Blood Grouping Reagent
IH-Card Anti-E
E-E-E-E-E-E

FOR IN VITRO DIAGNOSTIC USE
Gel card for use with the IH-System
MEETS FDA POTENCY REQUIREMENTS
U.S. LICENSE NUMBER: 1845

Product-Identification: 72020

IH-Card Anti-E:

<table>
<thead>
<tr>
<th>Volume</th>
<th>Reference Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 cards per box</td>
<td>813 220 100</td>
</tr>
<tr>
<td>48 cards per box</td>
<td>813 221 100</td>
</tr>
<tr>
<td>288 cards per box</td>
<td>813 222 100</td>
</tr>
</tbody>
</table>

INTENDED USE
The IH-Card Anti-E is intended for the detection of E (RH3) antigen on human red blood cells using the IH-System.

SUMMARY
Landsteiner and Wiener first described the Rhesus blood group system in 1940. More than 50 antigens belong to the Rhesus blood group system. The antigens C (RH2), E (RH3), c (RH4), e (RH5) and D (RH1) are the principle antigens of the Rh system. Although many other antigens have been identified, the antibodies associated with these five antigens are responsible for the majority of hemolytic transfusion reactions and cases of Hemolytic Disease of the Newborn associated with the Rh system.

The IH-Card Anti-E can be used for the detection of the E antigen on human red blood cells.

PRINCIPLES OF THE TEST
The test combines the principles of hemagglutination and gel filtration for detection of blood group antigen-antibody reactions.

The test sample (red blood cell suspension) is distributed into the microtubes containing the appropriate reagent(s). After centrifugation non-agglutinated red blood cells are collected at the bottom of the microtube while the agglutinates are dispersed throughout the length of the gel, depending upon their size. Their position in the gel determines the intensity of the reaction.

REAGENT

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Source</th>
<th>Antibody Class</th>
<th>Cell line</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-E</td>
<td>Human Monoclonal</td>
<td>IgM</td>
<td>DEM-1</td>
<td>Alba Bioscience Limited</td>
</tr>
</tbody>
</table>

Preservative: Sodium Azide (0.1%)

The bovine albumin used for the production of this reagent is purchased from BSE-free sources.

Each card contains six microtubes. Depending on the test profile, individual wells of this card can be used by carefully peeling off the aluminum foil from the individual microtubes.

STORAGE REQUIREMENTS
- Store at 18 to 25 °C.
- Do not use beyond expiry on the label, which is expressed as YYYY-MM-DD (Year-Month-Day).
- Store in an upright position.
- Do not freeze or expose cards to excessive heat.
- Do not store near any heat, air conditioning sources or ventilation outlets.
PRECAUTIONS

- All IH-System reagents and test samples must be brought to room temperature (18 to 25 °C) prior to use.
- Do not use cards showing signs of drying.
- Do not use cards with bubbles.
- Do not use cards with damaged foil strips.
- Use reagents as furnished.
- Cards with dispersed drops observed at the top of the microtube, due to improper storage or shipping conditions, have to be centrifuged with the IH-Centrifuge L with preset time and speed before use. If after one centrifugation drops are still observed on top of the microtube it is recommended not to use the card.
- The use of diluents other than IH-LISS for the red blood cell suspension may modify the reaction and lead to incorrect test results.
- The use of volumes and/or red blood cell suspension in concentrations other than those indicated in the method, may modify the reaction and lead to incorrect test results, i.e., false positive or false negative results.
- Once the IH-Card has been used for testing, it may contain infectious material and should therefore be handled and disposed of as biohazardous waste in accordance with local, state and national regulations.
- Warning: Contains sodium azide, which may react with lead or copper plumbing to form explosive azides. If discarded in the sink, flush with large amounts of water to prevent the buildup of explosive metal azides.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient or donor is required prior to specimen collection. Blood samples should be collected following general blood sampling guidelines.

Fresh blood samples collected in anticoagulant are acceptable. Samples should be tested as soon as possible post collection. If testing is delayed, EDTA samples may be stored at 2 to 8 °C for up to ten (10) days when tested manually and five (5) days when tested on automated systems.

On automated systems if testing is delayed, donor blood collected in CPD or CP2D may be tested up to expiration date of the unit when stored at 1 to 8 °C. Donor blood stored in additive solutions AS-1 or AS-3 may be tested up to thirty (30) days post collection when stored at 1 to 8 °C. Cord blood samples may be stored at 2 to 8 °C up to five (5) days post collection for automated testing.

