Comparison of Specimen Processing and Nucleic Acid Extraction by the Swab Extraction Tube System versus the MagNA Pure LC System for Laboratory Diagnosis of Herpes Simplex Virus Infections by LightCycler PCR

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A total of 563 specimens (234 dermal and 329 genital swabs) from patients suspected of having herpes simplex virus (HSV) infections were processed using two different extraction methods (the MagNA Pure LC system and the swab extraction tube system [SETS]); HSV DNA was amplified by LightCycler PCR. HSV DNA was detected in 157 of 563 specimens (27.9%) processed by the MagNA Pure LC system and in 179 of 563 specimens (31.8%) processed by SETS ($P < 0.0001$). There was no specimen processed by the MagNA Pure LC extraction method that was positive only for HSV DNA. Of 157 specimens positive by both methods, HSV DNA copy levels were higher (using cycle crossover points [cycle threshold $\{C_T\}$]) with SETS (mean C_T, 25.9 cycles) than with the MagNA Pure LC system (mean C_T, 32.0 cycles) ($P < 0.0001$). The time to process 32 samples was longer with the MagNA Pure LC extraction system (90 min) than with SETS (35 min). HSV DNA extraction using SETS is faster, less expensive, and more sensitive than the MagNA Pure LC system and could replace the latter for the laboratory diagnosis of HSV infections using LightCycler PCR.

Herpes simplex virus (HSV) is one of the most commonly detected viruses in diagnostic laboratories (11). It has the ability to establish latency with subsequent reactivation manifested usually by recurrent local disease (12). HSV infection of the genital tract, particularly with recurrent episodes, can be asymptomatic and is associated with ongoing risk of sexual and perinatal transmission. Thus, the importance of a sensitive, accurate, and rapid diagnostic test that can detect symptomatic or asymptomatic viral shedding that leads to early treatment in order to reduce the transmission of infection (10). The use of real-time PCR in the laboratory diagnosis and genotype determination of HSV has been shown in several studies to be more sensitive and rapid than the shell vial cell culture assay (K. Lublink, J. Welle, M. Campbell, and C. A. Gleaves, Abstr. 20th Annu. Clin. Virol. Symp., abstr. T30, 2004) (1–3, 5, 6, 15), and it has been proposed that real-time PCR should replace the shell vial cell culture assay as the new "gold standard" for routine laboratory diagnosis of HSV infection. However, high acquisition costs of automated DNA extraction systems and real-time PCR instruments may hinder the implementation of this technique as a routine procedure in many clinical laboratories.

Since May 2000, our laboratory has implemented the use of LightCycler PCR (Roche Molecular Biochemicals, Indianapolis, Ind.) for the routine diagnosis of dermal and genital HSV infections. Because of the large number of specimens processed in our laboratory, we also implemented the routine use of the MagNA Pure LC automated extraction system (Roche Molecular Biochemicals) for the detection of HSV DNA from dermal and genital tract swabs. The MagNA Pure LC system provided our laboratory with a standardized, efficient, and cost-effective method for extraction of nucleic acids from specimens compared to the manual IsoQuick system (Orca Research, Inc., Bothell, Wash.) and another automated extraction system (BioRobot 9604; QIAGEN, Inc., Chatsworth, Calif.) (4).

In an effort to optimize the performance characteristics of the LightCycler PCR assay, decrease cost, improve turnaround time, and increase the availability of our automated extraction system (MagNA Pure LC) to process other types of specimens, we evaluated the swab extraction tube system (SETS) (catalog no. 3 315 568; Roche Applied Science) as a potential effective method for extraction of HSV DNA from dermal and genital swabs that could replace the MagNA Pure LC system. In a study published earlier, a group in our laboratory has demonstrated the suitability of this extraction method for the detection of group A streptococcus target DNA from throat swabs using LightCycler PCR (14).

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MATERIALS AND METHODS

Study design. The study was approved by the Institutional Review Board of the Mayo Foundation. A difference of at least 2% between the two extraction methods (MagNA Pure LC system versus SETS) was predetermined as acceptable to implement a change in our standard practice. We calculated that 619 specimens processed by both methods would be needed to detect this 2% difference.

