JOURNAL OF CLINICAL MICROBIOLOGY, May 1996, p. 1166–1170 Vol. 34, No. 5
0095-1137/96/$04.00+0

A Three-Center European External Quality Control Study of PCR for Detection of Cytomegalovirus DNA in Blood†

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Received 29 June 1995/Returned for modification 17 November 1995/Accepted 14 February 1996

The presence of cytomegalovirus (CMV) in the blood has important consequences for patient management, and an external quality control study of its detection by the PCR was conducted by the Infectious Disease Working Party of the European Group for Blood and Marrow Transplantation. Forty-eight coded peripheral blood samples from bone marrow transplant recipients were processed in parallel in three European centers by using the routine in-house PCR assay. Protocols varied in choice of primers, specificity and amplificability controls, and sample processing. Results for 38 of 47 samples agreed, 35 being negative and 3 positive. Of the 12 samples reported as positive by at least one center, only 3 were found to be positive by all three centers, 1 was found to be positive by two centers, and the remaining 8 were found to be positive by one center only. The nine discrepant samples appeared to contain around 1,000-fold less viral DNA than the three concordant positive samples. CMV detection was affected both by the number of leukocytes from which DNA was extracted and by the number of cell equivalents added per PCR. External quality control schemes for CMV PCR are clearly necessary in order to compare data from different centers, and recommendations for standardizing the PCR detection of CMV in blood leukocytes are made.

Multicenter trials are often needed in order to enter a number of patients sufficient for the evaluation of antiviral agents. However, without appropriate standardization of clinical and laboratory practices between centers the data obtained in such trials can be uninterpretable. Lack of concordance between centers was highlighted in a recent survey by the European Group for Blood and Marrow Transplantation (EBMT) concerning current approaches for the diagnosis, prophylaxis, and treatment of cytomegalovirus (CMV) infection in bone marrow recipients; both the clinical definitions of disease states and the methods of laboratory diagnosis differed among the 42 participating centers (13). While some of this variation can be addressed by careful preparation of trial protocols, the issue of how to compare the results from the different types of tests currently in use, for example, virus isolation versus PCR, presents a more difficult problem. In addition, the predominant use of assays developed in-house for CMV diagnosis means that even the same type of test may be performed quite differently in different laboratories. Unfortunately, there are currently no external quality control schemes in operation to assess either the efficacies of the various methods to detect CMV in clinical specimens or the variation in their performance between centers. This makes it difficult not only to conduct multicenter trials but also to extrapolate the findings from trials or prospective studies of CMV infection in a particular center to other centers.

In bone marrow transplant (BMT) recipients, CMV viremia is a prognostic indicator of the subsequent development of CMV pneumonitis and CMV gastrointestinal disease (12, 14). The presence of CMV in the blood is therefore currently being used as the indication to initiate preemptive antiviral therapy, and CMV viremia is also used as a criterion for assessing the efficacy of antiviral therapy. The ability to detect CMV in the blood thus has important consequences for patient management, and sensitive techniques to detect CMV in the blood, including the antigenemia test (relying on the detection of the CMV pp65 protein in blood neutrophils [8, 17]) and the detection of CMV DNA in peripheral blood leukocytes by PCR (7), have recently been developed. The Infectious Disease Working Party of the EBMT initially applied external quality control to the PCR method and performed a pilot study to compare the sensitivities of detection of CMV DNA in fresh blood specimens between three EBMT centers. Samples were taken from bone marrow transplant (BMT) recipients attending clinic during the study period. Each center employed the assay routinely used at that center; no attempt to standardize PCR protocols before performing the study was made.

MATERIALS AND METHODS

Participating centers. Three EBMT centers participated in the study: Huddinge University Hospital, Huddinge, Sweden; University Hospital Tübingen, Tübingen, Germany; and the Royal Free Hospital and School of Medicine, London, England. The laboratory analyses were performed by the former Central Microbiological Laboratory of the Stockholm City Council, Stockholm, Sweden (now the Division of Clinical Virology at the Huddinge University Hospital); the University Hospital Tübingen, Tübingen, Germany; and the Department of Virology, Royal Free Hospital, London, England, respectively.

Patients and samples. Forty-eight peripheral venous blood samples were taken in triplicate from 34 patients, 13 from Tübingen, 12 from Huddinge/Stockholm, and 9 from London. Six had received autologous marrow, and 28 had received allogeneic marrow. Samples were taken between −1 and 17 weeks

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† A European Group for Blood and Marrow Transplantation Infectious Disease Working Party-sponsored study.

