



ATLAS ANTI-D BLEND SLIDE, MICROPLATE AND TUBE TEST

A slide, rapid tube and microplate test based on agglutination method for Rhesus Typing in serum using monoclonal/polyclonal blend reagent.

For *In-Vitro* and professional use only
Store at 2° to 8° C

INTRODUCTION & PRINCIPLES

ATLAS Anti-D reagent is prepared from carefully blended human monoclonal IgM and IgG Anti-D suitable for slide, tube and microplate test procedures. The reagent will directly agglutinate Rh D positive cells, including majority of variants (but not D^{VI}) and a high proportion of weak D (D^U) phenotypes. The reagent will agglutinate category D^{VI} and low grade weak D (D^U) phenotypes by the indirect antiglobulin techniques.

The reagent is diluted with a sodium chloride solution, sodium phosphate solution and bovine albumin (sodium caprylate free). The procedure is based on agglutination principle, where red cells in the reagent possessing the antigen agglutinates in the presence of the corresponding antibody in the reagent indicating that the result is positive. The test is considered negative when no agglutination appears.

PRECAUTIONS

1. The reagent was tested for HIV and HBsAg and was found negative. It should be noted, however, that there is no guarantee for products derived from

human and animal sources not to transmit infectious diseases and thus should be handled with care.

2. This reagent should be stored refrigerated between 2° to 8°C. Never Freeze or expose to elevated temperature.

3. Do not use the antibody reagent if it is marked with turbidity as this may indicate reagent deterioration or contamination.

4. The reagent contains 0.1% Sodium Azide which is toxic and can be absorbed through the skin. When drained, the drains should be thoroughly flushed with water.

5. The reagent should be used as supplied and in accordance to the procedure mentioned below. Do not use beyond expiration date.

PREPARING THE SPECIMEN

Blood collected in anticoagulated (EDTA, heparin or citrate) stoppered sterile tube, stored between 2 and 8, must be examined within 48 hours, insofar as no sign of Hemolysis is visible. At the time of the test, centrifuge the blood sample at 1200 g for 3 minutes

MATERIALS PROVIDED

ATLAS Anti-D Blend reagent.

MATERIALS NEEDED BUT NOT PROVIDED

Glass test tube, microplate or slide, isotonic buffered saline (pH 6.9), applicator sticks, centrifuge (900-1000 RFC), incubator, timer.

1) DIRECT METHOD IN A TUBE AT ROOM TEMPERATURE

- prepare a 5% suspension of red blood cells in isotonic solution.
- using the vial dropper, transfer a drop of reagent to a tube.
- add 50 µl of red blood cell suspension.
- shake to homogenize the mixture, then centrifuge at 500g for 1 minute.
- read macroscopically while gently shaking the tubes so as to detach the red blood cell pellet.
- note the appearance of any agglutinations.

2) ANTIGLOBULIN INDIRECT METHOD for ANTI-D

- after immediately centrifuging and reading as above, if the reaction is weak or negative, shake the tubes and incubate at 37 for 15 minutes.

-wash the red blood cells twice with isotonic saline solution and discard the last washing liquid.

- add 50 µl of ANTI-HUMAN GLOBULIN to the red blood cell pellet. Mix, then centrifuge at 120 g for 1 minute.

-conduct reading as indicated in section 1.

3) PLATE TECHNIQUE AT ROOM TEMPERATURE

except for ANTI-D (RH1) on a rigorously clean plate, using the vial dropper, apply 1 drop of reagent.

- take 25 µl of unwashed cell pellet and apply next to each drop of reagent, taking care not to create contact between the drops.
- mix the blood and reagent using a spiral movement with the end of the stirrer so as to create a regular lozenge of diameter 2 to 3 cm.
- incubate the plate at room temperature and without stirring for 30 seconds.
- hold the plate and give it a rolling movement for 3 minutes while macroscopically observing the possible appearance of agglutination.
- read the reaction immediately.

