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Quantification of Cytomegalovirus DNA in Peripheral Blood Leukocytes by a Branched-DNA Signal Amplification Assay

DAVID N. CHERNOFF,[1]* RICHARD C. MINER,[2] BRADLEY S. HOO,[1] LU-PING SHEN,[1] REED J. KELSO,[1] DRAGANA JEKIC-McMULLEN,[2] JACOB P. LALEZARI,[2] SUNWEN CHOU,[3] W. LAWRENCE DREW,[2] AND JANICE A. KOLBERG[1]

Chiron Diagnostics, Emeryville, California[1]; Mount Zion Hospital and Medical Center of the University of California, San Francisco, California[2]; and Veterans Affairs Medical Center, Portland, Oregon[3]

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Quantification of cytomegalovirus (CMV) DNA in blood may aid in the identification of patients at highest risk for developing CMV disease, the evaluation of new therapeutics, and the prompt recognition of drug-resistant CMV strains. A branched-DNA (bDNA) assay was developed for the reliable quantification of CMV DNA in peripheral blood leukocytes. The bDNA assay allowed for the highly specific and reproducible quantification of CMV DNA in clinical specimens. Furthermore, the bDNA assay was at least as sensitive as culture techniques and displayed a nearly 3 $\log_{10}$ dynamic range in quantification. Changes in CMV DNA levels measured by the bDNA assay in a human immunodeficiency virus-positive patient undergoing therapy were consistent with CMV culture, antigen, and genotype results and correlated with disease progression and resistance markers. The bDNA assay for the quantification of CMV DNA may provide a useful tool that can be used to aid physicians in monitoring disease progression, evaluating therapeutic regimens, and recognizing viral resistance and drug failure.

Cytomegalovirus (CMV) disease is an important cause of morbidity and mortality in immunocompromised patients, especially human immunodeficiency virus (HIV)-infected patients and transplant recipients. CMV is a common opportunistic viral pathogen, and CMV infection is associated with a broad spectrum of disease manifestations, including pneumonitis, allograft rejection, retinitis, hepatitis, vasculitis, and numerous neurological syndromes (for reviews, see references 24 and 30). Only three antiviral drugs are approved for use in the treatment of CMV disease—ganciclovir, foscarnet, and (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine (cidofovir). Although these drugs have been shown to be effective in delaying disease progression, their toxicity and the possibility of developing drug resistance limit their prolonged use in the management of patients with CMV disease (29). With the extended survival time of HIV-positive patients and the substantial rise in the number of transplant recipients in recent years, the incidence of CMV disease is expected to increase. It therefore is critical that methods be developed for the effective identification of patients at highest risk for developing clinically significant CMV disease and for managing patients with established disease.

Recently, the branched-DNA (bDNA) assay for the direct quantification of viral nucleic acid has been developed for hepatitis B, hepatitis C, and human immunodeficiency viral infections (9, 18, 28). The bDNA assay measures viral nucleic acids at physiological levels by boosting the reporter signal rather than amplifying target sequences as the means of detection. Inherently quantitative and amenable to routine use in a clinical setting, the bDNA assay may be useful in the management of patients with chronic viral diseases. Recent studies have illustrated the potential clinical utility of the bDNA assay in determining the prognosis and therapeutic monitoring of HIV infection (10, 20, 25). The development of a bDNA assay for the direct quantification of CMV DNA may aid in the identification of patients at highest risk for developing CMV disease, the evaluation of new therapeutic agents, and the prompt recognition of drug-resistant CMV strains that may lead to better long-term outcomes by allowing appropriate changes in therapy to be made early in the course of treatment.

In this report we describe the design and performance of a bDNA assay for the direct quantification of CMV DNA in blood. We analyzed the linearity, specificity, sensitivity, and reproducibility of the bDNA assay for CMV DNA quantification in clinical specimens. We investigated the correlation between CMV DNA quantification and other measures of CMV disease, including CMV antigenemia and cell culture. We also demonstrated the potential clinical utility of the bDNA assay by monitoring the virologic status of an AIDS patient with CMV retinitis undergoing therapy.

MATERIALS AND METHODS

Clinical specimens. Blood specimens from healthy CMV-seronegative and -seropositive blood donors were obtained from the Sacramento Blood Center. For sensitivity studies or comparison to culture, specimens from patients with CMV retinitis were obtained from the Clinical Virology Lab at Mt. Zion Medical Center and from Ella M. Swierkosz (Department of Pediatrics, St. Louis University School of Medicine, St. Louis, Mo.). Blood specimens were drawn into tubes containing EDTA (0.1 ml EDTA per 10-ml draw). Informed consent was obtained from all patients. The human experimentation guidelines of the U.S. Department of Health and Human Services and those of the Mount Zion Hospital and Medical Center of the University of California, San Francisco, and the St. Louis University School of Medicine were followed in the conduct of the clinical research.

