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 cause direct agglutination (clumping) of test red cells that carry the D antigen and indirect agglutination of red cells that are Category D. Agglutination of test red cells in an antibody (Anti D) phase. No agglutination generally indicates the absence of the D antigen (see Limitations).

CLINICAL SIGNIFICANCE

Levine and Stanton discovered the Rh blood group system in 1940. The D antigen is the most clinically significant of the Rh antigens, and the corresponding antibodies have been implicated in causing both Transfusion Reactions and Haemolytic Disease of the Newborn.

Weakened expression of the Rh D antigen

The term "D" is widely used to describe red cells which have a weaker expression of the D antigen than normal. The term weak D designates individuals with a reduced number of complete D antigen sites per red cell. The term partial D designates individuals with missing D antigen sites. DvI is a D partial 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 in the IAT phase.

REAENTS

SpiraReact Monoclonal Anti-D blood grouping reagent is a low protein, blended reagent containing human monoclonal IgG and IgG3 diluted in a phosphate buffer containing sodium chloride (0.9 g/l), bovine albumin (3%) and macromolecular polymers.

Precautions

- 1. This reagent is intended for in vitro diagnostic use only.
- 2. If a 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. Be aware when handling the reagent, as the flowing of water, as 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 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 ingestion.
- 8. Materials used to produce the products were tested at source and found to be negative for HIV 1-2 and HCV antibodies and no human viruses or animal blood were added during production.
- 9. No known tests can guarantee that products derived from human or animal sources are free from infectious agents. Carers 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 spill site see Material Safety Data Sheets, available on request.

NOTES

1. It is recommended a positive control (ideally Rr cells), a negative (rr) control red 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 monoclonal polymers in the reagent may cause false positive reactions with IgG coated cells.
3. Test samples for category D determination by the Indirect Antiglobulin and CoombsDialed ID Technique.
4. Weak and variant D antigens are poorly detected by gel card, microplate and slide techniques. It is recommended that all weak and partial variants are tested by the IAT technique.
5. The antiglobulins used in the technique can only be considered valid if all negative tests react negatively with IgG sensitised red cells.
6. In the experience of the 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 were used.
8. The user must determine suitability of the reagents for use in other techniques.

STORAGE

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

MATERIAL REQUIRED

- Glass test tubes (10 x 75 mm or 12 x 75 mm).
- Micro pipette.
- Water bath or dry heat incubator equilibrated to 37°C ± 2°C.
- Inoculum paper.
- Glass microscope slides.
- Apparatus for mixing of reagents.
- Phosphate Buffered Saline (PBS): NaCl 0.9%, pH 7.0 ± 0.2 at 22±2°C ± 1°C.
- Positive identity red (rr) and negative (rr) control red cells.

required. The samples collected are washed twice into AC, CPD or CPDA-1 may be used 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 Dg)

A. Tube Method

1. Prepare 3% suspension of washed test red cells in PBS.
2. Place 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 Dg 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. Mix thoroughly, preferably using a microplate shaker, taking care to avoid cross-contamination.
4. Incubate at room temperature for 15 minutes (time dependent on user).
5. Centrifuge the microslide for 1 minute at 400 rpm or for a suitable alternative time and force.
6. Resuspend the cell buttons using carefully controlled agitation on a microplate shaker.
7. 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-Dikant.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtubes 1 volume of test red cell suspension and 25µl of Anti-D reagent.
4. Incubate the ID-Card(s) for 15 minutes at 37ºC.
5. Read macroscopically for agglutination.

RECOMMENDED PROCEDURES (TO DETECT CATEGORY Dg)

A. Indirect Antiglobulin Technique (IAT)

1. Place in appropriate microtubes 1 volume of test red cell suspension and 25µl of Anti-D reagent.
2. Incubate the ID-Card(s) for 15 minutes at 37ºC.
3. Read macroscopically for agglutination.

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

1. Prepare 0.8% suspension of washed test red cells in ID-Dikant.
2. Remove aluminium foil from as many microtubes as needed.
3. Place in appropriate microtubes 1 volume of test red cell suspension and 25µl of Anti-D reagent.
4. Incubate the ID-Card(s) for 15 minutes at 37ºC.
5. Read macroscopically for agglutination.

INTERPRETATION OF TEST RESULTS

1. Positive: Agglutination of test red cells constitutes a positive test result and within accepted limitations of test procedure, indicate 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, indicate 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 probably caused by the effect of the macromolecular polymers in the reagent sensitised cells.

Stability of the reagents

1. Read all tube and microslide tests straight after centrifugation.
2. Complete washing steps without interruption and centrifugation and read tests immediately after centrifugation to avoid false positive or weak 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 recommended.

LIMITATIONS

1. Spirareact Anti-D reagent is not suitable for use with enzyme treated cells or cells suspended in LIS.
2. Stored blood may give weak reactions as sodium azide.
3. False positive agglutination may be seen when testing IgG sensitised cells.
4. False positive or false negative results may also occur due to:
   a. Contamination of test materials
   b. Improper storage, cell concentration, incubation time or temperature
   c. Improper or excessive centrifugation
   d. Deviation from the recommended techniques
5. The user is responsible for the performance of the tests by any method other than those here mentioned.
6. 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. Prior to release, each lot of Spirareact 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 91/582.
5. The manufacturer 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


PACKAGING

Anti-D IgG + IgM Monoclonal

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