PERFORMANCE
The performance study has been evaluated on 300 blood samples. The evaluation demonstrated 98.92% sensitivity of Matrix AHG in IAT and IAT testing. The evaluation demonstrated 100% specificity of Matrix AHG with IAT negative samples and with AP negative samples. The results obtained were similar to those obtained with established products of equivalent use.

BIBLIOGRAPHY
7. Data from: Tulip Diagnostics (P) Ltd.

SUMMARY
Generally, antibodies involved in transfusion reactions are of two types, namely the complete and the incomplete. The complete antibodies agglutinate human red blood cells in saline medium, whereas the incomplete type of antibodies sensitizes red blood cells without agglutination. Usually, the antibodies and IgG antibodies (IgG1 and IgG3 type) fix complement. Cell lysins, in vivo, are mediated through the complement system and the complement C3b is further acted upon to produce C4b. In the direct antiglobulin test, Anti-Human Globulin reagent is used to detect antibodies adsorbed to the red blood cells in vivo.

PRESENTATION
Matrix® AHG (Coombs) Test Card contains six microtubes, prefilled with a gel in a suitable buffer containing polyclonal Anti-Human IgG and monoclonal Anti-C3d (BIRC-8).

REAGENTS
The Matrix® AHG (Coombs) Test Card contains six microtubes, prefilled with a gel in a suitable buffer containing polyclonal Anti-Human IgG and monoclonal Anti-C3d (BIRC-8). The Matrix® AHG (Coombs) Test Card is suitable for Direct Coombs test, indirect Coombs test including compatibility testing, antibody screening and antibody identification.

STORAGE AND STABILITY
Store Matrix® gel cards in an upright position at 4-25°C. Do not freeze.

ADDITIONAL REAGENTS AND MATERIALS REQUIRED
Matrix® Diluent-2, USP for preparation of red cell suspension. (Refer package insert before use). Gel card centrifuge (85g), Incubator (23°C), Water bath, Micropipette capable of delivering 50-500μl of specimen, Bottle tip dispenser and Reagent red blood cell suspension for antibody screening and identification.

PRINCIPLE
As the Matrix® gel card containing red blood cells is centrifuged under specific conditions, the red blood cells sensitized with antibody will agglutinate in the presence of the Anti-Human Globulin reagent 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 matrix and are pelleted at the bottom of the column. The reactions are then read and graded according to their readability pattern.

SAMPLE COLLECTION
No special preparation of the patient is required prior to sample collection by approved techniques. For optimal results, freshly collected venous whole blood sample should be used. Anticoagulants like EDTA, CPD-A and Citrate can be used or serum or plasma samples can be used.

SAMPLE PREPARATION
Prepare a 1:50 red cell suspension in Matrix® Diluent-2, USP as follows:
1. Bring the Matrix® Diluent-2, USP to room temperature before use.
2. Dispense 1.0 ml of Matrix® Diluent-2, USP into a clean test tube.
3. Add 10μl of packed red cells to Matrix® Diluent-2, USP collected into test tube and mix gently.
4. Red blood cell suspension so obtained should be used for testing.

TEST PROCEDURE
For Direct Antiglobulin Test (DAT)
1. Label the appropriate microtubes of the Matrix® gel card with patient’s / donor’s name or identification number. Remove the aluminum foil if required from number of microtubes carefully by pulling it backwards.
2. Add 10μl of red blood cell suspension to the microtubes, taking care to ensure that the microspheres do not touch the microtubes.
3. Immediately centrifuge the Matrix™ gel card for 10 minutes in the gel card centrifuge.
4. Retain the card from centrifuge; read and record the results.

