Phenocell™ C Antibody Identification Panel

PRODUCT INFORMATION: 9

Product Description
bioCSL Phenocell™ C 3% and 0.8% Reagent Red Blood Cells (RRBCs) are ten-cell panels used for the identification of clinically relevant red cell alloantibodies (antibody identification). They are suitable for identifying antibodies detected during antenatal, donor screening and pretransfusion testing. Phenocell™ 3% (C Panel) is designed and validated for tube technique, as well as being validated for direct addition into Ortho-Clinical Diagnostics BioVue™ (BioVue™). Phenocell™ 0.8% (C Panel) is designed for Column Agglutination Technology (CAT) systems and is validated for direct addition into BioVue™ and DiaMed ID-Micro Typing System™ (ID-MTS™) and is suitable for direct addition into Grifols DG Gel™ CAT systems. bioCSL Phenocell™ C is shipped on a four week shipping cycle and has sufficient shelf life to ensure overlapping expiries.

<table>
<thead>
<tr>
<th>Catalogue No</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>96530201</td>
<td>10 x 3mL</td>
</tr>
<tr>
<td>86640201</td>
<td>10 x 3mL</td>
</tr>
</tbody>
</table>

Suitable Testing Platforms
✓ Column Agglutination Technology (CAT)
✓ Tube (Phenocell™ 3% - C Panel)

Product Design
• bioCSL Phenocell™ 3% (C Panel) is in an isotonic diluent designed for direct addition into tube technique and may also be used in OCD BioVue™ by the manufacturer’s method.
• bioCSL Phenocell™ 3% (C Panel) cells are suspended in bioCSL Celpresol™, and it is recommended that a LISS additive is used such as bioCSL RAM, which will allow a 10 minute incubation methodology.
• bioCSL Phenocell™ 0.8% (C Panel) is in a stabilised low ionic strength diluent for direct addition into commonly used CAT systems.
• Celpresol™ LISS is the diluent used in bioCSL Phenocell™ 0.8% (C Panel), which has a low ionic strength cell maintenance formulation that gives optimal specificity when used in OCD BioVue™, DiaMed ID-MTS™ and Grifols DG Gel™ CAT systems.

• Phenocell™ C 3% and 0.8% RRBCs consist of ten 3mL vials of Group O human red cell suspensions. These cells are from individual donors and are not pooled or frozen. Phenocell™ C 3% and 0.8% are designed to present antigens to detect antibodies of clinical significance globally. This includes variant MNS (MUT, Mur and Mi²) and Di².
• Phenocell™ C 3% and 0.8% will detect clinically significant anti-MUT, anti-Mur and anti-Mi², but will not detect clinically insignificant IgM antibodies. This reduces unnecessary work and costs by minimising the identification and subsequently crossmatching of clinically irrelevant antibodies.
• Where possible, rare or unusual phenotypes/antigens such as Fy(a-b-), Le (a-b-), Js¹, Kp¹, Co³, Lu¹, kk, R₁R₂ and R₂R₂ are represented.
• bioCSL Phenocell™ 3% and 0.8% are designed to be used with screening cells that are compliant with the ANZSBT Guidelines for Pretransfusion and Antenatal Testing and other international cell specifications eg. Guidelines for the Blood Transfusion Services in the United Kingdom and US FDA CFR660.33.

Benefits of bioCSL Phenocell™ C
• Global – Identifies red cell antibodies of clinical significance in Caucasian and Asian populations, as well as other ethnicities.
• Selective – Formulated from 10 donors carefully chosen to allow differential patterns for commonly encountered antibodies
• Specificity – Antigens chosen to optimally identify clinically significant antibodies Rh, Kell, Fy, Jk, MNS, P and Le blood groups, as well as variant MNS (vMNS) (Miltenberger) and Diego² (Di²) antigens
• Innovative – Utilising novel KODE™ technology to ensure accurate and enhanced antibody identification
• Improved Diluent Technology – Improved sensitivity, specificity and stability

This document includes:
• Product specifications
• Product design
• Recommended applications
• Technical performance notes
Phenocell™ C Antibody Identification Panel

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- **Flexibility** – Available in 0.8% concentration for Column Agglutination Technology systems and 3% for tube and BioVue™ CAT testing
- **Stability** – Made from fresh unfrozen red cells, for proven and consistent performance during the product’s shelf life
- **World’s First** – Identification panels designed to reliably detect antibodies of clinical significance in global and Asian-Pacific populations
- **Versatile** – May be used as daily identification panel or for performing specialised investigative work

