Atlas Medical CE 0459

Blood Grouping Reagents:
Anti-A Monoclonal Reagent, Anti-B Monoclonal Reagent, Anti-AB Monoclonal Reagent, Anti-D IgG/IgM blend Reagent, & Their variants
SLIDE AND TUBE TESTS

IVD **For *In-Vitro* and professional use only**

2℃ 8℃ **Store at 2- 8°C**

INTENDED USE

The blood grouping reagents are used to detect the presence or absence of A, B or Rhesus Antigens on the surface of human red blood cells based on hemaglutination using slide or tube test techniques in whole blood samples or anticoagulant blood samples collected in EDTA , citrate or heparin tubes.

INTRODUCTION & PRINCIPLES

Blood grouping 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 and slide procedures. **Anti-A monoclonal reagent is colored with acid blue (patent blue) dye, Anti-B monoclonal reagent is colored with acid yellow (tartrazine) dye, and Anti-AB monoclonal reagent is not colored.** The test procedure is based on hemaglutination 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.

Anti-D IgG/IgM blend reagent is prepared from carefully blended human monoclonal IgM and IgG. Anti-D IgG/IgM blend reagent is suitable for slide and tube 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 (Du) phenotypes. The reagent will agglutinate category D^{VI} and low grade weak D (D^{u}) phenotypes by the indirect anti-globulin techniques.

Anti-D IgG/IgM blend reagent is diluted with a sodium chloride solution, sodium phosphate solution and bovine albumin (sodium caprylate free). Anti-D IgG/IgM blend reagent is not colored. The procedure is based on hemaglutination principle, where red cells' 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.

MATERIALS

MATERIALS PROVIDED

Blood Grouping Reagents:

- Anti-A monoclonal reagent (10 ml/vial), Clone: (9113D10).
- Anti-B monoclonal reagent (10 ml/vial), Clone: (9621A8).
- Anti-AB monoclonal reagent (10ml/vial), Clone: (152D12+9113D10).
- Anti-D IgG/IgM Blend reagent (10 ml/vial), Clone: (P3X61 + P3X21223B10 + P3X290 + P3X35).

MATERIALS NEEDED BUT NOT PROVIDED

- Plastic test tube or glass.
- Isotonic saline solution (% 0.9) NaCl).
- Applicator sticks.
- Centrifuge (100-1200 (g) for tube test).
- Timer.
- Incubator
- Anti-Human Globulin Reagent (can be ordered from Atlas Medical).
- White or transparent glass slide.

PRECAUTIONS

- The reagents are intended for in vitro diagnostic use only.
- The test is for well trained professional healthy user not for lay user.
- These reagents are derived from animal and human 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.
- Do not use reagents if it is turbid or contain particles as this may indicate reagent deterioration or contamination.
- Protective clothing should be worn when handling the reagents.
- **The reagents contain (0.1-0.2%) Sodium Azide and 0.02% sodium arseniate which is toxic and can be absorbed through the skin. When drained, the drains should be thoroughly flushed with water.**
- The reagents should be used as supplied and in accordance to the procedure mentioned below. Don't use beyond expiration date.
- Avoid cross contamination of reagents or specimens.
- Visible signs of microbial growth in any reagent may indicate degradation and the use of such reagent should be discontinued.
- Don't use these reagents if the label is not available or damaged.
- Do not use dark glass slide.
- Don't use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.
- Wash hands and the test table top with water and soap once the testing is done.
- Heamolysed blood sample should not be used for testing.
- The test should be performed at room temperature in a well let area with very good visibility.
- Failure to follow the procedure in this package insert may give false results or safety hazard.
- Close the vial tightly after each test.
- The reagent is considered toxic, so don't drink or eat beside it.
- If spillage of reagent occurs clean with disinfectant (disinfectant used could be irritable so handle with care).

STORAGE CONDITIONS

- The reagents should be stored refrigerated between 2 - 8°C.
- Never Freeze or expose to elevated temperature.
- The reagent is stable until the expiry date stated on the product label. Do not use the reagents past the expiry date.

REAGENT PREPRATION

- The reagents are intended for use as supplied, no prior preparation or dilution of the reagent is required.
- All reagents should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

- Blood collected with or without anticoagulant (EDTA, Heparin or Citrate) can be used for Antigen typing.

Note: Blood collected without anticoagulant should be tested immediately.

- The specimens should be tested as soon as possible after collection. If testing is delayed, the specimens should be stored at 2- 8 ºC, Sample must be retained to room temperature prior to analysis. (Testing should be carried out within five days of collections).
- Insure that there is no sign of hemolysis.
- At the time of the test, centrifuge the blood sample at 1200 RCF for 3 minutes.
- Blood collection is to be done with great care.

