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BLOOD GROUPING REAGENT ANTI-A;ANTI-B;ANTI-A,B;(Murine Monoclonal) A LATEX TEST

SUMMARY AND EXPLANATION

The ABO blood group system first discovered and reported by Landsteiner, von DeCastello and Sturli in 1901 and 1902,^{1,2} is comprised of four major groups: A, B, AB and O. The ABO group of an individual is largely determined by the genetic inheritance of the A, B and H genes³.

Normal adult individuals whose red cells lack A and/or B antigens usually have the corresponding antibody in their serum. Anti-A and Anti-B can cause serious hemolytic transfusion reactions as well as hemolytic disease of the newborn. The potentially serious consequences of ABO incompatible transfusions requires that both transfusion recipient and donor red cells be reliably tested for the presence of A and B antigens, and that subsequently ABO group compatible donor blood be selected for transfusion. ABO grouping of adult patient red cells should always be supplemented by confirmatory serum grouping tests (i.e. testing the individual's serum with known A₁ and B Reagent Red Blood Cells).

The red cells of newborn infants do not have full expression of A and B antigens and slightly weaker ABO grouping tests may be encountered. Furthermore, serum from group A, B, or O newborn infants may not necessarily contain the expected anti-A and/or anti-B. In fact, passively acquired anti-A and/or anti-B from the mother's circulation may be present, resulting in unexpected reactions. Therefore, reverse grouping should not be performed on the sera of newborn infants, as it may not provide the expected confirmatory results.

Subgroups of A and B are known to exist and may result in weaker than expected or negative direct hemagglutination reactions with anti-A, anti-B and/or anti-A,B reagents. Although not extensively substantiated, it may be important to detect such weak expressions of the A antigen in donor blood units so that such blood is not transfused to group O recipients. Anti-A,B is a useful reagent for confirmation of test results obtained with anti-A and anti-B to further ensure accurate and reliable ABO grouping-essential for the maintenance of safe transfusion practice.

For reference purposes, the approximate frequencies in the ABO blood group system are as follows:

GROUP	FREQUENCIES (%) ⁴		
	White	Black	Oriental
Group A ₁	34	19	27
Group A ₂	10	8	Rare
Group B	9	19	25
Group A ₁ B	3	3	5
Group A ₂ B	1	1	Rare
Group O	44	49	43

BIOLOGICAL PRINCIPLE OF THE TEST

The test used with these reagents is based on the principle of direct hemagglutination⁵. Normal human erythrocytes will clump or agglutinate when mixed with CORTEZ DIAGNOSTICS, INC.Anti-A or Anti-B or Anti-A,B if they possess A and/or B antigens. Centrifugation may be applied in saline tube tests to increase the rate of agglutination and resultant strength of hemagglutination reaction. Absence of agglutination indicates a negative test result and, within the accepted limitations of the test procedure, indicates the absence of the corresponding antigen.

PRODUCT DESCRIPTION

CORTEZ DIAGNOSTICS, INC.Anti-A, Anti-b and Anti-A,B are prepared from murine monoclonal antibodies⁶ and designed for use in slide and saline tube tests to provide a specific qualitative test for the detection of the A and/or B antigens on human red blood cells. These reagents contain antibodies from cell lines prepared and developed exclusively by Dominion Biologicals Limited.

The diluent used for these reagents contains sodium chloride, bovine serum albumin, EDTA and phosphate buffer. Sodium azide at a final concentration of 0.1% is added as a preservative.

CAUTION: Sodium azide may react with copper and lead plumbing to form explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

CORTEZ DIAGNOSTICS, INC.Anti-A contains a blue dye-Patent Blue
CORTEZ DIAGNOSTICS, INC.Anti-B contains a yellow dye-Acid Yellow#9

CORTEZ DIAGNOSTICS, INC.Anti-A,B contains no colouring agent

CORTEZ DIAGNOSTICS, INC.Anti-A, Anti-B and Anti-A,B Blood Grouping Reagents should be stored at 2-8°C. Do not freeze.

