ANTI-A (ABO1), ANTI-B (ABO2), ANTI-A,B (ABO3) ANTI-D (RH1) IgM I, ANTI-D (RH1) IgM II, ANTI-D (RH1) TOTEM, ANTI-D (RH1) IgG, ANTI-DCE (RH1,2,3), NEG CONTROL, GROUPAKIT

DIAGAST

INTRODUCTION

These reagents are in vitro diagnostic medical devices (IVDMD) for professional use.
The ANTI-A (ABO1), ANTI-B (ABO2), ANTI-A,B (ABO3) are used in the red blood cell determination of blood ABO group. They enable determination of the presence of erythrocytic antigens A and/or B on the surface of human red blood cells.
The ANTI-D (RH1) IgM I, ANTI-D (RH1) IgM II, ANTI-D (RH1) TOTEM and ANTI-D (RH1) IgG are used for blood grouping. They enable the determination of the presence of antigen D (RH1) on the surface of human red blood cells.
ANTI-DCE (RH1,2,3) enable detection of the presence of at least one of erythrocytic antigens: D (RH1), C (RH2) and E (RH3).
The NEG CONTROL is used in blood ABO grouping. It is devoid of antibody activity. Tested under the same conditions as the reagent used, the control enables interpretation of the result obtained.

PRINCIPLE

The manual technique employed, on a plate or 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. The reaction occurs in two stages: the test red blood cells are exposed to the IgG antibody. The antibodies bind to the red blood cells carrying the corresponding antigen. After washing, addition of antiglobulin “AGH MAESTRIA IGG” induces agglutination of the sensitized red blood cells carrying the corresponding antigen.

ABO group determination is defined by both the demonstration of antigens A and/or B on the surface of human red blood cells and by the presence or absence or anti-A and/or anti-B antibodies in the plasma. It is therefore appropriate to identify the erythrocytic antigens using known anti-A, anti-B and anti-A,B reagents (red blood cell test), then to confirm the preceding results by verifying the presence of the corresponding antibodies in the plasma from the test blood using known red blood cells A1, B and, possibly, A2 and O (plasma test).
For blood RH1 grouping, the use of ANTI-D reagent (RH1) and NEG CONTROL reagent is necessary.

COMPOSITION

The reagents are prepared from monoclonal antibodies in a storage medium. The monoclonal antibodies produced by DIAGAST are derived from the supernatants of in vitro cultures of hybridomas of murine or human origin.
These reagents contain sodium azide (< 0.1 %), sodium arsenite (0.02 %) and bovine albumin.
The reagents are packaged in vials fitted with a calibrated dropper and are also included in the GROUPAKIT kit.
The **GROUPAKIT** kit (Ref. DIAGAST **70888**) consists of a vial of ANTI-A (ABO1), a vial of ANTI-B (ABO2), a vial of ANTI-A,B (ABO3), a vial of ANTI-D (RH1) IgM I and a vial of NEG CONTROL.
The **NEG CONTROL** produced by DIAGAST is devoid of antibodies.
Refer to the last page for a description of the reagents.

PRECAUTIONS

It is advisable to wear gloves and safety spectacles and handle test samples of human origin with caution. All substrates that have come into contact with the samples are to be handled as potentially infectious products. Special protective measures, conditions for disposal and disinfection should be implemented in accordance with local regulations.
Do not use damaged or leaking reagents.

STORAGE

The reagents must be stored between + 2 °C and + 8 °C. Their performance is guaranteed in the method recommended from first use to the expiry date indicated on the label. It must not be used beyond that date. It is advisable to minimize its time outside the refrigerator and to avoid leaving it at room temperature between two uses.

REAGENTS AND MATERIAL NECESSARY

- Isotonic saline solution (0.9 % NaCl).
- Incubator or water-bath at 37°C.
- Glass test tubes, 10 or 12 x 75 mm, tube rack.
- Mechanical stirrer.
- Opaline plate.
- Precision automatic adjustable pipettes.
- Centrifuge with a relative force of 100-1200 g.
- Control blood samples of known phenotypes such as HEMA CQI (DIAGAST ref.: see the catalogue).
- 'AGH MAESTRIA IGG' antiglobulins (DIAGAST ref.: see the catalogue).
- IgG-sensitized red blood cells
- Partial D identification kit for research use: D-SCREEN (DIAGAST ref.: see the catalogue).

SAMPLES – CONTROLS

Blood samples to be tested

Blood collected in anticoagulant: EDTA, heparin or citrate in a stoppered sterile tube, stored between 2 and 8°C, must be examined within 48 hours, insofar as no sign of hemolysis is visible.
At the time of the test, centrifuge the blood sample at 1200 g for 3 minutes.

Blood samples with known phenotypes: HEMA CQI

The analytical system must be validated using samples with known phenotypes:
- 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.

The use of those samples or HEMA CQI enables detection of anomalies (handling, reagents, apparatus and environment) and implementation of corrective actions.

