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 Cw, few of these antigens or their corresponding antibodies are encountered in routine testing. Rh antigens are probably 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. Testing red cell samples with anti-Rh antibodies is an essential procedure in the determination of antibody specificity and in selecting 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 RhD haemolytic disease of the foetus and newborn where Rh infants are likely to be more severely affected than are Rh 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

This reagent has been prepared from plasma collected from blood donors. ABO haemagglutinins were removed by adsorption. Conversion to serum was achieved by the addition of calcium chloride and where necessary, thrombin. Excess calcium was removed by the addition of sodium oxalate. The formulation also contains 1g/L sodium azide. The volume delivered by the reagent dropper bottle is approximately 40µl; 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 requirements 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.

STORAGE CONDITIONS

The reagent should be stored at 2°C - 8°C. Do not use if turbid. Do not dilute. The reagent is stable until the expiry date stated on the product label.

PRECAUTIONS FOR USE AND DISPOSAL

This reagent contains 0.1% sodium azide (EC No.247-852-1) and is classified as harmful. R22 - Harmful if swallowed. Sodium azide may react with lead and copper plumbing to form explosive compounds. If discarded into sink, flush with a large volume of water to prevent azide build-up.

CAUTION: SOURCE MATERIAL FROM WHICH THIS PRODUCT IS DERIVED WAS FOUND NON-REACTIVE FOR HBsAg, ANTI-HIV 1/2 AND ANTI-HCV. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS DISEASE. APPROPRIATE CARE SHOULD BE TAKEN IN THE USE AND DISPOSAL OF THIS PRODUCT.

This reagent is for in vitro professional use only.

SPECIMEN COLLECTION AND PREPARATION

Specimens should be collected by aseptic technique with or without an anticoagulant. The specimen should be tested as soon as possible after collection. If testing is delayed, the specimen should be stored at 2°C - 8°C. Blood specimens exhibiting gross haemolysis or contamination should not be used. Clotted samples or those collected in EDTA should be tested within seven days from collection. Donor blood stored in citrate anticoagulant may be tested until the expiry date of the donation.

TEST PROCEDURES

TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS DISEASE. APPROPRIATE CARE SHOULD BE TAKEN IN THE USE AND DISPOSAL OF THIS PRODUCT.

Additional Information

This reagent has been standardised for use by the techniques described below and therefore its suitability for use in other techniques cannot be guaranteed. Users are advised to carefully confirm reagent suitability before using alternative techniques.

UKEQAS 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.

Users should confirm that the batch of bromelin used in conjunction with this reagent produces satisfactory results eg, by testing the reagent and bromelin by the chosen recommended method against an antibody identification panel.

ADDITIONAL MATERIALS AND REAGENTS REQUIRED

- PBS pH 7.0 ± 0.2
- LISS
- 0.5% Bromelin
- Reagent Control
- Reagent red cells suitable for the control of Anti-Cw
RECOMMENDED TECHNIQUES

LOW IONIC STRENGTH (LISS) TESTS

LISS, One Stage Bromelin
- Add 1 volume of blood grouping reagent to a 12 x 75mm glass test tube.
- Add 1 volume of red cells suspended to 1.5-2% in LISS.
- Add 1 volume of bromelin.
- 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 in order to dislodge the cell button from the bottom.
- Observe macroscopically for agglutination.

LISS, One Stage Bromelin - Rapid Method
- Add 1 volume of blood grouping reagent to a 12 x 75mm glass test tube.
- Add 1 volume of red cells suspended to 1.5 - 2% in LISS.
- Add 1 volume of bromelin.
- Mix 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 in order to dislodge the cell button from the bottom.
- Observe macroscopically for agglutination.

NORMAL IONIC STRENGTH (NIS) TESTS

NIS, One Stage Bromelin
- Add 1 volume of blood grouping reagent to a 12 x 75mm glass test tube.
- Add 1 volume of red cells suspended to 2-3% in PBS pH 7.0 ± 0.2.
- Add 1 volume of bromelin.
- Mix 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 in order to dislodge the cell button from the bottom.
- Observe macroscopically for agglutination.

NIS, One Stage Bromelin - Rapid Method
- Add 1 volume of blood grouping reagent to a 12 x 75mm glass tube.
- Add 1 volume of red cells suspended to 2-3% PBS pH 7.0 ± 0.2.
- Add 1 volume of bromelin.
- Mix 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 in order to dislodge the cell button from the bottom.
- Observe macroscopically for agglutination.

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. As a minimum a positive and a negative control should be used.

C⁺ positive red cells should be used as a positive control.
R₁R₁ red cells should be used as a negative control.

PERFORMANCE LIMITATIONS

Since the antibodies from which this product has been prepared were stimulated by red blood cells, extensive tests have been undertaken to exclude the presence of additional contaminating blood group antibodies. However, it is impossible to state categorically that reagents of this nature will only contain antibodies of the required specificity.

The maximum recommended incubation time for this reagent should be strictly observed. Prolonged incubation with highly active enzyme reagents will result in cleavage of IgG antibody in the reagent and subsequent loss of activity.

Reagent controls must be incorporated to prevent the C⁺ group of autoagglutinated or IgG coated red cells being misinterpreted as a result of false positive reactions.

Driblocks and waterbaths promote better heat transfer and are recommended for 37°C tests, particularly where the incubation period is 30 minutes or less.

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

In tube tests 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.

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.

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.

UK frequencies: C⁺ pos 1%.