Rapid Diagnosis of Herpes Simplex Encephalitis Using Microchip Electrophoresis of PCR Products

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Background: Herpes simplex virus (HSV) is the most common cause of acute sporadic encephalitis in the United States. PCR of DNA extracted from cerebrospinal fluid (CSF) allows for reliable diagnosis of herpes simplex encephalitis (HSE). A faster turnaround time for HSE testing would improve patient management and lead to better outcomes. The aims of this study, therefore, were to develop a microchip-based electrophoretic method for rapid detection of HSV PCR products, and to compare the performance characteristics of liquid hybridization/gel retardation as an established clinical PCR product detection method with the new microchip-based method.

Methods: The study examined archival DNA from 33 selected CSF specimens submitted for HSV PCR testing to the clinical laboratory. One aliquot of the HSV PCR product was analyzed by liquid hybridization/gel retardation analysis, and a second PCR aliquot was analyzed directly with a microchip capillary electrophoresis system using an instrument built in-house. PCR samples were introduced directly into the microchip without a desalting step by use of a novel fluidic interface. Channel surfaces on the glass microchip were silanized, followed by derivatization with polyvinylpyrrolidone.

Results: Of the 33 CSF specimens tested by liquid hybridization analysis of HSV PCR products, 10 tested positive for HSV DNA, 2 gave a weakly positive result, and 21 tested negative. Total analysis time for detection of HSV DNA by gel retardation assay was 18 h. Microchip electrophoresis provided identical results in <110 s/sample, achieving 100% sensitivity and specificity compared with the established method.

Conclusions: Microchip-based electrophoresis can rapidly and accurately separate HSV PCR products, giving results identical to those obtained by liquid hybridization but with substantially decreased turnaround time. Clinical implementation of the new method will help to improve patient management and outcomes.

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Herpes simplex virus (HSV) is the most common cause of acute sporadic encephalitis in the United States, accounting for 10–20% of all cases (1, 2). A range of clinical presentations of herpes simplex encephalitis (HSE), including atypical mild disease course, relapsing encephalitis, or unusual neurological syndromes, has been described (3). The mortality rate in untreated cases of HSE is 70%, and permanent neurologic sequelae are the rule in survivors. The recommended antiviral treatment for HSE is a 10- to 14-day course of acyclovir given intravenously at a dosage of 10 mg/kg every 8 h (4–6). Although the treatment of HSE with acyclovir substantially decreases morbidity and mortality, antiviral therapy with acyclovir is successful only if the disease is diagnosed as early as possible (4, 5, 7). Acyclovir is usually well tolerated, but major adverse effects, including acute renal insufficiency and neurologic toxicity, have been observed (3, 8, 9). In clinical practice, the initiation of acyclovir therapy is mandatory as soon as a diagnosis of encephalitis, suggestive of HSE, is reached (3).

With the availability of effective therapies for HSE, the virology laboratory has acquired a role of primary importance in the early etiological diagnosis of this condition, which is essential to ensure appropriate patient treatment and management. Several retrospective and prospective studies have clearly established PCR as the method of choice for obtaining an early etiological diagnosis (7, 10–16). Although the technology underlying PCR is rel...
tively rapid, the PCR product must be identified definitively as the sequence of interest to provide adequate diagnostic specificity. Common techniques for this include hybridization of a specific probe to the PCR product after Southern blotting or by liquid hybridization followed by gel retardation analysis \((12, 14, 17)\). This step, however, adds 12–24 h to test performance, delaying the use of test results for clinical decisionmaking and intervention.

Obtaining an etiologic diagnosis as early as possible through improvements in turnaround time for HSE testing is, therefore, highly desirable. More rapid testing for HSE would allow antiviral treatment to be initiated earlier and help to extend the recognition of uncommon clinical presentations, leading to the best clinical outcomes for patients. Early availability of a negative PCR result for HSV DNA suggests an alternative etiological cause of encephalitis, demanding further laboratory tests and expansion of the diagnostic considerations for other causes of central nervous system (CNS) infection. In such cases, the withdrawal of acyclovir should be considered, leading to reduced drug costs and side effects, earlier hospital discharge, and more effective patient management \((3)\).

