Anti-Human Globulin

Anti-IgG, -C3d; Polyspecific
(Rabbit/ Murine Monoclonal)

FOR IN-VITRO DIAGNOSTIC USE
For Tube Testing
MEETS FDA POTENCY REQUIREMENTS
U.S. License Number: 1798

Package size
REF 804115100 VOL 10 x 10 mL Anti-Human Globulin, Anti-IgG, -C3d; Polyspecific

Intended Use
Anti-Human Globulin Anti-IgG, -C3d; Polyspecific is used for the direct antiglobulin test to demonstrate the in-vivo coating of red blood cells with antibody molecules and/or complement components (such as autoantibodies, maternal antibodies in hemolytic disease of the newborn, alloantibodies against red blood cells in transfusion reactions).

Anti-Human Globulin Anti-IgG, -C3d; Polyspecific is used for the indirect antiglobulin test to demonstrate the in-vitro coating of red blood cells with antibody molecules and/or complement components as in detection and identification of unexpected antibodies as well as crossmatch tests. Furthermore, blood group antigen typing (with the corresponding test reagent for the indirect antiglobulin-test) can be carried out.

Summary
Moreschi first described the use of Anti-Human Globulin in 1908. Coombs rediscovered the test in 1945. By injecting rabbits with human IgG, they were able to produce a protein (Anti-IgG) that reacted with "incomplete" antibodies (IgG). Most "incomplete" antibodies (IgG) fail to agglutinate red blood cells suspended in saline. Most clinically significant antibodies in red blood cell serology are of the IgG class and can only be detected by the use of Anti-IgG. A stable lattice structure is formed and agglutination occurs when Anti-IgG binds to the IgG sensitized red blood cells.

Biotest Anti-Human Globulin reagents are used to test for the presence or absence of unexpected red blood cell antibodies. Furthermore, blood group antigen typing (with the corresponding test reagent for the indirect antiglobulin test) can be carried out. Routine pretransfusion studies always include tests for antibody screening, crossmatch and antibody identification.

Principle of the Test
The test principle is a haemagglutination test. Anti-Human Globulin Anti-IgG, -C3d; polyspecific acts as a link between the antibody and/or complement coating of neighbouring red blood cells and induces agglutination. Uncoated red blood cells will not agglutinate.

Reagent
Anti-Human Globulin Anti-IgG, -C3d; Polyspecific is a blend of rabbit anti-IgG and murine monoclonal anti-complement (murine IgM Anti-C3d, Bric 8). The anti-IgG component contains antibody reactivity against light IgG chains and thus may also agglutinate IgA or IgM coated red blood cells. The anti-complement component consists of murine monoclonal IgM anti-C3d-antibody reactive with C3b- and C3d-coated red blood cells. Antibodies are diluted in an isotonic saline solution containing bovine albumin and as colorant Patent Blue and Tartrazine.

The following antibodies are produced using intermediate products produced for Biotest Medical Diagnostics GmbH in a shared manufacturing agreement with Millipore (UK) Ltd., 9 Fleming Road, Kirkton Campus, EH54 7BN, Livingston, UK; License Number 1721.

Anti-C3d clone BRIC 8 (IgM)
Preservative: 0.1% sodium azide.

Precautions
- For In-vitro diagnostic use.
- Store at 2 to 8°C.

Materials
Materials provided
- Anti-Human Globulin Anti-IgG, -C3d; Polyspecific

Material required but not provided
- Pipettes (drop volume 40 to 50 µL)
- Isotonic saline solution
- Reagent red blood cells (e.g. Biotest: Biotestcell® 1 & 2 REF 816014100, Biotestcell® 3 REF 816085100, Biotestcell®-I 8 [REF 816020100, Biotestcell®-I 11 REF 816021100])
- Donor or patient red blood cells
- IgG coated red blood cells (e.g. Biotest Coombscell-E REF 816030100)
- LISS (e.g. Biotest MLB2 [REF 805200100])
- Glass tubes 10 x 75mm or 12 x 75mm
- Serological Centrifuge
- Interval Timer
- Markers
- Optical aid (optional). The use of an optical aid for agglutination reading must be validated by the user.

Test Procedure
A. Indirect Antiglobulin Test (IAT)
If an enhancement medium (albumin, LISS) is used, please refer to the respective instructions for use.

