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Use of the designation "shedder" in mucosal detection of herpes simplex virus DNA involving repeated sampling

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Abstract

Objectives—We evaluated two methods to describe detection of HSV from the genital mucosa.

Methods—We assessed genital swabs from HSV-2 seropositive persons participating in longitudinal studies of HSV DNA detection at the University of Washington Virology Research Clinic. We determined the length of observation period necessary to ensure *some* HSV detection for most persons. We compared two measures to assess differences in shedding according to HIV status, the shedding rate ratio, defined as the proportion of total samples with detectable HSV in HIV-1 seropositive versus HIV-1 seronegative persons, and the ratio of "shedders", defined as the proportion of persons with any shedding over the interval in HIV-1 seropositive versus HIV-1 seronegative persons.

Results—While only 17% (51/308) of HSV-2 seropositive persons shed on their first day on study, 77% (238/308) had some genital shedding over 30 days (any HSV DNA detected on genital swabs). Shedding rate ratios for HIV-seropositive versus HIV-seronegative persons varied from SRR=1.42 using 10 samples to SRR=1.35 using 50 samples. The ratio of "shedders" approached 1 as the observation period increased (RS=1.13 using 10 samples to RS=1.01 using 50 samples). In a hypothetical case, the ratio of "shedders" was shown to exceed one when shedding rates were equal.

Conclusions—Most HSV-2 seropositive persons shed HSV from the genital mucosa. Dichotomization of persons into "shedders" and "nonshedders" or "high" and "low" shedders yields inferences that depend upon sampling interval length. Overall shedding rates provide consistent measures regardless of the number of swabs collected.

Keywords

HSV; shedder; shedding rate; misclassification; bias

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Contributions of the authors

AM performed the analyses and wrote the initial draft of the manuscript. CJ and AW contributed the clinical relevance of the paper and critical edits to the manuscript

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Introduction

Viral shedding (defined as having virus detected on swabs of the genital mucosa) is a common outcome in HSV-related studies. Detection of viral shedding can be used to confirm infection, to demonstrate infectiousness, and as a measure of disease severity. For example, the frequency of herpes simplex virus (HSV) reactivation shedding has been associated with disease severity (1,2) as well as risk of HSV transmission to an infant (3,4) or to sexual partners (5), and has been used as a means of demonstrating the effectiveness of antiviral therapy (6,7).

For those infected with HSV, mucosal shedding may occur infrequently and may last only a few days or hours (8). Frequency of HSV reactivations is difficult to predict and may depend on host immune status, time since acquisition of infection, and concurrent infections (9,10). As a consequence, not all persons will shed during short observation periods. Nevertheless, many studies summarize viral shedding patterns by assessing the presence of detectable virus at a single time point. In other cases, the term "shedder" has also been applied in studies of viral detection of HSV (4,8,11–17) and HIV (11,13,16), to describe an individual who has any detectable shedding over repeated sampling. While the term "shedder" may not be used explicitly, the dichotomous classification is often made between those who shed over the observation period and those who do not.

We consider the implications of the dichotomization of shedding patterns into *any* detection ("shedder") in describing shedding frequency both theoretically and using data from studies of HSV mucosal shedding. Then we consider the impact of this classification on detection of group-level differences in HSV shedding frequency and in other contexts.

Methods

Participants & Setting

HSV-2 seropositive participants enrolled in a variety of studies performed self-collection of genital mucosal swabs at home for HSV polymerase chain reaction (PCR) at the University of Washington Virology Research Clinic (UW VRC) between 1992 and 2002 (5,7,10,18–20). A single Dacron swab was collected daily for a period of at least 30 days by participants, swabbing the genital and perianal area. The Human Subjects Review Committee at the University of Washington approved each study and all persons gave written informed consent.

Laboratory Methods

HSV-2 serostatus was determined using Western blot (21). Quantitative, real-time HSV PCR was performed from swabs for HSV DNA as previously described (22,23).

Statistical Methods

Literature review

We performed a review of recently published studies whose procedures involved repeated measurement of HSV-2 via PCR. We compared number of swabs collected with measurements of the overall shedding rate (number of swabs positive out of swabs collected) and with the proportion of participants designated as "shedders" (proportion of persons with detectable HSV DNA at *any* time) using Spearman's correlation coefficient.

Clinical data evaluation

We assessed shedding rates, proportion of persons with any shedding, and shedding episode characteristics. An episode was defined as any period of HSV DNA detection including no more than one consecutive missed or negative swab. We computed the rate ratio (SRR) on all

available samples using Poisson regression (24) with HIV status as the only covariate. Using a fixed number of samples per person (between 1 and 50 samples) we estimated: 1) the ratio of shedders (RS) by dividing the proportion of participants with any (or “high”) shedding detected in one group by the other, and 2) the shedding rate ratio (SRR) using Poisson regression which estimates the ratio of group-specific overall shedding rates (swabs positive out of total swabs collected in one group divided by the other).

Theoretical model

In order to examine the implications of the dichotomization “shedder” on shedding rates and characteristics other than those observed, we modeled HSV-2 episode duration and the period between episodes using the geometric distribution (25). The geometric distribution is the discrete analogue to the exponential distribution used by others (26,27) to describe reactivations. We then constructed the probability of observing at least one episode (any shedding) for a given average episode length (μ) and average non-shedding interval length (λ) during a time period of length L . It is 1 minus the probability that the observation period begins during the interval between episodes multiplied by the probability that no episodes begin within the succeeding $L-1$ days:

$$1 - \left(\frac{\lambda}{\mu + \lambda} \right) * \left(\frac{\lambda - 1}{\lambda} \right)^{L-1}.$$

We computed this probability over a range of values for μ , λ , and L and computed the associated

shedding rate $\left[\frac{\mu}{\mu + \lambda} \right]$. We also computed the ratio of “shedders” and the shedding rate ratio between two hypothetical groups A and B varying numbers of samples and episode characteristics in order to assess their relative values.

Results

Review of HSV shedding studies

We first reviewed studies published between 2005 and 2008 in which HSV-2 detection by PCR was performed, using a Medline search on the keywords “shedding” and “HSV”. We selected studies whose procedures involved repeated measurement of HSV-2 (4,8,11–17). From each relevant paper, we recorded the overall shedding rate (swabs positive out of total swabs collected) and also the proportion of “shedders” (proportion of persons in whom HSV DNA was detected by PCR at *any* time). The probability of *any* HSV detection increased with observation length (Table 1, top portion). For example, 25% of HIV-1 seronegative HSV-2 seropositive persons in Burkina Faso shed HSV at least once over 4 samples (15) while 54% of HIV-1/HSV-2 seropositive persons in Burkina Faso shed HSV at least once over 6 samples (16), despite the overall shedding rate being lower in the latter study (9% and 4%, respectively). The proportion of persons having HSV DNA detection on at least one sample did not correlate with overall shedding rate (Spearman’s $\rho = .10$, $p = 0.78$). The rate of *any* HSV detection was, however, strongly correlated with total number of samples collected (Spearman’s $\rho = 0.70$, $p = .01$). Therefore, the detection of any shedding appeared more closely related to the length of sampling period than to the underlying viral shedding frequency. In some studies we reviewed (Table 1, bottom portion) the rate of any HIV detection was also available, and this varied by the number of samples collected (11,13,16).

Observed shedding characteristics among VRC study participants

Between 1992 and 2002, 308 HSV-2 seropositive participants performed daily home collection of genital swabs for at least 30 days (median 60 days, range 30–174) and were included in these analyses. Of these, 89% were white and 128 (42%) were women. None were on suppressive antiviral therapy for HSV, 156 (51%) were HSV-1 seropositive, and 83 (27%) were HIV-seropositive. Of 19,082 swabs collected, 3664 (19%) were HSV DNA positive by PCR.

We measured characteristics of HSV-2 reactivations or shedding episodes, defined as consecutive periods of HSV detection. We identified 912 episodes (mean duration=3.6 days, median=2.0). The mean and median interval between episodes of HSV-2 reactivation was 9.4 days and 7.0 days, respectively.

Observed shedding rate ratio versus ratio of shedders

We next evaluated demographic and clinical factors that influenced shedding rates from UW VRC study participants. We examined HIV status as a risk factor for increased shedding. The overall swab-specific shedding rate was 24% (1223/5030) among HIV-seropositive individuals and 17% (2441/14052) among HIV-seronegative individuals. Using Poisson regression on all available swabs, the shedding rate for HIV-seropositive persons was 1.39-fold higher than for HIV-negative persons (95% CI for RR: 1.13–1.74, $p = .002$). We then computed the shedding rate ratio and ratio of “shedders” over a range of observation periods (Figure 1a). Both methods were highly variable with less than 10 days of sampling. As the observation interval increased, the shedding rate ratio based on HIV status approached the value 1.39 obtained using all available data (from SRR=1.42 using 10 samples to SRR=1.35 at 50 samples). However, the ratio of shedders (proportion of HIV-seropositive persons with any shedding versus HIV-negative persons) approached 1 (RS=1.13 with 10 samples to RS=1.01 at 50 samples).

Observed dichotomization into high versus low shedding

We also examined the consequences of dichotomizing into “high shedders” versus low or no shedding using the data described above. We selected several cutoffs and defined those above the cutoff as “high shedders”. Figure 1b shows the results following dichotomization at 60% shedding. The shedding rate ratio prior to dichotomization remained 1.39 for HIV seropositivity as described above; however, there were 7.4 times as many “high shedders” among HIV seropositives versus seronegatives over 40 samples and 4.1 times as many “high shedders” among HIV seropositives over 50 samples.

Predicted proportion who shed (classified as “shedders”)

To confirm findings based on observed HSV shedding data, we computed shedding rates and estimates of group differences using expected values under the geometric distribution. Table 2 provides the probability of detecting *any* shedding for a range of average shedding characteristics: s (shedding rate), μ (episode duration) and λ (between-episode interval duration) and observation lengths L . The predicted probability of observing any shedding, or of classifying a participant as a “shedder”, increased with observation length and decreased with increasing episode duration and interval duration. Shown with triple borders are values corresponding to average shedding characteristics of VRC study participants: with a hypothetical shedding rate of 15%, average episode length of 4 days, 35% of participants were predicted to shed over 7 days and 77% over 30 days. These predicted rates matched the observed data in the cohort previously described: 17% (51/308) shed on the first day, 42% (130/308) shed at least once over 7 days and 77% (238/308) shed at least once over 30 days. Predicted rates also matched observed rates for a group of persons sampled four times daily (not shown) (8).

The episode durations and non-shedding interval lengths observed in the participants previously described corresponded well to the geometric distribution with the same mean.

Predicted shedding rate ratio versus ratio of shedders

We computed the shedding rate ratio and the ratio of "shedders" based on the geometric distribution with hypothesized average episode characteristics. For example, if groups A and B shed 10% of the time, but group A has shorter episodes on average than group B (1 day versus 3 days ($\mu_A=1$, $\lambda_A=9$, $\mu_B=3$, $\lambda_B=27$)), then the shedding rate ratio was 1 (10%/10%) but the ratio of shedders over 3, 10 and 20 samples was, respectively, 1.7 (29%/17%), 1.9 (69%/36%) and 1.6 (91%/58%) (Appendix 1). Persons in group A were more likely to be classified as "shedders" over a few samples since *any* shedding was more likely to be detected over an observation period when episodes were short and frequent versus long and rare (example data over a 30-day period is shown in figure 2). In this circumstance, the ratio of shedders created group differences where episode characteristics differ but shedding rate does not.

Predicted group differences with non-episodic viral shedding

We examined ratios of "shedders" when shedding patterns follow the binomial distribution. The probability of any shedding over an interval is shown in Appendix 2. Similar difficulties also occurred in this setting: the probability of being a "shedder" increased with the length of observation interval (Appendix 2), and the ratio of "shedders" in different groups was found to be either larger or smaller than the shedding rate ratio (Appendix 3).

Discussion

Most HSV-2 seropositive persons have genital HSV detected over observation periods lasting 30 days or longer, and the proportion classified as "shedders" increases with the number of samples collected. In fact it is likely that most if not all HSV-2 seropositive persons shed HSV in the genital tract if observed for long enough periods (20). Therefore, the term "shedder" is context specific.

It is appealing to dichotomize individuals into "shedders" and "not shedders" or into "high shedders" versus others since the computation is straightforward and may provide a qualitative distinction between those who shed frequently and those who do not. However, the data presented herein demonstrate the trouble with drawing conclusions based on the quantitative ratio of "shedders" or "high shedders". Real data examples using HIV status and HSV shedding show that dichotomization can cause group differences to appear larger or smaller than shedding rate ratios, depending on observation length. Theoretical examples also confirm these differences.

Conclusions based on dichotomization of the data may be misleading, particularly for studies that attempt to determine risk factors for viral transmission to others. The dichotomization may provide an arbitrary and context-dependent distinction between groups when defining risk factors for shedding; and it may not be predictive of disease status or infectiousness. A better summary measure is overall shedding rate: number of positive samples out of all swabs taken on persons in that group. Even the shortest observation intervals provide accurate information on shedding rate, though more samples increase precision. Group differences can then be described using Poisson regression. And zero-inflated models can be of use when most persons are not observed to shed (28,29).

Comparisons among "shedders" do not currently appear to be appropriate in any context as their value depends on the length of observation. However, some authors have provided group comparisons based both on "shedders" and the shedding rate ratio (11,16,17,30,31) and some

of the findings are discrepant. For example, in a crossover design with 6 samples per arm, Nagot (16) found a significant decrease in the rate of genital HIV-1 RNA detection while on valacyclovir using Poisson regression to compute rate ratios ($RR=.77$, $p=.006$) but found no effect of treatment using the ratio of shedders ($RS=.93$, $p=.30$). Mayaud (30) found a difference in HSV-2 shedding rate by HIV-1 serostatus and HAART use ($p<.001$) but no difference in the rates of “shedders” over 12 visits between these groups ($p=.17$). Cowan (11) found a 40% reduction in HSV-2 shedding with acyclovir using the ratio of shedders over 13 samples ($p=.004$) and a 76% reduction ($p<.001$) using the shedding rate ratio. Thus the difference in inference by method of measurement indicates that comparisons based on “shedders” may not be reliable.

Limitations of our methods include an imperfect ability to distinguish overlapping reactivations from the ganglia within a single shedding episode (27). Recent work has shown that frequent sampling allows identification of more distinct episodes (8). However, our purpose was to assess shedding frequency rather than reactivation duration. Further, we did not consider subject-level variability in episode characteristics. However, we anticipate the impact of this additional consideration to be negligible.

Dichotomization at a pre-determined cutoff, whether that cutoff is *any shedding* or some fixed rate, can result in non-differential misclassification and subsequent bias since those with lowest shedding rates or longest episode lengths are most likely to be classified as non- or low-shedders. Others have warned of the potential for bias in related contexts. Copeland demonstrated that non-differential misclassification can attenuate risk ratio estimates while differential misclassification can attenuate or exaggerate it (32). Irwig et al. showed the potential for attenuation of exposure-response relationship when dichotomizing a continuous outcome at cutoffs related to the observed standard error (33). We have found similar bias for cutoffs determined a priori in both episodic and non-episodic shedding patterns. Based on this evidence, dichotomization of shedding is discouraged and group comparisons based on proportion of “shedders” in each group are considered unreliable. Computation of overall shedding rates and group comparisons using Poisson regression is recommended.

Key messages

- Most HSV-2 seropositives persons are expected to shed HSV from the genital mucosa.
- Dichotomization of persons into “shedders” versus “nonshedders” over repeated samples leads to inference that varies by the number of samples collected.
- Poisson regression is recommended as it can accurately determine group differences in shedding rates regardless of the length of the sampling interval.

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Appendix

Appendix

1. Anti-conservative bias in ratio of shedders based on episodic shedding

For two groups designated by A and B, the ratio is shedders in group A versus B is

$$\left[1 - \left(\frac{\lambda_A}{\mu_A + \lambda_A} \right) * \left(\frac{\lambda_A - 1}{\lambda_A} \right)^{L-1} \right] / \left[1 - \left(\frac{\lambda_B}{\mu_B + \lambda_B} \right) * \left(\frac{\lambda_B - 1}{\lambda_B} \right)^{L-1} \right] \quad (\text{A1.1}), \text{ while the shedding rate}$$

ratio is $\left[\frac{\mu_A}{\mu_A + \lambda_A} \right] / \left[\frac{\mu_B}{\mu_B + \lambda_B} \right]$ (A1.2). If $(\text{A1.1}) > (\text{A1.2}) \geq 1$, then the ratio of shedders will be anti-conservatively biased relative to the shedding rate ratio. This can occur when $\mu_A < \mu_B$ and

$$\lambda_A \leq \lambda_B \left[\frac{\mu_A}{\mu_B} \right]. \text{ Similarly, If } (\text{A1.1}) < (\text{A1.2}) \leq 1, \text{ then the ratio of shedders will be anti-}$$

conservatively biased. This can occur when $\mu_A < \mu_B$ and $\lambda_A \geq \lambda_B \left[\frac{\mu_A}{\mu_B} \right]$.

2. Probability of observing any non-episodic shedding

If shedding is uncorrelated from sample to sample within individuals, then the probability of shedding is binomially distributed. The beta-binomial is a common way of describing variability in rates, assuming a variety of underlying person-level shedding rates described by a prior beta distribution (34). If individual shedding rates p_i are distributed beta with shape

parameters α and β such that $E(p_i) = \frac{\alpha}{\alpha + \beta}$, then the probability of observing any shedding in a given individual from this population over L days is:

$$\int_0^1 \left[1 - (1 - p_i)^L \right] * \left[\frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} * p_i^{\alpha-1} * (1 - p_i)^{\beta-1} \right] dp_i = 1 - \left[\frac{\Gamma(\alpha + \beta) * \Gamma(L + \beta)}{\Gamma(\beta) \Gamma(L + \alpha + \beta)} \right]. \text{ Since the}$$

limit of $\lim_{L \rightarrow \infty} \left[\frac{\Gamma(L + \beta)}{\Gamma(L + \alpha + \beta)} \right] = 0$, then the probability of detecting any shedding also approaches 1 as the observation interval increases.

3. Bias in ratio of shedders based on non-episodic shedding

The ratio of shedders based on the beta-binomial distribution described above can be expressed for group A (distributed beta with shape parameters α and β) versus B (beta with shape

parameters γ and δ) as: $\left[1 - \left[\frac{\Gamma(\alpha + \beta) * \Gamma(L + \beta)}{\Gamma(\beta) \Gamma(L + \alpha + \beta)} \right] \right] / \left[1 - \left[\frac{\Gamma(\gamma + \delta) * \Gamma(L + \delta)}{\Gamma(\delta) \Gamma(L + \gamma + \delta)} \right] \right]$, while the

shedding rate ratio has the expected value $\frac{\alpha}{\alpha + \beta} / \frac{\gamma}{\gamma + \delta}$. One can easily construct cases where the ratio of shedders differs from the shedding rate ratio. If $L=5$, and $(\alpha=3, \beta=3, \gamma=1, \delta=1)$, then the ratio of shedders ($1.1 = 92\%/83\%$) for group A versus B exceeds the shedding rate ratio ($1.0 = 50\%/50\%$). If $(\alpha=2, \beta=1, \gamma=1, \delta=2)$, then the ratio of shedders ($1.3 = 95\%/71\%$) is smaller than the shedding rate ratio ($2.0 = 67\%/33\%$).

References

1. Wald A, Huang ML, Carrell D, Selke S, Corey L. Polymerase chain reaction for detection of herpes simplex virus (HSV) DNA on mucosal surfaces: comparison with HSV isolation in cell culture. J Infect Dis 2003 Nov 1;188(9):1345–1351. [PubMed: 14593592]

2. Wald A, Zeh J, Selke S, Ashley RL, Corey L. Virologic characteristics of subclinical and symptomatic genital herpes infections. *N Engl J Med* 1995 Sep 21;333(12):770–775. [PubMed: 7643884]
3. Brown ZA, Wald A, Morrow RA, Selke S, Zeh J, Corey L. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. *JAMA* 2003 Jan 8;289(2):203–209. [PubMed: 12517231]
4. Andrews WW, Kimberlin DF, Whitley R, Cliver S, Ramsey PS, Deeter R. Valacyclovir therapy to reduce recurrent genital herpes in pregnant women. *Am J Obstet Gynecol* 2006 Mar;194(3):774–781. [PubMed: 16522412]
5. Corey L, Wald A, Patel R, Sacks SL, Tying SK, Warren T, et al. Once-daily valacyclovir to reduce the risk of transmission of genital herpes. *N Engl J Med* 2004 Jan 1;350(1):11–20. [PubMed: 14702423]
6. Diaz-Mitoma F, Sibbald RG, Shafran SD, Boon R, Saltzman RL. Oral famciclovir for the suppression of recurrent genital herpes: a randomized controlled trial. Collaborative Famciclovir Genital Herpes Research Group. *JAMA* 1998 Sep 9;280(10):887–892. [PubMed: 9739972]
7. Gupta R, Wald A, Krantz E, Selke S, Warren T, Vargas-Cortes M, et al. Valacyclovir and acyclovir for suppression of shedding of herpes simplex virus in the genital tract. *J Infect Dis* 2004 Oct 15;190(8):1374–1381. [PubMed: 15378428]
8. Mark KE, Wald A, Magaret AS, Selke S, Olin L, Huang ML, et al. Rapidly cleared episodes of herpes simplex virus reactivation in immunocompetent adults. *J Infect Dis* 2008 Oct 15;198(8):1141–1149. [PubMed: 18783315]
9. Koelle DM, Benedetti J, Langenberg A, Corey L. Asymptomatic reactivation of herpes simplex virus in women after the first episode of genital herpes. *Ann Intern Med* 1992 Mar 15;116(6):433–437. [PubMed: 1310837]
10. Schacker T, Zeh J, Hu HL, Hill E, Corey L. Frequency of symptomatic and asymptomatic herpes simplex virus type 2 reactivations among human immunodeficiency virus-infected men. *J Infect Dis* 1998 Dec;178(6):1616–1622. [PubMed: 9815213]
11. Cowan FM, Pascoe S, Barlow K, Langhaug L, Jaffar S, Hargrove J, et al. A randomised placebo controlled trial to explore the effect of suppressive therapy with acyclovir on genital shedding of HIV-1 and herpes simplex virus type 2 among Zimbabwean sex workers. *Sex Transm Infect.* 2008 Aug 6;
12. de Bruyn G, Vargas-Cortez M, Warren T, Tying SK, Fife KH, Lalezari J, et al. A randomized controlled trial of a replication defective (gH deletion) herpes simplex virus vaccine for the treatment of recurrent genital herpes among immunocompetent subjects. *Vaccine* 2006 Feb 13;24(7):914–920. [PubMed: 16213066]
13. Dunne EF, Whitehead S, Sternberg M, Thepamnuay S, Leelawiwat W, McNicholl JM, et al. Suppressive acyclovir therapy reduces HIV cervicovaginal shedding in HIV-and HSV-2-infected women, Chiang Rai, Thailand. *J Acquir Immune Defic Syndr* 2008 Sep 1;49(1):77–83. [PubMed: 18667923]
14. Kim HN, Meier A, Huang ML, Kuntz S, Selke S, Celum C, et al. Oral herpes simplex virus type 2 reactivation in HIV-positive and -negative men. *J Infect Dis* 2006 Aug 15;194(4):420–427. [PubMed: 16845624]
15. Nagot N, Foulongne V, Becquart P, Mayaud P, Konate I, Ouedraogo A, et al. Longitudinal assessment of HIV-1 and HSV-2 shedding in the genital tract of West African women. *J Acquir Immune Defic Syndr* 2005 Aug 15;39(5):632–634. [PubMed: 16044019]
16. Nagot N, Ouedraogo A, Foulongne V, Konate I, Weiss HA, Vergne L, et al. Reduction of HIV-1 RNA levels with therapy to suppress herpes simplex virus. *N Engl J Med* 2007 Feb 22;356(8):790–799. [PubMed: 17314338]
17. Sperling RS, Fife KH, Warren TJ, Dix LP, Brennan CA. The effect of daily valacyclovir suppression on herpes simplex virus type 2 viral shedding in HSV-2 seropositive subjects without a history of genital herpes. *Sex Transm Dis* 2008 Mar;35(3):286–290. [PubMed: 18157071]
18. Krone MR, Tabet SR, Paradise M, Wald A, Corey L, Celum CL. Herpes simplex virus shedding among human immunodeficiency virus-negative men who have sex with men: site and frequency of shedding. *J Infect Dis* 1998 Oct;178(4):978–982. [PubMed: 9806024]

19. Posavad CM, Wald A, Kuntz S, Huang ML, Selke S, Krantz E, et al. Frequent reactivation of herpes simplex virus among HIV-1-infected patients treated with highly active antiretroviral therapy. *J Infect Dis* 2004 Aug 15;190(4):693–696. [PubMed: 15272395]
20. Wald A, Zeh J, Selke S, Warren T, Ryncarz AJ, Ashley R, et al. Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. *N Engl J Med* 2000 Mar 23;342(12):844–850. [PubMed: 10727588]
21. Ashley RL, Militoni J, Lee F, Nahmias A, Corey L. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. *J Clin Microbiol* 1988 Apr;26(4):662–667. [PubMed: 2835389]
22. Magaret AS, Wald A, Huang ML, Selke S, Corey L. Optimizing PCR positivity criterion for detection of herpes simplex virus DNA on skin and mucosa. *J Clin Microbiol* 2007 May;45(5):1618–1620. [PubMed: 17329447]
23. Ryncarz AJ, Goddard J, Wald A, Huang ML, Roizman B, Corey L. Development of a high-throughput quantitative assay for detecting herpes simplex virus DNA in clinical samples. *J Clin Microbiol* 1999 Jun;37(6):1941–1947. [PubMed: 10325351]
24. Diggle, PJ.; Heagerty, PJ.; Liang, KY.; Zeger, SL. Analysis of longitudinal data. Vol. 2nd ed.. Oxford: Oxford University Press; 2002.
25. Hogg, RV.; Craig, AT. Introduction to Mathematical Statistics. Vol. 5th ed.. London: Prentice-Hall; 1995.
26. Blower S, Wald A, Gershengorn H, Wang F, Corey L. Targeting virological core groups: a new paradigm for controlling herpes simplex virus type 2 epidemics. *J Infect Dis* 2004 Nov 1;190(9):1610–1617. [PubMed: 15478066]
27. Crespi CM, Cumberland WG, Wald A, Corey L, Blower S. Longitudinal study of herpes simplex virus type 2 infection using viral dynamic modelling. *Sex Transm Infect* 2007 Oct;83(5):359–364. [PubMed: 17475687]
28. Hall DB. Zero-inflated Poisson and binomial regression with random effects: A case study. *Biometrics* 2000;56:1030–1039. [PubMed: 11129458]
29. Hall DB, Zhengang Z. Marginal models for zero inflated clustered data. *Statistical Modelling* 2004;4:161–180.
30. Mayaud P, Nagot N, Konate I, Ouedraogo A, Weiss HA, Foulongne V, et al. Effect of HIV-1 and antiretroviral therapy on herpes simplex virus type 2: a prospective study in African women. *Sex Transm Infect* 2008 Oct;84(5):332–337. [PubMed: 18596069]
31. Nagot N, Ouedraogo A, Konate I, Weiss HA, Foulongne V, Defer MC, et al. Roles of clinical and subclinical reactivated herpes simplex virus type 2 infection and human immunodeficiency virus type 1 (HIV-1)-induced immunosuppression on genital and plasma HIV-1 levels. *J Infect Dis* 2008 Jul 15;198(2):241–249. [PubMed: 18593294]
32. Copeland KT, Checkoway H. Bias due to misclassification in the estimation of relative risk. *Am J Epidemiol* 1977;105(5):488–495. [PubMed: 871121]
33. Irwig LM, Hennie TG, Simpson JM. Correcting for measurement error in an exposure-response relationship based on dichotomising a continuous dependent variable. *Australian Journal of Statistics* 1990;32(3):261–269.
34. Lehmann, EL.; Casella, G. Theory of point estimation. Vol. 2nd ed.. New York: Springer-Verlag; 1998.

Figure 1a.

Figure 1b.

Figure 1.

Figure 1a. Ratios indicating impact of HIV seropositivity on HSV shedding: measured using either ratio of "sheddters" (proportion having any shedding over the interval) or shedding rate ratio (number of swabs with detectable HSV DNA out of samples obtained). The dashed line indicates risk ratio for shedding based on Poisson regression (1.39) and the solid line at 1 indicates no difference.

Figure 1b. Ratios indicating impact of HIV seropositivity on HSV shedding: measured using either ratio of those having a shedding rate above 60% in each group (HIV seropositive persons versus HIV negative) or shedding rate ratio (number of swabs with detectable HSV DNA out

of samples obtained). The dashed line indicates risk ratio for shedding based on Poisson regression (1.39) and the solid line at 1 indicates no difference.

Case A: 10% shedding rate as three 1-day episodes in 30 days

Case B: 10% shedding rate as one 3-day episode in 30 days

Figure 2.

Classification rates of "shedders" depends on episode duration: 2 hypothetical cases are shown in which shedding occurs on exactly 3 of 30 days (10%). Viral shedding on each day is indicated by vertical gray bars, solid horizontal lines indicate detection of *any* shedding over the corresponding 10-day period and dashed lines indicate no shedding was detected. *Any* shedding is detected on a 18 of 21 intervals when episodes last a single day and on only 12 of 21 intervals when episodes last 3 days.

HSV or HIV viral detection for several recent studies both as overall shedding rate (total samples on which shedding was detected in any subject divided by total samples on all subjects) and as percent classified as "shedders" (percent of persons with any HSV or HIV detected over all samples). Also included are study characteristics and method and frequency of sampling. The top portion shows studies in which HSV DNA was measured via PCR for subjects *not* on antiviral medications. The bottom portion shows studies in which HIV RNA was measured by PCR in subjects *not* on antiretroviral medications.

Table 1

First author (citation)	Study Site	N	% female	% HSV-2+/HIV-1+	HIV-1 status	Sample collection method	Frequency of sampling	Overall HSV shedding rate	% HSV shedders
Andrews (4)	US	55	100%	100% / unk	not tested but HIV+ excluded	perineum and cervical sample	Single swab within 7 days of birth	11%	11%
Dunne (13)	Thailand	67	100%	100% / unk	positive	cervical lavage	3 times total	unk	42%
Nagot (15)	Burkina Faso	20	100%	100% / unk	negative	cervical lavage	Weekly, 4 times total	9%	25%
Nagot (15)	Burkina Faso	22	100%	100% / unk	positive	cervical lavage	Weekly, 4 times total	21%	32%
Nagot (16)	Burkina Faso	68	100%	100% / unk	positive	cervical lavage	Every 2 weeks, 6 times total	4%	54%
Cowan (11)	Zimbabwe	56	100%	100% / unk	positive	genital swab	Weekly, 12 times total	23%	65%
Sperling (17)	US	73	75%	100% / 56%	not tested	genital swab	Daily, 60 days	5%	46%
DeBruyn (12)	US	18	58%	100% / 100%	negative	genital swab	Daily, 60 days	16%	78%
Kim (14)	US	32	0%	100% / 100%	negative	oral swab	Daily, median 64 days (range, 30–127)	4%	50%
Kim (14)	US	59	0%	100% / 54%	negative	genital swab	Daily, median 64 days (range, 30–127)	12%	66%
Mark (8)	US	25	60%	100% / 48%	negative	genital swab	4× per day, median 62 days (range 5–74)	14%	84%
Mark (8)	US	18	33%	39% / 100%	negative	oral swab	4× per day, median 62 days (range 5–74)	7%	83%
First author (citation)	Study Site	N	% female	% HSV-2+/HIV-1+	HIV-1 status	Sample collection method	Frequency of sampling	Overall HSV shedding rate	% HSV shedders
Dunne (13)	Thailand	67	100%	100% / unk	Positive	cervical lavage	3 times total	60%*	80%
Nagot (16)	Burkina Faso	136	100%	100% / unk	positive	cervical lavage	Every 2 weeks, 6 times total	54%	85%
Cowan (11)	Zimbabwe	56	100%	100% / unk	Positive	genital swab	Weekly, 12 times total	63%	85%

* Approximated from figure. "unk" indicates measures which were not reported for that study.

Table 2
Predicted percent of observation periods in which shedding is observed over varying shedding rates (s), reactivation durations (μ) and non-shedding interval lengths (λ).

Percent of observation periods in which shedding is observed															
Shedding		L = Number of consecutive samples of observation													
s	μ	λ	1	2	7	14	21	30	60	90	120				
5%	1	19	5	10	31	53	68	80	96	99	100				
5%	2	38	5	8	19	33	44	56	80	91	96				
5%	4	76	5	6	12	20	27	35	57	71	80				
5%	8	152	5	6	9	13	17	22	36	47	57				
5%	16	304	5	5	7	9	11	14	22	29	36				
10%	1	9	10	20	56	81	91	97	100	100	100				
10%	2	18	10	15	36	57	71	83	97	99	100				
10%	4	36	10	12	24	38	49	60	83	93	97				
10%	8	72	10	11	17	25	32	40	61	74	83				
10%	16	144	10	11	14	18	22	26	40	52	61				
15%	1	6	15	30	73	93	98	100	100	100	100				
15%	2	11	15	23	51	74	87	94	100	100	100				
15%	4	23	15	19	35	53	66	77	94	98	100				
15%	8	45	15	17	26	36	46	55	77	88	94				
15%	16	91	15	16	20	26	32	38	56	68	77				
20%	1	4	20	40	86	98	100	100	100	100	100				
20%	2	8	20	30	64	86	94	98	100	100	100				
20%	4	16	20	25	46	65	78	88	98	100	100				
20%	8	32	20	22	34	47	58	68	88	95	98				
20%	16	64	20	21	27	35	42	49	68	80	88				
30%	1	2	30	60	98	100	100	100	100	100	100				
30%	2	5	30	45	84	97	99	100	100	100	100				
30%	4	9	30	37	65	84	93	97	100	100	100				
30%	8	19	30	34	50	66	77	86	97	99	100				
30%	16	37	30	32	41	51	59	68	86	94	97				

Gray shading shows where the predicted proportion is above 80%. Boxes triply outlined indicate rates of detection such as might be found in the VRC population where shedding is about 15% and the average reactivation duration is 4 days.