Anti-Human Globulin DG Gel 8 Anti-IgG (Rabbit)   REF 210394   3034960

Instructions for Use

INTENDED USE

The DG Gel 8 Anti-IgG (Rabbit) card is for the Direct and Indirect Antiglobulin Test of human blood samples.
This test does not contain antibodies to complement components.
For use with the DG Gel System.
For *in vitro* diagnostic use.

SUMMARY AND EXPLANATION

Carlo Moreschi described the principle of antiglobulin technique in 1908[1]. In 1945, Coombs and his co-workers Mourant and Race, unaware of this previous description, published and introduced the use of anti-human globulin for the detection of red blood cells coated with non-agglutinating antibodies[2]. After Coombs' publication the antiglobulin test was rapidly applied in regular clinical laboratory practice and must rank as almost as important as the discovery of the ABO groups[1]. The monospecific anti-IgG Antiglobulin Test is based on the use of anti-human globulin that allows the detection of red blood cells coated with immunoglobulin (IgG).
The Direct Antiglobulin Test allows the detection of red blood cells sensitized in vivo by immunoglobulin.
The Indirect Antiglobulin Test allows the detection of red blood cell antibodies present in the patient's serum or plasma by *in vitro* sensitization of red blood cells. The goal of screening for unexpected antibodies is detection of clinically significant antibodies present in the donor's or patient's sample. In a positive screening of unexpected antibodies, the autocontrol will indicate whether it is due to the presence of an autoantibody, an alloantibody or both. In the antiglobulin crossmatch test the donor's red blood cells combined with the recipient's serum or plasma will show the presence or absence of unexpected antibodies in the recipient's blood that are specific to the antigens of the donor's red blood cells.

PRINCIPLE OF THE TEST

The principle of the test is based on the gel technique described by Yves Lapierre[3] in 1985 for detecting red blood cell agglutination reactions. The DG Gel 8 plastic cards are composed of eight microtubes. Each microtube is made of a chamber, also known as incubator chamber, at the top of a long and narrow microtube, referred to as the column. Buffered gel solutions containing polyclonal anti-human globulin have been prefilled into the microtubes of the plastic card. The agglutination occurs when the red blood cell sensitized *in vivo* or *in vitro* by human IgG antibodies react with the anti-human globulin present in the gel solution. 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 reach the bottom of the microtube forming a pellet.

REAGENTS

Observable indications

Note: 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

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

Each microtube of the DG Gel 8 Anti-IgG (Rabbit) card contains reagent (polyclonal antibodies) mixed with a gel in buffered medium with preservative. The microtubes are identified on the front label of the card.

Microtubes **IgG**: buffered low ionic strength solution (LISS) with rabbit polyclonal anti-human globulin, is produced using intermediate products produced for Diagnostic Grifols S.A. in a shared manufacturing agreement with DIAGAST, Parc Eurasante, 251 av. Eugene Avinee-BP9, 59374 Loos Cedex France; US License Number 1744.

Reagent preparation

DG Gel 8 Anti-IgG (Rabbit) cards are supplied as ready to use. The gel cards and samples to be tested should be brought to room temperature (18-25 ºC) before testing.

Material required but not provided

- Automatic pipettes of 10 µL, 25 µL, 50 µL and 1 mL.
- Disposable pipette tips.
- Glass or plastic test tubes.
- Grifols Diluent.
- DG THERM incubator.
- DG SPIN centrifuge.
- Reagent Red Blood Cells at 0.8% from Medion Grifols Diagnostics AG or other validated RBCs at 0.8% for screening and identification of unexpected antibodies.

STORAGE AND STABILITY

- Do not use beyond the expiration date.
- Stored upright (as indicated by the two arrows on the outer packaging) with seal intact at 2-25 ºC.
- Do not freeze.
- Do not expose cards to excessive heat.

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.
- 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 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.
- 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.
- Do not use enzyme treated red blood cells.
- 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 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, consult the authorized distributor.

SPECIMEN COLLECTION AND PREPARATION

Blood samples collected in EDTA or sodium citrate should be used. The collection, separation and handling the blood should be performed by qualified technical personnel according to current standards[4-5], and following the instructions of the manufacturer of the material used for collecting the sample.

Do not use grossly hemolyzed, cloudy or contaminated samples.

Use serum or plasma collected for screening and/or identification of unexpected antibodies, crossmatch tests and autocontrol. If necessary, samples stored at 2-8 ºC can be used up to 72 hours after collection and frozen samples stored up to 5 years at -20 ºC or colder may be used after thawing.