Dextran settling procedure for collection of leukocytes. A dextran settling procedure was used to collect peripheral blood leukocytes (PBLs) for the direct quantification of CMV DNA. Specimens of whole blood, which had been held at room temperature for up to 8 h, were transferred to 15-ml centrifuge tubes. After the addition of 2 ml of 6% dextran (high-molecular-weight dextran 76,000; Sigma Chemical Co., St. Louis, Mo.) in phosphate-buffered saline, the tubes were mixed gently by inversion for 20 to 30 s and were then incubated without additional mixing at 37°C for 15 to 30 min to allow the blood to settle. The leukocyte-rich supernatant fraction (top layer) was collected, taking care not to disturb the erythrocyte layer, and transferred to a new 15-ml centrifuge tube. Culture medium (Eagle's minimal essential medium with 10% fetal calf serum, 2 mM

* Corresponding author. Mailing address: Nucleic Acid Diagnostics and New Markers, Chiron Diagnostics, 4560 Horton St., Emeryville, CA 94608-2916. Phone: (510) 923-2775. Fax: (510) 655-7733. E-mail: david_chernoff@cc.chiron.com.

L-glutamine, 27 mM sodium bicarbonate) was then added to bring the volume to 15 ml. The leukocyte suspension was centrifuged at 400 to 450 × *g* for 15 min, and the supernatant was decanted. The pellet was resuspended in 5 ml of sterile distilled H_2O with light vortexing to lyse the remaining erythrocytes, and culture medium was added to bring the volume to 15 ml. After centrifugation at 400 to 450 × *g* for 15 min, the supernatant was decanted and the pellet was resuspended to 2 ml with culture medium. Aliquots of 2×10^6 to 6×10^6 cells were then transferred to 1.5-ml conical screw-cap microcentrifuge tubes with O rings (catalog no. 72.692.005; Sarstedt, Newton, N.C.), and the tubes were centrifuged at 12,000 rpm for 10 min in a microcentrifuge. The supernatant was decanted and the PBL pellet was either processed immediately for the bDNA assay or frozen at −80°C for long-term storage.

bDNA assay. The bDNA assay for CMV DNA quantification was performed in a 96-well format similar to that described for hepatitis C virus RNA, HIV type 1 (HIV-1) RNA, and hepatitis B virus DNA quantification (8, 18, 28). Briefly, PBL pellets were lysed under denaturing conditions to release CMV DNA. CMV DNA-specific target probes mediated capture of the CMV DNA to capture probes on the microwell surface, as well as binding of the CMV DNA to bDNA amplifier molecules. Enhancement of the signal was achieved with bDNA amplifier molecules containing 45 sites available for binding to alkaline phosphatase-conjugated label probes. After introduction of a chemiluminescent dioxetane substrate which is activated by the alkaline phosphatase, the signal was measured as light emission in a luminometer. The amount of CMV DNA in each specimen was determined by using a standard curve (described below) which was run on every plate. Specimens generating light emission greater than the lowest calibrator of the standard curve were considered positive.

Quantification standard and controls. The CMV DNA standard consisted of the linearized pGEM vector containing a copy of the gB and UL56 genes (3, 34) of CMV laboratory strain Towne. The primary CMV DNA standard was rigorously quantified by four analytical methods including optical density determination, inorganic phosphate analysis, diphenylamine analysis, and hyperchromicity assay (7). Assay standards were assigned a value by comparison to the primary CMV DNA standard following the bDNA assay procedure described above. Four assay standards were prepared by serial dilution of linearized plasmid in Dulbecco's modified Eagle's medium and 10% fetal calf serum to 5.6×10^6 (standard A), 3.5×10^5 (standard B), 4.4×10^4 (standard C), and 4.4×10^3 (standard D) copies of CMV DNA per well. The concentration of CMV DNA in each specimen was calculated from the standard curve, which was defined by a quadratic curve-fit function. One copy was defined as the amount of CMV DNA that generates a level of light emission equivalent to that generated by 1 molecule of the primary CMV DNA standard. In addition to standards, both a positive control and a negative control were included in the bDNA assay. The positive control was a sonicated preparation of fibroblast cells infected with the Davis strain of CMV. The negative control was prepared from uninfected cells in a similar manner.

Oligonucleotide probes. A total of 43 target probes were designed: 9 to mediate capture of the CMV DNA to the microwells and 34 to mediate binding of the CMV DNA to bDNA amplifier molecules. Each target probe contains a 33-base sequence that hybridizes to either the gB or the UL56 region of the CMV genome. These regions of the CMV genome were used for probe design because they show a high degree of sequence conservation among multiple clinical isolates (5). For the gB gene, 21 target probes were designed with 33-base sequences complementary to nucleotides 1458 to 2150 of the coding sequence. For the UL56 gene, 22 target probes were designed with 33-base sequences complementary to nucleotides 508 to 1233 of the coding sequence. Each target probe also contains one of two 18-base sequences that hybridize either to the capture probes or to the bDNA amplifier molecules. The 3′ end of the target probes for capture is CTC TTG GAA AGA AAG TGA AGT G. The 3′ end of target probes for labeling is AGG CAT AGG ACC CGT GCT T.