For manual testing, if testing is delayed, donor blood collected in CPD, CP2D and CPDA-1 and donor blood stored in additive solutions AS-1 or AS-3 may be tested up to expiration date indicated on the label of the unit when stored at 2 to 8 °C. Cord blood samples may be stored at 2 to 8 °C up to ten (10) days post collection for manual testing.

Do not use grossly hemolyzed, lipemic or icteric samples.

A distinct separation of red blood cells and plasma is recommended for optimal results. This can be achieved through centrifugation for 10 minutes at 2000g or at a time and speed that consistently produces a distinct cell/plasma interface. Donor segments do not require centrifugation.

TEST PROCEDURE FOR MANUAL AND AUTOMATED SYSTEMS

Material provided

- IH-Card Anti-E
- IH-LISS Rack or IH-LISS Solution
- Dispenser pipette capable of delivering 1 mL
- Pipettes: 10 µL, 50 µL and 1 mL
- Disposable pipette tips
- Glass or plastic test tubes
- IH-Centrifuge L to centrifuge the IH-Cards at 85g with pre-set time or IH-1000

Method for automation

Please refer to the IH-1000 and IH-COM User Manual NA for testing and reagent handling instructions.

Method for manual testing

Refer to the IH-Centrifuge L User Manual NA for equipment operating instructions.

Prior to use prepare a red blood cell suspension of approximately 1% to be tested in IH-LISS

1. Transfer 1 mL of IH-LISS Solution to a labelled disposable tube
2. Add 10 µL of red blood cell pellet
3. Mix gently
4. The red blood cell suspension is ready for use

1. Allow reagents and samples to reach room temperature (18 to 25 °C) before use.
2. Inspect the condition of the cards before use (see Warnings and Precautions).
3. Label the gel card appropriately.
4. Withdraw the entire foil seal from the card or from the individual microtubes to be used for testing. Carefully peel off the aluminium foil to prevent cross-contamination of the microtube contents. 
   Note: Once the foil has been removed from the microtubes, testing must be initiated to prevent drying of the gel.
5. Ensure the resuspension of the red blood cells before use.
6. Distribute 50 µL of red blood cell suspension (approximately 1%) into the appropriate wells of microtubes. 
   Note: Carefully dispense the red blood cell suspension, avoiding contact of the pipette tip with the contents of the microtubes to prevent carryover.
7. Centrifuge in the IH-Centrifuge L at the pre-set conditions as determined by the manufacturer.
8. Read the reactions by visual inspection.
**INTERPRETATION OF RESULTS**

For visual interpretation

- **Positive result** - Agglutinates (on the surface of or dispersed through the gel) or hemolysis (in case of serum test) with very few or no red blood cells in the gel column. Report as a positive test result if hemolysis is present in the microtube but not in the sample column. Red blood cells may remain suspended on the top of the gel or are dispersed throughout the gel in varying degrees. A few cells may form a button in the microtube bottom in some positive reactions.

- **Negative result** - A compact button of red blood cells at the microtube bottom is a negative test result.

Refer to the IH-System Interpretation Guide for additional information

![Image of reaction grades]

**Well Reaction Grade**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Result Interpretation</th>
<th>Reaction Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Negative</td>
<td>A compact, pellet of RBCs* with a smooth surface at the bottom of the well with no visible agglutination.</td>
</tr>
<tr>
<td>+/-</td>
<td>Blood Grouping Antisera, and Phenotyping including Anti-D Blend = Not interpretable For Reverse (serum) ABO Testing = Positive Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive Antibody Identification= no overall result interpretation, only well result shown as +/- For Crossmatching = Incompatible</td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible</td>
<td></td>
</tr>
<tr>
<td>2+</td>
<td>For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible</td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible</td>
<td></td>
</tr>
</tbody>
</table>

*A very weak reaction is not an expected result for antigen testing. It may indicate that a false positive or a very weak/partial expression of the antigen is present. Further investigation of this sample should be performed before the antigen status is determined.

**For automated systems**

Below is a description of the various reaction grades and how the software uses that well reaction to determine the result interpretation. Please refer to the IH-1000 and IH-COM User Manual NA for further information.

