Titre: Titre is checked by tube method with 2.5% suspension. it is defined as the reciprocal of highest dilution of the antibodies which gives agglutination. The reagent is diluted up to 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 (D-).

Haemolysis: it is checked to ensure that cells do not haemolysed during checking for specificity.

Prozone: It is checked by tube method to ensure that no negative reactions are obtained with low dilution of the Antisera.

Rouleaux: It is checked to ensure that the Antisera does not give pseudo agglution in which the red blood cells look like pile of coins.

LIMITATION

The negative reactions are to be completed with a search of D^u antigen using the indirect Coomb's technique.

PERFORMANCE CHARACTERISTICS

The Performance of Anti-D (IgM+IgG) Monoclonal antibodies has been evaluated on more than 1500 samples (Donor, clinical, Antenatal and neonates) in comparison with established Antisera. The performance has been evaluated for Avidity, Intensity, Potency(Tire) and specificity on these samples. All samples have been correctly characterized with 100% sensitivity and specificity and results are in compliance to common technical specification of in-vitro medical devices under recommended methods. The performance has also been evaluated for Reactivity (Haemolysis, Prozone, Rouleaux) of Anti-D (IgM+IgG) Monoclonal and it complies the in-house specification.

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

- KHOLER G., MILSTEIN C., 1975 Continuous culture of fused cells secreting antibody of predefined specificity. Nature, 256, 495 -497
- Blood Group Serology, Boorman, Dodd & Lincoln, Churchill Livingstone, 6 th Edition
- 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 for use, 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 (IgM+IgG) Monoclonal Antibodies for Rh typing

FOR *IN VITRO* USE ONLY

INTRODUCTION

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

- It agglutinates in saline solution.
- Active at room temperature and 37ºC.
- Usable on glass slide as well as in tube.

INTENDED USE

The Anti-D (IgM+IgG) Monoclonal antibodies are used for determination of the presence or absence of "D" Antigen on Human Red blood cells . The Reagent is used in forward typing to detect the Rh blood group in human whole blood. The test is for in vitro diagnostic use only.

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. Agglutination of red cells with Anti-D (IgM + IgG) Monoclonal Antibodies indicates the presence of D-antigen and hence Rh positive result. Since the antibodies is a mixture of immunoglobulin class Monoclonal IgM and Monoclonal IgG, It can detect D antigen & higher and lower grade of D^u antigen i.e. the weak variant of Antigen 'D'. In case no agglutination is obtained with Anti-D (IgM + IgG) Monoclonal Antibodies on slide, the red cells should be further checked for the presence of D^u antigen using Anti Human Globulin serum (Coomb's Reagent) by Indirect Coomb's technique.

STORAGE AND PACK SIZE:

The Anti-D (IgM + IgG) Monoclonal Antibodies are packed in 10 ml 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 & 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 sysmbols used in or found on J. Mitra diagnostic products and packing. These sysbols 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	IVD	In vitro diagnostic medical device
REF	Catalogue Number		Instruction for use
LOT	Lot Number Batch Number	8°C 2°C	Temperature Limitation
	Manufacturing Date		Caution See instruction for use
	Expiry Date		Keep away from sunlight
	Do not use if package is damaged	BIO	Contains biological Material of Animal Origin
	Keep Dry	IN	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 professional 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. Test shall be performed at room temperature (20ºC to 30ºC).
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

Separate the RBCs from the serum or plasma by centrifuging at 5000 rpm for 5 mins.

The Rh Typing is performed at room temperature by:

(a) **Slide or tile method.**

(b) **Tube method.**

(c) **Microplate Method.**

(d) **Slide Method:** It is used mainly for emergency Rh typing especially in out door camps with whole blood sample. Place one drop each of Anti-D (IgM + IgG) Monoclonal Antibodies and negative control on a slide. Now add one drop of whole blood sample to both of them. Mix the cells & antibodies with clean mixing stick & spread the mixture over an area of 2 cm. Rock the slide gently from side to side & observe for the agglutination within one minute. Complete the test by a second observation after two minutes.

(e) **Reaction in tube by immediate centrifugation :**

Prepare a 3-4% suspension of red cells washed in isotonic saline solution. Place one drop of Anti-D (IgM + IgG) Monoclonal Antibodies reagent and one drop of 3-4% cells suspension in the tube. Shake to homogenise antibodies and red cells suspension, then 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 separate in one or more clumps, the reaction is positive. If the red cells return to a homogneous suspension, the reaction is negative.

REACTION IN TUBE (D^U testing) :
INDIRECT COOMB'S TECHNIQUE

After immediate centrifugation if the reaction is weak or negative, then shake the tubes and incubate for 30 minutes at 37ºC. After incubation, wash the cells thrice with Normal saline. Add two 2 drops of Anti Human Serum (Coomb's Antisera) to the red cells residue. Homogenise and centrifuge for one minute at 1000 rpm. Read the results as in “Immediate centrifugation” section. All negative reactions should be confirmed by adding known sensitized (O+) control cells, recentrifuge at 1000 rpm for 1 min. and look for agglutination. The presence of agglutination confirms the test results and no agglutination indicates invalid test.

INTERPRETATION

SLIDE AND TUBE METHOD

Agglutination of the red blood cells with Anti-D (IgM + IgG) Monoclonal Antibodies indicates cells are Rh positive & absence of agglutination indicates that the cells are either Rh negative or D^U positive.

D^U Testing : If agglutination is observed with Anti Human Serum (Coomb's Antisera) then the cells are said to be D^U positive and if no agglutination is observed, cells are interpreted as Rh negative.

QUALITY CONTROL

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

Avidity: It is defined as the reactivity time (in seconds) taken by the antibodies to show the agglutination in seconds and is tested by slide method with 40% red cells suspension.

Intensity: It shows the strength of the reaction i.e. clumping and is tested by slide method with 40 % red cells suspension. It is graded as:

+/- Doubtful for agglutination. Repeat test.

+1- Small clumps scattered in the test area

+2- Two or more clumps of equal size

+3- One big clump with some small clumps

+4- One big clump in the centre.