Intended Use
Anti D IgG/IgM is designed for in vitro diagnostic and professional use only. It is intended for detection of Rhesus D Antigen in Human Red Blood Cells.

Composition
Anti D IgG/IgM is a monoclonal IgG.IgM anti-D Antibody.

Source
Anti D IgG/IgM is obtained by raising lymphoblastoid cell lines, in vitro culture fro EBV transformed, antibody secreting Human B lymphocytes.

Storage
Anti D IgG/IgM will be well preserved within utility limit till the expiry dated, if stored at 2-8°C. do not freeze.

Principle
The procedure used within the reagent is based on the principle of agglutination. Red blood cells with Rhesus D antigens agglutinate when mixed with anti Rhesus (D) antibody, Phenotyping (grouping) of them is done by reacting the blood sample with Anti D IgG/IgM. Presence of haemagglutination determines the positive Anti D IgG/IgM antigen and the blood are categorized as Rh+ve.

Specimen
Properly stored anticogulated blood or 10% RBC-Saline suspension should be used.

Preparation of 10% RBC-Saline suspension.
1. Add approximately 5 volumes of isotonic saline to the whole blood (Washing if RBC's)
2. centrifugde for 2 minutes.
3. Remove the supertanant and wash the sedimente RBC/s three more times with normal salines.
4. After final washm take 100 μl of sedimented red cells dilute to 1ml with saline and mix thoroughly.

Procedure
Microscopic Slide Test
Bring the reagent and samples to room temperature .
1. Place 1 drop of Anti D IgG/IgM on a glass slide.
2. Label the respective areas as ‘D’ and also with name or code of the patient.
3. Add 1 drop whole blood sample of RBC-Saline suspension adjacent to each drop of the reagent.
4. Mix the reagent drop and the sample with an applicator stick and spread over an area of about 1 square inch within the circle.
5. Gently tilt the slide forward and backward at room temperature for a maximum of 2 minutes.
6. Read the slides for haemagglutination. Do not interpret fibrin Strands as agglutination.

Microscopic tube test ( for enchanced sensitivity).
Use 8x50 mm small glass test tube.
1. for each specimen, take a tube and label it with the name or code number of the patient.
2. Add one drop of Anti D IgG/IgM and saline to the respective tubes.
3. Add one drop of 2-3% RBC-Saline suspension to each tube.
4. Shake each tube thoroughly and centrifuge for 1 minute at 1000 rpm (125g) or 3400 RPM (1000 g) for 20 secs or incubate at Room Temperature for 1 hour
5. Gently dislodge the sedimeted cells and read for haemagglutination, either macroscopically or microscopically.

Interpretation
Agglutination of red blood cells are interpreted as:
Rh D +ve : Red cells sample positive for haemagglutination with Anti D IgG/IgM.
Rh D –ve : No agglutination of red cells with Anti D IgG/IgM.

Note
If any doubt arises in the interpretation, the entire test should be repeated after thoroughly washing the red cells in saline and resuspending them before use.

Anti D IgG/IgM agglutinates Rh D+ve cells and most of the weaker sub types of Du antigens. A few of the Du antigens may however be negative for direct haemagglutinating reaction .

To detect such weak variant of Du antigen, use a polyclonal anti-D sera or blend of polyclonal and monoclonal sera with IgG and IgM by Coomb’s test procedure.

Precautions
1. The blood drop on the slide should not be allowed to dry, partial drying of the blood could be misinterpreted as agglutination.
2. Centrifugation should be perfect. Over-Centrifugation or under- Centrifugation may result in false negative interpretation.
3. Dislodgement of sediment red cells in tube test should be done as gently as possible, rough dislodgement may disrupt small or weak agglutinates and hence may lead to false negative interpretation.
4. The entire procedure should be carried out at room temperature. Warm or cold antibodies in the tested blood can cause agglutination and may lead to wrong interpretation.
5. Haemolysed blood samples should not be used.
6. Improper antigen antibody concentration may cause false or delayed agglutination.
7. Coomb’s test should be carried out whenever necessary.

References