Anti-Human Globulin Anti-IgG,- C3d polyspecific – Green **REF 210546**

Anti-Human Globulin Anti-IgG – Green **REF 210547**

Anti-Human Globulin Anti-C3d **REF 210548**

Anti-Human Globulin Anti-IgG – Clear **REF 210549**

Anti-Human Globulin Anti-IgG,- C3d polyspecific – Clear **REF 210550**

(Murine Monoclonal)

For tube technique

- For *In Vitro* Diagnostic Use
- Meets FDA potency requirements
- Discard if turbid
- Preservative: 0.09% (w/v) sodium azide, 0.02% sodium arsenite

INTENDED USE

Anti-Human Globulins Anti-IgG, are intended use for use in the direct antiglobulin test to detect the *in vivo* coating on human red blood cells with IgG, and for indirect antiglobulin test for antibody screening and identification, and Crossmatch and for erythrocyte phenotyping with blood phenotyping reagents requiring an indirect antiglobulin test method.

Anti-Human Globulin Anti-C3d is intended for the use in the direct antiglobulin test to detect the *in vivo* coating on human red blood cells C3d components.

Anti-Human Globulins Anti-IgG,-C3d polyspecific are intended for the use in the direct antiglobulin test to detect the *in vivo* coating on human red blood cells with IgG and C3d components.

- **Anti-Human Globulin Anti-IgG** recognizes IgG antibodies.
- **Anti-Human Globulin Anti-C3d** only recognizes the complement fragment C3d and, consequently, cannot react with component C4.
- **Anti-Human Globulin Anti-IgG,-C3d polyspecific**: in addition to recognizing IgG antibodies and the complement fragment C3d, is able to recognize (like AHG anti-C3d) IgM antibodies on the surface of red blood cells since IgM antibodies always fix complement *in vivo* (and *in vitro* if the reaction occurs in the presence of complement: i.e. when using a fresh sample*).*

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 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 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.

These reagents enable the user to detect the presence of specific antibodies (immunoglobulins) bound to the corresponding antigens on the surface of red blood cells when direct agglutination of the red blood cells has not occurred.

Agglutination induced by the reagents also occurs if complement fragments are present on the surface of the red blood cells insofar as the reagents contain the specific antibody, such as Anti-C3d antibody (Anti-Human Globulin Anti-IgG,-C3d polyspecific and Anti-Human Globulin Anti-C3d).

PRINCIPLE OF THE TEST

The direct and indirect antiglobulin test methods are based on the principle of hemagglutination. The addition of the reagents induces agglutination of the red blood cells sensitized *in vivo* (direct antiglobulin test: direct 'Coombs' test) or *in vitro* (indirect antiglobulin test).

- The **Direct antiglobulin test** detects sensitization of the red blood cells *in vivo* either by autoantibodies (Autoimmune Hemolytic Anemia) or by alloantibodies (screening for Hemolytic Disease of The Newborn, transfusion incompatibility, diagnosis of drug-induced immunoallergic hemolytic complications).
  The red blood cells are washed and mixed directly with the reagent.

- The **Indirect antiglobulin test** is used in the determination of certain erythrocyte phenotypes. It demonstrates the presence of the antigen tested for on the surface of red blood cells. It may also be used in the tube method of testing for immune antibodies present in the patient's serum. In the test for the presence of immune antibodies, potentiators may be used according to the manufacturer's instructions for use (IFU).
  The reaction is two-stage. The red blood cells are exposed to the IgG antibodies. The antibodies bind to the red blood cells carrying the corresponding antigen. After washing, Anti-Human Globulin Anti-IgG is added, inducing agglutination of the sensitized red blood cells carrying the corresponding antigen.

REAGENTS

These reagents contain sodium azide (0.09%), sodium arsenite (0.02%) and bovine albumin. Any bovine albumin used in the manufacture of these products is sourced from donors' animals that have been inspected and certified by Veterinary Service inspectors to be disease free.

The reagents are produced by DIAGAST from monoclonal antibodies derived from the *in vitro* culture supernatant of murine hybridomas.

These reagents are provided with calibrated droppers.

