BHAT BIO-TECH INDIA (P) LTD.

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CE 1023

BHAT Bio-Scan™

COMBIPACK ABD

Blood grouping antibodies for
Slide and Tube test

For Professional Use IVD

READ THIS PACK INSERT
CAREFULLY BEFORE PERFORMING THE TEST

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CATALOGUE No.: CABD

INTENDED USE :

Monoclonal blood grouping reagents are intended for in vitro recognition of the blood group antigens A, B, and D(Rh) on human red blood cells (RBCs).

INTRODUCTION :

The human erythrocyte (RBC) has some 100 known blood group determinants that comprise 15 genetically distinct blood group systems. Of these, only two- the ABO blood group system and the rhesus (Rh) blood group system- have major clinical importance. According to ABO blood group system human red blood cells are classified into four groups A, B, AB, and O depending upon the presence or absence of inheritable blood group antigens on the erythrocytes.

In the Rh system of blood typing, human red blood cells are classified into two types based on the presence or absence of Rh factor (D-antigen). The term Rh positive is used to denote the presence of D antigen on the red cells. Anti A and Anti B are IgM class of monoclonal antibodies directed against human red blood cell antigens A and B respectively. Anti D is IgM & IgG class of monoclonal antibodies directed against human red blood cell antigen D. These antibodies are standardized for their potency, specificity and avidity.

PRINCIPLE :

Agglutination of red blood cells with Anti A, Anti B and Anti D is a positive test reaction and indicates the presence of corresponding antigens on the RBCs. Absence of agglutination of red blood cells with Anti A, Anti B and Anti D is a negative test result and it indicates the absence of the corresponding antigens on the RBCs.

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STORAGE AND STABILITY :

Storage :

Store the reagents at 2-8°C. Do not use the reagent beyond the expiry date mentioned on it. Before performing the test bring all the reagents to Room temperature. Replace the reagents to 2-8°C soon after performing the test. DO NOT FREEZE THE REAGENTS.

Stability :

1. The unopened kit is stable for 2 years from the date of manufacturing as indicated on the package.
2. The opened kit is stable for 6 months from the date of opening.
3. Repeated freeze thaw of reagents from 2-8°C to Room temperature several t i m e s will reduce the stability of the kit.

PACK SIZE : Available in packs of 2ml, 5ml, and 10ml.

Titre : Anti A - 1:256
Anti B - 1:256
Anti D - 1:512

Avidity : 2-7 Seconds

CONTENTS OF THE KIT :

Anti-A (Monoclonal IgM) =2ml/5ml/10ml.
Anti-B (Monoclonal IgM) =2ml/5ml/10ml.
Anti-D (Monoclonal IgM/IgG/IgM+IgG) =2ml/5ml/10ml.

MATERIALS REQUIRED BUT NOT PROVIDED :

1. Slide or Tube 2. Lancet 3. Applicator sticks 4. Isotonic saline

AUXILLARY REAGENT NOT PROVIDED : Normal saline

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SPECIMEN COLLECTION AND PREPARATION:

No special preparation of the patient is required prior to sample collection by aprroved technique. Samples should be stored at 2-8° C if not tested immediately. Do not use haemolysed sample. Anticoagulated blood various anti coagulants should be tested within the below mentioned time period .. EDTA or heparin sodium oxalate or sodium citrate 2 days ACD or CPD 28 days clotted whole blood should be tested with in 14 days

PRECAUTIONS :

1. For invitro diagnostic use only.
2. Bring all reagents and specimens to Room temperature, prior to testing.
3. Avoid using hemolysed sample.
4. Microbial contamination should be avoided.
5. All human serum and plasma samples should be considered potentially infectious. It is recommended that all specimens of human origin should be handled as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control / National Institute of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 1984.
6. Never pipette by mouth.
7. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
8. Wear disposable gloves while handling specimens and kit reagents. Afterwards wash hands carefully.
9. Avoid splashing or forming aerosols.
10. Discard all materials and specimens as if capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at 121°C. Liquid waste containing acid may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500mg/l available chlorine. Allow 30 minutes for decontamination to be completed.

