BLOOD GROUPING REAGENTS

Anti-D (RH1) IgG **REF 210524**

Anti-D (RH1) IgM **REF 210525**

(Human/Murine Monoclonal)

Neg Control **REF 210543**

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

These reagents are designed to determine the presence of the blood Rhesus antigen D (RH1) on the surface of human red blood cells by manual method.
The Neg Control (REF 210543) is used as a negative reagent control and has been designed to be tested under the same conditions and in parallel with DIAGAST Anti-D reagents (REF 210524, REF 210525 or REF 210526). The result of the Neg Control determination enables interpretation of the Rh(D) typing result obtained.

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 [1,2].

PRINCIPLE OF THE TEST

The manual technique employed in a tube, utilizes the principle of hemagglutination. Test red blood cells bearing an 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 IgG antibody.

REAGENTS

These reagents contain sodium azide (0.09%), sodium arsenite (0.02%) and bovine albumin. Any bovine materials used in the manufacture of these products are sourced from donor 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 human/murine heterohybridomas.

The Neg Control (REF 210543) produced by DIAGAST contains all components of the DIAGAST Anti-D reagents (REF 210524, REF 210525 or REF 210526) but not the antibodies.

These reagents are provided with calibrated droppers.

Code	Product Designation	Packaging
210524	Anti-D (RH1) IgG	5 x 10 mL
210525	Anti-D (RH1) IgM	5 x 10 mL
210543	Neg Control	5 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 to 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.
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.
Do not use blood specimens that exhibit contamination.

MATERIALS

Material provided:

- Anti-D (RH1) IgG (REF 210524): Monoclonal antibody. Anti-D IgG human/murine clone HM16.
- Anti-D (RH1) IgM (REF 210525): Monoclonal antibody. Anti-D IgM human/murine clone P3X61.

- The Neg Control (REF 210543) is devoid of antibodies.

Note: *All materials provided separately.*

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 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

Other required complementary reagents:

- Anti-Human Globulin Anti-IgG (such as REF 210547/REF 210549), only for indirect method
- Anti-D (RH1) IgM+IgG (REF 210526) (optional)

TEST PROCEDURES

- **For Anti-D (RH1) IgG (REF 210524) - Indirect Antiglobulin Weak D Test Method**

1. In a test 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 and incubate at 37°C ±1°C for 15 minutes.
5. Wash the red blood cells twice with isotonic saline solution and discard the remaining liquid from the last wash.
6. Using the vial dropper, add 1 drop of Anti-Human Globulin Anti-IgG to the red blood cell pellet.
7. Shake to mix, 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.

- **For Anti-D (RH1) IgM (REF 210525) - Direct agglutination method in tube at room temperature**

1. In a test 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 test 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.

RESULTS

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 (cf. 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 negative control.

With the direct hemagglutination tube method: if there is agglutination with Anti-D (RH1) IgM, antigen D is present. If there is no agglutination, it is possible to use Anti-D (RH1) IgM+IgG (REF 210526) or Anti-D (RH1) 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 (cf. Instructions for Use for the corresponding reagent).

QUALITY CONTROLS

The use of samples of guaranteed Rh(D) typing as control samples allows the user to detect anomalies with (handling, reagents, apparatus and the environment) and to implement corrective actions as required.
Known sample controls 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 each positive blood sample with DIAGAST Anti-D reagents (REF 210524 or REF 210525) a negative control should be performed. 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.

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 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.
- It is imperative to work with clean apparatus 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.

SPECIFIC PERFORMANCE CHARACTERISTICS

- These reagents meet FDA potency requirements for Blood Grouping 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 intensity of the reactions obtained with Anti-D (RH1) IgM may depend on the number of antigen sites present on the red blood cells.
- Anti-D (RH1) IgG enable screening for weak red blood cells D (RH1) in the indirect hemagglutination method with antiglobulin.
- 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 have the special feature of recognizing certain rare antigen motives of type RH33 (DHar) and may thus yield discordant reactions with polyclonal reagents that recognize them little or not at all.

- 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 –	+	+	+
HM16	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 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

Reagent	Nº of samples	Negative Percent Agreement (Lower 95% CI)	Nº of samples	Positive Percent Agreement (Lower 95% CI)
Anti-D IgM	496	100% (99.40%)	2538	100% (99.88%)
Anti-D IgG	488	100% (99.39%)	2546	100% (99.88%)

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.