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First experiences with an accelerated CMV antigenemia test: CMV Brite Turbo assay

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Abstract

Background: Cytomegalovirus disease is still a major problem in immunocompromised patients, such as bone marrow or kidney transplantation patients. The detection of viral antigen in leukocytes (antigenemia) has proven to be a clinically relevant marker of CMV activity and has found widespread application. Because most existing assays are rather time-consuming and laborious, an accelerated version (Brite Turbo) of an existing method (Brite) has been developed. The major modification is in the direct lysis of erythrocytes instead of separation by sedimentation. *Objective:* In this study the Brite Turbo method has been compared with the conventional Brite method to detect CMV antigen pp65 in peripheral blood leukocytes of 107 consecutive immunocompromised patients. *Results:* Both tests produced similar results. Discrepancies were limited to the lowest positive range and sensitivity and specificity were comparable for both tests. *Conclusions:* Two major advantages of the Brite Turbo method could be observed in comparison to the original method: assay-time was reduced by more than 50% and only 2 ml of blood was required. An additional advantage was the higher number of positive nuclei in the Brite Turbo method attributable to the increased number of granulocytes in the assay. Early detection of CMV infection or reactivation has become faster and easier with this modified assay. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: CMV; Antigenemia; pp65

1. Introduction

Cytomegalovirus (CMV) primary infections and reactivations are still responsible for major disease in transplant recipients and other immunocompromised patients. CMV causes a wide range of clinical manifestations varying from fever only to life-threatening organ failure (Baughman, 1997; Hebart and Einsele, 1998). In transplant recipients CMV infection can mimic the symptoms of allograft rejection, while differentiation is crucial because intensification of immunosuppression would only aggravate CMV infection. Early diagnosis and antiviral treatment is essential to

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prevent full-blown CMV disease and a fast, sensitive and specific assay is therefore instrumental to monitor patients at risk.

About a decade ago an assay came into use, based on the detection of the CMV lower matrix protein pp65 (The et al., 1990). The presence of this protein in peripheral blood polymorphonuclear cells (PMN) appeared strongly associated with an active CMV infection. This assay, commonly referred to as 'antigenemia assay', has found wide acceptance as a reliable tool for early detection of active CMV infection and is clearly more sensitive than culture of urine or throat swabs, and often also superior to culture of peripheral blood leukocytes (Erice et al., 1992; Boekh and Boivin, 1998). Results of this test are well comparable to various PCR methods detecting CMV nucleic acid, while its established correlation with CMV-associated disease is a particular advantage (Lo et al., 1997; Boekh and Boivin, 1998). PCR, however, might be more useful in patients with a low leukocyte count in the peripheral blood (Boekh et al., 1997). Although relatively easy to perform, a disadvantage of the assay is its rather time-consuming and laborious nature.

Many technical modifications have been introduced, since the introduction of this pp65 assay, in an effort to improve its performance (The et al., 1995). Recently, a modified version of an existing commercial assay, CMV Brite (IQ Products, Groningen, The Netherlands) became available. This assay, CMV Brite Turbo (IQ Products, Groningen, The Netherlands), mainly differs in the isolation procedure of the leukocytes. In the Brite Turbo method erythrocytes are lysed in contrast to the Brite in which leukocytes are isolated by dextran sedimentation of erythrocytes (Ho et al., 1998). The leukocyte input is higher in the Brite Turbo (2.0×10^6/ml compared with 1.5×10^6/ml in the original Brite method). Furthermore, fixation steps, permeabilization steps, washing steps and incubation times are kept to an absolute minimum. In this study the results of 107 consecutive blood samples of immunocompromised patients were compared, using the two different assays mentioned above.

