Real-Time Polymerase Chain Reaction Detection of Herpes Simplex Virus in Cerebrospinal Fluid and Cost Savings from Earlier Hospital Discharge

Kenneth Rand,* Herbert Houck,* and Robert Lawrence[†]

From the Departments of Pathology, Immunology, and Laboratory Medicine* and Pediatrics,[†] University of Florida, Gainesville, Florida

Neonatal herpes simplex virus (HSV) can be a devastating illness and may be difficult to diagnose in those cases without a typical skin rash. As a result, physicians often rely on HSV polymerase chain reaction of cerebrospinal fluid to rule out HSV encephalitis. We developed a real-time polymerase chain reaction assay for HSV using the SmartCycler II (Cepheid, Sunnyvale, CA). End point dilution studies showed sensitivity comparable to that of two national reference laboratories that use LightCycler. In-house turnaround time was ≈ 1.5 days versus ≈ 5.2 days for sending the test to a reference laboratory. We hypothesized that the rapid availability of a negative test result would allow physicians to discharge appropriate patients earlier. Six months after implementation, clinical case analysis identified 12 pediatric patients who were discharged earlier based on more rapid test results, with a projected savings of ≈ 55.2 hospital days throughout the first year. Actual length of stay for patients tested in-house was significantly less than that of historical controls and was projected to save ≈ 70.2 hospital days in the first year. Including projected annual laboratory cost/test savings of $\approx \$11,000$, a total savings of $\$38,000$ to $\$43,000$ was estimated for the first year of implementation, more than offsetting startup instrument and development cost. (*J Mol Diagn* 2005, 7:511–516)

Neonatal herpes simplex virus (HSV) infection is a life threatening infection occurring within the first several weeks after birth. Classically, ~ 60 to 70% of patients have a typical herpetic skin rash, with or without disseminated infection involving brain, liver, lung, and other organs.¹ Because central nervous system HSV can present with seizures, low-grade fever, and other non-specific symptoms in the absence of the vesicular skin rash, physicians often rely on a negative cerebrospinal fluid (CSF) HSV polymerase chain reaction (PCR) to rule out HSV encephalitis.^{2–4} In our hospital HSV CSF PCR

was sent to a reference laboratory with an average 5.2-day turnaround time. We attempted to show that performing HSV CSF PCR in the hospital laboratory would shorten this turnaround time and lead to an earlier discharge for a subset of neonates without other complications who were kept in the hospital for observation pending this test result.

Materials and Methods

Patient Population

All patients who had HSV PCR performed on CSF between May 7, 1996 and January 24, 2004 were included. This group was broken down into those who had the test performed in the hospital laboratory, on or after June 18, 2003 (in-house test group), and those whose test was performed at Quest Diagnostics, Teterboro, NJ (control group). These groups were also broken down into the following age categories: newborn (0 to 29 days), young child (30 days to 3 years), older child (4 to 17 years), and adult (≥ 18 years).

Estimation of Cost Savings

Preimplementation Study

To estimate hospital cost savings from more rapid reporting of HSV PCR results, we first set up an automatic fax-based notification to one of the authors (K.H.R.) whenever HSV CSF PCR was ordered. For all patients < 60 days of age, the author then contacted the attending physician personally 2 to 3 days after the test was ordered and before the report of a result. Each physician was asked, "If I were to tell you now at this time that the HSV CSF PCR was negative for your patient, would you discharge the patient today, yesterday, tomorrow, or would it have no effect on the patient's length of stay?" All patients were then followed to determine when they ac-

Supported in part by the Department of Pathology, Immunology, and Laboratory Medicine, Shands Hospital at the University of Florida, Gainesville, FL; and Cepheid, Sunnyvale, CA.

Accepted for publication May 16, 2005.

Address reprint requests to Kenneth H. Rand, M.D., University of Florida, College of Medicine, Department of Pathology, PO Box 100275, Gainesville, FL 32610. E-mail: rand@pathology.ufl.edu.

tually were discharged, and the net difference in days was recorded. If the patient was not discharged within a day of the actual report of a negative HSV CSF PCR, the physician was recontacted to determine whether other factors had delayed the patient's discharge.