Reagent control

For each sample, in RH1 group determination, a control reagent is tested under the same conditions by replacing ANTI-D (RH1) by **NEG CONTROL**.
In the event of an anomaly in blood ABO group determination, a control reagent is tested under the same conditions by replacing the ABO grouping reagent by **NEG CONTROL**.

PROCEDURE

a) Plate technique at room temperature (+ 18… + 25 °C) except for ANTI-D (RH1) IgG

- On a rigorously clean plate, using the vial dropper, apply 1 drop of reagent.
- Take 25 µL of unwashed cell pellet and apply next to each drop of reagent, taking care not to create contact between the drops.
- Mix the blood and reagent using a spiral movement with the end of the stirrer so as to create a regular lozenge of diameter 2 to 3 cm.
- Incubate the plate at room temperature and without stirring for 30 seconds.
- Hold the plate and give it a rolling movement for 3 minutes while macroscopically observing the possible appearance of agglutinates.
- Read the reaction immediately.

b) Direct method in a tube at room temperature except for ANTI-D (RH1) IgG

- Prepare a 5 % suspension of red blood cells in isotonic saline solution.
- Using the vial dropper, transfer a drop of reagent to a tube.
- Add 50 µL of red blood cell suspension.
- Shake to homogenize the mixture, then centrifuge at 500 g for 1 minute.
- Read macroscopically while gently shaking the tubes so as to detach the red blood cell pellet.
- Note the appearance of any agglutinates.

c) Antiglobulin indirect method for ANTI-D (RH1) TOTEM only

- After immediately centrifuging and reading as above, if the reaction is weak or negative, shake the tubes and incubate at 37 °C for 15 minutes.
- Wash the red blood cells twice with isotonic saline solution and discard the last washing liquid.
- Add 50 µL of “AGH MAESTRIA IGG” antiglobulin to the red blood cell pellet. Mix, then centrifuge at 120 g for 1 minute.
- Conduct reading as indicated in section b).

d) Antiglobulin indirect method for ANTI-D (RH1) IgG only

- Prepare a 5 % suspension red blood cells in isotonic saline solution.
- Using the vial dropper, transfer 1 drop of reagent to a tube.
- Add 50 µL of red blood cell suspension.
- Shake the tubes to homogenize the mixture and incubate at 37 °C for 15 minutes.
- Wash the red blood cells twice with isotonic saline solution and discard the last washing liquid.
- Add 50 µL of “AGH MAESTRIA IGG” antiglobulin to the red blood cell pellet. Mix, then centrifuge at 120 g for 1 minute.
- Conduct reading as indicated in section b).

INTERPRETATION

- If there is agglutination (the red blood cells form one or several clumps), the reaction is positive and the antigen or at least one of the antigens corresponding to the reagent used is present on the red blood cells tested. If there is no agglutination (the red blood cells reform a homogeneous suspension), the reaction is negative and the antigen is not present on the red blood cells.
- The ABO group of a subject can only unambiguously be determined if there is strict concordance between the results of the red blood cell test and those of the plasma test.
  If there is discordance, do not report the result and pursue blood group identification in compliance with current recommendations and protocols or forward the sample to an expert laboratory.
  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 *vis-à-vis* his own red blood cells.
  “Allo” control: under the same conditions, test the subject's plasma *vis-à-vis* 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 *vis-à-vis* the negative control.
- With the direct hemagglutination method on a plate or in a tube: if there is agglutination with ANTI-D (RH1) IgM or TOTEM, antigen D is present. If there is no agglutination, it is possible to use ANTI-D (RH1) TOTEM or ANTI-D (RH1) IgG in an indirect antiglobulin test if weak and/or partial antigens D are to be detected.
- A negative reaction obtained in an indirect antiglobulin test can be validated with IgG-sensitized red blood cells (cf. leaflet for the corresponding reagent).
- The reaction is only interpretable if:
  - the reagent control using the NEG CONTROL is negative,
  - the analytical system is validated using samples with known phenotypes.
- Furthermore, the reaction in indirect antiglobulin test is interpretable only if the direct antiglobulin test on the test red blood cells is negative.

LIMITATIONS OF THE METHOD

- Only qualified personnel should use the reagent.
- It is imperative to use the calibrated dropper provided in the IVDMD vial to dispense a reagent drop.
- The reactions must be read immediately after centrifuging and resuspending.
- It is imperative to work with clean equipment and non-contaminated products (bacterial or other contamination).
- The following points must be scrupulously observed:
  - storage conditions and expiry date,
  - procedures,
  - calibration of the recommended equipment.
- Weak phenotypes A and/or B may not be detected by the plate method since it is less sensitive than the tube method. In consequence, in the event of discordance between the red blood cell test and plasma test and since a weak phenotype may be present, the test should be repeated with the more sensitive method.
- It is imperative to use AGH MAESTRIA IGG for the indirect antiglobulin test.
- It is imperative to use NEG CONTROL as the negative control.
- ANTI-D (RH1) must not be used in methods involving enzymatic treatment of the red blood cells.
- ANTI-D (RH1) IgM cannot be used in the indirect antiglobulin test.
- Certain discordances (negative reaction for the direct hemagglutination method and positive reaction for the indirect antiglobulin method) may occur with ANTI-D (RH1) TOTEM. A weak and/or partial antigen D may be present.
- A false-positive reaction may occur:
  - when the reagent is used for the direct hemagglutination method with a subject who has cold agglutinins,
  - when the reagent is used in the indirect antiglobulin test with the red blood cells which present a positive reaction in the direct antiglobulin test.

