BLOOD GROUPING REAGENT

DG Gel 8 ABO/Rh (2D) **REF 210128**

Instructions for Use

INTENDED USE

The DG Gel 8 ABO/Rh (2D) card is for the determination of ABO forward and reverse group, and RhD antigen on the surface of red blood cells of human blood samples.

For use with the DG Gel System.

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 in 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 system. The determination of RhD is defined by the presence or absence of the D antigen in the red blood cells. The anti-A, anti-B, anti-AB, anti-D^{VI-} and anti-D^{VI+} reagents contained in the DG Gel 8 ABO/Rh (2D) card are used to perform the ABO and RhD blood group typing, and the determination of ABO reverse group confirms the ABO group.

PRINCIPLE OF THE TEST

The principle of the test is based on the gel technique described by Yves Lapierre[2] in 1985 for detecting red blood cell agglutination reactions. The DG Gel 8 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 antibody (anti-A, anti-B, anti-AB, anti-D^{VI-} or anti-D^{VI+}) 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

Each microtube of the DG Gel 8 ABO/Rh (2D) card contains a gel in buffered medium with preservative. The five first microtubes also contains antibody reagents and the last three microtubes have only the buffered medium. The different microtubes are identified on the front label of the card.

Microtube **A**: monoclonal antibody anti-A. Mixture of IgM and IgG antibodies of murine origin, clones 16243G2 and 16247E6.

Microtube **B**: monoclonal antibody anti-B. IgM antibody of murine origin, clone 9621A8. This reagent does not react with acquired B cells.

Microtube **AB**: monoclonal antibodies anti-AB. Mixture of IgM antibodies of murine origin, anti-A(B) clone ES-15, anti-A clone LA-2, and anti-B clone LB-2.

Microtube **D^{VI-}**: monoclonal antibody anti-D. IgM antibody of human origin, clone P3x61. This reagent does not detect partial DVI.

Microtube **D^{VI+}:** monoclonal antibody anti-D. Mixture of IgM antibodies of human origin, clones P3x61 and ESD1M. This reagent detects partial DVI.

Microtube **Ctl**.: buffered solution without antibodies (control microtube).

Microtubes **N**: buffered solution without antibodies for the ABO reverse group test.

Clones 16243G2, 16247E6, 9621A8 and P3x61 are produced using intermediate products produced for Diagnostic Grifols S.A. in a shared manufacturing agreement with DIAGAST; US License Number 1744.

Clones ESD1M and LA-2 are produced using intermediate products produced for Diagnostic Grifols S.A. in a shared manufacturing agreement with QUOTIENT; US License Number 1807.

Clones LB-2 and ES-15 are produced using intermediate products produced for Diagnostic Grifols S.A. in a shared manufacturing agreement with MILLIPORE (UK) LTD; US License Number 1761.

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

Warnings and precautions

- For *in vitro* diagnostic use.
- The results by themselves alone are not a clinical diagnosis. Evaluate the results together with the patient's clinical information and other data.
- 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.
- Do not use the card if opened or if the aluminum film seal is damaged.
- If you identify incorrect temperature conditions during storage or shipment, do not use the cards.
- If you identify improper storage or shipping conditions that results in dispersed drops observed at the top of the microtube, the card should be centrifuged with the DG SPIN before use. If after one centrifugation with the DG SPIN the drops do not descend, do not use the card.
- 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 positive or false negative results.
- The use of diluents other than Grifols Diluent for the red blood cell suspension may modify the reaction and lead to incorrect test results.
- Do not use a centrifuge other than the DG SPIN centrifuge.
- Samples collected in sodium citrate or sodium heparin should be tested by manual method.
- The reagents of the DG Gel 8 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 agents. Human blood products and samples should be handled as if they were potentially capable of transmitting 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.
- Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. If discarded into sink, flush with a large volume of water to prevent azide buildup.
- Once used, dispose 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.

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 2-25ºC.
- Do not freeze.
- Do not expose cards to excessive heat.

SPECIMEN COLLECTION AND PREPARATION

Blood samples collected in EDTA, sodium citrate or 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.

Use the red blood cells collected for the determination of the antigens of the ABO system and RhD antigen. Samples should be tested as soon as possible. If necessary, samples collected in EDTA and stored at 2 - 8 ºC can be used up to 7 days after collection. Samples collected in sodium citrate or heparin and stored at 2 - 8 ºC can be used up to 3 days after collection.

Red blood cells from bags collected in ACD, CPD, CPDA-1, CP2D or AS-1 (Adsol) or AS-3 can also be used up to 7 days after the expiration date indicated on the label of the bag. If red blood cells from the bag segment are

used, it is suggested that these be washed with physiological saline solution before preparing the suspension. Do not use if clots or hemolysis are observed.