Do not use if marked turbidity or other observable indications of product alteration occur. These signs may indicate bacterial contamination and/or product deterioration. Do not use beyond expiration date.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient/donor is required prior to specimen collection. Blood samples should be collected by approved medical procedure. Blood collected with or without anticoagulant is acceptable, if testing is performed without delay. If a delay in testing is unavoidable, red cells from clotted samples, EDTA or heparinized anticoagulated sample should be separated from the serum/plasma, washed and stored in a red cell preservative solution (such as CORTEZ DIAGNOSTICS, INC.Physio-Sol) at 2-8°C for no longer than 35 days. ACD, CPD and CPDA-1 anticoagulated blood samples may be tested up to their expiration date if storage is maintained at 2-8°C. Prolonged storage of red cells prior to testing may result in deterioration of red cell antigens and resultant weaker than expected test reactions.

MATERIALS AND EQUIPMENT NOT SUPPLIED

1. SLIDE TEST: Glass slides; pasteur pipettes; applicator sticks; isotonic saline.
2. SALINE TUBE TEST: Glass test tubes (10x75 mm or 12x75 mm); pasteur pipettes; centrifuge; isotonic saline.

TEST PROCEDURE

A. SLIDE TEST METHOD

1. Prepare a 35-45% suspension of test red cells. Cell suspensions may be prepared in autologous serum/plasma or saline.
2. Place one drop of the appropriate CORTEZ DIAGNOSTICS, INC.Blood Grouping Reagent (Anti-A or Anti-B or Anti-A,B) on a glass slide at room temperature (20-25°C).
NOTE: When anti-A and anti-B are used concurrently, one drop of each respective reagent may be applied to opposite ends of a single slide.
3. Add one drop of the prepared 35-45% suspension of red cells to each drop of reagent on the glass slide.
4. Mix the cells and reagent thoroughly over an approximate 20 mm circular area, using a separate, clean applicator stick for each reagent cell test.
5. Rock/rotate the slides gently and examine for macroscopic hemagglutination-Agglutination may begin within a few seconds, however observation should not continue beyond 2 minutes.

B. TUBE TEST METHOD

1. Prepare a 3-5% suspension of test red cells. Red cell suspensions may be prepared in autologous serum/plasma or saline.
2. Dispense one drop of CORTEZ DIAGNOSTICS, INC. Blood Grouping Reagent (Anti-A or Anti-B or Anti-A,B) into an appropriately labelled test tube.
3. Add one drop of the prepared 3-5% suspension of test red cells to the tube.
4. Mix tube contents thoroughly.
5. Incubate at room temperature (20-25°C) for 60 minutes (sedimentation technique) or centrifuge at room temperature for:
 - a. 15 seconds at 3200-3400 rpm (900-10000 rcf)
 - b. or centrifugation of equivalent force.

NOTE: The centrifugal force applied should be the minimum required to produce a clear supernatant and a clearly delineated red cell button that can be easily resuspended. No single centrifugation speed or time can be recommended for all types of available centrifuges or test applications. Centrifuges should be calibrated individually to determine the optimal time and speed required to achieve the desired results.

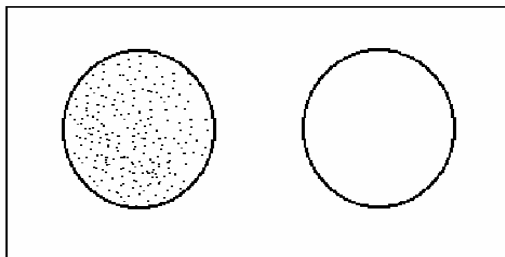
6. Gently resuspend the red cell button and examine macroscopically for agglutination.
7. Grade and record results.

QUALITY CONTROL

Appropriate control tests are essential for all laboratory test procedures.

1. Confirmation of forward (cell) ABO grouping results on adult samples must be confirmed by reverse (serum) grouping tests with known A₁ and B reagent red cells.
2. CORTEZ DIAGNOSTICS, INC. Anti-A,B Blood Grouping Reagent may be used as a confirmatory control for tests with CORTEZ DIAGNOSTICS, INC. Anti-A and Anti-B.
3. It is strongly recommended that the reactivity of Blood Grouping Reagents be confirmed each day of use by control tests with known antigen positive and negative red cells. Positive control cells should be selected to represent weak expression of the specific antigen.

