BLOOD GROUPING REAGENT

Anti-D (RH1) IgM/IgG REF 210526

(Human/Murine Monoclonal IgM/IgG)

For tube and gel 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-D (RH1) IgM/IgG is designed to determine the presence of the blood Rhesus antigen D (RH1) on the surface of human red blood cells, including weak D and partial D by tube method or by gel technique using Grifols gel cards.

SUMMARY AND EXPLANATION

After the ABO system, discovered by Landsteiner in 1900, the most important blood group antigen, first described in 1939, is the D antigen from the Rh blood group system. The determination of Rh(D) is defined by the presence or absence of the D antigen in the red blood cells.
Unlike antibodies of the ABO system, those of the Rh system do not occur naturally in the serum, but are most often the result of exposure to the antigen during pregnancy or through transfusion. The presence or absence of the D antigen is determined by testing the red blood cells with Anti-D. Agglutination indicates that the test cells are D positive. No agglutination indicates that the test cells are D negative. Approximately 85% of the white population and 94% of the black population are positive for the D antigen. The term “weak D” is used to describe forms of the D antigen that may not be agglutinated directly by Anti-D reagents. The red blood cells of donors are required to be further tested by performing indirect antiglobulin (weak D) test before being classified as D negative.

PRINCIPLE OF THE TEST

The techniques employed are based on the principle of hemagglutination. Test red blood cells bearing the antigen agglutinate in the presence of the reagent containing the corresponding antibody:
- either in the direct hemagglutination method, when they come into contact with the reagent containing the antibody (type IgM).
- or in the indirect hemagglutination method: antiglobulin test in the event of use of an Anti-IgG antibody.

When the Anti-D (RH1) IgM/IgG (REF 210526) is used with Grifols gel cards the technique combine the principle of hemagglutination and gel filtration. Please refer to Grifols gel cards Instructions for Use.

REAGENT

This reagent contains sodium azide (0.09%), sodium arsenite (0.02%) and bovine albumin. Bovine materials used in the manufacture of this product are sourced from donor animals that have been inspected and certified by Veterinary Service inspectors to be disease free.

The reagent is produced by DIAGAST from monoclonal antibodies derived from the *in vitro* culture supernatant of human/murine heterohybridomas.

This reagent is provided with calibrated dropper.

Code	Product Designation	Packaging
210526	Anti-D (RH1) IgM+IgG	5 x 10 mL

WARNINGS AND PRECAUTIONS

- This reagent contains 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.
- This reagent has 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."

NOTE: PLEASE INSPECT THE CONDITIONS OF THE CARDS BEFORE USE (SEE WARNINGS AND PRECAUTIONS SECTION OF GRIFOLS GEL CARDS).

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°C.
For tube method, they should be tested as follows:
- 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.

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.
For gel technique, blood samples collected in EDTA can be used and should be tested within 7 days. Red blood cells from bags collected in ACD, CPD, CPDA-1, CP2D or converted from ACD, CPD or CP2D and stored in AS-1 or AS-3 can also be used up to 7 days after 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. Do not use blood specimens that exhibit contamination.

MATERIALS

Material provided:
- Anti-D (RH1) IgM+IgG (REF 210526): Monoclonal antibodies IgM/IgG blend. Anti-D IgM human/murine clones P3x61 and P3x21223 B10 and Anti-D IgG human/murine clones P3x290 and P3x35.

Material required but not provided:

For tube method
- Test tubes and 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 control blood samples of guaranteed phenotype are required carrying the corresponding antigen and similarly for a negative control, blood samples should be used which lack the antigen corresponding to the reagent used.
- IgG-sensitized red blood cells.
- Anti-Human Globulin Anti-IgG (such as REF 210547/REF 210549) for indirect method
- Neg Control (REF 210543)

For gel technique

Please refer to the Instructions for Use of Grifols gel cards:
- DG Gel 8 Anti-IgG (Rabbit) (REF 210394).

TEST PROCEDURES

Tube Method

Direct agglutination method & Indirect antiglobulin method

1. In a tube, prepare a 3-5% red blood cell suspension in isotonic saline solution.
2. Using the vial dropper, transfer 1 drop of reagent to a test tube.
3. Add 1 drop or 50 µL of erythrocyte suspension.
4. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.
5. Gently swirl the tube to detach the erythrocyte pellet, observe macroscopically to detect the appearance of any agglutinates.
6. Read and record the reaction immediately. It is recommended grading positive reactions.
7. After immediately centrifuging and reading as above, if the reaction is weak or negative, shake the tubes and incubate at 37°C ± 1°C for 15 minutes.
8. Wash the red blood cells twice with isotonic saline solution and discard the remaining liquid from the last wash.
9. Using the vial dropper, add 1 drop of Anti-Human Globulin Anti-IgG to the red blood cell pellet.
10. Shake to mix, then centrifuge at 1000 rcf for 15 seconds or use a time and speed appropriate to the calibration of the centrifuge.
11. Gently swirl the 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.