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

PROCEDURE

Observable indications

Inspect the condition of the cards before use (see Warnings and Precautions).

Cards with an alteration or change in color, trapped bubbles in the gel, cracked gel or gel with fissures, drying gel, presence of other artifacts, and opened or damaged seals may indicate an alteration of the product.

Material provided

DG Gel 8 ABO/Rh (2D) cards are supplied as ready to use. Each DG Gel 8 ABO/Rh (2D) card contains 8 microtubes with different monoclonal antibodies anti-A, anti-B, anti-AB, anti-D^{VI-} or anti-D^{VI+} reagents in buffered medium or buffered gel without antibodies with preservative.

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.
- Grifols Diluent.
- DG SPIN centrifuge.
- A_1/B Reagent Red Blood Cells 0.8% from Medion Grifols Diagnostics AG or other validated A_1/B RBCs at 0.8%.
- DG Reader or DG Reader Net (optional).

For Fully Automated Methods

- Grifols Diluent.
- A_1/B Reagent Red Blood Cells 0.8% from Medion Grifols Diagnostics AG or other validated A_1/B RBCs at 0.8%.
- Grifols Wash Solution A and Grifols Wash Solution B.
- Erytra Eflexis, Erytra or WADiana Compact.

Test procedure

1. Allow DG Gel 8 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. Prepare a 5% red blood cell suspension in Grifols Diluent (50 µL of packed red blood cells in 1 mL of Grifols Diluent).
4. Carefully peel off the aluminum film that covers the microtubes to prevent cross-contamination of the microtube contents among them.
5. Ensure the re-suspension of the red blood cell suspension before use.
6. Add 10 µL of the 5% red blood cell suspension into each of the A/B/AB/D^{VI-}/D^{VI+}/Ctl microtubes.

   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. Thoroughly mix the vials of A_1/B Reagent Red Blood Cells 0.8% to ensure homogeneous suspension.
8. Dispense 50 µL of A1 Reagent Red Blood Cells into the first microtube **N**, and 50 µL of B Reagent Red Blood Cells into second microtube **N**.
9. Add 50 µL of serum or plasma in to the corresponding **N** microtubes.

   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.
10. Centrifuge the gel card in the DG SPIN centrifuge.
11. After centrifugation, remove the gel card from the centrifuge and read the results. Alternatively, use the DG Reader or DG Reader Net to read and to interpret the results.

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 ºC) and sealed with a laboratory covering film to avoid evaporation of the supernatant.

Quality Control

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 is 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 is visible in the microtube. In a positive result the agglutinated red blood cells may remain throughout the gel column showing different reaction grades (see Reaction Grades and Figure 1 for a picture of example of reaction grades). Some positive reactions may also form a pellet in the bottom of the microtube. Samples with normal expression of ABO and Rh(D) antigens provide strong positive reaction grades. Weaker reactions may indicate a weak or partial expression of ABO and Rh(D) antigens. Subgroup A_2 of the ABO system may also present a weak expression.

Notes:

1. Some fibrin, particulates or other artifacts may trap red blood cells at the top of the gel columns erroneously leading to an abnormal result (see limitation number 7).
2. Occasionally, red blood cell retention in the incubation chamber may occur with positive 4+ samples, without interfering in the result interpretation.

Reaction Grades

Negative:	0	Well-defined pellet of non-agglutinated red blood cells at the bottom of the gel column and no visible agglutinated cells in the rest of the gel column.
Positive:	w+	Barely visible small-sized clumps of agglutinated cells in the lower part of the gel column and a pellet of unagglutinated cells at the bottom.
	1+	Some small-sized clumps of agglutinated cells most frequently in the lower half of the gel column. A small pellet may also be observed at the bottom of the gel column.
	2+	Small or medium-sized clumps of agglutinated cells throughout the gel column. A few unagglutinated cells may be visible at the bottom of the gel column.
	3+	Medium-sized clumps of agglutinated cells in the upper half of the gel column.
	4+	A well-defined band of agglutinated red blood cells in the top part gel column. A few agglutinated cells may be visible below the band.
mf		Mixed-field. A band of red blood cells at the top part of the gel or dispersed throughout the gel column, and a pellet in the bottom as a negative result.
H		Hemolysis in the microtube with very few or no red blood cells in the gel column. Report if hemolysis is present in the microtube but not in the sample.

