



# CMV Control slides

*CMV pp65 positive and negative cytopots*

**REF**<sup>9</sup> VIR-CMV CS05    ▾ 5 slides    **i** Product Information File (PIF)

**IVD** **CE**0344 ***In Vitro Diagnostic medical device***

**Attachment: Package Insert CMV Brite™ Turbo kit**

This product is registered as "in vitro diagnostic use" in the countries that belong to the European Community. In all other countries it should be labeled "for research use only".

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## Control slides – CMV Brite™ Turbo kit

This product is registered as “in vitro diagnostic use only” as a component of the CMV Brite™ Turbo kit in the countries that belong to the European Community. All obligatory and necessary information as specified in Directive 98/79/EC needed to use this product safely and properly, can be found in the product manual of the CMV Brite™ Turbo kit (please see attachment).

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### Description

Control slides are CMV antigenemia control microscope slides. These slides are a component of the CMV Brite™ Turbo kit which can be used as a control in the fluorescence staining of the CMV Brite Turbo kit. Slides are provided in a sealed pouch with desiccant and are ready to use.

### Form

Microscope slides with a positive and negative cytospot. Positive cytospot contains pp65 positive sf9 cells. Cells are fixated.

### Applications

Slides can be used as a control in the fluorescence staining of the CMV Brite Turbo™ kit.

### Usage

The positive and negative control slides are provided with the CMV Brite Turbo™ 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 procedures are followed 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.

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### Handling and Storage

Control slides are stable for the period shown on the box label when stored properly.

**Warranty** Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product.

### Characterization

To ensure consistently high-quality, each batch of CMV control slides is tested for conformance.

**Warning** Product contains glass.

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## Attachment : Package Insert - CMV Brite™ Turbo Kit

Rapid CMV pp65 Antigenemia for the detection of active CMV infection

**REF** VIR-CMV 110

**▽** 110 Tests

**IVD** **CE**0344

### Intended use

The CMV Brite™ Turbo Kit is intended for the rapid qualitative detection of Cytomegalovirus (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 detection of CMV pp65 in human peripheral blood cells aids in the diagnosis of acute or reactivated CMV infection. This product is not FDA cleared (approved) for use in testing (i.e. screening) of blood or plasma donors.

### Summary and Explanation

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 asymptotically in the majority of immuno-competent persons, it can lead to serious complications in persons whose immune system has been weakened or is not yet fully developed (hepatitis, retinitis, pneumonie etc.). CMV is the most frequent pathogen 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 an acute CMV infection represents a serious threat are recipients of organ and bone-marrow transplants and AIDS patients. 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 antigen in peripheral blood polymorphonuclear (PMN) cells (CMV antigenemia) is both sensitive and rapid [1, 2]. This technique uses monoclonal antibodies to detect the CMV lower matrix phosphoprotein (pp65), an early antigen in virus replication, that is abundantly 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 erythrocytes [6]. Following lysis the leukocytes 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 antibody cocktail and visualized by means of a specific secondary FITC-labeled 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


<b>LYS</b>	200 ml	<b>Reagent A (A)</b> , Erythrocyte lysing solution (Ammonium chloride solution, sodium azide < 0,1%) Concentrate: dilute 1:10 with demineralized water ⚠ <b>WARNING</b>
<b>FIX</b>	290 ml	<b>Reagent B (B)</b> , Fixative solution (formaldehyde in PBS, sodium azide < 0,1% ) Concentrate: dilute 1:5 with PBS ⚠ <b>DANGER</b>
<b>PER</b>	290 ml	<b>Reagent C (C)</b> , Permeabilization solution (Igepal Ca-630, newborn calf serum in PBS, sodium azide < 0,1%), Concentrate: dilute 1: 5 with PBS
<b>MAB</b>	4 ml	<b>Reagent D (D)</b> , Monoclonal antibody (mouse), Mix of C10/C11 (IgG <sub>1</sub> /IgG <sub>1</sub> ) against lower matrix protein pp65. sodium azide < 0,1% (Ready to use)
<b>CONJ</b>	4 ml	<b>Reagent E (E)</b> , FITC-conjugated sheep anti-mouse-immunoglobulins with Evans Blue, sodium azide < 0,1%. (Ready to use)
<b>CONTR</b>	5 x 1	<b>Control Slide</b> , CMV antigenemia control microscope slides. Control slide in a sealed pouch with desiccant. (Ready to use)

