Evaluation of a Commercial PCR Kit for Diagnosis of Cytomegalovirus Infection of the Central Nervous System

ADRIANA WEINBERG,1, * DEBBIE SPIERS,1 GUAN-YUNG CAL,1 CHRISTOPHER M. LONG,2 RITA SUN,2 AND VINCE TEVERE2

Pediatric Infectious Diseases, University of Colorado School of Medicine, Denver, Colorado,1 and Roche Molecular Systems, Inc., Branchburg, New Jersey2

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We evaluated the AMPLICOR cytomegalovirus (CMV) PCR kit for the diagnosis of neurologic CMV infections on 43 positive and 112 negative archived cerebrospinal fluid specimens originally tested by an in-house PCR method. The AMPLICOR kit showed sensitivity and specificity of 95 and 100%, respectively, versus the home-grown assay, indicating its utility in this clinical setting.

Cytomegalovirus (CMV) infections of the central nervous system (CNS) are sources of morbidity and mortality frequently in AIDS patients and less often in normal hosts. CMV encephalitis is identified at autopsy in 12% of AIDS patients and in 2% of transplant recipients (3). Besides encephalitis, several other neurological syndromes have been associated with CMV infection, such as retinitis, myelitis/polyradiculopathy, meningitis, and mononeuritis multiplex (10). Although CMV infection of the CNS has been increasingly recognized at autopsy, its diagnosis in vivo has been limited by the lack of sensitivity of the most-traditional diagnostic procedures. CMV culture of the cerebrospinal fluid (CSF) is rarely positive (3, 10), and even in syndromes in which CSF invasion is the postulated mode of spread, such as polyradiculitis or ventriculoencephalitis, CMV is isolated only in half of the cases (8). Other techniques, such as detection of CMV antigen in CSF leukocytes (7, 11) or measurement of CSF/serum antibody ratios (6), have been successfully employed in small numbers of patients for the diagnosis of CMV encephalitis, but the most promising results reported have been obtained using qualitative CMV PCR on CSF (2, 4, 8, 13). With two exceptions (1, 9), the studies have shown that CMV PCR has both high sensitivity and specificity for the diagnosis of CMV infection of the CNS in vivo. Among other reasons, the discrepant results could be ascribed to a lack of standardization of CMV PCR among laboratories. In addition, the availability of CMV PCR has been limited to a few research laboratories. This study examines the accuracy of the commercial AMPLICOR CMV test (Roche Molecular Systems, Inc., Branchburg, N.J.) for the diagnosis of CMV infections of the CNS.

Clinical specimens were obtained from the archives of the Diagnostic Virology Laboratory at the University of Colorado Health Sciences Center. We selected 155 CSF specimens, 43 positive and 112 negative, that had been prospectively tested by in-house CMV PCR between the years 1994 and 1996 and had been stored in multiple aliquots at −70°C. Clinical information, collected prospectively for the positive specimens, revealed that 42 CSF samples were derived from human immunodeficiency virus (HIV)-infected patients with encephalitis (n = 23), polyradiculitis (n = 14), meningitis (n = 2), and undefined neurologic conditions (n = 3). The remaining specimen was from an HIV-negative patient with acute CMV infection, which was followed by Guillain-Barré syndrome. The 23 patients with CMV encephalitis were diagnosed by a neurologist and presented with progressive mental status alterations (n = 18), seizures (n = 5), cranial nerve palsies (n = 2), headache (n = 2), ataxia (n = 1), and fever (n = 5). The CD4 cell counts of these patients were lower than 37 cells/μl, with a median of 7 cells/μl (viral load values were not routinely available between 1994 and 1996). Ten of the 23 patients had concomitant or prior diagnosis of CMV infection at another site as follows: eight, retinitis; one, colitis; one, pneumonitis. The CSF showed pleocytosis and increased protein with normal glucose in two patients who also had radiologic evidence of ventriculoencephalitis. In the remaining 21 patients, the CSF was only mildly abnormal, consistent with previous descriptions of CMV diffuse micronodular encephalitis (3, 10). Computer tomography or magnetic resonance images were abnormal in 10 of the 14 patients for whom these studies were available for review. Other opportunistic infections or malignant processes were excluded. HIV dementia was considered less likely than CMV encephalitis based on previously established criteria (9). The 14 HIV-infected patients with polyradiculitis presented with sensorimotor abnormalities of the lower extremities and marked pleocytosis of the CSF. Other opportunistic infections or malignancies were ruled out by standard laboratory and radiologic CNS evaluations. The two patients diagnosed with CMV meningitis had intense headache and fever at presentation but normal mental status; the CSF examined after 1 month of ganciclovir therapy showed a marked decrease in cellularity (25 cells/μl) and negative CMV PCR. In the HIV-infected patients, PCR was the only CMV-specific test performed on CSF for the following reasons: it has been shown to...
be the method of choice for the diagnosis of CNS infections with CMV (3), CMV culture of the CSF is an insensitive method, and specific antibody production is grossly impaired in AIDS patients and cannot be relied upon for diagnostic purposes. In the normal host with Guillain-Barré syndrome following acute CMV infection, CMV-specific immunoglobulin M and G antibodies were found in serum but not in the CSF. Although the virus was isolated from the urine of this patient, CMV culture of the CSF was negative, which is consistent with the previously reported lack of sensitivity of CMV culture for CSF.