INTERPRETATION OF TEST RESULTS



Positive

Negative

POSITIVE: Within the accepted limitations of the test procedure, agglutination of test red cells with CORTEZ DIAGNOSTICS, INC. Anti-A or Anti-B indicates the presence of the corresponding antigen. Similarly, agglutination of test red cells with CORTEZ DIAGNOSTICS, INC. Anti-A,B indicates the presence of A and/or B antigen.

NEGATIVE: Within the accepted limitations of the test procedure, no agglutination of test red cells with CORTEZ DIAGNOSTICS, INC. Anti-A or Anti-B indicates the absence of the corresponding antigen. Similarly, no agglutination of test red cells with CORTEZ DIAGNOSTICS, INC. Anti-A,B indicates the absence of A and/or B antigens.

The expected reaction patterns (forward and reverse ABO grouping) of the common ABO phenotypes are as follows:

Forward Grouping (Cells)			Reverse Grouping (Serum)		ABO
Anti-A	Anti-B	Anti-A,B	A ₁ Cells	B Cells	Group
0	0	0	+	+	O
+	0	+	0	+	A
0	+	+	+	0	B
+	+	+	0	0	AB

NOTE: Hemolysis observed in forward (cell) ABO grouping tests should not necessarily be interpreted as a positive result-hemolysis may be caused by bacterial contamination. Discrepancies between ABO forward and reverse grouping tests must be resolved prior to assigning a blood group.

LIMITATIONS OF TEST PROCEDURE

1. False negative or unexpectedly weak reactions may occur with blood samples of weak A or B subgroups or with cord red cells from newborn infants.
2. False negative or unexpectedly weak reactions may occur with red cells that have been subjected to prolonged storage and/or inappropriate storage conditions.
3. Test variables such as improper technique, inappropriate centrifugation or incubation, improperly cleaned glassware and/or contaminated materials may cause false negative or false positive results.
4. Serological anomalies can occur that may result in unexpected reactions or discrepancies between forward and reverse ABO grouping tests^{3,5,7,8,9}.

SPECIFIC PERFORMANCE CHARACTERISTICS

CORTEZ DIAGNOSTICS, INC. Anti-A, Anti-B and Anti-A,B (Murine monoclonal) have been tested and found to specifically agglutinate human red cells if the corresponding antigen is present, when used according to the Recommended Directions for Use. Each lot of CORTEZ DIAGNOSTICS, INC. Anti-A, Anti-B and Anti-A,B has been tested to ensure potency and specificity according to methods recommended by U.S.-FDA and Canadian-C.G.S.B. standards.

Specificity tests for CORTEZ DIAGNOSTICS, INC. Anti-A, Anti-B and Anti-A,B are performed using extensive panels of red cells that lack A, B or both A and B antigens respectively.

Deviation from the Recommended Directions for Use may result in less than optimal product performance.

REFERENCES

1. Landsteiner K. Zur Kenntnis der antifermentativen, lytischen und agglutinierenden wirkungen des blutserums und der lymphfe. Zbl Bakt. 1900;27:357-362.
(Translation as published by the Blood Transfusion Research Division, U.S. Army Medical Research Laboratory. Fort Knox, Kentucky 40121).
2. von DeCastello A, Sturli A. Uber die isoagglutine in serum gesunder und kranker menschen. Munchen. Med. Wehnschr. 1902:1090-1095.
(Translation as published by the Blood Transfusion Research Division, U.S. Army Medical Research Laboratory. Fort Knox, Kentucky 40121).
3. Race RR and Sanger R. Blood groups in man. 6th edition. Oxford: Blackwell Scientific, 1975.
4. Pittiglio DH, Baldwin AJ, Sohmer PR. Modern blood banking and transfusion practices. Philadelphia: F.A. Davis, 1987, c. 1983.
5. Walker Rh, ed. Technical manual. 11th edition. Bethesda: American Association of Blood Banks, 1993.
6. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 1975:256-495.
7. Mollison PL. Blood transfusion in clinical medicine. 7th edition. Oxford: Blackwell Scientific, 1983.
8. Moore BPL. Serological and immunological methods of the Canadian Red Cross Blood Transfusion Service. 8th edition. Toronto: Hunter Rose, 1980.

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