Postimplementation Analysis

After implementation of the test in the hospital laboratory, all patients with HSV PCR ordered on CSF were recorded by the hospital's utilization department. Each case was discussed with the patient's attending physician (for adult patients) to ascertain whether the patient was believed to have been discharged from the hospital earlier because of the more rapid reporting of the HSV PCR result, and if so, by how many days. For pediatric patients, all patients were reviewed by one of the authors (R.M.L.), a pediatric infectious diseases specialist to estimate hospital days saved (if any) as a result of the more rapid availability of the result. Cost savings was calculated from the hospital's estimated actual cost/day multiplied by the total estimated days saved. We used 2002 direct variable costs of \$450/day for the low-intensity neonatal intensive care unit direct variable cost, \$502/day for pediatric, and \$609/day for adult non-day of admission or non-day of discharge cost/day.

Because the clinical case reviews were in part subjective, we also looked at the actual length of stay (LOS) in days for all patients with HSV CSF PCR ordered between May 7, 1996 through January 24, 2004, divided by age group and location of PCR test performance, as described above. Patients discharged on day 1 were excluded from this evaluation because the results of the test could not possibly have influenced the discharge decision. Patients discharged between 2 to 10 days after admission were included in the study, because it was possible that test results could have influenced a discharge decision within this time period. Patients discharged after ≥ 11 days were not included in the statistical evaluation because patients remaining in the hospital more than 10 days were assumed to have medical condition(s) that would not have been influenced by the results of the CSF HSV PCR performed in the first several days after admission. The distribution of LOSs between the group who had the test sent to a reference laboratory and the group whose test was performed in the hospital laboratory was analyzed by χ^2 test.

The LOS analysis was performed for all patients who had the test performed. In addition, we attempted to improve the comparability of the groups by matching for primary diagnosis. In this approach, only those in-house tested patients, whose primary diagnosis code was the same as that of one or more control patients, were divided into the same age groups and test location. The distribution of LOSs between the primary diagnosis matched group who had the test sent to a reference laboratory and the group whose test was performed in the hospital laboratory was analyzed by χ^2 test.

Viral Isolates

A total of 95 strains of HSV-1 and 95 strains of HSV-2 were obtained from the Diagnostic Virology Laboratory at Shands Hospital at the University of Florida, Gainesville, FL. All HSV strains were grown in MRC 5 or A 549 cell lines (obtained from both Viomed, Minnetonka, MN, and Diagnostic Hybrids, Athens, OH). All isolates were typed using fluorescent monoclonal antisera specific for HSV type 1 or type 2 (Bartels, Bellevue, WA).

PCR Development

HSV DNA extraction from tissue culture supernates was performed using Qiagen Blood Mini kit spin columns (Qiagen, Inc., Valencia, CA). Quantitated HSV-1 DNA (1.2×10^4 copies/ μ l), obtained from ABI (Columbia, MD) was used as a positive control. A TaqMan real-time PCR was developed for use on the Smartcycler II (Cepheid, Sunnyvale, CA). The primer sets were synthesized by GenoMechanix (Alachua, FL) and were all high performance liquid chromatography purified. Labeled probes were synthesized by BioSearch Technologies (Novato, CA). We modified a real-time FRET PCR assay developed by Espy and colleagues⁵ that targets the HSV DNA polymerase. We modified the sense primer,⁵ and substituted a unique anti-sense primer to achieve T_m s of ~ 60 to 62°C . The primers were: sense, CTCGAGTGCGAAAA-GACGTTTC and anti-sense, GTATCGTCGTAACAG-CAGGTC. The following unique probe was synthesized to have a T_m 8 to 10°C greater than the T_m of the primers; FAM-AAG GGC GTG GAT CTG GTG CG-BHQ-1. PCR reaction mixes consisted of OmniMix HS beads (Cepheid) (1.5 U TaKaRa hot start *Taq*, 200 μ mol/L dNTPs, 4 mmol/L MgCl_2 , HEPES, pH 8), additional MgCl_2 to 6 mmol/L, 0.4 μ mol/L primers, 0.2 μ mol/L probe, 5% dimethyl sulfoxide (DMSO), and 5 μ l of DNA per 25 μ l of reaction volume. After an initial denaturation step at 95°C for 2 minutes, a two-step PCR was performed with a 15-second denature step at 95°C , and a 30-second anneal step at 64°C , using a maximal ramp-down rate for 45 cycles. The assay detects both HSV-1 and HSV-2 and does not distinguish between them. A total of 190 strains of HSV were successfully amplified using these primers. All 95 HSV-1 and 94 of 95 strains of HSV-2 were detected by the probe. The HSV-2 strain not detected by the probe was sequenced. A specific probe was synthesized for this strain; Quasar 670-AAG GGC GTC GAC CTG GTG CG-BHQ-2. This probe was incorporated into the assay with no apparent loss of sensitivity.

