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Efficacy of Listerine® Antiseptic in reducing viral contamination of saliva

Timothy F. Meiller[1,2], Arley Silva[1,2], Sonia M. Ferreira[1,2], Mary Ann Jabra-Rizk[1], Jacqueline I. Kelley[1] and Louis G. DePaola[1]

[1]Department of Diagnostic Sciences and Pathology, Dental School, University of Maryland, Baltimore, MD, USA; [2]Institute of Human Virology, University of Maryland, Baltimore, MD, USA

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Abstract

Aim: The anti-viral efficacy of oral antimicrobial rinses has not been adequately studied in terms of potential clinical significance. As a follow-up to an in vitro study on the effect of oral antiseptics on *Herpes simplex* virus, Type 1, this study was undertaken to evaluate the in vivo effect of an essential oil containing oral antiseptic on the reduction of viral titer in saliva during active viral infection.

Method: Patients were recruited and evaluated in a single visit protocol at the onset of a perioral outbreak, consistent historically and clinically with recurrent *Herpes* labialis. Direct immunofluorescence of cytological smears of the lesions/oral fluids was used to confirm *Herpes simplex* virus types I or II. Patients were randomly assigned to one of two treatment groups: (1) active ingredient and (2) sterile water control. The viral lesion was evaluated as to clinical stage according to standard protocol. Salivary fluid samples were taken: (1) at baseline; (2) immediately following a 30 s rinse; (3) 30 min. after the 30 s rinse; and (4) on the repeat trial, also at 60 min. after the 30 s rinse. All samples were evaluated for viral titer and results compared.

Results: In Trial 1, the sample population consisted of 19 males and 21 females with an average age of 29.2 and in Trial 2, 21 males, 19 females with an average age of 28. In both Trials 1 and 2, recoverable infectious virions were reduced to zero after a 30 s experimental rinse; whereas, the control rinse resulted in a non-significant ($p > 0.05$) reduction. The experimental group also demonstrated a continued significant ($p < 0.05$) reduction 30 min. post rinse when compared with baseline while the control group returned to baseline levels. In Trial 2, the 60 min. post rinse follow-up demonstrated a 1–2 log residual reduction from baseline in the experimental group; however, this was not significant.

Conclusions: There is clinical efficacy in utilizing an oral rinse with the antimicrobial agent Listerine® Antiseptic in reducing the presence of viral contamination in oral fluids for at least 30 min. after oral rinse. The risk of viral cross contamination generated from these oral fluids in person to person contact or during dental treatment may be reduced.

Key words: anti-microbial oral rinse; essential oils; perioral recurrent *Herpes simplex* virus; saliva

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There are numerous sources of microbial contamination in the dental operatory. Dental unit water dispersed by three-way syringes, water coolant for ultrasonic instruments and aerosols from a patient's oral fluids produced by high-speed handpieces, represent initial sources of potentially infectious agents to the operatory. There are various methods currently being developed and in place for reduction of these sources of microbial contamination. Standard autoclaving procedures and ''standard precautions'' should preclude the transmission of potentially infectious microbial agents from these sources to the patient or to other individuals within the dental operatory. Concerning patients themselves, oral microorganisms, including numerous viral and bacterial pathogens, can potentially be mobilized into the bioaerosal by way of the high-speed manipulations of air and water in handpiece activity. This source of cross contamination has not been adequately studied to date. The anti-microbial efficacy of

oral rinses, although assumed in many instances, has also not been adequately studied in terms of potential clinical significance to reduce or eliminate this risk of such cross contamination.