Consecutive dermal and genital swabs from 642 adult and pediatric patients

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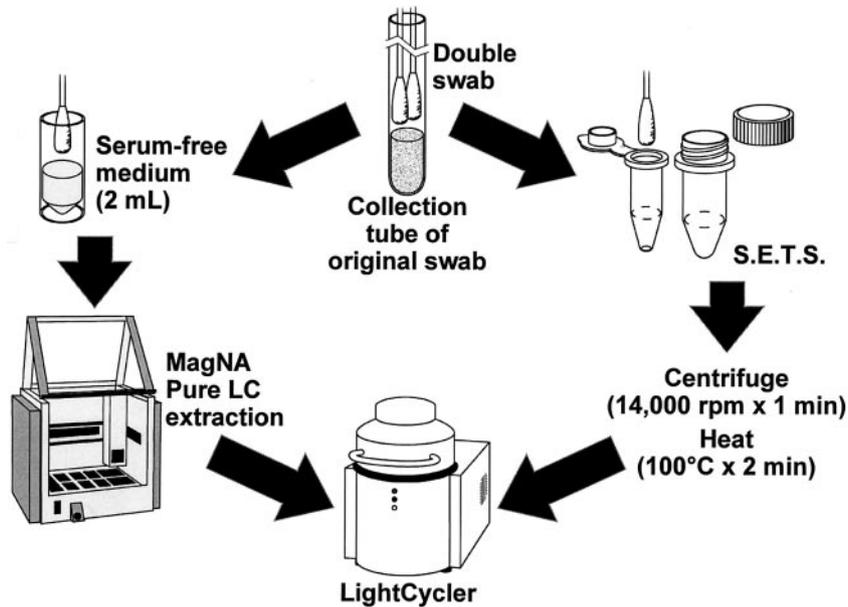
FIG. 1. Specimen collection and processing. Noted centrifuge speed is as on an Eppendorf 5417 (Brinkman Instruments).

were collected from December 2003 through January 2004. Seventy-nine of 642 (12.3%) specimens were excluded because patients declined to provide permission to use their specimens (Minnesota Statute 144.335) or because the specimens were taken from sources other than skin or genital tract. A total of 563 dermal and genital swabs sufficient to detect a 2.1% difference between the two methods were available for analysis.

Collection of specimens. Swabs from dermal specimens ($n = 234$) and genital swabs ($n = 329$) from patients suspected of having HSV infections were initially extracted by laboratory technologists, placed in 2 ml of serum-free medium, processed by the MagNA Pure LC system, and assayed by LightCycler PCR per our standard (laboratory) practice. The original swabs were discarded. However, the collection and transport tubes containing a gel or sponge with remaining fluid were then used as a specimen source for our study. For the (study) comparison, a double-swab (CultureSwab; Becton Dickinson Microbiology Systems, Cockeysville, Md.) was inserted into each collection tube (Fig. 1). The laboratory technologist made sure that both swabs were soaked with material; one swab was picked randomly and processed using the MagNA Pure LC system, and the other swab was processed by SETS. The time period between receiving and processing the specimens varied between 24 and 72 h.

Processing of the specimens. (i) MagNA Pure LC system nucleic acid extraction. A single swab was randomly selected and swirled in 2 ml of serum-free medium and extracted using the MagNA Pure LC automated system by using the Total Nucleic Acid isolation extraction kit produced by Roche Applied Science.

(ii) SETS. Two hundred microliters of swab neutralization buffer (2 mM EDTA and 30 mM Tris [pH 8.4]) was first placed in the outer tube of the SETS.

The remaining swab from the clinical specimen was inserted into the inner tube of the SETS. The exposed shaft of the CultureSwab was then broken above the swab portion contained in the inner tube using a gloved hand and Bio-Screen biohazard wipe (Fisher Scientific, Pittsburgh, Pa.). The SETS with the swab was capped and centrifuged at $14,000 \times g$ for 1 min using a microcentrifuge with an aerosol-tight fixed-angle rotor (Eppendorf 5417C; Brinkman Instruments, Westburg, N.Y.) to extract the fluid from the swab. The inner tube containing the dry swab was then discarded, and the outer tube containing fluid was covered with a screw cap, labeled, and placed in a 100°C heating block for 2 min.

