Agglutination assay for use on glass slide or test tube. Store at 2 - 8 °C. For "in vitro" use only.

PROPOSED USE

This method is valid for qualitative determinations of antigen D in human blood.

MATERIALS

Glass slide: glass slides, automatic pipette and applicator. Test tube: test tubes, automatic pipette and centrifuge.

FUNDAMENT OF THE METHOD

The presence of antigen D is determined by confronting problem red blood cells with monoclonal antibodies with known D specificity. Agglutination (or not) of the problem red blood cells with each of the reagents is indicative of the presence or absence of the relevant antigen in these cells.

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<th>Sodium azide 0.95 g/L</th>
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PREPARATION

The reagent is ready for use. Do not dilute.

CONSERVATION AND STABILITY

1. The reagents will remain stable until the date of expiry indicated on the label provided the vials have been properly closed and stored at a temperature of 2 - 8 °C and care is exercised when using the reagents in order to avoid microbial contamination.

2. The reagents are normally clear and transparent. Turbidity may be an indication of microbial contamination.

SAMPLES

Whole, anti-coagulated or coagulated blood in its own serum can be used if the test is performed immediately. Samples collected in EDTA or heparin should be processed before 48 hours. Samples collected in ACD, CPD, CPDA-1 remain active during 21 days. Store samples at 2 - 8 °C.

PROCEDURE

Qualitative Method

Glass slides method

1. Use a pipette to transfer 1 drop (~ 50 µL) of the problem red blood cells with a hematocrit of approximately 35-40%.

2. Add 1 drop (~ 50 µL) of the reagent.

3. Mix the blood and the reagent thoroughly with the applicator, forming a circle of 2 cm diameter.

4. Slowly move the slide with circular movements for 2 minutes.

Reading and interpretation

Reading: Wait two minutes ad check the presence or absence of agglutination.

Positive Reaction: Agglutination is observed.

Negative Reaction: Agglutination is not observed after two minutes.

Test Tube Method

1. Prepare a suspension of problem red blood cells after rinsing with 3 - 5 % NaCl 9g/L.

2. Add 1 drop of the reagent (~ 50 µL) in a test tube.

3. Add 1 drop (~ 50 µL) of the red blood suspension.

4. Centrifuge at 1000 r.p.m. during 1 minute.
**Reading and Interpretation**

Reading: Gently tap the tube in order to detach the sediment from the glass and examine macroscopically for the presence or absence of agglutination.  
*Positive Reaction*: Positive red blood cells remain agglutinated following resuspension.  
*Negative Reaction*: Resuspension of red blood cells is homogeneous.

In case of negative results, a Du Assay should be conducted.

**Du Assay:**  
1. Proceed as indicated in steps 1, 2 and 3 of the test tube method.  
2. Mix and incubate for 15 minutes at 37 ºC.  
3. Rinse the red blood cells with abundant NaCl 9g/L.  
4. Add 1 drop of Human Antiglobulin Serum and resuspend the red blood cells.  
5. Mix and centrifuge at 1000 r.p.m. for 1 minute.

**Reading and Interpretation**

*Positive Reaction*: Positive red blood cells remain agglutinated following re-suspension.  
*Negative Reaction*: Re-suspension of red blood cells is homogeneous.  
In order to confirm negativity, add 1 drop of red blood cells sensitized with IgG; if results are negative than the test is not valid.

**QUALITY CONTROL**

Users are recommended to verify the activity of the reagents whenever using with positive control red blood cells R1r and negative red blood cells r1r in parallel with the different assays.

**PRECAUTIONS**

Although the reagents are not of human origin and consequently free from HIV and Hepatitis B, both the reagents and the samples should be handled with the necessary precautions.

The preparation contains small amounts of sodium azide. Avoid spilling on irritated skin and on mucosa. Do not use if turbid.

**REFERENCE**


**PRESENTATION**

Code: 1700020 Anti-D 5 ml; 100 determ.  
Code.: 1700021 Anti-D 10 ml; 200 determ.