SBIOCAT™
Column Agglutination Test

REF	90225024
Pack Size	24 Cards

Forward and Reverse Grouping card with Subgrouping (8 Column)

+ 4°C + 25°C Store at 4-25°C	Manufacturer	IVD *In vitro* Diagnostic Medical Device	LOT Batch Number / Lot Number	Expiry date	F&R8C SBIOCAT™ Forward and Reverse Grouping card with Subgrouping (8 Column)
Consult Instructions for use	Date of Manufacture	REF Catalogue Number	This side up	Keep Away from Sunlight	

SUMMARY

Human red blood cell antigen can be divided into four groups A, B, AB and O depending on the presence or absence of corresponding antigens on the red blood cells. The Anti-A, Anti-B and Anti-D reagents are used to detect the presence of corresponding antigens on red blood cells.

Red blood cells used in Reverse grouping are of known ABO antigen, having the specificity to indicate the presence or absence of Anti-A and/or Anti-B, the result of which confirms the forward grouping results.

ABO subgroups are phenotypes that differ in the amount of antigen carried on red cells. Subgroups of A are more commonly encountered than subgroups of B. The two principal subgroups of A are A_1 and A_2. Red cells from A_1 and A_2 persons both react strongly with reagent Anti-A in direct agglutination tests. The serologic distinction between A_1 and A_2 cells can be determined by testing with Anti-A_1 Lectin (Dolichos biflorus).

On group O red cells, there is no A or B antigen, and the membrane expresses abundant H antigen. The H antigen is a precursor of A and B antigens, A and B persons have less H substance than O persons.Individuals whose red cells and secretions lack H, A and B antigens and whose plasma/serum contains potent Anti-H, Anti-A and Anti-B, are termed as O_h(Bombay) Phenotypes.

SBIOCAT™ Forward and Reverse Grouping Card with Sub Grouping facilitate the forward and reverse grouping, Anti-A_1 and Anti-H lectin for Sub grouping along with a control microtube on single card.

REAGENTS

SBIOCAT™ Forward and Reverse Grouping Card with Sub Grouping contains eight microtubes, prefilled with a gel in a suitable buffer containing Monoclonal Anti-A (Clone 11H5), Anti-B (Clone 6F9), Anti-D(IgM)(VI-)(Clone P3 x61+NaTH119), Anti-A_1 (Dolichos biflorus) and Anti-H Lectin (Ulex europaeus) with neutral gel for Control and reverse grouping in appropriate microtubes.

STORAGE AND STABILITY

Store SBIOCAT™ gel cards in an upright position at 4-25°C. Do not freeze. Avoid exposure of SBIOCAT™ gel cards to direct sunlight or any heat source. The shelf life of SBIOCAT™ gel cards is as per the expiry date mentioned on the label. Do not use beyond expiry date. Once the aluminium foil is removed from the microtube, it should be used immediately.

ADDITIONAL REAGENTS AND MATERIALS REQUIRED

SBIOCAT™ Diluent -2 LISS for preparation of red cell suspension. (Refer package insert before use), Lyophilized Papain, Gel card centrifuge (85g), Work station, Micropipette capable of delivering 5-50µl of specimen and Bottle top dispenser.

PRINCIPLE

As the SBIOCAT™ gel card containing red blood cells is centrifuged under specific conditions, the red blood cells possessing the corresponding antigen will agglutinate in presence of the specific antibody and will be trapped in the gel column. The red blood cells, which do not react are not trapped in the gel column and get settled at the bottom of the microtube. The reactions are then read and graded according to their reactivity pattern.

SAMPLE COLLECTION

No special preparation of the patient is required prior to sample collection by approved techniques. For optimal results, freshly collected sample should be used. Anticoagulants like EDTA, CPD-A and Citrate can be used. Serum or plasma samples can be used. Samples should be centrifuged at 1500g for 10 minutes to avoid fibrin residue which may interfere with results.