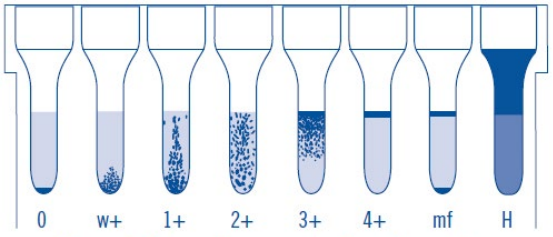

Figure 1. Picture of an example of reaction grades.

Interpretation of the results

ABO system. The expected reaction with microtubes A, B and AB, and reverse group with A_1 and B cells, and its interpretation are shown in the following table (+ = positive, and 0 = negative).

Microtube A	Microtube B	Microtube AB	Microtube Ctl.	Microtube N Reagent Red Blood Cells A_1	Microtube N Reagent Red Blood Cells B	Interpretation
0	0	0	0	+	+	0
+	0	+	0	0	+	A
0	+	+	0	+	0	B
+	+	+	0	0	0	AB

D antigen (Rh system). The expected reaction with microtubes D^{VI-} and D^{VI+}, and its interpretation are shown in the following table (+ = positive and, 0 = negative).

Microtube D^{VI-}	**Microtube D^{VI+}**	**Microtube Ctl.**	**Interpretation**
+	+	0	D positive
0	0	0	D negative
0	+	0	Weak or partial D
+	0	0	

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. The anti-D reagents detect most of weak D. However to ensure the detection of very weak and partial D antigen expressions, or if verification of D negative status is required, 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 D^{VI-} and D^{VI+}, it must be interpreted as a weak or partial D antigen. It is recommended to be analyzed the expression of this antigen.
6. Precaution should be taken in the interpretation of mixed-field 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 mixed-field[5].

Mixed-field 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.

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. Red blood cells from individuals with A or B variants may present a weak expression of the antigens, and may not be detected.
4. Antigen expression may be weakened in the red blood cells of persons with leukemia or other malignant diseases[5].
5. Abnormal concentrations of serum proteins, the presence of infused macromolecular solutions in the serum or plasma or the presence of Wharton's jelly in cord blood samples may cause non-specific agglutination of the red blood cells. It is suggested that red blood cells be washed before performing the test[5].
6. Samples with high-potency antibodies may coat the red blood cells completely, causing spontaneous agglutination[5].
7. If poorly anti-coagulated plasma or incompletely clotted serum is used, fibrin residues may trap non-agglutinated 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 mixed field 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 non- existent 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 dot or fleck. However, this nonspecific retention should not interfere with the interpretation of the result.

SPECIFIC PERFORMANCE CHARACTERISTICS

- Grifols DG Gel 8 Blood Grouping Reagents Anti-A, Anti-B, Anti-AB, Anti-D^{VI-}, Anti-D^{VI+} meet FDA potency requirements for Blood Grouping Reagents. There is no U.S. standard of potency for the DG Gel 8 Neutral and Control reagents, which contains no antibody reactivity specific for a blood group antigen.
- Every lot has been tested against a panel of positive and negative samples for the relevant antigens to assure reactivity and specificity in accordance with FDA requirements. Details of specificity test results submitted to the FDA for release of product will be furnished upon request.
- The performance of the reagents was confirmed against FDA-licensed reagents in a comparison study where reagents were tested in parallel at different clinical sites. The estimated percent agreements and their lower limits of 95% one-side confidence interval for all sites combined are indicated on the table below.

	Nº of samples	Negative Percent Agreement (Lower 95% CI)	Nº of samples	Positive Percent Agreement (Lower 95% CI)
Anti-A	1863	100.00% (99.84%)	1233	99.84% (99.49%)
Anti-B	2566	100.00% (99.88%)	540	100.00% (99.45%)
Anti-A,B	1420	100.00% (99.79%)	1685	100.00% (99.82%)
Anti-D^{VI-}	457	100.00% (99.35%)	2652	100.00% (99.89%)
Anti-D^{VI+} (1)	424	99.76% (98.89%)	2624	99.96% (99.82%)

Notes: (1) Two (2) discrepant results were obtained in the comparison study. One of these discrepancies was resolved in favor of DG Gel System but further investigation determined there was an operator error when performing the comparator test and this was not a reagent failure. The other discrepancy was resolved in favor of the comparator reagent, but the investigation determined that a wrong sample was added to the test card. Therefore, both discrepancies were attributed to operator error and not a reagent failure.

- Percent of Agreement only indicates agreement between the Diagnostic Grifols reagents and the FDA-licensed reagents and does not indicate which reagent gave the correct result(s).

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PRESENTATION

210128 DG Gel 8 ABO/Rh (2D) 50 Cards

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DATE OF ISSUE: 2022-XX-XX

SYMBOLS KEY

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