Comparative Evaluation of Colorimetric Microtiter Plate Systems for Detection of Herpes Simplex Virus in Cerebrospinal Fluid

YI-WEI TANG, PAUL N. RYS, BARBARA J. RUTLEDGE, P. SHAWN MITCHELL, THOMAS F. SMITH, AND DAVID H. PERSING*

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota 55905

Received 10 March 1998/Returned for modification 13 April 1998/Accepted 3 June 1998

In the past few years, application of the PCR to the detection of herpes simplex virus (HSV) DNA in the cerebrospinal fluid (CSF) from patients with encephalitis and meningitis has become standard laboratory practice. However, from an operational perspective, the true diagnostic value of PCR in this setting is yet to be realized because most laboratories subject the amplification products to lengthy probe hybridization procedures by Southern blotting. As alternatives to Southern blotting, we evaluated colorimetric microtiter plate (MTP) systems from ViroMed Laboratories, Inc. (PrimeCapture), CPG, Inc. (Quanti-PATH), and Incstar Corp. (GEN-ETI-K), in addition to a system developed at the Mayo Clinic with the PCR ELISA system (Boehringer Mannheim Corp.). We tested PCR products from 86 clinical CSF specimens submitted to our Molecular Microbiology Laboratory. The CSF specimens used had to have sufficient volume for comparative analysis. By conventional Southern blotting methods, 54 were positive and 32 were negative for HSV DNA. Compared with Southern blotting, the sensitivity and specificity were 63.0 and 100.0%, respectively, for the PrimeCapture system, 98.2 and 96.9%, respectively, for the Quanti-PATH system, 98.2 and 100.0%, respectively, for the GEN-ETI-K system, and 100.0 and 96.9%, respectively, for the Mayo system. All four MTP systems had turnaround times 12 to 24 h less than that for Southern blotting. There were no significant differences in costs or technologist time between the Mayo system and Southern blotting. Other features of the Mayo system include type-specific genotypic identification of HSV and the potential for determination of drug resistance by DNA sequencing. Overall, we found that colorimetric MTP systems were likely to improve test turnaround times and patient care at no additional cost.

Herpes simplex virus (HSV) is a ubiquitous agent responsible for a wide variety of human infections. In addition to epithelial infections such as gingivostomatitis, pharyngitis, genital herpes, whitlow, conjunctivitis, and keratitis, HSV is an important cause of central nervous system (CNS) infections and accounts for 2 to 19% of human encephalitis cases (9, 33, 38). The clinical spectrum of CNS diseases has recently been expanded; for example, most cases of benign recurrent aseptic meningitis (Mollaret’s meningitis) are caused by HSV (39), especially HSV type 2 (HSV-2) (36). Because specific antiviral therapy is available, the rapid, definitive laboratory diagnosis of HSV is important to support clinical findings. Moreover, in the setting of possible HSV encephalitis, patients are often managed as inpatients while awaiting test results.

Although cell culture is considered the standard method for laboratory diagnosis of ulcerative HSV infections, HSV is rarely recovered in cell cultures inoculated with cerebrospinal fluid (CSF). Brain biopsy specimens may yield culturable virus, but this invasive surgical procedure is controversial when performed solely to collect specimens for the laboratory diagnosis of infectious disease. The sensitivities of HSV antigen and antibody assays for CNS infections are very low (13). In addition, antibodies may appear in the CSF as a consequence of the breakdown in the blood-brain barrier, leading to false-positive results (23). Since 1990, several studies have shown that PCR detection of HSV DNA in CSF should be considered the new standard for the laboratory diagnosis of CNS disease caused by this virus (8, 11, 12, 15, 22, 24, 28–30).

Although the technology underlying PCR is relatively rapid, the PCR product (amplicon) must be identified definitively as the sequence of interest to provide adequate diagnostic specificity. The conventional technique for this is hybridization of a specific probe to a Southern blot, which increases both the sensitivity and the specificity of the test. This step, however, takes an additional 12 to 24 h to complete, delaying the use of test results for clinical intervention. The ideal postamplification detection system would combine the increased sensitivity and specificity of Southern blotting with a rapid turnaround time (2, 14, 19, 20, 32). For this purpose, enzyme-linked adsorbent microtiter plate (MTP) systems have been adapted for amplicon detection (40).

We report a comparison of four colorimetric MTP systems: the PrimeCapture system from ViroMed Laboratories, Inc., the Quanti-PATH system from CPG, Inc., the GEN-ETI-K system from Incstar Corp., and a system developed at the Mayo Clinic by PCR ELISA (Boehringer Mannheim Corp.). We tested all four systems against standard Southern blotting for the detection of HSV PCR products resulting from clinical CSF specimens.

(This study was presented in part at the 14th Annual Meeting of the Pan American Society of Clinical Virology, 26 to 29 April 1998, Clearwater Beach, Fla.)

MATERIALS AND METHODS

Specimens. Eighty-six CSF specimens submitted to the Molecular Microbiology Laboratory at the Mayo Clinic for the diagnosis of HSV CNS disease by PCR were selected retrospectively for the study.

