**INTENDED USE**

The DG Gel ABO/Rh (2D) card is used for determining the antigens of the ABO and Rh (D) system and the reverse ABO group, in gel technique.

**SUMMARY AND EXPLANATION**

The ABO system was the first human blood group system discovered by Landsteiner in 1900\(^1\) and is still the most important in transfusion practice. The ABO system is defined by the presence or absence of the A and/or B antigens on human red blood cells and by the presence of antibodies in the plasma or serum corresponding to the antigen or antigens missing on the red blood cells. In the field of transfusion medicine, after A and B antigens, the most important blood group antigen is the D antigen from the Rh blood group.

The determination of Rh (D) is defined by the presence or absence of the D (RH1) antigen on the red blood cells.

The anti-A, anti-B, anti-AB, anti-D\(^{VI}\) and anti-D\(^{VI+}\) reagents are used to perform the ABO and Rh (D) blood group typing, being complemented with the reverse group test (determination of the reverse ABO group).

**PRINCIPLE OF THE TEST**

The principle of the test is based on the gel technique described by Yves Lapierre\(^2\) for detecting red blood cell agglutination reactions. The DG Gel cards are composed of eight microtubes. Each microtube is made of a chamber, also known as incubation chamber, at the top of a long and narrow microtube, referred to as the column. Buffered gel solution containing specific monoclonal antibodies (anti-A, anti-B, anti-AB or anti-D) has been prefilled into the microtube of the plastic card. The agglutination occurs when the red blood cell antigens react with the corresponding antibodies, present in the gel solution or in the serum or plasma sample (in the case of reverse grouping test). The gel column acts as a filter that traps agglutinated red blood cells as they pass through the gel column during the centrifugation of the card. The gel column separates agglutinated red blood cells from non-agglutinated red blood cells based on size. Any agglutinated red blood cells are captured at the top of or along the gel column, and non-agglutinated red blood cells reach the bottom of the microtube forming a pellet.

**REAGENTS**

**Observable indications**

Inspect the condition of the cards before use.

- Do not use the card if you observe microbiological contamination, alterations or changes in color, or other artifacts.
- Do not use the card if you observe trapped bubbles in the gel, cracked gel or gel with fissures, drying gel, or gel without a visible fine line of supernatant.
- Do not use the card if opened or if the aluminum film seal is damaged.
- Do not use the card if dispersed drops are observed at the top of the microtube. In this case, the card should be centrifuged with the centrifuge for Grifols gel cards before use. If after one centrifugation the drops do not descend, do not use the card.

**Material provided**

Each microtube of the DG Gel ABO/Rh (2D) card contains a gel in buffered medium with preservative.

The microtubes are identified by the front label of the card:
- Microtube A: monoclonal anti-A (mixture of IgM and IgG antibodies of murine origin, clones 16243 G2+16247 E6).
- Microtube B: monoclonal anti-B (IgM antibodies of murine origin, clone 9621 A8).
- Microtube AB: monoclonal anti-AB (mixture of IgM antibodies of murine origin, clones 16245 F11 D8, 16247 E6 and 7821 D9).
- Microtube D^Rh-: monoclonal anti-D (IgM antibodies of human origin, clone P3x61). This anti-D monoclonal reagent does not detect the DVI variant.
- Microtube D^Rh+: monoclonal anti-D (mixture of IgG and IgM antibodies of human origin, clones P3x290, P3x35, P3x61 and P3x21223 B10). This anti-D monoclonal reagent detects the DVI variant.
- Microtube Ctl.: buffered solution without antibodies (control microtube).
- Microtubes N: buffered solution without antibodies (reverse ABO group test).

All microtubes contain sodium azide (NaN_3) as a preservative at a final concentration of 0.09%.

**Reagent preparation**

DG Gel ABO/Rh (2D) cards are supplied as ready to use. The gel cards should be brought to room temperature (18 - 25 ºC) before initiating the test.

**Material required but not provided**

**For Manual Method**
- Automatic pipettes of 10 μL, 50 μL and 1 mL.
- Disposable pipette tips.
- Glass or plastic test tubes.
- Diluent DG Gel Sol.
- Centrifuge for Grifols gel cards.
- Reagent Red Blood Cells (A^1/B) for reverse group test from Grifols.
- Reader for Grifols gel cards (optional).

