Abtectcell™ III C 3% and 0.8%

Reagent Red Blood Cells
Three Cell Antibody Screen

FOR TUBE, MICROPLATE AND COLUMN AGGLUTINATION METHODS

IVD

Caution:
Handle as if capable of transmitting infection

immulab

METHOD SUMMARY

Antibody Screening Cells	Tube	MTP†	CAT^
Abtectcell™ III C 3%	Yes	Yes	Yes*
Abtectcell™ III C 0.8%	No	No	Yes**

† Due to the wide variation in microplate methods and equipment, users should validate methods in routine use.
^ Refer to the test method recommended by the manufacturer of the Column Agglutination Technology (CAT) system.
* Validated for use in Ortho-Clinical Diagnostics BioVue™ System.
** Validated for use in Ortho-Clinical Diagnostics BioVue™ System, Bio-Rad ID-System (formerly DiaMed-ID Micro Typing System) and Grifols DG Gel® System.

REAGENT DESCRIPTION

Abtectcell™ III C 0.8% and 3% Reagent Red Blood Cells (RRBC) are three-cell panels for screening patient plasma or serum for clinically relevant red cell alloantibodies (antibody screening). Abtectcell™ III C 3% is designed and validated for tube technique and validated for direct addition on Ortho-Clinical Diagnostics BioVue™ (BioVue™) systems. Abtectcell™ III C 0.8% is designed for CAT Systems and is validated for direct addition into the BioVue™, Bio-Rad ID-System and Grifols DG Gel® System.

The Abtectcell™ III C 0.8% and 3% RRBC consist of three 10mL vials of Group O human red cell suspensions labelled Cell 1, Cell 2 and Cell 3. These consist of one R_1R_1 or $R_1^wR_1$ cell, one R_2R_2 cell and where possible one rr cell. As a minimum the following antigens are expressed: D, C, E, c, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, M, N, S, s, MUT, Mur, P1, Le^a, Le^b and Di^a.

Abtectcell™ III C 3% and 0.8% RRBC utilise KODE™ Peptide Antigen (PA) technology to add peptide antigens such as MUT and Mur. KODE™ technology is a novel, patented system that allows antigens to be expressed on human erythrocytes, creating cells with a precisely controlled antigen expression that is consistent and reproducible. Antigens added using KODE™ technology are resistant to Papain and other proteolytic enzymes but are sensitive to sulfhydryl reagents. Crossover events involving the red cell glycophorin genes can result in the expression of variant glycophorins with novel antigens. These antigens are detectable in patients who possess these variant glycophorins of which Gp. Mur (previously known as Mi III) is the most common example. IgG antibodies to MUT and/or Mur have been associated with both Haemolytic Transfusion Reactions (HTRs) and Haemolytic Disease of the Foetus and Newborn (HDFN) and are the most commonly detected in Asian populations.

Abtectcell™ III C 3% and 0.8% RRBC specifications are compliant with the Australian and New Zealand Society of Blood Transfusion Ltd (ANZSBT) Guidelines for Transfusion and Immunohaematology Laboratory Practice, 1st edition 2016 and other international screening cell specifications such as Guidelines for the Blood Transfusion Services in the United Kingdom and US FDA Code of Federal Regulations. At least one of the three cells in each panel will have the homozygous expressions of Fy^a, Fy^b, Jk^a and Jk^b. Abtectcell™ III C RRBC screening cells are from individual donors and are not pooled. The antigen specificities are recorded on the accompanying 'Antigen Composition Sheet'. All cells are tested by Direct Antiglobulin Test (DAT) by tube and CAT technology and where possible DAT negative cells are supplied.

Fresh donor units are used in Immulab RRBC products. The cells have been washed to remove plasma and any contaminating antibodies. Abtectcell™ III C 3% RRBC are suspended to 3% v/v in Celpresol™, an isotonic citrate phosphate buffered solution containing glucose and amino acids. Chloramphenicol and Neomycin Sulphate are included as antibacterial agents and Thiomersal is added as a preservative. The red cell suspensions are ready for direct addition in tube technique after gentle mixing to resuspend the cells. Abtectcell™ III C 0.8% RRBC are suspended to 0.8% v/v in Celpresol™ LISS, a low ionic strength phosphate buffered solution containing glucose and amino acids. Trimethoprim and Sulfamethoxazole are included as antibacterial agents. The red cell suspensions are ready for direct addition into CAT systems after gentle mixing to resuspend the cells. Washing and resuspension is not required prior to addition.

