ANTI-D (Rho) 
(IgM) 
MONOCLONAL BLOOD TYPING ANTIBODIES FOR SLIDE AND TUBE TESTS

SUMMARY
Monoclonal antibodies are derived from hybridoma cell lines, created by fusing mouse antibody producing B lymphocytes with mouse myeloma cells or are derived from a human B cell line through EBV transformation. Each hybridoma cell line produces homogenous antibodies of only one immunoglobulin class, which are identical in their chemical structure and immunological activity.
Human red blood cells are classified as Rho (D) positive or Rho (D) negative depending upon the presence or absence of D (Rho) antigen on them. Approximately 85% of the Caucasian population are Rho (D) positive. The D⁺ phenotype is a variant of D (Rho) antigen and is recognised by performing the antiglobulin test.
About 60% of the D⁺'s, now classified as weak or partial D's, may react with Anti-D (Rho) (IgM) in slide tests and about 90% may be detected by the tube technique.

REAGENT
Anti-D (Rho) (IgM) is a ready to use reagent, prepared from supernatants of cell cultures with antibody producing B lymphocytes obtained through EBV transformation and is a blend of monoclonal antibodies of immunoglobulin class IgM. These antibodies are a mixture of several monoclonal antibodies of the same specificity but having the capability of recognising different epitopes of the human red blood cell antigen D (Rho). Anti-D (Rho) (IgM) does not detect all weak and partial D's. For the confirmation of negative reactions with Anti-D (Rho)(IgM) further testing with an incomplete Anti-D (Rho) of IgG class is strongly recommended to confirm the presence or absence of weak/partial D's.
Each batch of reagent undergoes rigorous quality control at various stages of manufacture for its specificity, avidity and performance.

REAGENT STORAGE AND STABILITY
1. Store the reagent at 2-8°C, DO NOT FREEZE.
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

PRINCIPLE
Human red blood cells possessing the D (Rho) antigen will agglutinate in the presence of antibody directed towards the antigen. Agglutination of red blood cells with Anti-D (Rho)(IgM) reagent is positive test result and indicates the presence of D (Rho) antigen. No agglutination with the reagent is a negative test result and indicates the absence of D (Rho) antigen. All negative test results should be further tested for D⁺ (Presence of weak / partial D's) by performing the D⁺-test procedure using incomplete Anti-D (Rho) of IgG class, as described later.

NOTE
1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. Anti-D (Rho)(IgM) reagent is not from human source, hence contamination due to HBsAg and HIV is practically excluded.
3. The reagent contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. Extreme turbidity may indicate microbial contamination or denaturation of protein due to thermal damage. Such reagents should be discarded.

SAMPLE COLLECTION AND PREPARATION
No special preparation of the patient is required prior to sample collection by approved techniques. Samples should be stored at 2-8°C if not tested immediately. Do not use haemolysed samples.
Anticoagulated blood using various anticoagulants should be tested within the below mentioned time period.
EDTA or Heparin : 2 days
Sodium citrate or sodium oxalate : 14 days
ACD or CPD : 28 days
Clotted whole blood should be tested within 14 days.
ADDITIONAL MATERIAL REQUIRED FOR SLIDE AND TUBE TESTS

- Glass slides (50 x 75 mm)
- Test tubes (10 x 75 mm)
- Pasteur pipettes
- Isotonic saline
- Centrifuge
- Timer
- Mixing sticks
- Anti-Human Globulin (Coombs) reagent
- Anti-D (Rho)(IgG) or RHOFINAL Anti-D (Rho)(IgM+IgG)

TEST PROCEDURE

Bring reagent and samples to room temperature before testing.

Slide Test
1. Place one drop of Anti-D (Rho)(IgM) reagent on a clean slide.
2. Pipette one equal drop of whole blood on the slide.
3. Mix well with a mixing stick uniformly over an area of approximately 2.5 cm².
4. Rock the slide gently, back and forth.
5. Observe for agglutination macroscopically at two minutes.

Immediate Spin Tube Test
1. Prepare a 5% suspension of red cells to be tested in isotonic saline.
2. Place one drop of Anti-D (Rho)(IgM) reagent into a labelled test tube.
3. Pipette into the test tube one drop of 5% cell suspension and mix well.
4. Centrifuge for one minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
5. Gently resuspend the cell button, observing for agglutination macroscopically.

D⁺ TEST PROCEDURE
1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of any incomplete Anti-D (Rho) (IgG class) reagent such as Anti-D (Rho)(IgG) into a labelled test tube.
3. Add to the test tube one drop of the 5% cell suspension and mix well.
4. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g).
5. Very gently, resuspend the cell button and observe for agglutination macroscopically.

INTERPRETATION OF RESULTS

Slide and Tube Tests
a) Agglutination is a positive test result and indicates the presence of D (Rho) antigen. Do not interpret peripheral drying or fibrin strands as agglutination. No agglutination is a negative result and indicates the absence of D (Rho) antigen.

b) Cord cells heavily sensitized with Anti-D (Rho) may give a false negative immediate spin test result.

D⁺ Test Procedure
(a) Agglutination indicates the presence of D⁺ antigen (Presence of weak / partial D's). No agglutination indicates the absence of D⁺ antigen (Absence of weak / partial D's). (b) Mixed field agglutination in the D⁺ test on red cells from a recently delivered woman may indicate a mixture of maternal Rho (D) negative and fetal Rho (D) positive blood. (c) Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for D⁺ antigen (Presence of weak / partial D's).

REMARKS

As undercentrifugation and overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and the time required of achieving the results. It is strongly recommended that as a routine quality control measure known as Rho (D) positive and Rho (D) s negative red cells be occasionally run, preferably on a daily basis so as to control reagent performance and validation of test results. After usage, the reagents should be immediately recapped and replaced to 2-8°C storage.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.
BIBLIOGRAPHY

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