**For Fully Automated Methods**
- Diluent DG Gel Sol.
- Reagent Red Blood Cells (A^1/B) for reverse group test from Grifols.
- DG Fluid A and DG Fluid B.
- Automated instrument from Grifols.
STORAGE AND STABILITY

- Do not use beyond the expiration date.
- Store upright (as indicated by the two arrows on the outer packaging) with seal intact at 2 - 8°C.
- Do not freeze.
- Do not expose cards to excessive heat, air conditioning sources or ventilation outlets.
- Do not use the cards if you identify incorrect temperature conditions during storage or shipment.

WARNINGS AND PRECAUTIONS

- The results by themselves alone are not a clinical diagnosis. Evaluate the results together with the patient’s clinical information and other data.
- The product should only be used by qualified personnel.
- The use of volumes and/or red blood cell suspensions in concentrations other than those indicated in the method, may modify the reaction and lead to incorrect test results, i.e., false positives or false negatives.
- The use of diluents other than DG Gel Sol for the red blood cell suspension may modify the reaction and lead to incorrect test results.
- Do not use a centrifuge other than a centrifuge for Grifols gel cards.
- The reagents of the DG Gel ABO/Rh (2D) card of human monoclonal origin are manufactured using materials that have been tested and found non-reactive for the HBS antigen and for anti-HIV and anti-HCV antibodies. However, there is no known procedure to ensure that products of human origin will not transmit infectious diseases.
- All products with animal derived material, and human blood products and samples should be handled as if they were potentially capable of transmitting infectious diseases.
- Once used, dispose of the product in containers for biological waste, according to local, state and national regulations.
- If you have any questions or need further information on the use of this product, please contact your local Grifols service representative.

SPECIMEN COLLECTION AND PREPARATION

Blood samples collected in EDTA, sodium citrate or sodium heparin should be used. The collection, separation and handling of the blood should be performed by qualified technical personnel according to current standards\(^3\)\(^-\)\(^4\), and following the instructions of the manufacturer of the materials used for collecting the sample.

Do not use grossly hemolyzed, cloudy or contaminated samples.

Samples should be tested as soon as possible.

- For the determination of the antigens of the ABO/Rh, use the red blood cells. If necessary, samples stored at 2 - 8 °C can be used up to 7 days after their collection.
Red blood cells from bags collected in CPD, CPDA or SAG-Mannitol can also be used until the expiry date indicated on the label of the bag. If red blood cells from the bag segment are used, it is recommended to wash them with physiological saline solution before preparing the suspension.

- For the determination of the reverse ABO group, use serum or plasma. If necessary, samples stored at 2 - 8 ºC can be used up to 7 days after their collection; or frozen samples stored up to 5 years (from -20 ºC to -80 ºC), may be used after thawing.

**PROCEDURE**

1. Allow DG Gel ABO/Rh (2D) cards, additional reagents and the samples to reach room temperature (18 - 25 ºC).
   
   **Note:** For fully automated instruments, skip the next steps and refer to the Instructions for Use of the related instruments.

2. Identify the cards to be used and the samples to be tested.

3. Thoroughly mix the vials of Reagent Red Blood Cells (A1/B) for reverse group test from Grifols to ensure homogeneous suspension.

4. Carefully peel off the aluminum film, to prevent cross-contamination of the microtube contents among them.
   
   **Note:** Use the microtubes immediately once the seal has been opened.

5. Dispense 50 μL of Reagent Red Blood Cells A1 into the microtube N/A1, and 50 μL of Reagent Red Blood Cells B into microtube N/B.

6. Add 50 μL of serum or plasma in to the corresponding microtubes (N/A1 and N/B).
   
   **Note:** Carefully dispense the red blood cell suspension and the serum or plasma, avoiding contact of the pipette tip with the wall or the contents of the microtubes to prevent carryover.

7. Prepare a 5% red blood cell suspension in DG Gel Sol (50 μL of packed red blood cells in 1 mL of DG Gel Sol).

8. Ensure the homogeneity of the 5% red blood cell suspension before use.

9. Dispense 10 μL of 5% red blood cell suspension to each of the microtubes (A / B / AB / D* / D*+ / Ctl.)
   
   **Note:** Carefully dispense the red blood cell suspension, avoiding contact of the pipette tip with the wall or the contents of the microtubes to prevent carryover.

