INTRODUCTION

Since the description of the RhD antigen by Levine and Stetson in 1939, more than 40 other Rh antigen complexes have been identified. With the exception of C, c, E and e, and perhaps C\(^*\), few of these antigens or their corresponding antibodies are encountered in routine testing. Rh antigens are controlled by a series of closely linked loci on chromosome 1, the genetic contribution from each parent being inherited as a haplotype eg Cde, cDE etc. Used separately, anti-Rh blood grouping reagents will indicate whether an individual expresses the corresponding antigen - an essential procedure in the determination of antibody specificity and selection of blood for transfusion of patients with Rh antibodies. Testing red cell samples with anti-C, anti-D, anti-E, anti-c and anti-e will disclose the Rh phenotype from which the most probable genotype may be deduced. Knowing the probable paternal genotype can be of value in the management of Rh haemolytic disease of the foetus and newborn where R\(_D\) infants are likely to be more severely affected than are R\(_d\) infants. Probable genotype information can also be useful in establishing antibody specificity and in selecting blood for transfusion of patients with Rh antibodies.

REAGENT DESCRIPTION

The main component of this reagent is derived from the in vitro culture of the IgM secreting human/mouse heterohybridoma DEM1. The diluent formulation contains 0.8g/l EDTA, 20g/l BSA and 1g/l sodium azide in phosphate buffered saline. The volume delivered by the reagent dropper bottle is approximately 40\(\mu\)l; bearing this in mind, care should be taken to ensure that appropriate serum: cell ratios are maintained in all test systems. This reagent complies with the Common Technical Specifications for products defined in Annex II, List A of Directive 98/79/EC on in vitro Diagnostic Medical Devices and the recommendations contained in the Guidelines for Blood Transfusion Services in the United Kingdom.

TEST PROCEDURES

This reagent has been standardised for use by the techniques described below and therefore its suitability for use in other techniques cannot be guaranteed.

UKNEQAS exercises for blood group serology have demonstrated the importance of incorporating a reagent control in blood grouping tests where a potentiator is incorporated in the reagent formulation or is required to be added by the user. The reagent control should reflect the formulation of the reagent being used. For this reagent a satisfactory reagent control may be achieved by substituting inert AB serum, 8-10% BSA in saline or the patient's own serum for the blood grouping reagent in the procedure chosen for use.

ADDITIONAL MATERIALS AND REAGENTS REQUIRED

- PBS pH 7.0 ± 0.2
- LISS
- Reagent red cells suitable for the control of Anti-E
- 12 x 75mm glass test tubes
- Glass slides
- Pipettes
- Centrifuge
RECOMMENDED TECHNIQUES

Tube Technique - 5 Minute Incubation / Spin

. Add 1 volume of blood grouping reagent to a 12 x 75mm test tube.
. Add 1 volume of red cells suspended to 2-3% in PBS pH 7.0 ± 0.2 or 1.5-2% in LISS.
. Mix the test well and incubate at 37°C for 5 minutes.
. Centrifuge at 1000g for 10 seconds or at a suitable alternative g force and time.
. Gently shake the tube to dislodge the cell button from the bottom and observe macroscopically for agglutination.

Tube Technique - 15 Minute Incubation / Spin

. Add 1 volume of blood grouping reagent to a 12 x 75mm test tube.
. Add 1 volume of red cells suspended to 2-3% in PBS pH 7.0 ± 0.2 or 1.5-2% in LISS.
. Mix the test well and incubate at 37°C for 15 minutes.
. Centrifuge at 1000g for 10 seconds or at a suitable alternative g force and time.
. Gently shake the tube to dislodge the cell button from the bottom and observe macroscopically for agglutination.

Slide Technique

. Add 1 volume of blood grouping reagent to an appropriately prepared area of a glass slide eg a wax pencil oval.
. Add 1 volume of red cells suspended to 30-45% in PBS pH 7.0 ± 0.2 or in group homologous plasma/serum.
. Mix well by rocking the slide for approximately 30 seconds and incubate the test for 5 minutes at room temperature with occasional mixing.
. Observe macroscopically for agglutination. This may be facilitated by reading over a diffuse light source.

INTERPRETATION OF RESULTS

Agglutination = positive test result
No agglutination = negative test result

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 red cell samples are used to control the reactions of this reagent. Other red cell types may be suitable but should be selected with care.

O, R, red cells should be used as a positive control.
O, r red cells should be used as a negative control.

PERFORMANCE LIMITATIONS

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.

Slide tests are not recommended for detection of weak subgroups. All slide tests should be confirmed by tube grouping.

Tests should be read by a ‘tip and roll’ procedure. Excessive agitation may disrupt weak agglutination and produce false negative results.

It is important to use the recommended g force during centrifugation as excessive centrifugation can lead to difficulty in resuspending the cell button, while inadequate centrifugation may result in agglutinates that are easily dispersed.

False positive or false negative results can occur due to contamination of test materials, improper reaction temperature, improper storage of materials, omission of test reagents and certain disease states.

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For further information or advice please contact your local distributor.

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