ATLAS ANTI-A, ANTI-B, ANTI A, B SLIDE, MICROPLATE AND TUBE TESTS

Monoclonal ABO blood grouping reagents for slide, microplate and rapid tube test based on agglutination method

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

INTRODUCTION & PRINCIPLES

ATLAS ABO reagents are prepared from *In-Vitro* culture supernatants of hybridized immunoglobulin-secreting mouse cell lines. The reagents are diluted with phosphate buffer containing sodium chloride, EDTA and bovine albumin to give reagents that are optimized for use in tube, slide and microplate procedures. Anti-A is colored with acid blue (patent blue) dye, Anti-B is colored with acid yellow (tartrazine) dye, and Anti-A, B is not colored. The test procedure is based on agglutination principle, where red cells possessing the antigen agglutinate in the presence of the corresponding antibody indicating that the result is positive. The test is considered negative when no agglutination appears.

PRECAUTIONS

1. These reagents are derived from animal sources, thus, appropriate care must be taken in the use and disposal of these reagents, as there are no known test methods that can guarantee absence of infectious agents.
2. The reagents should be stored refrigerated between 2° to 8°C. Never Freeze or expose to elevated temperature.
3. Do not use reagents if it is marked with turbidity as this may indicate reagent deterioration or contamination.
4. The reagents contain 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 reagents 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

ABO grouping reagent.

MATERIALS NEEDED BUT NOT PROVIDED

Glass test tube, slide, or U-bottom microplate, isotonic buffered saline (pH 6.9), applicator sticks, centrifuge (900-1000 RCF for tube test and 140 RCF for microplate test), microplate shaker (for microplate procedure only), and timer.

PLATE TECHNIQUE AT ROOM TEMPERATURE

- 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.

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.

SLIDE PROCEDURE

1. Place one volume of ATLAS ABO blood grouping reagent on a slide at room temperature (18-25°C).
2. Add one volume of a 35-45% cells suspended in their own or group compatible plasma or serum.
3. Mix the reagent and the cells using a clean applicator stick over an area of approximately 20x40mm.
4. Slowly tilt the slides back and forth and observe for agglutination for a period not to exceed two minutes.

MICROPLATE TEST 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 (30-50ul) of ATLAS ABO blood grouping 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 140 RCF for 1 minute. Tests may be incubated at room temperature (18-25°C) for 15 minutes prior to centrifugation.
5. Tilt the plate at an angle of 60-90° 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 remains as a distinct button, either on the bottom of the well, or occasionally sliding down the side. Alternatively, the cell buttons can be resuspended using carefully controlled agitation on a microplate shaker, then examined for agglutination visually.

READING THE RESULT

POSITIVE: If Agglutination appears.
NEGATIVE: If no agglutination is observed.

Reactions:

Reagent			Cell Suspension				ABO
Anti-A	Anti-B	Anti-A,B	A_1	A_2	B	O	Group
+	-	+	-	-	+	-	A
-	+	+	+	+	-	-	B
+	+	+	-	-	-	-	AB
-	-	-	+	+	+	-	O

Tube tests and microplate tests should be read immediately following centrifugation. Slide tests should be interpreted within two minutes to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying reagents.

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 mentioned centrifugation times should be regarded as suggestions only. An appropriate centrifugation time should be determined by that which produces the strongest reaction of anti-body with antigen-positive cells, yet allows easy resuspension of antigen-negative red cells.
3. Weaker reactions may be observed with stored blood than with fresh blood.
4. ABO antigens are not fully developed at birth, weaker reactions may therefore occur with cord or neonatal red cells. Cord samples contaminated with Wharton's jelly may give false positive results.
5. Blood samples of weak A or B subgroups may give rise to false negative results or weak reactions.
6. To confirm the reactivity and specificity of ATLAS ABO blood grouping reagents, it is recommended that appropriate antigen-positive and antigen-negative cells be tested with the reagent on each day of use.
7. ABO blood grouping interpretation on individuals greater than 6 months old should be confirmed by testing serum or plasma of the individual against group A and group B red cells (reverse grouping).
8. If the results obtained with the serum do not correlate with the red cell test, further investigation is required.

SENSITIVITY

ATLAS ABO reagents are checked prior to release for their reactivity using the recommended test procedure on a wide variety of cells. The specificity of the source monoclonal antibodies from which these reagents are derived is demonstrated using a panel of antigen-negative cells.

REFERENCES

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ATLAS Medical
William James House,
Cowley Road, Cambridge, CB4 4WX, UK
Tel: ++44 (0) 1223 858 910
Fax: ++44 (0) 1223 858 524

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