INTENDED USE: Gamma-clone Anti-Human Globulin, Anti-IgG (Murine Monoclonal) Green or Uncolored is intended for use in direct or indirect antiglobulin tests where the detection of IgG is required.

SUMMARY OF THE TEST: The principle of the antiglobulin reaction, as described in 1908 by Moreschi [1], was first applied in blood group serology by Coombs and his associates in 1945 [2,3]. When the serum of animals immunized with human protein was used in the detection of so-called “incomplete” antibodies, the ability of antiglobulin serum to react with components of human complement bound to the red blood cells was reported in 1957 by Dacie and fellow-workers [4]. The antiglobulin (Coombs) test is an important procedure for the detection of immunoglobulin and/or complement bound to the red blood cells. The direct antiglobulin test is used to demonstrate antibodies and/or complement bound to the red blood cells in vivo, and the indirect antiglobulin test is used, after incubation of serum or plasma and red blood cells, to demonstrate antibodies and/or complement bound in vitro.

The following Table summarizes the applications and limitations of different Anti-Human Globulin reagents.

<table>
<thead>
<tr>
<th>Direct Antiglobulin Tests</th>
<th>Anti-IgG, C3d (Polyspecific)</th>
<th>Anti-IgG</th>
<th>Anti-C3b, C3d (Anti-C3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis of hemolytic disease of the newborn</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Investigation of transfusion reactions</td>
<td>Yes</td>
<td>Yes†</td>
<td>Yes†</td>
</tr>
<tr>
<td>Investigation of drug-induced red-cell sensitization</td>
<td>Yes</td>
<td>Yes†</td>
<td>Yes†</td>
</tr>
<tr>
<td>Investigation of auto-immune hemolytic anemia</td>
<td>Yes</td>
<td>Yes†</td>
<td>Yes†</td>
</tr>
<tr>
<td>Specific identification of cell surface coat (e.g., C3, IgG, IgM, IgA, etc.)</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Indirect Antiglobulin Tests</th>
<th>Compatibility testing</th>
<th>Screening for unexpected antibodies in donors</th>
<th>Screening for unexpected antibodies in patients</th>
<th>Identification of antibodies</th>
<th>Detection of antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>Yes†</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

†The red blood cells should be tested for the presence of both IgG and C3d, using either Anti-IgG,-C3d; (Polyspecific) or using Anti-Human Globulin reagents that contain these components separately (e.g. Anti-IgG and Anti-C3d).
‡Some literature reports indicate that Anti-IgG occasionally fails to detect antibodies that are demonstrable by means of an Anti-Human Globulin reagent containing an anti-C3 component. Antibodies not detected by Anti-IgG may be clinically significant in some cases.

PRINCIPLE OF THE TEST:
Anti-Human Globulin reagents are employed in direct and indirect antiglobulin testing. The direct antiglobulin test is used to detect red blood cell sensitization with IgG and complement components (C3b and C3d) that have occurred in vivo. Washed red blood cells are tested directly with Anti-Human Globulin reagent.

Key:
- Underline = Addition or significant change
- ▲ = Deletion of text

Antigen Antibody

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</tbody>
</table>

Indirect antiglobulin techniques are used to demonstrate in vitro sensitization of red blood cells with IgG and complement components. Serum or plasma containing antibody is first incubated with red blood cells to allow antigen-antibody binding to occur. The red blood cells are then washed and tested with Anti-Human Globulin reagent.

REAGENT: Gamma-clone Anti-Human Globulin, Anti-IgG (Murine Monoclonal) Green or Uncolored contains a murine monoclonal IgM antibody secreted by the hybridoma cell line 16H8 grown in fluid medium. The antibody has been determined to react with an epitope on the CH3 domain of the Fc region of human IgG. The tissue culture supernate containing the monoclonal antibody is diluted in a special proprietary buffer solution designed to facilitate the resuspension of the deposited red blood cell button after centrifugation. The final product is adjusted to a pH of 7.0±0.1. Any bovine albumin used in the manufacture of this product is sourced from donor animals of United States origin that have been inspected and certified by USDA Food Safety and Inspection Service inspectors to be disease-free. This human-based product is deemed to have low TSE (Transmissible Spongiform Encephalopathy) risk. Gamma-clone Anti-IgG, Green contains a mixture of Acid Blue #1 and Acid Yellow #23. Contains 0.1% sodium azide as a preservative.

