Blood Grouping Reagents: Anti-A Monoclonal Reagent, Anti-B Monoclonal Reagent, Anti-AB Monoclonal Reagent, Anti-D IgG/IgM blend Reagent

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. For *In-Vitro Diagnostic* Use only

ORDER INFORMATION

Pack Size	Anti-A	Anti-B	Anti-A/B	Anti-D
10 mL	PBGA 10	PBGB 10	PBAB 10	PBGD 10
30 mL	PBGA 30	PBGB 30	PBAB 30	PBGD 30
40 mL	PBGA 40	PBGB 40	PBAB 40	PBGD 40
100 mL	PBGA 100	PBGB 100	PBAB 100	PBGD 100
1000 mL	PBGA 1000	PBGB 1000	PBAB 1000	PBGD 1000
5000 mL	PBGA 5000	PBGB 5000	PBAB 5000	PBGD 5000

CLINICAL SIGNIFICANCE

ABO grouping is a test performed to determine an individual's blood type. It is based on the premise that individuals have antigens on their red blood cells (RBCs) that correspond to the four main blood groups: A, B, O, and AB. Antibodies (isohemagglutinins) in an individual's plasma are directed against blood group antigens that their own RBCs lack. These antibodies form early in life. ABO antigens are expressed on RBCs, platelets, and endothelial cells and are present in body fluids. The ABO blood group antigens remain of prime importance in transfusion medicine as they are the most immunogenic of all the blood group antigens. The most common cause of death from a blood transfusion is a clerical error in which an incompatible type of ABO blood is transfused. The ABO blood group antigens also appear to have been important throughout our evolution because the frequencies of different ABO blood types vary among different populations, suggesting that a particular blood type conferred a selection advantage (e.g., resistance against an infectious disease.) A person's ABO blood type can be used by lawyers in paternity suits, by police in forensic science, and by anthropologists in the study of different populations. 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, 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 reagent contains a blend of Anti-D human monoclonal IgM and IgG antibodies. **Anti-D IgG/IgM blend reagent is not colored**. The test is considered negative when no agglutination appears.

PRINCIPLE

Blood grouping reagents are prepared from in-Vitro culture supernatants of hybridized immunoglobulin-secreting mouse cell lines and diluted using suitable ingredients. 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-A and Anti-B reagents contain single monoclonal antibodies which agglutinate if A and B antigens are present of the RBCs respectively.

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.

CONTENTS

Blood Grouping Reagents:
- Anti-A monoclonal reagent (10 ml/vial)
- Anti-B monoclonal reagent (10 ml/vial)
- Anti-AB monoclonal reagent (10ml/vial)
- Anti-D IgG/IgM Blend reagent (10 ml/vial)

STORAGE & STABILITY

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

PRECAUTIONS

- The test is for well trained professionals only.
- 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 after testing is done and clean the testing area with 70% ethanol or appropriate agent.
- Hemolyzed 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).

SPECIMEN COLLECTION & PREPARATION

- Blood collected with or without anticoagulant (EDTA, Heparin or Citrate) can be used for Antigen typing.
- 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 two days of collections).
- Ensure that there is no sign of hemolysis.
- Blood collection is to be done with great care.

Note: Blood collected without anticoagulant should be tested immediately.

PROCEDURE

A.SLIDE METHOD

1. Bring the reagent and samples to room temperature.
2. Place 1 drop of Anti-A, Anti-B, Anti-AB and Anti-D IgG/IgM reagents on a glass slide.
3. Label the respective areas as 'A, B, AB and D' respectively. Also label the slide with name or code of the patient.
4. Add 1 drop (~40µl) whole blood or RBC-Saline suspension adjacent to each drop of the reagent.
5. Mix the reagent drop and the sample with an applicator stick and spread over an area of about 1 square inch within the circle.
6. Gently tilt the slide forward and backward at room temperature for a maximum of 2 minutes.
7. Read the slides for hemagglutination. Do not interpret fibrin Strands as agglutination.

B. TUBE METHOD (FOR ENHANCED SENSITIVITY)

1. Use 8x50 mm small glass test tube for each specimen, take a tube and label it with the name or code number of the patient.

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2. Add one drop of Anti-A, Anti-B, Anti-AB and Anti-D IgG/IgM reagents and 1 drop (~40µl) saline to the respective tubes.
3. Add one drop of 2-3% RBC-Saline suspension to each tube.
4. Shake each tube thoroughly and centrifuge for 1 minute at 1000 rpm (125g) or 3400 RPM (1000 g) for 20secs or incubate at Room Temperature for 1 hour.
5. Gently dislodge the sedimented cells and read for hemagglutination, either macroscopically or microscopically.

C. WEAK D (DU) TEST

1. Use 8x50 mm small glass test tube for each specimen, take a tube and label it with the name or code number of the patient.
2. Add one drop of Anti-D IgG/IgM and saline to the respective tubes.
3. Add one drop of 2-3% RBC-Saline suspension to each tube. Incubate at 37°C for 45mins.
4. Wash the contents of the tube 3 times with normal saline and discard the supernatant.
5. Add two drops of Anti-human globulin on the red cell button and incubate at 37°C for 30mins.
6. Centrifuge the tube for 1000 rpm for 1 min.
7. Gently dislodge the sedimented cells and examine under microscope.

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.
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.

PERFORMANCE CHARACTERISTICS

1. These reagents meet FDA potency requirements for Blood Grouping Reagents to be used in test tube technique.
2. Every lot of each product is tested to assure reliable reactivity and specificity in use in accordance with FDA requirements.
3. The intensity of the reactions obtained with Anti-D IgM may depend on the number of antigen sites present on the red blood cells.
4. Anti D IgM + IgG enable screening for weak red blood cells D (RH1) in the indirect hemagglutination method with antiglobulin.
5. The tests conducted on particular phenotypes,while satisfactory,cannot ensure recognition of all weak or variant subjects, due to the variability of antigen motifs.
6. Anti D IgM + IgG have the special feature of recognizing certain rare antigen motives of type RH33 (DHar) and may thus yield discordant reactions with polyclonal reagents that recognize them little or not at all. The performance of the reagents was confirmed against FDA licensed reagents in a comparison study where reagents were tested in parallel at different clinical sites

DISCLAIMER

Each facility should verify the optimum spin time for the specific centrifuge in use. False positive or false negative can occur due to improper centrifugation

BIBLIOGRAPHY

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GLOSSARY OF SYMBOL

	Consult Instruction for Use
	Catalog Number
	Store between
	Manufacturer
	Keep away from sunlight



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