Several different approaches to reduce turnaround time for HSE testing have been described. Detection of digoxigenin-labeled amplification products with the Enzymun-Test DNA assay on an ES 300 instrument \((18)\). Four different colorimetric microtiter plate systems were evaluated as alternatives to Southern blotting. All of them allowed for identification of HSV PCR products in <4 h \((19)\). Recently, a real-time quantitative fluorescence-based PCR assay \((TaqMan System)\) was used to rapidly identify HSV DNA in clinical samples from mucocutaneous sources \((20)\).

In the past 10 years, capillary electrophoresis (CE) has become an universally accepted method for the analysis of clinically relevant molecules \((21–23)\). This technique involves the high-efficiency separation of charged or uncharged molecules in a glass capillary by the application of high voltage. The high detection sensitivity, as well as the speed, efficiency, and potential for automation, make CE a particularly attractive analytical method for the laboratory performing DNA diagnostic analyses that use traditional high-resolution acrylamide gel electrophoresis \((24)\). However, free-standing CE systems may soon be replaced by microfabricated chips that incorporate CE separation.

Microchip electrophoresis includes the production and use of micron-sized channels fabricated by micromachining techniques \((25)\). Micromachining is a combination of film deposition, photolithography, and precise etching and bonding techniques, modified from silicon microfabrication technology. Fabrication of the microchip devices begins with the exposure of a photoresist mask that depicts the design of the channel geometry and is followed by etching and bonding techniques to achieve the desired three-dimensional structure. Microfabricated electrophoresis chips have been produced on glass substrates and used to perform separations of fluorescent dyes, amino acids, short oligonucleotides, DNA restriction fragment digestes, and PCR products \((26–30)\). The manipulation and transport of analytes in microchip devices is realized using electrokinetic phenomena, e.g., electrophoretic and electroosmotic effects. Buffer and sample flows within the channel network can be precisely controlled through high voltages applied at the buffer/sample reservoirs. The technique allows the manipulation of picoliter volumes with high precision that ultimately leads to separation equivalent to or exceeding current techniques. In contrast to conventional CE, the higher surface-to-volume ratio in the microchip devices allows better heat dissipation and, therefore, separations at higher field strengths. The increase in separation efficiency translates to separation times that are ~10-fold shorter than CE and 100-fold shorter than traditional gel electrophoresis \((31)\). The improved separation efficiency is most effectively realized in a clinical laboratory if microchip electrophoresis is integrated with other miniaturized analytical techniques. Efforts in our and other laboratories are under way to achieve the integration of microchip electrophoresis with sample preparation and miniaturized PCR to achieve the total analysis of clinical samples on a single microfabricated chip \((32–34)\).

The obvious advantages of microchip electrophoresis—the speed of analysis, the potential for automation and integration, as well as the sensitivity of detection—might be directly used to dramatically improve the turnaround time for the detection of HSV PCR products in the setting of suspected encephalitis. The goals of the present study were, therefore, to develop a microchip-based electrophoresis system for the rapid detection and diagnosis of HSE, and to compare the performance characteristics of the new microchip-based detection system for HSV PCR products with an established clinical liquid hybridization/gel retardation detection method using cerebrospinal fluid (CSF) specimens submitted to the clinical laboratory for HSE testing.

Materials and Methods

CSF specimens

Archived extracted DNA from 33 selected CSF specimens submitted for HSV PCR testing to the Division of Molecular Diagnostics at the University of Pittsburgh over a 3.5-year period was included in the study. Four of the 33 CSF specimens were obtained from College of American Pathologists proficiency testing samples. The study included all clinical CSF specimens that tested positive for HSV during the 3.5-year period.

HSV PCR

Extracted DNA was amplified by targeting part of the thymidine kinase gene of HSV. The primers used were HSV-A \((5’-ATACCGACGATATGCGACCT-3’)\) and HSV-B
(5’-TTATTGCCGTACATAGCGG-3’). Amplification was for 37 cycles in a Perkin-Elmer 9600 thermal cycler (Perkin-Elmer) programmed for a three-step protocol: 30 s at 94 °C for denaturation, 60 s at 55 °C for annealing, and 60 s at 72 °C for extension. The expected HSV PCR product lengths when primers HSV-A and HSV-B were used was 111 bp. After amplification, the PCR products were split for a comparison of detection methods. One aliquot was analyzed by liquid hybridization followed by gel retardation. The second PCR aliquot was analyzed by a different operator in a blinded fashion directly by microchip electrophoresis using an instrument built in-house from commercially available components. PCR products that were not used immediately were stored at −80 °C.