Code	Product Designation	Packaging
210546	Anti-Human Globulin Anti-IgG,-C3d polyspecific – Green	4 x 10 mL
210547	Anti-Human Globulin Anti-IgG – Green	4 x 10 mL
210548	Anti-Human Globulin Anti-C3d	4 x 5 mL
210549	Anti-Human Globulin Anti-IgG – Clear	4 x 10 mL
210550	Anti-Human Globulin Anti-IgG,-C3d polyspecific – Clear	4 x 10 mL

WARNINGS AND PRECAUTIONS

- These reagents contain sodium azide. Sodium azide may be toxic if ingested and may react with lead and copper plumbing to form explosive compounds. If discarded into a sink, flush with a large volume of water to prevent azide build-up. Handle and dispose of reagents as potentially infectious, in accordance with local, state, and national laws.
- Use proper Personal Protective Equipment according to local SOPs or guidelines.
- All materials that have come into contact with the samples are to be handled as potentially infectious products.
- Special protective measures and conditions for disposal and disinfection should be implemented in accordance with local regulations.
- For *In Vitro* Diagnostic Use.
- Do not use beyond expiration date.
- Do not use damaged or leaking reagents.
- Do not use if turbid.

- Do not dilute.
- The absence of all viruses has not been determined in these reagents.
- These reagents have components (Dropper bulb) containing dry natural rubber which may cause allergic reactions.
- This reagent contains material of human or animal origin and may transmit infectious agents and should be handled with extreme caution.

"CAUTION: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED FOR HIV, HBV AND HCV. NO KNOWN TEST METHODS CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS."

STORAGE AND STABILITY

- Store reagents at 2°C to 8°C when not use. Do not freeze.
- Do not use beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior the specimen collection.
The blood samples collected following standard blood sampling guidelines in EDTA, heparin or sodium citrate anticoagulant should be stored at 2-8 ºC.
They should be tested within the following validated hold times:
- Clotted specimens or blood drawn into sodium citrate or EDTA should be tested within 7 days.
- Blood drawn into heparin should be tested within 2 days.

Direct Antiglobulin Tests are recommended to be tested within 48 hours of collection.
Red blood cells from bags collected in ACD, ACD with AS-1, CPD, CPD with AS-1, CPDA-1, CP2D and CP2D with AS-3 can also be used up to 7 days after the expiration date indicated on the label of the bag.
Do not use blood specimens that exhibit contamination.

MATERIALS

Material provided:

- Anti-Human Globulin Anti-IgG (REF 210547 / REF 210549): Green or clear reagent. Monoclonal antibodies blend. Anti-IgG murine clones 18833 and 18896.
- Anti-Human Globulin Anti-C3d (REF 210548): Monoclonal antibody. Anti-C3d murine clone 12011D10.
- Anti-Human Globulin Anti-IgG,-C3d polyspecific (REF 210546 / REF 210550): Green or clear reagent. Monoclonal antibodies blend Anti-IgG murine clones 18833 and 18896, and Anti-C3d murine clone 12011D10.

Material required but not provided:

- Test tubes, tube rack.
- Pasteur pipettes (drop volume 40 to 50 µl) or Automatic pipettes with adjustable precision.
- Centrifuge of relative force from 100 to 1200 rcf.
- Timer.
- Incubator or water-bath at 37°C ± 1°C.
- Isotonic saline solution (0.9% NaCl).
- Positive and negative control blood samples suitable for antiglobulin test.
- IgG and/or complement-sensitized red blood cells.
- Optional enhancing media.
- 3-5% Reagent Red Blood Cells (optional)

TEST PROCEDURES

- **For Direct Antiglobulin Test (Direct 'Coombs' test): Anti-Human Globulins Anti-IgG, Anti-IgG,-C3d polyspecific and Anti-C3d.**

1. Identify the test tubes to be used and the samples to be tested.

2. Wash the test red blood cells 3 times with isotonic saline.
3. Prepare a 3-5 % red blood cell suspension (volume/volume) in isotonic saline solution.
4. Transfer 1 drop or 50 µL of the suspension and 2 drops or 100 µ of reagent to a test tube, using the vial dropper.
5. Shake to mix
6. If using Anti-Human Globulin Anti-C3d, allow the test tube to incubate at room temperature (18-25°C) for 5 minutes.
7. Then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.
8. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
9. Read and record the reaction immediately. It is recommended grading positive reactions.
10. Negative reactions should be validated using red cells sensitized *in vitro* with IgG or possibly complement. The use of those samples enables detection of anomalies (handling, reagents, apparatus and working environment) and the implementation of corrective actions.