Use the red blood cells collected for Direct Antiglobulin Test, crossmatch tests and autocontrol. If necessary, samples stored at 2-8 ºC can be used up to 72 hours after their collection, except for Direct Antiglobulin Test where sample storage less than 24 hours is recommended.

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 stored at 2-8 ºC. If red blood cells from a bag segment are used, it is recommended that these be washed with physiological saline solution before preparing the suspension. Do not use if clots or hemolysis are observed.

PROCEDURE

- For **Direct Antiglobulin** test:
  1. Allow DG Gel 8 Anti-IgG (Rabbit) cards, additional reagents and the samples to reach room temperature (18-25 ºC).
  2. Inspect the condition of the cards before use (see Warnings and Precautions).
  3. Identify the cards to be used and the samples to be tested.
  4. Prepare a 1% red blood cell suspension in Grifols Diluent (10 µL of packed red blood cells in 1 mL of Grifols Diluent).

5. Remove the foil seal from the complete DG Gel 8 card or from the individual microtubes to be used for testing. Carefully peel off the aluminum film to prevent cross-contamination of the microtube contents among them.
6. Ensure the re-suspension of the red blood cells before use.
7. Dispense 50 µL of the 1% red blood cell suspension into the corresponding microtube.

   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.
8. Centrifuge the gel card in the DG SPIN centrifuge.
9. After centrifugation, remove the gel card from the centrifuge and read the results.

- For **Indirect Antiglobulin** test:

Screening and identification of unexpected antibodies

1. Allow DG Gel 8 Anti-IgG (Rabbit) cards, additional reagents and the samples to reach room temperature (18-25 ºC).
2. Inspect the condition of the cards before use (see Warnings and Precautions).
3. Identify the cards to be used and the samples to be tested.
4. Remove the foil seal from the complete DG Gel 8 card or from the individual microtubes to be used for testing. Carefully peel off the aluminum film to prevent cross-contamination of the microtube contents among them.
5. Thoroughly mix the vials of Reagent Red Blood Cells for the screening and/or identification of unexpected antibodies to ensure homogeneous suspension of the red blood cells before use.
6. Dispense 50 µL of the Reagent Red Blood Cells (as supplied) into the microtubes.
7. Add 25 µL of serum or plasma into the 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.
8. Incubate 15 minutes at 37 ºC using DG THERM incubator.
9. Centrifuge the gel card in the DG SPIN centrifuge.
10. After centrifugation, remove the gel card from the centrifuge and read the results.

Antiglobulin Crossmatch tests

1. Allow DG Gel 8 Anti-IgG (Rabbit) cards, additional reagents and the samples to reach room temperature (18-25 ºC).
2. Inspect the condition of the cards before use (see Warnings and Precautions).
3. Identify the cards to be used and the samples to be tested.
4. Prepare a 1% donor's red blood cell suspension in Grifols Diluent (10 µL of packed red blood cells in 1 mL of Grifols Diluent).
5. Remove the foil seal from the complete DG Gel 8 card or from the individual microtubes to be used for testing. Carefully peel off the aluminum film to prevent cross-contamination of the microtube contents among them.
6. Ensure the re-suspension of the red blood cells before use.
7. Dispense 50 µL of the donor's 1% red blood cell suspension into the corresponding microtube.
8. Add 25 µL of the recipient's serum or plasma into the 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.

9. Incubate 15 minutes at 37 ºC using DG THERM incubator.
10. Centrifuge the gel card in the DG SPIN centrifuge.
11. After centrifugation, remove the gel card from the centrifuge and read the results.

Autocontrol

1. Allow DG Gel 8 Anti-IgG (Rabbit) cards, additional reagents and the samples to reach room temperature (18-25 ºC).
2. Inspect the condition of the cards before use (see Warnings and Precautions).
3. Identify the cards to be used and the samples to be tested.
4. Prepare a patient 1% red blood cell suspension in Grifols Diluent (10 µL of packed red blood cells in 1 mL of Grifols Diluent).
5. Remove the foil seal from the complete DG Gel 8 card or from the individual microtubes to be used for testing. Carefully peel off the aluminum film to prevent cross-contamination of the microtube contents among them.
6. Ensure the re-suspension of the red blood cells before use.
7. Dispense 50 µL of the patient's 1% red blood cell suspension into the corresponding microtube.
8. Add 25 µL of the patient's serum or plasma into the corresponding microtube.