Colorimetric MTP systems. We evaluated four colorimetric MTP systems. Three were commercial kits specifically designed for the detection of HSV DNA.
In one type of format (PrimeCapture [lot 9705309116; ViroMed Laboratories, Inc., Minneapolis, Minn.]; Quant-Path [lot 116; CPG, Inc., Lincoln Park, N.J.]), amplification of the HSV target was performed with the biotinylated primers included in the kit. The amplified product was denatured and hybridized to the MTP wells, which were precoated with a sequence-specific capture probe. Unbound amplicons were washed away. A streptavidin-enzyme conjugate was added following colorimetric detection (17, 20, 37). A second format (GEN-ETI-K [lot 95SB04; Instar Corp., Stillwater, Minn.]) also had HSV-specific probes bound to the MTP wells. In this system, however, the target (also amplified with primers included in the kit) was not biotinylated. Instead, an enzyme-linked antibody recognized target DNA that had hybridized to the specific probes on the MTP well surface (21). In another format, developed by Boehringer Mannheim Corp. (the PCR ELISA kit), digoxigenin-dUTP was incorporated with the PCR product. A sequence-specific, biotinylated capture probe was hybridized to the denatured amplicon, and the complexes were captured in avidin-coated MTP wells. Detection was completed with enzyme-linked anti-digoxigenin antibodies (10, 16, 27). We developed the Mayo system with the PCR ELISA kit. Type-specific 5'-biotinylated capture probes for HSV-1 (TK-G, 5'-ACAAACATCGTGTTGGGGGC-3') and HSV-2 (TK-H, 5'-AGCAACCTGTGGCTTGGGTG-3') were designed to differentiate HSV genotypes (7, 34).

**TABLE 1. Sensitivities and specificities of colorimetric MTP systems for HSV amplicon identification**

<table>
<thead>
<tr>
<th>Test source</th>
<th>No. of specimens with the following results:</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPG, Inc.</td>
<td>SB+, MTP+ SB–, MTP+ SB–, MTP–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Instar Corp.</td>
<td>53</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>Mayo</td>
<td>53</td>
<td>1</td>
<td>32</td>
</tr>
<tr>
<td>ViroMed Laboratories, Inc.</td>
<td>34</td>
<td>20</td>
<td>32</td>
</tr>
</tbody>
</table>

*SB, Southern blotting followed by probe hybridization; MTP, colorimetric MTP systems; +, positive result; −, negative result.

**RESULTS**

**Performance evaluation.** Of 86 CSF specimens tested, 54 were HSV DNA positive and 32 were HSV DNA negative, as determined by detection of an amplification product in ethidium bromide-stained agarose gels or by film exposure after Southern blotting with probe hybridization. This technique is referred to as the “conventional method” in the present study. All four MTP systems had high specificities (≥96.9%) compared with the results of conventional methods (Table 1). The MTP systems from CPG, Inc., Instar Corp., and Mayo also had excellent sensitivities (≥98.2%) compared with the sensitivity of the system from ViroMed Laboratories, Inc., which was significantly lower (63.0%; 95% confidence interval, 51 to 77%). With MTP assays, the difference in OD between positive and negative controls is the objective basis for interpreting colorimetric data. All four MTP systems had mean ratios of the OD for a specimen versus the average OD for a negative control (S/N ratios) of >10 for specimens positive by conventional methods and mean S/N ratios of ≤1 for specimens negative by conventional methods (Fig. 1). Thus, the mean cutoff values which distinguished positive results from negative results were well separated for all four systems evaluated.

The Mayo system was able to differentiate HSV genotypes by using type-specific capture probes. Among 55 CSF specimens which yielded positive tests with the Mayo system, 20 (36.4%) were infected with HSV-1, 32 (58.2%) were infected with HSV-2, and 3 (5.5%) were infected with both genotypes of the virus. Of the 54 CSF specimens identified as positive both with the Mayo system and by the conventional method, the MTP system from ViroMed Laboratories, Inc., was positive for 13 (65.0%) of 20 HSV-1-positive specimens, 20 (64.5%) of

![FIG. 1. S/N ratios determined with four MTP systems. HSV DNA positivity or negativity was determined by Southern blotting followed by probe hybridization. The large dots represent 10 specimens, and the small dots represent 1 specimen. A short bar stands for the cutoff value for each test.](http://jcm.asm.org/)

---

<|image> Downloaded from [http://jcm.asm.org/](http://jcm.asm.org/) on October 20, 2017 by guest |
antiviral therapy could be provided. In addition, negative PCR results for HSV DNA in some cases provide sufficient evidence to mitigate against unnecessary intravenous acyclovir treatment ($180.00/day) and would expand the diagnostic consideration for other etiologic causes of CNS infection.

Colorimetric MTP systems and Southern blotting use a sequence-specific probe that provides two technical enhancements to PCR (10, 20, 21). First, the sensitivity for the detection of PCR products with MTP systems is 10- to 100-fold greater than that with ethidium bromide-stained agarose gels. Second, the use of a specific probe confirms the specificity of the PCR product. Unlike Southern blotting and probe hybridization, use of the MTP allows the simultaneous rapid analysis of multiple PCR mixtures. Importantly, the assay is potentially automatable when performed in a 96-well format. Finally, the physical containment of the amplification products in the MTP is superior to that by Southern blotting-based methods and thus may be less prone to contamination problems.