STORAGE AND CONDITIONS OF USE

Protect from light.
Store at 2° to 8°C (Refrigerate. Do Not Freeze).
Opened product is suitable for use until expiry, provided no haemolysis or evidence of contamination present.

PRINCIPLE OF THE TEST

Agglutination and/or haemolysis of one or more of the Abtectcell™ III C RRBC, in one or more technique will demonstrate the presence of unexpected clinically relevant antibodies in the serum/plasma. The identification of antibodies detected in an antibody screen should be performed using an extended panel of fully typed reagent red cells (Phenocell™ RRBC).

BACKGROUND

The purpose of antibody screening is to detect clinically relevant antibodies other than those naturally occurring (IgM) antibodies such as those of the ABO blood group system. These antibodies, commonly called unexpected red cell antibodies, are usually formed by immune exposure to foreign antigens found on red cells during blood transfusion or from foetal red cells during pregnancy. Screening for the detection of clinically relevant blood group antibodies is an integral part of the immunohaematological investigations performed on blood donor samples and on samples from patients undergoing antenatal and pretransfusion testing. Abtectcell™ screening cells are used for both patient and donor antibody screening. More specifically, they are used to detect unexpected red cell alloantibodies in the patient or donor serum. Abtectcell™ screening cells are carefully selected to express the antigens associated with most clinically relevant antibodies. Some antibodies react more strongly with cells displaying homozygous antigen expression than heterozygous cells, an effect called dosage. Antibodies in the Rh, Duffy and Kidd systems most commonly manifest dosage. Correct detection and identification of red blood cell antibodies is very important for the selection of appropriate blood for transfusion and in the investigation of HDFN, immune haemolytic anaemias and transfusion reactions.

SPECIMEN COLLECTION AND PREPARATION

Blood samples should be withdrawn by aseptic technique. As per the Guidelines for Transfusion and Immunohaematology Laboratory Practice, 1st edition 2016, serum or EDTA plasma may be used. EDTA whole blood samples can be stored at 18-25°C for up to 2 days from the time of venepuncture. EDTA whole blood or separated plasma/serum can be stored at 2-8°C for up to 7 days from the time of venepuncture. Separated EDTA plasma or serum for storage may be stored at –30°C for up to 3 months. Note: the use of stored serum or EDTA plasma may result in failure to detect complement-dependent antibodies.
For Abtectcell™ III C 0.8% refer to the CAT manufacturer's recommended sample collection requirements.

RECOMMENDED METHODS

Tube Method

Abtectcell™ III C 3% is designed for use in tube methods and may be used directly from the vial without further modification. Abtectcell™ III C 3% may be used by any of the validated tube methods in routine use in immunohaematology laboratories.

Generally methods used for antibody screening are those that are capable of detecting clinically relevant red cell antibodies. These include a direct 37°C incubation phase and an Indirect Antiglobulin Test (IAT), although alternative test methods and temperatures may be substituted if the method has been appropriately validated and documented.

A commonly used Low Ionic Strength Additive method is:

1. Appropriately label 3 separate, clean test tubes.
2. Add 2 drops of test serum to each tube.
3. Add 1 drop of the appropriate Abtectcell™ III C 3% screening cell to the appropriately labelled tube.
4. Centrifuge at low speed (500rcf) for 15 to 20 seconds*.
5. Gently resuspend the cell button. Read and record results.
6. Add 2 drops of enhancement reagent Immulab RAM (Rapid Antibody Medium) to each tube (refer to manufacturer's instructions).
7. Incubate at 37°C for 10 to 30 minutes (refer to manufacturer's instructions).
8. Centrifuge at low speed (500rcf) for 15 to 20 seconds*.
9. Gently resuspend the cell button. Read and record results.
10. Wash a minimum of three times in buffered saline.

11. Add 1 or 2 drops of Epiclone™ AHG Poly reagent to each tube.
12. Centrifuge at low speed (500rcf) for 15 to 20 seconds*.
13. Gently resuspend the cell button. Read and record results.
14. Add 1 drop of Immulab AHG Control Cells 3% to all negative tests tubes to validate the result.
15. Centrifuge at low speed (500rcf) for 15 to 20 seconds*. Read and record results.