2. Materials and methods

One hundred and seven blood samples (EDTA) were drawn from 71 solid organ transplantation recipients, 30 bone marrow transplantation or peripheral stem cell transplantation recipients and six otherwise immunocompromised patients receiving immunosuppressive therapy. All samples were evaluated for the presence of pp65 in both the original CMV Brite and the CMV Brite Turbo assay. Both assays were performed according to the manufacturer's instruction. The procedure for the original Brite was described earlier (Landry et al., 1996). In the Brite Turbo 2 ml EDTA blood was mixed with 30 ml of lysing solution (ammonium chloride containing 0.01% sodium azide) and incubated for 5 min at room temperature. This mixture was centrifuged at $1000 \times g$ and the cells were washed in phosphate buffered saline (PBS) and resuspended in 1 ml PBS. Leukocytes were counted and the percentages of granulocytes were determined in an automated cell-counter (Cell-Dyn® 1700, Abbott Laboratories, North Chicago, IL) and adjusted to a concentration of 2×10^6 leukocytes/ml. One hundred microliters of this cell suspension was cytocentrifuged on glass slides (two spots/slide) for 4 min at $54 \times g$. Slides can be kept overnight at room temperature before fixation. Prior to fixation the spots were marked. Fixation was established by immersing the slides for 5 min in paraformaldehyde solution containing 0.05% sodium azide and slides were then washed for 3 min in PBS. Cells were permeabilized by immersion in permeabilization solution (nonidet P-40 and fetal calf serum in PBS containing 0.05% sodium azide) for 1 min and washed for 5 min in PBS. Subsequently, 35 μl of C10/C11 monoclonal antibody, as supplied with this kit, was applied to each spot and slides were incubated for 20 min at 37°C in a humid chamber. Slides were washed with PBS for 3 min prior to applying 35 μl of conjugate (FITC-labelled sheep anti-mouse Ig) to each spot, followed by incubation for 20 min at 37°C in a humid chamber. Finally the slides were rinsed twice in fresh PBS and three times with tap water. After drying and applying a cover glass over the spots, slides were ready for reading by using a fluorescence micro-

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scope. A minimum of one positive nucleus was regarded a positive result. The negative cells in the background of these slides were not counted.

3. Results

Ninety-nine of the total of 107 samples, processed in both assays, produced an identical result; with 26 positive and 73 negative results. There were eight discrepancies; four samples were negative in the original Brite method but positive in the Brite Turbo and four were positive in the original Brite method but negative in the Brite Turbo. All discrepancies were in the low positivity range (one to two nuclei per slide). Sensitivity and specificity were similar for both tests (Table 1).

Of the 26 samples positive in both tests the Brite Turbo revealed significantly higher numbers of positive nuclei (Table 2). This refleots a higher input of granulocytes in the Brite Turbo method compared with the original Brite method (Table 3). The time needed to process a blood sample was reduced by more than 50%, from 4.5 to less than 2 h in the Brite Turbo compared to the original method.

Table 1
Sensitivity and specificity of the original Brite and the Brite Turbo assays

	Brite Turbo		Sensitivity 87%[a] Specificity 95%[a]
Brite	Positive	Negative	Total
Positive	26	4	30
Negative	4	73	77
Total	30	77	107
Sensitivity 90.9%[b]			
Specificity 99.0%[b]			

[a] Sensitivity and specificity compared to original Brite method.
[b] Landry et al., 1996.

Table 2
Number of positive cells

	Brite	Brite Turbo
Mean	27.9	34.5*
Median	3.5	6.0*

* $P = 0.006$ according to the Wilcoxon Signed Rank test.

Table 3
Number of granulocytes on slides based on a 100% recovery

	Brite	Brite Turbo
Mean	227 215	303 241*
Median	231 300	315 600*

* $P < 0.0001$ according to the Wilcoxon Signed Rank test.

4. Discussion

The major advantages of the Brite Turbo assay are the reduced processing time and the smaller amount of blood required (2 ml in the Brite Turbo method and 5 ml in the original Brite method). Samples can be prepared and examined within 2 h after withdrawal.

Apart from a few discrepancies the results produced by both test were comparable and sensitivity and specificity were similar (Table 1). These discrepancies can most likely be explained by sampling error, as they were present only in the lowest positive range (one to two nuclei per slide). This explanation is supported by the similar distribution of the discrepancies (four in either test). Similar results were recently presented in another study, based on a lower number of positive samples (Landry and Ferguson, 2000).

The fact that positive results showed higher number of positive nuclei in the Brite Turbo method in comparison to the original method reflects the higher input of granulocytes in the first method (Tables 2 and 3). The sensitivity of the Brite Turbo method was not increased in comparison to the original Brite method. This can be explained by the fact that a mean increase of ~80 000 granulocytes per slide will not substantially increase the number of positive cells when the load is one positive cell per 300 000 cells.

In conclusion, the results of this small-scale study suggest that this new technical development, leading to a significant reduction in preparation-time and in which a minimum of blood is re-

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quired, increases the practical value of antigenemia detection in the management of CMV infections.

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