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

Titre: Titre is checked by tube method. It is defined as the reciprocal of the 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 (O-).

LIMITATION
The negative reactions are to be completed with a search of low grade D antigen using the indirect Coomb’s technique.

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

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 thereof.

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

- The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
- The Rh Typing is performed at room temperature by:
  - **Microplate 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) Monoclonal Antibodies and negative control on a slide and 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.
  - **Tube Method (Immediate centrifugation)**: Prepare a 3-4% suspension of red cells washed in isotonic saline solution. Put respectively one drop of Anti-D (IgM) 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 homogeneous suspension, the reaction is negative.
  - **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-D (IgM) Monoclonal Antibodies & negative control to two different wells & add one drop of 2-4% cell suspension to both the wells. Gently shake to mix the antibodies and cells. Incubate at room temperature for 30 mins. Gently shake the plate either by tapping the side of the plate or on microplate shaker. Results are read as in tube method.

**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 blood at 5000 rpm for 5 mins.

- The Rh Typing is performed at room temperature by:
  - **Slide or tile method**.
  - **Tube method**.
  - **Microplate Method**.
- **Microplate 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) Monoclonal Antibodies and negative control on a slide and 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.
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