Gel technique (for US market only)

Anti-D (RH1) IgM+IgG can be used in Grifols gel manual method or with Grifols automatic instruments. For the automatic instruments, refer to the Instructions for Use of the related instrument.

Indirect Antiglobulin Test

1. Allow the gel cards, additional reagents and the samples to reach room temperature (18-25ºC).
2. Inspect the conditions of the cards before using (see Warnings and Precautions of the DG Gel 8 Anti-IgG cards).
3. Identify the card to be used and the samples to be tested.
4. In a tube, prepare a 1% red blood cell suspension in the diluent (10µL of packed red blood cells in 1mL of Grifols Diluent).
5. Remove the foil seal from the complete gel card of from the individual microtubes to be used for testing.
6. Ensure the re-suspension of the red blood cells before use.
7. Dispense 50µL of the 1% RBC suspension into the corresponding microtube.
8. Add 25µL of the Anti-D (RH1) IgM+IgG to the microtube.
   Note: Carefully dispense the red blood cell suspension and the reagent, avoiding contact of the pipette tip with the wall or the contents of the microtubes to prevent carryover.
9. Incubate 15 minutes at 37ºC using DG Therm incubator.
10. Centrifuge the gel card in the DG Spin centrifuge.
11. After the centrifugation, remove the gel card from the centrifuge and read and record the results according to the Instructions for Use of DG Gel 8 Anti-IgG cards. Alternatively, use the Grifols reader to read and to interpret the results.

RESULTS

Tube Method

- **Positive Result:** If there is agglutination (the red blood cells form one or several clump(s)), the reaction is positive and the D antigen is present on the tested red blood cells.
- **Negative Result:** If there is no agglutination (the red blood cells reform a homogeneous suspension), the reaction is negative and the D antigen is not present on the tested red blood cells.

Interpretation: The reaction can only be interpreted if:
- the result of the Neg Control (REF 210543) with the subject's red blood cells is negative,
- the analytical system has been validated with control samples of guaranteed phenotype,
- for indirect antiglobulin test, the direct antiglobulin test on the red blood cells is negative,
- a negative reaction obtained in an indirect antiglobulin test can be validated with IgG-sensitized red blood cells (see Instructions for Use for the corresponding RBC reagent).

If there is discordance, do not report the result and pursue blood group identification in compliance with current recommendations and protocols.

The “auto” control, “allo” control and “reagent” control, and the clinical context may help elucidate the anomaly.
“Auto” control: under the same conditions, test the subject's plasma with his own red blood cells.
“Allo” control: under the same conditions, test the subject's plasma with a panel of test known O red blood cells (detection of anti-erythrocytic antibodies other than Anti-A or Anti-B).
“Reagent” control: under the same conditions, test the subject's red blood cells with the Neg Control (REF 210543).

With the direct hemagglutination tube method: if there is agglutination with Anti-D (RH1) IgM+IgG, antigen D is present. If there is no agglutination, it is possible to use Anti-D (RH1) IgM+IgG in an indirect antiglobulin test if weak and/or partial antigens D are to be detected (blood donations).

A negative reaction obtained in an indirect antiglobulin test can be validated with IgG-sensitized red blood cells (see Instructions for Use for the corresponding reagent).

Gel technique

- **Positive Result:** Agglutination of 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 in the Instructions for Use of the Grifols gel cards.
- **Negative Result:** No agglutination 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.

Interpretation: A positive result indicates the presence of antigen D (Rh system).

A positive reaction can only be interpreted if the direct antiglobulin test on the red blood cells is negative.

The reading and interpretation of the gel cards has to be done according to the manufacturer's instructions. See Grifols gel cards Instructions for Use for more information.

QUALITY CONTROLS

The use of samples of guaranteed Rh(D) typing as control samples allows the user to detect anomalies with (handling, reagents, instruments and the environment) and to implement corrective actions as required.
Known samples control should be run in parallel on each day of use.
- a sample possessing the antigen corresponding to the antibody in the reagent used,
- a sample devoid of the antigen corresponding to the antibody in the reagent used.

If an unexpected control result is obtained, a complete assessment of the reagents and material used should be made.

For tube method, a negative control should be performed for each positive blood sample with DIAGAST Anti-D reagent (REF 210526). The use of the Neg Control (REF 210543) as a reagent control to be performed alongside using the same conditions but replacing the anti-D reagent with Neg Control allows the detection of anomalies and to implement corrective actions as required.

In gel technique, for each positive blood sample with DIAGAST Anti-D reagent (REF 210526) a direct antiglobulin test on the red blood cells should be performed. Do not use the Neg Control in gel testing.