In-house qualitative PCR was performed according to a technique previously published (5) using primers 625 and 461 and probe 626 from the EcoRI fragment D region of CMV strain AD 169 (13). DNA was extracted from 30-µl aliquots with the InstaGene purification matrix (Bio-Rad Laboratories Inc., Hercules, Calif.) according to the manufacturer’s instructions. The PCR was carried out in a 50-µl final volume containing 20 µl of extracted DNA, 1.25 U of PfuM (Stratagene, La Jolla, Calif.), 100 nM each of four deoxynucleoside triphosphates, and 1 µM each primer in PfuM buffer (Stratagene). The samples were amplified in duplicate for 45 cycles. The amplified DNA was separated by polyacrylamide gel electrophoresis and transferred to nylon membranes. The identity of the DNA bands was confirmed by hybridization with the digoxigenin-labeled probe 626 (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) followed by an antidigoxigenin antibody conjugated to alkaline phosphatase (Boehringer Mannheim) and CSPD chemiluminescent substrate for phosphatases (Tropix, Bedford, Mass.). Each assay included a negative water control and two positive sensitivity controls, a detergent lysate of CMV AD 169 at 1 PFU/ml and a known positive clinical specimen. The test was considered valid if the controls yielded the expected results and if the patient replicates agreed. This method has an analytical sensitivity of two to three copies per PCR, which corresponds to 270 to 400 copies/ml of CSF. The sensitivity of the in-house PCR, evaluated on different specimens, was 100% for 30 CMV culture-positive blood and bronchoalveolar lavage specimens, 95% for 20 culture-positive urine samples, and 100% for 20 CSF samples spiked with 400 CMV DNA copies/ml. These results demonstrated that the PCR assay could detect a diversity of CMV clinical strains and that CMV processed according to the described procedure did not inhibit the PCR. A direct comparison of CMV PCR and culture of CSF was not available due to the extremely low number of positive CMV cultures of the CSF (3, 10). The specificity of the in-house PCR was 100% measured on 50 CSF samples from HIV-infected and uninfected patients, in whom an etiologic diagnosis different from CMV had been established, including infections with herpes simplex virus, varicella-zoster virus, Epstein-Barr virus, enterovirus, JC virus, CNS lymphoma, and bacterial meningitis.

The AMPLICOR CMV PCR test, which targets a region of the CMV DNA polymerase gene (UL54), was performed according to the manufacturer’s instructions. Briefly, CMV DNA is isolated by lysis of the virus at 100°C in a proteinase K-containing detergent solution and amplified by using the biotinylated primers LC383 (5’-GGCCAGGTGTCACTTGGCAGG-3’) and LC342 (5’-GCGCTATCGGTGTCGCTGGTACTC-3’), Taq thermostable polymerase, and deoxynucleotide triphosphates in a buffered solution. Selective amplification of the target DNA was achieved with dUTP and uracil-N-glycosylase. The amplified product was hybridized to a complementary oligonucleotide probe, LC359 (5’-GGCCCGAACGAACCGCAACAC-3’), used to coat on a microwell plate. Bound amplicon was detected with avidin-conjugated horse-radish peroxidase and colorimetric substrate. The AMPLICOR CMV test has an analytical sensitivity of five copies per PCR, which corresponds to 1,000 copies/ml.

