CMV Brite™ Turbo Kit

Rapid CMV pp65 Antigen for the detection of active CMV infection

Intended use
Infections with human cytomegalovirus (CMV), a β-herpes virus, are widespread throughout the world (rate of prevalence 40-100%). While an infection with CMV proceeds asymptomatically in the majority of immunocompetent persons, it can lead to serious complications in persons whose immune system has been weakened or is not yet fully developed (hepatitis, retinitis, pneumonia etc.). CMV is the most frequent pathogens of congenital infections. Approximately 10% of children congenitally infected with CMV show symptoms at birth (icterus, hepatosplenomegaly, petechial bleeding and chorioretinitis). Further groups of patients for whom acute CMV infection represents a serious threat are recipients of organ and bone-marrow transplants and AIDS patients. The CMV Brite™ Turbo Kit is intended for the rapid qualitative detection of CMV lower matrix protein pp65 by indirect immunofluorescence using microscopy in isolated peripheral blood leukocytes obtained from ethylenediaminetetraacetic acid (EDTA) or heparin anti-coagulated human peripheral blood. The CMV antigenemia assay has been developed using a cocktail of two monoclonal antibodies (C10/C11) directed against pp65 [2]. The CMV antigenemia assay is valuable in the diagnosis of active CMV infection. Shell vial cultures provide a result within 1 to 2 days, but are not sensitive for detection of CMV in blood specimens. In contrast, detection of CMV due to peripheral blood polymorphonuclear leukocytes (PMN) (CMV antigenemia) is both sensitive and rapid [1, 2]. This technique uses monoclonal antibodies to detect the CMV lower matrix phosphoprotein (pp65), an active antigen associated with replication, that is abundant present in antigen-positive PMNs [2-5]. The CMV Brite Turbo assay can be completed within 2 hours of sample collection.

Principle of the test – immunofluorescence test -

The CMV Brite™ Turbo method consists of:

a. direct lysis of peripheral blood erythrocytes
b. preparation of cytospin slides
c. fixation and permeabilization
d. indirect immunofluorescence staining using monoclonal antibodies directed against CMV pp65 protein.
e. reading and evaluation of results

The first step in the CMV Brite™ Turbo method involves direct lysis of the peripheral blood leucocytes [6]. Following leucocytes are cytocentrifuged onto a slide, fixed and permeabilized to allow subsequent detection of pp65 antigen. The presence of pp65 is detected by the C10/C11 anti-CMV antibody. CMV antigen-positive leukocytes exhibit homogeneous yellow-green polylobate nuclear staining when observed using a fluorescence microscope. The number of CMV antigen-positive cells is counted per duplicate stain.

Kit contains

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A (A)</td>
<td>200 ml</td>
</tr>
<tr>
<td>Fixative solution (formaldehyde in PBS, sodium azide &lt; 0.1%)</td>
<td>290 ml</td>
</tr>
<tr>
<td>Reagent B (B)</td>
<td>290 ml</td>
</tr>
<tr>
<td>Reagent C (C), Permeabilization solution</td>
<td>4 ml</td>
</tr>
<tr>
<td>Reagent D (D), Monoclonal antibody (mouse), Mix of C10/C11 (IgG1) and C11 (IgG3)</td>
<td>4 ml</td>
</tr>
<tr>
<td>Control Slide, CMV antigenia control microslide</td>
<td>1 x 14</td>
</tr>
<tr>
<td>Control Slide, CMV antigenia control microslide</td>
<td>5 x 1</td>
</tr>
</tbody>
</table>

Laboratory material required and not included in the Kit

Laboratory centrifuge; 50 ml conical bottomed centrifuge tubes; Sterile pipettes, micropipette and tips; phosphate buffered saline (PBS), pH 7.4, Ca, Mg free; Hemocytometer; Automated cell counter; Cytocentrifuge slides; Cytocentrifuge (such as Shandon Southern Products, Ltd., model Cytopsin 3); Coplin jars or histology staining jars; Humid chamber; 37 °C incubator; Fluorescence microscope capable of 2500x, 600x and 400x magnification; Mounting medium (non-fluorescent, such as Citifluor, Glycerol PBS solution, UKC Chem Ltd); Immunocytocent Mounting Medium, Catalogue # 111); Fume Hood; Micro cover glass; Stop watch/Timer.

Warning and precautions
Do not incorporate reagents. Avoid contact with eyes and skin. All samples and materials used for the test must be treated as being potentially infectious and appropriate safety precautions taken. In the preparation of the CMV Brite™ Turbo Control Slides, leukocytes have been used obtained from healthy human blood donor. Each donor sample has been tested and found reactive for the presence of antibodies to HIV-1, HIV-2, HCV and CMV. In contrast, the CMV Brite™ Turbo Control Slide, leukocytes have been used obtained from a healthy human blood donor.

