104530.90 Grifols DG Gel 8 Manual Antibody Screen

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Comments for version 1.0 (last major revision)
Initial version

Comments for version 1.2 (this revision)
Added related documents and graded reaction chart.

Approval and Periodic Review Signatures

Type	Description	Date	Version	Performed By	Notes
Approval	Lab Director	12/14/2017	1.1	Anita Martin	

Version History

Version	Status	Type	Date Added	Date Effective	Date Retired
1.2	Approved and Current	Minor revision	12/18/2017	12/20/2017	Indefinite
1.1	Retired	Minor revision	12/7/2017	12/14/2017	12/20/2017
1.0	Retired	Initial version	12/7/2017	12/7/2017	Indefinite

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STATEMENT OF PURPOSE

The purpose of this procedure is to describe how to perform antibody screen testing using Grifols Anti-IgG gel card methodology.

SCOPE

All blood bank departments using the Grifols DG Gel 8 Manual System

PRINCIPLE

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 principle of this test is based on the gel technique described by Yves Lapierre 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 cells sensitized *in vivo* or *in vitro* by human IgG antibodes 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 or along the gel column, and non-agglutinated red blood cells reach the bottom of the microtube forming a pellet.

OWNERS

Director, Transfusion Services

RELATED DOCUMENTS

IAT – Indirect Antiglobulin Testing

Flow Chart for Positive Antibody Screen

Antibody Screening in Obstetrical Patients Treated with Antenatal RHIG – "r set"

SPECIMEN

1.0 Use centrifuged whole blood samples stored at 2 – 8°C, test within 72 hours of collection.
   A. Serum from freshly clotted blood is preferred.
   B. Plasma collected in EDTA may be used.
   C. Samples frozen at ≤-20°C may be used up to 5 years after thawing.

2.0 Do not use samples that are grossly hemolyzed, lipemic, icteric or cloudy.

MATERIALS and REAGENTS

1.0 Grifols Anti-Human Globulin DG Gel 8 Anti-IgG cards
   A. Do not use beyond expiration date.
   B. Store upright with seal intact at 2 – 25°C. Do not freeze or expose to excessive heat.
   C. Each microtube of the Anti-IgG card contains buffered low ionic strength solution (LISS) with rabbit polyclonal anti-human globulin. Each microtube also contains polyclonal antibodies mixed with a gel in a buffered medium with preservative (sodium azide.)

2.0 Search-Cyte® Reagent Red Blood Cells 0.8 ± 0.1%
   A. The expiration date of each lot is no longer than 61 days from the collection date of red blood cells from any donor in the lot.
   B. Store at 2 – 8°C. Bring to room temperature (20 – 25°C) prior to testing.
   C. Do not freeze.
   D. Do not use if there is notable hemolysis, darkening of the reagent red blood cells or spontaneous clumping.

EQUIPMENT

1.0 Grifols DG Spin
2.0 Grifols DG Therm
3.0 Grifols Dispenser Plus
4.0 Grifols DG Pipette

QUALITY CONTROL

Reagent quality control must be performed each day of use for each IgG card lot number as well as all Search-Cyte lot numbers. Refer to separate Grifols DG Gel 8 Manual Quality Control procedure.

Grifols DG Gel 8 Manual Antibody Screen

PROCEDURE

Step	Action	Notes
1	Inspect condition of the card prior to use.	Do not use gel cards with bubbles trapped in the gel, cracked gel, gel with fissures, drying gel, other defects present, and opened and/or damaged foil seals.
2	Remove the foil seal from 2 microtubes (per patient) of the Anti-IgG gel card. • Label the microtubes SI and SII • Label the microtubes with patient identification	
3	Dispense 50 µL of Search-Cyte I and II into the corresponding microtubes.	
4	Dispense 25 µL of patient serum or plasma into each of the microtubes.	
5	Incubate the gel card(s) for 15 minutes at 37°C using the DG Therm.	
6	At the end of the 15 minutes, centrifuge the gel card(s) for 9 minutes using the DG Spin.	
7	After centrifugation remove the gel cards and read the results. Grade reactions 0 to 4+.	Refer to DG Gel 8 Cards Reaction Guide for assistance with reaction grading.

GRADING REACTIONS

Refer to DG Gel 8 Cards Reaction Guide for assistance with reaction grading or refer to the chart below.

Reaction Grade	Description	.	Reaction Grade	Description
0	Well defined pellet on non-agglutinated red blood cells at the bottom of the gel column and no visible agglutinated cells in the rest of the gel column.		**3+**	Medium sized clumps of agglutinated cells in the upper half of the gel column.
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.		**4+**	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.
1+	Some small-sized clumps of agglutinated cells most frequently in the lower half of		**Mf**	(Mixed field) A band of red blood cells at the top part of the gel or dispersed through the gel column,

	the gel column. A small pellet may also e observed at the bottom of the gel column.			and a pellet in the bottom as a negative result.
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		**H**	(Hemolysis) 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.

REPORTING RESULTS

Record reactions in the laboratory information system or on the appropriate MACL computer downtime form immediately after reading the reactions.

PROCEDURE NOTES

If the antibody screen is positive, refer to Antibody Identification procedure to determine if an ID is required at this time.

LIMITATIONS

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

2.0 Aged or hemolyzed specimens may cause weaker reactions compared to those obtained with fresh samples.

3.0 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.

4.0 Samples with high-potency antibodies may coat the red blood cells completely, causing spontaneous agglutination.

5.0 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. This may also be seen if the sample was improperly centrifuged. 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 samples, it is recommended to reclot the serum and repeat the test.

6.0 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.

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

8.0 On occasion, 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 test.

REFERENCES

Grifols DG Gel 8 Manual System Operator Training Manual, 2017 Grifols Diagnostic Solutions Inc.

Grifols Anti-Human Globulin DG Gel 8 Anti-IgG (Rabbit) package insert, Diagnostic Grifols, SA

Grifols Search-Cyte package insert, Medion Grifols Diagnostics AG