



Cat. No.	10259024	102940024
Presentation	24 Cards	System Pack

Matrix™ ABO/Rho (D)/AHG Neonate Group Card

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-AB reagents are used to detect the presence or absence of corresponding antigens on the red blood cells.

In Newborn's the A and B antigens are not completely developed, thus weaker reaction may be shown when compared to adults, when tested. In adults the antigens and respective antibodies are present, but in newborns the antibodies appear after 4 to 6 months of birth. Thus reverse grouping cannot be done on newborn samples, but it is possible after 2 to 4 years of birth.

Also human red blood cells are classified as Rho(D) positive or Rho(D) negative depending upon the presence or absence of Rho(D) antigen. The D antigen and weak D are fully developed at birth. If the mother is Rho(D) negative, it is very much important to determine the D antigen and weak D of the newborn's.

The Direct Antiglobulin Test is very important to detect the antibodies adsorbed to the red blood cells in-utero.

The determination of ABO, Rho(D) blood groups and the Direct Coombs Test can be made on newborn's blood cells using the Matrix™ Neonate Group Card. This technique will assist in prompt treatment of the Neonate.

REAGENTS

The Matrix™ Neonate Group Card contains six microtubes, pre-filled with gel in a suitable buffer containing specific Monoclonal Anti-A (Clone 11H5), Anti-B (Clone 6F9), Anti-AB (Clone ES-15), Anti-D (IgM) (VI-) (Clone P3x61 + TH-28) antibodies and Neutral gel in appropriate microtube. The Anti Human Globulin reagent is a blend of Anti Human IgG and Monoclonal Anti-C3d (Clone 12011D10).

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. (Refer package insert before use). Gel card centrifuge (85 g), Work station, Micropipette capable of delivering 5-50µl of specimen and Bottle top dispenser.

PRINCIPLE

As the Matrix™ card containing red blood cells is centrifuged under specific conditions, the red blood cells possessing corresponding antigen will agglutinate in presence of the specific antibody directed towards the antigen in the gel matrix 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 column. 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. Cord or heel prick samples may be used. (Cord blood samples must be washed at least 3 times with normal saline before testing).

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 use.
2. Dispense 1.0 ml of Matrix™ Diluent -2 LISS into a clean test tube.
3. Add 10µl of packed red cells to Matrix™ Diluent -2 LISS collected in test tube and mix gently.
4. Red blood cell suspension so obtained should be used for testing.

TEST PROCEDURE

1. Label the appropriate Matrix™ gel card with patient's name / identification number. Remove the aluminium foil carefully by pulling it backwards.
2. Pipette 50 µl of 0.8% red blood cell suspension to all the microtubes. Take care to ensure that the micropipette tip does not touch the reagent in the microtube.
3. Centrifuge the cards for 10 minutes in the gel card centrifuge.
4. 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

The control microtube (Ctrl) must be negative to validate the forward grouping 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.

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.

Expected reactivity pattern for ABO grouping with Matrix™ gel card:

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

Expected reactivity pattern for Rho (D) typing with Matrix™ gel card:

Anti-D	Rho(D) Type
± to 4+	Positive
Negative	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 Matrix™ Coombs Anti-IgG card.

For Direct Antiglobulin Test:

Negative reaction indicates absence of detectable IgG antibodies or Complement component C3d on the newborn's red blood cells.

Positive reaction indicates that newborn's red blood cells are sensitized with IgG or Complement component C3d.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. 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.
3. All Matrix™ gel cards should be centrifuged for one complete cycle (10 minutes) in gel card centrifuge before use.
4. Visually inspect the Matrix™ gel cards before use.
5. 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.
6. 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.
7. Matrix™ gel cards with damaged aluminium foil seal should not be used.
8. Freezing of Matrix™ 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 top of gel column presenting a pink line. To avoid, samples should be well centrifuged at 1500g for 10 minutes before taking samples and RBCs should be washed 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 Matrix™ gel cards.
14. Do not use hemolysed samples.
15. Exterme turbidity or discoloration may indicate microbial contamination or denaturation of protein due to thermal damage. Such Matrix™ 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 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.
19. Do not use lipemic and icteric samples.
20. To avoid contamination always use fresh tips before dispensing into each microtube.
21. Since A and B antigens are not fully developed at birth, weak reactions may occur with neonate samples and subgroups can not be identified.
22. Some pathological conditions are reported as causing non-specific reactions in AHG procedures.
23. Matrix™ ContaVoid can be used to avoid contamination of reagents in microtubes while usage. For details refer pack insert of Matrix™ ContaVoid (Catalogue no.102770100).

REMARKS

1. Known positive and negative control should be tested as per Good Laboratory Practices.
2. Agtro™ (Cat. No. 10252010) can be used for quality control procedures related to AHG.
3. ERYWELL (Catalogue no. 10253020) can be used as red blood cell preservative solution for preservation of known cells.
4. The Anti-D does not detect the D VI variant.

BIBLIOGRAPHY

1. Human Blood Groups by Geoff Daniels, 2nd Ed., Blackwell Science, Oxford 2002.
2. HMSO, Guidelines for the Blood Transfusion Services, 2nd Ed., 1993.
3. H. Malyska & D. Weiland, The Gel Test. Laboratory Medicine Vol. 25, No.2 February 1994, pp. 81-85.
4. M.C.Z. Novaretti et al. Comparison Of Tube And Gel Techniques For Antibody Identification. Immunohematology 2000;16:138-141.
5. D. Voak, New Developments in Blood Group Serology, Infusion Therapy Transfusion Medicine 1999;26:258-260.
6. Data on file: Tulip Diagnostics (P) Ltd.