Preparation of panels of potentially interfering substances. Panels of plasma specimens containing known amounts of CMV DNA and negative controls were seeded with microorganisms and viruses commonly found in the blood of immunocompromised patients and drugs commonly used in the management of immunocompromised patients. Microorganisms were tested at 10^6 cells per well and included *Bacillus subtilis*, *Bacteroides fragilis*, *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Peptostreptococcus magnus*, *Peptostreptococcus anaerobius*, *Propionibacterium acnes*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas fluorescens* I, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* group D species, *Streptococcus pneumoniae*, *Yersinia enterocolitica*, *Cryptococcus neoformans*, *Mycobacterium tuberculosis*, *Mycobacterium kansasil*, and *Candida albicans*. Viruses tested at $\geq 10^5$ viral particles per well included human HIV-1, herpes simplex virus type 1, herpes simplex virus type 2, hepatitis B virus, and human herpesvirus 6. Hepatitis A virus was tested at 10^4 viral particles per well. All drugs were tested at 10 times the maximum concentration of drug in plasma (C_{max}). Drugs and concentrations were as follows: itraconazole (Sporanox), 30 μg/ml; ketoconazole (Nizoral), 30 μg/ml; ciprofloxin, 30 μg/ml; azithromycin (Zithromax), 10 μg/ml; clarithromycin (Biaxin), 30 μg/ml; fluconazole (Diflucan), 100 μg/ml; didanosine, 30 μg/ml; foscarnet, 40 μg/ml; rifabutin, 10 μg/ml; ganciclovir, 60 μg/ml; acyclovir, 700 μg/ml; zalcitabine (ddC), 10 μg/ml; dapsone, 700 μg/ml; ethambutol, 700 μg/ml; sulfamethoxazole-trimethoprim, 700 μg/ml; and zidovudine, 20 μg/ml.

TABLE 1. CMV DNA quantification in peripheral blood mononuclear cells from healthy blood donors and CMV-seropositive subjects

Group and CMV status of specimen	No. of subjects	No. (%) of specimens with the following CMV DNA quantification value:	
		$>4.4 \times 10^3$ copies per 10^6 cells	$<4.4 \times 10^3$ copies per 10^6 cells
Healthy blood donors			
CMV seronegative	72	4 (6)[a]	68 (94)
CMV seropositive	27	0 (0)	27 (100)
CMV-seropositive subjects			
CMV culture negative	149	21 (14)[b]	128 (86)
CMV culture positive	62	59 (95)	3 (5)

[a] Upon retesting all samples yielded values below the quantification limit of the assay.

[b] The majority of these specimens with discordant results came from patients with an established diagnosis of CMV disease who were undergoing therapy (19 of 21 specimens tested) and who were CMV antigen positive (10 of 16 specimens tested).

Other assays. Specimens were classified as CMV culture negative or positive by standard tissue culture techniques by the institutions from which they were obtained. CMV antigenemia was determined by using CMV-vue (Incstar Corporation, Stillwater, Minn.) according to the manufacturer's instructions. Phenotypic ganciclovir susceptibility testing was performed by plaque reduction assay as described previously (2). UL97 resistance mutations at codons 460, 591, 592, 594, and 595 were tested by PCR amplification of leukocytes or infected cell extracts with specific primers, followed by digestion with selected restriction enzymes as described previously (4, 6). The UL97 coding region of DNA extracted from CMV isolates was also partially sequenced to screen for mutations at codons 460, 520, and 590 to 607, which are the loci where ganciclovir resistance mutations are known to occur (6).

Statistical analysis. Comparison of the distribution of CMV DNA quantification values was performed by the Student two-tailed *t* test. The percent coefficient of variance (CV) was evaluated according to the proposed guidelines of the National Committee for Clinical Laboratory Standards (26). The 95% prediction limits were calculated by using the linear model (17).

RESULTS

Standard curve and linearity of quantification. The standard curve for the bDNA assay covered the dynamic range from 4.4×10^3 to 5.6×10^6 CMV DNA copies (data not shown). An essentially linear response between CMV DNA concentration and the relative luminescence (relative light units [RLUs]) was observed throughout the nearly 3-$\log_{10}$ range of the standard curve. Since even the most concentrated standard was measured, the upper quantification limit of the bDNA assay was not defined by this analysis.

The linearity of the bDNA assay was evaluated further by testing serial dilutions of cultured CMV virus (Davis strain) in specimen diluent. Four replicates of each dilution were tested in two assay runs. The relationship between CMV DNA quantification values and dilution was defined by the following equation: $y = 0.39 + 1.00x$. An R^2 value of 0.99 was calculated by linear regression analysis, indicating that 99% of the observed variation can be explained by the linear relationship between dilutions of CMV DNA-positive specimens and quantification values. These results also demonstrated that the bDNA assay yielded accurate quantification values for all dilutions tested throughout the dynamic range of the standard curve.

