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Attachment LAB.1:
Proposed Draft Package Insert for DG Gel 8 Direct Coombs card

Anti-Human Globulin

DG Gel 8 Direct Coombs REF 210129

Instructions for Use.

INTENDED USE

The DG Gel 8 Direct Coombs card is used for the evaluation of the Direct Antiglobulin Test of two different human blood samples. It allows to differentiate red blood cells sensitized *in vivo* by IgG type immunoglobulins or the complement C3d fractions.
For use with the DG Gel System.

SUMMARY AND EXPLANATION

Carlo Moreschi described the principle of antiglobulin technique in 1908[1]. In 1945, Coombs published and introduced the use of anti-human globulin for the detection of red blood cells coated with non-agglutinating antibodies[2]. The antiglobulin test is applied in regular clinical laboratory practice and must rank as almost as important as the discovery of the ABO groups[1]. A positive Direct Antiglobulin test (DAT) with polyspecific anti-human globulin (AHG) generally indicates that the red cells are coated *in vivo* with immunoglobulin and/or complement. The DG Gel 8 Direct Coombs card allows differentiation between IgG and C3d coating of red cells using monospecific AHG anti-IgG and anti-C3d.

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 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 a polyspecific AHG anti-IgG,-C3d reagent, monospecific AHG anti-IgG reagent or monospecific AHG anti-C3d reagent has been prefilled into the microtubes of the plastic card. The agglutination occurs when the red blood cell sensitized *in vivo* by human IgG antibodies and/or complement fraction react with the AHG reagents 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

Each microtube of the DG Gel 8 Direct Coombs card contains a gel in buffered medium with preservative. The microtubes are identified on the front label of the card.
Microtubes **IgG,-C3d**: polyspecific anti-human globulin in buffered low ionic strength solution (LISS). Mixture of rabbit polyclonal anti-IgG and monoclonal anti-C3d antibodies (IgM antibodies of murine origin, clone 12011 D10).
Microtubes **IgG**: rabbit polyclonal anti-human globulin anti-IgG in buffered low ionic strength solution (LISS).
Microtubes **C3d**: monoclonal anti-human globulin anti-C3d (IgM antibodies of murine origin, clone 12011 D10) in buffered low ionic strength solution (LISS). This reagent detects C3d and/or C3b.
Microtubes **Ctl.**: buffered solution without antibodies (control microtube).

Anti-IgG,-C3d, anti-IgG and anti-C3d are produced using intermediate products produced for Diagnostic Grifols S.A. in a shared manufacturing agreement with DIAGAST, US License Number 1744.
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 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.
- 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 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.

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 - 8 ºC.
- Do not freeze.
- Do not expose cards to excessive heat.

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.
Samples should be tested as soon as possible. If necessary, samples stored at 2 - 8 ºC can be used up to 24 hours after their 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 the expiration date indicated on the label of the bag. If red blood cells from a 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.

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 required

Each DG Gel 8 Direct Coombs contains 8 microtubes with polyspecific AHG anti-IgG,-C3d, monospecific AHG anti-IgG, monospecific AHG anti-C3d reagents in buffered low ionic strength solution 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.
- DG Reader or DG Reader Net (optional).

For Fully Automated Methods

- Grifols Diluent.
- Grifols Wash Solution A and Grifols Wash Solution B.
- Erytra Eflexis, Erytra or WADiana Compact.

Test procedure

1. Allow DG Gel 8 Direct Coombs cards, additional reagents and the samples to reach room temperature (20 - 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 1% red blood cell suspension in Grifols Diluent (10 µL of packed red blood cells in 1 mL of Grifols Diluent).
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. Ensure the re-suspension of the red blood cells before use.
6. Dispense 50 µL of the 1% red blood cell suspension into each of the IgG,-C3d/ IgG/ C3d/ Ctl. microtubes.
   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.
7. Centrifuge the gel card in the DG Spin centrifuge.
8. 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. A positive result indicates the presence of IgG antibodies and/or complement fraction 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.
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	-	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.

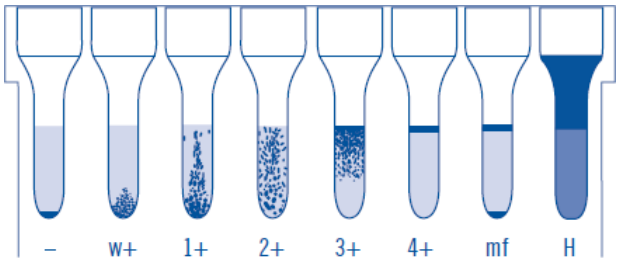

Figure 1. Picture of an example of reaction grades.

Interpretation of the results

Direct Antiglobulin tests. Tests determined by the result obtained in the microtubes anti-IgG,-C3d, anti-IgG and anti-C3d.

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. The positive results obtained in microtube anti-IgG,-C3d should be contrasted with those obtained in microtubes anti-IgG and anti-C3d. It is recommended that any discrepant result obtained should be checked /investigated.
4. 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.
5. Drug-induced antibodies can cause a positive Direct Antiglobulin Test[6].
6. 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. 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].
4. 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.
5. A positive Direct Antiglobulin Test result has a poor predictive value in a patient without hemolytic anemia[6].
6. 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].
7. If an unexpected result is obtained, it is recommended to repeat the determination after washing the red blood cells with physiological saline solution and prepare a new suspension of the washed red blood cells.
8. 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

- Every lot of anti-IgG,-C3d, anti-IgG and anti-C3d meets FDA potency requirements.
- The potency of anti-IgG,-C3d, anti-IgG and anti-C3d are verified according to methods approved by FDA with sensitized red blood cells.
- 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:

	Overall Statistical Analysis Results of the comparison study			
	Negative Agreement		Positive Agreement	
	Nº of samples	Percent Agreement (Lower 95% CI)	Nº of samples	Percent Agreement (Lower 95% CI)
Anti-IgG,-C3d	993	98.39% (97.56%)	179	99.44% (97.38%)
Anti-IgG	202	100.00% (98.53%)	73	100.00% (95.98%)
Anti-C3d	1071	100.00% (99.72%)	118	99.15% (96.04%)

- 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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5. CLSI H18-A04: Procedures for the handling and processing of blood specimens; Approved Guideline, 4rd edition, 2010.
6. Technical Manual, 20th edition, American Association of Blood Banks, Bethesda, Maryland, 2020.

PRESENTATION

210129 DG Gel 8 Direct Coombs 25 Cards

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

Diagnostic Grifols, S.A.
Response to the Complete Response for anti-IgG, -C3d
Section 1: Labeling