

ANTI-D
IgG & IgM
MONOCLONAL BLEND
FOR SLIDE AND TUBE TESTS

INTENDED USE

Vitro Anti D reagent is a blood grouping reagent intended for the detection of the D antigen on human red cells.



METHOD

Method is based on the direct hemagglutination reaction between monoclonal Anti-D antibodies and erythrocytes, containing D-antigen of human rhesus system. These reagents are also suitable for use in automated test systems, spin tube or microplate and should be standardized and validated by the user.

BACKGROUND

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 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 and 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 Du phenotype is a traditional definition to describe the weak / partial D's that can be detected with Anti-D (Rho) (IgM+IgG). About 60% of the Du (weak / partial D's) may react with Anti-D (Rho) (IgM+IgG) in slide tests and about 90% may be detected by tube technique.

ASSAY PRINCIPLE

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

REAGENTS

Anti-D Rho (IgM+IgG) is a ready to use high protein reagent, prepared from supernatants of cell cultures with antibody producing B lymphocytes obtained through EBV transformation and is a blend of monoclonal antibodies of the immunoglobulin class IgM and IgG. These antibodies are a mixture of several monoclonal antibodies of the same specificity but having the capability of recognizing different epitopes of the human red blood cell antigen D (Rho). Anti-D (Rho) (IgM+IgG) is a blend of IgM and IgG class of Anti-D (Rho) monoclonals, a characteristic which accords versatility to the reagent. It gives an avid saline reacting slide / tube test reagent the capability of detecting Du (weak/partial D's) in the Anti Human Globulin Phase. 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.

SPECIMEN

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.

PROCEDURE

Bring reagent and sample to room temperature before testing.

Slide Test

1. Place one drop of Anti-D (Rho) (IgM+IgG) reagent on a clean glass 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. **Tube Test**

1. Prepare a 5% cell suspension of the red cells to be tested in isotonic saline. 2. Place one drop of Anti-D (Rho) (IgM+IgG) reagent into labeled test tube. 3. Pipette into each of the test tubes, one drop of test red 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. **DU TEST PROCEDURE**

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of Anti-D (Rho) (IgM+IgG) reagent into a labeled test tube.
3. Add to the test tube one drop of cell suspension under test, mix well and incubate at 37°C for 15 minutes.
4. Wash the contents of the tube thoroughly, at least three times, with isotonic saline and decant completely after the last wash.
5. Add two drops of ® Anti Human Globulin reagent and mix well.
6. Centrifuge for 1 minute at 1000 rpm (125 g) or 20 seconds at 3400 rpm (1000 g). 7. Very gently, resuspend the cell button and observe for agglutination macroscopically.

INTERPRETATION OF RESULTS

Slide and Tube Tests

- 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 with reagent and control is a negative test result and indicates the absence of D antigen.
- Cord Cells heavily sensitized with Anti-D (Rho) may give false negative immediate spin test result.

Du Test Procedure

- Agglutination with reagent and no agglutination with the control indicates the presence of Du antigen (weak / partial D's). No agglutination with reagent and control indicates the absence of Du antigen.
- Mixed field agglutination in the Du 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 Du antigen (weak / partial D's).

PROCEDURAL NOTES

- As under centrifugation and over centrifugation 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 Rho (D) positive and Rho (D) 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.
- Unexpected positive results due to: pseudoagglutination, autoagglutination, mixed field reaction, the presence of Whartons jelly together with umbilical cord cells.
- Unexpected negative or weak results due to: weak antigens, mixed field reaction, decreased activity of the reagent.
- False positive or false negative results may occur through contamination of test materials or any deviation from the recommended technique.
- Red cells that have a positive direct antiglobulin test (DAT) produce false positive test result.

WARNINGS & PRECAUTIONS

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

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.

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
	Batch code (Lot #)
	Catalog number
	Temperature limitation
	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
2041	10 X 10 ml