(iii) LightCycler PCR assay. Five microliters of extracted specimen processed by the MagNA Pure LC system and SETS was added, each separately, to 15 µl of LightCycler FastStart Master Hybridization probe mix containing the following: 6.6 µl of water, 2.4 µl of 25 mM magnesium chloride, 2.0 µl of LightCycler-herpes simplex virus 1/2 primer/hyb probes (catalog no. 03355632001; Roche Diagnostics), 2.0 µl of LightCycler FastStart DNA Master hybridization probes (catalog no. 3003248; Roche Diagnostics), and 2.0 µl of a 1:8 dilution of Light-Cycler-herpes simplex virus 1/2 recovery template (catalog no. 03355624001; Roche Diagnostics) per reaction mixture. The LightCycler PCR was performed using the following protocol: (i) an initial step of 10 min at 95°C; and (ii) 45 cycles, with 1 cycle consisting of 10 s of denaturation at 95°C, 15 s of annealing at 55°C, and 15 s at 72°C with fluorescence measurement. Melting curve analysis was performed after PCR as follows: 95°C for 0 s, 40°C for 1 min, and 85°C at 0.2°C/s with continuous measurement of the fluorescence. LightCycler software was used to determine the genotype and cycle threshold (C_T) for each specimen processed by either method.

TABLE 1. Detection of HSV DNA by LightCycler PCR after extraction of DNA from dermal and genital swabs by the MagNA Pure LC system and by SETS[a]

SETS result	MagNA Pure LC system result (no. of swabs)	
	Pos	Neg
Pos	157	22
Neg	0	384

[a] Positive (Pos) and negative (Neg) test results are shown. The positivity rate of the MagNA Pure LC system was 27.9% (157 of 563 swabs), and the 95% confidence interval was 24.2 to 31.8%. The positivity rate of SETS was 31.8% (179 of 563 swabs), and the 95% confidence interval was 28.0 to 35.8%. The positivity rates of the two methods were significantly different ($P < 0.0001$) by the sign test.

TABLE 2. Detection of HSV DNA by LightCycler PCR after extraction of DNA from dermal and genital swabs by the laboratory (original swabs) and by SETS[a]

SETS result	Laboratory result (original swabs) (no. of swabs)	
	Neg	Pos
Pos	170	9
Neg	11	373

[a] Negative (Neg) and positive (Pos) test results are shown. The positivity rate of the laboratory result (original swabs) was 32.2% (181 of 563 swabs), and the 95% confidence interval was 28.3 to 36.2%. The positivity rate of SETS was 31.8% (179 of 563 swabs), and the 95% confidence interval was 28.0 to 35.8%. The positivity rates of the two methods were not significantly different ($P = 0.82$) by the sign test.

Statistical analysis. The positivity rates for the MagNA Pure LC system and SETS were estimated, and 95% exact binomial confidence intervals were calculated for each HSV genotype and specimen source (dermal and genital swabs) separately. The positivity rates of the two different methods (MagNA Pure LC system and SETS) were compared in pairs by the sign test. The numbers of cycles before the detection of HSV DNA were compared by the paired t test or Wilcoxon signed-rank test as appropriate.

RESULTS

HSV DNA was detected in 157 (27.9%) of 563 specimens processed by the MagNA Pure LC system, 179 (31.8%) of 563 specimens processed by SETS ($P < 0.0001$), and 181 (32.2%) of 563 original specimens processed by the laboratory (Tables 1, 2, and 3). Among the 157 specimens positive by both methods, the specimens processed using SETS had a mean C_T of 25.9 cycles by the LightCycler PCR assay (median, 26.0; range, 19 to 39) compared to a mean C_T of 32.0 cycles for specimens processed by the MagNA Pure LC system (median, 31.0; range, 23 to 44) ($P < 0.0001$) (Fig. 2). There were no specimens for which only the MagNA Pure LC assay gave a positive result. HSV type 1 (HSV-1) was detected in 84 (14.9%) of 563 specimens, HSV-2 was detected in 89 (15.8%) specimens, and 6 (1.1%) specimens were positive for HSV DNA but were nontypeable (intermediate) by melting curve analysis. There was no HSV genotype discordance between specimens extracted by the MagNA Pure LC system and SETS. HSV-1 and HSV-2 DNA was detected more frequently by SETS ($P = 0.0005$) than by the MagNA Pure LC system ($P = 0.002$) (Table 4). The detection rates of HSV DNA in dermal speci-