FOR ANTI-BODY SCREENING / ANTI-BODY IDENTIFICATION (SAT)

1. Label the appropriate number of microtubes of Matrix™ gel card with patient’s / donor’s name or identification number. Remove the aluminum foil to allow release of antibodies carefully by pulling it backwards.
2. Pipette 50µl of each reagent red blood cell suspension (0.5%) to appropriate labeled microtubes taking care to ensure that microtube tip does not touch the microtube.
3. If an autotest 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 Matrix™ gel card for 15 minutes at 37°C in an incubator.
6. After incubation, centrifuge the Matrix™ gel card for 15 minutes in the gel card centrifuge.
7. Retain the card from centrifuge; read and record the results.

FOR COMPATIBILITY TEST (MAJOR)

1. Label the appropriate number of microtubes of Matrix™ gel card with patient’s / donor’s name or identification number. Remove the aluminum foil to allow release of antibodies carefully by pulling it backwards.
2. Pipette 50µl of 0.8% donor’s red blood cell suspension to appropriate labeled microtubes of the Matrix™ gel card, taking care to ensure that microtube tip does not touch the microtube.
3. If an autotest 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 serum or plasma to the above microtubes of the Matrix™ gel card. The interval between cells and serum or plasma transfer should not exceed 10 minutes.
5. Incubate the Matrix™ gel card for 15 minutes at 37°C in an incubator.
6. After incubation, centrifuge the Matrix™ gel card for 15 minutes in the gel card centrifuge.
7. Retain the card from centrifuge; read and record the results.

INTERPRETATION OF RESULTS

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

DIRECT ANTIGLOBULIN TEST

Negative reaction indicates absence of detectable IgG antibodies or Complement component C3b on the red blood cells.
Positive reaction indicates that red blood cells are sensitized with IgG or Complement component C3b.

ANTIBODY SCREENING / ANTI-BODY IDENTIFICATION

Positive reaction indicates presence of IgM antibodies. Negative reaction indicates absence of detectable IgM antibodies in the patient’s / donor’s serum or plasma.

COMPATIBILITY TEST

Negative reaction indicates compatibility of the donor blood with the patient. Positive reaction indicates incompatibility of the donor blood with the patient, due to presence of antibodies directed against antigens on the donor’s red blood cells. Further investigation to identify the antibody specificity should be performed.

The autotest microtube must be negative to validate results. Positive reaction in autotest may indicate autoantibodies.

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

The reaction strength may be recorded as follows:

<table>
<thead>
<tr>
<th>Strength of reaction</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Agglutinated red blood cells form a line at the top of the gel microtube.</td>
</tr>
<tr>
<td>3+</td>
<td>Most agglutinated red blood cells remain in the upper half of the gel microtube.</td>
</tr>
<tr>
<td>2+</td>
<td>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.</td>
</tr>
<tr>
<td>1+</td>
<td>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.</td>
</tr>
<tr>
<td>a</td>
<td>Most agglutinated red blood cells are in the lower third part of the gel microtube.</td>
</tr>
<tr>
<td>Negative</td>
<td>All the red blood cells pass through and form a compact button at the bottom of the gel microtube.</td>
</tr>
</tbody>
</table>

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.

NOTES AND LIMITATIONS

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The Matrix™ gel cards contain no 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 visible(s) setpapped within the gel can be centrifuged for two complete cycles in gel card centrifuge to remove the bubble. Bubbles are not removed the card should not be used.
6. Matrix™ gel cards that exhibit any sign 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 aluminum 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. Fibres or particulate matter in the sample may lead to erroneous results.
10. Fibre or particulate matter in the sample 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. Cold cell panels may give an unclear background with Matrix™ gel cards.
14. Do not use hemolyzed, lipemic, icteric and hyperproteinemic samples.
15. Extreme turbidity or discolouration 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 with the passage.
18. Aluminium foil seal of Matrix™ gel cards should be removed gently and carefully by pulling the full 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.
20. Some pathological conditions are reported as causing non-specific reactions in ABO procedures.

REMARKS

1. Known positive and negative control should be tested as per Good Laboratory Practices, Annex V (Cat. No. 10252010) can be used for quality control procedures related to ABO.
2. ERYWELL (Catalogue no. 1025300) can be used for stored red blood cell preservation solution for preservation of known cells.