- 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 testing and RBCs should be washed if not collected properly in an anticoagulant.
- 11. Use of red blood cell 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 show an unclear background with Matrix<sup>™</sup> gel cards.
- 14. Do not use hemolysed, lipemic or icteric samples.
- 15. Extreme turbidity or discoloration may indicate microbial contamination or denaturation of protein due to thermal damage. Such Matrix<sup>™</sup> 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 blood cell suspension may interfere the passage.
- 18. Aluminium foil seal of Matrix<sup>™</sup> gel cards should be removed gently and carefully by pulling the aluminium foil seal backwards to avoid contamination of reagents from one microtube to another.
- 19. Do not use lipemic, icteric and hyperproteic samples.
- 20. To avoid contamination always use fresh tips before dispensing into each microtube.
- 21. Matrix<sup>™</sup> Octoblus ContaVoid can be used to avoid contamination of reagents in microtubes while usage. For details refer pack insert of Matrix<sup>™</sup> Octoplus ContaVoid (Catalogue no.102830100).

### REMARKS

- Known positive and negative controls should be tested as per Good Laboratory Practices.
- ERYWELL (Catalogue no. 10253020) can be used as red cell preservative solution for preservation of known cells.
- 3. Anti-D does not detects D VI variant.

## **BIBLIOGRAPHY**

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- 3. H. Malyska & D. Weiland, The Gel Test. Laboratory Medicine Vol. 25, No.2 February 1994, pp. 81-85.
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- D. Voak, New Developments in Blood Group Serology, Infusion Therapy Transfusion Medicine 1999;26:258-260.
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Cat. No.	102560192
Presentation	24 Cards

# Matrix<sup>™</sup> Octoplus Forward and Reverse Grouping Card with Sub Grouping

# 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-D 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, and A, and A, and A, persons both react strongly with reagent Anti-Ain direct agglutination tests. The serologic distinction between A<sub>1</sub> and A<sub>2</sub> cells can be determined by testing with Anti-A, Lectin (Dolichos biflorus).

Matrix™ Octoplus Forward and Reverse Grouping Card with Sub Grouping facilitate the forward and reverse grouping, Anti-A₁ and Anti-H lectin for Sub grouping along with a control microtube on single card.

### REAGENTS

The Matrix<sup>™</sup> Octoplus 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 8F9), Anti-D (IgM)(VI-) (Clone P3x61 + TH-28), Anti-A, and Anti-H Lectin with neutral gel for Control and reverse grouping in appropriate microtubes.

# STORAGE AND STABILITY

Store the Matrix<sup>™</sup> gel cards in an upright position at 4-25°C. Do not freeze.

Avoid exposure of Matrix<sup>™</sup> gel cards to direct sunlight or any heat source. The shelf life of Matrix<sup>™</sup> 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<sup>™</sup> Diluent -2 LISS for preparation of red cell suspension, Matrix<sup>™</sup> Erygen RG for Reverse Grouping (Refer package insert before use). Gel card centrifuge (85g), Work station, Micropipette capable of delivering 5-50µl of specimen and Bottle top dispenser.

As the Matrix<sup>™</sup> gel card containing red blood cells is centrifuged under specific conditions, 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. Anticoaquiants like EDTA, CPD-A and Citrate 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 Matrix<sup>™</sup> Diluent- 2 LISS as follows:

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

10256/0714/AE/VER-02

Manufactured by:

**TULIP DIAGNOSTICS (P) LTD.** 

Plot Nos. 92/96, Phase II C, Verna Industrial Estate, Verna Goa - 403 722 India Website: www.tulipgroup.com

# For Reverse Grouping

Use of Matrix<sup>™</sup> Erygen RG for Reverse Grouping or alternatively prepare a 0.8% red blood cell suspension in Matrix<sup>™</sup> Diluent-2 LISS as follows:

- 1. Collect known A, and B cells from at least three donors and pool in respective test tubes labeled as A, & B.
- 2. Wash the cells with 0.9% saline till the supernatant is clear.
- 3. Dispense 1ml of Matrix<sup>™</sup> Diluent-2LISS into clean labeled test tubes (A<sub>1</sub>&B).
- 4. Add 10µl of packed red blood cells (pooled and washed known A,& B cells) into respective test tubes and mix gently.
- 5. Red blood cell suspensions so obtained should be used for reverse grouping.

#### **TEST PROCEDURE**

- Label the "Matrix™ Octoplus 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<sub>1</sub> cell suspension to the microtube 7.
- 3. Pipette 50ul 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.
- Pipette 50µl of 0.8% patient's red cell suspension to the microtubes 1-6 (A-B-D-Ctrl-A<sub>1</sub>-H), taking care to ensure that micropipette tip does not touches the microtube.
- 6. Add 25µl of Enzyme (LYOPAP) to microtubes 5 & 6 (A<sub>1</sub> & 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.

# INTERPRETATION OF RESULTS

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

Positive reaction: Agglutinated red blood cells forming a clear line at top of the 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 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.	
Н	Hemolysis of red blood cells	

# Expected reactivity pattern for ABO grouping:

Anti-A	Anti-B	Blood Group
± to 4+	Negative	A
Negative	± to 4+	В
± to 4+	± to 4+	AB
Negative	Negative	0

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 Matrix™ Coombs Anti-IgG card.

# Reactions for different blood groups with Anti-A, and Anti-H Lectin.

	Anti-A₁	Anti-H
A,	++ to ++++	+ to +++
A <sub>2</sub>	Neg to +	++ to ++++
A₁B	++ to ++++	+ to +++
A <sub>2</sub> B	Neg to +	+ to +++
В	Neg	++ to +++
0	Neg	+++ to ++++
O <sub>h</sub>	Neg	Neg

# Reaction for reverse grouping:

<b>A</b> ,	В	Blood group
± to 4+	Negative	В
Negative	± to 4+	A
± to 4+	± to 4+	0
Negative	Negative	AB

#### NOTE

- 1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
- The Matrix<sup>™</sup> gel card 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<sup>™</sup> gel cards before use.
- Matrix de cards having bubble(s) entrapped within the gel can be centrifuged for two complete cycles in gel card centrifuge to remove the bubbles, if bubbles are not removed the card should not be used.
- Matrix<sup>™</sup> 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 or cracked gel should not be used.
- 7. Matrix<sup>™</sup> gel cards with damaged aluminium foil seal should not be used.
- Freezing of Matrix<sup>™</sup> gel cards or evaporation of gel or reagent buffer due to exposure to heat may lead to erroneous results