Notes: *Or centrifuge at a speed and time appropriate for the centrifuge in use.

There is no requirement for the use of enhancement reagents in antibody detection tests however, routine procedures in the majority of laboratories utilise these reagents because they decrease incubation times and increase the sensitivity of the tests. Various enhancement reagents are available but the most widely used are Low Ionic Strength Solution (LISS) additives such as Immulab RAM.

Microplate Method
Due to the variation in methods and equipment, microplate users should validate these cells using their methods.

CAT Method
Abtectcell™ III C 0.8% RRBC are suitable and validated for BioVue™, Bio-Rad ID-System and Grifols DG Gel® CAT systems and may be used directly from the vial without further modification.

Patient or donor samples should be tested with each cell in the Abtectcell™ III C 0.8% RRBC screening cells according to the method recommended by the manufacturer of the CAT system in use.

Note: Abtectcell™ III C 3% may also be used by the CAT methods. The manufacturer's instructions should be followed and the correct diluent should be used, ensuring the final red cell concentration is correct.

INTERPRETATION OF RESULTS
Agglutination or haemolysis of one or more of the Abtectcell™ III C cell suspensions represents a positive result and indicates the presence of one or more antibodies in the test sample. Identification of such antibodies should be performed using an extended panel of fully typed RRBCs (Phenocell™). Absence of agglutination or haemolysis indicates the lack of detectable antibodies against antigenic determinants present on the screening cells.

CONTROLS
When performing antibody screening tests using Abtectcell™ III C 3%, all negative tube antiglobulin tests should be validated by the addition of cells weakly sensitised with IgG antibody (Immulab AHG Control Cells 3%). The inclusion of the appropriate controls is necessary to validate test results. It is recommended that users of Abtectcell™ III C 0.8% RRBCs follow CAT manufactuer's recommended procedures.

LIMITATIONS OF PROCEDURE
Antibody detection tests may be affected by:
1. Use of plasma or serum stored unfrozen for long periods.
2. Antibodies against low incidence or other antigens not represented on the screening cells may not be detected.
3. In very rare cases, the test serum may contain interfering antibodies active against components in the RRBC suspending medium.
4. RRBC should not be used for antibody detection beyond their expiry date.
5. RRBC may show some loss of antigen reactivity during storage. The rate at which reactivity is lost is partially dependent upon individual donor characteristics that are neither controlled nor predicted by the manufacturer. The P1, Le^a and Le^b antigens are those most likely to deteriorate during storage, but antibodies to these antigens are seldom of clinical significance. This loss of reactivity may increase if the cells are stored under conditions other than those recommended. The antigenic strength of the cell suspensions may be monitored during the dating period by testing against antisera of known strength.
6. Serum samples collected using tubes containing clot activators may result in complement activation.

False results may occur due to:
1. Incorrect technique.
2. Presence of gross rouleaux.
3. Use of aged blood samples, reagents or supplementary materials.
4. Contaminated blood samples, reagents or supplementary materials.
5. Red cells that have a positive DAT.
6. Other deviation from the recommended test methods.

PRECAUTIONS
1. For *in vitro* diagnostic use only.
2. CAUTION: HANDLE AS IF CAPABLE OF TRANSMITTING INFECTION. The material from which this product was derived was found to be non-reactive for specified markers for HIV 1 and 2, Hepatitis B and C, HTLV and Syphilis by currently approved methods. However no known method can assure that products derived from human blood will not transmit infectious agents.
3. Abtectcell™ III C 3% contains Neomycin Sulphate and Chloramphenicol as antibacterial agents and Thiomersal (0.001% w/v) as a preservative. Abtectcell™ III C 0.8% contains Trimethoprim and Sulfamethoxazole to retard bacterial contamination. Users should take appropriate precautions when handling and discarding these reagents.
4. Do not use if any of the red cell suspensions show signs of haemolysis. Do not use past expiry date. Take care to avoid contamination.

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	Consult instructions for use	IVD	*In vitro* diagnostic medical device	REF	Catalogue number		Temperature limitation		Manufacturer


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