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TABLE 1. Comparison of PCR methods used in the three centers

Characteristic of PCR method	STOCKHOLM	TÜBINGEN	LONDON
Anticoagulant used	EDTA	EDTA	Heparin
DNA extracted from:	Leukocyte fraction	Whole blood	Whole blood
Amt of sample added to PCR based on:	Leukocyte no.	Amt of DNA	Blood vol
CMV primer	167 bp from IE gene[a]	147 bp from IE gene	149 bp from gB gene[b]
PCR solution	10 mM Tris HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin; 200 μM each dTNP[c]; 2 pmol of each primer (PCR 1) or 50 pmol of inner primers (PCR 2); 1.25 U of *Taq* polymerase	10 mM Tris HCl (pH 9.6), 10 mM $MgCl_2$, 50 mM NaCl, 10 μg of bovine serum albumin; 1 mM each dTNP; 0.25 μg of each primer; 1 U of *Taq* polymerase	25 mM Tris HCl (pH 8.3), 2 mM $MgCl_2$, 17 mM $(NH_4)_2SO_4$, 0.002% gelatin, 10 mM 2-mercaptoethanol; 200 μM each dTNP; 0.5 μM each primer; 1 U of *Taq* polymerase
PCR vol	25 μl	50 μl	100 μl
PCR cycles	PCR1, 20 cycles of 1 min at 94°C, 2 min at 52°C, and 4 min at 72°C; PCR2, 30 cycles of 1 min at 94°C, 2 min at 52°C, and 3 min at 72°C	5 min at 94°C and 32 cycles of 3 min at 66°C and 1 min at 94°C	4 min at 94°C and 40 cycles of 0.5 min at 94°C, 0.5 min at 60°C, and 0.5 min at 72°C
Detection of PCR product	Ethidium bromide staining of agarose gel	Slot blot hybridization with digoxin-labelled 40-bp probe	Ethidium bromide staining of agarose gel
Amplificability control	PCR for DQ alpha gene	PCR for DP beta gene	None
Control of specificity	Use of nested PCR	Specific hybridization	Retesting of positives
Sensitivity	7 genomes	10 genomes	10 genomes

[a] IE, immediate-early.
[b] gB, glycoprotein B.
[c] dNTP, deoxynucleoside triphosphate.

posttransplantation with two exceptions (one taken at 6 months and one taken at 2.5 years). Additional data relating to the patients involved (e.g., information concerning use of antiviral drugs and routine CMV surveillance results) were collected from the three centers before the code on the study samples was broken.

On six different sampling days, blood samples from approximately eight of the patients described above were drawn at a particular center and processed in parallel in all three centers. Each center shipped 16 samples in total. From each patient three samples were drawn, two with EDTA as the anticoagulant and the other with preservative-free heparin. Samples kept at approximately 4°C were transported to all participating laboratories within 24 h and were processed on arrival. All samples were coded and processed blindly in the various laboratories.

Processing of samples and PCR methods. Details of the methods used at the three centers are summarized in Table 1 for ease of comparison. In Stockholm samples in EDTA were processed as described in detail elsewhere (4, 5). Briefly, leukocytes were harvested from 5 ml of peripheral blood (10 ml for leukopenic patients) by using a sodium metrizoate gradient and lysed in a solution containing detergent and proteinase K. Samples of 2×10^6 and 0.5×10^6 leukocytes from each patient were processed in parallel. The sample of 2×10^6 leukocytes was processed further, and the result obtained was reported as the study result; 1/10 of the total volume, corresponding to DNA from 200,000 leukocytes, was used in the PCR. When the result was positive, the Stockholm center then processed 1/4 and 1/10 dilutions of the sample, representing 50,000 and 20,000 leukocyte equivalents per PCR, respectively, together with the stored material from the sample of 0.5×10^6 leukocytes initially processed in parallel (also equivalent to 50,000 leukocytes per PCR). These results were reported after the code for the study samples was broken. The primer pairs described by others (15) were purchased from Lund University, Lund, Sweden, and resulted in amplification of a 723-bp fragment of exon 4 of the immediate-early 1 gene. A nested PCR was used as described in detail elsewhere (4, 5) and gave a second PCR product of 167 bp. Primers located in the cellular DQ alpha gene were used as a positive control for amplificability of the sample.

