



ANTI-D IgM

PRINCIPLE:

Human red blood cells possessing D antigen will agglutinate in the presence of antibody directed towards the antigen. All negative test results should be further tested for D by performing the Du test procedure as described later.

REAGENT:

Anti-D (IgM) is a ready to use reagent Prepared from the supernatants of cell culture with antibody producing B lymphocytes obtained through EBV transformation and then forced with mouse Myeloma cells to form a heterohybridoma. These Antibodies of the immunoglobulin class IgM 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).

REAGENT STORAGE & STABILITY:

Store the reagent at 2° - 8° C. DO NOT FREEZE.

The shelf life of the reagent is as per the expiry date mentioned on the reagent vial label.

SPECIAL NOTE:

In Vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.

Monoclonal Anti-D reagent is not from human source, hence contamination due to HBsAg and HIV is practically eliminated.

SAMPLE:

No special preparation of the patient is required prior to sample collection by approved techniques. Sample should be stored at 2° - 8° C, if not tested immediately. Do not use haemolysed samples.

PROCEDURE:

1. On a clean glass slide place 1 volume of Anti-D (IgM).
2. Add 1 volume of whole blood (40% to 50% PCV) to one volume of anti-D reagent.
3. With a clean applicator stick, thoroughly mix the reagent and blood over an area of approximately 20mm square.
4. Gently tilt the slide back and forth. Over an illuminated viewing box examine for agglutination.

NOTE: Agglutination of D positive cells will usually commence within 10 to 20 seconds. Reading should not be prolonged beyond 3 minutes as false positives may occur due to peripheral drying. If longer incubation is performed, the slide or tile should be placed in a moist container.

TUBE METHOD:

1. Place 1 volume of Anti-D (IgM) into a labelled 75mm x 10mm glass test tube.
2. Add 1 volume of 5% suspension of red cells in physiological saline.
3. Mix well and centrifuge the tube for 1 minute at 200 rpm or 20 seconds at 1000 rpm.
4. Gently agitate to dislodge the packed button of cells from the bottom of the tube and examine macroscopically for agglutination.
5. If no agglutination is observed, incubate for 15 minutes in 37° C water bath and repeat steps 3 and 4.

INTERPRETATION OF RESULTS:

Agglutination of red cells Anti-D (IgM) in either the rapid tube or slide test constitutes a positive result and indicates the presence of the D antigen in the test red cells.

Lack of agglutination with Anti-D (IgM) indicates the lack of normal strength D antigen in the test red cells. In such cases, patients may safely be classified as "Rh (D) Negative", but donors should be further tested with an IgM anti-D reagent by indirect antiglobulin test for the presence of the Du phenotype.

Factors which may cause false results include:

1. Contaminated blood samples, reagents or supplementary materials.
2. Deviation from recommended procedures.
3. Aged red cells which may give weaker reactions.
4. Red cells strongly coated with IgG anti-D may give false negative results in the rapid tube test.

REFERENCES:

1. Landsteiner K, Wiener AS. An agglutinating factor in human blood recognised by immune sera for Rhesus blood. Proc Soc Exp Biol Med (N.Y.) 1940; 43: 223.
2. Levine P, Stetson RE. An unusual case of intragroup agglutination. JAMA 1939; 113: 126-7.
3. Melamed MD, Gordon J, Ley SC, Edgar D, Hughewes O, Jones NC. Senescence of a human lymphoblastoid clone producing anti-Rhesus (D). Eur J Immunol 1985; 15: 742-6.
4. Thompson KM, Melamed MD, Eagle K, Gorick BD, Gibson T, Holburn AM, Hughes-Jones NC. Production of human monoclonal IgG and IgM antibodies with anti-D (Rhesus) specificity using heterohybridomas. Immunol 1986; 58: 157-60.

For in vitro diagnostic use only, not for medical use

Manufacturing For:

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