PROCEDURES

A. DIRECT TUBE METHOD AT ROOM TEMPERATURE

1. Prepare a 5% suspension of red blood cells in isotonic solution.
2. Using the vial dropper, transfer a drop (40±10µl) of each reagent into a separate and appropriately marked tube.
3. Add 50 µl of red blood cell suspension prepared in step 1.
4. Shake to homogenize the mixture, then centrifuge at 500g for **1 minute.**
5. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
6. Read the reaction immediately.
7. For Anti-D tube, if the reaction is weak or negative, shake the tubes and incubate at 37°C for **15 minutes.**
8. Wash the red blood cells twice with isotonic saline solution (NaCl 0.9%) and discard the last washing liquid.
9. Add one drop (50µl) of the AHG reagent into the tube. Mix and centrifuge at 120g for **1 minute.**
10. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
11. Read the reaction immediately.

B. ANTIGLOBULIN INDIRECT METHOD for ANTI-D

1. After immediately centrifuging and reading as above, if the reaction is weak or negative, shake the tubes and incubate at 37°C for 15 minutes.
2. Wash the red blood cells twice with isotonic saline solution (NaCl 0.9%) and discard the last washing liquid.
3. Add one drop (40 µl ± 10 µl) of ANTI-HUMAN GLOBULIN to the tube. Mix and centrifuge at 120 (g) for **1 minute**.
4. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
5. Read the reaction immediately.

C. DIRECT SLIDE METHOD AT ROOM TEMPERATURE

1. Bring reagents and samples to room temperature (18-25°C).
2. Using the wax pen divide the slide into appropriate numbers of divisions.
3. Using the provided dropper, place one drop (40 µl ± 10 µl) of each reagent onto its correspondent division on the slide.
4. Add 25µl of the precipitated cells next to each drop of reagents.
5. Mix the reagent and the cells using a clean stirring stick over an area with a diameter of approximately 20-40mm.
6. Incubate the slide at room temperature (18-25°C) without stirring for **30 seconds**.
7. Hold the slide and gently rock the slide for **3 minutes** and observe macroscopically for any agglutination.
8. Read the reaction immediately.

READING THE RESULT

POSITIVE: If Agglutination appears.

NEGATIVE: If no agglutination is observed.

Use the below table to determine the blood group:

Result of each reaction				ABO Group
Anti-A monoclonal reagent	Anti-B monoclonal reagent	Anti-AB monoclonal reagent	Anti-D IgG/IgM blend reagent	
+	-	+	+	**A+**
+	-	+	-	**A-**
-	+	+	+	**B+**
-	+	+	-	**B-**
+	+	+	+	**AB+**
+	+	+	-	**AB-**
-	-	-	+	**O+**
-	-	-	-	**O-**

STABILITY OF THE REACTIONS

- ABO Blood Grouping Tube tests should be read immediately following centrifugation.
- Slide tests should be interpreted within three minutes to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of reagents.
- Delay in reading and interpreting results may result in weekly positive or falsely negative reactions. Slide tests should be interpreted at the end of the three minutes.

PROCEDURE LIMITATION

1. False positive/ negative results may occur due to:
   - Contamination from test materials.
   - Improper storage, cells concentration, incubation time or temperature.
   - Improper or excessive centrifugation.
   - Deviation from the recommended technique.
   - Blood samples of weak A or B subgroups may give rise to false negative results or weak reactions when tested using slide test method. It is advisable to re-test weak subgroups using tube test method.
2. Weaker reactions may be observed with stored blood than with fresh blood.
3. ABO antigens are not fully developed at birth, weaker reactions may therefore occur with cord or neonatal red cells.
4. 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). If the results obtained with the serum do not correlate with the red cell test, further investigation is required.
5. Return the kit to the agent if it does not function properly.
6. Anti-D IgG/IgM blend Reagent tests conducted on particular weak-D phenotypes, while satisfactory, cannot ensure recognition of all weak variants, due to the variability of antigen patterns.

DIAGNOSTIC PERFORMANCE CHARACTERISTICS

The following tables compare the results in slide and tube techniques of 3 lots of Atlas Medical reagents and the results of a CE marked device.

