confirmatory tests are performed. Antibodies to Le^c and Le^d which are most unlikely to occur as contaminants in blood grouping serum are not necessarily excluded.

Deviation from the Recommended Directions for Use may result in less than optimal product performance.

CORTEZ DIAGNOSTICS, INC. Anti-D Blood Grouping Serum meets U.S.-F.D.A. and Canadian C.G.S.B. potency requirements.

REFERENCES

1. Levine P, Stetson RE. An unusual case of intragroup agglutination. J Amer Med Assoc 1939; 113:126-127.
2. Mollison PL. Blood transfusion in clinical medicine. 6th edition. Oxford, Blackwell Scientific 1979.
3. Moore BPL. Serological and immunological methods of the Canadian Red Cross Blood Transfusion Service. 8th edition. Toronto, Hunter Rose 1980.
4. Widmann FK. (Ed.) Technical Manual. 9th edition. Washington, D.C. American Association of Blood Banks, 1985.
5. Race RR, Sanger R. Blood groups in man. 6th edition. Oxford, Blackwell Scientific 1975.
6. Garratty G. et al. Spontaneous agglutination of red cells with a positive direct antiglobulin test in various media. Transfusion 1984; 24:214-217.

Revised: 03/06