

Reagent Red Blood Cells

Biotestcell® Pool

Biotestcell® 1 & 2

Biotestcell® 3

3.0 to 3.4 %

FOR USE IN DETECTION OF UNEXPECTED ANTIBODIES WITH TANGO® INSTRUMENTS AND BY TUBE TEST*

*Tube test is not approved by Health Canada

FOR IN VITRO DIAGNOSTIC USE
NO U.S. STANDARD OF POTENCY
U.S. License Number: 1845
Rx only

PACKAGE SIZE

[REF] 816065100	[VOL] 1 x 10 mL	Biotestcell® Pool
[REF] 816014100	[VOL] 2 x 10 mL	Biotestcell® 1 & 2
[REF] 816085100	[VOL] 3 x 10 mL	Biotestcell® 3

INTENDED USE

Biotestcell® Pool, Biotestcell® 1 & 2 and Biotestcell® 3 are used for the detection of unexpected antibodies in tube test and solid phase test Solidscreen® II with TANGO® instruments. Tube test is not approved by Health Canada.

SUMMARY

The detection of clinically significant antibodies is an important component of pre-transfusion and donor testing. This is to ensure that the donor red blood cells chosen for transfusion are those that will not cause harm to the recipient and will have optimum survival once transfused. Unexpected red blood cell alloantibodies are found in 0.3% to 38% of the population, depending on the group studied and the detection method used.¹

Bio-Rad Reagent Red Blood Cells Biotestcell® 1 & 2, Biotestcell® 3 and Biotestcell® Pool are used to test for the presence or absence of the unexpected red blood cell antibodies in antibody screening. Routine pre-transfusion studies always include tests for the detection of unexpected antibodies to red blood cells.

PRINCIPLES OF THE TEST

The test principle is a hemagglutination test or solid phase test. Antigens on 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 uniform layer of red blood cells on the micro test plate wells will occur.

REAGENT

[IVD]

OBSERVABLE INDICATIONS.

Do not use if markedly hemolyzed or discolored.

Do not use damaged vials.

Biotestcell® 1 & 2 and Biotestcell® 3 are Reagent Red Blood Cells with polyvalent antigens of two or three single blood donors in separate vials for the detection of red blood cell antibodies. Biotestcell® Pool is a single vial containing Reagent Red Blood Cells with polyvalent antigens of two single blood donors. Biotestcell® 1 & 2, Biotestcell® 3 and Biotestcell® Pool contain the following antigens: D, C, E, c, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, M, N, S, s, Le^a, Le^b, P₁, Xg^a. For the exact antigen content of each production lot, please refer to the enclosed table. Biotestcell® 1 & 2 and Biotestcell® 3 are also suited for use with enzymes (papain, ficin, bromelain, trypsin) or enhancement reagents (albumin, LISS). The shelf life of enzyme treated Reagent Red Blood Cells is listed in the instructions for use of the respective enzymes. Biotestcell® 1 & 2, Biotestcell® 3, and Biotestcell® Pool are suspended 3.0 to 3.4% in a modified Alsevers solution and can be used immediately following careful resuspension.

Preservative: 0.01% Neomycin, 0.033% Chloramphenicol, 5 ppm Amphotericin B

PRECAUTIONS

- For *in vitro* diagnostic use.
- Store at 2 to 8°C.
- Do not use beyond expiration date.
- Do not use damaged vials.
- When using Biotestcell® 1 & 2 and Biotestcell® 3 do not mix vials of different lots as reactions patterns for result interpretation vary.
- Handle and dispose of reagents as potentially infectious.
- Caution: Do not pipette by mouth. The absence of all viruses has not been determined.
- Caution: ALL BLOOD PRODUCTS SHOULD BE TREATED AS POTENTIALLY INFECTIOUS. SOURCE MATERIAL FROM WHICH THIS PRODUCT WAS DERIVED WAS FOUND NEGATIVE WHEN TESTED WITH FDA LICENSED EIA/ELISA TESTS. NAT TESTING WAS NOT PERFORMED. NO KNOWN TEST METHOD CAN OFFER ASSURANCE THAT PRODUCTS DERIVED FROM HUMAN BLOOD WILL NOT TRANSMIT INFECTIOUS AGENTS.

