**Anti-D Monoclonal (IgG+IgM) Reagent**

**PRINCIPLE**
The presence of the D antigen is determined by testing the test blood cells against the antibody with a known anti-D specificity. The reagent will cause direct agglutination of test red cells that carry the D antigen and indirect agglutination of test red cells that are Category D VI in the antiglobulin (AHG) phase of testing. The agglutination of the red blood cells being tested indicates the presence in them of the corresponding antigen. No agglutination generally indicates the absence of the D antigen.

<table>
<thead>
<tr>
<th>Anti-D</th>
<th>Phenotype</th>
<th>Caucasians %</th>
<th>Afro-Americans %</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Rh D positive</td>
<td>85</td>
<td>72</td>
</tr>
<tr>
<td>0</td>
<td>Rh D negative</td>
<td>15</td>
<td>28</td>
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</tbody>
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The D antigen is the most clinically significant non-ABO red blood cells antigen and has been implicated in causing Haemolytic Transfusional Reactions and Haemolytic Disease of the Newborn.

**REAGENT COMPOSITION**
Rapid Labs Monoclonal Anti-D blood grouping reagent is a low protein, blended reagent containing a human monoclonal IgM and IgG anti-D:

This reagent will directly agglutinate Rh D positive cells, including majority of variants (but not D⁰ and a high proportion of weak D (D¹).

**Precautions:** Components of different human origin have been tested and found to be negative for the presence of antibodies anti-HIV 1+2 and anti-HCV, as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious. Protective clothing should be worn when handling the reagent, such as disposable gloves. **Warning:** The reagents in this kit contain sodium azide. Do not allow to contact with skin or mucous membranes.

**WEAKENED EXPRESSION OF THE RhD ANTIGEN**
The collective term D” is widely used to describe red cells, which have a weaker expression of the D antigen than normal. The term weak D denotes individuals with a reduced number of complete D antigen sites per red cell. The term partial D denotes individuals with missing D antigen epitopes. D VI is a partial D category, which misses most D epitopes. The reagent will detect most examples of partial and weak D red cells by direct agglutination, but will not detect D VI cells. This reagent will detect D VI and partial D cells in the AHG phase.

**Reagent Preparation**
The Rapid Labs anti-D reagent is ready to use.

**STORAGE AND STABILITY**
1. The reagents will remain stable until the expiry date printed on the label, when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not use the reagents after the expiration date.
2. Do not freeze or expose to elevated temperatures. Prolonged storage outside the recommended temperature range may result in accelerated loss of reagent reactivity.

3. This product should be clear. Turbidity may indicate microbial contamination. Do not use the reagents if a precipitate is present.
4. If a vial is cracked or leaking, discard the contents immediately.

**SAMPLES**
The blood samples can be collected with or without anticoagulant. They must be tested as soon as possible. Samples collected into EDTA or heparin should be tested within 48 hours. Blood collected into ACD, CPD, CPDA-1 may be typed up to 35 days from the date of withdrawal. Store at 2-8°C.

**MATERIAL REQUIRED**
- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Pasteur pipettes.
- Centrifuge or similar.
- Glass slides.
- Applicator sticks.

**ADDITIONAL REAGENT REQUIRED**
Phosphate Buffered Saline (PBS): 8.5 to 9.0 g/L NaCl (0.145-0.154 mol/L) pH 7.0±0.2 at 22 ±1°C.

Test red cells. Positive (ideally group R, r) and negative (group rr)

Bring all reagents up to room temperature.

**TEST PROCEDURES**

1. **SLIDE TEST**
   1. Place 1 drop (40-50 μL) of resuspended whole blood (approx. 35-45% cell concentration) to be tested on a slide.
   2. Add 1 drop (40-50 μL) of reagent next to the blood sample.
   3. Mix the reagent and cells with an applicator stick, over an area of about 2 cm. in diameter.
   4. Rotate the slide gently and continuously during a 3-minute period.