- **For Indirect Antiglobulin Test : Anti-Human Globulin Anti-IgG.**

a. Screening and identification of unexpected antibodies

1. Identify the test tubes according to the number of cells to be used in the test and the samples to be tested.
2. With a clean transfer pipette, add 2 drops or 100 µl of the sample serum to be tested to each test tube.
3. Thoroughly mix the vials of 3-5% Reagent Red Blood Cells for the screening and/or identification of unexpected antibodies to ensure homogeneous suspension of the red blood cells before use.
4. Add 1 drop or 50 µl of the corresponding thoroughly mixed Reagent Red Blood Cells in each test tube.
5. Gently shake the test tubes to homogenize the mixture.
6. If desired, add antibody enhancement solution to each test tube in the amount specified in the manufacturer's directions.
7. Incubate the test tubes at 37°C ± 1°C. Incubation time depends on the antibody enhancement solution used, but incubation should be extended to 30-60 minutes if no additive is used.
8. Centrifuge the test tubes at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.
9. Examine the supernatants for hemolysis. Complete hemolysis must be interpreted as a positive test result. If partial hemolysis is observed, record and continue.
10. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
11. Read and record the reaction immediately. It is recommended grading positive reactions.
12. Wash the cell/serum mixture 3 times with isotonic saline solution and discard the remaining liquid from the last wash.
13. Using the vial dropper, add 1 drop of Anti-Human Globulin Anti-IgG to the red blood cell pellet.
14. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.
15. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
16. Read and record the reaction immediately. It is recommended grading positive reactions.
17. Negative reactions should be validated using red blood cells sensitized in vitro with IgG or possibly complement. The use of those samples enables detection of anomalies (handling, reagents, apparatus and working environment) and the implementation of corrective action.

b. Antiglobulin Crossmatch tests

1. Identify the test tubes to be used and the samples to be tested.
2. Wash the donor's red blood cells with isotonic saline
3. Prepare a 3-5 % suspension of the red blood cells (volume/volume) in isotonic saline solution.
4. Ensure the re-suspension of the red blood cells before use. Dispense 1 drop or 50 µL of the diluted donor's red blood cell suspension into the corresponding test tube.
5. Add 2 drops or 100 µL of the recipient's serum or plasma into the test tubes.
6. Gently shake the test tubes to homogenize the mixture.
7. If desired, add antibody enhancement solution to each test tube in the amount specified in the manufacturer's directions.
8. Incubate the test tubes at 37°C ± 1°C. Incubation time depends on the antibody enhancement solution used, but incubation should be extended to 30-60 minutes if no additive is used.
9. Centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.

10. Examine the supernatants for hemolysis. Complete hemolysis must be interpreted as a positive test result. If partial hemolysis is observed, record and continue.
11. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
12. Read and record the reaction immediately. It is recommended grading positive reactions.
13. Wash the cell/serum mixture 3 times with isotonic saline solution and discard the remaining liquid from the last wash.
14. Using the vial dropper, add 1 drop of Anti-Human Globulin Anti-IgG to the red blood cell pellet.
15. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.
16. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
17. Read and record the reaction immediately. It is recommended grading positive reactions.
18. Negative reactions should be validated using red blood cells sensitized in vitro with IgG or possibly complement. The use of those samples enables detection of anomalies (handling, reagents, apparatus and working environment) and the implementation of corrective action.

c. Autocontrol (when the use of an autocontrol is desired)

Follow the "**Antiglobulin Crossmatch tests"** procedure with patient's red blood cells and patient's serum or plasma.

d. Phenotyping

1. Refer to the Instructions for Use on the erythrocytic phenotyping reagent used.
2. After washing, add 1 drop of Anti-Human Globulin Anti-IgG to the red blood cell pellet.
3. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.
4. Gently swirl the test tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
5. Read and record the reaction immediately. It is recommended grading positive reactions.

RESULTS

Positive Result: If there is agglutination (the red blood cells form one or several clump(s)), the reaction is positive.

Negative Result: If there is no agglutination (the red blood cells reform a homogeneous suspension), the reaction is negative.

Reaction validation: In order to validate negative reactions, add IgG- and/or possibly complement- sensitized red blood cells (refer to the Instructions for Use of the corresponding RBC reagents):

- If the reaction is positive, the activity of Anti-Human Globulin Anti-IgG,-C3d polyspecific Anti-Human Globulin Anti-IgG and Anti-Human Globulin Anti-C3d is confirmed and the negative reaction prior to addition of the sensitized red blood cells is validated.
- If the reaction is negative, the antiglobulin test and validation are to be repeated.

Direct Antiglobulin Test interpretation:

- If agglutination occurs (the red blood cells form one or several clumps), the reaction is positive. The agglutination of red blood cells in the presence of Anti-Human Globulin Anti-IgG,-C3d polyspecific, Anti-Human Globulin Anti-IgG and Anti-Human Globulin Anti-C3d, is a positive reaction indicating that the red blood cells have been sensitized by human IgG and/or complement components or fragments.
- The absence of agglutination indicates that IgG and/or complement components or fragments have not been detected on the surface of the red blood cells.
- The test requires concomitant and independent use of Anti-Human Globulin Anti-IgG and Anti-Human Globulin Anti-C3d and the appropriate controls.

