Specificity : It is checked to ensure whether the reagent is specific for the purpose or not. For example specificity of Anti-B Monoclonal Antibodies is checked with 'A', 'B' 'A,B' & 'O' cells.

Haemolysis: It is checked to ensure that cells do not get haemolysed during specificity testing.

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.

Avidity, Intensity, titre, specificity, Haemolysis, prozone and rouleaux of Anti-A, Anti-B and Anti-D (IgM+IgG) Monoclonal Antibodies are as per I.P standards.

PROBLEMS IN ABO GROUPING:

The ABO and Rh Group of an individual can only be clearly determined if the results of forward & reverse typing match properly. Following factors are responsible for discrepancies in ABO and Rh Grouping :

1. Improper identification of specimen.
2. Improper techniques like,
   - Cell to reagent ratio.
   - Failure to identify haemolysis
   - Improper storage of Reagents.
3. Improper centrifuge calibration resulting in over/ under centrifugation.
4. Patients may fail to express ABO antigens on red cells due to diseases like Leukemia & lymphoma.
5. Acquired B antigen can occur due to Infections; gram negative septicemea, carcinoma colon, Blood Group chimera ie an individual with two population of cells which may occur as a result of either Bone marrow transplantation or Transfusion of group 'O' blood to 'A' or 'B' patient.
6. Rouleaux formation occurs in patients with abnormal Albumin/globulin concentration (mutliple myelona) or in cord blood samples due to Whartons Jelly contamination and may lead to false positive results.
7. Acquired antibodies ie
   - Anti- A1 in A2 persons
   - Anti- H in Bombay phenotype
   - Cold auto - antibodies
   - Unexpected allo-antibodies.
8. 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.

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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'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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COMBINE ABD (IgM+IgG)

Reagent Combipack for monoclonal antibodies ABO & Rh Grouping

FOR *IN VITRO* USE ONLY

INTRODUCTION

The Combined ABD (IgM+IgG) Monoclonal Antibodies are *in vitro* culture supernatant of hybrids obtained by cellular fusion (Hybridoma Technology).

INTENDED USE

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

GROUPING PRINCIPLE

There are two most important blood group system in blood transfusion ; ABO and the Rhesus system. There are four major blood groups ; A,B,AB and O in ABO System and are defined by the presence of 'A' and/or 'B' Antigens on the surface of the red blood cells and by the simultaneous presence of anti-A and / or anti-B antibodies in the serum. An individual has in his serum the antibodies corresponding to the antigens which are not on his Red Blood cells. It is thus necessary to identify the erythrocyte antigens by the known ANTI- A, ANTI-B and ANTI-AB Monoclonal Antibodies (forward typing) and then confirm the results by verifying the presence of corresponding antibodies in the blood serum to be tested with control cells: 'A'1, 'A'2, 'B' and 'O'. (Reverse Typing). Human Red blood cells, possessing 'A' and/or 'B' Antigen will agglutinate in the presence of antibody directed towards the antigen.

The Rhesus system is the second major system of the blood group and "D" Antigen is the main antigen of this system.; 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. It can detect D antigen & higher and lower grade of D^u antigen i.e. the weak variant of Antigen 'D'. In case no

aggluntination 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-D (IgG) & Anti Human Globulin serum (Coomb's Reagent) by Indirect Coomb's technique.

STORAGE AND PACK SIZE:

The combine ABD (IgM+IgG) Monoclonal Antibodies are packed in 10 ml dropper vial. The antibodies are stable at 2-8°C 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 2-8°C & 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	IVD	*In vitro* diagnostic medical device
REF	Catalogue Number		Instruction for use
LOT	Lot Number Batch Number	2°C 8°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 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 (20°C to 30°C) before use.
8. All materials used in the testing and samples should be disposed off 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" (Centre for Disease Control, Atlanta, Georgia, April 30, 1976.)

ADDITIONAL MATERIALS REQUIRED

Glass slides, Test tubes (10X75mm), Pasteur Pipettes,Normal saline, Beakers, Centrifuge, Timer & Mixing sticks.

GROUPING TECHNIQUE

Separate the RBCs from the serum or plasma by centrifuging at 5000 rpm for 5 min. The ABO and Rh Grouping is performed at room temperature by:

(a) Slide or tile method.
(b) Tube method.
(c) Microplate method.

(a) Slide Method: It is used mainly for emergency ABO and Rh Grouping especially in out door camps with whole blood sample.

Place one drop each of Anti-A, Anti-B and Anti-D (IgM+IgG) Monoclonal Antibodies on a slide.Now add one drop of whole blood sample to each of the antibodies. Mix the cells and 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.

(b) Tube Method: Take one drop each of Anti-A, Anti-B & Anti-D (IgM+IgG) Monoclonal Antibodies in three different test tubes. Make 3-4% red cell suspension of washed RBCs (to be tested) & add one drop to each tube. Shake well to mix the antibodies & cell suspension.Incubate for two minutes at room temperature & then centrifuge at 1000 rpm for one minute. Gently shake the tube in such a way to dislodge the pellet. If the red cells separate in one or more clumps, the reaction is positive. If shaking gives a homogeneous suspension again, the reaction is interpreted as negative.

(c) Microplate Method: Microplate method is ideal for testing large no. of blood samples. Microplates are polystyrene plates having 96 small wells (either 'v' shaped type, flat bottom or 'U' type). Add one drop each of Anti-A, Anti-B & (IgM+IgG) Monoclonal Antibodies to different wells & add one drop of 2 - 4% cell suspension to all the 3 wells. Gently shake to mix the antibodies and cells. Incubate at room temperature for 30 mins.
Gently shake the plate by tapping the side of the plate & read the results.

INTERPRETATION

SLIDE AND TUBE METHOD : Agglutination of the red blood cells with the antibodies is a positive test indicating the presence of 'A' and /or 'B' / 'D' antigens on the cells and no agglutination indicates the absence of 'A' and / or 'B' / 'D' antigens on the cells.

QUALITY CONTROL : Each batch of Anti - A, Anti - B & (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 and is tested by slide method with 40% red cells suspension.

Intensity: It shows the strength of the reaction ie clumping. It is tested by slide method with 40% red cells suspension & 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.

Titre : Titre is checked by tube method with 2-5% cell suspension. It is defined as the reciprocal of highest dilution of antibodies which gives agglutination. The reagent is diluted up to 512 dilution by two fold serial dilution.