The studies reported here were performed without the addition of an internal control for extraction and amplification. For purposes of determining hospital cost savings, this approach is justified. We currently use a multiplex assay that includes an internal control consisting of 17.5 pg of mouse DNA/reaction mixture and final concentrations of the mouse primers (sense, GTTGTAGGA-CAGGGTTTGGGT and anti-sense, CTCTGAATTTCCG-GTTCCTGTT; each at 0.1 μ mol/L) and 0.2 μ mol/L probe Cal-Orange-CGGCCTCCGGTAGCCTCTCCA-BHQ-1

Table 1. Cycle Threshold Results of 10 Replicate Assays of HSV-1 and HSV-2 DNA of Known Copy Number with and without the Internal Control DNA

No. copies/ reaction	Without Internal Control		With Internal Control*	
	Mean $\pm$ SD	No. replicates positive	Mean $\pm$ SD	No. replicates positive
HSV-1				
1000	30.9 $\pm$ 0.6	10	31.1 $\pm$ 0.3	10
100	34.2 $\pm$ 0.7	10	34.5 $\pm$ 0.5	10
10	37.8 $\pm$ 1.4	10	38.2 $\pm$ 1.7	9
2	38.6 $\pm$ 1.6	4	40.7 $\pm$ 2.2	9
HSV-2				
1000	30.1 $\pm$ 0.3	10	30.0 $\pm$ 0.2	10
100	33.4 $\pm$ 0.5	10	33.4 $\pm$ 0.4	10
10	36.5 $\pm$ 0.9	10	36.3 $\pm$ 0.4	10
2	39.0 $\pm$ 1.5	5	39.4 $\pm$ 2.3	7

Total of 10 runs.

*The internal control was positive in all PCR reactions with ≤ 100 copies/reaction mixture. The mean $\pm$ SD of the internal control was 36.0 ± 2.1 cycles and the 95% confidence interval of the mean was 35.3 to 36.6 cycles.

(courtesy of Perry Chan, Ph.D., Esoterix, Calabasas Hills, CA). The addition of the internal control had no effect on the end point sensitivity of the assay for HSV as shown in Table 1.

Validation Studies

Because validation studies for HSV PCR have not previously been reported for the SmartCyclerII, we extracted DNA from 15 HSV-1 and 15 HSV-2 strains. Dilutions of these DNA were prepared in water, aliquoted, and frozen at -70°C . Dilutions were designed to give an estimated cycle threshold (C_T) by our method, of between 25 and 40 per 5- μl sample volume after being frozen at -70°C . In addition, a series of twofold end point dilutions of the ABI HSV-1 DNA standard in water was prepared, and six aliquots of each dilution were frozen at -70°C . Aliquots of the DNA from the 30 HSV strains and two aliquots of each dilution of the ABI standard HSV-1 DNA were shipped on dry ice to two national reference laboratories. The two remaining aliquots of each dilution of the ABI standard HSV DNA were tested in parallel in our laboratory. Both reference laboratories used Lightcycler and a 5- μl sample input volume. Discordant results for the detection of the HSV strains were resolved by sending repeat aliquots and a 10-fold concentrated sample to the appropriate reference laboratory. No resolution of discordant results was attempted for the dilution study, because the number of copies was known.

Assay Optimization

Tween-20, bovine serum albumin (BSA), and Trehalose were tested alone and in combination at various concentrations with or without 2% DMSO to improve the sensitivity of the assay.