Specificity and sensitivity. The specificity of the bDNA assay was evaluated by testing a panel of PBL specimens from 72 CMV-seronegative healthy blood donors (Table 1). The RLU values for 68 of the CMV-seronegative specimens fell below that of the assay's quantification limit (4.4×10^3 CMV DNA

TABLE 2. Reproducibility of CMV DNA quantification by the bDNA assay

Panel and specimen no.[a]	Mean no. of copies of CMV DNA per 10^6 cells	% CV	95% Prediction limits	Fold range
Panel A				
1	1.93×10^6	14.4	1.42×10^6–2.54×10^6	1.78
2	9.67×10^5	23.3	7.65×10^5–1.19×10^6	1.55
3	1.80×10^5	13.3	1.42×10^5–2.31×10^5	1.62
4	2.04×10^4	25.1	1.27×10^4–3.27×10^4	2.57
5	9.12×10^3	43.4	4.40×10^3–1.89×10^4	4.29
6	7.11×10^3	46.2	3.19×10^3–1.58×10^4	4.97
Panel B				
1	8.68×10^5	2.4	7.94×10^5–9.42×10^5	1.18
2	1.78×10^5	3.0	1.67×10^5–1.89×10^5	1.13
3	2.39×10^4	9.2	1.95×10^4–2.83×10^4	1.45

[a] Panel A, 96 replicates tested on-site by Chiron Diagnostics; panel B, 19 replicates tested at reference laboratory site.

copies), resulting in a 94% specificity for this population. Upon retesting these four specimens yielded RLU values below the quantification limit. A two-tailed *t*-test analysis of CMV-seronegative specimens and replicates of standard D, the lowest calibrator of the standard curve, demonstrated that the distribution of RLU values for these two populations do not overlap. In addition, specimens from 27 CMV-seropositive blood donors were tested, and all were found to yield RLU values below that of the assay's quantification limit. A two-tailed *t* test performed with a subset of the CMV-seronegative and CMV-seropositive specimens indicated that there was no significant difference in RLU values between these two populations. Thus, normal healthy individuals, even those with latent CMV infection, were not detected as CMV DNA positive by the bDNA assay.

The sensitivity of the bDNA assay was compared to that of CMV cell culture assays (Table 1). Of 62 CMV culture-positive specimens, 59 tested positive for CMV DNA by the bDNA assay, thereby yielding a 95% detection rate. Testing of the three culture-positive specimens that were negative by bDNA assay could not be repeated because of insufficient sample volume. While there is no obvious explanation for the discordance between the negative bDNA assay results and the positive culture results, one possibility is that the level of CMV DNA in these specimens may have been below the detection limit of the bDNA assay. In this regard, it is not known how long it took for these specimens to become culture positive. Of 149 CMV culture-negative specimens, 21 (14%) tested positive for CMV DNA by the bDNA assay. It is unlikely that these culture-negative specimens yielded false-positive results by the bDNA assay since further investigation of these 21 specimens revealed that 17 were from patients undergoing ganciclovir or foscarnet therapy and 2 were from patients later diagnosed with CMV-related disease. Also, the majority of these discordant specimens, 10 of 16 tested, were found to be CMV antigen positive. A potential explanation for the culture negativity of these specimens may be the presence of anti-CMV drugs which might sterilize the cultures. Hence, the discordance between the positive bDNA assay and negative culture results may be due to the false-negative results obtained in culture assays.

Reproducibility. The reproducibility of the bDNA assay was evaluated by testing 96 replicates of specimen panels (panels A and B) in assay runs by three different operators over a 4-day period with two different lots of reagents (Table 2, panel A). Panel A was tested on-site at Chiron Diagnostics, included six specimens prepared from a tissue cultured stock of CMV, and contained concentrations of CMV DNA that spanned the range of the standard curve. Mean quantification values ranged from 7.11×10^3 to 1.93×10^6 CMV DNA copies per 10^6 cells. The CVs for overall assay precision ranged from 14.4 to 46.2%. Analysis of these data to calculate the 95% prediction limits demonstrated that the reproducibility of the bDNA assay is sufficient to discern 1.5- to 5.0-fold changes in CMV DNA levels as statistically significant. As expected, smaller fold changes discerned as statistically significant were observed at higher CMV DNA levels. These values reflect assay reproducibility encountered in real-time testing in which specimens are tested on different days, by different operators, and with different lots of reagents.

The reproducibility of the bDNA assay was assessed further by testing 19 replicates of specimen panels by one operator at the reference laboratory site (Table 2, panel B). Panel B included three specimens with mean quantification values ranging from 2.39×10^4 to 8.68×10^5 CMV DNA copies per 10^6 cells. As was observed with replicates tested on site at Chiron Diagnostics, replicates tested at the reference laboratory yielded low CVs for overall assay precision, ranging from 2.4 to 9.2%. Even lower CVs, ranging from 1.5 to 5.7%, were observed for within-run assay precision (data not shown). Also consistent with the results of on-site testing, analysis of replicates tested at the reference laboratory demonstrated that the reproducibility of the bDNA assay is sufficient to discern 1.1- to 1.5-fold changes in CMV DNA levels as statistically significant, with smaller fold changes discerned at higher CMV DNA levels. These values reflect the assay reproducibility encountered in batch testing, such as that used in longitudinal clinical studies.

