



ATLAS ANTI-HUMAN GLOBULIN (CLEAR OR GREEN)

Polyspecific Blended Rabbit Anti-Human IgG and Murine Monoclonal Anti-Human C3d for the direct and indirect antiglobulin tests

For *In-Vitro* and professional use only
Store at 2° to 8°C

INTRODUCTION & PRINCIPLES

ATLAS Anti-Human Globulin (AHG) is prepared from serum of rabbits immunized with purified human IgG to provide the Anti-IgG. Non-specific activity in the rabbit serum is absorbed and removed. The anti-IgG is then blended with anti-C3d (BRIC 8) which is an IgM antibody derived from spleen cells of an immunized mouse. The blend is maintained in a bovine albumin based buffered solution producing the clear version. Green AHG is made by adding eriochlorine and naphthol yellow S to the blend. The test procedure is based on the agglutination principle, where human immunoglobulins and/or complement attached to the red cell surface agglutinates in the presence of the polyspecific AHG indicating a positive result. The test is considered negative when no agglutination appears indicating the presence of only un-sensitized cells. In order to get rid of unbound globulins, the red cells are washed thoroughly with buffer salines during the test. The test is mainly intended for use in tube antiglobulin tests. There are two methods of testing. The direct method, which is used to demonstrate *in-vivo* sensitization of cells from patients or donors. The indirect method is used to demonstrate *in-vitro*

sensitization of cells, tests include antibody detection and identification, compatibility testing, the D^u test and other antigen detection tests. The indirect method is characterized by the incubation phase before the addition of antiglobulin. Red cells shown to be positive with the direct method should not be used in the indirect method. Both methods are outlined below.

PRECAUTIONS

1. This reagent is derived from animal sources, thus, appropriate care must be taken in the use and disposal of this reagent, as there are no known test methods that can guarantee absence of infectious agents.
2. The reagent should be stored refrigerated between 2° to 8°C. Never Freeze or expose to elevated temperature.
3. Do not use the reagent if it is marked with turbidity as this may indicate reagent deterioration or contamination.
4. The reagent contain 0.1% Sodium Azide which is toxic and can be absorbed through the skin. When drained, the drains should be thoroughly flushed with water.
5. The reagents should be used as supplied and in accordance to the procedure mentioned below. Do not use beyond expiration date.

PREPARING THE SPECIMEN

Any acceptable phlebotomy technique can be used to draw specimens. For direct test procedure, specimens should be drawn into EDTA, ACD, CPD or CPDA-1 and tested within 24 hours. Clotted samples are not recommended and results should be interpreted with caution when using clotted samples. For indirect procedures, clotted specimen obtained from fresh serum should be used. In order to avoid false results due to improper storage and handling, testing should be performed as soon as possible. If testing is delayed samples should be stored at 2° to 8°C and tested within two days provided there is no evidence of haemolysis or it should be stored frozen. In this case red cells should be removed from the serum.

MATERIALS PROVIDED

ATLAS Anti-Human Globulin Clear or Green reagent.

MATERIALS NEEDED BUT NOT PROVIDED

Test tubes, isotonic buffered saline (pH 6.9), centrifuge (900-1000 RCF), incubator, and timer.

DIRECT PROCEDURE

- wash the test red blood cells 3 times with isotonic saline (NaCl 0.9%) and prepare a 5% suspension (v/v) in isotonic saline solution (NaCl 0.9%).
- Transfer 50 µl of the suspension and 100 ml of the product to a tube.
- If using AHG anti-C3D, allow the tube to incubate at room temperature 18- 25 for 5 minutes.
- gently shake the tubes to homogenise the mixture.
- centrifuge at 120 g for 1 minute.
- read macroscopically, gently shake the tubes so as to detach the erythrocyte pellet.

INDIRECT PROCEDURE

- Prepare a 5% suspension of 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 37C for 15 minutes .
- Wash the red blood cells twice with isotonic saline solution and discard the last washing liquid .
- Add 50 µl of anti-Human Globulin to the red blood cell pellet . Mix, then centrifuge at 120 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.

READING THE RESULT

POSITIVE: If Agglutination appears.

NEGATIVE: If no agglutination is observed.

Positive results indicate the presence of human IgG or components of complement on the red blood cells. In the direct test, a negative result does not necessarily rule out HDN especially where ABO incompatibility is suspected. In the indirect test, false negative results have been noticed when washing with isotonic saline that have been prepared in sterile plastic bags designed primarily for irrigation and I.V. use or washing with solutions intended for cell counting and sizing experiments.

All negative or weak positive results must be confirmed by using IgG sensitized red cells (e.g. Coombs Control Cells).

PROCEDURE LIMITATION

1. False positive/ negative results may occur from contamination from test materials, improper storage of test materials, improper washing, improper incubation time or temperature, delayed reading of results, or any deviation from the recommended test procedures.
2. Improper centrifugation may also cause false results. The mentioned centrifugation times should be regarded as suggestions only. An appropriate centrifugation time should be determined by that which produces the strongest reaction of anti-body with antigen-positive cells, yet allows easy re-suspension of antigen-negative red cells.
3. To confirm the reactivity and specificity of ATLAS AHG, it is recommended that IgG sensitized and unsensitized red cells be tested with the reagent on each day of use.

SENSITIVITY

ATLAS AHG reagents are checked prior to release for their reactivity to ensure that it contains enough levels of anti-bodies to meet/exceed the standards of major world regulatory authorities. These checks includes performing the above mentioned procedures with patient red cells sensitized with IgG, C3 and a variety blood group antibodies such as anti-Fy^a, anti-Fy^b, anti-Jk^a, anti-Kell, anti-M and anti-S.

REFERENCES

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