### Detailed Cell Antigen Specifications

- Expresses: D, C, E, c, e, Cw, K, k, Kp*, Fyα, Fyβ, Jkα, Jkβ, M, N, S, s, MUT, Mur, Lea, Leβ, P1 and Di antigens.
- The MUT and Mur (Miltenberger) antigens are added using KODE™ technology.
- Homozygous expression for D, C, E, c, e, K, Fya, Fyb, Jka, and Jkb.
- Where possible Le(a-b-) and Fy(a-b-) phenotypes will be provided.
- Where possible Js, Lu, Co and k (cellano) negative cells will be provided.
- Where possible, one or more R,R or R,R cells will be provided. This will allow the differentiation of anti-E in the presence of anti-C antibodies, and anti-C in the presence of anti-e respectively.
- Where possible red cells will not express Bgα and Bgβ.
- All cells Direct Antiglobulin Test (DAT) tested by tube and Column Agglutination Technology (CAT).

### Background

Antibody screening is designed to detect clinically relevant (IgG) antibodies. These IgG antibodies are commonly called unexpected red cell allo-immune antibodies and are usually formed by immune exposure to foreign antigens found on red cells during blood transfusion or from foetal red cells during pregnancy. The unexpected red cell antibodies are usually either allo-antibodies or auto-antibodies and are normally only found in 0.3% to 2% of the population.

Once an unexpected antibody is detected in the antibody screening cells using bioCSL Abtectcell™, an antibody identification is performed to determine the antibody specificity and the clinical relevance of the antibody or antibodies found. In practice, this requirement is most easily met by the use of a carefully selected panel of fully phenotyped Group O red cells from different individuals that provides a specific antigenic pattern, such as those in bioCSL Phenocell™. The red cells are selected so that, a distinctive pattern of positive and negative reactions exists for each antigen.

bioCSL Phenocell™ makes it possible to identify clinically relevant alloantibodies that are most frequently encountered, such as Rh, Kell, Duffy and Kidd. Correct identification of red cell antibodies is important for the selection of appropriate blood for transfusion and in the investigation of potential haemolytic disease of the newborn, immune haemolytic anaemias and transfusion reactions. Patients with clinically relevant antibodies should, where possible, receive red cells that have been tested and found to lack the corresponding antigen.

### Special Notes on Variant MNS Antigens & Antibodies

Miltenberger is an obsolete name for a related group of glycophorin variant-based phenotypes that are common in Asian populations, but are somewhat rarer in Caucasian populations. These phenotypes are the result of mutated Glycophorin A and B molecules on human red blood cells which derive from genetic crossover events, where the resultant glycophorin can be a mixture of glycophorins A and B or are produced by a point mutation.

The ISBT recently modified the nomenclature of these phenotypes to the term GP (glycophorin) followed by a dot and the first 3 letters of the family name of the case e.g. GP.Mur from Murell. There is currently no formal name given to the group of variant MNS antigens that are rare in Caucasian populations but are common in Asian populations. For the purposes of this document we will use the term vMNS to describe this group of serologically related antigens formerly called Miltenberger or the Miltenberger series.

There are 11 Miltenberger phenotypes currently recognised and each phenotype has been shown to express 1 to 6 antigens on their red cells. Many antigens are common to more than one phenotype. The antigens that arise include: Mur, MUT, Hl, M1, Vw and others. These antigens have been found in relatively high incidences in every Asian population that has been studied so far. Table 1 shows the older Mi class phenotype nomenclature on the left followed by the new notation on the right. The antigens that are present on each phenotype are tabulated. As an example, the most common glycophorin variant phenotype found in Asian populations is GP.Mur (formerly Mi.III). This phenotype expresses Mi*, Mur, Hl, MUT and MINY antigens. The other common phenotype is GP.Bun (formerly Mi.VI) and this cell will express all of these antigens, but also Hop.
When an antigen negative individual is exposed to these vMNS cells by pregnancy or transfusion they may make antibodies to one or more of these antigens. The common alloantibodies detected in these cases are anti-MUT, anti-Mur and anti-Mia. IgM class antibodies are often seen and like an IgM anti-M these antibodies appear to be clinically insignificant. There are a significant number of published clinical cases of anti-MUT, anti-Mur and anti-Mia causing pathological effects including transfusion reactions and severe Haemolytic Disease of the Foetus and Newborn (HDFN). These clinical cases implicate IgG class antibodies. There are no published cases of clinical disease associated with IgM antibodies to antigens arising from genetic crossover events on GP.Mur positive cells.