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NOTE: 1. The source material (tissue culture supernatant) used to produce this reagent has been tested and found to be negative for HIV and HCV antibodies and HBsAG in Micro biological test required. No known regime of testing can completely guarentee that any product derived from human blood is incapable of transmitting infections.
2. Liquid waste containing acid must be neutralized with a proportional amount of base prior to the addition of sodium hypochlorite.
   Spills should be wiped thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe up spills should be added to biohazardous waste matter for proper disposal.
3. Deterioration is indicated by a significant decrease by weak agglutination.
4. Do not use reagents after the expiration date printed on the label.
5. When removing reagents from the bottles, use aseptic technique to avoid contamination
2. Mix the reagent bottle gently before use.
3. Do not use clotted blood sample for testing.
4. Ensure used glass slide is disinfected, washed thoroughly and rinsed free of detregents.

TEST PROCEDURE :

1. ABO BLOOD GROUPING :

A. Slide test :

1. Place one drop of blood grouping reagent Anti-A & Anti-B on a glass slide.
2. To each of the reagent drop, add one drop of whole blood. Mix well with applicator stick or tooth pick.
3. Rock the slide gently back and forth.
4. Observe for agglutination at the end of two minutes. Peripheral drying should not be interpreted as a positive test result.

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B. Tube test :

1. Prepare a 2-5% suspension of the cells to be tested in isotonic saline.
2. To the two small test tubes ("2x3/8") labeled test (T) and control (C). Add one drop of above cell suspension using a Pasteur pipette.
3. Add one drop of Anti-A & Anti B reagent to tube (T) and Normal saline to tube (C) and mix well.
4. Centrifuge for one minute at 1000rpm or allow the tubes to stand at RT (25-30°C) for 15-60 minutes.
5. Gently dislodge cell button and observe for agglutination.

2. Rh TYPING :

A. Slide test :

1. Use whole blood or prepare 40% suspension of red blood cells in individuals own plasma or normal saline.
2. Palce one drop each of Anti-D and whole blood or above cell suspension (from step 1) on a glass slide using a Pasteur pipette.
3. Mix well using an applicator stick or tooth pick.
4. Rock the slide gently back and forth.
5. Observe for agglutination macroscopically or using a hand lens within two minutes.

B. Tube test:

1. Prepare a 5% suspension of red blood cells in individuals own plasma or in normal saline.
2. To the two small test tubes (2"x3/8") labeled test (T) and control (C) , add one drop of above cell suspension (from step 1) using a Pasteur pipette.

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3. Add one drop of Anti-D to tube (T) and one drop of 22% Bovine Albumin to tube (C).
4. Mix well and centrifuge both the tubes at 1000rpm for one minute. Alternatively the tubes can be incubated at 37°C for 30minutes.
5. Gently dislodge the cell button and examine macroscopically for agglutination.

INTERPRETATION OF RESULTS :

1. Agglutination of red blood cells in presence of Anti-A indicates the presence of A antigen on red blood cells (Group A).
2. Agglutination of red blood cells in presence of Anti-B indicates the presence of B antigen on red blood cells (Group B).
3. Agglutination of red blood cells in presence of Anti-A as well as Anti-B indicates the presence of both A and B antigens on red blood cells(Group AB).
4. Absence of agglutination of red blood cells in presence of Anti-A and Anti-B indicates absence of both A & B antigens(Group O).
5. Agglutination of the red blood cells in presence of Anti-D antibody with both slide test and tube test is a positive (+) test and indicates the presence of D (Rho) antigen on red blood cells.
6. Absence of agglutination of the red blood cells in presence of Anti-D antibody with both slide test and tube test is a negative test. It generally indicates that D (Rho) antigen is not demonstrable. However, this requires to be confirmed by indirect Coomb's test to rule out the possibility of the presence of D antigen, using Anti-D(Rho) Monoclonal (IgM/IgG/IgM+IgG) reagent.
7. No interpretation should be made if the agglutination appears in negative control with either slide test or tube test.

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TROUBLE SHOOTING:

FALSE POSITIVE	
Cause	**Remedy**
1. Contaminated blood specimen or reagents	Make sure that there is no contamination of blood specimen or reagent. Use clean slide or tube for testing. Do not read the result after 2 minutes.
2. Drying in slide test	Do not read the result after 2 minutes
3. Clotting of blood	Test the sample immediately if anti-coagulant is not added to the sample

FALSE NEGATIVE	
Cause	**Remedy**
1. Contamination of blood specimens or reagents	Make sure that there is no contamination of blood specimen or reagent Use clean slide or tube for testing. Do not read the result after 2 minutes.

