purpose or not. For example specificity of Anti-A Monoclonal Antibodies is checked with 'B' & 'O' cells.

Avidity, Intensity & titre of Anti-A, Anti-B & Anti AB Monoclonal Antibodies are as per I.P standards.

PROBLEMS IN ABO GROUPING:
The ABO 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 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 septicemia, carcinoma colon, Blood Group chimera in an individual with two populations 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: It occurs in patients with abnormal Albumin/globulin concentration or in cord blood samples due to Wharton's Jelly contamination.
7. Acquired antibodies ie
   - Anti-A in A2 persons
   - Anti-H in Bombay phenotype
   - Cold auto - antibodies
   - Unexpected allo-antibodies.

BIBLIOGRAPHY
- VOAK D., LENNOX E., SACKS, MILSTEIN C., DARNBOROUGH, J. 1982
- Anti- A1 in A2 persons
- Unexpected allo-antibodies.
- Cold auto- antibodies
- For in-vitro diagnostic use only, not for medicinal use

Limited Expresed 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 thereof.
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 British and European Standard BS EN 15223-1:2012.

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 procedures.
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 onl.
5. Do not pipette by mouth.
6. 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 121°C 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.
7. 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.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.
9. 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 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

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

(a) Slide Method: It is used mainly for emergency ABO Grouping especially in out door camps whole blood sample. Place one drop each of Anti-A, Anti-B and Anti-AB 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-AB 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 & Anti-AB Monoclonal Antibodies to different wells & add one drop of 2-4% cell suspension to all the 3 wells. Gently shake to mix the antibodies & 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' antigens on the cells and no agglutination indicates the absence of 'A' and/or 'B' antigens on the cells.

QUALITY CONTROL: Each batch of Anti - A, Anti - B & Anti - AB 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 10% red cells suspension.

Intensity: It shows the strength of the reaction ie clumping. It is tested by slide method with 10% 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. 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.

Specificity: It is checked to ensure whether the reagent is specific for the