1. Prepare a 3 to 5 % suspension of red blood cells in isotonic saline solution.
2. Place 1 drop of red blood cell suspension in an appropriately marked tube and add 2 drops of serum to be tested (or as directed for test reagent).
3. Incubate at 37°C for 30 to 60 minutes or as appropriate to the enhancement reagent used.
4. Wash the red blood cells 3 times with isotonic saline. Decant supernatant saline completely.
5. Add 2 drops of Anti-Human Globulin Anti-IgG, C3d; Polyspecific to the packed red blood cells and mix.
6. Centrifuge for 20 seconds at 800 - 1000 x g.
7. Gently dislodge the red blood cell button and observe for agglutination.
8. Record results

B. Direct Antiglobulin Test (DAT)
1. Prepare a 3 to 5% suspension of the red blood cells in isotonic saline.
2. Wash 1 drop of this red blood cell suspension 3 times, with isotonic saline. Decant supernatant saline completely.
3. Add 2 drops of Anti-Human Globulin Anti-IgG, C3d; Polyspecific to the packed red blood cells and mix.
4. Centrifuge for 20 seconds at 800 - 1000 x g.
5. Gently dislodge the red blood cell button and observe for agglutination.
6. Record results

Stability of the Reaction
Following centrifugation, all tube tests should be read immediately and results interpreted without delay. Time delays may cause a dissociation of the antigen-antibody complexes resulting to false negative or more often weak positive reactions.

Quality Control
The reactivity of all reagents should be confirmed by testing with known positive and negative red blood cells on each day of use. To confirm the reactivity or specificity of Biotest Anti-Human Globulin Anti-IgG, -C3d; Polyspecific, the reagent should be tested with IgG coated (and if possible complement coated) and non coated red blood cells respectively. The reagent is satisfactory for use if it reacts only with the IgG (and complement) coated red blood cells. Negative results in an antiglobulin test should be verified with IgG coated red blood cells: Add 1 drop of IgG coated red blood cells, mix and centrifuge for 20 seconds at 800 - 1000 x g. Positive result: The negative reaction in the indirect antiglobulin test is valid, reactive Anti-Human globulin is present. Negative result: A technical error was made and the test must be repeated. It is recommended that a positive and a negative control be performed in parallel with testing.

Interpretation of results
Agglutination of the red blood cells with the indirect antiglobulin test is a positive result and indicates the presence of an unexpected antibody(ies). Agglutination of the red blood cells with the direct antiglobulin test is a positive result and indicates an auto-agglutinin or auto antibodies. No agglutination is a negative result and indicates the absence of an unexpected antibody or the absence of the corresponding antigen or lack of an auto-agglutinin. An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technical Manual, 15th edition).

Limitations
- Low frequency antigens may not always be present on reagent red blood cells and a double dose of antigen may be required to detect very weakly reacting antibodies. Therefore, negative reactions with the screening red blood cells do not always indicate the absence of unexpected antibodies.
- Insufficient or inappropriate washing can lead to false negative or false positive reactions. Small amounts of residual patient sera/plasma can neutralize the Anti-Human Globulin Anti-IgG, -C3d: Polyspecific.
- Some conditions that may cause false positive results are:
  - Contamination of sample or reagents
  - Autoantibodies
  - Improper storage or preparation of red blood cells
  - Antibodies to antibiotics or other reagents
  - Cold Antibodies
- Positive reactions may be seen from individuals who have received Rh Immune globulin.
- Do not use frozen/deglycerolized and enzyme treated red blood cells

Specific Performance Characteristics
Testing is performed in accordance with FDA recommended methods. The final release testing is performed according to the product specific SOPs. Each lot of Biotest Anti- Human Globulin reagent is tested in the Quality control by package insert method against IgG and complement coated red blood cells to insure suitable reactivity. The products meet FDA potency requirements. The specificity testing for the presence of contaminating antibodies is performed according to the product specific SOPs.

For the product performance it is necessary to adhere to the recommended method in the instructions for use.

The performance of the Biotest Anti-Human Globulin Anti-IgG, -C3d; Polyspecific was confirmed against a FDA approved reference reagent in a Multi Center Field Trial.

For Technical Support or further product information, contact Biotest Diagnostics Corporation at 800-522-0090.

Note
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 national regulations.

Glossary of Symbols

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<tr>
<th>Symbol</th>
<th>Definition</th>
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<th>Definition</th>
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<tbody>
<tr>
<td>[LOT]</td>
<td>Batch Code</td>
<td>[IVD]</td>
<td>In vitro diagnostic medical device</td>
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<tr>
<td>△</td>
<td>Caution, consult accompanying documents</td>
<td>[J]</td>
<td>Consult instructions for use.</td>
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<tr>
<td><em>PEER</em></td>
<td>Manufacturer</td>
<td>[Y]</td>
<td>Use by YYYY-MM-DD</td>
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<td>⬇️</td>
<td>Contains sufficient quantity for &lt;n&gt; tests.</td>
<td>[REF]</td>
<td>Catalog number</td>
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<tr>
<td>⬆️</td>
<td>Temperature limitation</td>
<td>[VOL]</td>
<td>Volume</td>
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Bibliography
1. Moneschi C. Neue Tatsache über die Blutkörperchen Agglutinationen, Zbl Bakt 1908; 46:49,456