LIMITATIONS OF THE PROCEDURE

- These reagents are not to be used in a method not described in this Instructions for Use.
- The use of complementary reagents other than the ones cited in the section entitled “MATERIAL - Other required complementary reagents “ is of the entire responsibility of the user and must be validated.
- It is imperative to work with clean instrumentation and uncontaminated products (bacterial or other contamination).
- Strict compliance with the following is required:
  - storage conditions,
  - equipment calibration is recommended.
- No reagent can guarantee the detection of all the antigenic profiles rare, weak or variants.
- Antigen expression may be weakened in the red blood cells of persons with leukemia or other malignant diseases.
- Direct antiglobulin test on the red blood cells shall be negative to interpret the results of the indirect antiglobulin test with Anti-D (RH1) IgM+IgG in gel technique and tube method. Red blood cells coated with allo- or autoantibodies (DAT positive) may produce false positive results.

Tube method

- It is recommended to use the calibrated dropper provided in the vial to dispense a reagent drop.
- The reactions are to be read immediately after centrifuging and resuspending.
- False positive or false negative can occur due to improper centrifugation.

Grifols gel cards techniques:

- Please refer to the Grifols gel cards Instructions for Use: take under consideration all limitation statements appearing in the Instructions for Use of the used gel cards.

SPECIFIC PERFORMANCE CHARACTERISTICS

- These reagents meet FDA potency requirements for Blood Grouping Reagents.
- Every lot of each product is tested to assure reliable reactivity and specificity in use in accordance with FDA requirements.
- Anti-D (RH1) IgM+IgG enable screening for weak red blood cells D (RH1) in the indirect hemagglutination method with antiglobulin in test tube or in Grifols gel cards system.
- The tests conducted on particular phenotypes, while satisfactory, cannot ensure recognition of all weak or variant subjects, due to the variability of antigen motifs.
- Anti-D (RH1) IgM+IgG have the special feature of recognizing certain rare antigen motifs of type RH33 (DHar) and may thus yield discordant reactions with polyclonal reagents that recognize them little or not at all.
- Anti-D (RH1) IgM+IgG may enable detection of D partial DVI in direct or indirect techniques.
- In addition, the clones of Anti-D may specifically recognize certain epitopes of antigen D:

Clones	Type	DII	DIIIa DIIIb DIIIc	DIVa	DIVb	DVa	DVI	DVII	DFR	DBT	DHAR	DHMi
P3X61	IgM	+	+	+	+	+	–	+	+ or –	+	+	+
P3X21223B10	IgM	–	+	–	–	+	+	+	+	–	–	+
P3X290	IgG	+	+	+ or –	–	+	+ or –	+	+	–	–	+
P3X35	IgG	+	+	+	+	–	–	+	–	–	–	+

\+ indicates a positive result whose intensity may vary as a function of the number of antigen sites present on the test red blood cells.
+ or - indicates that a positive or negative result may be obtained. The result depends on the antigenicity.

- The performance of the reagent was confirmed in comparison studies where the reagent and comparator method were tested in parallel at different clinical sites. The estimated percent agreements and the lower limits of 95% one-side confidence interval (CI) for all sites combined are indicated in the table below:

Table 1. Overall Statistical Analysis results of the comparison study

Method	Nº of samples	Negative Percent Agreement (Lower 95% CI)	Nº of samples	Positive Percent Agreement (Lower 95% CI)
Tube	488	100% (99.39%)	2546	100% (99.88%)
Manual Gel (Operator Read)	143	100% (97.93%)	870	100% (99.66%)
Manual Gel (DG Reader)	143	98.60% (95.66%)	870	100% (99.66%)
Automated Gel (Erytra)	183	98.91% (96.60%)	834	100% (99.64%)
Automated Gel (Wadiana)	137	99.27% (96.58%)	883	99.89% (99.46%)
Automated Gel (Erytra Eflexis)	477	100% (99.37%)	2629	100% (99.89%)

Percent of Agreement only indicates agreement between the reagent and the comparator method and does not indicate which one gave the correct result(s).

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- Aramburu E., Rabasa P., Esquiroz R., Galarreta T. and Olcoz B. Valoración de un Antisuero Anti-D IgM-IgG monoclonal (DIAGAST) en donantes de sangre con expresividad débil del antígeno D. Hematology Congress, Madrid, October 1990.
- Mannessier L. The use of monoclonal Antibodies as blood grouping reagents: applications, advantages and problems. Congress of the Italian society for blood transfusion, Rome, June 1992.

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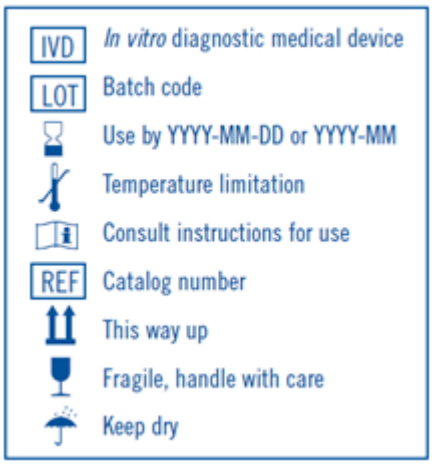
SYMBOLS KEY

One or more of the following 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