2. **Reading**
   - Examine macroscopically for agglutination.
   - **Negative reaction:** No visible agglutination after 5 minutes
   - **Positive reaction:** Positive red cells agglutinate in a few seconds. Do not mistake fibrin strands as agglutination.
   - Any weak reactions should be repeated by the tube technique.

3. **Tube Test (Not recommended Category D VI**
   1. Prepare a 2-3% suspension of red cells washed (twice with PBS) in PBS.
   2. Place 1 drop (40-50 μL) of reagent into an appropriate test tube.
   3. Add 1 drop (40-50 μL) of washed red cells.
   4. Mix and centrifuge for 20 sec. at 1000 r.c.f. or for a suitable alternative time and force.

5. **Reading**
   - Gently resuspend each cell button and read macroscopically for agglutination.
   - **Negative reaction:** A smooth homogeneous suspension indicates a negative reaction.
   - **Positive reaction:** Agglutination of the red cells indicates a positive reaction.

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**CATALOGUE NUMBERS**
BG-DBlend10 Anti-D Monoclonal (IgG+IgM) 10ml
BG-DBlend1L Anti-D Monoclonal (IgG+IgM) 1000ml

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Any tube showing a negative or a questionable test result should be incubated for 15 minutes at 18-25°C.
Following incubation, repeat steps 4 and 5.

Tube test (Recommended Category D) AGH (D Test)

1. Follow steps 1, 2 and 3. Tube test.
2. Mix thoroughly and incubate at 37°C for 15 minutes. Wash test red cells 4 times with PBS, taking care to:
3. Decant saline between washes and resuspend each cell button after each wash. Completely decant saline after last wash.
4. Add 1 drop of anti-human globulin to each dry cell button.
5. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or a suitable alternative time and force.
6. Reading
See test Tube 2. The antiglobulin tube technique can only be considered valid if all negative tests react positively with IgG sensitised red cells.

Interpretation

1. Positive reaction: Agglutination of the test red cells constitutes a positive test result within accepted limitations of test procedure, and indicates the presence of D antigen on the test red cells.
2. Negative reaction: No agglutination of the test red cells constitutes a negative result within the accepted limitations of the test procedure, and indicates the absence of the D antigen on the test red cells.
3. Test results of cells that are agglutinated using the reagent negative control shall be excluded, as the agglutination is most probably caused by the effect of the macromolecular determinants in the reagent on sensitised cells.

Quality controls and advice

- It is recommended a positive control (ideally group R1 cells) and a negative control (ideally group R cells) be tested in parallel with each batch of tests.
- Tests must be considered invalid if controls do not show expected results.
- When typing red cells from a patient it is important that a reagent negative control is included since the macromolecular determinants in the reagent may cause false positive reactions with IgG coated cells.
- Read all tests tube straight after centrifugation.
- Complete washing steps without interruption and centrifuge and read tests immediately after addition of anti-human globulin because delays may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.
- Slide tests should be interpreted within two minutes to ensure specificity and to avoid the possibility a negative result may be incorrectly interpreted as positive due to drying of the reagent.
- Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.
- Use of reagents and interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagents are in use.
- The user must determine the suitability of the reagents for use in other techniques.

Analytical performance

- The reagent has been characterised by all the procedures mentioned in the procedure.
- Prior to release, each lot of monoclonal Anti-D is tested by the procedure against a panel of antigen-positive red cells to ensure suitable reactivity.
- The potency of the reagents is tested for against the following minimum potency reference standards obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-D reference standard 91/592

Limitations of the procedure

- Anti-D is not suitable for use with enzyme treated cells or cells suspended in LSS.
- Stored blood may give weaker reactions than fresh blood.
- False positive or false negative results may occur due to:
  - Contamination of test materials
  - Improper incubation time or temperature
  - Improper or excessive centrifugation
  - Improper storage of test materials or omission of reagents

References


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Rev 01
www.rapidlab.co.uk Email: medical@rapidlabs.co.uk 21/04/2012

For in vitro diagnostic use
Expiry date CCYY-MM-DD
Lot number
Catalog number
Storage temperature limitation
Consult Instruction for use (IFU)
Manufacturer