SAMPLE PREPARATION

For Forward Grouping:

Prepare a 0.8% red blood cell suspension in SBIOCAT™ Diluent- 2 LISS as follows:

1. Bring the SBIOCAT™ Diluent- 2 LISS to room temperature before testing.
2. Dispense 1.0 ml of SBIOCAT™ Diluent- 2 LISS into a clean test tube.
3. Add 10µl packed red cells or 20µl whole blood and mix gently.
4. Red blood cell suspension so obtained should be used for forward grouping & subgrouping.

For Reverse Grouping

Prepare a 0.8% red blood cell suspension in SBIOCAT™ Diluent- 2 LISS as follows:

1. Collect known A_1 and B cells from at least three donors and pool in respective test tubes labeled as A_1 & B.

2. Wash the cells with 0.9% saline till the supernatant is clear.
3. Dispense 1ml of SBIOCAT™ Diluent- 2 LISS into clean labeled test tubes (A_1 & B).
4. Add 10µl of packed red blood cells (pooled and washed known A_1& B cells) into respective test tubes and mix gently.
5. Red blood cell suspensions so obtained should be used for reverse grouping.

TEST PROCEDURE

1. Label the "SBIOCAT Forward and Reverse Grouping Card with Sub Grouping" with patient's/ donor's name or identification number. Remove the aluminium foil carefully by pulling it backwards.
2. Pipette 50µl of 0.8% known A_1 cell suspension to the microtube 7.
3. Pipette 50µl of 0.8% known B cell suspension to the microtube 8.
4. Pipette 50µl of patient's plasma or serum to the microtubes 7 and 8.
5. Pipette 50µl of 0.8% patient's red cell suspension to the microtubes 1-6 (A-B-D-Ctrl-A_1-H), taking care to ensure that micropipette tip does not touches the microtube.
6. Add 25µl of Enzyme (papain) to microtubes 5 & 6 (A_1 & H).
7. Allow the card to incubate for 10 minutes at room temperature.
8. Centrifuge the cards for 10 minutes in the gel card centrifuge.
9. Retrieve the card from centrifuge, read and record the results.

Note: For applications on SBIOCAT™ HEXA, 50µl of 0.8-1.0% red cell suspension can be used instead of 10µl of 5% red cell suspension.

INTERPRETATION OF RESULTS

The control microtube (Ctrl) must be negative to validate the test results. If not, then repeat the test after washing the patient's red blood cells with warm saline.

Positive reaction: Agglutinated red blood cells forming a clear line on the surface of gel column or agglutinates dispersed in the gel column.

Negative reaction: Non agglutinated red blood cells settle at the bottom of the microtube forming a compact button.

Note: A positive reaction indicates presence of the corresponding antigen. Weaker reactions may indicate weaker antigen expressions or antigen variants.

The reaction strength may be recorded as follows:

Strength of reaction	Comments
4+	Agglutinated red blood cells form a line on the surface of the gel microtube.
3+	Most agglutinated red blood cells remain in the upper half of the gel microtube.
2+	Agglutinated red blood cells are observed throughout the length of the gel microtube. A small button of red blood cells may also be visible at the bottom of the gel microtube.
1+	Most agglutinated red blood cells remain in the lower half of the gel microtube. A button of cells may also be visible at the bottom of the gel microtube.
±	Most agglutinated red blood cells are in the lower third part of the gel microtube.
Negative	All the red blood cells pass through and form a compact button at the bottom of the gel microtube.
Mixed field agglutination	Agglutinated red blood cells form a line on the surface of the gel and non-agglutinated red blood cells form a compact button at the bottom of the gel microtube.
H	Hemolysis of red blood cells

Note : Visual reading of reactions in a card may differ from the reactions read by any automated software through image processing. However this may not change the final result interpretation.