mens were similar for the MagNA Pure LC system (54 of 234 [23.1%]) and SETS (56 of 234 [24.0%]); however, with genital swabs, the MagNA Pure LC extraction method yielded a lower detection rate (103 of 329 [31.3%]) than that of SETS (123 of 329 [37.4%]) ($P = 0.0001$) (Table 4).

There were 34 discrepant results between and among specimens processed by the MagNA Pure LC system, SETS, and laboratory (original swabs). The discrepant specimens and the corresponding C_T by each method are shown in Table 5.

DISCUSSION

Real-time PCR has become the gold standard for the routine laboratory diagnosis of HSV infections. More than 10

TABLE 3. Detection of HSV DNA by LightCycler PCR after extraction of DNA from dermal and genital swabs by the laboratory (original swabs) and by the MagNA Pure LC system (dual swab)[a]

MagNA Pure LC system result	Laboratory result (original swabs) (no. of swabs)	
	Pos	Neg
Pos	152	29
Neg	5	377

[a] Positive (Pos) and negative (Neg) test results are shown. The positivity rate of the laboratory result (original swabs) was 27.9% (157 of 563 swabs), and the 95% confidence interval was 24.2 to 31.8%. The positivity rate of the MagNA Pure LC system was 32.2% (181 of 563 swabs), and the 95% confidence interval was 28 to 36.2%. The positivity rates of the two methods were significantly different ($P < 0.0001$) by the sign test.
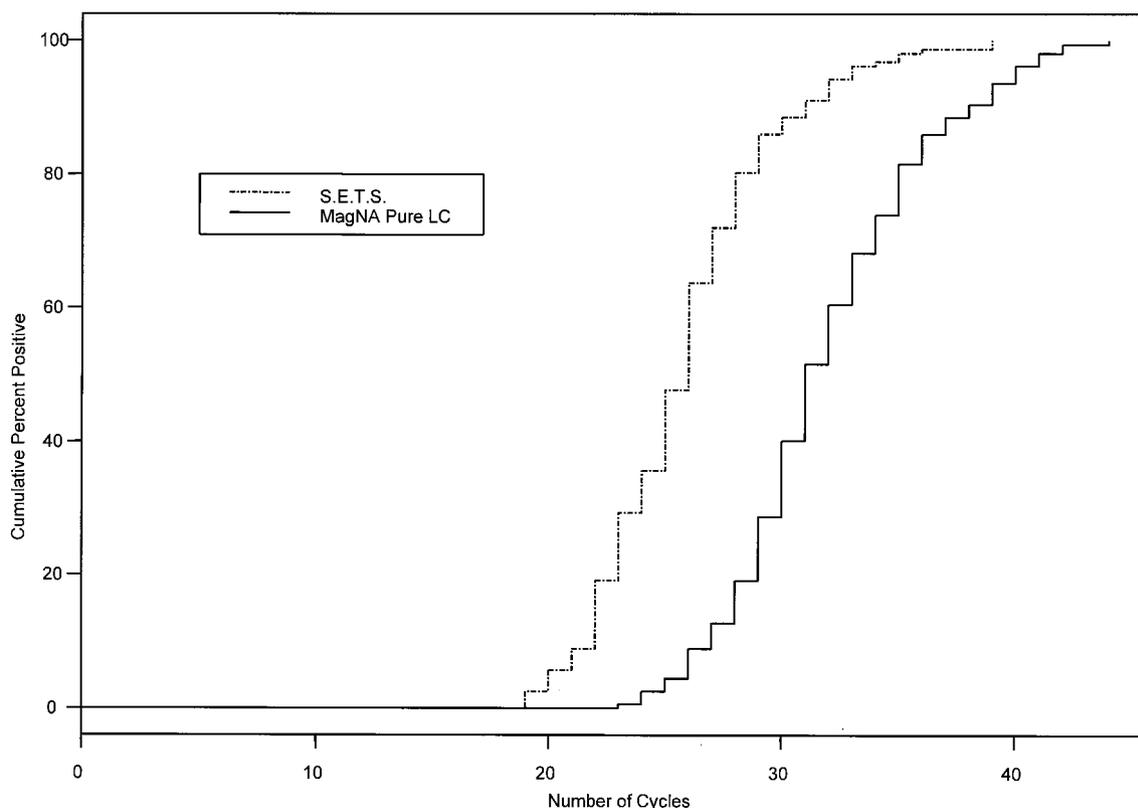

