

PACKAGING

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| Ref. 101-0521 | Cont.: 10 mL |
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Store at 2-8° C

CLINICAL SIGNIFICANCE

Levine and Stetson discovered the Rh blood group system in 1940. The D antigen is the most clinically significant non-ABO red blood cell antigen and the corresponding antibodies have been implicated in causing both Transfusion Reactions and Haemolytic Disease of the Newborn.

| Anti-D | Phenotype | Caucasians % | Afro-Americans % |
|--------|-----------|--------------|------------------|
| + | Rh D +ve | 85 | 72 |
| 0 | Rh D -ve | 15 | 28 |

Weakened expression of the RhD antigen

The collective term D^w 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 IAT phase.

PRINCIPLE

The reagent will cause direct agglutination (clumping) of test red cells that carry the D antigen and indirect agglutination of test red cells that are Category D^{VI} in the antiglobulin phase of testing. No agglutination generally indicates the absence of the D antigen (see **Limitations**).

REAGENTS

Monoclonal Anti-D blood grouping reagent is a low protein, blended reagent containing human monoclonal IgM and IgG diluted in a phosphate buffer containing sodium chloride (0.9 %), bovine albumin (3 %) and macromolecular potentiators. When typing patient samples, this reagent will directly agglutinate Rh D positive cells, including majority of variants (**but not D^{VI}**) and a high proportion of weak D (D^w) phenotypes when using the recommended techniques. The reagent is supplied at optimal dilution for use on patient samples with all recommended techniques stated below without need for further dilution or addition. For lot reference number and expiry date see **Vial Label**.

| IgM / IgG | Cell Line / Clone |
|-----------|-------------------|
| IgM | RUM-1 |
| IgG | MS-26 |

STORAGE

Do not freeze. Reagent vials should be stored at 2 - 8° C on receipt. Prolonged storage at temperatures outside this range may result in accelerated loss of reagent reactivity.

MATERIAL REQUIRED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Test tube centrifuge.
- Water bath or dry heat incubator equilibrated to 37° C ± 2° C.
- Volumetric pipettes.
- Glass microscope slides.
- Applicator sticks.
- Phosphate Buffered Saline (PBS): NaCl 0.9 %, pH 7.0 ± 0.2 at 22° C ± 1 °C.
- Positive (ideally R_{1r}) and negative (rr) control red cells.
- DiaMed ID-Cards (Neutral).
- DiaMed ID-Cards (AHG/Coombs).
- DiaMed ID-Centrifuge.
- DiaMed ID-Diluent: e.g. ID-CellStab.
- DiaMed ID-Incubator equilibrated to 37° C ± 2° C.
- Plate shaker.
- Automatic plate reader.
- Validated "U" well microplates.
- Microplate centrifuge.

SAMPLE

Blood samples drawn with or without anticoagulant may be used for antigen typing. If testing is delayed then store specimens at 2-8° C. EDTA and citrate samples should be typed within 48 hours. Samples collected into ACD, CPD or CPDA-1 may be tested up to 35 days from the date of withdrawal. All blood samples should be washed at least twice with PBS before being tested. Blood samples showing evidence of lysis may give unreliable results.

PRECAUTIONS

1. The reagents is intended for *in vitro* diagnostic use only.
2. If a reagent vial is cracked or leaking, discard the contents immediately.
3. Do not use the reagent past the expiration date (see **Vial Label**).
4. Do not use the reagent if a precipitate is present.
5. Protective clothing should be worn when handling the reagent, such as disposable gloves and a laboratory coat.
6. The reagents have been filtered through a 0.2 µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
7. The reagents contain < 0.1 % sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive metal azides. On disposal flush away with large volumes of water.
8. Materials used to produce the products were tested at source and found to be negative for HIV 1+2 and HCV antibodies and HBsAg using approved microbiological tests.
9. No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Care must be taken in the use and disposal of each vial and its contents.
10. For information on disposal of the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

NOTES

1. It is recommended a positive control (ideally R_{1r} cells), a negative control (ideally rr cells) and a reagent negative control be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. 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.
3. Test samples for category D^{VI} determination by the **Indirect Antiglobulin and Coombs DiaMed-ID Techniques** only.
4. Weak and variant D antigens are poorly detected by gel card, microtitre plate and slide techniques. It is recommended that that weak and partial variants are tested using the tube test technique.
5. The antiglobulin tube technique can only be considered valid if all negative tests react positively with IgG sensitised red cells.
6. In the here recommended techniques one volume is approximately 40µl when using the vial dropper provided.
7. The 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.
8. The user must determine suitability of the reagents for use in other techniques.

PROCEDURE (NOT CATEGORY D^{VI})

A. Tube Method

1. Prepare a 2-3 % suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume of Anti-D reagent and 1 volume test red cell suspension.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf or for a suitable alternative time and force.
4. Gently resuspend red cell button and read macroscopically for agglutination
5. Any tubes, which show a negative or questionable result (which can happen with D^w or weak D samples), should be incubated for 15 minutes at room temperature.
6. Following incubation, repeat steps 3 and 4.