Potentially interfering substances. A wide variety of microorganisms and viruses commonly found in the blood of immunocompromised patients and drugs commonly used in the management of immunocompromised patients (listed in Materials and Methods) were tested in the bDNA assay to assess whether these potentially interfering substances would augment or dampen the relative luminescence. No significant difference in quantification values between specimens with and without these potentially interfering substances was indicated by paired *t*-test analysis. Thus, these substances had no effect on the relative luminescence generated in the bDNA assay.

Quantification of CMV DNA in a patient undergoing therapy. The bDNA assay was used to monitor CMV DNA levels in PBL specimens in an HIV-positive patient with CMV retinitis undergoing therapy. Figure 1 presents the CMV DNA profile of this patient over time. For comparison, CMV culture and antigen results also are indicated. Upon the initial diagnosis of CMV retinitis in this patient, he was treated with intravenous (i.v.) ganciclovir for ~5 months. Subsequently, this patient received adjunctive therapy with intravitreal foscarnet for 3 months. Because of disease progression, the i.v. ganciclovir was stopped and the patient was enrolled in a research protocol with an experimental intravitreal drug. CMV DNA levels remained relatively low during the approximately 2 months that this patient was off i.v. ganciclovir. Because disease progression was noted in this patient, i.v. ganciclovir was reinitiated. However, despite the reinitiation of i.v. ganciclovir therapy and the subsequent addition of oral ganciclovir therapy, disease progression continued, with a concomitant increase in CMV DNA levels as measured by the bDNA assay. Again, the bDNA assay results correlated with the CMV culture, antigen, and genotype data. The patient's lack of response to ganciclovir is explained by the presence of a mutation in the UL97 coding region at position I460, which confers

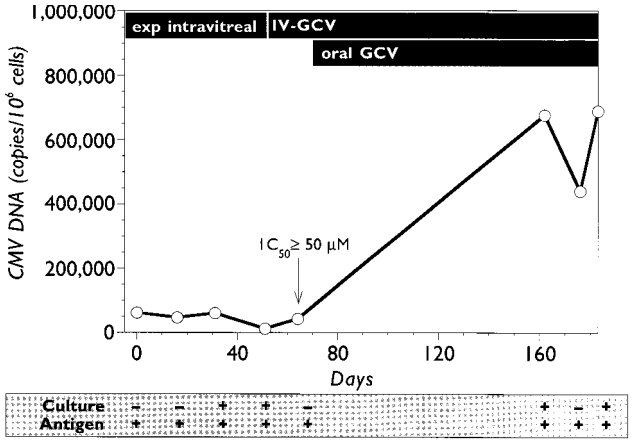

FIG. 1. Profile of an HIV-positive patient with CMV retinitis undergoing therapy. exp, experimental; IV-GCV, i.v. ganiclovir; IC_{50}, 50% inhibitory concentration.

genotypic resistance to ganciclovir (6). The phenotypic assays done at this point in time indicate a high level of ganciclovir resistance, i.e., a 50% inhibitory concentration of >50 μM (12). The evolution of resistance was predictable given that this immunosuppressed patient had been on prolonged drug therapy. Consistent with this finding, CMV DNA levels increased and remained high throughout the period of ganciclovir retreatment, while CMV antigen results continued to be positive. Thus, in this patient, there was a sustained increase in CMV DNA levels measured by the bDNA assay that correlated with disease progression and resistance markers.

DISCUSSION

The morbidity and mortality caused by CMV disease remain important concerns in the clinical management of immunocompromised patients. Given the potential for prophylactic treatment to prevent the expression of CMV disease, there is a need to develop markers for CMV which will have utility in identifying those individuals at greatest risk for the development or actual presence of CMV disease. While cell culture and antigen-staining methods allow for the detection of CMV in patient specimens, these methods are at best semiquantitative, are subject to variability in their results, are difficult to standardize, and are not always sufficient for predicting the development of symptomatic disease (37). More promising are methods for the direct detection and quantification of CMV DNA.

Recent studies by PCR and in situ hybridization techniques have indicated that CMV DNA can be detected in specimens from individuals with CMV infection and that the detection of CMV DNA correlates with the detection of pp65 antigen (16, 32) and with the presence or subsequent development of CMV disease (see, for example, references 13, 23, and 31). Furthermore, studies that use these sensitive techniques to monitor therapeutic responses have shown that early detection of CMV DNA may help to improve the efficacy of therapy (14, 27, 35). While the results of those studies are encouraging, techniques for qualitative CMV DNA detection are not sufficient since the presence of CMV DNA does not necessarily indicate active infection (for a review, see reference 38). Methods for CMV DNA quantification should allow for greater realization of the full prognostic and therapeutic value of this marker (1, 21).

