ACCUCARE™

DIRECT AND INDIRECT COOMB'S TESTS
Blood Grouping

Qualitative test for determination of human anti-IgG and anti-C3 on red blood cells. For In Vitro Diagnostic Test use only.

ORDER INFORMATION

REF	Pack Size
AHG 10	1 X 10mL
AHG 100	10 X 10 mL
AHG 1000	1 X 1000mL

CLINICAL SIGNIFICANCE

In 1945, Coombs, Mourant and Race described the use of anti-human globulin serum for detecting red cell-bound non-agglutinating antibodies. In 1957, Dacie et al showed that the antibodies present in antiglobulin sera were directed against certain components of complement.

Antihuman globulin reagents detect non-agglutinating antibody molecules as well as molecules of complement attached to red cells following *in vivo* or *in vitro* antigen-antibody reactions. Accordingly, Anti-Human Globulin is used for compatibility testing, antibody detection, antibody identification, testing for the variant of the Rho (D) antigen (D^U tests), and umbilical cord red blood cell testing.

METHOD

Hemagglutination technique.

PRINCIPLE

The procedures used with this reagent are based on the principle of heteroagglutinins directed against components of human serum as originally described by Moreschin and agglutination as described by Landsteiner. Normal human red blood cells, in the presence of antibody directed toward an antigen they possess, may become sensitize but fail to agglutinate due to the particular nature of the antigen and antibody involved. Anti-Human Serum will react with immunoglobulins and/or complement attached to the red cell surface, resulting in agglutination (clumping) of adjacent sensitized cells. Cells not sensitized will not be agglutinated (See **Limitations**).

REAGENT

Anti-Human Globulin Elite Green reagents contain anti-IgG derived from rabbits with nonspecific activity removed by absorption and mouse monoclonal IgM anti-C3d, Clone BRIC-8. The antibodies are diluted in a buffered solution containing bovine albumin. Each reagent is supplied at optimal dilution, for use with all the recommended techniques stated below without need for further dilution or addition.

Reagent	Colour	Dye Used
Coomb's Sera (AHG)	Green	Patent Blue + Tartrazine

REAGENT PREPARATION

The reagent supplied is ready to use. Protect from Bright Light.

REAGENT STORAGE AND STABILITY

This product will be well-preserved within utility limit till the expiry date, if stored at temperature between +2°C and +8°C.

Caution: Do not freeze.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- If the reagent vial is cracked or leaking, discard the contents immediately.
- Do not use the reagents if a precipitate is present.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagents have been filtered through a 0.2µm capsule to reduce the bio-burden. Once a vial has been opened the contents should remain viable up until the expiry date as long as there is no marked turbidity, which can indicate reagent deterioration or contamination.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- Consider Blood specimen as potentially infectious, handle and dispose it as per national applicable guideline.
- For information on disposal of the reagent and decontamination of a spillage site see **Material Safety Data Sheets**, available on request.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- Test tubes (8X50mm),
- Pipettes,
- Centrifuge
- (0.9% NaCl) saline.
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

- Blood should be drawn by an aseptic technique with an anticoagulant. The specimen should be tested as soon as possible after collection.
- If delay in testing should occur, the specimen must be stored at 2°C to 8°C. Bacterial contamination may cause false test results.
- Blood drawn into EDTA should be use within 24 hours. If EDTA is unavailable, samples drawn into ACD, CPD or CPDA-1 are preferable to clotted ones. If only clotted samples are available, do not refrigerate them before testing. All blood samples should be washed at least twice with PBS before being tested.

ASSAY PROCEDURE

Direct Antiglobulin Technique (DAT)

1. Wash test red cells 4 times with PBS, taking care to decant saline between washes and resuspend each cell button after each wash. Completely decant saline after last wash.
2. Add 2 volumes of Anti-Human Globulin to each dry cell button.
3. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000rcf or for a suitable alternative time and force.
4. Gently resuspend red cell button and read macroscopically for agglutination

Indirect Antiglobulin Technique (NISS IAT)

1. Prepare a 2-3% suspension of washed test red cells in PBS.
2. Place in a labeled test tube: 2 volumes of test serum and 1 volume of test red cell suspension.
3. Mix thoroughly and incubate at 37oC for 15 minutes.
4. Wash test red cells 4 times with PBS, taking care to decant saline between washes and resuspend each red cell button after each wash. Completely decant saline after last wash.
5. Add 2 volumes of Anti-Human Globulin to each dry cell button.
6. Mix thoroughly and centrifuge all tubes for 20 seconds at 1000rcf or for a suitable alternative time and force.
7. Gently resuspend red cell button and read macroscopically for agglutination

LISS Indirect Antiglobulin Technique (LISS IAT)

1. Prepare a 1.5-2% suspension of washed test red cells in LISS.
2. Place in a labeled test tube: 2 volumes of test serum and 2 volumes of test red cell suspension.

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3. Mix thoroughly and incubate at 37oC for 15 minutes.
   Follow steps 4 to 7 of **NISS IAT** above.