Most studies have been completed on bacterial pathogens leaving the in vivo anti-viral effectiveness of commercial mouthrinses less well documented. This project was undertaken to evaluate the efficacy of Listerine® Antiseptic (LA, Morris Plains, NJ, USA) mouthrinse *versus* a sterile water control in the reduction of infectious viral levels in saliva during an active viral infection. The human *Herpes simplex* virus (HSV-1 or -2) was selected as the test virus in order to establish a model for showing reduction of infectious virions in saliva following a 30 s rinse as recommended by the manufacturer with an antiseptic mouthrinse containing essential oils. It is well established that viral shedding and hence recovery occurs at variable levels in human saliva from the prodromal period through at least the full vesicular stages of recurrent herpetic outbreaks (Arvin & Prober 1999).

This is primarily because of variations in clinical protocol in determining the stage of the lesion and in recovery techniques, which have not been standardized in every study. In previous work in our laboratories, five stages of clinical variation have been defined, see Table 1. Other researchers have determined that early recurrent *Herpes* labialis lesions (Stage 1 and 2 in our clinical evaluation) represent the time when the greatest viral shedding will likely be occurring (Arvin & Prober 1999). Since Stage 3 is a variable period with some new vesicle formation potentially still occurring, the degree to which infectious virions can be recovered from saliva or from lesions themselves is also variable. This study was undertaken to determine if, in patients known to suffer with *Herpes*, a reduction in salivary viral titer might be accomplished by an oral rinse during clinical Stages 1–3.

Table 1. The five stages of clinical variation during a *Herpes* outbreak

Stage	Perioral tissue sensation and visual display
1	Lesions are not visibly present; patients report as much as a 24–48 h period of sensation, usually including tingling and sometimes burning or itching. Mild erythema may be present at the site of usual recurrence. Clinicians usually call this period the prodrome.
2	Increasing edema and redness is generally present at the site that the patient suffers normal recurrences with vesicle formation. These vesicles generally continue to form for a variable period of time, usually 2–5 days and, as the vesicles continue to develop older areas will begin to burst.
3	A combination of vesicles and crusted areas with an exudate usually resembling serum is present.
4	New vesicles do not appear. However, the lesions are crusted and often coalesced into a larger scabbed area.
5	The last scabbed area will be sloughed and the tissue begins to return to normal with only minor erythema left.

Material and Methods

Two complimentary trials testing the efficacy of LA were completed. In each trial, 40 adult patients, age 18–65 years, and known by history to suffer with viral recurrences, were recruited and advised to report to our clinical facility at the first sign of prodrome, or if a perioral viral lesion occurred. The advertisement stated that the patient should have an established history of viral recurrences consistent with *Herpes*. The clinicians examining the patients and referring to the clinical site utilized standard criteria for determining the likelihood of a herpetic infection. Since the study design was a one-visit protocol, testing of the virus to determine its consistency with HSV-1 or -2 by direct immunofluorescence (DIF) microscopy was performed at the first outbreak and used to determine if further analysis of samples was warranted.

For each of the two trials, following informed consent, each patient was examined, a medical history was obtained and soft-tissue examination was performed. Patients were randomly assigned to either the experimental or the control group until a 20 patient sample in each group was met. The viral lesion, usually on the patient's perioral tissues, was staged according to standard procedures (see Table 1).

Following examination and the determination that the patient's history corresponded with clear evidence of likely recurrent *Herpes* labialis, a sterile swab was rolled against the labial tissue adjacent to the area of the lesion. In instances where the lesion was Stage 3 with evidence of vesicular rupture, the swab touched this area as well. The swab was then immediately rolled onto a slide and transported to the laboratory for DIF viral identification. In addition, a second sample was obtained from the patient and consisted of 1–2 ml of pooled saliva. The sample was collected in a sterile 15 ml centrifuge tube. The samples were immediately transported to the laboratory. The volume was determined and the sample was aliquoted for later testing.

The general procedure included sampling for viral identification at baseline, followed by the patients being instructed to rinse for 30 s with either 20 cm^3 of the experimental mouthrinse (Cool Mint LA), or in the case of the control patients with 20 cm^3 of sterile distilled water. Following the 30 s rinse, the patient expectorated and then a second sample of saliva was collected 30 s post rinse. The patient was then instructed to wait for 30 min. and a third sample of pooled saliva was collected. The second trial, carried out on 40 additional patients, was identical to the first trial with the exception that a fourth oral sample was taken 60 min. post rinse. All samples were transported to the laboratory for testing.