DETECTION OF HSV PCR PRODUCTS BY LIQUID HYBRIDIZATION

For detection by liquid hybridization, 30 μL of HSV PCR product was mixed with 20 μL of hybridization solution containing a [32P]dATP-labeled DNA probe (5’-GTTCTTTCCGGTATTGTCTC-3’) complementary to a sequence internal to the expected HSV PCR product. After boiling for 5 min and a 10-min incubation at 55 °C, the samples were loaded on an 8% polyacrylamide gel and run at 200 V for 1 h in Tris-borate-EDTA buffer. The gels were then subjected to autoradiography, using Kodak XAR-2 films for 16 h. Positive samples containing HSV show a characteristic “retarded” band on the autoradiogram of the electrophoresis gel. The total time needed for detection of HSV PCR products with the liquid hybridization/gel retardation method was ~18 h.

DETECTION OF HSV PCR PRODUCTS BY MICROCHIP ELECTROPHORESIS

The HSV PCR products were analyzed on an instrument that was constructed in-house from commercially available components. The high voltage power supply consisted of four high-voltage modules (EMCO), several high voltage relays, and relay drivers (Kilovac; CIITechnologies). The output voltage of the high-voltage modules was programmed and controlled via a computer driven D/A output. Several computer-controlled relays allowed high voltage settings in individual buffer reservoirs to be either high voltage (~5000 to 5000 V) or grounded/floating. Software drivers for voltage control were integrated into the data acquisition program written in Labview Software (National Instruments). A diagram of the laser-induced fluorescence detection system used with the microchip electrophoresis system is shown in Fig. 1. The computer-controlled power supply, the data acquisition software, and the detection system are similar to instrumentation published previously (35) and a detailed description of the setup used in these experiments will be described elsewhere (36). Data acquired with Labview were electronically filtered using a Chebyshev Labview filter module and subsequently imported to a graphical program.

GLASS MICROCHIPS FOR ELECTROPHORESIS

Glass microchips (10.16 × 1.905 cm) were fabricated at the Alberta Microelectronics Center, using a modified silicon micromachining technique (25). Channels were etched in one glass plate to which a top plate was bonded. The layout and dimensions of the electrophoretic microchip are depicted in Fig. 2. Single-lane chips were used throughout the entire study. The channels were 0.05 mm wide and 0.02 mm deep, and detection occurred 60 mm downstream from the injection cross in the separation channel. Holes (1 mm) were drilled in the top glass plate to allow access to the channels. The glass microchips were mounted on a cassette to provide the fluidic interface to the microchip and electrical connections between the...
buffer reservoirs of the microchip and the high-voltage power supply. As described previously, buffer reservoirs consisted of flangeless HPLC fittings that were threaded over the access holes (35). The fittings were also used as connectors to an external HPLC pump to fill the channels. The microchip cassette allowed the placement of the microchip onto the detection system at various predefined (e.g., 60 mm) detection distances. The microchip cassette was constructed in-house from Plexiglas, and additional details will be published elsewhere (Hühmer et al., manuscript in preparation). Channel surfaces were coated using a modified two-step process of a method described previously (37). In brief, hydrolyzed channel surfaces were silanized with chlorodimethyloctylsilane and chlorotrimethylsilane. Subsequently, a layer of polyvinylpyrrolidone was covalently bonded to the silanized channel surface.