WEAKLY / DELAYED REACTION	
Cause	**Remedy**
1. Prolonged storage of red blood cells	Store the blood sample with anticoagulant at 2-8°C for less than 30 days
2. Expired reagent	Check the expiry date on the reagent bottle

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ACCURACY :

Bhat-Bioscan Anti-A, Anti-B, Anti-D Test meets the requirements when tested against DCI approved kit.

Sensitivity :

1. Blood Group A:

No. of Blood Group A samples tested	132
No. of Positive results by Anti-A (IgM) kit	132

2. Blood Group AB:

No. of Blood Group AB samples tested	78
No. of Positive results by Anti-A (IgM) kit	78

Sensitivity of Anti-A (IgM) Kit is estimated to be 100% (132/132), (78/78), Assuming 100% reactivity by comparing with other kits

1. Blood Group B:

No. of Blood Group B samples tested	98
No. of Positive results by Anti-B (IgM) kit	98

2. Blood Group AB:

No. of Blood Group AB samples tested	84
No. of Positive results by Anti-B (IgM) kit	84

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Sensitivity of Anti-B (IgM) Kit is estimated to be 100% (98/98), (84/84), Assuming 100% reactivity by comparing with other kits

1. Rh Positive

No. of Rh Positive samples tested	88
No. of Positive results by Anti-D (IgM/IgG/IgM+IgG) kit	88

2. Rh Negative

No. of Rh Negative samples tested	93
No. of Positive results by Anti-D (IgM/IgG/IgG+IgM) kit	93

Sensitivity of Anti-D (IgM/IgG/IgM+IgG) Kit is estimated to be 100% (88/88), (93/93), Assuming 100% reactivity by comparing with other kits

Specificity :

1. Blood Group B

No. of Blood Group B samples tested	78
No. of Positive results by Anti-A (IgM) kit	0

2. Blood Group O

No. of Blood Group O samples tested	55
No. of Positive results by Anti-A (IgM) kit	0

Specificity of Anti-A (IgM) Kit is estimated to be 100% (0/78), (0/55).

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1. Blood Group A

No. of Blood Group A samples tested	145
No. of Positive results by Anti-B (IgM) kit	0

2. Blood Group O

No. of Blood Group O samples tested	68
No. of Positive results by Anti-B (IgM) kit	0

Specificity of Anti-B (IgM) Kit is estimated to be 100% (0/145), (0/68).

1. Blood Group A

No. of Blood Group A samples tested	105
No. of Positive results by Anti-D (IgM/IgG/IgM+IgG) kit	0

2. Blood Group B

No. of Blood Group B samples tested	79
No. of Positive results by Anti-D (IgM/IgG/IgM+IgG) kit	0

3. Blood Group AB

No. of Blood Group AB samples tested	98
No. of Positive results by Anti-D (IgM/IgG/IgM+IgG) kit	0

Specificity of Anti-D (IgM/IgG/IgM+IgG) Kit is estimated to be 100% (0/105), (0/79), (0/98).

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LIMITATIONS OF THE TEST :

1. It is recommended that with every set of tests, positive and negative controls should be included. The result of positive and negative controls should be read before reading the test results. The satisfactory result of positive and negative control indicates that the reagents are working well.
2. Factors other than reagents, which might affect the performance of the test, include cleanliness of glassware, meticulous follow up of the procedure and rouleaux formation.
3. Blood obtained by finger prick may be tested directly by the slide method, but to avoid clotting, blood should be immediately mixed with the reagent.
4. Contaminated blood specimen or reagents may interfere with test results. Peripheral drying in slide test should not be misinterpreted as agglutination.

REFERENCE :

1. Race, R.R & Sanger. R. (1975) Blood groups in man, Sixth edition, Blackwell publications.p.813.
2. Technicals manual of the American Association of Blood Banks (1981) Eight edition.p.124.
3. Molison.p.l. (1977) Blood Transfusion in clinical Medicine, Sixth edition.p.292.

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EN 980:2008 (E) MEDICAL DEVICES SYMBOL

2°C 30°C	Temperature Limitation	2001-06	Date of Manufacture	IVD	In vitro Diagnostic Device	LOT	Batch Code
	Company name & address		Refer Operating Instructions		Use by	Company	Company Name
EC REP	Authorised Representative in European Community		Do Not Reuse	Σ	Sufficient for		KEEP AWAY FROM SUNLIGHT
	KEEP DRY	NON STERILE	NON-STERILE	CONTROL −	NEGATIVE CONTROL	CONTROL +	POSITIVE CONTROL

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