Test Procedure

I. Preparation of leucocyte suspension

- Dilute Reagent A 1:10 in demineralized (distilled) water and allow to cool to 4 °C.
- Mix 2 ml blood with 30 ml of cold (+4 °C) diluted erythrocyte lysing solution in a 50 ml conical bottomed tube and incubate for 5 minutes at 4 °C.
- Centrifuge for 2 minutes at 1000xg (2500 rpm). Discard the supernatant.
- Repeat the cold lysing step if lysing of erythrocytes is not sufficient after the first time.
- Resuspend the cell pellet in 30 ml PBS.
- Centrifuge for 2 minutes at 1000xg (2500 rpm). Discard the supernatant.
- Resuspend the pellet in 1 ml PBS. (In patients with severe neutropenia re-suspension in as low as 0.2 ml may be required.)

II. Cell counting

- Count cells using a Hemocytometer or automated cell counter.
- Adjust concentration to 2 x 10⁶ cells/ml by diluting in PBS.

III. Preparation of cytocentrifuge slides

- Centrifuge 100 µl of the 2.0 x 10⁶ cells/ml suspension against CMV pp65 protein.
- Prepare at least 3 slides per patient specimen (2 testing, plus an additional slide for quality control).
- Let slides dry for approximately 5 minutes.
- Circle cell area on the slide using a laboratory marker pen.
- Slides can be kept room temperature overnight before fixation.

IV. Fixation and permeabilization

- Dilute fixative (reagent B): 1: 5 in PBS in a fume hood prior to use.
- Dilute permeabilization solution (reagent C): 1: 5 in PBS in a fume hood prior to use. (Do not reuse.)
- Immune 2 slides in diluted reagent B for 5 minutes at room temperature in the fume hood.
- Dip slides 3 times in PBS (washing solution) and leave in the washing solution for 3 minutes.
- Dip slides in diluted reagent C for 1 minute at room temperature.
- Dip slides 3 times in washing solution and place in fresh washing solution for 5 minutes (or any time up to 60 minutes). If staining with monoclonal antibody is to follow directly then proceed to step V.
- If slides are to be stored then rinse in demineralized (distilled) water for 15 seconds. Let slides air dry in a fume hood.
- Once dry, slides should be packed in aluminum foil and stored at 4 °C for 24 hours, or frozen at -80 °C.

V. Immunofluorescence staining

- From this point on, do not allow the cell preparations to dry out during the remainder of the staining procedure.
- Place 5 µl of C10/C11 (IgG1) and C11 (IgG3) respectively on the cell spot. Place C11-4 µl (mouse), Mix of C10/C11 (IgG1 and IgG3) after lower matrix protein and sodium azide 0.1% (Ready to use)
- Apply 35 µl conjugate (reagent E), incubate for 20 minutes at 37 °C in a humid chamber.
- Dip slides 3 times in washing solution (PBS) and put in fresh washing solution for 3 minutes.
- Remove one slide at a time from the washing solution, carefully dry the area surrounding the cell spot.
- Apply 35 µl conjugate (reagent E), incubate for 20 minutes at 37 °C in a humid chamber.
- Wash twice in fresh PBS and carefully rinse with tap water (3 times) and mount with mounting medium and a micro cover glass.

VI. Reading

Perform reading as soon as possible. Slides may be stored for up to 8 hours at 4 °C, protected tightly. Perform microscopic evaluation using an immunofluorescence microscope at 400x magnification. A higher magnification of 1000x may be used to increase resolution. (See note on recommendations for the use of high power objective and fluorescence cube under “Labware and material and equipment”). Scan the whole surface of the spot. Two spots should be scanned per patient. Positive cells show homogeneous yellow-green polychromatic nuclear staining and negative cells show no yellow-green nuclear staining. Equivocal readings due to artifacts may occur rarely (less than 5%). The most common artifact is due to eosinophils. They are recognized by a characteristic “snowflake” nuclear appearance. If all

and consult a doctor. The test must be performed by well-trained and authorized laboratory technicians. Testing is performed under aspetic and microbiologically controlled conditions. Please contact the manufacturer if the original test kit is damaged.

Storage

Upon receipt, store reagents at 2-8 °C. Avoid direct sunlight. Reagents stored according to stated storage instructions are stable until the expiration date indicated on the label. For repeatedly testing store the reagents immediately after usage at 2-8 °C.

Processing of the blood sample

Collect CMV antigenemia assay is valuable in the diagnosis of active CMV infection. Shell vial cultures provide a result within 1 to 2 days, but are not sensitive for detection of CMV in blood specimens. In contrast, detection of CMV due to peripheral blood polymorphonuclear leukocytes (PMN) (CMV antigenemia) is both sensitive and rapid [1, 2]. This technique uses monoclonal antibodies to detect the CMV lower matrix phosphoprotein (pp65), an active antigen associated with replication, that is abundant present in antigen-positive PMNs [2-5]. The CMV Brite Turbo assay can be completed within 2 hours of sample collection.

