Reagent Red Blood Cells

**Intended Use**

Biotestcell®-I 8 and Biotestcell®-I 11 are used for the identification of unexpected antibodies which are found in an antibody screening test using Reagent Red Blood Cells such as Biotestcell® 1 & 2, Biotestcell 3 and/or Biotestcell Pool. Biotestcell®-I 8 and Biotestcell®-I 11 are used in tube test and solid phase test Solidscreen II with the TANGO® optimo and is suited for all routine testing.

**Summary**

Antibody identification is used to determine specificity and possible clinical significance of antibodies against red blood cell antigens detected in the method used for detection of unexpected antibodies. This determination is important for the selection of products for transfusion as well as for pre-natal care.

**Principle of the Test**

Antigens of the Reagent Red Blood Cells react with the corresponding antibodies in the serum or plasma directly or after addition of Anti-Human Globulin. In a tube test agglutination will occur. In solid phase test Solidscreen II a reddening of red blood cells on the microtest plate wells will occur.

**Reagent**

Biotestcell®-I 8 and Biotestcell®-I 11 are Reagent Red Blood Cells with polyvalent antigens. Biotestcell®-I 8 and Biotestcell®-I 11 contain the following antigens: D, C, CC, E, c, e, K, k, Fy*, Fy, Lu*, Lu, Jk*, Jk, Js, M, N, s, s, Le*, Le, Xg*, Xg, Co.

**Materials provided**

- Biotestcell®-I 8 or Biotestcell®-I 11

**Material required but not provided**

A. 3-phase-tube test

- Pipettes (drop volume 40 to 50 μl)
- Isotonic saline solution
- Anti-Human Globulin Anti-IgG (e.g. Biotest REFR 804175100)
- Anti-Human Globulin Anti-IgG,-C3d Polyspecific (e.g. Biotest REFR 804115100)
- IgG coated red blood cells (e.g. Biotest Coombscell-E REFR 816030100)
- Biotest MLB2 (Modified LISS Biotest) II [REF] 806516100
- Glass tubes 18 x 75mm or 12 x 75mm
- Serological centrifuge
- Test tubes
- Optical aid (optional). The use of an optical aid for agglutination reading must be validated by the user.

**Test Procedure**

1. Prepare reagents: E. coli suspension to tube labelled for it and mix.
2. Add 1 drop of corresponding Biotestcell® Reagent Red Blood Cell suspension to tube labelled for it and mix.
3. Centrifugation for 20 seconds at 800 -1000 x g.
4. Gently dislodge the red blood cell button and observe for agglutination.
5. Record results

**Specimen Collection**

- Fresh samples of clotted or EDTA anticoagulated whole blood collected following general blood sampling guidelines are acceptable.
- Blood specimens exhibiting gross hemolysis or contamination should not be used. Do not use specimens collected with gel separators.
- Samples older than seven days can be tested, however antibody reactivity has been shown to decrease in older samples. Stored samples should be allowed to reach room temperature (18 to 26°C) prior to testing.

**Materials**

- Biotestcell®-I 8 or Biotestcell®-I 11
8. Gently dislodge the red blood cell button and observe for agglutination.
9. Record results.
With the centrifugation test after incubation mainly Rh-antibodies as well as some incomplete antibodies are detected.

Phase 3: Indirect Antiglobulin-Test
10. Wash the red blood cells 3 times with isotonic saline. Decant supernatant saline completely.
11. Follow the directions of the Anti-Human Globulin manufacturer.
12. Centrifuge for 20 seconds at 800 - 1000 x g.
13. Gently dislodge the red blood cell button and observe for agglutination.
14. Record results.
This test shows IgG antibodies such as anti-Duffy, anti-Kidd, all Rh-antibodies.

B. Solidscreen® II and TANGO® optimo
Detailed test procedure instructions as well as details for the evaluation of test results are given in the instructions for use of Solidscreen II and TANGO® optimo, User’s Guide.