We have developed a method for the direct quantification of CMV DNA in clinical specimens using bDNA technology. A rapid, sensitive, chemiluminescent method that does not rely on the amplification of target sequences, the bDNA assay exhibits several of the performance criteria necessary for routine use in a clinical laboratory. Our results demonstrate that the bDNA assay is at least as sensitive as culture techniques, is linear over the nearly 3-$\log_{10}$ dynamic range, and exhibits a high level of reproducibility. Our results also indicated that the bDNA assay was specific for CMV DNA and was not affected by the presence of other viruses, microorganisms, or common drugs. Furthermore, the bDNA assay was not influenced by latent CMV infection since specimens from both healthy seronegative and seropositive individuals consistently tested negative.

The potential clinical utility of the bDNA assay was illustrated in this study by monitoring CMV DNA levels in PBLs from an HIV-positive patient undergoing therapy. Other studies further illustrate the potential clinical utility of CMV DNA quantification by the bDNA assay. For example, in evaluating the efficacy of the antiviral nucleotide analog cidofovir, (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]cytosine, the bDNA assay was used to measure CMV DNA levels in serial semen samples from HIV-positive men undergoing therapy (22). The bDNA assay also has been used to measure CMV DNA levels in serial samples of cerebrospinal fluid from patients with CMV neurologic infections to monitor the response to antiviral therapy (15). These studies not only provide examples of the use of the bDNA assay in clinical laboratories but also illustrate the application of the bDNA assay for measuring CMV DNA levels in various clinical specimens.

Recent studies have focused on the potential clinical utility of drugs such as ganciclovir and acyclovir for CMV prophylaxis and preemptive therapy in immunocompromised patients, including HIV-positive patients (11) and transplant recipients (19, 33). Although these therapies can be effective, they are not without toxicity and cost. It may be possible to use these drugs more effectively by identifying those patients at the very highest risk of developing CMV disease by using molecular biology-based methods such as the bDNA assay for CMV DNA quantification. For example, it has recently been shown that oral ganciclovir is effective for CMV prophylaxis in HIV-positive patients at high risk of CMV disease, i.e., <100 $CD4^+$ cells (36). To avoid unnecessary toxic effects and expense, it would be clinically useful to identify within this subset of patients the highest-risk patients for selective prophylaxis. The bDNA assay may be useful in defining a threshold CMV DNA level associated with an extremely high risk of CMV disease and thereby provide information that could be used for more selective prophylaxis and preemptive therapy.

Another area in which the bDNA assay may prove to be especially useful is in the management of severely immunocompromised patients, such as HIV-infected individuals, who frequently need to be on chronic suppressive therapy for viral infections like herpes simplex virus or CMV. In this particular population, viral resistance often emerges, leading to drug failure and clinical progression (12). Since viral resistance could be inferred by increases in viral load during therapy (4, 6), the ability to monitor viral load in the blood of these patients may allow physicians to detect viral resistance and drug failure earlier. Thus, the monitoring of therapy both from a clinical perspective and from a quantitative perspective might lead to better patient management, since adjustments to therapy could be made prior to clinical deterioration of the patient.

In summary, we have developed a bDNA assay for the quantification of CMV DNA in clinical specimens. With its high degree of reproducibility and broad linear dynamic range, the bDNA assay for the quantification of CMV DNA may provide a useful tool that can be used to aid physicians in monitoring

disease progress, evaluating therapeutic regimens, and facilitating prompt recognition of viral resistance and drug failure.