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cells appear greenish, this represents an artifact, which may be associated with some types of patients and is very rare. A greenish staining at cells at the periphery of the spot only, is not considered positive. This may occur if the spot has started to dry out during incubation with either monoclonal antibody solution or conjugate solution. Be sure to incubate in a humid chamber and check that the whole cell spot is covered with the reagent. If slides are not interpretable due to equivocal readings of artifacts, stain backup slides or test an additional patient specimen. In order to determine that a result is negative. If equivocal readings due to artifacts occur or if positive staining, the results should not be interpreted. Stain back-up slides or obtain and test an additional patient specimen.

**Quality Control**

The positive and negative control slides are provided with the kit. The slides are only used to check the staining procedure and do not influence the diagnostic value of the kit. The positive control must demonstrate appropriate staining before patient specimens can be evaluated. The positive control should exhibit homogeneous yellow-green staining of positive cells with round morphology (nucleus is not visible). The negative control should show no yellow-green staining. Despite the fact that the quality procedure is strictly, single positive cells could be observed in the negative control. In such cases the staining run should not be considered invalid. The single positive cells on the negative control do not mean that the slide with patient material cannot be interpreted.

**Choice of microscope**

Selecting the correct filter cube configuration, which matches the fluorochrome in use, is important. This will vary for each microscope manufacturer. Select the correct combination for FITC use, such as the Olympus BX-40 or BX-60 microscope with: Fluorescence cube (U-MNB), Excitation wavelength (470-490 nm), Emission wavelength (> 515 nm).

**Reading and interpretation of result**

Results of evaluation of patient specimen slides are qualitative. Positive result: one or more CMV antigen-positive cells present per duplicate slide. Negative result: no CMV antigen-positive cells present. Stain. Negative result: no CMV antigen-positive cells present per duplicate slide. A minimum of approximately 50,000 specimen cells should be present in order to determine that a result is negative. If equivocal readings due to artifacts occur or if positive staining, the results should not be interpreted. Stain back-up slides or obtain and test an additional patient specimen.

**Limitations of the procedure**

Detection of CMV pp65 lower matrix, early structural protein should be performed by laboratories experienced in immunocytochemical techniques. Leukocyte preparation should be performed by personnel experienced in aseptic techniques. The efficacy of the CMV Brite™ Turbo Kit with samples other than human peripheral blood leukocytes has not been established. Test performance characteristics have been established using the CMV Brite™ Kit (EDTA and heparin specimens) and validated in internal studies using the CMV Brite™ Turbo Kit. The CMV Brite™ Turbo Kit is intended for use with either monoclonal antibody solution or conjugate solution.

**Cross reactivity**

For the analysis of cross reactivity clinical isolates of the following viruses were tested: Herpes virus type 1 and type 2, Varicella-zoster virus, Adenovirus 2, 4 and 5, Parainfluenza virus 1, 2 and 3, Respiratory syncytial virus, Poliovirus 3 (wild type 3), Echovirus 11, Echovirus 30, Epstein-Barr virus (laboratory strain P3HR1). Human immunodeficiency virus type 1 and 2 (laboratory strain 89GR). Human inactivated virus type 6 (laboratory strain GS). Human immunodeficiency virus type 1 (type 1 commercially available IF slides). No cross reactivity was observed except for a weak positive reaction with six of the seven HSV-1 isolates in the shell vial assay. The weak staining observed with the six HSV-1 isolates was cytoplasmic (i.e., seen only outside the nucleus, in a limited number of small foci), similar to the IF pattern usually found with HSV-1 monoclonal antibodies. The weak staining observed may have been due to Fe receptors being expressed by the HSV-1 infected cells in the shell vial assay. Subsequent analysis concluded that there is no evidence of cross reactivity between HSV and the CMV antigenemia assay using the C10/C11 monoclonal antibody cocktail.

**Literature**


The performance of the CMV Brite™ Turbo Kit was validated in a study of 183 patient samples. The patient samples included 173 organ transplant patients, 9 immunocompetent patients and 1 HIV positive patient sample. Each patient was tested in parallel with the CMV Brite™ and the CMV Brite™ Turbo Kits. The results of this validation study are provided in the table below.

**Comparison of CMV Brite Kit with CC and SV**

<table>
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<th>-</th>
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<tbody>
<tr>
<td>+</td>
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<td>-</td>
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<td>205</td>
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</tr>
<tr>
<td>total</td>
<td>93</td>
<td>239</td>
<td>331</td>
<td>524</td>
</tr>
</tbody>
</table>

**Sensitivity:** 31/34 = 91.2% (95% CI = 76.3 to 98.1%)

**Specificity:** 205/239 = 86.6% (95% CI = 70.2 to 81.5%)

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**CMV Brite™ Turbo Kit**

<table>
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<tr>
<td>total numbers</td>
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