In Tübingen samples were processed as described previously (6, 7). Five milliliters of blood in EDTA was subjected to lysis of erythrocytes by hypotonic shock, followed by proteinase K digestion. Proteins were precipitated with saturated Na acetate and removed by centrifugation. Nucleic acids were precipitated from the supernatant with ice-cold isopropanol and collected by centrifugation at 4°C. The resulting pellet was washed with alcohol, dried in air, and resuspended in distilled water at 100 ng/μl, as determined by spectroscopy. One microliter, corresponding to 100 ng of DNA, was used in the PCR. The primers used amplified a 147-bp fragment in exon 4 of the immediate-early 1 gene (6, 7) and were synthesized with a Pharmacia DNA Synthesiser. The PCR protocol is described in detail elsewhere (6, 7), and key features are shown in Table 1. A CMV-specific hybridization step using a 40-bp digoxin-labelled probe and an enzyme-linked immunoassay detection system was employed. Primers located in the cellular DP beta gene were used as a positive control for amplificability of the sample.

In London total DNA was extracted from heparinized peripheral blood as described elsewhere (11) except that the Quiagen affinity column system (Hybaid, Middlesex, England) was used and 1 ml of whole blood was added to the affinity column. All extracted DNA was collected into 250 μl, and 5 μl of this DNA material, representing the equivalent of 0.02 ml of whole blood, was added to the PCR mixture. The primers amplified a 149-bp fragment in the glycoprotein B gene (3, 11). The PCR protocol was as described previously (11) except that specificity was controlled by retesting of positive samples rather than by Southern hybridization; key features of the method are shown in Table 1.

All laboratories included positive controls and interspersed their clinical samples with negative controls; in Stockholm negative controls were inserted between every other sample, and in London and Tübingen they were inserted between every fifth sample.

CMV antigenemia assay. In Stockholm, where sufficient cells were available, samples were also processed for the detection of CMV pp65 (UL83) antigen by the antigenemia test as described elsewhere (4, 5). Forty-three samples were initially processed for this test at the same time as they were processed for the PCR analysis, but after fixation the cytospin slides were stored and the final staining and reading of the results were performed after the study code for the PCR results was broken.

RESULTS

Results for 47 samples were available from all three centers. Of these, results for 38 samples were in agreement, while those for 9 samples were discrepant. Of the 38 samples for which results agreed between centers, 35 were negative and 3 were positive. Of the 12 samples reported to be positive by at least one center, only 3 samples (25%) were found to be positive by all three centers, 1 sample (8%) was found to be positive by two centers (Stockholm and Tübingen), and the remaining 8 samples (67%) were reported to be positive by one center only (Stockholm).

The three samples which were positive in all three centers were from two seropositive patients, who were both known to be positive for CMV in the blood by routine PCR screening in the home center, and all three samples were positive by the antigenemia assay performed in Stockholm (with 88, 23, and 8 antigen-positive cells per 200,000 cells examined). Both patients had received antiviral therapy, initiated 5 and 11 days (for one patient) and 9 weeks (for the other) before the three study samples were drawn.

TABLE 2. Analysis of the disparate samples

Sample	PCR result in:						No. of cell equivalents added/ PCR in London	Timing of sample in relation to antiviral therapy[a]	Patient's clinical status	Result of routine PCR testing of patient (home center)[b,c]	Patient's pretransplant CMV serological status[c]
	Stockholm[d]				Tübingen[e]	London					
	A	B	C	D							
1	+	−		−	+	−	54,000	Pre-Fosc		+ ve (L)	+ ve
2	+	+	−	+	−	−	16,800	Pre-GCV	Symptomatic	+ ve (S)	+ ve
3	−				−	+	5,200	None	3 wk post-autologous BMT	− ve (S)	− ve
4	+	−	−	−	−	−	8,600	None		− ve (L)	+ ve
5	+	+	−	−	−	−	19,600	None		− ve (T)	+ ve
6	+	+	−	−	−	−	7,000	3 wk post-GCV	CMV IP[f]	+ ve (T)	+ ve
7	+	−		−	−	−	17,400	2 wk post-GCV	Symptomatic	+ ve (T)	+ ve
8	+	−	−	−	−	−	100,600	6 wk post-GCV	Symptomatic	+ ve (S)	+ ve
9	−				+	−	51,600	Pre-GCV		+ ve (S)	+ ve

[a] Fosc, foscarnet; GCV, ganciclovir.
[b] Routine testing was performed at the home center. L, London; S, Stockholm; T, Tübingen.
[c] + ve, positive; − ve, negative.
[d] Numbers of leukocytes processed and cell equivalents added per PCR for methods A to D were 2×10^6 and 200,000, 2×10^6 and 50,000, 2×10^6 and 20,000, and 5×10^5 and 50,000, respectively.
[e] Approximately 10 to 50,000 cell equivalents were added per PCR.
[f] IP, interstitial pneumonitis.