### 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 or automated cell counter; Cytocentrifuge slides; Cytocentrifuge (such as Shandon Southern Products, Ltd., model Cytospin 3); Coplin jars or histology staining jars; Humid chamber; 37 °C incubator; Fluorescence microscope capable of 250x to 1000x magnification; Mounting medium (non-fluorescent, such as Citifluor, Glycerol PBS solution, UKC Chem Lab; or Immunoconcept 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 a healthy human blood donor. Each donor sample has been tested and found non-reactive for the presence of antibodies to HIV-1 , HIV-2, HCV and CMV as well as for HBsAg. Do not pipette with mouth. According to good laboratory practice wear gloves, laboratory coat and safety glasses. Liquids and non-combustible materials should be decontaminated with sodium hypochlorite (final concentration: 3%, activity time at least 30 minutes). Liquid waste which contains acids must be neutralized before disposal. All materials that are to be reused must be autoclaved for 1 hour at 121 °C. **LYS** contains Ammonium chloride. ⚠ H302 Harmful if swallowed; H319 Causes serious eye irritation. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **FIX** contains less than 9.3% formaldehyde. Formaldehyde is a highly toxic allergenic and potentially carcinogenic reagent, which should be handled in accordance

with good laboratory practices using appropriate precautions. Avoid skin or eye contact.  H301 + H311 + H331 Toxic if swallowed, in contact with skin or if inhaled; H314 Causes severe skin burns and eye damage; H317 May cause an allergic skin reaction; H335 May cause respiratory irritation; H351 Suspected of causing cancer; H370 Causes damage to organs. P260 Do not breathe dust/ fume/ gas/ mist/ vapours/ spray; P280 Wear protective gloves/ protective clothing/ eye protection/ Face protection; P301 + P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician; P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P310 Immediately call a POISON CENTER or doctor/ physician. **CONJ** contains Evans Blue. Avoid contact with eyes, skin, clothing. Keep container tightly closed. Wash thoroughly after contact with **FIX** and **CONJ** and consult a doctor. The test must be performed by well-trained and authorized laboratory technicians. Testing is performed under aseptic 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 between 3 to 5 ml venous blood into an EDTA-treated tube, using aseptic venipuncture. Send the sample to the laboratory without delay. The blood sample should be kept at room temperature (20-25 °C) until processing. Processing should be performed within 6 to 8 hours of sample collection since it has been shown that a decrease of antigen-positive cells (collected in heparin) can be found after storage [7-8]. Therefore, immediate preparation of the slides should always be attempted. In patients with severe neutropenia (absolute neutrophil count less than 200/mm<sup>3</sup>) at least 10 ml of blood may be required. If samples are to be transported, they must be packed in accordance with legal requirements for the transportation of infectious materials.

### Test Procedure

**The protocol has to be followed strictly.** Unless stated otherwise, reagents (including PBS) should be at room temperature when used in the test procedure. Room temperature is defined as 20-25 °C.

#### I. Preparation of leukocyte suspension

- Dilute Reagent A 1:10 in demineralized (distilled) water and allow to cool to 2-8 °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 2-8 °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 2.0 x 10<sup>6</sup> cells/ml by diluting in PBS.

#### III. Preparation of cytocentrifuge slides

- Centrifuge 100 µl of the 2.0 x 10<sup>6</sup> cells/ml suspension at approx. 600 rpm (54x g) for 4 minutes onto glass slides by cytocentrifuge.
- Prepare at least 3 slides per patient specimen (2 for testing, plus an additional slide for backup).
- Let slides dry for approximately 5 minutes.
- Circle cell area on the slide using a laboratory marker pen.
- Slides can be kept at 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.)
- Immerse 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.
- Immerse 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.

##### To store slides:

If slides are to be stored then rinse in demineralized (distilled) water for 15 seconds. Let slides dry under the ventilator for approximately 20 minutes. Once dry, slides should be packed in aluminum foil and stored at 2-8 °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.
- CMV Brite™ Turbo Control slide. Rehydrate control slide in PBS for 1 or 2 minutes.
- Remove one slide at a time from the washing solution, carefully dry the area surrounding the cell spot.
- Apply 35 µl of C10/C11 MoAb solution (reagent D) 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 2-8 °C covered tightly in order to minimize fading. 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 microscope and fluorescence cube under "Laboratory material and equipment".) Scan the whole surface of the spot. Two spots should be scanned per patient. Positive cells show homogenous yellow-green polylobate nuclear staining. 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 nonspecific cytoplasmic staining, with the nucleus appearing as black holes, often spectacle-shaped. The cytoplasm may appear dull and more yellow. If all cells appear greenish, this represents an artifact, which may be associated with some types of patients and is very rare.