Slide Technique				
Group A				
Positive with anti-A monoclonal reagent and anti-AB monoclonal reagent Negative with anti-B and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
232	232	232	232	100%
Tube Technique				
Group A				
Positive with anti-A monoclonal reagent and anti-AB monoclonal reagent Negative with anti-B and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
212	212	212	212	100%

Slide Technique				
Group B				
Positive with anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with anti-A and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
61	61	61	61	100%
Tube Technique				
Group B				
Positive with anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with anti-A and Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
61	61	61	61	100%

Slide Technique				
Group O				
Negative with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
241	241	241	241	100%
Tube Technique				
Group O				
Negative with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
243	243	243	243	100%

Slide Technique				
Group AB				
Positive with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
33	33	33	33	100%
Tube Technique				
Group AB				
Positive with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent Negative with Negative control				
CE marked device	Lot A	Lot B	Lot C	Compliance
24	24	24	24	100%

No inversion in diagnosis has been shown: from a qualitative point of view we have observed 100% compliance in direct group testing in slide and tube techniques for determination of A, B, AB and O groups for the three lots of Atlas Medical.

QUALITY CONTROL

The reactivity of all blood grouping reagents should be confirmed by testing known positive and negative red blood cells on each day of use.

To confirm the specificity and sensitivity, Blood grouping reagents should be tested with antigen-positive and antigen-negative red blood cells.

REFERENCES

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CE 0459

LIST OF VARIENTS:

Product Code	Product Name
8.02.00.0.0010	Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 1 vial/Carton Box
8.02.00.1.0100	Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial. 10 vials / Plastic Pack
8.02.00.1.0180	Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial. 18 vials / Carton Box
8.02.01.0.0010	Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, / Carton Box
8.02.01.1.0100	Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 10 vials / Plastic Pack
8.02.01.1.0180	Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 18 vials / Carton Box
8.02.02.0.0010	Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 1 vial/ Carton Box
8.02.02.1.0100	Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 10 vials/Plastic Pack
8.02.02.1.0180	Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 18 vials/Carton Box
8.02.03.0.0010	Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 1 vial/ Carton Box
8.02.03.1.0100	Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 10 vials / Plastic Pack
8.02.03.1.0180	Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 18 vials / Carton Box
8.02.04.0.0010	Anti-A Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1 Vial/Carton Box
8.02.04.0.0100	Anti-A Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 10 vials / Plastic Pack
8.02.05.0.0010	Anti-B Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1vial/Carton Box
8.02.05.0.0100	Anti-B Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 10 vials /Plastic Pack
8.02.05.6.0030	ABO Set (Anti-A (1/256), Anti-B (1 /256), Anti-D (1/64)),3x10ml / plastic Pack
8.02.05.7.0020	ABO Set: Anti-A (1/256), Anti-B (1 /256), 2x10ml /Plastic Pack
8.02.06.0.0010	Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1vial/Carton Box
8.02.06.1.0100	Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial,10 vials /Plastic Pack
8.02.06.1.0180	Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial,18 vials / Carton Box
8.02.07.0.0010	Anti-D IgG/IgM Blend Reagent (Titer: 1 /64), 10ml/vial, 1Vial/ Carton Box
8.02.07.1.0100	Anti-D IgG/IgM Blend Reagent (Titer: 1 /64), 10ml/vial, 10 vials / Plastic Pack
8.02.47.0.0030	ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-D (1 /128)),3x10ml/Plastic Pack
8.02.47.1.0030	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /64)), 3x10ml /Carton Box.
8.02.47.3.0030	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /64)), 3x10ml /Plastic Pack
8.02.47.5.0030	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /128)), 3x10ml/Plastic Pack
8.02.49.0.0040	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-AB (1 /256), Anti-D (1 /64)), 4x10ml/Carton Box
8.02.49.2.0040	ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-AB (1 /256), Anti-D (1 /128)), 4 x 10ml, 4 vials/Plastic Pack
8.02.53.0.0040	ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-AB (1 /512) Anti-D (1 /128)), 4x10ml/Plastic Pack
8.02.53.1.0040	ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-AB (1 /512) Anti-D (1 /128)), 4x10ml, 4vials/Plastic Pack
8.02.70.0.0010	Anti-A monoclonal reagent , Titer (1/1024), 10 ml/vial, 1Vial/ Carton Box
8.02.71.0.0010	Anti-B Monoclonal reagent (Titer: 1 /1024) , 10 ml/vial ,1Vial/ Carton Box
8.02.72.0.0010	Anti-AB Monoclonal reagent (Titer: 1 /1024) , 10 ml/vial , 1Vial/ Carton Box
8.02.85.0.0010	Anti-D IgG/IgM Blend reagent (Titer 1 /256), 10ml/vial, 1Vial/ Carton Box

REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry