DG Gel Coombs
Anti-Human Globulin
Instructions for Use. For in vitro diagnostic use.

INTENDED USE
The DG Gel Coombs card is used for the Direct and Indirect Antiglobulin Test of human blood samples, in gel technique.
The Indirect Antiglobulin Tests include screening and identification of unexpected antibodies, crossmatch tests, autocontrol and red blood cell typing.

SUMMARY AND EXPLANATION
Carlo Moreschi described the principle of antiglobulin technique in 1908. 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. 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. The Polyspecific Anti-human Globulin Test is based on the use of anti-human globulin that allows the detection of red blood cells coated with immunoglobulin (IgG) and/or complement fractions.
The Direct Antiglobulin Test allows the detection of red blood cells sensitized in vivo by immunoglobulins and/or complement fractions.
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 the 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:
- Major crossmatch test: the donor's red blood cells combined with the patient's serum or plasma will show the presence or absence of unexpected antibodies in the patient's blood that are specific to the antigens of the donor's red blood cells.
- Minor crossmatch test: the patient's red blood cells combined with the donor's serum or plasma will show the presence or absence of unexpected antibodies in the donor's blood that are specific to the antigens of the patient's red blood cells.
The Indirect Antiglobulin Test is also used for investigation purposes such as titration of antibodies against red blood cell antigens. In this case the serum or plasma sample should be diluted in the appropriate buffer (e.g. DG Gel Sol) in order to prepare the set of dilutions before performing the Indirect Antiglobulin Test.

PRINCIPLE OF THE TEST
The principle of the test is based on the gel technique described by Yves Lapierre in 1985 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 mixture of polyclonal anti-human globulin (anti-IgG) with monoclonal anti-C3d antibodies has been prefilled into the microtube of the plastic card. The agglutination occurs when the red blood cell sensitized in vivo or in vitro by human IgG antibodies or complement fraction react with the antibodies 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 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 Coombs card contains a gel in buffered medium with preservative. The microtubes are identified by the front label of the card.
- Microtubes AHG: buffered low ionic strength solution (LISS) with polyspecific anti-human globulin.
- Mixture of rabbit polyclonal anti-IgG and monoclonal anti-C3d antibodies (IgM antibodies of murine origin, clone 12011 D10).
All microtubes contain sodium azide (NaN₃) as a preservative at a final concentration of 0.09%.

Reagent preparation
DG Gel Coombs 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, 25 μL, 50 μL and 1 mL.
- Disposable pipette tips.
- Glass or plastic test tubes.
- Diluent DG Gel Sol.
- Incubator for Grifols gel cards.
- Centrifuge for Grifols gel cards.
- Reagent Red Blood Cells at 0.8% from Grifols.
- Haemoclassification sera.
- Reader for Grifols gel cards (optional).

For Fully Automated Methods
- Diluent DG Gel Sol.
- Reagent Red Blood Cells at 0.8% from Grifols.
- Haemoclassification sera.
- 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 - 25 ºC.
- 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.
- Do not use enzyme-treated red blood cells.
- All products with animal derived material 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 or sodium citrate should be used. The collection, separation and handling of the blood should be performed by qualified technical personnel according to current
standards\textsuperscript{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.

Samples should be tested as soon as possible.

- For Indirect Antiglobulin test, use serum or plasma. Frozen samples stored up to 5 years at -20\textdegree\tiny{C} or colder may be used after thawing. If the patient has been pregnant or transfused within the previous three months, samples stored at 2 - 8\textdegree\tiny{C} should be used within 72 hours after collection.

- For Direct Antiglobulin Test, crossmatch tests and autocontrol, use the red blood cells. If necessary, samples stored at 2 - 8 \textdegree\tiny{C} can be used up to 72 hours after their collection, except for Direct Antiglobulin Test where sample storage less than 48 hours is recommended.

Red blood cells from bags collected in CPD, CPDA or SAG-Mannitol can also be used up until the expiration date indicated on the label of the bag. 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.

- For red blood cell typing, follow the instructions for use of the haemoclassification serum used.

**PROCEDURE**

For Direct Antiglobulin test:

1. Allow DG Gel Coombs cards, additional reagents and the samples to reach room temperature (18 - 25 \textdegree\tiny{C}).

   \textbf{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 1\% red blood cell suspension in DG Gel Sol (10 \textmu L of packed red blood cells in 1 mL of DG Gel Sol).

4. Remove the foil seal from the complete DG Gel card or from the individual microtubes to be used for testing.

   \textbf{Note}: Use the microtubes immediately once the seal has been opened.

5. Ensure the homogeneity of the 1\% red blood cell suspension before use.

6. Dispense 50 \textmu L of the 1\% red blood cell suspension into the corresponding microtube.

7. Centrifuge the gel card in the Grifols centrifuge.

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

- For Indirect Antiglobulin tests:

1. Allow DG Gel Coombs cards, additional reagents and the samples to reach room temperature (18 - 25 \textdegree\tiny{C}).

   \textbf{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. For **Crossmatch test and Autocontrol**, prepare a 1% red blood cell suspension in DG Gel Sol (10 μL of packed red blood cells in 1 mL of DG Gel Sol). Ensure the homogeneity of the 1% red blood cell suspension.

   For **Screening and/or Identification of unexpected antibodies**, thoroughly mix the vials of Reagent Red Blood Cells to ensure homogeneous suspension of the red blood cells before use.

4. Remove the foil seal from the complete DG Gel card or from the individual microtubes to be used for testing.

   **Note:** Use the microtubes immediately once the seal has been opened.

5. Dispense 50 μL of the 1% red blood cells suspension for **Crossmatch test and Autocontrol** or 50 μL of Reagents Red Blood Cells for **Screening and Identification of antibodies** into the microtubes.

6. Add 25 μL of serum or plasma into the same microtubes.

7. Incubate 15 minutes at 37 ºC using Grifols incubator.

8. Centrifuge the gel card in the Grifols centrifuge.

9. After centrifugation, remove the gel card from the centrifuge and read the results. Alternatively, use the Grifols reader to read and to interpret the results.

**Red blood cell typing**

Follow the instructions for use of the haemoclassification serum used.

**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 is visible in the microtube. In negative results, 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 positive results, 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. A positive result indicates the presence of IgG antibodies in the serum or plasma sample or coated on the red blood cells.
**Reaction Grades**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>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</td>
</tr>
<tr>
<td>+/-</td>
<td>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</td>
</tr>
<tr>
<td>1+</td>
<td>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</td>
</tr>
<tr>
<td>2+</td>
<td>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</td>
</tr>
<tr>
<td>3+</td>
<td>Medium-sized clumps of agglutinated cells in the upper half of the gel column</td>
</tr>
<tr>
<td>4+</td>
<td>A well-defined band of agglutinated red blood cells in the top part of the gel column. A few agglutinated cells may be visible below the band</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Double Population</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>A band of red blood cells at the top part of the gel or dispersed through the gel column, and a pellet in the bottom as a negative result</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hemolysis</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>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</td>
</tr>
</tbody>
</table>

![Figure 1. Pattern of Reaction Grades](image)

**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.

**Note:** In the 24-hour delayed reading of processed cards with weak positive samples, a loss in the agglutination intensity may be observed.

