

Matrix[™] Coombs and Neutral Gel Card

SUMMARY

In screening test, enzyme tests are considered to be very useful especially when clubbed with Anti-human globulin procedures. The polyspecific Anti-human globulin contains Anti-human IgG and Anti-C3d. In AHG test presence of IgG antibody is detected by Anti-human IgG and Anti-C3d component of AHG detects the complement (C3d) activity. Alongwith 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. This property is also useful in identifying an antibody when more than one antibodies are present.

Matrix[™] Coombs and Neutral Gel Card offers a combination of Polyspecific AHG and Neutral Gel in one card.

REAGENTS

Matrix[™] Coombs and Neutral Gel Card contains six microtubes prefilled with a gel in a suitable buffer containing Polyspecific Anti-human globulin in microtubes 1-3, and Neutral Gel in microtubes 4-6.

STORAGE AND STABILITY

Store Matrix[™] gel cards in an upright position at 4-25°C. Do not freeze.

Avoid exposure of Matrix[™] gel cards to direct sunlight or any heat source. The shelf life of Matrix[™] 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

Matrix[™] Diluent -2 LISS for preparation of red cell suspension. Papain solution suitable for serological applications LYOPAP (Cat. No. 102500061). Reagent Red Blood Cells for antibody screening and identification (Refer package insert before use). Gel card centrifuge (85g), Incubator (37°C), Work station, Micropipette capable of delivering 5-50µl of specimen and Bottle top dispenser.

PRINCIPLE

As the Matrix[™] 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 samples 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 0.8% red cell suspension in Matrix[™] Diluent- 2 LISS as follows:

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

TEST PROCEDURE

For Coombs Test

1. Label the Matrix[™] Coombs and Neutral Gel Card with patient's name / identification number. Remove the aluminium foil of required number of microtubes carefully by pulling it backwards.

- Pipette 50µl of 0.8% reagent red cell suspension to the appropriate microtubes (or donor's cell suspension for compatibility test).
- Add 25µl of patient's serum or plasma to the above microtubes.
- Incubate the Matrix™ gel card for 15 minutes at 37°C in an incubator.
- Centrifuge the Matrix™ gel card for 10 minutes in the gel card centrifuge.
- Retrieve the card from centrifuge, read and record the results.

For Enzyme Test

- Label the "Matrix™ Coombs and Neutral Gel Card" with patient's name or identification number. Remove the aluminium foil of required number of microtubes carefully by pulling it backwards.
- Pipette 50µl of 0.8% reagent red cell suspension to the appropriate microtubes (or donor's cell suspension for compatibility test).
- Add 25µl of patient's serum or plasma to the above microtubes.
- Add 25µl of Enzyme (LYOPAP) to the above microtubes.
- Allow the card to incubate for 15 minutes at 37°C.
- Centrifuge the Matrix™ gel card for 10 minutes in the gel card centrifuge.
- Retrieve the card from centrifuge, read and record the results.

Note: For applications on Matrix™ Automax-80, 50µl of 0.8-1.0% red cell suspension can be used instead of 10µl of 5% red cell suspension.

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 at the top 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 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 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 at the top 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.

ANTIBODY SCREENING / ANTIBODY 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.

COMPATIBILITY TEST

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

A positive reaction indicates incompatibility of the donor's blood with the patient, due to presence of antibodies directed against 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 results. Positive reaction in autocontrol may indicate autoantibodies.

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

NOTE

- In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
- The Matrix™ gel cards contains sodium azide <0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantity of water.
- All Matrix™ gel cards should be centrifuged for one complete cycle (10 minutes) in gel card centrifuge before use.
- Visually inspect the Matrix™ gel cards before use.
- Matrix™ 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.
- Matrix™ 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.
- Matrix™ gel cards with damaged aluminium foil seal should not be used.
- Freezing of Matrix™ gel cards or evaporation of gel or reagent buffer due to exposure to heat may lead to erroneous results.
- Fibrin or particulate matter if present in the sample may lead to erroneous results.
- Fibrin if present in the sample may trap red blood cells on top of 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.
- Use of red blood cells concentration/ volume and reagents other than those described may lead to erroneous results. Follow the instructions carefully.
- Aged or stored red blood cells may exhibit weaker reactivity than freshly collected cells.
- Old cell panels may give an unclear background with Matrix™ gel cards.
- Do not use hemolysed, lipemic, icteric and hyperproteic samples.
- Extreme turbidity or discoloration may indicate microbial contamination or denaturation of protein due to thermal damage. Such Matrix™ gel cards should be discarded.
- Contamination of reagents during usage may cause false positive or negative results.
- Red cell aggregation in the red cell suspension may interfere with the passage.
- Aluminium foil seal of Matrix™ gel cards should be removed gently and carefully by pulling the foil seal backwards to avoid contamination of reagents from one microtube to another.
- To avoid contamination always use fresh tips before dispensing into each microtube.
- Some pathological conditions are reported as causing non-specific reactions in AHG procedures.

REMARKS

- Known positive and negative controls should be tested as per Good Laboratory Practices.
- ERYWELL (Cat. No. 10253020) can be used as red blood cell preservative solution for preservation of known cells.
- Agtrol™ (Cat. No. 10252010) can be used for quality control procedures related to AHG.
- LYOPAP (Cat. No. 102500061) can be used as Papain for serological applications.

BIBLIOGRAPHY

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