SEPARATION OF HSV PCR PRODUCTS BY MICROCIP ELECTROPHORESIS

HSV PCR product identification was carried out in a single blind fashion, where the operator of the microchip electrophoresis system had no knowledge of prior results obtained on the same specimens by liquid hybridization. The microchip was loaded with a sieving matrix consisting of 10 g/L hydroxyethylcellulose, 89 mmol/L HEPES, 89 mmol/L borate, and 200 μmol/L EDTA (pH 8.0) containing YO-PRO-1 fluorescent intercalator (Molecular Probes) at a concentration of 1 μmol/L by means of a HPLC pump. Samples were loaded onto the microchip sample reservoir (∼40 μL), using a disposable micropipette. Aliquots of the PCR solution were loaded undiluted onto the microchip into the sample reservoirs for electrokinetic injection. Sample injection on the microchip was performed by applying a +200 V (167 V/cm) potential across the sample and sample waste reservoirs, with the sample reservoir at ground. For separations, the sample and sample waste reservoirs were grounded while −200 V was applied to the inlet and +1700 V to the outlet (243 V/cm). Samples that were removed after the injection were replaced with doubly distilled water for a 5-min electrokinetic injection (333 V/cm) of doubly distilled water to remove residual DNA from the injection path. DNA marker HaeIII digest of pBR322 (Boehringer Mannheim Biochemicals) was diluted with doubly distilled water to a final concentration of 4.6 mg/L and routinely injected electrokinetically to measure electrophoretic migration times of DNA fragments and to adjust for day-to-day variations in migration time.

DETECTION LIMIT DETERMINATION OF THE MICROCIP ELECTROPHORESIS SYSTEM

HSV PCR product from a CSF specimen positive for HSV was serially diluted in doubly distilled water, and each dilution was then split for a detection limit comparison of the liquid hybridization method vs microchip electrophoresis. In a second approach, a known quantity of pBR322 HaeIII plasmid DNA digest (4.6 mg/L) was injected into the microchip electrophoresis system, and the limit of detection was calculated for the peak of a specific DNA fragment of interest with a signal-to-noise ratio of >10.

VERIFICATION OF RESULTS WITH CONVENTIONAL CE

To verify the results obtained with microchip electrophoresis, selected HSV PCR products were also analyzed with a conventional CE system. PCR product analysis was carried out on a Beckman P/Ace System 5510 (Beckman Instruments) equipped with a single-wavelength (488 nm) argon-ion laser for the detection of intercalated DNA at 510 nm. The CE system was interfaced to an IBM-compatible computer using System Gold software for instrument control and data collection. Separation of PCR products was carried out in a fluorocarbon-coated capillary (J&W Scientific) with an effective separation length of 20 cm. Separations were conducted in a 50 μm × 27 cm (total length) fluorocarbon-coated capillary equilibrated with 10 g/L hydroxyethylcellulose as a sieving matrix in HEPES-borate-EDTA buffer, pH 8.1 (89 mmol/L HEPES, 89 mmol/L borate, 10 mmol/L EDTA). Fluorescent intercalator YO-PRO-1 (Molecular Probes) was added to the running buffer at a final concentration of 1 μmol/L. Aliquots of the HSV PCR products were electrokinetically injected at 130 V/cm for 40 s directly without further dilution or desalting. The electrophoretic separation was carried out at 5 kV (185 V/cm) with a typical system current of ∼14 μA at a capillary temperature of 20 °C.

RESULTS

PERFORMANCE CHARACTERISTICS OF MICROCIP ELECTROPHORESIS

For initial characterization of the microchip electrophoresis system, we performed a separation of PCR product from an HSV-positive control specimen that was co-injected with a plasmid DNA restriction enzyme digest. The expected size of the HSV PCR product was 111 bp, whereas the pBR322 HaeIII digest (Sigma) contained DNA fragments from 8 to 587 bp with good fragment coverage in the size range of the expected PCR product. Native PCR product (5 μL) from an HSV-positive control specimen was mixed with 15 μL of diluted pBR322 HaeIII digest with a final plasmid DNA concentration of 1 mg/L. The microchip electropherogram resulting from co-injection is shown in Fig. 3. The fragments of the plasmid digest ranging from 51 to 587 bp are clearly baseline separated in 195 s at a field strength of 220 V/cm. The peak visible between the 104- and 124-bp fragments corresponds to the expected 111-bp HSV PCR product. The additional broad peak observed at ~100 s contains PCR side products. The migration time for the 111-bp HSV PCR product in the microchip electrophoresis was 110 s under the separation conditions used.

The separations of HSV PCR-positive and -negative CSF specimens by microchip electrophoresis are shown in Fig. 4, A and B. The PCR products were injected undiluted
into the microchip electrophoresis system without prior purification or desalting steps. The unincorporated primers contained in the PCR mixture created a peak preceding the expected 111-bp HSV PCR product. At the field strength of 243V/cm used in the experiments shown, separation of primer-dimer from HSV PCR product was completed in <95 s. The negative CSF specimen shows only the peak corresponding to DNA primer-dimer fragments. The autoradiographs of the respective CSF specimens obtained by the routine clinical liquid hybridization method are shown in the insets on the right of the panels in Fig. 4.