Results

HSV PCR Assay

The in-house HSV PCR assay detected 95 of 95 strains of HSV-1 and 94 of 95 strains of HSV-2. One strain of HSV-2

was sequenced and found to differ by 2 bp from the probe. A probe with this sequence was included in the assay without loss of sensitivity (see Materials and Methods for details). Analytic sensitivity was determined using HSV-1 and HSV-2 DNA of known copy number (ABI). Ten separate runs of HSV-1 and HSV-2 with and without the internal control were tested at 1000, 100, 10, and 2 copies per PCR reaction mixture. The results are shown in Table 1. There is essentially no difference in the mean cycle threshold at any level of input copy number with or without the internal control. At two copies/reaction mixture in the presence of the internal control, HSV-1 was detected in 9 of 10 runs and HSV-2 in 7 of 10 runs.

In addition, replicate end point dilutions of the ABI HSV-1 reference DNA were sent blinded to two national reference laboratories and compared with in-house results. Figure 1 shows the interlaboratory comparison of the end point dilution study. The y axis shows the number of copies/reaction mixture and the x axis shows the measured cycle threshold, C_T . All laboratories detected both replicates at four copies/reaction mixture, but as expected detection below that level was less inconsistent. ND, not detected.

Figure 1. Interlaboratory comparison of blinded duplicate end point dilutions of quantitated HSV-1 DNA (1.2×10^4 copies/ μl ; ABI). The y axis shows the number of copies/reaction mixture and the x axis shows the measured cycle threshold, C_T . All laboratories detected both replicates at four copies/reaction mixture, but as expected detection below that level was less inconsistent. ND, not detected.

Table 2. Improvement in End-Point Detection by PCR Additives

Additive(s)	No. runs 10^{-6} end-point dilution detected/total runs
No additives	1/11 (9%)
0.2% Tween-20/0.2 mg/ml BSA/150 mmol/L Trehalose	3/9 (33%)*
2% DMSO	33/69 (48%)†

* $P = \text{NS}$ versus no additives.

† $P = 0.02$, Fisher's exact probability versus no additives.

At the time of this comparison, Tween-20/BSA/Trehalose was incorporated into our assay, but 2% DMSO was not. Sensitivity of the original assay was improved with the addition of a combination of Tween-20/BSA/Trehalose. Further improvement seemed to occur using 2% DMSO (with or without BSA), in place of the Tween-20/BSA/Trehalose combination. Table 2 shows the improvement in end point dilution detection of an in-house HSV-positive control, although the difference was only statistically significant for the 2% DMSO versus no additives ($P = 0.02$, χ^2). Of the 15 strains each of HSV-1 and HSV-2 sent to the two national reference laboratories, reference laboratory A did not detect one strain and laboratory B did not detect 2; however, both were detected on repeat testing with a subsequent aliquot. We also tested 30 HSV-1 and HSV-2 culture-positive and 30 culture-negative clinical samples (respiratory, skin, and genital) using the original material submitted for culture. All 30 culture-positive specimens were positive by PCR and 1 of 30 culture-negative specimens was also positive by PCR.

Preimplementation Estimate of Cost Savings

Before HSV PCR test development, attending physicians contacted 2 to 3 days after ordering HSV CSF PCR, stated that four (of a total of 13) infants would have been discharged at the time they were told HSV CSF PCR was (hypothetically) negative. Compared with the time the patient was actually discharged, this would have resulted in a savings of 9 full days (2.25 days per patient) at a direct variable cost of \$450 per day, or $\approx$ \$4050 throughout a 4-month period, or $\approx$ \$12,150 on an annual basis.

HSV PCR Turnaround Time

Before in-house testing, the time from test order until result reporting from the outside reference laboratory was an average of 5.2 ± 7.2 days with a median of 4.1 days and 95% CI of 4.5 to 5.9 days. When performed in-house on Mondays, Wednesdays, and Fridays, the turnaround time was 1.5 ± 1.0 days, 95% CI 1.3 to 1.7 days for the first 9 months after implementation.