PRECAUTIONS:
For in vitro diagnostic use. Store at 1° to 10°C when not in use. Do not freeze. Do not dilute. Do not use after the expiration date. Effort should be made to minimize contamination during use of the product. Do not use if turbid.

**CAUTION:** DO NOT Pipette this product by mouth, as the absence of murine virus has not been determined. The packaging of this product (dropper bulb) may contain dry natural rubber.

This reagent contains 0.1% sodium azide. Warning: H302 Harmful if swallowed.

Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sinks, flush with a large volume of water to prevent azide build-up.

Handle and dispose of reagent as potentially infectious.

The format for the expiration date is expressed as CCYY-MM-DD (year-month-day).

SPECIMEN COLLECTION AND PREPARATION: No special preparation of the patient is required prior to specimen collection. Blood should be drawn by aseptic technique. If delay in testing should occur, the specimen must be stored at 1° to 10°C for not longer than the storage limits given below for particular applications.

For the Direct Antiglobulin Test blood drawn into EDTA and testing within 48 hours of collection is recommended. Blood collected into other anticoagulants (ACD, CPD, CPDA-1, CPD2, or oxalate) may be used. Clotted blood may be used.

For the Indirect Antiglobulin Test separated serum or plasma may be tested. In the case of potential blood transfusion recipients, the specimen should be stored for no longer than is permitted by the relevant regulatory agencies. Red blood cells from donor units, and Reagent Red Blood Cells may be tested throughout their dating period.

PROCEDURE:
Materials Provided: Gamma-clone Anti-Human Globulin, Anti-IgG (Murine Monoclonal) Green or Uncolored.

Additional Materials Required: Test tubes (12×75 mm or 10×75 mm), pipettes, isotonic saline or phosphate-buffered (approximately 15 mM) isotonic saline pH 6.5-7.5, 37°C waterbath or incubator, timer, centrifuge, an optical aid such as a hand lens, a concave mirror or a microscope, Reagent Red Blood Cells and IgG-
sensitized red blood cells. Use of an antibody potentiator (e.g. 22% or 30% Bovine Albumin, Gamma LO-ION™, Gamma N-HANCE®, or Gamma PeG™) for the Indirect Antiglobulin Test is optional.

TEST METHODS:

Direct Antiglobulin (Coombs) Test
1. Place one drop of an approximate 3-4% suspension of red blood cells to be tested in an appropriately labeled test tube.
2. Wash the red blood cells at least 3 times with the tube full of saline, being careful to decant the saline between washes and to resuspend the red blood cells thoroughly when adding saline for the next wash. Decant the saline completely following the last wash.
3. Add 1 or 2 drops of Gamma-clone Anti-IgG to each ‘dry button’ of red blood cells.
4. Mix well and centrifuge IMMEDIATELY for:
   (a) 1 minute at 1,000 rpm (rcf 100 to 125), or
   (b) 15 seconds at 3,400 rpm (rcf 900 to 1,000), or
   (c) a time appropriate to the calibration of the centrifuge.
5. Resuspend the red blood cells by gentle shaking and examine for agglutination. Negative reactions may be examined with an optical aid. Record results.

Indirect Antiglobulin (Coombs) Test

The test procedure detailed below is one that is in common use. It may be varied according to the requirements of the particular laboratory. If potentiating agents are employed, they should be used according to the manufacturer’s directions.

1. Label an appropriate number of test tubes for each red blood cell suspension to be tested.
2. Place 2-3 drops of the serum or plasma to be tested into each of the tubes.
3. Add one drop of an approximate 3-4% red blood cell suspension in saline to each appropriate tube. Reagent Red Blood Cells may be used directly from the vial or according to manufacturer’s directions. NOTE: If desired, tubes may be incubated at room temperature for 5-30 minutes.
4. Centrifuge all tubes for:
   (a) 1 minute at 1,000 rpm (rcf 100 to 125), or
   (b) 15 seconds at 3,400 rpm (rcf 900 to 1,000), or
   (c) a time appropriate to the calibration of the centrifuge.
5. Examine for hemolysis and record if present.
6. Resuspend the red blood cells by gentle shaking and examine for agglutination. Record results.
7. Add potentiator, if used, according to manufacturer’s directions.
8. Incubate the tubes at 37°C±1°C for 30-60 minutes or according to the manufacturer’s directions for the potentiator being used.
9. Centrifuge all tubes as described in step 4.
10. Repeat steps 5 and 6.
11. Wash the red blood cells in the tube at least 3 times with the tube full of saline, being careful to decant the saline between washes and to resuspend the red blood cells thoroughly when adding saline for the next wash. Decant the saline completely following the last wash.
12. Add 1 or 2 drops of Gamma-clone Anti-IgG to each ‘dry button’ of red blood cells.
13. Mix well and centrifuge immediately as described in step 4.
14. Resuspend the red blood cells by gentle shaking and examine for agglutination. Negative reactions may be examined with an optical aid. Record results.