DIF for identification of HSV-1

While complement fixation, haemagglutination and other immunological tests have been developed to assist in the diagnosis of active herpetic infections, the fluorescent antibody test also called the DIF antibody test, for HSV is generally considered to be an extremely reliable assay available for the detection of viral antigens in patient samples (Arvin & Prober 1999). The approach is more sensitive and specific than the visualization of TZANCK cells and a specific diagnosis can be made earlier in the infection (Arvin & Prober 1999). In this test, the smears taken and applied to clinical laboratory slides, after fixation in 95% ethanol, were stained with a 1–2000 dilution in phosphate buffered saline (PBS) of monoclonal antibodies to HSV-1or HSV-2 (DAKO Corp, Carpinteria, CA, USA) that had been

conjugated with fluorescein. Staining was for 20 min. in a moist chamber at room temperature. The viral antigen was evaluated for evidence of the HSV-1 or -2 antigens following several washings with PBS using fluorescence microscopy.

Cell culture systems and stock viral cultures

Primary African monkey kidney cell cultures (Vero) are excellent indicator cells for evaluation of cytopathic effects (CPE) for HSV (Crane et al. 1980). Vero cells (American Type Culture Collection, ATCC) were grown in monolayer in 75 cm^2 plastic tissue culture flasks (Corning, NY) in RPMI-1640 complete medium (RPMI-1640 CM) with the addition of the antibiotics penicillin G sodium (100 U/ml) and streptomycin sulfate (100 µg/ml) and supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), HEPES buffer (10 mM) and MEM sodium pyruvate (1 mM). Cells were incubated at 37°C in a humidified atmosphere consisting of 5% CO_2. As a positive control of the test system, Vero cells were inoculated with 1×10^6/ml of HSV-1 McIntyre strain (ATCC) (titer previously determined by standard methods). In the case of the experimental patient samples, the Vero cells were allowed to adsorb with 1 ml of 10-fold serial dilutions of the sample (10^{-1}–10^{-7}) for 1 h at 37°C with gentle rocking and supernatant fluids were then discarded.

Estimation of HSV-1 concentration using plaque assay

A standardized procedure was used for the plaque assays using Vero cells. Three-day grown monolayers of Vero cells, in triplicate on Falcon Petri dishes (60 × 15 mm) were overlayed with either 1 ml of the dilutions (10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}) of the HSV-1 McIntyre strain aliquot to be titrated or the diluted salivary samples from the patients. A Petri dish with a 3-day monolayer of uninfected Vero cells was included as a negative control. Following adsorption and aspiration of supernatant fluids, the plates were overlayed with RPMI-1640 CM (3 × concentration) in 1% methyl cellulose in a 1:2 v/v solution. The plates were incubated undisturbed at 37°C with 5% CO_2 and examined daily for 5 days. Supernatant fluids were aspirated from the plates at the end of 5 days and plaques were counted as clear zones within the monolayer of cells and expressed as plaque forming units per ml (PFU/ml).

Statistical analysis

Statistical analyses were performed on the data using a paired Student's *t* test to compare differences between the control and experimental groups at the pre-treatment, post treatment, 30 min. post treatment and, in the case of trial 2, 60 min. post treatment time periods. A *p*-value of less than 0.05 was considered significant.