Expected reactivity pattern for ABO grouping:

Anti-A	Anti-B	Blood Group
± to 4+	Negative	A
Negative	± to 4+	B
± to 4+	± to 4+	AB
Negative	Negative	O

NOTE: Human red blood cells that show weak reaction with Anti-A and/or Anti-B probably indicate subgroups of A and/or B and further testing is recommended.

Expected reactivity pattern for Rho (D) typing:

Anti-D	Rho(D) Type
± to 4+	Rho (D) Positive
Negative	Rho (D) Negative

NOTE: Weak D/ Partial D type human red blood cells may give a weaker or negative reaction. Such cells should be retested for weak D confirmation with SBIOCAT™ Coombs Anti-IgG card.

Reactions for different blood groups with Anti-A_1 and Anti-H Lectin.

	Anti-A_1	Anti-H
A_1	++ to ++++	+ to +++
A_2	Neg to +	++ to ++++
A_1B	++ to ++++	+ to +++
A_2B	Neg to +	+ to +++
B	Neg	++ to +++
O	Neg	+++ to ++++
O_h	Neg	Neg

Reaction for reverse grouping:

A_1	B	Blood group
± to 4+	Negative	B
Negative	± to 4+	A
± to 4+	± to 4+	O
Negative	Negative	AB

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The SBIOCAT™ gel cards contains sodium azide < 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantity of water.
3. Since A & B antigens are not fully developed at birth,weak reactions may occur with new born cells and sub groups cannot be identified.
4. All SBIOCAT™ gel cards should be centrifuged for one complete cycle (10 minutes) in gel card centrifuge before use.
5. Visually inspect the SBIOCAT™ gel cards before use.
6. SBIOCAT™ gel cards having bubble(s) entrapped within the gel can be centrifuged for two complete cycles in gel card centrifuge to remove the bubble, if bubbles are not removed the card should not be used.
7. SBIOCAT™ gel cards that exhibit any signs of drying (i.e. absence or reduced level of reagent buffer above the gel column), decreased volume of gel, cracked gel should not be used.
8. SBIOCAT™ gel cards with damaged aluminium foil seal should not be used.
9. Freezing of SBIOCAT™ gel cards or evaporation of gel or reagent buffer due to exposure to heat may lead to erroneous results.
10. Fibrin or particulate matter if present in the sample may lead to erroneous results.
11. Fibrin if present in the sample may trap red blood cells on surface of the gel column presenting a pink line. To avoid, samples should be well centrifuged at 1500g for 10 minutes before taking serum or plasma and RBCs should be washed if not collected properly in an anticoagulant.
12. Use of red blood cells concentration/ volume and reagents other than those described may lead to erroneous results. Follow the instructions carefully.
13. Aged or stored red blood cells may exhibit weaker reactivity than freshly collected cells.
14. Old cell panels may give an unclear background with SBIOCAT™ gel cards.
15. Do not use hemolysed samples.
16. Extreme turbidity or discoloration may indicate microbial contamination or denaturation of protein due to thermal damage. Such SBIOCAT™ gel cards should be discarded.
17. Contamination of reagents during usage may cause false positive or negative results.
18. Red cell aggregation in the red cell suspension may interfere the passage.
19. Aluminium foil seal of SBIOCAT™ gel cards should be removed gently and carefully by pulling the foil seal backwards to avoid contamination of reagents from one microtube to another.
20. Do not use lipemic, icteric and hyperproteic samples.
21. To avoid contamination always use fresh tips before dispensing into each microtube.
22. SBIOCAT™ ContaVoid (Cat. No. 903300100) can be used to avoid contamination of reagents in microtubes while usage.

REMARKS

1. Known positive and negative control should be tested as per Good Laboratory Practices.
2. SBIOCAT™ Red Cell Preserving Solution (Cat. No.90262020) can be used as red blood cell preservative solution for preservation of known cells.
3. The Anti-D does not detect the D VI variant.

BIBLIOGRAPHY

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