STABILITY OF REACTION:
The washing phases of the antiglobulin test must be carried out without interruption, and final test results must be interpreted immediately upon completion of the test.

QUALITY CONTROL:
1. All negative antiglobulin tests should be confirmed by adding IgG-sensitized red blood cells, such as Checkcell®, and then repeating centrifugation and reading. A positive test result at this point confirms that active antiglobulin (anti-IgG) was added to the test system and was present when the original antiglobulin test was interpreted as negative.
2. An autologous control (patient serum or plasma plus own cells) is recommended for antibody identification tests.
3. False-positive reactions in the direct antiglobulin test may be recognized by carrying out a control test on each red blood cell suspension tested, in which two drops of saline are added at step 3, instead of Anti-Human Globulin. The control will facilitate the recognition of aggregates caused by Wharton’s Jelly, or of agglutinates produced by ‘complete’ (IgM) autoantibodies that have not dissociated during the washing phases.
4. Confirmation that satisfactory levels of Anti-IgG are present in this reagent should be included as part of the daily quality control routine. IgG-sensitized red blood cells, such as Checkcell, can be used for daily quality control of this product.

INTERPRETATION OF TEST RESULTS: Agglutination of the test red blood cells in either the direct or the indirect antiglobulin test constitutes a positive test result and indicates the presence on the red blood cells of IgG. No agglutination in either the direct or the indirect antiglobulin test constitutes a negative test result and indicates the absence of detectable IgG on the red blood cells, subject to satisfactory control tests. NOTE: A negative direct antiglobulin test may not necessarily exclude hemolytic disease of the newborn, especially if ABO incompatibility is suspected as the cause. The test with this reagent provides no information as to whether or not the test red blood cells are coated with complement components.

LIMITATIONS: As in all serological tests, such factors as contaminated materials, improper incubation time or temperature, improper centrifugation and improper examination for agglutination may give rise to false test results. In addition:
1. The sensitivity of antiglobulin tests is greatly impaired if human protein is introduced into the test system after washing the red blood cells (even when the amount is very small).
2. It is important to centrifuge the test without delay after adding the Gamma-clone Anti-IgG to the washed red blood cell button. Progressively diminishing agglutination may accompany delayed centrifugation, and this effect may be even more pronounced if two drops of Gamma-clone Anti-IgG are used instead of one drop. If for any reason centrifugation is postponed beyond one minute, the test must be regarded as invalid and repeated accordingly, even though the Coombs Control test yield a positive result.
3. Examples of pure IgG4 subclass antibodies may not be detected by this reagent. NOTE: Pure IgG4 antibodies are very uncommon.
4. Some literature reports indicate that Anti-IgG occasionally fails to detect antibodies that are demonstrable by means of an Anti-Human Globulin reagent containing an anti-C3 component. Antibodies not detected by Anti-IgG may be clinically significant in some cases.
5. The lack of anti-complement activity in this product will give rise to a negative result whenever the test red blood cells are coated with complement alone.

SPECIFIC PERFORMANCE CHARACTERISTICS: Each lot of Gamma-clone Anti-Human Globulin, Anti-IgG (Murine Monoclonal), Green or Uncolored, contains an appropriate level of anti-lgG to meet FDA potency requirements. Potency is determined by parallel titration with the anti-IgG reference preparation supplied by the Food and Drug Administration. Block titration against red blood cells coated with anti-D and with anti-Fy® assures that anti-IgG potency at least equals that of the reference preparation. Each lot is further tested against red blood cells coated with a variety of representative antibodies from different blood group systems, in order to assure adequate reactivity in use. The absence of anti-human species antibodies is proved by testing against uncared red blood cells of all ABO groups. The product contains no anti-IgM, anti-IgA, anti-C3 or anti-C4 reactivity. The performance of this product is dependent on adhering to the recommended methods found in this insert.

For additional information or for technical support, contact Immucor at 855-IMMUCOR (466-8267).

BIBLIOGRAPHY:

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Revised: 2/2013

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