FIG. 2. Comparison of the detection of HSV DNA extracted by SETS and MagNA Pure LC system.

TABLE 4. Positive results of specimens or swabs processed by the
MagNA Pure LC system and SETS by HSV subtype and
specimen source

Method	No. of positive specimens or swabs				
	HSV-1	HSV-2	HSV intermediate	Dermal	Genital
MagNA Pure LC system	74	77	6	54	103
SETS	84	89	6	56	123
P value[a]	0.002	0.0005		0.5	0.0001

[a] The P values shown compare the values (number of positive specimens or swabs for the HSV subtype or specimen or swab source by the MagNA Pure LC system and SETS.

years ago, PCR was used in molecular microbiology laboratories to detect HSV DNA in cerebrospinal fluid specimens from patients with central nervous system infections caused by HSV. Apart from invasive brain biopsy, HSV was very rarely recovered in cell cultures inoculated with cerebrospinal fluid speci-

TABLE 5. Discrepant results between specimens processed by the
MagNA Pure LC system, SETS, and laboratory result
(original specimens)

Sample no.	Discrepant result[a]		
	Original specimen, laboratory result	Study specimen	
		SETS	MagNA Pure LC
38	Neg	Pos (38)	Neg
42	Pos (31)	Pos (35)	Neg
46	Pos (28)	Pos (32)	Neg
50	Pos (45)	Pos (36)	Neg
67	Pos (31)	Pos (32)	Neg
86	Pos (24)	Pos (27)	Neg
107	Pos (39)	Neg	Neg
128	Pos (35)	Pos (34)	Neg
131	Pos (39)	Pos (38)	Neg
162	Neg	Pos (35)	Neg
178	Pos (32)	Pos (32)	Neg
184	Pos (31)	Pos (30)	Neg
188	Pos (40)	Neg	Neg
260	Pos (34)	Neg	Neg
264	Neg	Pos (36)	Neg
291	Pos (45)	Neg	Neg
300	Pos (45)	Pos (39)	Neg
301	Pos (34)	Neg	Neg
315	Pos (36)	Neg	Neg
327	Pos (29)	Pos (34)	Neg
368	Pos (33)	Pos (31)	Neg
388	Pos (43)	Neg	Neg
403	Pos (45)	Pos (38)	Neg
404	Pos (33)	Pos (33)	Neg
411	Pos (40)	Neg	Neg
434	Pos (28)	Pos (33)	Neg
444	Neg	Pos (36)	Pos (38)
448	Pos (33)	Pos (35)	Neg
459	Pos (33)	Pos (31)	Neg
462	Pos (45)	Neg	Neg
476	Pos (33)	Pos (30)	Neg
483	Neg	Pos (40)	Neg
556	Pos (37)	Neg	Neg
612	Pos (36)	Neg	Neg

[a] Negative (Neg) and positive (Pos) test results are shown. The numbers in parentheses are the C_T values.

mens (7, 13). PCR was prototypic for the laboratory diagnosis of viral infections, especially those that replicated slowly or not at all in cell cultures (8).

