Qualitative test for determination of D antigen on human red blood cells.

IVD

Store at 2-8°C

**PRINCIPLE OF THE METHOD**

The reagent will react directly agglutinate (clumping) of test red cells that carry the D antigen and indirectly agglutinate of test red cells that lack the D antigen in presence of the homologous D reagent. No agglutination generally indicates the absence of the D antigen (see Limitations).

**CLINICAL SIGNIFICANCE**

Levine and Staton discovered the Rh blood group system in 1940. The D antigen is the most clinically significant antigen on red blood cells and the corresponding antibodies have been implicated in causing both transfusion Reactions and Haemolytic Disease of the Newborn.

Weakened expression of the RhD antigen

The collective term D-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. D-d is a partial D category which misses most D epitopes. The reagent will detect most examples of partial and weak red cells by direct agglutination, but will not detect Dv red cells. This reagent will detect Dv and partial D cells in the IAT phase.

**REAGENTS**

Spinreact Monoclonal Anti-D blood grouping reagent is a low protein, blended reagent containing human microglobulin (6% w/v) and IgG diluted in a phosphate buffer containing sodium chloride (0.9 g%), bovine albumin (3 g%) and macromolecular potentiators. When typing patient samples, this reagent will directly agglutinate Rh D positive cells, including majority of variants (not D-d) and a high proportion of weak D (Dp) 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 further dilution or addition. For lot reference number and expiry date see Vial Label.

**PRECAUTIONS**

1. The reagents are intended for in vitro diagnostic use only.
2. If a reagent vial is broken or cracked or any other indication of contamination is immediately visible, do not use.
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. This reagent should be warmed when handling the reagent, such as disposable gloves and a laboratory coat.
6. The reagents have been filtered through a 0.2 µm filter to reduce the bioburden. Once a vial has been opened the contents should remain viable up to 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 biological materials containing free histidine, cysteine, glutamine, or arginine.
8. Materials used to produce the products were at source and found to be negative for HIV-1 and HIV-2, hepatitis B and C, and Mycobacteria and other microorganisms.
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 Rh-D positive whole blood sample) be tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. It is recommended that weak and partial variants are tested in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
3. The collective term D-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. D-d is a partial D category which misses most D epitopes. The reagent will detect most examples of partial and weak red cells by direct agglutination, but will not detect Dv red cells. This reagent will detect Dv and partial D cells in the IAT phase.

**STORAGE**

Do not freeze. Reagent vials should be stored at 2-8°C on receipt. Prolonged storage at temperatures above or below these ranges may result in accelerated loss of reagent reactivity.

**MATERIAL REQUIRED**

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Test microtubes.
- Water bath or dry heat incubator equilibrated to 37°C ± 2°C.
- Volumetric pipettes.
- Water bath or dry heat incubator equilibrated to 37°C ± 2°C.
- Centrifuge.
- Test tube centrifuge.
- CellStab.
- DiaMed ID Micro Typing Technique card(s).
- Positive (ideally Rh-D positive whole blood sample) and 1 volume test red cell suspension.

**SAMPLE**

Blood samples drawn with or without anticoagulant may be used for antigen typing. If typing is delayed then store whole blood at 3-6°C, EDTA and serum should be kept refrigerated until tested, and serum samples collected into ACD, CPD or CPDA-1 may be kept 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.

**PROCEDURE (NOT CATEGORY D)**

**A. Tube Method**

1. Prepare 2% suspension of washed test red cells in PBS.
2. Place in a labelled glass slide: 1 volume of Anti-D reagent and 1 volume test red cell suspension.
3. Mix thoroughly and centrifuge all tubes for 2 minutes at 1000 rcf 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-d 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. Incubate the ID slide at 37°C for 20 minutes.
4. Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2-minute incubation period.
5. Read macroscopically after 2 minutes of diffusion time and do not mistake fibrin strands as agglutination.
6. Any weak reactions should be repeated by the tube technique.

**C. DiaMed ID Micro Typing Technique**

1. Prepare a 0.8% suspension of washed test red cells in an ID-Diak.
2. Remove aluminium foil from as many microtubes as needed.
3. Prepare in appropriate microtubes: 50 µl of test red cell suspension and 25 µl of Anti-D reagent.
4. Incubate the ID-Cards for 15 minutes at 37°C.
5. Place the ID-Cards 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 or manually by gentle swirling or simply by tapping on the table.
7. Any weak reactions should be repeated by the tube technique.

**RECOMMENDED TECHNIQUES (TO DETECT CATEGORY D)**

A. Indirect Antiblood group (IAT)

1. Prepare 0.8% suspension of washed test red cells in ID-Diak.
2. Place in a labelled test tube: 1 volume of Anti-D reagent and 1 volume of test red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-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 each cell button and read macroscopically.

**B. DiaMed-ID Micro Typing Technique**

1. Prepare a 2.3% suspension of washed test red cells in PBS.
2. Place in the appropriate well 1 volume of test red cell suspension.
3. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-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. Read macroscopically for agglutination.

**INTERPRETATION OF TEST RESULTS**

1. Positive: when any trace of agglutination of 2-3% of test cells constitutes a positive test result and within accepted tolerances of test procedure, indicates the presence of the D antigen on the test antigen.
2. Negative: when no agglutination of the test cells constitutes a negative result and within the acceptable tolerances of the test procedure, indicates the absence of the D antigen on the test antigen.
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 in sensitised cells.

**Stability of the reagents**

1. Read all tube and microplate tests straight after centrifugation.
2. Complete washing steps without interruption and centrifuge and read tests immediately after centrifugation as any washific steps will likely result in loss of agglutination or weak positive results 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.
3. Care should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

**LIMITATIONS**

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

**PERFORMANCE CHARACTERISTICS**

1. The reagent has been characterised by all the procedures here mentioned.
2. To prior release, each lot of Spinreact Anti-D, is tested by the here recommended techniques against a panel of antigen positive red cells to ensure suitable reactivity.
3. Anti-D grouping reagents for D grouping of patients should not react with Dv cells using the methods recommended for use. Follow-on tests of negative results using an antigen-antibody procedure are not recommended.
4. Results of source monoclonal antibodies is demonstrated using a panel of antigen-negative cells.
5. The potency of the reagent has been tested against the following minimum potency reference standard obtained from National Institute of Biological Standards and Control (UK).
6. The Quality of the controls was performed using reagent cells that had been washed twice with PBS prior to use.
7. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

**BIBLIOGRAPHY**

5. The user is responsible for performance of the agents by any method other than those here mentioned.
6. Any deviations from the here recommended techniques should be validated prior to use.

**PACKAGING**

Anti-D IgG + IgM

Ref: GSIS04

10 ml