10. Centrifuge the gel card in the Grifols centrifuge.

11. After centrifugation, remove the gel card from the centrifuge and read the results. Alternatively, use a reader for Grifols gel cards to read and to interpret the results.

**QUALITY CONTROL**

It is recommended to include positive and negative controls with testing on each day of use. If an unexpected control result is obtained, a complete assessment of the instrument, reagents and material used should be made.
RESULTS

Report results as an agglutination grade, absence of agglutination or hemolysis.

**Negative results**: no agglutination and no hemolysis of red blood cells are visible in the microtube. In a negative result, the red blood cells are located in the bottom of the gel column.

**Positive results**: agglutination and/or hemolysis of the red blood cells are visible in the microtube.

In a positive result, the agglutinated red blood cells may remain throughout the gel column showing different reaction grades as described below. Some positive reactions may also form a pellet in the bottom of the microtube.

Samples with normal expression of A, B and D antigens provide strong positive reaction grades. Weaker reactions may indicate a weak or partial expression of A, B and D antigens. Subgroup A₂ of the ABO system may also present a weak expression.

### Reaction Grades

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative:</strong></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
</tr>
<tr>
<td></td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>4+</td>
</tr>
<tr>
<td><strong>Double Population:</strong></td>
<td>DP</td>
</tr>
<tr>
<td><strong>Hemolysis:</strong></td>
<td>H</td>
</tr>
</tbody>
</table>

![Figure 1. Pattern of reaction grades.](image-url)
Stability of the results
After centrifuging the cards, it is recommended that the results be read immediately. Do not leave processed cards in a horizontal position. If necessary, a delayed reading can be performed up to 24 hours after processing the cards if they are kept in an upright position, refrigerated (2-8 °C) and sealed with a laboratory covering film to avoid evaporation of the supernatant.

Interpretation of the results
**ABO system.** The expected reaction with microtubes A and B, and its interpretation are shown in the following table (+ = positive, and 0 = negative).

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>O group</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>A group</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>B group</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>AB group</td>
</tr>
</tbody>
</table>

**D antigen.** The expected reaction with microtubes DVI− and DVI+, and its interpretation are shown in the following table (+ = positive and, 0 = negative).

<table>
<thead>
<tr>
<th>Microtube DVI−</th>
<th>Microtube DVI+</th>
<th>Microtube Ctl.</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>0</td>
<td>D positive</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>D negative</td>
</tr>
<tr>
<td>0</td>
<td>+</td>
<td>0</td>
<td>Weak or partial D</td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Notes:
1. The acronym “Ctl.” means Control.
2. The Ctl. microtube should be negative. If it is positive, due to the formation of rouleaux, to strong cold autoagglutinins or other causes, invalidate the test. Repeat the determination after washing the red blood cells with physiological saline solution and preparing a new suspension of the washed red blood cells. If the Ctl. microtube of the repeat test is negative, the results of the test can be interpreted; if it is positive, invalidate the test.
3. Forward (cell grouping) and reverse (serum grouping) discrepancies should be investigated before releasing the result.
4. To verify D negative status or to ensure detection of weak and partial D, other reagents and techniques (e.g. Indirect Antiglobulin testing) which may detect different weak and partial D variants should be used.
5. In the event of obtaining discrepant results in microtubes DVI− and DVI+, it must be interpreted as a weak or partial D antigen. It is recommended the expression of this antigen be analyzed.
6. Precaution should be taken in the interpretation of Double Population events. Not all mixed-cell situations are detected. Additional information on patient history and additional testing will be necessary for resolution. Transfused patients or those subjected to bone marrow transplant may present images of Double Population\(^5\). Double Population is also observed in some ABO subgroups (A\(3\)), in Tn cryptantigens, in blood group chimerism in fraternal twins, and in the very rare case of mosaicism arising from dispermy\(^5\)\(^6\).

7. The observation of complete or partial hemolysis (pinkish supernatant and/or gel column) in microtubes should be interpreted as a positive result, after verifying that it is not due to a problem of collection and/or handling of the sample.

