### Problems in Rh Typing

1. Improper identification of specimen.
2. Improper techniques like:
   - Cell to reagent ratio.
   - Failure to identify haemolysis
   - Improper storage of Reagents.
   - Fibrin clots
   - Over incubation of cells and reagents.
3. Improper centrifuge calibration resulting in over/under centrifugation.
4. Problems in Donor/Patients.
   - Weak expression of D antigens.
   - Immunoglobulin coating of red blood cells.
   - Increased abnormal proteins in patients (multiple myeloma) resulting in rouleaux and thus giving false positive results.
   - Poly agglutination.

### BIBLIOGRAPHY


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- Practical Blood Transfusion , Douglas W. Huestis , Bove & Coso, 4th Edition 1988, Little Brown and Co.
- Blood Tranfusion in Clinical Medicine , P.L. Mollison, C. T. Engelfreit, Marcela Contreras, 9 th Edition, 1993.
- Basic Blood Group Serology , Makroo R.N. 1st Edition 1994.


### LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, in as much as that the test kit will function as an *in vitro* diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose.The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

*in vitro* diagnostic Reagent, not for medicinal use


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**J. Mitra & Co. Pvt. Ltd.**

# Anti-D (IgG) Monoclonal Antibodies for Rh Typing

**FOR *IN VITRO* USE ONLY**

The Anti-D (IgG) Monoclonal Antibodies are *in vitro* culture supernatant of hybrids obtained by cellular fusion (Hybridoma technology). The Anti-D (IgG) Monoclonal Antibodies has the following features:

- Usable in the preparation of control cells for Direct & Indirect Antiglobulin test.
- Active at 37ºC.

### PRINCIPLE

Human red cells are classified as Rh positive ( $Rh^+$) or Rh negative ($Rh^-$) depending upon the presence or absence of " D" antigen on them. Major percentage of the population is Rh positive. Human red blood cells possessing D antigen will agglutinate in the presence of corresponding antibody. The red blood cells are first checked using Anti-D (IgM) Monoclonal Antibodies.

Agglutination of red cells with Anti-D (IgM) Monoclonal Antibodies indicates the presence of D-antigen and hence Rh positive result. If no agglutination is obtained then the red blood cells are checked with Anti-D (IgG) Monoclonal Antibodies as it sensitize the Rh positive / $D^u$ positive cells which will give agglutination on addition of coomb's reagent (Indirect coomb's technique)

### STORE AND PACK SIZE:

The Anti-D (IgG) Monoclonal antibodies are packed into 5ml & 10ml dropper vials. The antibodies are stable at 2-8ºC until the expiry date mentioned on the reagent vial label. Sodium azide is added to the antibodies at 0.1% concentration as preservative.

## SAMPLE COLLECTION

Blood sample should be collected with a suitable anticoagulant in a sterile stoppered container & should be tested immediately. If testing is delayed, blood should be stored at 2-8ºC and must be examined not later than 48 hours. Haemolysed Samples should not be used for testing and clotted blood should be used within 24 hours of collection.

## DESCRIPTION OF SYMBOLS USED

The following are graphical symbols used in or found on J. Mitra diagnostic products and packing. These symbols are the most common ones appearing on medical devices and their packing. They are explained in more detail in the European Standard EN ISO 15223-1:2021.


Manufactured By


Catalogue Number

Lot Number
Batch Number


Manufacturing Date


Expiry Date


Do not use if package is damaged

Keep Dry

*In vitro* diagnostic medical device


See Instruction for use

Temperature Limitation


Caution
See instruction for use

Keep away from sunlight


Contains biological Material of Animal Origin

Country of Manufacture

## WARNING FOR USERS

***CAUTION:*** ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for *in vitro* diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. Bring the antisera and specimen at room temperature (20ºC to 30ºC) before use.
8. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121ºC at 15psi for 60 min. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed of in accordance with established safety procedures.
9. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
10. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
11. The monoclonal antibodies contain Sodium Azide as a preservative. If these material are to be disposed off through a sink or other common plumbing systems, flush with generous amounts of water to prevent accumulation of potentially explosive compounds. In addition, consult the manual guideline "Safety Management No. CDC-22", Decontamination of Laboratory Sink Drains to remove Azide salts" (Center for Disease Control, Atlanta, Georgia, April 30, 1976.)

## GROUPING TECHNIQUE (Tube Method)

Separate the RBCs from plasma by centrifuging at 5000 rpm for 5 minutes.

The Rh Typing is performed at room temperature and 37ºC by tube method as follows:

Prepare a 4-5% suspension of test red cells washed in isotonic saline solution. Add one drop of Anti-D (IgG) Monoclonal Antibodies reagent and one drop (50$\mu$l) of 4-5% cells suspension in the tube. Shake to homogenise antibodies and red cells suspension and incubate at 37ºC for 30 minutes for sesitization.

Take out the tube from 37ºC and look for agglutination. If no agglutination is observed, wash the sensitized cells 3-4 times with normal saline and decant the last washed completely.

Add two drops of Anti human Serum (Coomb's Antisera) and mix gently. Centrifuge for one minute at 1000 rpm. The reaction is read macroscopically by shaking gently the tube so as to loosen the cells pellet. If the red cells spearate in one or more clumps, the reaction is positive. If the red cells return to a homogeneous suspension, the reaction is negative.

All negative reactions should be confirmed by adding known sensitized O positive control cells, recentrifuge at 1000 rpm for 1 minute and look for agglutination.

The presence of agglutination confirms the test results and no agglutination indicates invalid test.

## INTERPRETATION

Agglutination of the sensitized red blood cells with the coomb's reagent is a positve test indicating cells are $D^u$ positive & absence of agglutination indicates that the cells are Rh negative.

## QUALITY CONTROL

Each batch of Anti-D (IgG) Monoclonal Antibodies is subjected to stringent internal quality control regarding specificity and titre to ensure constant quality of Antibodies.

**Titre :** Titre is checked by tube method. It is defined as the reciprocal of highest dilution of the antibodies which gives agglutination. The reagent is diluted upto 512 dilution by two fold serial dilution in tubes.

**Specificity :** It is checked to ensure whether the reagent is specific for the purpose or not with Rh negative cells as it should not sensitize Rh negative cells.