ACCUCARE®

Monoclonal Antibodies for Phenotyping Human Red Blood Cells

Anti-A, Anti-B and Anti-AB are monoclonal antibodies of IgM type specific against red blood antigens A and B.

The monoclonal test reagents are used to determine the antigens of the ABO blood group system by agglutination of Human red blood cells.

ORDER INFORMATION

REF	Pack Size
A 10 / B 10 / AB 10	1 X 10mL
A 100 / B 100 / AB 100	10 X 10 mL
A 1000 / B 1000 / AB 1000	1 X 1000mL

CLINICAL SIGNIFICANCE

Between 1900 and 1902, Landsteiner and associates discovered the ABO system of red blood cell antigens. The importance of this discovery is the recognition that antibodies are present when the corresponding antigens are lacking. The ABO system is the only blood group system in which the reciprocal antibodies are consistently and predictably present in most people. Due to this reciprocity, an ABO blood type determination is considered valid if serum typing corresponds with the red blood cell antigen grouping.

The test reagents are produced from supernatant of hybridoma cells which obtained by immunizing Balb/C mouse with red blood cells of blood groups A and B and fusion of spleenocytes from mouse with myeloma (SP2/0) cells.

Mouse monoclonal antibody raised against Human blood group antigens A & B.

Accucare Anti-A, Anti-B and Anti-AB Blood Grouping Reagents are used to test for the presence or absence of the corresponding antigens. Routine pretransfusion studies always include tests for the ABO antigens and reverse grouping.

METHOD

Hemagglutination technique.

PRINCIPLE

The human ABO blood group system consists in the fact that persons lacking the A and/or B antigens from the red cells regularly have antibodies in the serum at the missing antigens. The following table shows the principle antigens and antibodies of ABO system.

Blood Group	Antigens present on the RBC	Antibodies present in the serum
O	-	Anti-A and Ant-B
A	A	Anti-B
B	B	Anti-A
AB	A and B	None

The specific monoclonal antibodies of the reagents agglutinate red cells possessing the relevant antigen. Phenotyping (grouping) of the blood sample is determined by presence of haemagglutination with Anti A or Anti B.

REAGENT

Accucare® Anti-A, Accucare® Anti-B, and Accucare® Anti-A,B contain as reactive components monoclonal antibodies of the immunoglobulin class IgM.

Antibodies are diluted in a buffered protein solution containing bovine albumine, ethylenediamine tetraacetate (EDTA) and as colorant Patent Blue (Anti-A) or Tartrazin (Anti-B).

REAGENT PREPARATION

The reagent supplied is ready to use. Protect from Bright Light.

REAGENT STORAGE AND STABILITY

This product will be well-preserved within utility limit till the expiry date, if stored at temperature between +2°C and +8°C.

Caution: Do not freeze.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.
- Consider Blood specimen as potentially infectious, handle and dispose it as per national applicable guideline.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- Test tubes (8X50mm),
- Slides,
- Pipettes,
- Applicator stick,
- Centrifuge
- (0.9% NaCl) saline.
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

- Blood should be drawn by an aseptic technique with an anticoagulant. The specimen should be tested as soon as possible after collection.
- If delay in testing should occur, the specimen must be stored at 2°C to 8°C. Bacterial contamination may cause false test results.
- Blood drawn into heparin or Sodium citrate or EDTA should be use within 2 days or 14 days respectively.

ASSAY PROCEDURE - PHENOTYPING

Macroscopic Slide Test

1. Label two glass slides with name or number of the patient and make two circles on each slide. Label the circles as A, B, AB and S.
2. Add one drop of Anti-A antibody in Circle A, Anti-B antibody in circle B, Anti-AB antibody in circle AB and a drop of saline in the fourth circle.
3. Add one drop of patient's whole blood or red cell saline suspension to each circle.
4. Mix the red cells and the antibody immediately with an applicator stick and spread it over an area of about one square inch within the circle.
5. Gently tilt the slides forward and backward at room temperature for a maximum of two minutes.
6. Read the slides for haemagglutination.