Rapid real-time PCR cycling, and most importantly the amplification and detection of amplicons in a closed vessel in automated platforms, has allowed integration of this seminal technology into the mainstream clinical laboratory for the diagnosis of many viral and other microbial infections. Since May 2000, our laboratory implemented real-time PCR as a replacement test for cell culture technology for the detection of HSV DNA from dermal and genital swabs (3).

Using real-time PCR, we and others have achieved higher sensitivities ranging from 28 to 108% in the clinical laboratory compared to the sensitivities of conventional cell culture methods (2, 3, 9). Although test costs and reimbursement of real-time PCR are similar or better than cell culture methods, the associated capital equipment expenses, especially for nucleic acid extraction technology, are a potential drawback for laboratories wanting to incorporate this new level of viral diagnosis into the clinical laboratory.

We previously evaluated the technical performance of three nucleic acid extraction methods (IsoQuick, MagNA Pure LC system, and BioRobot 9604) for recovery of nucleic acid prior to amplification of target DNA of HSV by real-time LightCycler PCR (4). In the present study, we compared a manual nucleic acid extraction method, SETS, to the MagNA Pure LC system in order to improve work flow, increase turnaround time, and potentially decrease the costs associated with automated technology particularly for laboratories which process fewer of these specimens daily than larger reference centers.

Interestingly, in our evaluation, the SETS extraction method was more sensitive than the MagNA Pure LC system in that HSV DNA was detected in more specimens (179 versus 157, respectively; $P < 0.0001$) than the automated method when the two methods were compared head to head under exactly the same conditions. The higher positivity rate detected by the laboratory (original swabs) compared to that of SETS (although not statistically significant [$P = 0.82$]) results from the fact that the laboratory had the advantage of processing the original specimen. The high C_T for the positive specimens detected by the laboratory indicates a low initial HSV DNA copy number in the original swab, and the copy number of HSV DNA could have been even lower in the remaining fluid that was used to test the SETS method.

The technical processing for comparing SETS with the MagNA Pure LC system was experimentally designed to reflect existing methods for nucleic acid extraction. Thus, for MagNA Pure LC extraction, one of two dual swabs was initially swirled in 2 ml of serum-free medium prior to real-time PCR amplification. Alternatively, the comparison swab for extraction of nucleic acid by SETS was centrifuged in 0.2 ml of swab neutralization buffer; the resultant extract was then heated at 100°C for 2 min before real-time PCR amplification. Therefore, the volume of the SETS extraction was concentrated 10-fold relative to the MagNA Pure LC method. The MagNA Pure LC system can process a variable number of samples (1 to 32) in one run. To process 30 samples, it requires approximately 30 min of hands-on time to aliquot the samples, prepare the machine, and load the reagents and 1.5 h to complete the nucleic acid extraction. The cost of MagNA Pure LC extraction

in our laboratory is $5.69 per sample (excluding the cost of acquiring the MagNA Pure LC instrument).

The SETS method requires less than 1 min per sample to insert the swab, break the shaft, and close the inner tube. The centrifugation time is 1 min, and the heating time is 2 min. Therefore, it takes between 25 and 35 min to complete the DNA extraction of 30 specimens with a cost of $0.5 per sample.

Overall, our study shows that HSV DNA extraction using SETS is faster, less expensive, and more sensitive than the MagNA Pure LC system and that SETS could replace the MagNA Pure LC system in the laboratory diagnosis of HSV infections using LightCycler PCR.

