Spectrum For Diagnostic Industries

Anti-D (Rho)
Monoclonal IgM & IgG

IVD

REF.	Pack size
437 10 010	10 x 10 ml

Intended Use

Anti-D (Rho) plus reagent is intended for the detection of Rho (D) Type in human Blood.

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 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 on the presence or absence of Rho (D) antigen on them. Approximately 85 % of the Caucasian population is 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 Dus (weak / partial D´s) may react with Anti-D (Rho) plus reagent in slide test and about 90 % may be detected by the tube technique.

Assay Principle

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

Note

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

Reagents

Anti-D (Rho) plus is 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 and IgG. 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) plus reagent 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 quality control at various stages of manufacture for its specificity, avidity and performance.

Reagent Storage and Stability

1. Store the reagent at 2-8 ^{O}C. Don't freeze.
2. The shelf life of reagent is as per the expiry date stated on the vial label.Open vial is stable for 6 months at the specified temperature. on the reagent vial label

Specimen Collection and Storage

No special preparation of the patient is required prior to sample collection by approved techniques. Samples should be stored at 2-8 ^{O}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 T

Glass slides (50x75 mm), Test tubes (12x75 mm), Pasteur pipettes,isotonic saline, Centrifuge, timer, mixing sticks. Anti Human Globulin (Coombs) reagent

Procedure

Bring reagent and samples to room temperature before testing.

Slide Test

1. Place one drop of Anti-D (Rho) plus reagent on a clean glass slide.
2. Add 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. Rock the slide gently, back and forth.
4. Observe for agglutination macroscopically at two minutes.

Tube Test

1. Prepare a 5% suspension of the red cells to be tested in isotonic saline.
2. Place one drop of Anti-D (Rho) plus into the labelled test tubes.
3. Pipette into each of the test tubes , one drop of the 5% red cell suspension and mix well.
4. Centrifuge for 1 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^{u}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) plus into labelled test tube.
3. Add to the test tube, one drop of the 5 % red cell suspension and mix well, incubate at 37 ^{o}C for 15 minutes.
4. Wash the contents of the tube at least three times with isotonic saline and decant completely after the last wash.
5. Add 2 drops of Anti Human Globulin reagent and mix well.
6. Centrifuge for one 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

(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 test result and indicates absence of D (Rho) antigen.

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

Procedure

(a) Agglutination with the Anti Human Globulin reagent and no agglutination with the control test indicates the presence of the Du antigen (weak / partial D´s). No agglutination with both indicates the absence of the Du antigen (weak/partial D´s)
(b) Red cells demonstrating a positive direct antiglobulin test cannot be accurately tested for Du antigen (weak/partial D´s)

Remarks

1. As undercentrifugation or overcentrifugation could lead to erroneous results, it is recommended that each laboratory calibrate its own equipment and determine the time required for achieving the desired results.
2. It is strongly recommended that red cells with known Rho (D) positive and Rho (D) negative be occasionally run, preferably on a daily basis so as to control reagent performance and validate test results.
3. 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.

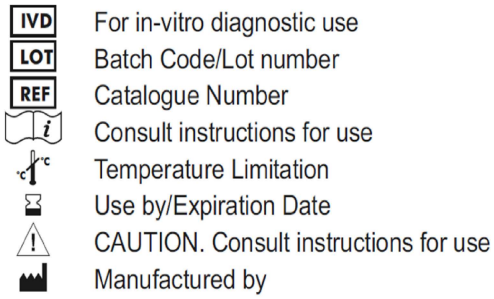
Waste Disposal

This product is made to be used in professional laboratories.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment. refer to special instructions/safety data sheets.

Bibliography

1. Kohler C. & Milstein C. (1975), Continuous cultures of fused cells secreting antibody of predefined specificity, Nature, 256, 495-497.
2. Lee H.H., Rouger P, Germain C., Muller A. & Salmon C. (1983), The production and standardisation of monoclonal antibodies as AB blood group typing reagents. Symposium of International Association of Biological Standardisation on Monoclonal antibodies.
3. Human Blood Groups by Geoff Daniels, 1st Ed., Blackwell Science,Oxford 1995.
4. HMSO, Guidelines for the Blood Transfusion Services, 2nd Ed., 1994.
5. AABB Technical manual, 13th Ed., 1999.

SYMBOLS IN PRODUCT LABELLING



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