

MONOCLONAL ANTI-D (IgM) BLOOD GROUPING SERA

For use by slide, tube and microplate methods

INTRODUCTION

First described in 1939, the RhD antigen is surpassed in importance only by the antigens of the ABO blood group system. Transfusion of RhD positive blood to a RhD negative recipient or failure to administer prophylactic anti-D to an RhD negative woman can result in the production of anti-D. Consequently, establishing the correct RhD group is fundamental to safe transfusion practice. Certain individuals exhibit a quantitative reduction in the expression of their RhD antigen and are categorised as weak D (D^w). Others display a qualitative variation in D antigen expression and are referred to as D category or partial D. Weak D individuals may also be partial D.

The recent availability of potent high quality IgM monoclonal anti D reagents and a greater awareness of the clinical importance of partial D phenotypes, especially D^{VI}, has resulted in a change to RhD testing policies in the UK

Anti-D Human Monoclonal IgM Sera is a low protein sera formulated for use by slide, rapid tube and microplate methods. Use of the sera by suitable methods will detect D_{weak} (D^w) phenotypes.

PRINCIPLE OF THE SERA AND TEST PROCEDURE

The test procedures recommended for the use of this sera are based upon the agglutination (clumping) of red blood cells carrying the D antigen in the presence of an IgM Anti-D antibody. The sera has been optimised for use as supplied by the recommended techniques without further dilution or additions.

PRECAUTIONS

1. All blood products should be treated as potentially infectious. The source used to produce this sera has been tested and found to be negative for HIV and HCV antibodies and HBsAg in microbiological tests required by the UK. No known regime of testing can completely guarantee that any product derived from human blood is incapable of transmitting infectious agents. Care should be exercised in the use and the disposal of the container and its contents.
2. The sera contains less than 0.1% w/v sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form highly toxic explosive salts. On disposal flush with large quantities of water.
3. The reagent should be stored at 2-8°C at all times. The product should be clear. Turbidity may indicate bacterial contamination. The product should not be used beyond the stated expiry date.
4. For *in vitro* diagnostic use only.
5. **UNDER NO CIRCUMSTANCES SHOULD THE PRODUCT BE DILUTED PRIOR TO USE.**

COMPOSITION OF REAGENT

The main component of this reagent is derived from the *in vitro* culture of the IgM secreting human/mouse heterohybridoma LDM1. The formulation also contains EDTA and less than 0.1% sodium azide.

ADDITIONAL REAGENTS REQUIRED

PBS pH 7.0 +/- 0.2

LISS, Low Ionic Strength Saline

Reagent red cells for use in RhD grouping

RhD reagent control

ADVICE TO USERS

It is recommended that a positive control (ideally R_{1r} cells) and a negative control (rr cells) should be tested in parallel with each batch of tests. It is not required to use a diluent control in parallel with all tests using this sera. Only in typing the red cells of patients known to have auto-antibodies or protein abnormalities or apparently low grade D^w is use of a diluent control recommended. This should be tested in parallel with the sera.

RECOMMENDED TECHNIQUES

1. Slide Techniques

- 1.1 Prepare a 30–45% suspension of red blood cells in homologous plasma/serum or PBS pH 7.0 may be used.
- 1.2 To one volume of sera on a clean, labelled glass slide add an equal volume of the test red cell suspension.
- 1.3 Mix well by rocking slide for 30 seconds and incubate the test for 5 minutes with occasional mixing.
- 1.4 Read macroscopically for agglutination. This may be facilitated by the use of a diffuse light source.

Note: Do not confuse drying effects with weak positive results.

2. Tube Technique – Immediate spin

- 2.1 To one volume of sera in a labelled test tube, add an equal volume of a 2–3% suspension of test red blood cells in PBS pH 7.0 or 1.5 – 2% in LISS.
- 2.2 Mix the test well.
- 2.3 Centrifuge immediately at 1000g for 10 seconds or for a suitable alternative force and time.
- 2.4 Gently agitate the tube to dislodge the red cells and examine macroscopically for agglutination.

3. Tube Technique – LISS

- 3.1 To one volume of sera in a labelled test tube add an equal volume of a 1.5-2% suspension of test red blood cells in LISS.
- 3.2 Mix the test well and incubate at 37°C for 15-20 minutes.
- 3.3 Centrifuge immediately at 1000g for 10 seconds or for a suitable alternative force and time.
- 3.4 Gently agitate the tube to dislodge the cells and examine macroscopically for agglutination.

QUALITY CONTROL

Quality control of reagents is essential and should be performed with each series of groups and with single groups. It is recommended that the following cell samples are used to control the reactions of these reagents. Other cell types may be suitable but should be selected with care.

OR_{1r} red cells should be used as positive control.

Orr red cells should be used as negative control.

A reagent control is required for this anti D

TECHNICAL NOTES

The quantity of D antigen expressed by weak D individuals varies considerably. Whilst this anti D reagent will directly agglutinate red cells from most weak D individuals, if it is considered important to test for weak D, a reagent specifically prepared for that purpose should be used.

This reagent is designed to be used in conjunction with Anti D Blend reagent to optimise the detection of weak D (D^u) and D variant red cells.

Some weak D and/or partial D samples will not react with monoclonal anti D reagents.

The expression of certain red cell antigens may diminish in strength during storage, particularly in EDTA and clotted samples. Better results will be obtained with fresh samples.

LIMITATIONS

Some examples of D_{weak} or partial D may not be directly agglutinated when using the slide method. Indeterminate results in the slide method should be confirmed using a tube method.

False positive or false negative results may occur through contamination of test materials or any deviation from the recommended technique.

Certain tests performed on unwashed samples (e.g. cord) or samples stored and tested at 20C or below may exhibit false positive reactions due to the potentiators used in the formulation of this reagent.

REFERENCES:

1. Widmann F.K. ed Technical Manual 10th ED Washington DC, American Association of Blood Banks 1990, Chapter 11.
2. Race R.R and Sanger R. Blood Groups in Man, 6th Edition Oxford Blackwell Scientific Publishers 1975:178 3.Issit P.D. Applied Blood Group Serology 3rd Edition, Montgomery Scientific Publications, Miami, Florida, USA, 1985, Chapter 10.

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