Evaluation of PCR Primers for Cytomegalovirus Detection

We read with great interest the article by Mendez et al. (2) published in a recent issue evaluating different PCR primers for cytomegalovirus (CMV) detection. As CMV PCR becomes a frequently used assay in the management of immunocompromised patients, such as transplant recipients and human immunodeficiency virus-infected individuals, an increasing number of laboratories are establishing in-house CMV PCR assays. In designing a PCR assay, the choices of specimen, sample preparation method, primers, probes, and amplification and detection conditions play a critical role in the sensitivity and specificity of the assay. Mendez et al. (2) compared primer pairs for HindIII-X fragment, EcoRI fragment D, immediate-early antigen 1 gene (IEA1), and major immediate-early gene (MIE) and found sensitivities of 94, 87, 32, and 20%, respectively. Prompted by their paper, we evaluated the HindIII-X primer pair versus EcoRI fragment D (4) and MIE 2783-3114 (1), both of which are routinely used in our laboratory. For this comparison, we used 10 plasma samples containing a low level of CMV DNA (<1,000 copies/ml). DNA was extracted from each plasma sample by using four protocols: protocols A and B involved DNA extraction from 5 and 10 μl of plasma, respectively, with Chelex resin (Bio-Rad); protocol C started with 300 μl of plasma, which was centrifuged at 24,000 × g for 1 h after which DNA was extracted from the pellet with Chelex resin; and in protocol D, plasma samples were heated at 100°C for 50 to 55 s. The amounts of template DNA used in each PCR tube in protocols A, B, C, and D corresponded to 1, 2, 60, and 2 μl of plasma, respectively. The amplification was performed as previously described (3), by using 1 U of Pyrococcus furiosus (Pfu) DNA polymerase (Stratagene) in appropriate buffer, 50 μM each deoxynucleoside triphosphate (dNTP), and 0.5 μM each primer in 50-μl reaction volumes. PCR products were separated according to their molecular weight by agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV light. Positive and negative controls were included in each run. In contrast to Mendez et al., we found a sensitivity of 78% for the EcoRI primers, which was superior to the MIE and HindIII primers’ sensitivities of 18 and 5%, respectively. All the specimens in which CMV was detected by HindIII primers also had CMV detected by EcoRI and MIE primers. The differences between primer pair sensitivities were stable across sample preparation protocols. Protocol D, which used 2 μl of heat-inactivated plasma sample/reaction tube, yielded the highest number of positive results across primer pairs, whereas protocol A, Chelex extraction of DNA from 10 μl of plasma, had the lowest yield. There are several differences between our assay and that described by Mendez et al. which might account for the discrepant results, mainly the utilization of different specimens, plasma versus peripheral-blood leukocytes (PBL), and different DNA polymerases, buffers, dNTP and primer concentrations, and cycling conditions. In addition, for this comparison we did not perform a Southern blot, although the assay used for selecting the 10 specimens relied on Southern blot confirmation. These discrepancies emphasize the importance of performing extensive evaluations of in-house PCR methods and adapting assay conditions to the primers used and the DNA template area that is interrogated.

REFERENCES


Adriana Weinberg
Shaobing Li
University of Colorado Health Sciences Center
4200 East Ninth Ave.
Denver, Colorado 80262

Authors’ Reply

Optimization and standardization of molecular diagnostic laboratory assays for the detection of CMV infection in organ transplantation have been important goals at our institution for effective management of these patients. Using primers directed to the EcoRI fragment D region of CMV, infection was detected a mean of 13 days before the onset of symptomatic infection with high sensitivity (92%) and specificity (100%), but the method lacked specificity (57%) for predicting disease due to this virus (5). Based on these results, a strategy for implementation of preemptive therapy of CMV infection in liver transplant recipients was performed by using the EcoRI primers, once more demonstrating their high sensitivity for the detection of early CMV infection. However, we had to abandon these primers for clinical use because isosporal incorporation into the small, 152-bp amplicon was not effective for the control of carryover contamination (2, 4, 6).

Using the specimens collected in such a prospective and interrupted preemptive-therapy trial, we sought primers selected by computer analysis of the MIE gene. In practice, these primers failed to detect CMV DNA early in the posttransplantation period. These results prompted us to evaluate several primer sets for early detection of CMV DNA which were described by other investigators and successfully implemented into their transplant programs; this was the basis for the published study to which Weinberg and Li refer.

Weinberg and Li compared results with only two of the four primer sets that we evaluated (406 bp [1] and 152 bp [6]). CMV primers directed to the MIE gene were different in the two studies (332 bp in the study by Weinberg and Li and 370 bp in our study). In addition, Weinberg and Li cited different conditions for almost every variable in the PCR protocol (specimen type, nucleic acid extraction method, DNA polymerase enzyme, etc.); nevertheless, the sensitivities for detection of CMV DNA with primers to the EcoRI fragment D gene were surprisingly similar in the two studies (87% in our study and 78% in that of Weinberg and Li). Other variables that need to be highlighted are the type and number of samples and method of sample collection, that is, plasma versus PBL (a few plasma samples [10 samples] in the study of Weinberg and Li versus many PBL samples [148 samples] in our study) and preselected stored samples versus prospectively collected and stored PBL specimens from a much larger number of individuals (21 individuals in our study). These factors likely account for the ob-
served differences in the sensitivities of primers for detecting CMV DNA.

We believe that valid comparison of PCR results between laboratories requires use of the same experimental conditions, as an absolute prerequisite, to expect results that can be accurately evaluated. In our study, we obtained equivalent results using our standard PCR protocol versus conditions customized in our laboratory that complied exactly with those described by investigators such as Drouet et al. for the primers directed to the HindIII-X locus (1). Moreover, we have continued to use HindIII-X locus primer sets in our ongoing trial demonstrating, once again, high sensitivity and specificity.

Clinical management of organ transplant patients, AIDS patients, and other immunocompromised populations susceptible to CMV infections is extremely complex. We agree with Weinberg and Li that in-house PCR protocols need to be carefully standardized to accurately detect and quantitate CMV DNA, at least for use in these local patient populations (3). Most importantly, several commercial systems are being developed and evaluated for the qualitative and quantitative detection of CMV DNA or RNA. In the future, implementation of these tests will allow for accurate and meaningful comparisons of data among laboratories.

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Thomas F. Smith
Mark J. Espy
Carlos V. Paya
Division of Clinical Microbiology
Division of Infectious Diseases
Mayo Clinic
Rochester, Minnesota 55905