AGH MAESTRIA IGG+C3D
AGH MAESTRIA IGG
AGH MAESTRIA C3D

DIAGAST

INTRODUCTION

The reagents are in vitro diagnostic medical devices (IVDMD) for professional use. They are intended for the analysis of human biological specimens.
AGH MAESTRIA IGG+C3D and AGH MAESTRIA IGG are used for erythrocyte phenotyping with blood phenotyping reagents, in testing for immune antibodies requiring an indirect antiglobulin test method.
AGH MAESTRIA IGG+C3D, AGH MAESTRIA IGG and AGH MAESTRIA C3D are used in the direct antiglobulin test (direct Coombs' test) on human red blood cells.

- **AGH MAESTRIA IGG** recognizes IgG antibodies.
- **AGH MAESTRIA C3D** only recognizes the complement fragment C3d and, consequently, cannot react with component C4.
- **AGH MAESTRIA IGG+C3D**: in addition to recognizing IgG antibodies, is able to recognize (like AGH MAESTRIA 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).

PRINCIPLE AND OVERVIEW

- The direct and indirect antiglobulin test methods are based on the principle of haemagglutination. 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).
- 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 (AGH MAESTRIA IGG+C3D and AGH MAESTRIA C3D).
- **The direct antiglobulin test** detects sensitisation of the red blood cells in vivo either by autoantibodies (Autoimmune Haemolytic Anaemia) or by alloantibodies (screening for Haemolytic Disease of The Newborn, transfusion incompatibility, diagnosis of drug-induced immunoallergic haemolytic 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.
  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, AGH MAESTRIA IGG+C3D and AGH MAESTRIA IGG is added, inducing agglutination of the sensitised red blood cells carrying the corresponding antigen.

COMPOSITION

The reagents are prepared from monoclonal antibodies in a storage medium. The monoclonal antibodies are derived from the supernatants of in vitro cultures of hybridomas of murine origin.
These reagents contain sodium azide (< 0.1 %), sodium arsenite (0.02 %) and bovine albumin.
The reagents are packaged in kits containing 4 vials fitted with calibrated droppers.

REFERENCE	PACKAGING	LABEL	CLONES
76318	4 x 10 mL	**AGH MAESTRIA IGG+C3D**	18833 + 18896 + 12011D10
76218	4 x 10 mL	**AGH MAESTRIA IGG**	18833 + 18896
76118	4 x 5 mL	**AGH MAESTRIA C3D**	12011D10

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PRECAUTIONS

It is advisable to wear gloves and safety spectacles, and handle 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 and conditions for disposal and disinfection should be implemented in accordance with local regulations.
Do not use damaged or leaking reagents.

STORAGE

The reagents are to be stored between +2 °C…+8 °C. Their performances are 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 minimize their time outside the refrigerator and to avoid leaving them at room temperature between use.

REAGENTS AND MATERIALS NECESSARY

- Isotonic saline solution (0.9 % NaCl).
- Incubator or water-bath at +37 °C.
- 10 or 12 x 75 mm glass tubes, tube rack.
- Automatic pipettes with adjustable precision.
- Centrifuge with a relative force of 100-1200 g.
- IgG- and/or complement- sensitised red blood cells.
- Negative controls suitable for direct antiglobulin test
- Internal Quality Controls suitable for associated devices for direct antiglobulin test (red blood cells panels for antibody screening and phenotyping reagents)

SAMPLES – CONTROLS

Blood collected in anticoagulant EDTA, heparin or citrate anticoagulant, in a stoppered sterile tube, and stored between +2 °C and +8 °C, should be tested as soon as possible after collection and within 48 hours providing that no haemolysis is visible.
At the time of testing, centrifuge the blood sample at 1200 g for 3 minutes.

PROCEDURE

Direct antiglobulin test (direct 'Coombs' test)

- Wash the test red blood cells 3 times with isotonic saline and prepare a 5 % suspension (volume/volume) in isotonic saline solution.
- Transfer 50 µL of the suspension and 2 drops of reagent to a tube, using the vial dropper.
  If using AGH MAESTRIA C3D, allow the tube to incubate at room temperature (+18… +25 °C) for 5 minutes.
- Gently shake the tubes to homogenise the mixture.
- Centrifuge at 120 g for 1 minute.
- Read macroscopically, gently shaking the tubes so as to detach the erythrocyte pellet.

The analytical system should be validated using red blood cells sensitised 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.

Indirect antiglobulin test (indirect 'Coombs' test)

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

INTERPRETATION

Reaction validation

In order to validate negative reactions, add IgG- and/or possibly complement-sensitised red blood cells (c.f. leaflets on the corresponding reagents).

- if the reaction is positive, the activity of AGH MAESTRIA IGG+C3D, AGH MAESTRIA IGG and AGH MAESTRIA C3D is confirmed and the negative reaction prior to addition of the sensitised red blood cells is validated.
- if the reaction is negative, the antiglobulin test and validation are to be repeated.

Direct antiglobulin test

- 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 AGH MAESTRIA IGG+C3D, AGH MAESTRIA IGG and AGH MAESTRIA C3D, is a positive reaction indicating that the red blood cells have been sensitised 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 AGH MAESTRIA IGG and AGH MAESTRIA C3D and the appropriate controls.

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

LIMITATIONS OF THE METHOD

- Only suitably qualified personnel should use the reagent.
- It is obligatory to use the calibrated dropper of the IVDMD vial to transfer the reagent.
- False negatives may be observed if the red blood cells are not sufficiently washed.
- It is necessary to validate the negative reactions with IgG- and/or complement-sensitised red blood cells.
- In the event of Haemolytic Disease of The Newborn, the direct antiglobulin test (or direct 'Coombs' test) may be negative, particularly in the event of ABO incompatibility.
- It is essential to work with clean equipment and uncontaminated products (bacterial or other contamination).
- Strict compliance with the following is required:
  - storage conditions and expiry date,
  - procedures for use,
  - calibration, preventive maintenance and maintenance of the recommended equipment.

PERFORMANCE

- The specificity studies of the anti-IgG fractions of AGH MAESTRIA IGG+C3D and AGH MAESTRIA IGG vis-à-vis human immunoglobulin G, conducted by the haemagglutination 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 AGH MAESTRIA C3D, vis-à-vis human red blood cells labeled or not labeled with various fragments of human complement or red cell antibodies demonstrated perfect specificity of the reagent. AGH MAESTRIA 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 evaluation of the performances of AGH MAESTRIA IGG+C3D and AGH MAESTRIA IGG with respect to screening and identifying immune antibodies using the indirect antiglobulin tube test, conducted on 15000 random serum and plasma samples (blood donors, patients, pregnant women), which included 18.40 % positive samples, showed 99.97 % reaction specificity relative to the reference polyclonal anti-human globulin.
- The tests, conducted on a panel of 304 samples from donors and patients of which 49 (16.12 %) were known to be positive for the direct antiglobulin test (IgG or C3d), showed 100 % reaction specificity relative to the reference polyspecific polyclonal anti-human globulin.
- Over 500 tests were conducted on a panel of random samples (blood donors, patients and neonates) drawn on the recommended anticoagulants using the DIAGAST IVDMD (ANTI-D (RH1) IgG, ANTI-D (RH1) TOTEM...) intended for erythrocytic Rhesus D (RH1) phenotyping in the indirect antiglobulin test and combined with AGH MAESTRIA IGG. These tests showed 100 % reaction specificity vis-à-vis common Rhesus D (RH1) phenotypes.

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