Postimplementation Estimate of Cost Savings

A clinical expert case-based estimate of hospital days saved from the more rapid availability of HSV CSF PCR was performed by one of us (R.M.L., a pediatric infectious diseases specialist) for all pediatric cases (<18 years), and by discussion with the patient's attending

physician for adult cases (≥ 18 years). Of 32 infants, 5 were thought to have been discharged an average of 2.1 ± 0.55 (95% CI 1.4 to 2.8) days per patient sooner, than if the PCR test had been sent to the reference laboratory (10.5 total hospital days). Of 23 children aged 30 days to 3 years, 4 were thought to have been discharged an average of 2.9 ± 0.75 (95% CI 1.7 to 4.1) days (11.5 days total) sooner. Two older children and one adult were judged to have been discharged 2.5, 2.5, and 2 days, respectively, sooner. In total, 29 hospital days were estimated to have been saved throughout a 6.3-month period, which would be projected to save a total of 55.2 days for 1 year or $\approx$ \$27,011 (assuming a direct variable hospital cost per day of \$450 per day for low-intensity neonatal intensive care unit, \$502 per day for pediatric, and \$609 per day for adults; see Materials and Methods).

Because of the inherent subjectivity of the expert case analysis, we also analyzed the actual LOS before and after implementation of the in-house test. When all patients across all age groups were combined (excluding those with a LOS of 1 day or ≥ 11 days; see Materials and Methods), the LOS of the patients whose test was performed in-house was significantly shorter than that of those whose test was sent to the reference laboratory ($P < 0.05$; Figure 2A). When broken down by age group, the shorter LOS was statistically significant only for the newborn group (Figure 2B), but not for older children or adults. Because health care practices could potentially have changed throughout the 7-year historical control period, we also compared the LOS after the introduction of HSV CSF PCR with that of the year immediately before its implementation. For the newborn age group the LOS was significantly shorter when compared with the 1-year historical controls ($P < 0.01$; data available on request). When LOS was compared for those patients matched by primary diagnosis, the LOS was again significantly shorter for the entire group (Figure 2C) and for the newborn age group (Figure 2D), $P < 0.01$ for both, but not for any of the older child or adult age groups.

To estimate the hospital days saved, we calculated the difference between observed and expected LOS for all patients. On this basis, we estimated that a total of 42.1 days were saved throughout the first 7 months of implementation, which projected to an annualized total of 70.2 days. This figure is in reasonable agreement with the 29 days saved throughout 6.3 months by the clinical analysis, which annualized to a total of 55.2 days. Using the above cost per day estimates, the 70.2-day total shorter length stay would result in an annual direct variable cost savings of $\approx$ \$31,590.

Figure 2. **A:** The distribution of hospital LOS for all patients with CSF HSV PCR performed divided into those whose test was sent to a reference laboratory before June 18, 2003 (■) and those whose test was performed in-house on or after June 18, 2003 (□). The mean LOS for all patients before in-house HSV CSF PCR testing was 5.4 days (median, 5 days) compared with 4.7 days (median, 4 days) after in-house implementation. The difference in the distributions is statistically significant using a $2 \times 9 \chi^2$ test ($P < 0.05$). Patients discharged on day 1 or after day 10 were excluded because it was reasonable to assume that the test results would not have influenced patient discharge. **B:** The distribution of hospital LOS for neonates (<30 days of age at admission) with CSF HSV PCR performed divided into those whose test was sent to a reference laboratory before June 18, 2003 (■) and those whose test was performed in-house on or after June 18, 2003 (□). The distributions are statistically different ($P < 0.01$, χ^2). The mean LOS for newborns before in-house CSF HSV PCR testing was 6.2 days (median, 6 days) compared with 4.5 days (median, 3 days) after CSF HSV PCR in-house testing. **C:** The distribution of hospital LOS for all patients matched by primary diagnosis with CSF HSV PCR performed divided into those whose test was sent to a reference laboratory before June 18, 2003 (mean, 4.9 days; median, 5.0 days) (■) and those whose test was performed in-house on or after June 18, 2003 (mean, 3.7 days; median, 3.0 days) (□). The distributions are statistically different ($P < 0.01$, χ^2). **D:** The distribution of hospital LOS for neonates <30 days of age on admission matched by primary diagnosis with CSF HSV PCR performed divided into those whose test was sent to a reference laboratory before June 18, 2003 (mean, 5.7 days; median, 7.0 days) (■) and those whose test was performed in-house on or after June 18, 2003 (mean, 2.7 days; median, 3.0 days) (□). The distributions are statistically different ($P < 0.01$, χ^2).