REFERENCES

1. **Aldea, C., C. P. Alvarez, L. Folgueira, R. Delgado, and J. R. Otero.** 2002. Rapid detection of herpes simplex virus DNA in genital ulcers by real-time PCR using SYBR green I dye as the detection signal. J. Clin. Microbiol. **40:**1060–1062.
2. **Burrows, J., A. Nitsche, B. Bayly, E. Walker, G. Higgins, and T. Kok.** 2002. Detection and subtyping of herpes simplex virus in clinical samples by Light-Cycler PCR, enzyme immunoassay and cell culture. BMC Microbiol. **2:**12.
3. **Espy, M. J., T. K. Ross, R. Teo, K. A. Svien, A. D. Wold, J. R. Uhl, and T. F. Smith.** 2000. Evaluation of LightCycler PCR for implementation of laboratory diagnosis of herpes simplex virus infections. J. Clin. Microbiol. **38:**3116–3118.
4. **Espy, M. J., P. N. Rys, A. D. Wold, J. R. Uhl, L. M. Sloan, G. D. Jenkins, D. M. Ilstrup, F. R. Cockerill III, R. Patel, J. E. Rosenblatt, and T. F. Smith.** 2001. Detection of herpes simplex virus DNA in genital and dermal specimens by LightCycler PCR after extraction using the IsoQuick, MagNA Pure, and BioRobot 9604 methods. J. Clin. Microbiol. **39:**2233–2236.
5. **Espy, M. J., J. R. Uhl, P. S. Mitchell, J. N. Thorvilson, K. A. Svien, A. D. Wold, and T. F. Smith.** 2000. Diagnosis of herpes simplex virus infections in the clinical laboratory by LightCycler PCR. J. Clin. Microbiol. **38:**795–799.
6. **Koenig, M., K. S. Reynolds, W. Aldous, and M. Hickman.** 2001. Comparison of Light-Cycler PCR, enzyme immunoassay, and tissue culture for detection of herpes simplex virus. Diagn. Microbiol. Infect. Dis. **40:**107–110.
7. **Mitchell, P. S., J. J. Germer, and R. Patel.** 2004. Nucleic acid amplification methods: laboratory design and operations, p. 85–93. *In* D. H. Persing, F. C. Tenover, J. Versalovic, Y. W. Tang, E. R. Unger, D. A. Relman, and T. J. White (ed.), Molecular microbiology. Diagnostic principles and practice. ASM Press, Washington, D.C.
8. **Rowley, A. H., R. J. Whitley, F. D. Lakeman, and S. M. Wolinsky.** 1990. Rapid detection of herpes-simplex-virus DNA in cerebrospinal fluid of patients with herpes simplex encephalitis. Lancet **335:**440–441.
9. **Ryncarz, A. J., J. Goddard, A. Wald, M. L. Huang, B. Roizman, and L. Corey.** 1999. Development of a high-throughput quantitative assay for detecting herpes simplex virus DNA in clinical samples. J. Clin. Microbiol. **37:**1941–1947.
10. **Schomogyi, M., A. Wald, and L. Corey.** 1998. Herpes simplex virus-2 infection. An emerging disease? Infect. Dis. Clin. N. Am. **12:**47–61.
11. **Smith, T. F., M. J. Espy, and M. F. Jones.** 2004. Molecular virology: current and future trends, p. 543–548. *In* D. H. Persing, F. C. Tenover, J. Versalovic, Y. W. Tang, E. R. Unger, D. A. Relman, and T. J. White (ed.), Molecular microbiology. Diagnostic principles and practice. ASM Press, Washington, D.C.
12. **Sucato, G., A. Wald, E. Wakabayashi, J. Vieira, and L. Corey.** 1998. Evidence of latency and reactivation of both herpes simplex virus (HSV)-1 and HSV-2 in the genital region. J. Infect. Dis. **177:**1069–1072.
13. **Tang, Y. W., P. S. Mitchell, M. J. Espy, T. F. Smith, and D. H. Persing.** 1999. Molecular diagnosis of herpes simplex virus infections in the central nervous system. J. Clin. Microbiol. **37:**2127–2136.
14. **Uhl, J. R., S. C. Adamson, E. A. Vetter, C. D. Schleck, W. S. Harmsen, L. K. Iverson, P. J. Santrach, N. K. Henry, and F. R. Cockerill.** 2003. Comparison of LightCycler PCR, rapid antigen immunoassay, and culture for detection of group A streptococci from throat swabs. J. Clin. Microbiol. **41:**242–249.
15. **van Doornum, G. J., J. Guldemeester, A. D. Osterhaus, and H. G. Niesters.** 2003. Diagnosing herpesvirus infections by real-time amplification and rapid culture. J. Clin. Microbiol. **41:**576–580.