Results

Ninety-two patients demonstrating clinical evidence of recurrent *Herpes* labialis and/or reporting a history consistent with recurrent herpetic perioral lesions were evaluated. In Trial 1, four of these patients and in Trial 2, eight of these patients were non-reactive by DIF and, therefore, no further evaluation of their samples was performed. In Trial 1, the sample population consisted of 19 males and 21 females with an average age of 29.2 years. Lesions were clinically evaluated according the staging scale described in Tables 1 and 2. Twenty-five of the patients demonstrated lesions consistent with the Stage 2, seven of the patients were in Stage 3 with some early rupture of vesicles and eight of the

Table 2. Trial #1 in vivo efficacy of LA against *Herpes simplex* virus *(PFU/ml)*

Pt #	Sex	Age	Stage	Pre	Post	30 min. post	DIF
1	F	24	3	1.6 × 10 (3)	0	1.0 × 10 (1)	HSV-1
2	F	36	2	3.8 × 10 (5)	0	6.7 × 10 (2)	HSV-1
3	M	27	1	6.1 × 10 (4)	0	5.8 × 10 (1)	HSV-1
4	F	41	1	4.0 × 10 (5)	0	0	HSV-1
5	M	29	2	5.4 × 10 (4)	0	1.6 × 10 (1)	HSV-1
6	M	21	2	7.1 × 10 (5)	0	8.9 × 10 (2)	HSV-1
7	F	29	2	9.0 × 10 (5)	1.0 × 10 (1)	5.7 × 10 (1)	HSV-1
8	F	40	3	4.7 × 10 (4)	0	0	HSV-1
9	M	38	2	9.8 × 10 (4)	0	0	HSV-1
10	M	32	1	5.1 × 10 (6)	3.7 × 10 (1)	3.6 × 10 (2)	HSV-1/2
11	F	20	3	8.7 × 10 (5)	0	0	HSV-1
12	M	28	3	4.6 × 10 (3)	0	0	HSV-1
13	M	34	1	7.4 × 10 (4)	0	0	HSV-1
14	F	29	2	2.6 × 10 (4)	0	0	HSV-1
15	M	44	2	3.7 × 10 (4)	0	1.9 × 10 (1)	HSV-1
16	F	21	2	6.1 × 10 (5)	0	8.4 × 10 (1)	HSV-1
17	F	20	1	1.8 × 10 (5)	0	0	HSV-1
18	M	39	2	3.1 × 10 (3)	0	0	HSV-1
19	M	22	2	4.6 × 10 (5)	0	2.6 × 10 (1)	HSV-1
20	F	35	2	5.5 × 10 (6)	0	1.0 × 10 (2)	HSV-1
21	F	26	1	2.2 × 10 (5)	7.4 × 10 (3)	9.4 × 10 (4)	HSV-1
22	M	38	2	1.9 × 10 (4)	1.0 × 10 (4)	1.0 × 10 (4)	HSV-1
23	M	33	2	2.7 × 10 (4)	9.1 × 10 (3)	3.1 × 10 (5)	HSV-1
24	M	25	1	8.6 × 10 (4)	6.4 × 10 (3)	4.6 × 10 (3)	HSV-1
25	F	44	2	7.4 × 10 (5)	6.1 × 10 (4)	9.1 × 10 (4)	HSV-1
26	M	23	3	6.8 × 10 (4)	7.8 × 10 (4)	5.4 × 10 (4)	HSV-1
27	M	37	3	6.8 × 10 (4)	7.8 × 10 (4)	5.4 × 10 (4)	HSV-1
28	F	29	2	7.8 × 10 (5)	3.7 × 10 (4)	8.1 × 10 (4)	HSV-1
29	F	25	2	6.1 × 10 (5)	2.9 × 10 (4)	5.1 × 10 (5)	HSV-1/2
30	F	30	2	1.8 × 10 (6)	1.0 × 10 (6)	1.0 × 10 (6)	HSV-1
31	F	23	2	1.7 × 10 (5)	9.1 × 10 (4)	7.0 × 10 (5)	HSV-1
32	M	24	2	2.9 × 10 (4)	8.4 × 10 (4)	3.0 × 10 (4)	HSV-1
33	F	32	2	3.8 × 10 (4)	2.6 × 10 (4)	6.4 × 10 (4)	HSV-1
34	F	19	1	9.6 × 10 (5)	3.1 × 10 (4)	5.0 × 10 (5)	HSV-1
35	M	28	3	4.1 × 10 (4)	3.7 × 10 (4)	1.9 × 10 (4)	HSV-1
36	M	20	2	3.7 × 10 (5)	7.8 × 10 (4)	9.0 × 10 (4)	HSV-1
37	F	31	2	6.7 × 10 (5)	5.5 × 10 (4)	4.1 × 10 (5)	HSV-1
38	F	18	2	4.0 × 10 (4)	2.8 × 10 (4)	4.9 × 10 (4)	HSV-1
39	F	26	2	6.8 × 10 (5)	1.2 × 10 (4)	6.1 × 10 (5)	HSV-1
40	M	23	2	7.3 × 10 (5)	6.0 × 10 (4)	9.8 × 10 (4)	HSV-1