Quantitative CMV PCR was performed by a competitive method. The internal standard (IS) for this assay was synthesized by the following procedure: a 335-bp fragment in the CMV major immediate-early (MIE) region MIE2783 to MIE3117 was amplified by PCR; a 92-bp internal segment was deleted from the 335-bp fragment resulting in a 243-bp fragment, the IS, which shared primer sites with the parent fragment (MIE2783 to MIE3117). The 243-bp IS was amplified by PCR and inserted in the vector PCR-Script SK (+) (Stratagene). Escherichia coli XL1-Blue (Stratagene) transformed with the IS-containing plasmid was grown in Luria-Bertani medium (Gibco BRL, Grand Island, N.Y.) and selected with an ampicillin marker. The plasmid DNA was purified with a Qiagen tip 500 column (Qiagen, Valencia, Calif.) and quantitated with a spectrophotometer (DU 62; Beckman). DNA extracted from the clinical specimen was mixed with six 10-fold dilutions of the IS between 10² and 10⁸ copies and amplified with PfuM as described for qualitative PCR. The PCR products were separated by gel electrophoresis, stained with vista green, scanned with a Storm instrument (Molecular Dynamics, Sunnyvale, Calif.), and quantitated by using ImageQuant software.

The limit of detection of the two PCR methods in our laboratory was compared using CMV AD 169, a laboratory-adapted strain of CMV, and two CMV clinical isolates. All three CMV strains were grown in human embryonic lung fibroblast tissue cultures. CMV cultures were harvested at 80% cytopathic effect and stored in liquid nitrogen. Serial 10-fold dilutions of CMV AD 169 and of the two clinical isolates were tested by in-house PCR and with the AMPLICOR CMV kit (Table 1). The endpoint dilution sensitivity of the AMPLICOR CMV test was lower than that of the in-house PCR in proportion to the different limits of detection of the two methods. The two PCR assays differed with respect to the target DNA, extraction, amplification, and hybridization procedures. Any of these variables might have contributed to the limit-of-detection difference.

AMPLICOR CMV evaluation of clinical specimens showed agreement between the two PCR methods for the 112 negative specimens, but 4 of the 43 positive samples were repeatedly negative in the AMPLICOR assay. Repeat testing by the in-house method of these four discrepant CSFs showed two positive and two negative results, indicating that the CMV DNA had degraded in two samples. The sensitivity and specificity of the AMPLICOR CMV test compared with the in-house CMV PCR was 95% and 100%, respectively, and the overall agreement between the two methods was 99%.

The two persistently discrepant CSF specimens, tested by quantitative CMV PCR, had 735 and 400 copies of CMV DNA/ml, which was below the limit of detection of the AMPLICOR CMV test. Although the CMV DNA copy num-

<table>
<thead>
<tr>
<th>CMV strain</th>
<th>CMV DNA copies/ml</th>
<th>Last positive dilution</th>
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<tbody>
<tr>
<td>AD 169</td>
<td>3.45 × 10⁹</td>
<td>10⁻⁷</td>
</tr>
<tr>
<td>CMV 1827</td>
<td>6.99 × 10⁹</td>
<td>10⁻⁷</td>
</tr>
<tr>
<td>CMV 238</td>
<td>9.29 × 10⁹</td>
<td>10⁻⁸</td>
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Quantitative CMV PCR compared with that of in-house PCR for CMV clinical and laboratory strains.
ber was low in these specimens, both results were clinically relevant. In contrast to plasma or circulating leukocytes, where low numbers of CMV DNA copies are frequently found in the absence of symptomatic disease (11), the presence of CMV DNA in the CSF appears to correlate closely with clinical disease. This is in accordance with autopsy findings (2, 13) which, except for one study (1), showed a high correlation between positive CMV PCR with CSF and histopathologic evidence of CMV-associated inflammation in the CNS.

Besides the immediate patient care benefit of a user-friendly CMV PCR assay, a readily available, reproducible, sensitive, and specific diagnostic test for CMV infection of the CNS will facilitate better definition of the spectrum of this disease in immunocompromised and immunocompetent patients. We showed that the AMPLICOR CMV test had a high sensitivity and specificity and its limit of detection was adequate to identify CMV DNA in the CSF of 95% of patients with neurologic CMV disease.

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REFERENCES