Stability of the Reaction
Tube Test
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.
TANGO® optimo
The results are read immediately and a digital image is stored for review by the operator. Image algorithms contained in the TANGO® optimo evaluate, and provide an interpretation (positive or negative) for the well. The operator performs verification of the final results.

Quality Control
A series of quality control samples must be run each day before testing or according to local requirements to ensure that the reagents and analyzer are functioning properly.

Refer to TANGO® optimo instructions for recommended instrument quality control.
Controls should be run whenever:
• Lot numbers change (plate, reagent).
• A new bottle or preparation is placed on the system.
• After service/repair of the analyzer.

Negative results in a tube antiglobulin test should be verified with IgG coated red blood cells in a tube test: 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.

Interpretation of QC
The tests are considered valid if the expected results for the controls are obtained. If the controls do not give the expected results, you must determine the cause for the failed QC.

Follow institutional SOP for repeat testing of QC samples, repeat testing of patient/donor samples and documentation of QC results and corrective action if required.
Please contact Biotest Diagnostics Corporation (800-522-0090) if controls repeatedly fail to give expected results.

Interpretation of results
Tube Test
Agglutination of the red blood cells is a positive result and indicates the presence of an unexpected antibody(ies). No agglutination is a negative result and indicates that no unexpected antibody was detected.

An agglutination viewer may facilitate the reading of tube tests (as recommended by the AABB Technical Manual, 15th edition).

The positive and negative reactions are compared to the Biotestcell®-I8 and Biotestcell®-I 11 antigen pattern and are read accordingly. For verification an additional antigen test of the red blood cells may be performed.

TANGO® optimo
When used for testing with Solidscreen II and the TANGO® optimo, a smooth monolayer of red blood cells is indicative of a positive reaction. A compact button of red blood cells in the middle of the well is indicative of a negative reaction.

The results are read immediately and a digital image is stored for review by the operator. Image algorithms contained in the TANGO® optimo evaluate, and provide an interpretation (positive/negative) for the well. The operator performs verification of the final results.

Limitations
• Negative reactions and subsequent positive reactions with IgG coated red blood cells indicate that the serum contains no detectable antibodies against one of the antigens present on the Reagent Red Blood Cells (enclosed antigen pattern).
• Because some antibodies show dosage effect, the antigen density on the Reagent Red Blood Cells needs to be considered when evaluating the test results (homozygous or heterozygous hereditary disposition). A heterozygous expression of the antigen may result in non-detection of weak antibodies depending on the used test method.
• In very rare cases HLA-antigens within the product may lead to false positive reactions.
• The reactivity of the product may decrease during the dating period and therefore should not be used after the expiration date. The rate of decrease in reactivity is partially dependant on individual donor characteristics that are neither controlled nor predicted by the manufacturer.
• Negative reactions will be obtained if the sample contains antibodies present in concentrations too low to be detected by the test method employed. No test method is capable of detecting all red cell antibodies.

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 Reagent Red Blood Cells is tested in the Quality control by package insert method against a panel of blood grouping reagents to insure suitable reactivity. To exclude a mixup of the Reagent Red Blood Cells with identical Rh phenotype at least one differential antigen has to be tested. The result must react appropriately positive or negative.
No FDA Standard of potency. For the product performance it is necessary to adhere to the recommended method in the instructions for use.
For Technical Support or further product information, contact Biotest Diagnostics Corporation at 800-522-0090.

Bibliography

Glossary of Symbols

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<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>[LOT]</td>
<td>Batch Code</td>
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<td>Volume</td>
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<td>[T]</td>
<td>Temperature limitation</td>
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Symbol Definition

- **Caution, consult accompanying documents**
- **Consult instructions for use.**
- **Use by YYYY-MM-DD**
- **Contains sufficient quantity for () tests.**
- **Catalog number**
- **Temperature limitation**
- **In vitro diagnostic medical device**

Key: Underline = Addition of changes  ❌ = Deletion of text