<table>
<thead>
<tr>
<th>Well Reaction Grade</th>
<th>Result Interpretation</th>
<th>Reaction Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Negative</td>
<td>A compact, pellet of RBCs* with a smooth surface at the bottom of the well with no visible agglutination.</td>
</tr>
<tr>
<td>+/-</td>
<td>Blood Grouping Antisera, and Phenotyping including Anti-D Blend = Not interpretable For Reverse (serum) ABO Testing = Positive Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive Antibody Identification= no overall result interpretation, only well result shown as +/- For Crossmatching = Incompatible</td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible</td>
<td></td>
</tr>
<tr>
<td>2+</td>
<td>For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible</td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive For Reverse (serum) ABO Testing = Positive For Antibody Detection and DAT = Positive For Antibody Identification= no overall result interpretation, only well result shown as positive For Crossmatching = Incompatible</td>
<td></td>
</tr>
</tbody>
</table>

Agglutinated RBCs distributed throughout the entire length of the gel column, with no line of RBCs on the top of the well.

Most agglutinated RBCs concentrated at the top of the gel or upper half of the gel column.
### Well Reaction Grade

<table>
<thead>
<tr>
<th>Well Reaction Grade</th>
<th>Result Interpretation</th>
<th>Reaction Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>For Blood Grouping, Antisera and Phenotyping including Anti-D Blend = Positive</td>
<td>Agglutinated RBCs concentrated as a line on the top of the gel column with a few agglutinated RBCs just underneath the gel surface.</td>
</tr>
<tr>
<td></td>
<td>For Reverse (serum) ABO Testing = Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For Antibody Detection and DAT = Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For Antibody Identification = no overall result interpretation, only well result shown as positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For Crossmatching = Incompatible</td>
<td></td>
</tr>
<tr>
<td>Mixed Field (DP)</td>
<td>Blood Grouping, Antisera, and Phenotyping including Anti-D Blend, Not interpretable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For Reverse (serum) ABO Testing = Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Direct Antiglobulin Test, Antibody Detection, Autocontrol = Positive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antibody Identification = no overall result interpretation, only well result shown as DP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For Crossmatching = Incompatible</td>
<td></td>
</tr>
<tr>
<td>?</td>
<td>For Blood Grouping including Reverse ABO Testing, Antisera, and Phenotyping</td>
<td>Ambiguous result.</td>
</tr>
<tr>
<td></td>
<td>including Anti-D Blend, Antibody Detection and Identification, Direct Antiglobulin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Testing = Not interpretable</td>
<td></td>
</tr>
<tr>
<td></td>
<td>For Crossmatching = Incompatible</td>
<td></td>
</tr>
</tbody>
</table>

* RBCs = Red Blood Cells

Expected reactions with Anti-E and the interpretation are shown in the following table:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Well Reaction</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-E</td>
<td>positive</td>
<td>E+</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>E-</td>
</tr>
</tbody>
</table>

- A control test to detect spontaneous agglutination is not essential in routine testing because the IH-System Monoclonal Blood Grouping Reagents do not contain ingredients that enhance spontaneous agglutination of immunoglobulin-coated red blood cells. In some circumstances, a false positive test result may occur due to strong cold autoagglutinins or a protein imbalance causing the formation of rouleaux. In certain circumstances, a control may be indicated. The IH-Card Control can be used for this purpose. If the control test is positive, laboratories are advised to consult their approved site-specific procedures. The test cells can be washed several times in warm saline and retested. If the control test again gives a positive reaction, a valid interpretation of the results obtained cannot be made. Additional testing will be necessary to resolve the false positive reaction according to site-specific procedures.

- Caution must be taken in interpreting a reaction as a mixed field. Additional patient history and testing may be necessary for resolution. Not all mixed field populations have a sufficient minor population to be detected.

### STABILITY OF REACTIONS

For visual reading of reactions, best results are obtained within six (6) hours of centrifugation. Interpretation may be affected by drying of the gel, hemolysis of red blood cells and slanting of reaction patterns due to storage in a non-upright position. Processed cards that are stored in the refrigerator (2 to 8 °C) and properly sealed to protect from evaporation may be interpreted for up to one (1) day. Gel cards should not be interpreted after the first sign of drying, or if hemolysis is observed. The age and condition of red blood cells, as well as the temperature at which the card is stored, will affect how long cards can be stored. The presence of sodium azide in the gel may cause the red blood cells to become dark in color over time. This darkening does not interfere with the test result.