For 2 of the 33 HSV PCR products obtained from CSF specimens, the microchip electrophoresis showed a very small but reproducible peak at the separation time point expected for the 111-bp HSV PCR product consistent with being weakly positive for HSV DNA (Fig. 4C). The routine liquid hybridization assay for both specimens was also very weakly positive in one of two replicates only, indicating that these specimens contained a very low concentration of HSV PCR product that was near the detection limit of the liquid hybridization/gel retardation assay.

DETECTION LIMIT OF THE MICROCHIP ELECTROPHORESIS SYSTEM

Two different approaches were used for assessment of the detection limit with the microchip electrophoresis system. The results of the detection limit comparison by both methods for the detection of HSV PCR products using serial dilutions of a known positive specimen are shown in Fig. 5. The microchip electrophoresis system was able to unequivocally detect a 1:500 dilution of HSV PCR product, which compared favorably with the detection limit of the liquid hybridization/gel retardation method.

Fig. 3. Microchip electrophoresis (229V/cm) after co-injection of PCR product from an HSV-positive control with pBR322 HaeIII plasmid DNA digest.

The individual DNA fragment sizes of the pBR322 digest in order of decreasing fragment sizes are 587, 540, 504, 458, 434, 267, 234, 213, 192, 184, 124/123, 104, 89, 80, 64, 57, and 51 bp. The additional peak between the 104- and 124-bp pBR322 fragments corresponds to the expected 111-bp HSV PCR product. The migration times are indicated in seconds; the signal intensity obtained with laser-induced fluorescence detection is given in relative fluorescence units (RFU), sec, seconds.

Fig. 4. Separation (243V/cm) of HSV PCR positive (A), negative (B), and weakly positive (C) CSF specimens by microchip electrophoresis.

Unincorporated primers contained in the PCR reaction create a peak preceding the expected 111-bp HSV PCR product, which is indicated by arrows. The autoradiographs of the respective CSF specimens obtained by the routine clinical liquid hybridization method are shown in the insets. Liquid hybridization was performed in duplicate for each specimen, RFU, relative fluorescence units.
(1:500). The signal observed with the liquid hybridization/gel retardation method for the 1:500 dilution was extremely faint and could easily be overlooked when examining routine clinical autoradiographs. In a second approach, the detection limit of the microchip electrophoresis system obtained by the dilution series was verified by calculating the detection limit of a 21-bp DNA fragment obtained from the injection and separation of a known quantity of pBR322 HaeIII plasmid DNA digest (4.6 mg/L). Among the peaks of the digest on the electropherogram, a peak corresponding to the 21-bp fragment was clearly visible with a signal-to-noise ratio of >10, which translates to a calculated detection limit for DNA of the microchip electrophoresis system of 264 fmol of DNA. This detection sensitivity compares favorably with the detection sensitivity routinely achieved by radiography under the conditions described (Fig. 5). It is noteworthy that comparison of microchip electrophoresis detection limits with the liquid hybridization/gel retardation method corresponds very well with the comparison of CE and Southern blotting reported previously (23).

VERIFICATION OF MICROCHIP ELECTROPHORESIS RESULTS WITH CONVENTIONAL CE
Randomly selected HSV PCR products that were positive, weakly positive, or negative by microchip electrophoresis were analyzed with a conventional CE system to verify the results obtained by microchip electrophoresis. A total of nine positive, two weakly positive, and nine negative specimens underwent control CE on the conventional system. Examples of separations by conventional CE of a positive and a negative HSV PCR product are shown in Fig. 6. The results obtained by conventional CE confirmed the results obtained by microchip electrophoresis in all examined cases. The separation time for the HSV PCR product on the conventional CE system was 8.3 min, which is approximately fivefold slower than the separa-

Fig. 5. Detection limit of the microchip electrophoresis system for HSV PCR products compared with the liquid hybridization method. The microchip electrophoresis was able to detect the HSV PCR product in a 1:500 dilution, which was comparable to the detection limit of the liquid hybridization method. A very faint signal, which is not visible on this reproduction of the autoradiograph, was also observed with the liquid hybridization assay for the 1:500 dilution. The signals corresponding to the HSV PCR product on the autoradiogram and microchip electropherograms are indicated by arrows.