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REFERENCES

1. **Arribas, J. R., D. B. Clifford, C. J. Fichtenbaum, D. L. Commins, W. G. Powderly, and G. A. Storch.** 1995. Level of cytomegalovirus (CMV) DNA in cerebrospinal fluid of subjects with AIDS and CMV infection of the central nervous system. J. Infect. Dis. **172:**527–531.
2. **Biron, K. K., S. C. Stanat, J. B. Sorrell, J. A. Fife, P. M. Keller, C. U. Lambe, and D. J. Nelson.** 1985. Metabolic activation of the nucleoside analog 9-{[2-hydroxy-1-(hydroxymethly)ethoxy]methyl}guanine in human diploid fibroblasts infected with human cytomegalovirus. Proc. Natl. Acad. Sci. USA **82:**2473–2477.
3. **Bogner, E., M. Reschke, B. Reis, T. Mockenhaupt, and K. Radsak.** 1993. Identification of the gene product encoded by ORF UL56 of the human cytomegalovirus genome. Virology **196:**290–293.
4. **Boivin, G., S. Chou, M. R. Quirk, A. Erice, and M. C. Jordan.** 1996. Detection of ganciclovir resistance mutations and quantitation of cytomegalovirus (CMV) DNA in leukocytes of patients with fatal disseminated CMV disease. J. Infect. Dis. **173:**523–528.
5. **Chou, S., and K. M. Dennison.** 1991. Analysis of interstrain variation in cytomegalovirus glycoprotein B sequences encoding neutralization-related epitopes. J. Infect. Dis. **163:**1229–1234.
6. **Chou, S., S. Guentzel, K. R. Michels, R. C. Miner, and W. L. Drew.** 1995. Frequency of UL97 phosphotransferase mutations related to ganciclovir resistance in clinical cytomegalovirus isolates. J. Infect. Dis. **172:**239–242.
7. **Collins, M. L., C. Zayati, J. J. Detmer, B. Daly, J. A. Kolberg, T. A. Cha, B. D. Irvine, J. Tucker, and M. S. Urdea.** 1995. Preparation and characterization of RNA standards for use in quantitative branched DNA hybridization assays. Anal. Biochem. **226:**120–129.
8. **Davis, G. L., J. Y. Lau, M. S. Urdea, P. D. Neuwald, J. C. Wilber, K. Lindsay, R. P. Perrillo, and J. Albrecht.** 1994. Quantitative detection of hepatitis C virus RNA with a solid-phase signal amplification method: definition of optimal conditions for specimen collection and clinical application in interferon-treated patients. Hepatology **19:**1337–1341.
9. **Detmer, J., R. Lagier, J. Flynn, C. Zayati, J. Kolberg, M. Collins, M. Urdea, and R. Sánchez-Pescador.** 1996. Accurate quantification of HCV RNA from all HCV genotypes using branched DNA (bDNA) technology. J. Clin. Microbiol. **34:**901–907.
10. **Dewar, R. L., H. C. Highbarger, M. D. Sarmiento, J. A. Todd, M. B. Vasudevachari, R. J. Davey, J. A. Kovacs, N. P. Salzman, H. C. Lane, and M. S. Urdea.** 1994. Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. J. Infect. Dis. **170:**1172–1179.
11. **Drew, W. L., D. Ives, J. P. Lalezari, C. Crumpacker, S. E. Follansbee, S. A. Spector, C. A. Benson, D. N. Friedberg, L. Hubbard, M. J. Stempien, A. Shadman, and W. Buhles.** 1995. Oral ganciclovir as maintenance treatment for cytomegalovirus retinitis in patients with AIDS. Syntex Cooperative Oral Ganciclovir Study Group. N. Engl. J. Med. **333:**615–620.
12. **Drew, W. L., R. C. Miner, D. F. Busch, S. E. Follansbee, J. Gullett, S. G. Mehalko, S. M. Gordon, W. F. Owen, Jr., T. R. Matthews, W. C. Buhles, and B. DeArmond.** 1991. Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection. J. Infect. Dis. **163:**716–719.
13. **Drouet, E., R. Colimon, S. Michelson, N. Fourcade, A. Niveleau, C. Ducerf, A. Boibieux, M. Chevallier, and G. Denoyel.** 1995. Monitoring levels of human cytomegalovirus DNA in blood after liver transplantation. J. Clin. Microbiol. **33:**389–394.
14. **Einsele, H., G. Ehninger, H. Hebart, K. M. Wittkowski, U. Schuler, G. Jahn, P. Mackes, M. Herter, T. Klingebiel, J. Loffler, S. Wagner, and C. A. Müller.** 1995. Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. Blood **86:**2815–2820.
15. **Flood, J., W. L. Drew, R. Miner, D. Jekic-McMullen, L.-P. Shen, J. Kolberg, J. Garvey, S. Follansbee, and M. Poscher.** 1997. Detection of cytomegalovirus (CMV) polyradiculopathy and documentation of in vivo anti-CMV activity in cerebrospinal fluid using branched DNA signal amplification and CMV antigen assays. J. Infect. Dis. **176:**348–352.
16. **Gerna, G., M. Furione, F. Baldanti, and A. Sarasini.** 1994. Comparative quantitation of human cytomegalovirus DNA in blood leukocytes and plasma of transplant and AIDS patients. J. Clin. Microbiol. **32:**2709–2717.
17. **Graybill, F.** 1976. Theory and application of the linear model, p. 606–648. Prindle, Weber and Schmidt, Boston, Mass.
18. **Hendricks, D. A., B. S. Stowe, B. S. Hoo, J. Kolberg, B. S. Irvine, P. D. Neuwald, M. S. Urdea, and R. P. Perillo.** 1995. Quantitation of HBV DNA in human serum using a branched DNA (bDNA) signal amplification assay. Am. J. Clin. Pathol. **104:**537–546.