- Caution: This Product Contains Natural Rubber Latex Which May Cause Allergic Reactions.
- Refer to the instrument operator's manual for on-board shelf life.
- Do not use samples collected with gel separators of any kind.
- Biotestcell® Pool is not recommended for pretransfusion testing of patient samples.
- Do not use beyond seven days when loaded on TANGO® instruments.
- Consult downloads.bio-rad.com to download the valid version of this instruction for use.

SPECIMEN COLLECTION

Tube Test and Automated Methods

Fresh samples of clotted or EDTA anticoagulated whole blood collected following general blood sampling guidelines are acceptable.

The specimen should be tested as soon as possible after collection. If testing is delayed, specimens should be stored at 2 to 8°C or the plasma or serum can be separated from the red blood cells and frozen. Stored samples should be allowed to reach room temperature prior to testing.

Note: Blood specimens exhibiting gross hemolysis or contamination should not be used.

Note: Do not use specimens collected with gel separators.

A distinct separation between plasma and red blood cells must be visible for testing. Samples may be centrifuged or allowed to settle. Clotted or EDTA samples older than ten days for tube methods and older seven days for automated methods may be tested, however antibody reactivity has been shown to decrease in older samples.

MATERIALS

Material provided

- Biotestcell® 1 & 2, Biotestcell® 3 or Biotestcell® Pool

Material required but not provided

A. Tube Test

- Pipettes
- Isotonic saline
- Anti-Human Globulin Anti-IgG (e.g. Bio-Rad [REF] 804175100)
- Anti-Human Globulin Anti-IgG, -C3d Polyspecific (e.g. Bio-Rad [REF] 804115100)
- IgG coated red blood cells (e.g. Bio-Rad Coombscell-E [REF] 816030100)
- Potentiator (e.g. MLB 2 Bio-Rad Modified LISS [REF] 805200100 50 mL glass bottle or MLB 2 Bio-Rad Modified LISS [REF] 805205100 10 x 10 mL glass bottle (not for use with TANGO® instruments) or albumin (optional). The 10 mL MLB 2 glass bottle is not approved for tube testing by Health Canada).
- Glass tubes 10 x 75mm or 12 x 75mm
- Serological centrifuge
- Interval timer
- Markers
- Agglutination viewer (optional).

B. TANGO® instruments

- TANGO® optimo Bio-Rad [REF] 848900010
- TANGO infinity™ Bio-Rad [REF] 850000010
- Solidscreen® II plates Bio-Rad [REF] 806521100
- Anti-Human Globulin, Anti-IgG Solidscreen® II [REF] 806516100
- Bio-Rad MLB2 (Modified LISS Bio-Rad) [REF] 805200100
- Solidscreen® II Control [REF] 806514100
- Solidscreen® II Control B [REF] 806519100
- Solidscreen® II Negative Control [REF] 806509100
- Alsevers Solution [REF] 806510100
- Washing Solution Concentrate [REF] 848000091
- Sodium hydroxide solution (0.5 N NaOH)
- Cell mixers
- Deionized water

TEST PROCEDURE

Resuspend Reagent Red Blood Cells prior to use and allow to reach room temperature.

The 3-phase-test (tube technique) has not been approved by Health Canada for use on the Canadian market.

A. 3-phase-tube-test

If an enzyme or enhancement reagents (albumin, LISS) is used, please refer to the respective instructions for use.

Phase 1: Immediate Spin

- Place two drops (approx. 40 to 50 µL each) of serum/plasma to be tested into each tube labelled for a selected red blood cell.
- Add one drop of corresponding Biotestcell® Reagent Red Blood Cells suspension to the appropriate tube and mix.
- Centrifuge for:
 - 20 seconds at 800 to 1000 x g or
 - at a time and speed appropriate for the centrifuge calibration.
- Gently dislodge the red blood cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer, however, microscopic examination is not recommended.
- Record results.