  These possibilities constitute the rationale for concomitant use of NEG CONTROL.

PERFORMANCE DATA

- In the recommended methods, these reagents comply with the Common Technical Specifications of IVDMD.
- A performance assessment of ANTI-A (ABO1), ANTI-B (ABO2) and ANTI-A,B (ABO3) was conducted on over 15,000 all-comer samples (blood donors, patients and neonates) drawn on each of the recommended anticoagulants (EDTA, heparin, citrate). The assessment demonstrated 100 % specificity of each of the reagents versus the expected results vis-à-vis common known phenotypes A1, A2, A1B, A2B, B and O.
  The tests conducted on particular red blood cells of weak phenotype ABO showed good specificity *vis-à-vis* phenotypes A3 and B3.
- ANTI-A,B (ABO3) recognizes red blood cells Ax.
- ANTI-B (ABO2) does not agglutinate the “acquired B” red blood cells tested.
- In certain cases (transfusion recipients, certain weak phenotypes A or B (A3, B3...), certain hemopathological modifications, mosaics or chimeras, etc.), an image of a double population may be observed.
- Antibody ANTI-A and, accessorily, antibody ANTI-A,B yield a cross-reaction with antigen Tn which gives rise to an image of a double population (exceptional phenomenon).
- A performance assessment of ANTI-D (RH1) IgM I, IgM II, IgG, TOTEM and ANTI-DCE (RH1,2,3) was conducted on a panel of 1,000 to 200,000 all-comer samples (blood donors, patients and neonates). The samples were collected on each of the recommended anticoagulants (EDTA, heparin, citrate). The assessment demonstrated 100 % specificity of each of the reagents versus the expected results vis-à-vis known common Rhesus phenotypes.
- 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) TOTEM and 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 I and TOTEM 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.
- Only ANTI-D (RH1) TOTEM may enable detection of D partial DVI in the tube direct hemagglutination method.
- In addition, the clones of ANTI-D may specifically recognize certain epitopes of antigen D (cf. table on the last page).
- In general, for the identification of partial D, the use of the D-SCREEN kit is recommended.

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- MANNESSIER L. Blood Transfusion Centre, Lille, France. The use of monoclonal antibodies as blood grouping reagents: applications, advantages and problems. Congress of the Italian society for blood transfusion, Rome, June 1992.

REAGENT		REFERENCE	CLONE	TYPE	ORIGIN
ANTI-A (ABO1)	5 x 10 ml	70501	9113D10	IgM	Murine
	100 x 10 ml	70540			
ANTI-B (ABO2)	5 x 10 ml	70502	9621A8	IgM	Murine
	100 x 10 ml	70541			
ANTI-A,B (ABO3)	5 x 10 ml	70503	9113D10 + 152D12	IgM	Murine
	100 x 10 ml	70542			
ANTI-D (RH1) IgM I	5 x 10 ml	71000	P3X61	IgM	Human
	100 x 10 ml	70543			
ANTI-D (RH1) IgM II	5 x 10 ml	71005	HM10	IgM	Human
ANTI-D (RH1) TOTEM	5 x 10 ml	71010	P3X61 + P3X21223B10 + P3X290 + P3X35	IgM IgM IgG IgG	Human
	100 x 10 ml	70544			
ANTI-D (RH1) IgG	5 x 10 ml	71020	HM16	IgG	Human
ANTI-DCE (RH1,2,3)	5 x 5 ml	74111	P3X61 + P3X25513G8 + P3X234	IgM	Human
NEG CONTROL	5 x 10 ml	79000			
	100 x 10 ml	70545			

Clones	Type	DII	DIIIa DIIIb DIIIc	DIVa	DIVb	DVa	DVI	DVII	DFR	DBT	DHAR	DHMi
P3X61	IgM	+	+	+	+	+	–	+	+/–	+	+	+
HM10	IgM	+	+	+	+	+	–	+	–	+	–	+
HM16	IgG	+	+	+	+	+	–	+	–	+	+	+
P3X21223B10	IgM	–	+	–	–	+	+	+	+	–	–	+
P3X290	IgG	+	+	+/–	–	+	+/–	+	+	–	–	+
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.

+/- indicates that a positive or negative result may be obtained. The result depends on the antigenicity.

REVISION HISTORY

Description of the change	Impact on the Verification of method according to standard NF EN ISO 15189
§ PERFORMANCE DATA : Correction of the term “indirect hemagglutination” by “direct hemagglutination” for the detection of DVI in the Spanish version.	No

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0459

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