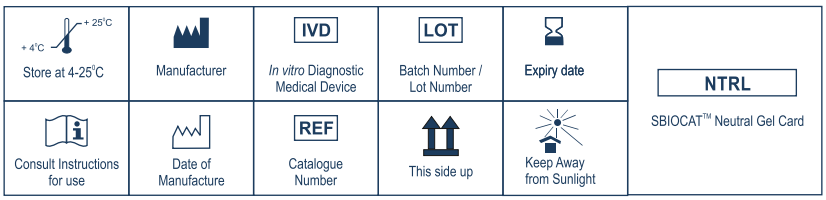
SBIOCAT™
Column Agglutination Test

REF	90212024
Pack Size	24 Cards

Neutral Gel Card



SUMMARY

Along with Coombs techniques, Saline and Enzyme techniques are also very important to detect antibodies which predominantly react at 4°C or at room temperature. Enzyme techniques are very useful when increased sensitivity in detecting an antibody is required. Enzymes enhances the reactions of certain antibodies like Rh, Kell and Kidd system and at the same time some antigens like M, N, S of MNS system and Fya and Fyb of Duffy system are destroyed by Enzyme treatment. Saline techniques are used to detect antibodies that react predominantly at 4°C or at room temperature such as Anti-M, N, P1, Lea, Leb and I. Saline techniques are very useful in detecting autoimmune hemolytic anemia associated with cold antibodies. Saline techniques are also used for ABO reverse grouping and compatibility testing.
SBIOCAT ™ Neutral Gel Card facilitates Enzyme and Saline phase tests in gel techniques.

REAGENTS

SBIOCAT™ Neutral Gel Card contains six microtubes prefilled with a gel in a suitable buffer.

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. Papain solution suitable for serological applications (Refer package insert before use). Gel card centrifuge (85g), Incubator (37°C), Work station, Micropipette capable of delivering 5-50µl of specimen, Bottle top dispenser and Reagent red blood cell panels.

PRINCIPLE

As the SBIOCAT™ gel card containing the 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

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 of packed red cells to SBIOCAT™ Diluent -2 LISS collected in test tube and mix gently.
4. Red blood cell suspension so obtained should be used for testing.

For reverse grouping, collect known cells (A_1, B and O group) from at least three donors and pool in respective pre labeled test tubes. Wash the cells with 0.9% saline till the supernatant is clear and prepare 0.8% red blood cell suspension as described above

TEST PROCEDURE

A) **FOR ANTIBODY SCREENING / IDENTIFICATION - ENZYME TEST**

1. Label the appropriate number of microtubes of SBIOCAT™ Neutral Gel Card with patient's / donor's name or identification number. Remove the aluminium foil of required number of microtubes carefully by pulling it backwards.
2. Pipette 50µl of each 0.8% reagent red blood cell suspension to appropriate labeled microtube, taking care to ensure that micropipette tip does not touch the microtube.

3. If an autocontrol is to be included, pipette 50µl of 0.8% patient's / donor's own red cell suspension in an appropriate labeled microtube.
4. Add 25µl of patient's / donor's serum or plasma to be tested in all the microtubes. The interval between cells and serum or plasma transfer should not exceed 10 minutes.
5. Add 25µl of Enzyme (Papain) to all the microtubes.
6. Incubate the SBIOCAT ™gel card for 15 minutes at 37°C in an incubator.
7. After incubation, centrifuge the SBIOCAT™ gel card for 10 minutes in the gel card centrifuge.
8. Retrieve the card from centrifuge, read and record the results.

B) **FOR ANTIBODY SCREENING/ IDENTIFICATION - SALINE TEST AT 4°C**.

The SBIOCAT™ Neutral Gel Card and other test components should be kept in refrigerator (2-8°C) for at least 2 hours before use. Refrigerated test components should be used for testing.

1. Label the appropriate number of microtubes of SBIOCAT ™ Neutral Gel Card with patient's / donor's name or identification number. Remove the aluminium foil of required number of microtubes carefully by pulling it backwards.
2. Pipette 50µl of each 0.8% reagent red blood cell suspension to appropriate labeled microtube, taking care to ensure that micropipette tip does not touches the microtube.
3. If an autocontrol is to be included, pipette 50µl of 0.8% patient's / donor's own red cell suspension in an appropriate labeled microtube.
4. Add 25µl of patient's / donor's serum or plasma to be tested in all the microtubes. The interval between cells and serum or plasma transfer should not exceed 10 minutes.
5. Incubate the SBIOCAT™gel card for 30 minutes at 2-8°C.
6. After incubation, centrifuge the SBIOCAT™ gel card for 10 minutes in the gel card centrifuge.
7. Retrieve the card from centrifuge, read and record the results.