- The common antibodies characterised as clinically relevant are MUT, Mur and Mia.
- Published clinical cases implicate strong examples of IgG class antibodies.
- IgG class antibodies cause HDFN and Haemolytic Transfusion Reactions (HTRs).
- IgM antibodies are not implicated in disease.

### bioCSL Phenocell™ C Technical Performance Notes

bioCSL Phenocell™ C antibody identification cells are manufactured with MUT and Mur antigens added to select cells using novel KODE™ technology. Cells modified in this way are known as kodecytes. This technology allows the specific addition of a functional red cell antigen epitope to a cell that does not naturally carry this antigen. bioCSL Phenocell™ C carry two of these epitopes (MUT and Mur) on separate cells to allow antibody identification. These epitope constructs are created in a way that can selectively detect IgG class antibodies. IgM class antibodies will not be detected and this greatly improves the specificity by failing to detect antibodies considered clinically irrelevant. Published studies demonstrate that less than 50% of MUT and Mur antibodies detectable with “natural” Mi are IgG and most examples detected with natural cells will be IgM and are considered clinically irrelevant.

- bioCSL Abtectcell™ III (C Panel) includes a kodecyte on a select cell that expresses MUT and Mur epitopes. The MUT/Mur cell will detect IgG MUT, Mur and Mi antibodies. The antibody presence can be confirmed and identified using the Phenocell™ C identification cell panel that has the MUT epitope on one cell and the Mur epitope on another.

### Table 1. Current Miltenberger phenotypes

<table>
<thead>
<tr>
<th>Mi Class</th>
<th>New Notation</th>
<th>Antigens</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>GP.Vw</td>
<td>Mi⁺ Vw</td>
</tr>
<tr>
<td>II</td>
<td>GP.Hut</td>
<td>-</td>
</tr>
<tr>
<td>III</td>
<td>GP.Mur</td>
<td>Mur⁺ HIL</td>
</tr>
<tr>
<td>IV</td>
<td>GP.Hop</td>
<td>Hut⁺</td>
</tr>
<tr>
<td>V</td>
<td>GP.Hil</td>
<td>-</td>
</tr>
<tr>
<td>VI</td>
<td>GP.Bun</td>
<td>-</td>
</tr>
<tr>
<td>VII</td>
<td>GP.Nob</td>
<td>-</td>
</tr>
<tr>
<td>VIII</td>
<td>GP.Joh</td>
<td>-</td>
</tr>
<tr>
<td>IX</td>
<td>GP.Dane</td>
<td>-</td>
</tr>
<tr>
<td>X</td>
<td>GP.HF⁺</td>
<td>-</td>
</tr>
<tr>
<td>XI</td>
<td>GP.JL</td>
<td>-</td>
</tr>
</tbody>
</table>

Adapted from Daniels G, Human Blood Groups, 2002.

*Originally called Hut                ¹GP.HF previously named GP.Mor                NT Not Tested
Published cases show that harmful antibodies are IgG and have a high titre (of 8 up to 1024), therefore clinically significant VMNS antibodies are considered to always contain strong examples of IgG class antibodies. The MUT and Mur cells are designed to identify these IgG class antibodies.

Papain has been shown to destroy many MNS antigens including M, N, S, s, MUT, Mur and Mi. The MUT and Mur antigens on bioCSL Phenocell™ (C Panel) are NOT sensitive to Papain and serological reactions are instead enhanced. This can be a very useful diagnostic feature.

DTT (Dithiothreitol) and 2-ME (2-Mercaptoethanol) are sulphydryl reagents that are sometimes used to denature the structure of IgM and prevent its ability to directly agglutinate red cells and these reagents may be used in autoabsorption procedures. They have been shown to denature antigens in the Kell system and act on the site of a cysteine residue to cleave disulphide bonds.

As KODE™ peptide constructs like MUT and Mur on select Abtectcell™ III and Phenocell™ panels utilise cysteine in the addition of the peptide onto the linker molecule, the MUT and Mur epitopes are denatured by the DTT treatment of the kodecytes either directly or by addition of DTT in sample preparations. The use of DTT treated plasma samples or the use of ZZAP eluates will yield negative serological reactions when tested against kodecytes.

What is KODE™ Technology?

KODE™ technology, incorporating CA (Carbohydrate Antigen) was developed by KODE Biotech Limited and the Biotechnology Research Institute at Auckland University of Technology and further developed and commercialised by bioCSL Immunohaematology.