Fig. 6. Verification of microchip electrophoresis results with conventional CE. The specimens are similar to those shown in Fig. 4. The results obtained by conventional CE confirmed the results obtained by microchip electrophoresis. The separation times for the HSV PCR products on the conventional CE system were ~8.3 min, which is fivefold longer than the separation times achieved with the microchip platform. Relative fluorescence (RFU) values for the upper trace in the overlaid electropherograms were modified to preserve clarity. Double peaks in the sample negative for HSV are peaks corresponding to primer-dimer.
tion times in the 100-s range accomplished with microchip electrophoresis.

**Correlation of HSV PCR Product Detection by Microchip Electrophoresis and Conventional Liquid Hybridization/Gel Retardation**

PCR products obtained from a total of 33 CSF specimens tested for HSV because of suspected HSE were split into two aliquots and analyzed by both detection methods, liquid hybridization and microchip electrophoresis. The results of the method comparison are shown in Table 1. The conventional liquid hybridization/gel retardation assay was interpreted as positive for HSV DNA in 10 cases, weakly positive in 2 cases, and negative in 21 cases. Microchip electrophoresis was able to unequivocally identify all positive, weakly positive, and negative cases correctly. The sensitivity and specificity of the microchip electrophoresis were, therefore, 100% when compared with liquid hybridization as the established clinical method for HSV PCR product detection.

**Discussion**

A cornerstone in further improvements in the speed of analysis and throughput capability of DNA-based clinical tests is miniaturization of instrumentation. Miniaturization and microchip-based technology have revolutionized the computer and semiconductor industry and will revolutionize the technology used for DNA analysis and other clinically important molecules. CE has laid the groundwork for this development.

Several studies have shown that conventional CE is a useful technique for high-efficiency, automated separation of a variety of molecules of medical importance (21, 24, 31). Miniaturization of CE will maximize the benefits of decreased sample consumption, decreased separation time, increased resolving power, and the potential for automated high-throughput analysis (29, 31, 40). Complexes of specific antibody and labeled cortisol could be separated on a microchip to determine cortisol in a competitive assay over the range of clinical interest (10–600 µg/L) (41). Theophylline determinations in a free-solution assay and separations of serum proteins have been carried out using on-chip separation (42, 43). Channels filled with polyacrylamide microfabricated in a planar glass structure have also been used for the size separation of single-stranded oligonucleotides with 10–25 bases and to carry out DNA separations on a microchip (26, 44).

Despite the widespread interest in microchip electrophoresis, few of the reports to date have shown the applicability of this new technique to the clinical laboratory setting with its strict requirements for reproducibility of test results, demands on sensitivity, and the requirement of validation against established clinical methods. Microchip electrophoresis can rapidly and accurately separate and detect HSV PCR products in a clinical environment. The results of microchip electrophoresis were comparable in sensitivity and specificity to results obtained with a liquid hybridization/gel retardation method used widely in clinical virology laboratories for the detection of HSV and other infectious agents in clinical specimens (17, 45, 46). The use of a microchip cassette presents a consistent interface between the operator and the microchip during PCR product analysis. This facilitates the handling of multiple specimens, if required, at substantially reduced time and effort. Channel walls in the microchip were coated with a hydrolytically stable layer of polymer to provide consistent suppression of electrophoretic migration and substantially decreased nonspecific adsorption of analyte in the microchip channels. Therefore, no PCR sample treatment (e.g., sample desalting, DNA dilution, or DNA concentration) before injection onto the microchip was necessary, markedly simplifying the analysis of actual PCR products varying in concentration and quality.