C) COMPATIBILITY TEST – ENZYME TEST

1. Label the appropriate number of microtubes of SBIOCAT™ Neutral Gel Card with the patient's name or identification number. Remove the aluminium foil of required number of microtubes carefully by pulling it backwards.
2. Pipette 50µl of 0.8% donor's red blood cell suspension to appropriate microtubes, taking care to ensure that micropipette tip does not touches the microtube.
3. If an autocontrol is to be included, pipette 50µl of 0.8% patient's own red cell suspension in an appropriate labeled microtube.
4. Add 25µl of patient's serum or plasma to the above microtubes. The interval between cells and serum or plasma transfer should not exceed 10 minutes.
5. Add 25µl of Enzyme (Papain) to all the microtubes.
6. Incubate the SBIOCAT™gel card for 15 minutes at 37°C in an incubator.
7. After incubation, centrifuge the SBIOCAT™ gel card for 10 minutes in the gel card centrifuge.
8. Retrieve the card from centrifuge, read and record the results.

D) COMPATIBILITY TEST – SALINE TEST

1. Label the appropriate microtubes of SBIOCAT™ Neutral Gel Card with the patient's name or identification number. Remove the aluminium foil of required number of microtubes carefully by pulling it backwards.
2. Pipette 50µl of 0.8% donor's red blood cell suspension to appropriate microtubes, taking care to ensure that micropipette tip does not touches the microtube.
3. If an autocontrol is to be included, pipette 50µl of 0.8% patient's own red cell suspension in an appropriate labeled microtube.
4. Add 25µl of patient's serum or plasma to the microtubes. The interval between cells and serum or plasma transfer should not exceed 10 minutes.
5. Incubate the SBIOCAT ™gel card for 15 minutes at room temperature.
6. After incubation, centrifuge the SBIOCAT™ gel card for 10 minutes in the gel card centrifuge.
7. Retrieve the card from centrifuge, read and record the results.

E) Reverse Grouping

1. Label the appropriate microtubes of SBIOCAT™ Neutral Gel Card with patient's name or identification number. Remove the aluminium foil carefully by pulling it backwards.
2. Pipette 50 µl of 0.8% known A,, B and O cell suspension to the appropriate labeled microtube.
3. Pipette 50 µl of patient's plasma or serum to all the microtubes, taking care to ensure that micropipette tip does not touches the microtube.
4. Incubate the SBIOCAT™ gel card for 10 minutes at room temperature.
5. After incubation, centrifuge the SBIOCAT™ gel card for 10 minutes in the gel card centrifuge.
6. Retrieve the card from centrifuge, read and record the results.

INTERPRETATION OF RESULTS

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.

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

Antibody Screening / Identification

Positive reaction indicates the presence of irregular antibodies.

Negative reaction indicates absence of detectable irregular antibodies in the patient's / donor's serum or plasma.

Further testing is recommended to identify the antibody specificity.

Compatibility testing

A negative reaction indicates compatibility of donor's blood with the patient.

A positive reaction indicates incompatibility of donor's blood with the patient, due to presence of antibodies directed against the antigens on the donor's red blood cells. Further investigations to identify the antibody specificity should be performed.

The autocontrol microtube must be negative to validate the results. Positive results in autocontrol may indicate autoantibodies.

After incubation if hemolysis is observed in upper part of the gel column, it should be interpreted as a positive reaction.

Reverse Grouping

Reactions for reverse grouping

A_1	B	O	Blood Group
± to 4+	Negative	Negative	B
Negative	± to 4+	Negative	A
± to 4+	± to 4+	Negative	O
Negative	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. All SBIOCAT™ gel cards should be centrifuged for one complete cycle (10 minutes) in gel card centrifuge before use.
4. Visually inspect the SBIOCAT™ gel cards before use.
5. 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.
6. 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.
7. SBIOCAT™ gel cards with damaged aluminium foil seal should not be used.
8. Freezing of SBIOCAT™ gel cards or evaporation of gel or reagent buffer due to exposure to heat may lead to erroneous results.
9. Fibrin or particulate matter if present in the sample may lead to erroneous results.
10. Fibrin if present in the sample may trap red blood cells on the surface of the gel column presenting a pink line. RBCs should be washed with normal saline if not collected properly in an anticoagulant
11. Use of red blood cells concentration/ volume and reagents other than those described may lead to erroneous results. Follow the instructions carefully.
12. Aged or stored red blood cells may exhibit weaker reactivity than freshly collected cells.
13. Old cell panels may give an unclear background with SBIOCAT™ gel cards.
14. Do not use hemolysed samples.
15. Extreme turbidity or discoloration may indicate microbial contamination or denaturation of protein due to thermal damage. Such SBIOCAT™ gel cards should be discarded.
16. Contamination of reagents during usage may cause false positive or negative results.
17. Red cell aggregation in the red cell suspension may interfere the passage.
18. 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.
19. To avoid contamination always use fresh tips before dispensing into each microtube.

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.

PERFORMANCE CHARACTERISTICS

The performance characteristics of SBIOCAT™ Neutral gel card is suitable for the intended use of the product. The performance study which has included saline and enzymatic tests where results obtained were similar to those obtained with established product of equivalent intended use. The reverse grouping results showed 100% specificity and sensitivity.

BIBLIOGRAPHY

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