Acyclovir Usage

Mean pediatric acyclovir usage decreased from 58.6 ± 20 g/month for the 2 years before implementation of in-house HSV PCR testing to 37.6 ± 15.8 g for the subsequent 5 months after, $t = 2.2$, $P = 0.018$, one-tail. There was no change in adult usage of acyclovir.

Discussion

A TaqMan real-time PCR was developed for HSV-1 and -2 using the Cepheid SmartCycler II. By end point dilution the assay sensitivity was comparable to that of two national reference laboratories that use the Roche LightCycler. We found that 189 of 190 strains of HSV-1 and HSV-2 were detected by the assay, but that the assay could be multiplexed to include a probe for the errant strain without loss of sensitivity. Optimization of the assay to maximize sensitivity required addition of 2% DMSO, possibly because of the relatively high GC content of our probe (65%). DMSO is one of a variety of agents such as betaine, formamide, glycerol, nonionic detergents, bovine serum albumin, polyethylene glycol, and tetramethylammonium chloride that have been reported to improve efficiency of PCR reactions. DMSO is believed to act through disruption of base pairing, whereas agents such as betaine are thought to provide their effect through equalizing the contribution of GC- and AT-base pairing to

the stability of the DNA duplex.⁶ The amides represented by the most widely used, formamide, exert their effect by binding to the major and minor grooves of DNA and destabilizing the template helix.⁷⁻⁹ The choice of additives must be determined experimentally, and is highly sequence- and reaction-dependent. In this study, addition of a mixture of 0.2% Tween-20, 0.2 mg/ml BSA, and 150 mmol/L Trehalose did improve the sensitivity of the assay compared with the OmniMix HS beads alone, but this was not statistically significant because of the small number of assays involved. Although the 2% DMSO was not statistically better than the Tween, BSA, Trehalose mixture, it did appear to be more sensitive in head-to-head comparisons (data not shown), and was statistically better than the OmniMix alone ($P = 0.02$, Table 2).

The HSV PCR assay detects both HSV-1 and HSV-2 with apparent equivalent sensitivity, but does not distinguish between the two. We attempted multiplexing with several other primer/probe combinations that would have distinguished between the two types within the assay, but a loss of sensitivity was inevitably observed usually in the range of two to three cycles. Because the assay was designed for the detection of neonatal herpes encephalitis, and because the treatment would be the same regardless of HSV type, we opted to pursue the most sensitive methodology. In the first 9 months since implementation, we have had 7 of 184 (3.8%) patients with a positive test, and all have been typed using type-specific primers and probes from other published assays in follow-up testing.

One national reference laboratory failed to detect one and the other failed to detect 2 of the 30 total HSV-1 and HSV-2 viral isolates submitted under code. Both labs could detect these strains in follow-up specimens, so it is unlikely sequence variation accounted for the problem. Because the analytic sensitivity of their assays was essentially one to two copies per reaction, we can only speculate that the negative results may represent failure of DNA extraction. We did not specifically inquire if these laboratories included an internal control for extraction; however, the end point dilution study samples were discussed with the laboratory directors and were to be tested without nucleic acid extraction.

As in many institutions, cost was a major consideration in the introduction of new technology into routine clinical laboratory usage. At the time the decision to implement was made, comparable systems such as the LightCycler and ABI real-time PCR instruments cost between \$60,000 to \$80,000 with accessories, compared with the cost of the SmartCycler ($\approx$ \$29,000). We therefore justified the capital outlay on the expectation that more rapid availability of negative CSF HSV PCR results would lead to earlier patient discharge, especially neonates and young children. Analysis of the patient impact in the first 6 to 7 months after implementation of the PCR, support this conclusion. On the basis of individual case analysis by a pediatric infectious disease specialist and consultation with the attending physicians caring for the adult cases, an estimated 12 patients were discharged a total of 29 days sooner than would have been expected if the test had been sent to a reference laboratory. When annual-

ized and adjusted for hospital ward differences in direct variable cost, an estimated savings of 55.2 hospital days or ≈\$27,011 in the first year would be achieved. This does not take into consideration savings from the lower cost/test by performing the test in-house, nor the savings due to decreased use of acyclovir. Because of the inherently subjective nature of the case analysis, the actual LOS of the patients who had the CSF HSV PCR performed in-house was compared with those whose test was sent to a reference laboratory during the previous 7 years. This analysis also showed statistically earlier discharge and the estimated number of hospital days based on the difference between observed and expected in the χ^2 distribution was 70.2 days or ≈\$31,590 annually. When the lower cost of the in-house test compared with reference laboratory cost was included, an additional annual savings of ≈\$11,000 was realized, bringing the total savings to \$42,590 in the first year. Thus, the estimated actual savings (\$42,590) was greater than the cost of the instrument (\$29,000) plus the estimated \$8000 in test development costs (mostly technologists time).

