Monoclonal Blood Grouping Reagents
Anti-D (IgG + IgM)

INTENDED USE
These reagents are suitable for use by the slide and tube techniques and are designed for the use by operators trained in serological techniques.

PRINCIPLE
The presence of the D antigen is determined by testing the test blood cells against the antibody with 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 DVI 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>
</tr>
</tbody>
</table>

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.

WEAKENED EXPRESSION OF THE RH D ANTIGEN
The collective term Da 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. DVI 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 DVI cells. This reagent will detect DVI and partial D cells in the AHG phase.

MATERIALS
Anti-D blood grouping reagent is a low protein blended reagent containing a human monoclonal IgM and IgG Anti-D (Anti-D Blend). This reagent is made in a phosphate buffer solution containing bovine albumin. The reagent is supplied at the optimal dilution for use on patient samples with all recommended techniques. There is no need for further dilution or addition of this reagent.

Materials required but not provided:
- Glass test tubes (10x75mm or 12x75mm)
- Pasteur pipettes
- Centrifuge (or similar)
- Glass slides
- Applicator sticks

Additional reagent required:
- Phosphate Buffered Saline (PBS): 8.5/9.0g/L NaCl (0.145-0.154mol/L) – pH 7.0±0.2 at 22±1°C
- Test red cells – Positive (ideally group Rh’r) and negative (group r)

All reagents must be brought up to room temperature before use.

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.

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.

SPECIMEN COLLECTION AND PREPARATION
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.

INSTRUCTIONS FOR USE
1. Slide Test
   1.1 Place 1 drop (40-50 µL) of re-suspended whole blood (approx. 35-45% cell concentration) to be tested on a slide.
   1.2 Add 1 drop (40-50 µL) of reagent next to the blood sample.
   1.3 Mix the reagent and cells with an applicator stick, over an area of about 2 cm in diameter.
   1.4 Rotate the slide gently and continuously during a 3-minute period.
   1.5 Reading
      Examine macroscopically for agglutination.

Negative reaction:
No visible agglutination after 3 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.

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

Reading
Gently resuspend each cell button and read macroscopically for agglutination.
Monoclonal Blood Grouping Reagents
Anti-D (IgG + IgM)

**Negative reaction:**
A smooth homogeneous suspension indicates a negative reaction.

**Positive reaction:**
Agglutination of the red cells indicates a positive reaction.

Any tube showing a negative or a questionable test result should be incubated for 15 minutes at 18-25°C. Following incubation, repeat steps 2.4 and 2.5.

3. Tube test (Recommended Category D(IgG) AHG (D(+) Test)

3.1 Follow steps 2.1, 2.2 and 2.3 from the Tube test 2.

3.2 Mix thoroughly and incubate at 37°C for 15 minutes. Wash test red cells 4 times with PBS, taking care to decant saline between washes and re-suspend each cell button after each wash. Completely decant saline after last wash.

3.4 Add 2 drops of AHG to each dry cell button.

3.5 Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or a suitable alternative time and force.

3.6 Reading
See Tube test 2.

The AHG tube technique can only be considered valid if all negative tests react positively with IgG sensitised red cells.

**INTERPRETATION OF RESULTS**

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.

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 potentiators in the reagent on sensitised cells.

**QUALITY CONTROL**

- It is recommended a positive control (ideally group Rio cells) and a negative control (ideally group ri 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 potentiators 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 centrifugation and re-test 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 technique.

**ANALYTICAL PERFORMANCE**

The performance of this reagent has been validated by the test procedures defined in the instructions for use. Any deviations from these techniques are the responsibility of the user.

**LIMITATIONS**

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

**BIBLIOGRAPHY**


**Index of Symbols**

- Consult instructions for use
- For in vitro diagnostic use only
- Catalogue Number
- Lot Number
- Store between 2-8°C
- Use by
- Date of manufacture
- Manufactured by: Rapid Labs Ltd

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Colchester • Essex CO7 8SD • United Kingdom

Email: medical@rapidlabs.co.uk Website www.rapidlabs.co.uk

Revision 4
Monoclonal Blood Grouping Reagents
Anti-D (IgG + IgM)

INTENDED USE
These reagents are suitable for use by the slide and tube techniques and are designed for the use by operators trained in serological techniques.

PRINCIPLE
The presence of the D antigen is determined by testing the test blood cells against the antibody with 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 D⁶ 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.

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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. D⁶ 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 D⁶ cells. This reagent will detect D⁴ and partial D cells in the AHG phase.

MATERIALS
Anti-D blood grouping reagent is a low protein blended reagent containing a human monoclonal IgM and IgG Anti-D (Anti-D Blend). This reagent will directly agglutinate Rh D positive cells, including a majority of variants (not D⁴) a high proportion of weak D (D⁴). Rapid Labs Anti-D reagent is ready to use.

Materials required but not provided
- Glass test tubes (10x75mm or 12x75mm)
- Pasteur pipettes
- Centrifuge (or similar)
- Glass slides
- Applicator sticks

Additional reagent required
- Phosphate Buffered Saline (PBS): 8.5/9.0g/L NaCl (0.145-0.154mol/L) – pH7.0±0.2 at 22±1°C
- Test red cells – Positive (ideally group R¹r) and negative (group rr)
All reagents must be brought up to room temperature before use

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1.4 Rotate the slide gently and continuously during a 3-minute period.
1.5 Reading
Examine macroscopically for agglutination.
Negative reaction:
No visible agglutination after 3 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.
2. Tube test (Not Recommended Category D⁴)
2.5 Prepare a 2-3% suspension of red cells (washed twice with PBS) in PBS.
2.6 Place 1 drop (40-50μL) of reagent into an appropriate test tube.
2.7 Add 1 drop (40-50μL) of washed red cells.
2.8 Mix and centrifuge for 20 sec. at 1000 r.c.f. or for a suitable alternative time and force.
2.9 Reading
Gently resuspend each cell button and read macroscopically for agglutination.
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3.8 Mix thoroughly and incubate at 37º C for 15 minutes. Wash test red cells 4 times with PBS, taking care to
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3.11 Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or a suitable alternative time and force.
3.12 Reading
See Tube test 2.
The AHG tube technique can only be considered valid if all negative tests react positively with IgG sensitised red cells.

INTERPRETATION OF RESULTS
3. 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.

4. 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.

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QUALITY CONTROL
- It is recommended a positive control (ideally group R1r cells) and a negative control (ideally group rR 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 potentiators in the reagent may cause false positive reactions with IgG coated cells.
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BIBLIOGRAPHY

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<table>
<thead>
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<th>Description</th>
</tr>
</thead>
<tbody>
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<td>Consult instructions for use</td>
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<tr>
<td>2</td>
<td>Manufacturer</td>
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<td>Store between 2-8°C</td>
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<td>4</td>
<td>For in vitro diagnostic use only</td>
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Email: medical@rapidlabs.co.uk Website www.rapidlabs.co.uk

Revision 4