**Clinical Significance**

D(IgG+IgM) is designed for in-vitro diagnostic and professional use only. It is intended for phenotyping of Rhesus D antigen and its weaker variants including DU. The IgM monoclonal Anti D, a component of this reagent directly agglutinates Rh D positive Red Blood Cells including the higher antigens of DU and the IgG component of this reagent will agglutinate low grade DU antigens by the indirect Coomb's test.

**Source**

D(IgG+IgM) is a blend of two human monoclonal antibodies produced from clones of EBV transformed Human B lymphocytes.

**Storage**

D(IgG+IgM) will be well preserved within utility limit till the expiry date, if stored at temperature between 2 to 8 degree Celsius. Caution: Do not freeze.

**Principle**

The procedure used for the use of this reagent is based on the principle of agglutination of Red blood cells carrying the Rh D antigen in the presence of Anti Rh D antibody.

**Specimen**

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

**Preparation of 10% RBC-Saline Suspension**

**Step 1:** Add approximately 5 volumes of isotonic saline to the whole blood. (Washing of RBCs).

**Step 2:** Centrifuge for 2 minutes.

**Step 3:** Remove the supernatant and wash the sedimented RBC three more times with normal saline as above.

**Step 4:** After final wash, take 100 µl of sedimented red cells and dilute to 1 ml with saline and mix thoroughly before use.

**Procedure**

1. **Macroscopic Slide Test**

   Bring the reagents and samples to room temperature.
   
   **Step 1:** Place 1 drop of D(IgG+IgM) on a glass slide.
   
   **Step 2:** Label the respective area as ‘D’ and also with name or code number of the patient.
   
   **Step 3:** Add 1 drop of whole blood sample or RBC-Saline suspension adjacent to each drop of the reagent.
   
   **Step 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.

   **Step 5:** Gently tilt the slide forward and backward at room temperature for a maximum of 2 minutes.
   
   **Step 6:** Read the slides for haemagglutination. Do not interpret fibrin strands as agglutination.

2. **Microscopic Tube Test (For enhanced sensitivity)**

   **Step 1:** For each specimen, take a tube and label it with the name or code number of the patient. Also mark the tubes for D and saline.
   
   **Step 2:** Add one drop of D(IgG+IgM) and saline to the respective tubes.
   
   **Step 3:** Add one drop of 10% RBC-Saline suspension to each tube.
   
   **Step 4:** Shake each tube thoroughly and centrifuge for 1 minute at 1000 rpm, at room temperature.
   
   **Step 5:** Gently dislodge the sedimented cells and read for haemagglutination, either macroscopically or microscopically.

**Interpretation**

Agglutination of red blood cells are interpreted as:

- **Rh D+ve:** Red cell sample positive for haemagglutination with D(IgG+IgM).
- **Rh D-ve:** No agglutination of red cells with D(IgG+IgM).

**Note 1:** If any doubt arises in the interpretation, the entire test should be repeated after thoroughly washing the red cells in saline and re-suspending them before use.

**Note 2:** 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.

**Note 3:** To detect such weaker variant of DU antigen, do the following indirect DU detection test (Coomb’s test).

**D TEST**

1. Add one drop of D(IgG+IgM) antibody to labelled tube.
2. Add one drop of 10% RBC-saline suspension of the test cell to the above tube.
3. Mix and incubate at 37°C for 15 minutes.
4. Wash the cells once with isotonic saline for 3 times.
5. Add two drops of Anti Human Serum (Coomb's Serum) mix and centrifuge at 1000 rpm for one minute.
6. Gently dislodge the sedimented cells and examine macroscopically or microscopically for agglutination.
**D(IgG+IgM)**

Monoclonal antibodies (Blood Grouping Sera) for Phenotyping Human

**SLIDE AND SALINE TUBE**  

**10 mL**

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**Confirmation**

To confirm the test results use IgG sensitized and non-sensitized Red Blood Cells. Run the tests simultaneously as above. If sensitized cell test is negative or non-sensitized cell test is positive, then the DU test is incorrect and it should be repeated.

**RBC Sensitization**

Confirmed Rh D positive and Rh D negative RBC are individually mixed with D (IgG) to serve as positive and negative control cells.

1. Take 2 volumes of Anti D (IgG) and 2 volumes of washed Rh positive and negative cells individually in two tubes.
2. Incubate at 37°C for one hour.
3. Remove the supernatant carefully and wash the cells gently with saline for 3 times.
4. Cells are ready for D test control.

**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. Dislodgment of sedimented red cells in tube test should be done as gently as possible, rough dislodgment 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. Most of the weaker sub types of DU antigens. A few of the DU antigens may however be negative for direct reaction.

**Presentation**

Ready to use vials/bottles containing antibody solution preserved in 0.1% sodium azide.

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<th>Code No</th>
<th>Reagent</th>
<th>Pack Size</th>
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<tbody>
<tr>
<td>MBG 032</td>
<td>D(IgG+IgM)</td>
<td>10 mL</td>
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**For Further Readings**


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