NOTES

1. It is recommended a positive control (weak Anti-D 0.1 IU/ml) and a negative control (an inert serum) be test in parallel with each batch of tests. Tests must be considered invalid if controls do not show expected results.
2. The antiglobulin techniques can only be considered valid if all negative tests react positively with IgG sensitized red cells.
3. In the techniques, here mentioned, one volume is approximately 40µl when using the vial dropper provided.
4. Use of the reagents and the interpretation of results must be carried out by properly trained and qualified personnel in accordance with requirements of the country where the reagents are in use. User must determine the suitability of the reagents for use in other techniques.

Stability of the reactions

1. Washing steps should be completed without interruption and tests centrifuged and read immediately after addition of the reagent.
2. Delays may result in dissociation of antigen-antibody complexes, causing false negative or weak positive results. Caution should be exercised in the interpretation of results of tests performed at temperatures other than those recommended.

INTERPRETATION

Positive: Agglutination of test red cells constitutes a positive test result and within the accepted limitations of the test procedure, indicates the presence of IgG and/or complement (C3) on the test red cells.

Negative: No agglutination of the test red cells constitutes a negative result and within the accepted limitations of the test procedure, indicates the absence of IgG and/or complement (C3) on the test red cells.

PERFORMANCE CHARACTERISTICS

1. The reagents have been characterized by all the procedures here described.
2. Prior to release, each lot of Accucare's Anti-Human Globulin is tested, by the techniques here mentioned, against red cells coated with Anti-D, Anti-K and Anti-Fya to check suitable reactivity.
3. Potency of anti-IgG and anti-C3d have been tested against the following minimum potency reference standard obtained from National Institute of Biological Standards and Controls (NIBSC): Anti-AHG reference standard 96/666
4. Anti-C3d potency is demonstrated in tests employing cells coated with C3.
5. The presence of contaminating heterospecific agglutinins or antibodies to C4d has been excluded in tests employing red cells of all ABO groups and cells coated with C4d.
6. The reactivity of any Anti-IgM, Anti-IgA or Anti-light chain components that might be present has not been established.
7. The Quality Control of the reagents was performed using red cells that had been washed twice with PBS prior to use.
8. The reagents comply with the recommendations contained in the latest issue of the Guidelines for the UK Blood Transfusion Services.

DISCLAIMER

- Each facility should verify the optimum spin time for the specific centrifuge in use.
- Manual techniques are to be performed according to the manufacturer's instructions.
- Each deviation from these instructions is the sole responsibility of the user.
- Used tests must be discarded as hazardous material. Manage waste according to local, state and national regulations.

LIMITATION OF THE PROCEDURE

- Red cells that have a positive DAT due to a coating of IgG cannot be typed by the Indirect Antiglobulin Techniques.
- A positive DAT due to complement sensitization may not reflect *in vivo* complement fixation if test cells are from a refrigerated clotted specimen.
- Inadequate washing of red cells in the indirect antiglobulin techniques may neutralize the anti-human globulin reagent.
- Following completion of the wash phase excess residual saline may dilute the anti-human globulin, reducing its potency.
- A negative direct antiglobulin test result does not necessarily preclude clinical diagnosis of ABO Hemolytic Disease of the Newborn or Auto Immune Hemolytic Anemia. It also does not necessarily rule out HDN, especially if ABO incompatibility is suspected.
- False positive or false negative results may also occur due to:
  - Improper storage, cell concentration, incubation time or temperature
  - Improper or excessive centrifugation
- The user is responsible for the performance of the reagents by any method other than those here mentioned.
- Any deviations from the techniques here recommended should be validated prior to use Contamination of test materials.

BIBLIOGRAPHY

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3. Howard JE, Winn LC, Gottlieb CE, Grumet FC, Garratty G, Petz LD. Clinical significance of the anti - complement components of antiglobulin antisera. Transfusion 1982: 22:269.
4. Howell P, Giles CM. A detailed serological study of five anti-Jka sera reacting by the antiglobulin technique. Vox. Sang. 1983; **45**: 129-138.
5. Issitt PD, Smith TR. Evaluation of antiglobulin reagents. A seminar on performance evaluation. Washington, DC. American Association of Blood Banks. 1976; 25-73.
6. The anti-complement reactivity low ionic methods as published by FDA. Recommended Methods for Anti - Human Globulin Evaluation (revision October 1984).

GLOSSARY OF SYMBOL

	Consult Instruction for Use	LOT	Lot Number
REF	Catalog Number		Date of Manufacturing
	Store between		Use By or Expiration Date
	Manufacturer	IVD	For *in vitro* Diagnostic use only
	Keep away from sunlight	CONT	Content of the kit

LAB-CARE DIAGNOSTICS (INDIA) PVT. LTD.
C1 Type, Shed No.: 3225, Chemical Zone,
GIDC Sarigam – 396155, Dist. Valsad, Gujarat, India.
Tel.: +91 22 2554 2109 /1558
Email: accucarediagnostics.com; Website: www.labcarediagnostics.com

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