The application of KODE™ technology in bioCSL Phenocell™ C enables MUT and Mur antigen epitopes to be added to select red cells that do not naturally carry these antigens. This technology creates unique FSL constructs that have a Functional group or epitope, a Spacer and a Lipid tail. The lipid tail inserts stably into the red cell bilipid membrane, allowing the functional epitope to project out from the red cell surface and be available for antibody interaction. This enables controlled antigen expression on red blood cells that do not naturally carry the antigen. Cells modified with KODE™ Technology are known as kodecytes and retain their normal vitality and functionality while gaining the new function of the inserted constructs.

Precautions

The material from which bioCSL Phenocell™ C antibody identification cells are derived is 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, therefore good laboratory practice requires safe handling procedures.

bioCSL Phenocell™ 3% (C Panel) contains Neomycin Sulphate and Chloramphenicol as antibacterial agents. bioCSL Phenocell™ 0.8% (C Panel) contains Trimethoprim and Sulfamethoxazole to retard bacterial contamination. Users should take appropriate precautions when handling and discarding these reagents.

For in vitro diagnostic use only.

Incorrect reactions may occur due to:

1. Failure to comply with the recommended procedures
2. Variations in time and temperature of incubation, centrifuge speeds and reaction reading methods
3. Contamination of test samples, reagents or supplementary materials
4. Use of aged or expired samples or reagents
5. Incorrect red blood cell suspension strengths
6. Use of DTT will denature KODE™ MUT and Mur constructs resulting in negative reactions.
Antibody Identification Flowchart

1. Antibody screen
   - Neg
   - Pos

2. ID Panel by same method
   - No
   - Yes

3. Is Auto negative?
   - Yes
   - No

4. Autoantibody
   - Consider autoadsorption

5. An appropriate exclusion method

6. Have you identified an antibody?
   - No
   - Yes

7. Have you excluded all other clinically relevant antibodies?
   - No
   - Yes

8. Phenotype patient
   - Can the patient make the alloantibody?
     - Yes
     - No

9. Phenotype donor units
   - Select antigen negative units
     - Yes
     - No

10. IAT crossmatch
    - Issue blood

Notes on the flowchart
1. Antibody screen is positive
2. Perform antibody ID panel test by IAT technique with an Autocontrol, Positive control and AntiH Control Cells added to each negative test
3. Results recorded on antigen composition sheet and appropriate exclusion method performed
4. Antibody screen is positive
5. Antibody screen is positive
6. Antibody screen is positive
7. Antibody screen is positive
8. Antibody screen is positive
9. Antibody screen is positive

- Papain
- Cell selection for antibody exclusion
- PEG
- Other panels
- Cell selection for antibody exclusion
- Long incubation
- Other IAT methods (eg. CAT/tube)
- AET
- DTT
- Other temperatures
- Cord cells
- Inhibition

Still confused?
Send sample to reference laboratory
If blood is required, refer to documented laboratory policy

6. Have you met the ‘Rule of 3’s’ – 3 positive reactions on antigen positive cells and 3 negative reactions on antigen negative cells – Yes
7. Can the patient make this allo-antibody? Phenotype the patient – patient is antigen negative – Yes
8. If blood is required for transfusion, phenotype and select antigen negative donor units
9. Crossmatch using an IAT method and issue if compatible.
Storage and Handling
Store at 2° to 8°C (Do Not Freeze)
Refrigerate at 2° to 8°C when not in use
Take appropriate precautions to maintain sterility
Do not use if:
1. Signs of gross haemolysis are present in red cell suspensions or the simulated plasma component is turbid
2. Expiration date has passed

bioCSL Phenocell™ suspensions do not need to be washed and are ready for use after gentle mixing to resuspend the cells

Sample Collection
Blood samples should be withdrawn by aseptic technique. Serum or EDTA plasma may be used. Serum should be separated from the clot as soon as possible and stored at −20°C if testing is delayed.

Note: the use of stored serum or EDTA plasma may result in failure to detect complement-dependent antibodies. Use of serum may result in in vitro activation of complement resulting in positive reactions in Polyspecific AHG CAT systems. Please refer to the CAT manufacturer’s recommended sample collection requirements.

October 2013

References
5. Australian and New Zealand Society of Blood Transfusion Inc. Guidelines for Pretransfusion Laboratory Practice. 5th Ed. 2007.
13. DiaMed CAT method, please refer to the DiaMed ID-MTS™ ID-Card “LISS/Coombs” or appropriate product leaflets.
14. BioVue™ CAT method, please refer to the Ortho BioVue™ “Poly Cassette” or appropriate product leaflets.