8. Occasionally, a red blood cell retention in the incubation chamber may occur with positive 4+ samples, without interfering in result reading.

LIMITATIONS OF THE PROCEDURE

1. Grossly hemolyzed, cloudy or contaminated samples or samples with presence of a clot, may cause false positive or false negative results.

2. Aged or hemolyzed specimens may cause weaker reactions compared to those obtained with a fresh sample.

3. Samples with high-potency antibodies may coat the red blood cells completely causing spontaneous agglutination\(^5\).

4. Abnormal concentrations of serum proteins, the presence of macromolecular solutions in the serum/plasma or the presence of Wharton’s jelly in cord blood samples may cause the nonspecific agglutinations of the red blood cells. It is recommended to wash the red blood cells before performing the test\(^5\).

5. Red blood cells from individuals with A or B variants may present a weak expression of the antigens and may not be detected.

6. Antigen expression may be weakened in red blood cells of people with leukaemia or other malignant diseases\(^5\).

7. If poorly anti-coagulated plasma or incompletely clotted serum is used, fibrin residues may trap nonagglutinated red blood cells at the top of the gel, appearing as a pinkish or reddish layer. Although the results could be correctly interpreted, in a negative reaction the false appearance of a Double Population could lead to a misinterpretation. In case of incompletely clotted serum, it is recommended to re-clot the serum and repeat the test\(^5\).

8. Discrepancies between forward and reverse groups may be observed in patients with low or nonexistent levels of isoagglutinins: newborns up to the age of 4-6 months, elderly persons, patients with immunodeficiency or with very diluted antibodies due to plasma exchange procedures\(^5\).

9. A very weak expression or variants of the D antigens may not be detected.

10. The anti-A reagent contained in this card could react with Tn cryptantigens.

11. On occasions, unagglutinated red blood cells may be retained somewhere in the gel column with the appearance of very minute red dots or flecks. However, this nonspecific retention should not interfere with the interpretation of the result.
SPECIFIC PERFORMANCE CHARACTERISTICS

Diagnostic sensitivity and specificity:

ABO/Rh system

The diagnostic sensitivity and specificity of the antibodies present in the DG Gel ABO/Rh (2D) card, for the determination of the antigens of the ABO and Rh system, have been studied in a representative number of positive and negative samples.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>No. of Samples</th>
<th>Sensitivity%</th>
<th>Specificity%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>3041</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-B</td>
<td>3046</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-AB</td>
<td>3038</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-D**</td>
<td>3054**</td>
<td>99.6%</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-D***</td>
<td>3057***</td>
<td>99.50%</td>
<td>100%</td>
</tr>
</tbody>
</table>

(a) Sensitivity: (number of true positive results / (number of true positive results + number of false negative results)) x 100

(b) Specificity: (number of true negative results / (number of true negative results + number of false positive results)) x 100

(c) These samples include 51 samples with weak expression of the D antigen. If only these samples are considered, the anti-D** reagent of microtube D** has a sensitivity of 80.40%.

(d) These samples include 52 samples with weak expression of the D antigen. If only these samples are considered, the anti-D*** reagent of microtube D*** has a sensitivity of 73.10%.

Reverse ABO group

The DG Gel ABO/Rh (2D) card owns performance characteristics suitable for the determination of the reverse group, backed up by a study where the obtained results were similar to those obtained with other established products of equivalent intended use.

Precision

The precision of the reagents present in the DG Gel ABO/Rh (2D) card has been determined, including repeatability, inter-lot reproducibility and intra-laboratory reproducibility tests. No false positive or false negative results were obtained and differences between agglutination intensities in positive samples were 1 agglutination grade or less in all assays.

BIBLIOGRAPHY

3. CLSI H3-A6: Procedures for the collection of diagnostic blood specimens by venipuncture; Approved

PRESENTATION
210338 DG Gel ABO/Rh (2D) 50 cards Profile: A/B/AB/DVI/DDVI+/Ctl./N_A1/N_B

Manufactured by:
Diagnostic Grifols, S.A.
Passeig Fluvial 24, 08150 Parets del Vallès (Barcelona), Spain

Date of last version: January 2017

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SYMBOLS KEY
One or more of these symbols may have been used in the labeling/packaging of this product.