### QUALITY CONTROL

On each day of use, the reactivity of all Blood Grouping Reagents should be confirmed by testing with known positive and negative samples. The Blood Grouping Reagent contained on this card could be controlled by testing E negative and E positive samples (heterozygous when available). Each reagent is satisfactory for use if positive and negative samples react as expected. For additional information, please consult the IH-1000 User Manual NA and the IH-COM User Manual NA, Quality Control Sections

### LIMITATIONS

Erroneous and abnormal results may be caused by:
- Bacterial or chemical contamination of the blood specimens, reagents, supplementary materials and/or equipment.
- Patient medication or disease yielding a cross-reaction.
- A red blood cell concentration or suspension medium different from that recommended.
- Incomplete resuspension of the red blood cells.
- Sample hemolysis prior to testing.
- Contamination between microtubes through pipetting errors.
- Use of procedure other than the one described above.
- Grossly icteric blood samples, blood samples with abnormally high concentrations of protein or blood samples from patients who have received plasma expanders of high molecular weight may give false positive results.
- Fibrin, clots, particulates or other artifacts may cause some red blood cells to be trapped at the top of the gel and may cause an anomalous result. That may appear as a pinkish layer. In a negative reaction the false appearance of a mixed field could lead to misinterpretation.
• If red blood cells (pellet at the bottom of the microtube) are too low in concentration they become difficult to visualize, and, in certain cases, a weak positive reaction can fail to be detected.
• A weak reaction is not an expected result for antigen typing and may be indicative of a false positive or weak/partial expression of the antigen. Further investigations may be warranted per site specific procedures.
• The performance characteristics of these reagents have not been established with chemically modified, frozen/thawed or enzyme treated red blood cells.

Please refer to the IH-1000 and IH-COM User Manual NA for instrument-specific assay limitations.

**SPECIFIC PERFORMANCE CHARACTERISTICS**

The final release testing is performed according to the productspecific Standard Operating Procedures. As part of the lot release process, each lot of Bio-Rad Blood Grouping Reagents is tested against antigen positive and negative samples to ensure suitable reactivity and specificity.

**Performance characteristics on the IH-1000 Analyzer**

Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagent Anti-E was performed at four different US clinical sites and included patient and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH Blood Grouping Reagents in comparison to the FDA-licensed reference reagents. Microtube results for a given reagent were combined across applicable IH-Cards.

Results of the positive percent agreement and negative percent agreement, with the one-sided Exact 95% Lower Confidence Limit (LCL) are listed in the data table below. Note: See the IH-1000 User Manual NA and IH-COM User Manual NA for more information on verification of results.

<table>
<thead>
<tr>
<th>Test</th>
<th>Results from Clinical Trials</th>
<th>Negative Agreement</th>
<th>Positive Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Point Estimate</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(one-sided Exact 95% LCL)</td>
<td></td>
</tr>
<tr>
<td>Anti-E</td>
<td>1,078</td>
<td>99.72% (99.28%)</td>
<td>431</td>
</tr>
</tbody>
</table>

Agreement between the methods does not imply which method obtained the correct result. The above results do not reflect any discrepancy resolution between the methods.

Reproducibility was evaluated at two external sites and one internal site by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 1 operator x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days using the IH-1000 Analyzer. Reproducibility was demonstrated for the Blood Grouping Reagent Anti-E within runs, between runs and between sites.

A precision study was conducted internally using three reagent lots x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days using the IH-1000 Analyzer. Precision was demonstrated with all three lots of Blood Grouping Reagent Anti-E.

**Performance characteristics for manual testing**

Testing to determine the performance characteristics of the Bio-Rad IH Blood Grouping Reagent Anti-E was performed at five different US clinical sites and one internal site and included patient, cord blood and donor samples. The positive and negative percent agreements were calculated for the Bio-Rad IH Blood Grouping Reagents in comparison to the FDA licensed reference reagents. Microtube results for a given reagent were combined across applicable IH-Cards.

Results of the positive percent agreement and negative percent agreement, with the one-sided Exact 95% Lower Confidence Limit (LCL) are listed in the data table below.

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<td></td>
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<td>Point Estimate</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(one-sided Exact 95% LCL)</td>
<td></td>
</tr>
<tr>
<td>Anti-E</td>
<td>882</td>
<td>99.43% (98.81%)</td>
<td>372</td>
</tr>
</tbody>
</table>

Agreement between the methods does not imply which method obtained the correct result. The above results do not reflect any discrepancy resolution between the methods.

Reproducibility was evaluated at three external sites by testing a reproducibility panel according to the following scheme: one lot of reagent x 3 sites x 2 operators x 5 non-consecutive days x 2 runs x 2 replicates over a period of 20 days. Reproducibility for the Blood Grouping Reagent Anti-E using the IH-Centrifuge L was demonstrated within runs, between runs and between sites.

For technical support or further product information, contact Bio-Rad Laboratories, Inc. at 800-224-6723.

**GLOSSARY OF SYMBOLS**

Key: **Underline** = Addition of changes ◄ = Deletion of text

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