The one advantage of microchip electrophoresis in the setting of HSE is a substantial decrease in turnaround time for detection of PCR product in <100 s. The routine clinical liquid hybridization/gel retardation assay takes ~18 h including autoradiography for the detection of the HSV PCR products. More lengthy autoradiographic procedures have limited the true diagnostic value of PCR in the setting of HSE (19). Although dozens of samples can be processed simultaneously with the liquid hybridization/gel retardation assay, the successive characterization of a limited number of CSF samples submitted to the laboratory for urgent HSV analysis is still substantially faster by microchip electrophoresis of PCR products. For example, 20 CSF samples could be analyzed for the presence of HSV in <35 min after PCR amplification. The detection of HSV by microchip electrophoresis in combination with amplification by conventional PCR can be achieved in ~3 h. The total analysis time for the amplification and detection of HSV in a CSF sample is therefore reduced by a factor of ~6 for the microchip electrophoresis format. Microchip electrophoresis clearly has the resolving power to separate specific PCR fragments from byproducts, making identification by specific hybridization probes unnecessary. However, if the identification of a HSV product is difficult because of variations in the electrophoretic migration time, the addition and comigration of an internal standard in microchip electrophoresis might be useful. We have clearly demonstrated that PCR product detection and identification can be achieved simultaneously by microchip electrophoresis using a co-injection of PCR product with an internal DNA sizing

| Table 1. Comparison of results for HSV PCR product detection obtained with liquid hybridization vs microchip electrophoresis. |
|---|---|---|
| Method             | Positive | Weakly positive | Negative |
| Liquid hybridization | 10       | 2              | 21       |
| Microchip electrophoresis | 10       | 2              | 21       |


marker. The dramatic improvement in diagnostic speed with microchip electrophoresis allows earlier diagnosis of HSE with improvements in effective patient management and outcomes. Atypical presentations of HSV infections of the CNS, including atypical mild disease course, relapsing encephalitis, or unusual neurological syndromes, can also be recognized earlier (3). In the scenario of suspected viral CNS infection, the rapid availability of a negative HSV PCR result helps refocus the clinical differential diagnosis on other causes of CNS infection. Therapy with antiviral agents such as acyclovir may also be discontinued earlier in some cases, thereby reducing the risk of potentially serious drug side effects and cost. In the setting of possible HSE, patients are often managed as inpatients while awaiting HSV test results. Potential earlier discharge from the hospital in some cases will allow additional cost savings.

Other strategies to reduce testing time for HSE have been described, using microtiter plate formats to carry out hybridization and HSV PCR product detection (18, 19). Microchip electrophoresis is orders of magnitude faster than these methods, which currently take ~4 h to detect PCR product. The real-time quantitative fluorescence-based PCR assay (TaqMan System) recently described for rapid identification of HSV DNA in clinical samples from mucocutaneous sources is elegant, fast, and eliminates the hybridization steps usually required after PCR amplification (20). However, use of this technology requires the purchase of expensive instrumentation for the sole purpose of specifically testing specimens by a PCR-based assay. Microchip-based technologies, on the other hand, can detect a variety of fluorescently labeled clinical markers and have the potential to integrate additional laboratory functions other than separation and detection of clinical markers. In the present study, one important objective was to identify routine clinical samples from a clinical laboratory correctly and to demonstrate the validity of microchip electrophoresis against an established method. DNA amplification for both detection assays was performed on a conventional PCR device to allow the valid comparison of the detection methods, but other investigators have demonstrated that rapid separation of DNA analytes on electrophoretic microchips can be combined with ultrafast, miniaturized PCR amplification (28). In addition to the ultrafast amplification of DNA fragments of interest, miniaturized PCR has been shown to increase specificity during amplification, thereby dramatically reducing the formation of PCR side products (33, 47). The combination of ultrafast, miniaturized PCR with microchip electrophoresis might, therefore, replace hybridization assays for the detection and characterization of PCR products without compromising sensitivity and specificity. In an effort to integrate several steps of DNA analysis in a single microchip system, microfabricated silicon PCR reactors and glass electrophoresis microchips have been successfully coupled, and the DNA amplification with subsequent detection of DNA product by electrophoresis has been demonstrated in <20 min (28). In addition to rapid DNA amplification and separation, on-chip sample pretreatment, labeling, and enzymatic reactions have been demonstrated successfully. The construction of a multichannel microchip for simultaneous amplification and analysis of clinical samples is imminent, and will further increase throughput (34). This substantiates microchip electrophoresis technology as a versatile analytic platform that can be used for the analysis of diverse clinically relevant molecules, including separation of DNA fragments for oncology, genetic testing, and DNA research (40, 48).

We thank Ralph Anderson, Ronald Busch, Karen Freilino, and Leann Stringos for their help with HSV PCR testing. We acknowledge Nicole Munro for development of the optical setup for microchip electrophoresis.

References


