DIAGAST

		REF	CLONE
ANTI-Fya (FY1)	3 ml	**78014**	DG-FYA-02
ANTI-S (MNS3)	3 ml	**78007**	P3S13JS123
ANTI-s (MNS4)	3 ml	**78008**	P3YAN3
ANTI-Fyb (FY2)	3 ml	**78112**	
ANTI-k (KEL2)	3 ml	**78016**	P3A118OL67
POLY CONTROL	10 ml	**78200**	

INTRODUCTION

These reagents are in vitro diagnostic medical devices (IVDMD) for professional use. They are intended for the analysis of human biological specimens. The reagents are used in the phenotyping of human red blood cells. They determine the presence of antigens Fy^a (FY1), Fy^b (FY2), S (MNS3), s (MNS4) and k (KEL2) on the surface of red blood cells.
POLY CONTROL is devoid of all antibody activity. It is tested under the same conditions as the polyclonal reagent so that the result obtained is validated.

PRINCIPLE

The manual tube method used is based on the principle of haemagglutination. The red blood cells for testing express an antigen which indirectly cause agglutination in the presence of the corresponding antibodies contained in the reagent (indirect haemagglutination method : antiglobulin test).
The reaction is conducted in two stages. The red blood cells for testing are exposed to the antibody. The antibodies bind to the red blood cells bearing the corresponding antigen. After washing, addition of 'AGH MAESTRIA IGG' anti-IgG antiglobulin or 'AGH MAESTRIA IGG+C3D' polyspecific antiglobulin (anti-IgG + anti-C3d) induces agglutination of the sensitised red blood cells bearing the corresponding antigen.

COMPOSITION

ANTI-Fya (FY1), ANTI-S (MNS3), ANTI-s (MNS4) and k (KEL2) are prepared from red cell monoclonal antibodies and are presented in a storage medium. These monoclonal antibodies, of type IgG, are derived from the in vitro culture supernatant of hybridomas of human origin.
ANTI-Fyb (FY2) is polyclonal reagent prepared from human sera.
POLY CONTROL, prepared from human sera of group AB, is devoid of antibodies.
All the reagents contain sodium azide (< 0.1%). All the reagents except ANTI-Fyb (FY2) and ANTI-k (KEL2) contain sodium arsenite (0.02 %). ANTI-Fya (FY1), ANTI-Fyb (FY2) and ANTI-k (KEL2) contain bovine albumin.
ANTI-k (KEL2) contains plasma from human origin and Proclin (0.05%).

The reagents are packaged in vials fitted with calibrated droppers.

PRECAUTIONS

WARNING (Only for ANTI-k (KEL2) containing Proclin 0.05%)
Particulars of danger **:**
H317 May cause an allergic skin reaction.
Advice of caution **:**
P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P272 Contaminated work clothing should not be allowed out of the workplace.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P302+P352 IF ON SKIN: Wash with plenty of water.
P501 Dispose of contents/container in accordance with the local regulations, regional, national and international.

ANTI-Fyb (FY2) and POLY CONTROL have been tested and found negative for anti-HIV 1,anti-HIV 2, and anti-hepatitis C antibodies, and HBs antigen, but nonetheless must be handled as potentially infectious products.
It is advisable to wear gloves and safety spectacles and handle samples of human origin with caution. 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 compliance with local regulations. Do not use damaged or leaking reagents.

STORAGE

All the reagents except ANTI-Fyb (FY2) are to be stored between +2°C and +8°C. ANTI-Fyb (FY2) is to be stored between –18°C and –30°C. Do not refreeze a thawed reagent. After thawing, ANTI-Fyb (FY2) can be stored between +2 ºC and +8 ºC for up to 6 months.
The performance of the reagents is guaranteed in the recommended methods from first use to the expiry date indicated on the label. The reagents must not be used after the expiry date. It is advisable to minimise their time outside the refrigerator and to avoid leaving them at room temperature between uses.

REAGENTS AND MATERIAL NECESSARY

- Plastic and glass test tubes, tube rack.
- Automatic pipettes with adjustable precision.
- Centrifuge of relative force from 100 to 1200 g.
- Incubator or water-bath at 37°C.
- Isotonic saline solution (0.9 % NaCl).
- AGH MAESTRIA IGG or AGH MAESTRIA IGG+C3D antiglobulin (DIAGAST ref.: see catalogue).
- LISS (DIAGAST ref.: see catalogue).
- Negative controls : NEG CONTROL and POLY CONTROL (DIAGAST ref.: see catalogue).
- Positive control blood samples of guaranteed phenotype are required expressing the corresponding heterozygous antigen. Similarly for a negative control, blood samples should be used which lack the antigen corresponding to the reagent used.

SAMPLES – CONTROLS

TEST BLOOD SAMPLES
The blood sample, collected in EDTA heparin or citrate anticoagulant, in a stoppered sterile tube, and stored between +2°C and +8°C, should be tested within 72 hours, providing that no haemolysis is visible.
At the time of testing, centrifuge the blood sample at 1200 g for 3 minutes.