Microscopic Tube Test (For Enhanced Sensitivity)

1. Use 8x50 mm small glass test tubes. For each specimen, take 4 tubes and label them with the name or number of patient. Mark the tubes as A, B, AB and saline.
2. Add one drop of Anti-A, Anti- B, Anti-AB antibody and saline to the respective tubes.
3. Add one drop of 2-3% RBC-saline suspension to each tube.

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(To prepare 2-3% of RBC saline suspension, add approximately 5 volumes of isotonic saline to the whole blood and centrifuge for 2 minutes. Remove the supernatant and wash the sedimented red cells three more times with normal saline as above. After final wash, take 20-30µl of sedimented red cells, makeup with 1ml of saline and mix thoroughly before use).

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 sediment cells and read for haemagglutination, either macroscopically or microscopically.

Stability of the Reaction

Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting to false negative or more often weak positive reactions.

INTERPRETATION

Agglutination of red blood cells within two minutes indicates the corresponding antigens in the patient's red blood cells. Absence of agglutination indicates the absence of such antigens on the red blood cells.

Agglutination results are as interpreted follows for phenotyping.

Sample red cell reacted with				
Anti-A	Anti-B	Anti-AB	Saline	Results
+	-	+	-	'A' Group
-	+	+	-	'B' Group
+	+	+	-	'AB' Group
-	-	-	-	'O' Group
-	-	+	-	Weaker variants of 'A' or 'B' Group
+	+	+	+	(*)
(*) suggestive of antibody in the blood giving a non specific reaction. The entire test is to be repeated using 10% saline suspension of red cells.				

Eventhough Anti-A and Anti-B antiseras are sufficient for ABO phenotyping 'O' blood it is advisable to use Anti-AB sera to rule out any doubt arising due to weaker variants of subgroups of A and B.

QUALITY CONTROL

Run positive and negative test controls for each batch of blood grouping sera every time before proceeding with the actual test samples.

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. Anti-B does not agglutinate the "acquired B" red blood cells tested.
4. In certain cases (transfusion recipients, certain weak phenotypes A or B (A3, B3...), certain hemopathological modifications, mosaics or chimeras, etc.), an image of a double population may be observed.
5. Antibody Anti-A and, accessorily, Antibody Anti-A,B yield a cross-reaction with Antigen Tn which gives rise to an image of a double population (exceptional phenomenon).

DISCLAIMER

- Each facility should verify the optimum spin time for the specific centrifuge in use.
- Manual techniques are to be performed according to the manufacturer's instructions.
- Each deviation from these instructions is the sole responsibility of the user.
- Used tests must be discarded as hazardous material. Manage waste according to local, state and national regulations.

LIMITATION OF THE PROCEDURE

- The blood drop on the slide should not be allows to dry, partial drying of the blood could be misinterpreted as agglutination.
- Centrifugation should be perfect. Over-centrifugation or under-centrifugation may result in false positive or false negative interpretation.
- Dislodgement of sedimented red cells in tube test should be done as gently as possible, rough dislodgement may disrupt small or weak agglutinates and hence may lead to false negative interpretation.
- The entire procedure should be carried out at room temperature. Warm or cold antibodies in the tested blood can cause agglutination and may lead to wrong interpretation.
- Haemolysed blood samples should not be used.
- Improper antigen antibody concentrations may cause false or delayed agglutination.
- On plates, blood samples may occasionally react by rouleaux formation, which can be mistaken for a weak agglutination and may incorrectly be read as a positive result. This phenomenon has non-immunological causes. Rouleaux formation also occurs in heparin blood, blood from patients treated with plasma expanders (e.g. dextran or hydroxylethyl starch), as well as blood from patients with plasmacytoma (high protein content, changes in protein composition), ontological disease (abnormal blood count) or coagulation dysfunction. For testing these samples, use the tube test, which usually avoid this phenomenon.

BIBLIOGRAPHY

Vox sanguinuis, 1989, 56, 122.
Bio test bulletin, 1988, 3, 117

GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit

LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
C1 Type, Shed No.: 3225, Chemical Zone,
GIDC Sarigam – 396155, Dist. Valsad, Gujarat, India.
Tel.: +91 22 2554 2109 /1558
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