   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.
9. Incubate 15 minutes at 37 ºC using DG THERM incubator.
10. Centrifuge the gel card in the DG SPIN centrifuge.
11. After centrifugation, remove the gel card from the centrifuge and read the results.

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. A positive result indicates the presence of IgG antibodies in the serum or plasma sample or coated on the red blood cells.

Note:

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 5).
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.

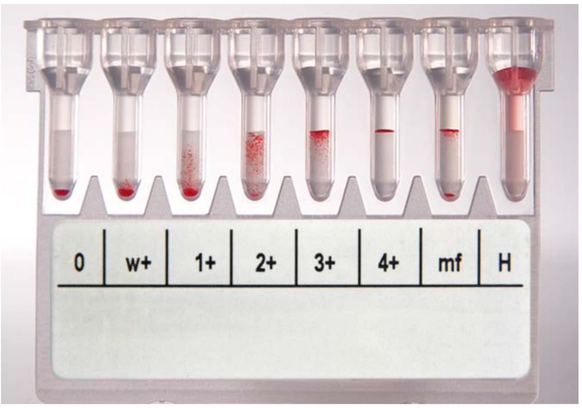

Figure 1. Picture of an example of reaction grades.

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.

Interpretation of the results

Indirect and Direct Antiglobulin **tests.** Tests determined by the result obtained in the microtube. The interpretation of the results depends on the sample and the reagents added to the microtube.

Notes:

1. A Direct Antiglobulin Test with a negative result does not mean absence of hemolytic disease of the newborn, especially in cases where ABO incompatibility is suspected.
2. Drug-induced antibodies can cause a positive Direct Antiglobulin Test[6].
3. 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[6].
4. 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 cell 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 fresh sample.
3. Abnormal concentrations of serum proteins, the presence of infused macromolecular solutions in the serum or plasma may cause non-specific agglutination of the red blood cells[6].
4. Samples with high-potency antibodies may coat the red blood cells completely, causing spontaneous agglutination[6].
5. 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 filed could lead to a misinterpretation. In case of incompletely clotted serum samples, it is recommended to re-clot the serum and repeat the test[6].
6. No single method is able to detect all unexpected antibodies. The optimum reaction conditions (e.g. sample volume, incubation times) may vary for different antibody specificities.
7. The presence of a high concentration of IgG paraproteins in the sample can neutralize the polyspecific anti-human globulin and lead to a false negative result in the antiglobulin test[6].
8. Rare antibodies, notably some anti-Jk^a or anti-Jk^b, may be detected only when polyspecific AHG is used and when active complement is present[6].
9. The Indirect Antiglobulin Test at 37 ºC in gel or glass sphere techniques have been reported to show a lower level of sensitivity than results obtained with the tube technique, in the detection of weak agglutination reactions of the ABO system[7].
10. A false positive result in the Direct Antiglobulin Test can be due to the complement attached to red blood cells in specimens collected from infusion lines used to administer dextrose-containing solutions[6] or in specimens collected in tubes containing silicone gel[6].
11. In the Direct Antiglobulin Test not all positive reactions infer that clinically significant antibodies are present. Specific anti-IgG reagent and elution techniques may be used for additional investigation of positive results.

12. Nonspecifically adsorbed proteins (e.g., high-dose intravenous immune globulin, multiple myeloma, autoimmune disorders and other diseases associated with elevated serum globulin) and modification of red cell membrane by some drug, can cause positive Direct Antiglobulin test[6].
13. Red blood cell samples with a positive Direct Antiglobulin Test should not be used for Indirect Antiglobulin testing.
14. Antibody activity may decrease in the elderly, infants or persons with disease.
15. 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

Contains Anti-IgG with no anti-complement activity.

Every lot has been tested against a panel of positive and negative samples for the relevant antigens to assure Anti-IgG sensitivity and absence of contaminating antibodies. Details of specificity test results submitted to the FDA for release of the product will be furnished upon request.

The potency of Anti-IgG is verified by tests with red blood cells sensitized with Anti-D and Anti-Fy^a according to methods approved by FDA. The Anti-IgG meets FDA potency requirements.

BIBLIOGRAPHY

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PRESENTATION

210394 DG Gel 8 Anti-IgG (Rabbit) 50 Cards

Manufactured by:

Diagnostic Grifols, S.A.

Passeig Fluvial 24 08150 Parets del Vallès (Barcelona), Spain

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SYMBOLS KEY

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