Of the 34 samples (72%) which gave negative results in all three centers, 8 samples were from seronegative recipients (two autologous marrow recipients and six recipients with seronegative donors) and 8 samples were from seropositive recipients but were outside the time interval of increased risk for CMV detection (1 from −1 week, 4 from 1 to 3 1/2 weeks, 2 from 6 months, and 1 from 2 1/2 years post-BMT). Thus, 47% (16 of 34) of the concordant negative results were from samples predicted most likely to be negative. Thirty-one samples were negative when analyzed in Stockholm by the antigenemia assay, the results for two samples were not available because of insufficient cell numbers, and the remaining one sample contained 1 antigen-positive cell per 200,000 cells examined.

The nine discrepant samples were analyzed as shown in Table 2. Samples 1, 2, and 6 to 9 were taken from patients whose blood was known to be CMV positive on the basis of PCR in routine screening in the host center. Samples 1 and 6 were positive in the antigenemia assay (with 3 and 1 antigen-positive cells per 200,000 cells examined, respectively), and samples 2, 4, 5, 7, and 8 were antigenemia negative, while results were not available for samples 3 and 9 because of insufficient cell numbers. Samples 6 to 8 were from patients who had previously received antiviral therapy and who probably had declining titers of CMV in the blood. Samples 1, 2, and 9 were from patients who subsequently received antiviral therapy and who may have had rising titers of CMV in the blood at the time of the study. Samples 4 and 5 were from seropositive recipients with seropositive donors, who were not found to be positive in routine testing by PCR in London and Tübingen, respectively, and thus it is not possible to determine whether the positive results found in Stockholm are the correct results. Sample 3 was taken from a seronegative recipient 3 weeks after that individual received an autologous BMT, and it tested negative in Stockholm and Tübingen but positive in London. The patient was never found to be positive for CMV in routine testing in Stockholm, and we conclude that the result from London was probably a false positive. We believe that this is likely to be the only true false-positive result of the study, representing a 0.7% (1 of 141) false-positive rate.

As mentioned in Materials and Methods, the Stockholm center processed parallel preparations from 2×10^6 and 0.5×10^6 leukocytes for each sample, corresponding to DNA from 200,000 and 50,000 leukocytes, respectively, per PCR, as well as 1/4 and 1/10 dilutions of the sample from 2×10^6 leukocytes (representing 50,000 and 20,000 leukocyte equivalents, respectively, per PCR). The results discussed above were obtained with the preparations representing 200,000 equivalents per PCR, whose results were reported as the study results, and those obtained with the other preparations are shown in Table 2. All seven of the study samples which were positive in Stockholm but negative in Tübingen and/or London were negative when tested in Stockholm at a 1/10 dilution (20,000 cell equivalents per PCR). Only three of seven were positive at the 1/4 dilution (50,000 cell equivalents), with only one of these being positive with the parallel preparation from 0.5×10^6 leukocytes (also 50,000 cell equivalents). Thus, six of seven would have been recorded as negative if the Stockholm center had run only preparations processed from 0.5×10^6 leukocytes and not preparations processed from 2×10^6 leukocytes. With the preparation from 2×10^6 leukocytes, the addition of 200,000 cell equivalents per PCR picked up four more positive samples than did the 1/4 dilution with 50,000 cell equivalents.

In contrast to the case with the discrepant samples just discussed, of the three samples which were found to be positive in all three centers, two were still positive in Stockholm when diluted down to 1/10,000 (20 cell equivalents) and the other was still positive at 1/1,000 (200 cell equivalents). Thus, the positive samples which agreed between centers appeared to have more CMV DNA present as detected by PCR than the discrepant samples.