Often the immediate centrifugation test shows expression of anti-M, -N, -P and cold reactive antibodies.

Phase 2: Incubation

Refer to instructions for the enhancement reagent being used.

6. Incubate at 36 to 38°C for 30 to 60 minutes or as appropriate to the enhancement reagent used.
7. Centrifuge for:
 - a. 20 seconds at 800 to 1000 x g or
 - b. at a time and speed appropriate for the centrifuge calibration.
8. Gently dislodge the red blood cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer, however, microscopic examination is not recommended.
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 (IAT)

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:
 - a. 20 seconds at 800 to 1000 x g or
 - b. at a time and speed appropriate for the centrifuge calibration.
 13. Gently dislodge the red blood cell button and observe for macroscopic agglutination. Negative reactions may be examined with an agglutination viewer, however, microscopic examination is not recommended.
 14. Record results.
 15. To control all antiglobulin negative tests, add red blood cells sensitised with IgG antibody, e.g. Coombscell E (see Package insert for procedure).
- This test shows IgG antibodies such as anti-Duffy, anti-Kidd, all Rh-antibodies.

B. TANGO® instruments

Detailed test procedure instructions as well as details for the evaluation of test results are given in the respective instrument User Manual.

STABILITY OF 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.

QUALITY CONTROL

Positive and negative quality control samples should be run each day according to local requirements to ensure that the reagents are functioning properly. Refer to instrument User Manual for recommended instrument quality control.

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 Bio-Rad Laboratories, Inc., (800-224-6723) 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.

The positive and negative reactions may be compared to the Biotestcell® antigen pattern and read accordingly. Antibody identification can be performed with Biotestcell®-I 8, Biotestcell®-I 11 or Biotestcell®-I 11 Plus.

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

TANGO® instruments

Refer to the respective instrument User Manual and Solidscreen® II package insert for detailed instructions on the interpretation of reactions.

LIMITATIONS

- Low frequency antigens may not always be present on Biotestcell® 1 & 2, Biotestcell® 3 and Biotestcell® Pool. Therefore, negative reactions with the screening Reagent Red Blood Cells do not always indicate the absence of unexpected antibodies.
- 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).
- Reagent Red Blood Cells not adequately resuspended prior to placing on the instrument may result in false positive result interpretation when performing testing with TANGO® instruments.
- 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 test method used.
- In very rare cases HLA-Class I Antigens or low frequent antigens within the product may lead to 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.

- False positive reactions might occur due to:
 - Cold antibodies
 - HTLA antibodies
 - Auto antibodies
 - Panagglutinins
 - patient's medication (e.g. antibiotics, plasma expanders of high molecular weight)
- Do not use if markedly hemolyzed, slight hemolysis before the expiry date does not affect the reactivity.
- ◀
- Fibrin, clots, particulates or other artifacts may cause anomalous results.
- 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.
- The 3-phase-test (tube technique) has not been approved by Health Canada for use on the Canadian market.

SPECIFIC PERFORMANCE CHARACTERISTICS

Testing is performed in accordance with FDA recommended methods.

The final release testing is performed according to the product specific SOPs. As part of the release process, each lot of Bio-Rad Reagent Red Blood Cells is tested according to the package insert method against a panel of blood grouping reagents to insure suitable reactivity. To exclude a mix-up 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 U.S. 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 Bio-Rad Laboratories, Inc., at 800-224-6723.

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 test material must be discarded as hazardous material. Manage waste according to local, state and national regulations.

GLOSSARY OF SYMBOLS

Symbol	Definition	Symbol	Definition
[LOT]	Batch Code	[IVD]	<i>In vitro</i> diagnostic medical device
!	<u>Consult the instructions for use for important cautionary information such as warnings and precautions</u>	!	Consult instructions for use
M	Manufacturer	e	Use by YYYY-MM-DD
S	Contains sufficient quantity for <n> tests.	[REF]	Catalog number
t	Temperature limitation	[VOL]	Volume

BIBLIOGRAPHY

1. John D. Roback ,MD et al. Technical Manual 17th Edition, Bethesda, MA: AABB, 2011.

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