BLOOD SAMPLES OF GUARANTEED PHENOTYPES
The use of control samples allows the user to detect anomalies with (handling, reagents, apparatus and the environment) and to implement corrective actions as required.
The analytical system should be validated using control samples of guaranteed phenotype:
- sample possessing the antigen corresponding to the antibody contained in the reagent used,
- sample not possessing the antigen corresponding to the antibody contained in the reagent used.

CONTROLS
For each blood sample to be tested, a reagent control is used and tested under the same conditions replacing:
- the monoclonal reagents by the NEG CONTROL,
- the polyclonal reagents by the POLY CONTROL.

PROCEDURE

- In a plastic tube, prepare a 5 % unwashed red blood cell suspension in LISS.
- Using the vial dropper, transfer 1 drop of reagent to a glass tube.
- Add 50 µL of erythrocyte suspension.
- Shake the tubes to mix and incubate at +37°C for 10 minutes.
- Wash the red blood cells with isotonic saline solution 3 times and discard the remaining liquid from the last wash.
- Using the vial dropper, add 1 drop of 'AGH MAESTRIA IGG' or 'AGH MAESTRIA IGG+C3D' antiglobulin to the red blood cell pellet.
- Shake the tubes to mix. Centrifuge at 120 g for 1 minute.
- Immediately read macroscopically, swirling the tubes gently to detach the erythrocyte pellet.
- Report any agglutinates.

INTERPRETATION

If there is agglutination (the red blood cells form one or several clumps), the reaction is positive and the antigen corresponding to the reagent used is present on the red blood cells.
If there is no agglutination (the red blood cells go back into homogeneous suspension), the reaction is negative and the antigen is absent from the red blood cells.
The reaction is only validated if:
- the result of the NEG CONTROL or POLY CONTROL mixed with the subject's red blood cells is negative,
- the analytical system has been validated using samples of guaranteed phenotypes,
- the direct antiglobulin test on the red blood cells is negative.

LIMITATIONS OF THE METHOD

Only suitably qualified personnel should use the reagent.
It is imperative to use the calibrated dropper of the IVDMD vial to transfer 1 drop of reagent.
Only use the complementary reagents cited in the section entitled 'Reagents and materials necessary'.
These devices are not to be used in an enzymatic method.
The reactions are to be read immediately after centrifuging and resuspension.
It is imperative to work with clean apparatus and uncontaminated products (bacterial or other contamination).
Strict compliance with the following is required:

- storage conditions and expiry date,
- the procedure indicated above,
- equipment calibration is recommended.

No reagent can guarantee the detection of all the antigenic profiles rare, weak or variants.

PERFORMANCE DATA

A performance assessment of the ANTI-Fya (FY1), ANTI-S (MNS3) and ANTI-s (MNS4) was conducted on a random samples panel of known common phenotypes including clinical and neonatal samples. The samples were drawn in the recommended anticoagulants (E.D.T.A., heparin, citrate). The expert assessment demonstrated 100 % specificity for each of the reagents with respect to the expected results.
A performance assessment of the ANTI-Fyb (FY2) was conducted on a random samples panel of known common phenotypes including clinical and neonatal samples. The samples were drawn in the recommended anticoagulants (E.D.T.A., heparin and citrate). The expert assessment demonstrated 98.4 % specificity for the reagent with respect to the gel filtration technique.
A performance assessment of the ANTI-k (KEL2) using the recommended associated reagents and materials, was conducted on a random panel of 294 samples (donors, clinical and newborn samples). These studies have shown 100% of concordance comparing to awaited results of the reference method.

Each batch of reagent is submitted to a strict internal quality control in order to ensure a constant quality.
However, ANTI-Fyb (FY2) and POLY CONTROL can contain antibodies specific for the extremely rare "private antigen", as can be the case with polyclonal reagents.
The device performance is guaranteed only if they are used in the proposed technique and reagents to be used in combination mentioned in this technical insert (ex : AGH MAESTRIA IGG).
Use and validation of other reagents used in combination with the devices, other than those indicated in paragraph titled "Reagents and material necessary", is possible but only on the user's responsibility.

DIAGAST denies all responsibility in cases where the devices are not used in accordance with this leaflet.

REVISION HISTORY

Description of the change	Impact on the Verification of method according to standard NF EN ISO 15189
§ « Precautions » : Sentences of risks according to CLP regulation N°1272/2008 only for ANTI-k (KEL2)	No

CE 0459

The identifier, "0459", of the notified organisation (LNE/G-MED) which validated the conformity evaluation procedure, cited in appendix IV of Directive 98/79/EC, is only applicable to the ANTI-Fya (FY1), ANTI-Fyb (FY2) and POLY CONTROL reagents. The ANTI-Fya (FY1), ANTI-Fyb (FY2) and POLY CONTROL antibodies are included in list B of appendix II to that directive.

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