19. **Hibberd, P. L., N. E. Tolkoff-Rubin, D. Conti, F. Stuart, J. R. Thistlethwaite, J. F. Neylan, D. R. Snydman, R. Freeman, M. I. Lorber, and R. H. Rubin.** 1996. Preemptive ganciclovir therapy to prevent cytomegalovirus disease in cytomegalovirus antibody-positive renal transplant recipients. Ann. Intern. Med. **123:**18–26.
20. **Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature **373:**123–126.
21. **Kühn, J. E., T. Wendland, P. Schafer, K. Mohring, U. Wieland, M. Elgas, and H. J. Eggers.** 1994. Monitoring of renal allograft recipients by quantitation of human cytomegalovirus genomes in peripheral blood leukocytes. J. Med. Virol. **44:**398–405.
22. **Lalezari, J. P., W. L. Drew, E. Glutzer, C. James, D. Miner, J. Flaherty, P. E. Fisher, K. Cundy, J. Hannigan, J. C. Martin, and H. S. Jaffe.** 1995. (*S*)-1-[3-Hydroxy-2-(phosphonylmethoxy)propyl]cytosine (cidofovir): results of a phase I/II study of a novel antiviral nucleotide analogue. J. Infect. Dis. **171:**788–796.
23. **Mañez, R., S. Kusne, C. Rinaldo, J. M. Aguado, K. St. George, P. Grossi, B. Frye, J. J. Fung, and G. D. Ehrlich.** 1996. Time to detection of cytomegalovirus (CMV) DNA in blood leukocytes is a predictor for the development of CMV disease in CMV-seronegative recipients of allografts from CMV-seropositive donors following liver transplantation. J. Infect. Dis. **173:**1072–1076.
24. **McCutchan, J. A.** 1995. Clinical impact of cytomegalovirus infections of the nervous system in patients with AIDS. Clin. Infect. Dis. **21**(Suppl. 2)**:**S196–S201.
25. **Mellors, J. W., C. R. Rinaldo, Jr., P. Gupta, R. M. White, J. A. Todd, and L. A. Kingsley.** 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science **272:**1167–1170.
26. **National Committee for Clinical Laboratory Standards.** 1992. Evaluation of precision, performance of clinical chemistry devices; tenative guidelines, vol. 12. NCCLS publication EP5-T2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
27. **Nolte, F. S., R. K. Emmens, C. Thurmond, P. S. Mitchell, C. Pascuzzi, S. M. Devine, R. Saral, and J. R. Wingard.** 1995. Early detection of human cytomegalovirus viremia in bone marrow transplant recipients by DNA amplification. J. Clin. Microbiol. **33:**1263–1266.
28. **Pachl, C., J. A. Todd, D. G. Kern, P. J. Sheridan, S.-F. Fong, M. Stempien, B. Hoo, D. Besemer, T. Yeghiazarian, B. Irvine, J. Kolberg, R. Kokka, P. Neuwald, and M. S. Urdea.** 1995. Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA (bDNA) signal amplification assay. J. Acquired Immune Defic. Syndr. Hum. Retrovirol. **8:**446–454.
29. **Polis, M. A., and H. Masur.** 1995. Promising new treatments for cytomegalovirus retinitis. JAMA **273:**1457–1459.
30. **Pollard, R. B.** 1988. Cytomegalovirus infections in renal, heart, heart-lung, and liver transplantation. Pediatr. Infect. Dis. J. **7**(Suppl. 5)**:**S97–S102.
31. **Rasmussen, L., S. Morris, D. Zipeto, J. Fessel, R. Wolitz, A. Dowling, and T. C. Merigan.** 1995. Quantitation of human cytomegalovirus DNA from peripheral blood cells of human immunodeficiency virus-infected patients could predict cytomegalovirus retinitis. J. Infect. Dis. **171:**177–182.
32. **Schmidt, C. A., H. Oettle, R. Peng, P. Neuhaus, G. Blumhardt, R. Lohmann, F. Wilborn, K. Osthoff, J. Oertel, H. Timm, and W. Seigert.** 1995. Comparison of polymerase chain reaction from plasma and buffy coat with antigen detection and occurrence of immunoglobulin M for the demonstration of cytomegalovirus infection after liver transplantation. Transplantation **59:**1133–1138.
33. **Singh, N., V. L. Yu, L. Mieles, M. M. Wagener, R. C. Miner, and T. Gayowski.** 1994. High-dose acyclovir compared with short-course preemptive ganciclovir therapy to prevent cytomegalovirus disease in liver transplant recipients. A randomized trial. Ann. Intern. Med. **120:**375–381.
34. **Spaete, R. R., R. M. Thayer, W. S. Probert, F. R. Masiarz, S. H. Chamberlain, L. Rasmussen, T. C. Merigan, and C. Pachl.** 1988. Human cytomegalovirus strain Towne glycoprotein B is processed by proteolytic cleavage. Virology **167:**207–225.
35. **Spector, S. A., K. Hsia, D. Wolf, M. Shinkai, and I. Smith.** 1995. Molecular detection of human cytomegalovirus and determination of genotypic ganciclovir resistance in clinical specimens. Clin. Infect. Dis. **21**(Suppl. 2)**:**S170–S173.
36. **Spector, S. A., G. F. McKinley, J. P. Lalezari, T. Samo, R. Andruczk, S. Follansbee, P. D. Sparti, D. V. Havlir, G. Simpson, W. Buhles, R. Wong, M. J. Stempien, and for the Roche Cooperative Oral Ganciclovir Study Group.** 1996. Oral ganciclovir for the prevention of cytomegalovirus disease in persons with AIDS. N. Engl. J. Med. **334:**1491–1497.
37. **The, T. H., A. P. van den Berg, M. C. Harmsen, W. van der Bij, and W. J. van Son.** 1995. The cytomegalovirus antigenemia assay: a plea for standardization. Scand. J. Infect. Dis. Suppl. **99:**25–29.
38. **Weber, B., and H. W. Doerr.** 1994. Diagnosis and epidemiology of transfusion-associated human cytomegalovirus infection: recent developments. Infusther. Transfusmed. **1:**32–39.