Indirect Antiglobulin Test interpretation:

Refer to the Instructions for Use on the erythrocytic phenotyping reagent used or the supplier's Instructions for Use on the ready-for-use red blood cell panel for testing for immune antibodies.

QUALITY CONTROLS

The reactivity of all reagents should be confirmed by testing with known positive and negative red blood cells on each day of use. To confirm the reactivity or specificity of DIAGAST Anti-Human Globulin Anti-IgG,-C3d polyspecific, Anti-Human Globulin Anti-IgG and Anti-Human Globulin Anti-C3d, the reagents should be tested with IgG coated (and if possible complement coated) and non-coated red blood cells respectively. The reagent is satisfactory for use if it reacts only with the IgG (and complement) coated red blood cells.

LIMITATIONS OF THE PROCEDURE

- These reagents are not to be used in a method not described in this Instructions For Use.
- It is recommended to use the calibrated dropper provided in the vial to dispense a reagent drop.
- False negatives may be observed if the red blood cells are not sufficiently washed.
- False positive or false negative can occur due to improper centrifugation.
- It is necessary to validate the negative reactions with IgG- and/or complement-sensitized red blood cells.
- In the event of Hemolytic Disease of the Newborn, the Direct Antiglobulin Test (or Direct 'Coombs' test) may be negative, particularly in the event of ABO incompatibility.
- It is imperative to work with clean apparatus and uncontaminated products (bacterial or other contamination).
- Strict compliance with the following is required:
  - storage condition,
  - equipment calibration is recommended.

SPECIFIC PERFORMANCE CHARACTERISTICS

- These reagents meet FDA potency requirements for Anti-Human Globulin Reagents to be used in tube technique.
- Every lot of each product is tested to assure reliable reactivity and specificity in use in accordance with FDA requirements.
- The specificity studies of the anti-IgG fractions of Anti-Human Globulin Anti-IgG,-C3d polyspecific and Anti-Human Globulin Anti-IgG with human immunoglobulin G, conducted by the hemagglutination inhibition method and on BIACORE®, demonstrated perfect antibody specificity for the alleles, G1m, G2m, G3m and G4m of subclasses IgG1, IgG2, IgG3 and IgG4.
- The specificity studies on Anti-Human Globulin Anti-C3d, with human red blood cells labeled or not labeled with various fragments of human complement or red cell antibodies demonstrated perfect specificity of the reagent. Anti-Human Globulin Anti-C3d is specific for component C3 and fragments C3d, iC3b and C3d of human complement. The reagent does not recognize red blood cells labeled with component C4, or fragments C4b or C4d of human complement or with red cell antibodies not fixing complement. 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.

Table 1. Overall Statistical Analysis results of the comparison study

Method / Reagent	N° of samples	Negative Percent Agreement (Lower 95% CI)	N° of samples	Positive Percent Agreement (Lower 95% CI)
Ab. Screening Anti-IgG	817	100% 99.63%	302	99.67% 98.44%
Ab. Identification Anti-IgG	NA	NA	108	100% 97.26%
AHG Crossmatch Anti-IgG	185	100% 98.39%*	185	100% 98.39%*
DAT Anti-IgG-C3d	184	100% 98.39%	104	100% 97.16%
DAT Anti-IgG	193	99.48% 97.57%	95	100% 96.90%
DAT Anti-C3d	243	100% 98.77%	45	97.78% 89.89%**

NA: Not Applicable NPA: Negative percent agreement PPA: Positive percent agreement

*Both PPA and NPA had 100% of agreement but the smaller sample size meant that the lower confidence bound was under the acceptance criteria.

**Lower value for the PPA lower confidence bound was obtained due to the limited quantity of samples with complement activated and bound to the red blood cells.

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

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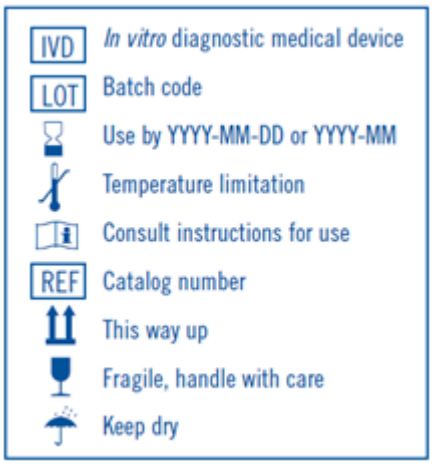
SYMBOLS KEY

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


IVD
In vitro diagnostic medical device
LOT
Batch code
Use by YYYY-MM-DD or YYYY-MM
Temperature limitation
Consult instructions for use
REF
Catalog number
This way up
Fragile, handle with care
Keep dry