SUMMARY
Red cell reagents used in Reverse Grouping Card are of known ABO antigen, having the specificity to indicate the presence or absence of Anti-A and Anti-B, the result of which determines the reverse group. Classification of blood groups must be based on both forward and reverse grouping. The Matrix ABO Reverse Grouping Card helps to determine the reverse group.

REAGENTS
Matrix ABO Reverse Grouping Card contains Neutral gel in all the six microtubes. Preservative: 0.1% sodium azide.

REAGENT STORAGE
Store the matrix gel cards at 4°C to 25°C in upright position. Do not freeze. Avoid exposure of the cards to any heat source, direct air-conditioning sources or any area receiving direct sunlight. The shelf life of the matrix gel card is as per the expiry date mentioned on the label. Do not use beyond expiry date.

ADDITIONAL REAGENTS AND MATERIAL REQUIRED
1. Matrix LISS solution (Diluent-2) for preparing red blood cell suspension (refer Diluent-2 pack insert before use).
4. 12x75 mm test tube for the preparation of red blood cell suspension.
5. Matrix sample pipette or equivalent (5-50 µl) (MP-0550).
6. Matrix bottle Top Dispenser (0.5-2.5 ml) (BD-5250).
7. Known test cells A and B (Erygen RG).

PRINCIPLE
Human red blood cells possessing known A and/or B antigen are used for Reverse Grouping. The red blood cells will agglutinate in the presence of antibody directed towards the antigen. Agglutinated cells forming a red button on the surface of the gel or agglutinates dispersed in the gel is a positive result and indicates the presence of the corresponding antibody. Compact button of cells at the bottom of the microtubes indicates the absence of the corresponding antibody. The reactions are visually read and graded according to their reactivity pattern.

NOTE
1. In-vitro diagnostic reagent for laboratory and for professional use only. Not for medicinal use.
2. The matrix gel card contains sodium azide 0.1% as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
3. Extreme turbidity or discoloration may indicate microbial contamination or denaturation of protein due to thermal damage. Such matrix gel card should be discarded.
4. Do not use matrix gel cards, which show signs of drying.
5. Matrix gel cards having bubbles or damaged seals should be discarded.
6. Known samples must preferably be tested before start of work each day as a good laboratory practice using respective batch of matrix gel card.
7. The Aluminium foil on the matrix gel card should be removed carefully and gently so as to ensure that no carry over takes place.
8. For every sample a new micropipette tip should be used.
9. Fibrin residue in the serum or red cell aggregation in the red cell suspension can trap non-agglutinated cells, forming a pink line on top of the gel, whereas most cells pellet to the bottom after centrifugation.
10. Mixed field agglutination is observed in case of the mix up of samples or in case of incompatible transfusion. In such cases the results are considered invalid and further testing is recommended using a fresh sample.
SAMPLE COLLECTION
Plasma or serum for reverse grouping. No special preparation of the patient is required prior to sample collection by approved techniques. Samples should preferably be tested as soon as possible. If delay in testing occurs sample should be stored at 2-8°C and may be used up to 48 hrs after collection, or stored frozen at -20°C to -80°C. If serum is used instead of plasma, it must be cleared by centrifuging it at 1500 g for 10 minutes, so as to avoid presence of fibrin residues, which might interfere with the test results.

SAMPLE PREPARATION
Do not use haemolysed or contaminated blood samples, or those containing clots. Collect known A, and B cells. Wash the cells twice with 0.9% saline. Discard the supernatant and prepare 0.8% cell suspension.
In Matrix Diluent -2.
Preparation of 0.8 % red blood cell suspension is as follows:
1. Bring the Matrix Diluent-2 to room temperature before testing.
2. Add 1 ml of Matrix Diluent-2 into a clean glass test tube.
3. Add 10 µl of packed cells to the Matrix Diluent-2 and mix gently.
4. The red blood cell suspension so obtained should be used for testing.
5. Set up as many Matrix gel cards as may be required.

TEST PROCEDURE
1. Allow samples and reagent to reach room temperature.
2. Label the appropriate microtubes of the Matrix Gel with patient name / identification number.
3. Pipette 50 µl of 0.8%A cell suspension to the appropriate microtube.
4. Pipette 50 µl of 0.8%B cell suspension to the appropriate microtube.
5. Pipette 50 µl of patient’s serum or plasma to both the microtubes.
6. Allow the cards to incubate for 10 minutes at room temperature (18 - 25°C).
7. Centrifuge the cards for 10 minutes in the Matrix Card Centrifuge.
8. Read and record the results immediately.

REACTION FOR REVERSE GROUPING

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>Blood group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+ to 4+</td>
<td>Negative</td>
<td>B</td>
</tr>
<tr>
<td>Negative</td>
<td>1+ to 4+</td>
<td>A</td>
</tr>
<tr>
<td>1+ to 4+</td>
<td>1+ to 4+</td>
<td>O</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>AB</td>
</tr>
</tbody>
</table>

INTERPRETATION OF RESULTS
Reading and interpretation of results must be done after centrifugation process only.
Positive Reaction: Agglutinated red blood cells form a clear line on the surface of the matrix gel column or get dispersed in the gel column.
Negative Reaction: Unagglutinated red blood cells settle at the bottom of the microtube.
The reaction strength may be recorded as follows:

<table>
<thead>
<tr>
<th>Strength of reaction</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>Agglutinated red blood cells form a band at the top of the gel column.</td>
</tr>
<tr>
<td>3+</td>
<td>Most agglutinated red blood cells remain in the upper half of the gel column.</td>
</tr>
<tr>
<td>2+</td>
<td>Agglutinated red blood cells are observed throughout the length of the column. A small button of red blood cells may also be visible at the bottom of the gel column.</td>
</tr>
<tr>
<td>1+</td>
<td>Most agglutinated red blood cells remain in the lower half of the column. A button of cells may also be visible at the bottom of the gel column.</td>
</tr>
</tbody>
</table>
±  Most agglutinated red blood cells are in the lower third part of the column.

Negative (0) All the red blood cells pass through and form a compact button at the bottom of the gel column.

Mixed field agglutination Agglutinated red blood cells form a band at the top of the gel and non-agglutinated red blood cells form a compact button at the bottom of the gel column.

H Hemolysis of the red blood cells

REMARKS
1. It is important that known positive and negative controls should be included as per the guidelines of Good Laboratory Practices (GLP).
2. Freezing or evaporation of the matrix gel card due to exposure to heat may impede the passage of unagglutinated red blood cells through matrix gel.
3. Matrix gel cards that exhibit drying, if used, can lead to erroneous results.
4. Use of red blood cell concentrations other than those described may interfere with the final reporting of results.
5. Aged or stored red blood cells may exhibit weaker reactivity than freshly collected cells.
6. Bubble entrapped in the matrix gel may hamper the passage of unagglutinated red blood cells. Matrix gel cards that have bubble entrapped in the matrix gel may be centrifuged before performing the test. Even after the centrifugation process, if the cards show the presence of bubble then they have to be discarded.
7. If any of the matrix gel cards show the presence of decreased volume or cracked gel, they should be discarded.
8. Usage of haemolysed samples may interfere with the final interpretation of results.
9. Fibrin or particulate matter or incompletely washed red blood cells can give rise to erroneous results.
10. Bacterial or other contamination of reagents may cause false positive or false negative results.

WARRANTY
This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY