Polymerase Chain Reaction for Detection of Herpes Simplex Virus (HSV) DNA on Mucosal Surfaces: Comparison with HSV Isolation in Cell Culture

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This study compared the rate of isolation of herpes simplex virus (HSV) from >36,000 samples of mucosal secretions obtained from 296 HSV-infected persons versus the rate of detection of HSV DNA, by means of a real-time quantitative polymerase chain reaction (PCR) assay. Overall, HSV was isolated in 3.0% of samples, and HSV DNA was detected in 12.1% of samples. The mean number of HSV DNA copies was $10^{4.9}$ in samples obtained on days when HSV lesions were present and $10^{4.4}$ in samples from days when HSV lesions were absent. There was a linear relationship between the ability to isolate virus in culture and the log number of copies of HSV DNA in the sample; this relationship persisted in samples from men or women, in samples from human immunodeficiency virus–negative or –positive participants, and in samples obtained on days when lesions were present or absent. In home-collected specimens, the ratio of PCR positivity to viral-culture positivity rose from 3.8:1 in the winter to 8.8:1 in the summer months, reflecting the lability of viral-culture specimens transported during warm weather.

The development of methods to amplify nucleic acids has provided a way of identifying and quantifying infectious pathogens on mucosal surfaces or in tissues [1–3]. Accumulating evidence indicates that DNA detection by polymerase chain reaction (PCR) can be a reproducible and sensitive method, even for pathogens that can be isolated in viral culture [4–6].

In the present study, we compare our experience with isolation of herpes simplex virus (HSV) in culture and detection of HSV DNA by PCR of >36,000 mucosal swab specimens obtained from a variety of persons, clinical settings, and anatomic sites. This study evaluates the association between quantitative HSV DNA and standard viral culture for this common pathogen, in specimens that have been processed for both assays, and, to our knowledge, it provides the most extensive comparison between virus isolation and detection of a pathogen by PCR.

SUBJECTS, MATERIALS, AND METHODS

Participants, Setting, and Sample Collection

The specimens included in this study were collected during research projects conducted at the University of Washington Virology Research Clinic, between March 1994 and November 2001, from study subjects enrolled in a variety of studies of HSV infection, including natural history, therapeutic vaccine, and treatment evaluation [7–11]. At enrollment in the clinic, all subjects signed informed-consent forms, underwent a standardized questionnaire that included demographic characteristics, clinical history of genital and oral herpes, and other medical history, and provided sera for HSV serology by Western blot [12].

Viral-culture samples and PCR samples were collected both by participants at home and by clinicians in the research clinic. For the collection of specimens at home, subjects were instructed how to swab their

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genital area to obtain a sample of secretions for viral culture and PCR. Women collected cervicovaginal, vulvar, and perianal samples, whereas men collected penile and perianal samples, as described elsewhere [7, 8, 13]. In addition, a separate swab of lesions, including lesions outside the genital area, was collected. In some studies, additional samples were collected from oral mucosa as well as from other sites, such as the nares. A separate Dacron swab pair was used to sample each anatomic site daily; the swabs were held together and were used at the same time. One swab, for viral culture, was placed into a vial containing 1 mL of virus-transport medium and was delivered to the laboratory ⩾3 times/week; the other swab, for HSV DNA PCR, was placed into vials containing 1 mL of PCR digestion buffer and was delivered to the PCR laboratory at regular intervals. All samples were refrigerated until transported. Samples were collected at the research clinic by use of the same techniques, and samples were delivered from these clinics to the respective laboratories twice daily. Swabs for HSV DNA PCR were stored at −20°C until processed.

In studies involving collection of samples at home, the participants maintained daily diaries of genital lesions and recorded whether they took antiviral medication. Clinicians also noted the presence of lesions and antiviral treatment during clinic visits. All clinical and laboratory information was recorded on standardized data-collection forms.

Laboratory Methods

Virus isolation and HSV-antibody testing. Specimens removed from virus-transport medium (veal infusion broth with antibiotics) were inoculated onto microtiter plates containing human diploid fibroblasts, and all isolates were typed by monoclonal antibodies, as described elsewhere [14, 15]. The same virus-isolation method was used throughout the study period. The HSV type–specific serological tests were done by Western blot [12].

PCR assay. The PCR assay was a modification of the real-time quantitative fluorescent–probe assay described elsewhere [16, 17]. The swab samples in PCR buffer were thawed and opened, the swab was discarded, and 200 μL of the swab sample was extracted by use of Qiagen columns, as described elsewhere. Ten microliters of the extracted DNA was used in each assay. The primers and probes were directed to the HSV glycoprotein B gene.

To control for pipetting errors and other causes of well-to-well variability, an internal reference control, consisting of the passive fluorescent dye 6-carboxy-X-rhodamine conjugated to the 5′ end of 5′-GATTAG-3′, was included in the master mix for each reaction. This reagent was used at a working concentration of 60 nmol/L. The PCR instrument used to read the assay (ABI Prism 7700 Sequence Detection System; Applied Biosystems) standardizes each sample to the quantity of the reference dye in each reaction. The real-time data generated are analyzed with sequence-detector software, version 1.6.3 (Applied Biosystems). The threshold of detection is set at the point that is >10 SD above the background and when the PCR reaction enters the exponential phase. Each PCR run contained several negative controls including 2 reactions without DNA as well as several clinical specimens that were known to contain no HSV DNA, a positive amplicon control, and a standard dilution curve of amplicon DNA. Each specimen was run in duplicate, and only those specimens in which the results of both replications were above the cutoff value were considered to be positive. We reported as positive only those samples in which >10 copies of HSV DNA/reaction (500 copies of HSV DNA/mL of transport medium) were detected. Sequence-detector software determined the standard curve, which was then used to calculate precise quantities of starting template molecules for the unknown sample. By standard curve and by validation with clinical samples in this trial, this assay is linear from 10 to 10^9 copies of HSV DNA.

Data Analyses

For inclusion in this study, PCR specimens and viral-culture specimens were matched according to the following criteria: subject, body site, collection mode (at home vs. in clinic), and collection day. Swabs obtained on days during which antiviral medication was administered were excluded from the analyses. Days when lesions were present were defined as days on which lesions were noted by the clinician or the patient. Additional analyses were done on a small number of specimens that were collected directly from genital lesions. All data are expressed as number of copies of HSV DNA per milliliter of PCR buffer. The rates of HSV detection, both by the 2 methods and between the groups of participants, were compared by use of Wilcoxon signed-rank test or Mann-Whitney test, as appropriate. Because the number of specimens per person varied widely, statistical comparisons were limited to participants who contributed ⩾10 specimens.

RESULTS

Study population. We evaluated samples from 296 subjects, of whom 137 were women and 159 were men (table 1); 83% of the subjects were white, with a median age of 34 years, and 89 (30%) of them had human immunodeficiency virus (HIV) infection. All subjects had HSV-2 infection and/or HSV-1 infection: 45% had only HSV-2 antibodies, 43% had both HSV-1 antibodies and HSV-2 antibodies, and 13% had only HSV-1 antibodies. Overall, 36,471 separate pairs of samples were included in the analyses, with a mean number of 123 pairs/person (table 1). To provide an objective measure of specificity of the PCR method and the viral-culture method, we also collected

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Table 1. Sex, human immunodeficiency virus (HIV) status, and number specimens contributed by study participants.

Sex, HIV status	No. (% of total) of subjects	Mean no. of specimens per participant	No. (%) of specimens
Women			
Negative	125	65.5	8191 (22.5)
Positive	12	231.9	2783 (7.6)
Subtotal	137 (46.3)		
Men			
Negative	82	103.4	8478 (23.2)
Positive	77	221.0	17,019 (46.7)
Subtotal	159 (53.7)		
Total	296	123.2	36,471

samples from 5 persons who were persistently HSV seronegative and submitted them in blinded fashion to the laboratory. All 1082 paired viral-culture/PCR samples from seronegative persons were negative for HSV DNA by PCR and negative for HSV by virus isolation.

Rates of detection of HSV by virus isolation and of HSV DNA by PCR. The rates of virus isolation and of detection by PCR are summarized in table 2. Of 36,471 specimens, 1087 were viral-culture positive and 4415 were PCR positive (3.0% vs. 12%; $P < .001$), so that the overall ratio of PCR positivity to culture positivity was 4.1:1. The frequency of HSV isolation by culture was higher among women than among men (3.9% vs. 2.6%; $P = .18$) and also was higher on days when lesions were present than on days when lesions were absent (12% vs. 1.7%; $P < .001$). The rate of detection of HSV DNA by PCR followed a similar pattern (16% of samples from women vs. 10% of samples from men [$P = .003$] and 37% of samples obtained on days when lesions were present vs. 8.4% of samples of obtained on days when lesions were absent [$P < .001$]). The mean number of HSV DNA copies was $10^{4.9}$ on days when lesions were present and $10^{4.4}$ on days when lesions were absent. The ratio of PCR positivity to viral-culture positivity varied from 3.1:1, on days when lesions were present, to 5.1:1, on days when lesions were absent.

Of 4464 samples that were positive by ⩾1 of the tests, 3377 (75.7%) were positive by PCR only, 49 (1.1%) were positive by viral culture only, and 1038 (23.3%) were positive by both tests. Thus, compared with PCR, viral culture yielded false-negative results for 76% of samples; conversely, compared with viral culture, PCR yielded false-negative results for 1.1% of samples. Of 1665 samples that were positive by ⩾1 of the tests on days when genital lesions were present, both viral culture and PCR yielded positive results on 32.0% of the days, PCR alone yielded positive results on 67.0% of the days, and viral culture alone yielded positive results 1.0% of the days.

We observed a linear relationship between the frequency of HSV isolation in culture and the number of HSV DNA copies detected (figure 1). Negative HSV culture results occurred even with high copy numbers of HSV DNA in the sample, and, conversely, even low copy numbers of HSV DNA (500–1000 copies of DNA/mL) could be associated with isolation of virus in culture. The relationship between the proportion of viral culture–positive samples and the number of HSV DNA copies detected appeared to be similar regardless of lesion status, sex, or HIV serostatus. When HSV DNA was detected in amounts ⩾10^4 copies/mL of swab specimen, 45% of samples resulted in virus isolation, versus only 6% of samples with fewer copies of HSV DNA. Virus-isolation rates above and below 10^4 copies/mL were similar for samples obtained on days when lesions were present (53% vs. 5%) and days when lesions were absent (38% vs. 6%), for women (46% vs. 4%) and men (44% vs. 6%), and for HIV-seropositive patients (46% vs. 4%) and HIV-seronegative patients (43% vs. 7%). HSV DNA detection was more sensitive than viral culture, in detecting HSV on mucosal surfaces in HIV-seropositive persons, both during episodes of lesions and during subclinical shedding (table 3).

Comparison of PCR and viral culture, during episodes of lesions. The distribution of HSV DNA copies, by sex and by presence or absence of genital lesions, is shown in figure 2 and table 4. As expected, the proportion of samples with high amounts of HSV DNA was greater in samples obtained on days when lesions were present than in samples obtained on days when lesions were absent (figure 2). Because HSV was more likely to be isolated in viral culture on days when high amounts of HSV DNA were present, the ratio of PCR positivity to viral-culture positivity was lower on days when lesions were present than on days when lesions were absent. However, in the distribution of HSV DNA copies, little difference was noted between men and women: 14% of samples from men and 12% from women had HSV DNA detected at ⩾10^7 copies/mL. Additional

Table 2. Rates of virus isolation and herpes simplex virus (HSV) DNA detection by polymerase chain reaction (PCR), by sex, presence of lesions, and human immunodeficiency virus (HIV) status.

	No. (%) of specimens				
		Positive			
	Total	By PCR	By viral culture	Positivity ratio[a]	Mean copies of HSV DNA, log
Sex					
Men	25,497	2636 (10)	664 (2.6)	4.0	4.6
Women	10,974	1779 (16)	423 (3.9)	4.2	4.5
Lesions					
Present	4670	1735 (37)	559 (12)	3.1	4.9
Absent	31,801	2680 (8.4)	528 (1.7)	5.1	4.4
HIV status					
Negative	16,669	1818 (11)	447 (2.7)	4.1	4.5
Positive	19,802	2597 (13)	640 (3.2)	4.1	4.6
Overall	36,471	4415 (12)	1087 (3.0)	4.1	4.6

[a] PCR positivity:viral-culture positivity.

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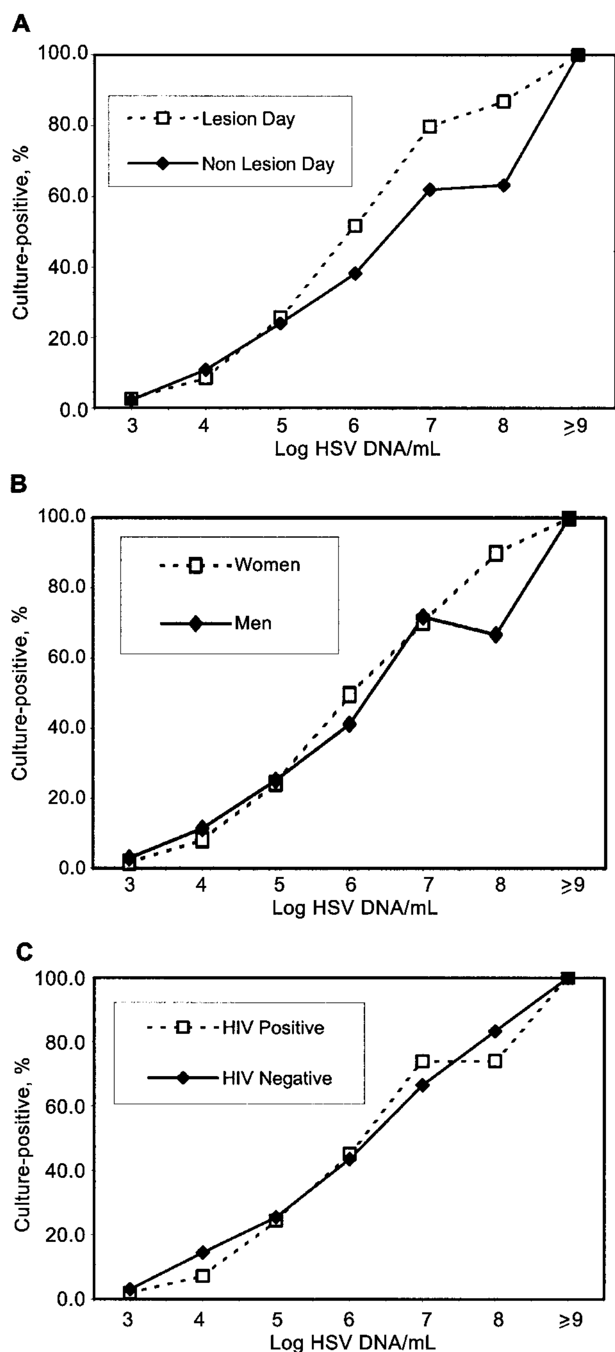

Figure 1. Relationship between rate of virus isolation in culture and number of herpes simplex virus (HSV) DNA copies detected by polymerase chain reaction, in samples collected on days when lesions were present versus samples collected on days when lesions were absent *(top),* in men versus women *(middle),* and in human immunodeficiency virus (HIV)–seropositive subjects versus HIV-seronegative subjects *(bottom).*

analyses of 183 samples that were collected directly from a lesion revealed a 25% rate of virus isolation, compared with a 66% rate of HSV detection by PCR, for a ratio of 2.7:1.

Anatomic site and HSV detection. The anatomic site from which the sample was obtained influenced the rates of viral-culture positivity and detection by PCR (table 4). The mean number of copies of HSV DNA detected was 0.5–1 log higher when lesions were present than when lesions were absent (table 4, figure 2). Of interest, HSV DNA was detected in cervical swabs on days with lesions far more frequently than culture. This 11-fold-higher rate of DNA to culture in the rate of detection at the cervix compares with the 2–4-fold rate difference at the other genital sites on the days with lesions.

Variability in isolation rates in home-collected samples, by season of the year. As noted above, a substantial number of samples were viral-culture negative, despite the presence of large amounts of HSV DNA; for example, among the 2004 samples with $\geqslant 10^5$ copies of HSV DNA, 55% were viral-culture negative (also see figure 1). To identify factors associated with high rates of false-negative viral-culture results, we examined the effect that season of collection of daily home-collected swabs had on our ability to isolate virus in tissue culture. The ratio of PCR-positive to viral culture–positive specimens, for 2116 swabs collected at home during January and February of 1999 through 2001, was 4.5:1, compared with a ratio of 8.8:1 among 1578 swab pairs collected during July and August of the same years (figure 3). In contrast, the rate of HSV DNA detection in the same winter swabs was 8.8%, compared with 8.9% for the summer swabs. Thus, as much as a 50% reduction in virus isolation by culture appears to be attributable to a seasonal effect on specimen viability, because the ratio of PCR positivity to viral-culture positivity varied throughout the calendar year, in concert with local mean daily high temperatures [18].

DISCUSSION

Our study clearly showed the higher frequency of detection of HSV DNA by PCR, compared with virus isolation. Our data indicate that, regardless of whether samples are obtained from HSV lesions or from genital or oral secretions during a period of subclinical shedding, detection of HSV DNA by PCR is more

Table 3. Rates of virus isolation and detection of herpes simplex virus (HSV) DNA by polymerase chain reaction (PCR), in human immunodeficiency virus (HIV)–seropositive and HSV-2–infected persons, stratified by presence of lesions.

	No. (%) specimens				
		Positive			
Lesion status	Total	By PCR	By viral culture	Positivity ratio[a]	Mean copies of HSV DNA, log
Present	1476	705 (48)	275 (19)	2.6	5.2
Absent	11,809	1339 (11)	290 (2.5)	4.6	4.6
Overall	13,285	2044 (15)	565 (4.3)	3.6	4.7

[a] PCR positivity:viral-culture positivity.

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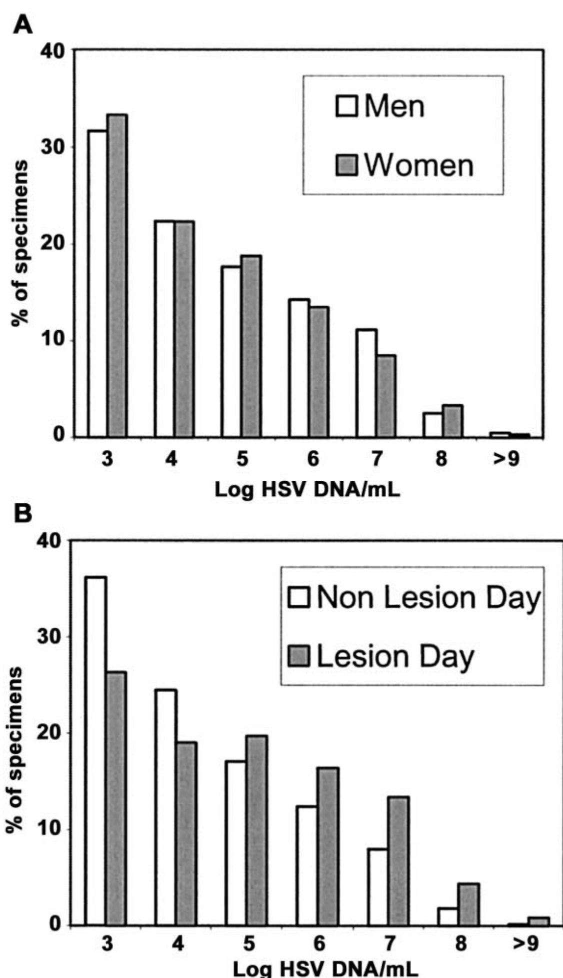

Figure 2. Distribution of specimens, by herpes simplex virus (HSV) DNA copy count, stratified by sex *(top)* and by days when lesions were present versus days when lesions were absent *(bottom)*.

sensitive than virus isolation, for detection of HSV on mucosal surfaces. Of >1000 paired samples in which HSV was isolated by viral culture, only 4.5% did not have HSV DNA detected in them. In contrast, in >4000 samples in which HSV DNA was detected, only 24% yielded HSV by viral culture. Besides increased sensitivity, detection of HSV DNA by PCR had excellent specificity: none of >1000 mucosal samples from HSV-seronegative persons had HSV DNA detected in them. This high specificity relates to the meticulous quality-control procedures used in our laboratory and illustrates the reliability of high-throughput laboratories skilled in the use of PCR.

Perhaps the most surprising but convincing demonstration of the utility of PCR compared with viral culture is the decrease in virus isolation rate that occurred during the summer months. Despite the cool summer weather in the Puget Sound area and the provision of cold packs to subjects for the packing of home-collected samples, the viability of virus was clearly higher in swabs collected during the winter. The genesis of this analysis related to a drop in our virus-isolation rate, which our data manager (S.S.) noticed in 2 studies in which only viral cultures were obtained. This initiated an extensive reevaluation of HSV-isolation procedures, including cell lines, transport media, and culture reagents used for virus isolation. However, we subsequently analyzed data from studies in which PCR assays were done and noted no relative seasonal loss in sensitivity. This led to the more detailed analysis presented in this report. That the sensitivity of virus isolation varies with ambient temperature is of more than local interest. A review of the literature on genital ulcer disease in tropical countries in which only viral culture was used has shown a relatively low frequency of HSV as an etiology of genital ulcer disease (GUD), even in populations in which HSV-2 seroprevalence was high [19–22]. More-recent studies that used PCR-based methods have shown that HSV is the most frequent cause of GUD in these regions of the world [23–25]. Thus, it is likely that much of the 3–4-fold increase in identification of HSV as a pathogen of GUD in tropical regions that has been demonstrated in studies using PCR reflects the lability of HSV during transport. This lability

Table 4. Rates of isolation of virus and detection of herpes simplex virus (HSV) DNA by polymerase chain reaction (PCR), from genital-area specimens, stratified by sex, presence of lesions, and genital site, in human immunodeficiency virus (HIV)–seronegative and HSV-2–infected persons.

	No. (%) of specimens				
		Positive			
Sex, lesion status, site	Total	By PCR	By viral culture	Positivity ratio[a]	Mean copies of HSV DNA, log
Women					
Present					
Cervix	290	122 (42)	11 (3.8)	11.1	4.1
Vulvar	454	265 (58)	94 (21)	2.8	5.1
Perianal	330	164 (50)	41 (12)	4.0	4.4
Buttock[b]	50	36 (72)	13 (26)	2.8	5.3
Absent					
Cervix	1477	155 (10)	11 (0.7)	14.1	4.2
Vulvar	1697	223 (13)	21 (1.2)	10.6	4.0
Perianal	1646	166 (10)	28 (1.7)	5.9	4.1
Men					
Present					
Penile	188	81 (43)	30 (16)	2.7	5.3
Perianal	121	41 (34)	21 (17)	2.0	5.6
Buttock[b]	14	14 (100)	5 (36)	2.8	5.0
Absent					
Penile	2407	95 (4.0)	16 (0.7)	5.9	4.1
Perianal	2259	131 (5.8)	32 (1.4)	4.1	4.9
Total	10,933	1493 (14)	323 (3.0)	4.6	4.5

[a] PCR positivity:viral-culture positivity.
[b] Swabs from buttocks were collected only when lesions were present.

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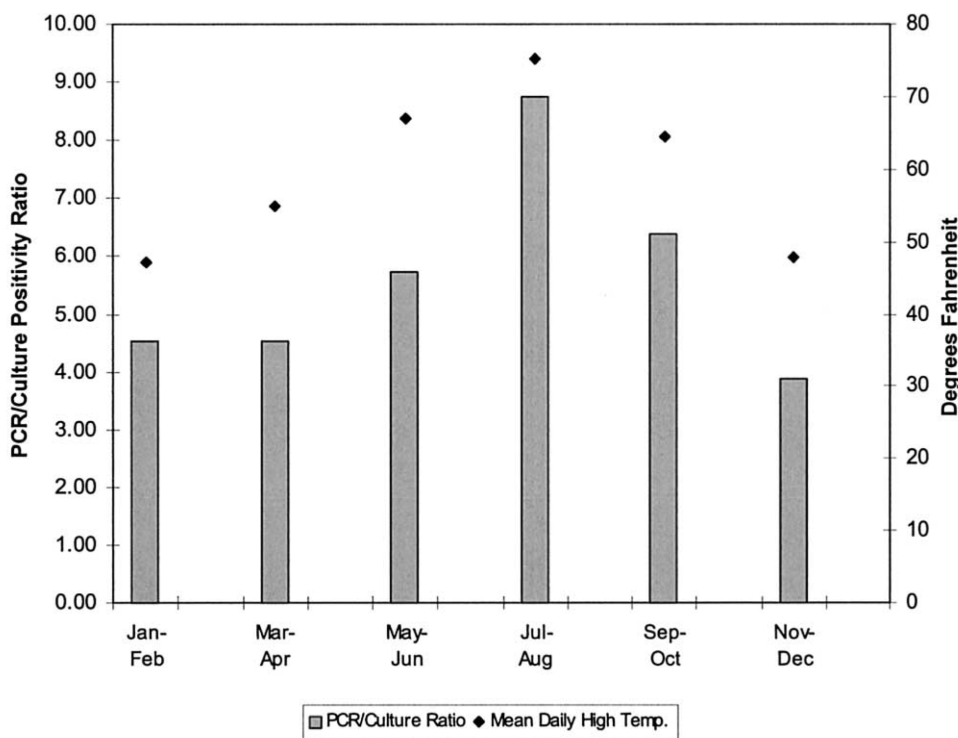

Figure 3. Ratio of polymerase chain reaction (PCR) positivity to viral-culture positivity, by season of the year, with mean daily high temperatures for Seattle.

of HSV during transport, even when swabs were put in an optimal carrying medium, is also shown by the relatively high number of samples that were viral-culture negative but that had $\geqslant 10^5$ copies of HSV DNA detected in the same sample (1099/2004).

What levels of HSV DNA reflect replication and transmission? Our sampling method involves the swabbing of mucosal surfaces. HSV reactivation results from release of virus from nerve endings in the submucosa; as such, isolation of HSV from a mucosal surface or lesion requires either a break in the epithelium or cell-to-cell transfer of virus from submucosa to mucosa [26, 27]. Because HSV is lytic for epithelial cells, a mucosal ulceration seems likely. This possibility is corroborated by studies that have shown that subclinical shedding is associated with microscopic or invisible ulcerations, in mucosal or skin surfaces, that are not noted by the patient or the clinician [28]. In addition, detection of HSV DNA is markedly reduced by antivirals, which inhibit viral proteins present only during the replication cycle of the HSV life-cycle [8, 9, 29]. Hence, it is evident that detection of HSV DNA from a mucosal surface indicates viral replication in most, if not all, cases.

Defining the minimum HSV DNA copy count associated with risk of transmission is a more difficult task. We have previously reported negative results of viral culture, but positive results of PCR (with HSV DNA counts of 500–10^4 copies/mL), for genital swabs collected from delivering women who transmitted HSV to the neonate [30]. The minimum level of detection that we report in mucosal samples is 500 copies/mL, and we initiate our assay with 20 μL of swab sample and use 10 μL of the extracted DNA. Modification of the assay could detect even lower copy numbers of HSV DNA. Defining the DNA levels required for sexual transmission would require daily sampling of genital secretions and highly detailed sexual histories among discordant couples not using barrier forms of protection. These data, we believe, are unlikely to be obtainable either practically or ethically. It is of interest that data from a recent study of valacyclovir used to reduce transmission of HSV among heterosexual couples suggest that even low copy numbers of HSV DNA may result in transmission [31].

Perhaps the most important implication of these data relates to the development of resources for regional diagnostic laboratories. Our data indicate that, for the detection of mucosal HSV infections, including genital ulcers, PCR amplification technology is superior to virus isolation. In addition, PCR has improved diagnostic utility for invasive HSV infection [32] and offers the advantage of more-stable transport. Thus, we believe that health planners and regional reference laboratories should consider using their resources for PCR-based detection methods rather than for virus isolation. Currently, the sole advantage of viral culture is that it addresses the need for an isolate as a requirement in determining antiviral sensitivity, which is an issue in a small subset of diagnostic samples. In the future,

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PCR-based methods for defining relevant mutations are likely to supplant, or replace, phenotypic assays, as they have in the cases of cytomegalovirus and HIV [33, 34].

Although, on a per-specimen basis, PCR is more expensive than HSV isolation, capital costs of tissue culture are greater in the long run. Most important, the yield of virus positivity is 4 times greater and the results are more reliable, especially in settings in which transport or climate may interfere with the yield from viral culture.

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References

1. Pereira BJ, Milford EL, Kirkman RL, et al. Prevalence of hepatitis C virus RNA in organ donors positive for hepatitis C antibody and in the recipients of their organs. N Engl J Med **1992**; 327:910–5.
2. Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. N Engl J Med **1992**; 327:293–301.
3. Josefsson AM, Magnusson PK, Ylitalo N, et al. Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma in situ: a nested case-control study. Lancet **2000**; 355:2189–93.
4. Bassiri M, Mardh PA, Domeika M. Multiplex AMPLICOR PCR screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in women attending non–sexually transmitted disease clinics. The European Chlamydia Epidemiology Group. J Clin Microbiol **1997**; 35:2556–60.
5. Yates MD, Drobniewski FA, Wilson SM. Evaluation of a rapid PCR-based epidemiological typing method for routine studies of *Mycobacterium tuberculosis.* J Clin Microbiol **2002**; 40:712–4.
6. Noordhoek GT, Kolk AH, Bjune G, et al. Sensitivity and specificity of PCR for detection of Mycobacterium tuberculosis: a blind comparison study among seven laboratories. J Clin Microbiol **1994**; 32:277–84.
7. Wald A, Zeh J, Selke S, Ashley RL, Corey L. Virologic characteristics of subclinical and symptomatic genital herpes infections. N Engl J Med **1995**; 333:770–5.
8. Wald A, Zeh J, Barnum G, Davis LG, Corey L. Suppression of subclinical shedding of herpes simplex virus type 2 with acyclovir. Ann Intern Med **1996**; 124:8–15.
9. Wald A, Corey L, Cone R, Hobson A, Davis G, Zeh J. Frequent genital HSV-2 shedding in immunocompetent women. J Clin Invest **1997**; 99: 1092–7.
10. Wald A, Zeh J, Selke S, et al. Reactivation of genital herpes simplex virus type 2 infection in asymptomatic HSV-2 seropositive persons. N Engl J Med **2000**; 342:844–50.
11. Straus S, Wald A, Kost R, et al. Immunotherapy of recurrent genital herpes with recombinant herpes simplex virus type 2 glycoproteins D and B: results of a placebo-controlled vaccine trial. J Infect Dis **1997**; 176:1129–34.
12. Ashley RL, Militoni J, Lee F, Nahmias A, Corey L. Comparison of Western blot (Immunoblot) and G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. J Clin Microbiol **1988**; 26:662–7.
13. Wald A, Zeh J, Selke S, Warren T, Ashley R, Corey L. Genital shedding of herpes simplex virus among men. J Infect Dis **2002**; 186(suppl 1): S34–9.
14. Langenberg A, Zbanyszek R, Dragavon J, Ashley R, Corey L. Comparison of diploid fibroblast and rabbit kidney tissue culture and diploid and fibroblast microtiter plate system for the isolation of herpes simplex virus. J Clin Microbiol **1988**; 26:1772–4.
15. Lafferty WE, Krofft S, Remington M, et al. Diagnosis of herpes simplex virus by direct immunofluorescence and viral isolation from samples of external genital lesions in a high prevalence population. J Clin Microbiol **1987**; 25:323–6.
16. Ryncarz AJ, Goddard J, Wald A, Roizman B, Corey L. Development of a high throughput quantitative assay for detecting HSV DNA in clinical samples. J Clin Microbiol **1999**; 37:1941–7.
17. Jerome KR, Huang ML, Wald A, Selke S, Corey L. Quantitative stability of DNA after extended storage of clinical specimens as determined by real-time PCR. J Clin Microbiol **2002**; 40:2609–11.
18. National Climatic Data Center. Available at: http://lwf.ncdc.noaa.gov/oa/ncdc.html
19. Dickerson MC, Johnston J, Delea TE, White A, Andrews E. The causal role for genital ulcer disease as a risk factor for transmission of human immunodeficiency virus: an application of the Bradford Hill criteria. Sex Transm Dis **1996**; 23:429–40.
20. Behets FM, Liomba G, Lule G, et al. Sexually transmitted diseases and human immunodeficiency virus control in Malawi: a field study of genital ulcer disease. J Infect Dis **1995**; 171:451–5.
21. O'Farrell N, Hoosen A, Coetzee K, van den Ende J. Sexual behaviour in Zulu men and women with genital ulcer disease. Genitourin Med **1992**; 68:245–8.
22. Htun Y, Morse SA, Dangor Y, et al. Comparison of clinically directed, disease specific, and syndromic protocols for the management of genital ulcer disease in Lesotho. Sex Transm Infect **1998**; 74(suppl 1):S23–8.
23. Risbud A, Chan-Tack K, Gadkari D, et al. The etiology of genital ulcer disease by multiplex polymerase chain reaction and relationship to HIV infection among patients attending sexually transmitted disease clinics in Pune, India. Sex Transm Dis **1999**; 26:55–62.
24. Morse SA. Etiology of genital ulcer disease and its relationship to HIV infection. Sex Transm Dis **1999**; 26:63–5.
25. Beyrer C, Jitwatcharanan K, Natpratan C, et al. Molecular methods for the diagnosis of genital ulcer disease in a sexually transmitted disease clinic population in northern Thailand: predominance of herpes simplex virus infection. J Infect Dis **1998**; 178:243–6.
26. Penfold M, Armati P, Cunningham A. Axonal transport of herpes simplex virions to epidermal cells: evidence for a specialized mode of virus transport and assembly. Proc Natl Acad Sci USA **1994**; 91:6529–33.
27. Holland DJ, Miranda-Saksena M, Boadle RA, Armati P, Cunningham AL. Anterograde transport of herpes simplex virus proteins in axons of peripheral human fetal neurons: an immunoelectron microscopy study. J Virol **1999**; 73:8503–11.
28. Koutsky LA, Ashley RL, Holmes KK, et al. The frequency of unrecognized type 2 herpes simplex virus infection among women: implications for the control of genital herpes. Sex Transm Dis **1990**; 17:90–4.
29. Elion GB, Furman PA, Fyfe JA, deMiranda P, Beauchamp L, Schaeffer H. The selectivity of action of an antiherpetic agent, 9-(2-hydroxyethoxymethyl) guanine. Proc Natl Acad Sci USA **1977**; 74:5716–20.
30. Cone R, Hobson A, Brown Z, et al. Frequent detection of genital herpes simplex virus DNA by polymerase chain reaction among pregnant women. JAMA **1994**; 272:792–6.
31. Corey L, Tyring S, Sacks S, et al. Once daily valacyclovir reduces transmission of genital herpes [abstract LB-3]. In: Program and abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy (San Diego). Washington, DC: American Society for Microbiology, **2002**.
32. Whitley R, Lakeman F. Herpes simplex virus infections of the central nervous system: therapeutic and diagnostic considerations. Clin Infect Dis **1995**; 20:414–20.
33. Chou S, Waldemer RH, Senters AE, et al. Cytomegalovirus UL97 phosphotransferase mutations that affect susceptibility to ganciclovir. J Infect Dis **2002**; 185:162–9.
34. Shafer RW. Genotypic testing for human immunodeficiency virus type 1 drug resistance. Clin Microbiol Rev **2002**; 15:247–77.

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