Cimolai and colleagues¹⁰ reported on the clinical impact of the introduction of CSF HSV PCR and also achieved ≈2-day turnaround time for in-house test reporting. In their study negative test results led to the discontinuation of acyclovir in 34 of 81 patients. They also noted that negative results were likely to lead to earlier hospital discharge, but did not provide cost estimates for the savings from either reduced use of acyclovir or earlier hospital discharge. Several other groups have analyzed the clinical impact of real-time PCR testing for CSF enterovirus. Projected direct variable cost savings were achieved by earlier discharge of patients and decreased use of antibiotics,^{11–13} although some of the cost reductions depend on the prevalence of enterovirus meningitis exceeding a certain threshold.¹³

In conclusion, we developed a real-time PCR for HSV using the Cepheid SmartCycler with sensitivity comparable to that of national reference laboratories using LightCycler. Cost savings achieved through decreased LOS and reduced cost per test were projected to more than offset the capital and development costs within a year.

Acknowledgment

We thank the laboratory staff of the Virology Laboratory of the Shands Hospital at the University of Florida for their generous support.

References

1. Whitley RJ: Herpes simplex virus infection. *Semin Pediatr Infect Dis* 2002, 13:6–11
2. Kimberlin DW, Lakeman FD, Arvin AM, Prober CG, Corey L, Powell DA, Burchett SK, Jacobs RF, Starr SE, Whitley RJ: Application of the polymerase chain reaction to the diagnosis and management of neonatal herpes simplex virus disease. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. *J Infect Dis* 1996, 174:1162–1167
3. Kimberlin DW: Herpes simplex virus infections of the central nervous system. *Semin Pediatr Infect Dis* 2003, 14:83–89
4. Lakeman FD, Whitley RJ: Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. *J Infect Dis* 1995, 171:857–863
5. Espy MJ, Uhl JR, Mitchell PS, Thorvilson JN, Svien KA, Wold AD, Smith TF: Diagnosis of herpes simplex virus infections in the clinical laboratory by LightCycler PCR. *J Clin Microbiol* 2000, 38:795–799
6. Frackman S, Kobs G, Simpson D, Storts D: Betaine and DMSO: enhancing agents for PCR. *Promega Notes* 1998, 65:27–30
7. Cheng S, Fockler C, Barnes WM, Higuchi R: Effective amplification of long targets from cloned inserts and human genomic DNA. *Proc Natl Acad Sci USA* 1994, 91:5695–5699
8. Chakrabarti R, Schutt CE: The enhancement of PCR amplification by low molecular weight amides. *Nucleic Acids Res* 2001, 29:2377–2381
9. Varadaraj K, Skinner DM: Denaturants or cosolvents improve the specificity of PCR amplification of a G + C-rich DNA using genetically engineered DNA polymerases. *Gene* 1994, 140:1–5
10. Cimolai N, Thomas EE, Tan R, Hill A: Utilization of herpes simplex PCR assays for cerebrospinal fluid in a pediatric health care setting. *Can J Microbiol* 2001, 47:392–396
11. Stellrecht KA, Harding I, Woron AM, Lepow ML, Venezia RA: The impact of an enteroviral RT-PCR assay on the diagnosis of aseptic meningitis and patient management. *J Clin Virol* 2002, 25(Suppl 1):S19–S26
12. Robinson CC, Willis M, Meagher A, Giesecke KE, Rotbart H, Glode MP: Impact of rapid polymerase chain reaction results on management of pediatric patients with enteroviral meningitis. *Pediatr Infect Dis J* 2002, 21:283–286
13. Nigrovic LE, Chiang VW: Cost analysis of enteroviral polymerase chain reaction in infants with fever and cerebrospinal fluid pleocytosis. *Arch Pediatr Adolesc Med* 2000, 154:817–821