DISCUSSION

The results of the study showed that there was relatively good agreement (97%) between centers with regard to the negative samples, since 35 of 36 samples considered to be true negatives were found to be negative in all three centers. The discrepant sample, which was from a seronegative autologous BMT recipient, tested negative in two centers but was reported to be positive by the third. We believe this to be the only probable false-positive result in the study. It is interesting that it occurred in the center not using a nested PCR or a hybridization step to ensure specificity and that repeat testing of the sample there had given equivocal results. In routine practice

clinical decisions are not usually made on the basis of single PCR results, and it is likely that in view of the patient details, subsequent samples from this patient would have been processed before any therapy was considered. Thus, overall the study demonstrated that false-positive PCR results did not appear to be a major problem in the centers concerned. It should be noted, however, that all three centers are experienced in PCR technology and have stringent measures in place to prevent contamination; false-positive results may prove to be a problem for less-experienced laboratories or for those without the appropriate facilities. False-positive results have been shown to be a greater problem than false-negative results in recent multicenter studies of PCR detection of hepatitis C virus in plasma (19) and a reference panel of human immunodeficiency virus type 1 (HIV-1) template DNA (1).

While the agreement between centers for the negative samples was good, there was considerable discrepancy with regard to the positive samples, with only 3 (25%) of the 12 positive results being reported by all three centers. These 3 samples appeared to contain large amounts of CMV DNA, and they also had many (6 to 88) antigen-positive cells as determined by the antigenemia assay. In contrast to the three samples which were positive in all three centers, the seven discrepant samples which were positive only in Stockholm appeared to contain smaller amounts of viral DNA and had few (one to three), if any, antigen-positive cells as determined by the antigenemia test.

The number of cell equivalents added per PCR was found to profoundly influence the results, and this may partly explain the discrepancies between centers. In Tübingen the amount of DNA added corresponded to around 10 to 50,000 cell equivalents per PCR, while in London a specified amount of whole blood was processed, so that the number of cell equivalents per PCR would depend on the peripheral blood white cell count. The leukocyte counts for the study samples ranged from 0.1×10^6/ml to 50.3×10^6/ml, representing 200 to 106,000 (mean, 27,340) cell equivalents per PCR. Thus, both the Tübingen and London centers were adding on average 4- to 10-fold less sample material per PCR than the Stockholm center was, and for neutropenic patients, up to 1,000-fold less material was added in London. The London and Tübingen protocols would also have processed any CMV DNA present in the plasma; however, it is not clear whether this would have been significant.

The findings from Stockholm also showed that the number of leukocytes initially processed had a profound influence on the final result for a significant number of samples. Different results were obtained when two different sources of 50,000 cell equivalents per PCR were tested, namely, the processing of 2×10^6 and 0.5×10^6 leukocytes. It would be expected that the former source, representing a dilution of a CMV DNA-positive preparation extracted from two million cells with the CMV DNA dispersed throughout, would yield a higher frequency of positives than the latter, a dilution of the number of cells from which the DNA was initially extracted, since any CMV DNA-positive cell probably contains more than one DNA copy. We know from studies using the antigenemia test that expression of the CMV pp65 protein is restricted to a very small proportion of blood neutrophils (9), with often as few as 1 of 200,000 leukocytes being pp65 positive. If this is also true for the number of leukocytes containing CMV DNA, then the number of cells from which the DNA was initially extracted will prove to be a critical factor in determining the sensitivity of the PCR test, as suggested by our findings here. In addition, the Poisson distribution suggests that when this critical limit of detection is approached repeat testing of the same sample or testing of the same sample in different laboratories is likely to yield different results.

We conclude that both the extraction of DNA from a greater number of leukocytes and the addition of more leukocyte DNA per PCR contributed to the increased sensitivity of the Stockholm assay. On the other hand, the study did not address the question of whether the Tübingen and London centers would have reported the discrepant samples to be positive if they had processed a larger number of cell equivalents in their PCR assays. Larger amounts of DNA can nonspecifically inhibit the PCR. Such inhibition was seen for 1 of the 48 study samples (2%) processed in Stockholm, and this inhibition could be seen in 5 to 10% of samples in routine practice at that center. All the study samples contained amplifiable DNA as processed by the Tübingen protocol, while the London protocol does not test for this parameter. Clearly, increased sensitivity must be balanced by stringent controls for amplificability, so that samples in which the control gene cannot be amplified can be diluted and retested. In addition, samples from very neutropenic patients may have small amounts of DNA which may thus be difficult to amplify, again stressing the need for a control gene.