**Interpretation of the results**

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

**Red blood cell typing.** Follow the instructions for use of the haemoclassification serum used.
Notes:
1. It is recommended that any discrepant result obtained should be checked /investigated.
2. 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.
3. Drug-induced antibodies can cause a positive Direct Antiglobulin Test\(^6\).
4. 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\(^6\).
5. 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.
6. 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\(^6\).
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 non-specific agglutination of the red blood cells. It is recommended to wash the red blood cells before performing the test\(^6\).
5. The presence of some drugs, dextran solutions or remains of silicone gel from the extraction tube in the sample, can induce a positive result in the Direct Antiglobulin test\(^6\).
6. Antibody activity may decrease in the elderly, infants or persons with disease.
7. If plasma is used, complement-dependent hemolytic reactions may not be detected.
8. The presence of high concentrations of immunoglobulins and other serum proteins in the sample can neutralize the polyspecific anti-human globulin, even after numerous washes and lead to a false positive result in the antiglobulin test\(^6\).
9. 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 Double Population could lead to a misinterpretation. In case of incompletely clotted serum samples, it is recommended to re-clot the serum and repeat the test\(^6\).
10. If Indirect Antiglobulin Test is used for antibody titration studies, the laboratory should validate the titration procedure with clinical findings and laboratory data to ensure meaningful interpretation based on its own titration values.
11. 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. For screening and identification of unexpected antibodies, crossmatch, autocontrol and titration tests is acceptable increasing the volumes of serum or plasma from 25 µL to 50 µL. This variation in the concentration of antibodies brings the antigen/antibody ratio down and can improve the detection of antibodies at very low concentration.

12. Rare antibodies, notably some anti-Jka or anti-Jkb, may be detected only when polyspecific AHG is used and when active complement is present.

13. The Indirect Antiglobulin Test at 37 °C 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.

14. 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 or in specimens collected in tubes containing silicone gel.

15. 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.

16. 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 drugs can cause positive Direct Antiglobulin test.

17. Blood cell samples with a positive Direct Antiglobulin Test should not be used for Indirect Antiglobulin testing.

18. 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**

There is no described procedure or technique capable of detecting all the possible unexpected antibodies present in a sample population with absolute certainty. Occasional unexpected positive or negative results may be obtained due to the special characteristics of the sample tested, without resulting in a deviation of the expected performance of the products. Performance evaluation studies were carried out with DG Gel Coombs card showing that the performance characteristics conforms the intended use of the product. The studies included Indirect Antiglobulin test and Direct Antiglobulin testing and were performed in manual gel testing using multiple lots of Reagent Red Blood Cells from Diagnostic Grifols. The results were comparable to those obtained with other established products of equivalent intended use. The lower confidence bounds for the positive and negative percentages of agreement were estimated with a 95% level of confidence.
<table>
<thead>
<tr>
<th>Antibody</th>
<th>Technique</th>
<th>N(^{(a)})</th>
<th>Lower 95% CI</th>
<th>Percent Agreement(^{(b)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHG (Anti-IgG, -C3d)</td>
<td>DAT(^{(c)})</td>
<td>PPA(^{(d)}) 92</td>
<td>94.9%</td>
<td>98.91%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NPA(^{(f)}) 416</td>
<td>97.2%</td>
<td>98.56%</td>
</tr>
<tr>
<td></td>
<td>IAT(^{(d)}) - Investigation</td>
<td>PPA(^{(d)}) 151</td>
<td>98.0%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NPA(^{(f)}) 3181</td>
<td>99.9%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>IAT(^{(d)}) - Crossmatch</td>
<td>PPA(^{(d)}) 20</td>
<td>86.1%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NPA(^{(f)}) 58</td>
<td>95.0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

(a) N: Nº of samples or tests
(b) Percent of Agreement only indicates the agreement obtained in a specific study between Diagnostic Grifols reagents and the comparative method using a specific panel of positive and negative samples
(c) DAT: Direct Antiglobulin test
(d) IAT: Indirect Antiglobulin test
(e) PPA: Positive percent agreement (number of true positive results / (number of true positive results + number of false negative results)) x 100
(f) NPA: Negative percent agreement (number of true negative results / (number of true negative results + number of false positive results)) x 100

**Precision:**

The precision of the reagents present in the DG Gel Coombs card was 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**


**PRESENTATION**

210342 DG Gel Coombs 50 Cards Profile: 8x (AHG)

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

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This document is available in several languages. The translations have been made from the master document in English. In the event of doubts or discrepancies, the wording in the master document in English shall take precedence.

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