4) SLIDE PROCEDURE

1. Place one volume of ATLAS Anti-D blend on a labelled slide prewarmed to 40°-50°C on a lighted viewbox.
2. Add one volume of a 35-45% suspension of cells in isotonic buffered saline (pH 6.9), serum or plasma to the slide. Mix the serum-cell mixture with separate clean applicator stick over an area of approximately 20x40mm.
3. Tilt the slides back and forth and observe for agglutination.
4. If no agglutination appears within two minutes, the result is interpreted as negative. Do not mistake peripheral drying or fibrin strands as agglutination.
5. If the test is negative and a D^U test is required, proceed to the D^U test procedure below.

MICROPLATE PROCEDURE

1. Prepare a 2-3% suspension of red cells in isotonic buffered saline (pH 6.9).
2. In a well of a U-bottom microplate place one volume (30-50µl) of ATLAS Anti-D blend reagent and one volume of the 2-3% cell suspension.

3. Mix well, preferably with a microplate shaker, taking care to avoid cross-well contamination.
4. Centrifuge the microplate at 45 RCF for 1-2 minutes.
5. Tilt the plate at an angle of 60-75° to the bench top and observe for streaming for up to 3 minutes. Negative reactions allow the cells to flow downwards in a uniform stream. Positive reactions remain as a distinct button, either on the bottom of the well, or occasionally sliding down the side. Positive tests may be confirmed by tapping the plate gently until the cell button is dislodged, then examine immediately for agglutination. If the test is negative and a D^U test is required, proceed to the D^U test procedure below.

D^U TEST PROCEDURE

1. Repeat steps 1-3 in the test tube procedure.
2. Mix tube well and incubate at 37°C for 15-30 minutes.
3. Wash cells with isotonic buffered saline (pH 6.9) for at least three times after the incubation. Decant saline completely after each wash.
4. Add two volumes of ATLAS Anti-Human Globulin.
5. Mix gently to resuspend the cells and Centrifuge at 900-1000 RCF for 15 seconds.
6. Gently resuspend cells and immediately observe for agglutination.
7. Confirm validity of negative tests with IgG sensitised cells according to the manufacturer's directions.

READING THE RESULT

POSITIVE: If Agglutination appears.

NEGATIVE: If no agglutination is observed.

Tube tests should be read immediately following centrifugation. Delay in reading and interpreting results may result in weekly positive or falsely negative reactions. Slide test should be interpreted at the end of the two minutes.

PROCEDURE LIMITATION

1. False positive/ negative results may occur from contamination from test materials, improper cell concentration, improper incubation time or temperature, or any deviation from the recommended test procedures.
2. Improper centrifugation may also cause false results. The centrifugation time mentioned in the

procedures is a suggestion. The appropriate centrifugation time is that which produces the strongest reaction of anti-body with antigen positive red cells, yet allows easy resuspension of antigen-negative red cells.

3. Red cells showing a positive direct antiglobulin test cannot be typed for D^U.
4. Weaker reactions may be observed with stored blood than with fresh blood.
5. To confirm the reactivity and specificity of ATLAS Anti-D reagent, it is recommended that negative cells and cells showing a diminished expression of the D antigen be tested with the reagent on each day of use.

SENSITIVITY

ATLAS Anti-D for slide and Rapid tube test is manufactured within the guidelines of the U.S. FDA potency requirements. The specificity is shown by the recommended test procedure with a panel of antigen positive red cells. Antibodies to Le^c, Le^d, Yt^b, Mg and Wr^a may not be excluded in routine specificity testing. These and other low frequency antigens can be tested with reagents available from ATLAS.

REFERENCES

1. Race R.R. and Sanger R., Blood Groups in Man, 6th edition, Oxford, Blackwell Scientific Publications, 1975.
2. Standards for Blood Banks and Transfusion Services. 11th Ed., Washington D.C., AABB 1984:25.
3. Widmann F.K. ed Technical Manual, 9th Ed., Washington D.C.: AABB 1985:9.

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PPI485A01
Revision A (01.10.2007)