In our study design no attempt to standardize PCR protocols before analyzing the samples was made, since we wished to compare the PCR assays routinely in use at each center and the three protocols used had many differences. The disparate sensitivities observed might also be due to factors other than the different amounts of leukocyte DNA added to the PCR, such as the anticoagulant used, the choice of primers, and the specificity controls employed. For example, the collection of blood into heparin has been shown to profoundly inhibit the PCR detection of HIV and hepatitis C virus and the PCR amplification of cellular genes (9, 16, 18), while additional hybridization steps might amplify the signal. Transport of the samples did not appear to have adversely affected the results, since there was no bias towards detecting positives in the center shipping the specimen.

Whatever the underlying factors, it was clear that the sensitivities of the PCRs routinely used for detection of CMV in the blood were different between the centers. Samples containing relatively low levels of virus were missed by some centers. The question of whether use of a lower sensitivity assay would cause clinically important samples to be missed cannot be addressed by the present study, but it needs to be answered. On the other hand, a more sensitive PCR could also increase the detection of CMV in patients who would not go on to develop disease, thereby resulting in unnecessary treatment of such patients with potentially toxic antiviral drugs. In addition, the existence of differing PCR sensitivities between centers could potentially have a number of effects on antiviral trials. First, it could affect the choice of patients and/or the time at which they are entered into preemptive antiviral therapy. Second, the length of treatment could be affected if the decision to cease antiviral therapy is made on the basis of the presence or absence of PCR positivity for CMV in the blood. Third, the perceived effectiveness of antiviral therapy could differ between centers if PCR positivity is used as a laboratory endpoint in the trial (with treatment appearing to be more effective in centers with the least sensitive PCR). This latter point also applies to prophylactic trials, in which centers with low PCR sensitivities might fail to detect viral DNA at all, moving patients from an infected category to a noninfected category.

Although it appears to be possible to ship blood samples without affecting the ability to detect CMV DNA by PCR, it may be more difficult to establish external quality control schemes for CMV PCR than for that with some other viruses.

When the virus and/or viral nucleic acid is associated with the plasma fraction, reference panels can be set up by "spiking" plasma with different amounts of viral nucleic acid and/or virus (19). For HIV, reference panels have been set up by spiking blood with lymphoid cell lines infected with HIV in vitro (10), as well as by using whole blood specimens from well-characterized patients known to be consistently PCR positive (10). Neither of these latter approaches is easily adopted for CMV, since we do not have an in vitro model which reflects the physiologic state of CMV in the blood and CMV viremia is usually of shorter duration and of lower magnitude than viremia with viruses such as hepatitis C virus. However, even when such a reference panel is available, the comparison of in-house PCR assays between centers can prove to be disappointing, as was the case with hepatitis C virus (19), for which erroneous results were reported by 52% of the laboratories. Moreover, even when centers used the same commercially available kit for the PCR detection of HIV, the results were not always in agreement (10), emphasizing the fact that staff expertise and laboratory facilities can also affect the outcome.

The issues of external and internal quality control for virus detection should be given high priority in both routine diagnostic practice and the performance of antiviral trials. Standardization of PCR protocols would undoubtedly be desirable. At the present time, for the PCR detection of CMV in blood leukocytes the EBMT Infectious Disease Working Party recommends that (i) peripheral blood be collected into EDTA (or citrate), (ii) a standard number of leukocytes be processed, (iii) DNA from a standard number of leukocyte equivalents or a standard amount of DNA be added per PCR, (iv) cellular gene controls of amplificability of the sample be included, (v) a specificity control (nesting or specific hybridization step) be included, and (vi) primers in a conserved part of the CMV genome be chosen (since the amplification sensitivity with sequence-mismatched strains can be reduced by up to 100-fold [2]). The results from the present study lead us to suggest that it might be appropriate to standardize on the processing of 2×10^6 leukocytes from peripheral blood for the PCR and on the addition of DNA from the equivalent of 200,000 leukocytes per PCR. However, if standardization of the number of leukocytes processed is achieved, then we would recommend subsequent standardization of the actual amount of DNA added per PCR, rather than the number of leukocyte equivalents, since the former would allow for differences in the yield of DNA when different extraction procedures are used.

ACKNOWLEDGMENT

The financial support of the Wellcome Foundation, UK Limited for this study is gratefully acknowledged.

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