B. Slide Method

1. Prepare a 35-45 % suspension of test red cells in serum, plasma or PBS.
2. Place on a labelled glass slide: 1 volume of Anti-D reagent and 1 volume of test red cell suspension.
3. Using a clean applicator stick, mix reagent and cells over an area of about 20 x 40 mm.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2-minute period, maintaining slide at room temperature.
5. Read macroscopically after 2 minutes over a diffuse light and do not mistake fibrin strands as agglutination.
6. Any weak reactions should be repeated by the tube technique.

B. DiaMed-ID Micro Typing Technique

1. Prepare a 0.8% suspension of washed test red cells in an ID-Diluent.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50µl of test red cell suspension and 25µl of Anti-D reagent.
4. Centrifuge the ID-Card(s) for 10 minutes at 90 rcf or for a suitable alternative time and force.
5. Read macroscopically for agglutination.

C. Microplate Technique, using "U" wells

1. Prepare a 2-3 % suspension of washed test red cells in PBS.
2. Place in the appropriate well: 1 volume of Anti-D reagent and 1 volume test red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-well contamination.
4. Incubate at room temperature for 15 minutes (time dependant on user).
5. Centrifuge the microplate for 1 minute at 140 rcf or for a suitable alternative time and force.
6. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker
7. Read macroscopically or with a validated automatic reader.
8. Any weak reactions should be repeated by the tube technique.

RECOMMENDED TECHNIQUES (TO DETECT CATEGORY D^V)**A. Indirect Antiglobulin Technique (IAT)**

1. Prepare a 2-3 % suspension of washed test red cells in PBS.
2. Place in a labelled test tube: 1 volume of Anti-D reagent and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at 37° C for 15 minutes.
4. Wash test red cells 4 times with PBS, taking care to decant saline between washes and resuspend each cell button after each wash. Completely decant saline after last wash.
5. Add 2 drops of anti-human globulin or anti-IgG to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000 rcf for a suitable alternative time and force.
7. Resuspend each cell button and read macroscopically.

B. DiaMed-ID Micro Typing Technique (AHG/Coombs cards)

1. Prepare 0.8 % suspension of washed test red cells in ID-Diluent.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtube: 50 µl of test red cell suspension and 25 µl of Anti-D reagent.
4. Incubate the ID-Card(s) for 15 minutes at 37° C.
5. Centrifuge the ID-Card(s) for 10 minutes at 1000 rpm.
6. Read macroscopically for agglutination.

INTERPRETATION OF TEST RESULTS

1. **Positive:** Agglutination of the test red cells constitutes a positive test result and within accepted limitations of test procedure, indicates the presence of the D antigen on the test red cells.
2. **Negative:** No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, 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 potentiators in the reagent on sensitised cells.

Stability of the reactions

1. Read all tube and microplate tests straight after centrifugation.
2. Complete washing steps without interruption and centrifuge and read tests immediately after reagent addition because delays may result in dissociation of antigen-antibody complexes, leading to false negative or weak positive reactions.
3. 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.
4. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

LIMITATIONS

1. Anti-D reagent is not suitable for use with enzyme treated cells or cells suspended in LISS.
2. Stored blood may give weaker reactions than fresh blood
3. False positive agglutination may be seen when testing IgG sensitised cells.
4. False positive or false negative results may also occur due to:
 - Contamination of test materials
 - Improper storage, cell concentration, incubation time or temperature
 - Improper or excessive centrifugation
 - Deviation from the recommended techniques
5. The user is responsible for the performance of the reagents by any method other than those here mentioned.
6. Any deviations from the here recommended techniques should be validated prior to use⁹.

PERFORMANCE CHARACTERISTICS

1. The reagent has been characterised by all the procedures here mentioned.
2. Prior to release, each lot of Anti-D, is tested by the here recommended techniques against a panel of antigen-positive red cells to ensure suitable reactivity.
3. Specificity of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
4. The potency of the reagent has been tested against the following minimum potency reference standard obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-D reference 91/592.
5. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS prior to use.
6. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

BIBLIOGRAPHY

1. Kholer G, Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity. Nature 1975, **256**, 495-497.
2. Race RR, Sanger R. Blood Groups in Man 6th Edition, Oxford, Blackwell Scientific Publishers 1975, Chapter 2.
3. Issitt PD. Applied Blood Group Serology, 3rd Edition, Montgomery Scientific, Miami, 1985, Chapter 10.
4. Mollison PL. Blood Transfusion in Clinical Medicine, 8th Edition, Oxford, Blackwell Scientific Publications, 1987, Chapter 7.
5. Tippett P. Sub-divisions of the Rh (D) antigen. Medical. Laboratory Science 1988; **45**, 88-93
6. Thompson KM, Hughes-Jones NC. Production and characteristics of monoclonal anti-Rh. Bailliere's Clinical Haematology 1990; April
7. Jones J, Scott ML, Voak D. Monoclonal anti-D specificity and Rh D structure: criteria for selection of monoclonal anti-D reagents for routine typing of patients and donors. Transfusion Medicine 1995. **5**, 171-184
8. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.
9. British Committee for Standards in Haematology, Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of new techniques for blood grouping, antibody screening and cross matching. Transfusion Medicine, 1995, **5**, 145-150.