Additional advantages of the Mayo-developed system include identification of virus genotypes and the recognition of polymorphisms that may be responsible for drug resistance. The TK gene of HSV has been targeted by the Mayo MTP system. Because significant heterogeneity between HSV-1 and HSV-2 exists in the 335-bp amplicon, allele-specific probes can be used for genotype determination. Interestingly, our results have indicated that some HSV-amplified DNAs react with both type-specific probes, suggesting mixed infections with the two genotypes. Point mutations in the TK gene may also be responsible for acyclovir resistance (3, 4, 25), and polymorphisms in this locus may be associated with neurotropism (31). Determination of acyclovir resistance or neurotropism by detection of these point mutations may be important for patients undergoing long-term therapy and immunocompromised hosts (6, 26). A single test that includes amplification of the TK gene followed by determination of sequence polymorphisms may be able to predict the HSV genotype and possibly drug resistance and neurotropism without the need for a second amplification reaction. Therefore, by using separate MTP wells for HSV-1- and HSV-2-specific hybridizations, the viral type can be identified immediately. Acyclovir resistance could be determined by direct sequencing of the amplicon based on clinical management.

**TABLE 2. Contrast of test time among colorimetric MTP systems for amplicon identification**

<table>
<thead>
<tr>
<th>Test source</th>
<th>Time (min) required for the following step:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Denaturation</td>
</tr>
<tr>
<td>CPG, Inc.</td>
<td>2</td>
</tr>
<tr>
<td>Incstar Corp.*</td>
<td>15</td>
</tr>
<tr>
<td>Mayo</td>
<td>10</td>
</tr>
<tr>
<td>ViroMed Laboratories, Inc.</td>
<td>2</td>
</tr>
</tbody>
</table>

*Additional precoating step not included.

### DISCUSSION

The generation of rapid laboratory test results has had a major impact on the clinical management of patients with CNS infections. This is especially true for HSV infections since early treatment with acyclovir is very effective, reducing the rate of mortality from HSV encephalitis from 70% to 19 to 30% (33, 38). In the present study, we used Southern blotting followed by probe hybridization as our evaluation standard, which has a demonstrated sensitivity of 97.7 to 98.1% compared to the estimated laboratory volume of 3,040 procedures in 1996. Costs and technologist times were similar for the Mayo assay and the conventional methods (Table 3).

**TABLE 3. Cost analysis of HSV PCR: Southern blotting versus the Mayo colorimetric MTP system**

<table>
<thead>
<tr>
<th>Test</th>
<th>Direct cost ($/procedure)</th>
<th>Total annual cost</th>
<th>Fixed effort</th>
<th>Variable paramedical effect</th>
<th>Total technologist time (h) annually</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBf</td>
<td>53.75</td>
<td>58.05</td>
<td>176,472.00</td>
<td>13.30</td>
<td>782.29</td>
</tr>
<tr>
<td>Mayo MTP</td>
<td>52.75</td>
<td>56.95</td>
<td>173,128.00</td>
<td>7.03</td>
<td>772.27</td>
</tr>
</tbody>
</table>

*Volume and expenses are based on information for 1996.

b The estimated annual volume is 3,040 procedures.

SB, Southern blotting.
Our data indicate that one need not sacrifice sensitivity to obtain rapid results. Three of the four MTP systems tested had sensitivities of $\geq98\%$ compared with the results of Southern blotting followed by probe hybridization. The Mayo system had the best sensitivity (100%) of the four systems tested. We noted that among 36 CSF specimens determined to be HSV DNA negative by conventional methods, one was positive by MTP analysis with the systems from both CPG, Inc., and Mayo. This result was obtained for a patient who was clinically diagnosed with aseptic meningitis on the basis of a headache, increased leukocyte counts, and elevated total protein levels in her CSF, suggesting a likely false-negative result by the conventional method.

Colorimetric MTP analysis for HSV PCR amplicon identification can be performed in less than 4 h. These assays do not require toxic chemical agents or an electrophoretic apparatus. Substitution of MTP for Southern blot analysis is cost neutral, with no compromise in test sensitivity. MTP systems may be adapted for automation, which is a compelling requirement for PCR testing, which, in many laboratories, is expanding at a rapid rate. Collectively, these factors make the implementation of this technology in routine diagnostic testing a fundamental goal in our laboratory.

ACKNOWLEDGMENTS
We thank Jill Thorverson, T. J. Berg, Carl Greiner, Heather Skarhus, Paul Heimgartner, Ann Wimmer, and David Majewski for technical assistance and Jonathan Hibbs for critically reviewing the manuscript.

ADDITION IN PROOF
The data generated with the PrimeCapture HSV DNA detection system were derived using a system lot number recalled by Paul Heimgartner, Ann Wimmer, and David Majewski for technical interpretation of this technology in routine diagnostic testing a fundamental goal in our laboratory.

REFERENCES