A greenish staining of 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 slide(s) or if unresolved obtain and test an additional 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 procedures are followed 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 stain. **Negative result:** no CMV antigen-positive cells present per duplicate stain. 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 on both duplicate stains, 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 personal experienced in aseptic techniques. The efficacy of the CMV Brite™ Turbo Kit with samples other than human 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 (EDTA specimens only). The CMV Brite™ Turbo Kit is intended for use only with immunofluorescent microscopy and not for use with flow cytometry. The type and condition of the instrumentation used will influence the visual appearance of the image obtained. The reaction may vary due to the type of microscope employed, the light source, age of the bulb, filter assembly and filter thickness, differences in sensitivity of the antigen substrate, or the assay procedure used. Each laboratory should establish its own criteria for the reading of patient specimens using appropriate controls. The detection and confirmation of CMV pp65 lower matrix, early structural protein in peripheral blood leukocytes is not diagnostic of symptomatic illness, since CMV pp65 may be present for a significant period following acute infection and patients with CMV antigenemia (especially low levels) may have asymptomatic illness. There are many published reports of patients who are antigenemia-negative and viremia-positive and some of these patients may have CMV disease. Thus, a negative antigenemia test does not absolutely exclude CMV infection or disease. The CMV Brite™ Turbo Kit is not intended for antiviral drug monitoring.

Test performance characteristics have not been established using neonatal specimens. A decrease can be noted of antigen-positive cells (collected in heparin) per slide after storage [7-8]. Therefore, immediate preparation of the slides should always be attempted. The maximal specimen storage time at room temperature has not been determined. In patients with severe neutropenia (absolute neutrophil count less than 200/mm<sup>3</sup>) at least 10 ml of blood may be required. Since the monoclonal antibodies have been prepared using a prototype strain, they may not detect all antigenemic or new strains of CMV. For example, monoclonal antibodies may fail to detect strains of CMV which have undergone minor amino acid changes in the target epitope region. Test performance characteristics have not been established using specimens collected in heparin.

### Performance characteristics

The CMV Brite™ Kit was compared to CMV virus detection in conventional culture (CC) and shell vial culture (SV) using human peripheral blood leukocytes. 300 clinical samples were evaluated in comparison to conventional culture (CC) and shell vial culture (SV). A total of 300 venous blood samples were collected from transplant recipients (159 allogeneic bone marrow and 47 solid organ), 85 HIV positive/AIDS patients, and 9 immunocompetent patients. 92 samples (30.7%) were determined to be CMV positive by the CMV Brite™ Kit. In random blood donor populations, the number of specimens found repeatedly reactive for CMV antigenemia by the CMV Brite™ Kit has typically been 0%. Note: The performance characteristics have been established using the CMV Brite™ Kit and validated in internal studies using the CMV Brite™ Turbo Kit.

### Comparison of CMV Brite™ Kit with CC and SV

CC/SV	CMV Brite Kit		total
	+	-	
+	31	3	34
-	61	205	266
total	92	208	300

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

Specificity:  $205/266 = 77.1\%$  (95% CI = 70.2 to 81.1%)

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.

CMV Brite™ Turbo Kit	CMV Brite™ Kit		Total numbers
	+	-	
+	43	7	50
-	6	127	133
Total numbers	49	134	183







### 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 herpes virus type 6 (laboratory strain GS), Human immunodeficiency virus (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 Fc 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

1. Van der Bij, W., Schirm, J., Torensma, R., Van Son, W.J., Tegzess, A.M., The, T.H. (1988) Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. *J. Clin. Microbiology* 26, 2531 - 2535.
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9. NEN EN ISO 15223-1 Medical devices - Symbols to be used with medical device labels, labeling and information to be supplied - Part 1: General requirements.

### Explanation of used symbols

	Consult instructions for use
	Catalogue number
	Sufficient for
	In Vitro Diagnostic medical device
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
	For Research Use Only
	Batch code
	Use by yyyy-mm-dd
	Manufacturer
	Authorized Representative in the European Community
	Conformité Européenne (European Conformity)

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ENG

VIR-CMV 110 Version 5