DIF, direct immunofluorescence assay; LA, Listerine® Antiseptic; HSV, *Herpes simplex* virus; M, male; F, female; PFU, plaque forming units.

patients demonstrated mild erythema only with evidence of prodrome (Stage 1). The patients were randomly assigned to either the control or the experimental group and samples were taken.

The results of Trial 1 experimentation are illustrated in Table 2 and Fig. 2. Thirty-eight of the patients, by DIF, reacted with HSV-1 monoclonal antibodies; two of the patients reacted with HSV-1 and -2, a male and a female patient. A clinical photograph of a typical Stage 2/3 lesion is shown in Fig. 1. Although patients were accrued randomly, the tables are presented with those patients receiving the experimental rinse listed as numbers 1–20 and controls as 21–40. The data indicate that 18 of the experimental patients demonstrated zero recoverable virions following the 30 s rinse and nine of those patients remained at zero following the 30 min. delay. The other 11 patients returned to a 1–2 log recoverable sample. The pre-evaluation of recoverable virions was consistent with the literature (Arvin & Prober 1999) utilizing viral shedding in saliva.

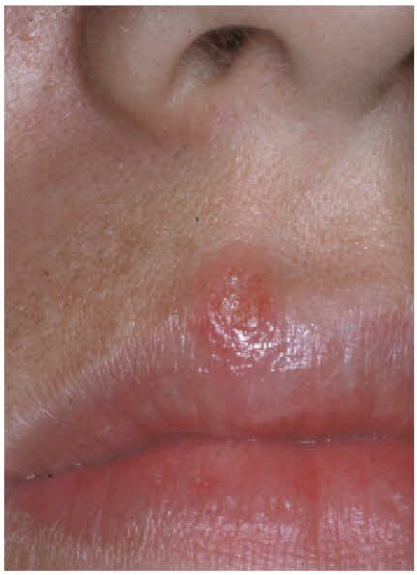

Fig. 1. A typical stage 2/3 recurrent *Herpes* labialis lesion.

In Trial 2, the average age was 28 years with 19 females and 21 males. Twenty-nine stage 2, three stage 1 and eight stage 3 lesions were evaluated. Two HSV-1 and HSV-2 reactions were noted on DIF. Eighteen patients had zero recoverable virions post rinse, 12 remained zero after 30 min. and the log reductions were comparable with Trial 1. At 60 min. post rinse, all 20 patients were shedding 1–2 logs lower than their pre-rinse levels. In Trial 2, control patient data were comparable with Trial 1.(Fig. 3, Table 3)

Our recovery rate was consistent between the control sample and the experimental sample. The control patients were well distributed in gender and age distribution with the experimental patients. The rinsing with sterile distilled water resulted in a 1–2 log reduction in recoverable virions at the 30 s sampling, however, the return to numbers comparable with the pre-rinse state were found at 30 min. These data indicate a direct effect of LA on viral kill and some residual effect at 30 min. generally to the 3–4 log level of reduction.

Following statistical analysis, no significant differences were found between the control and the experimental groups at baseline. A significant difference ($p<0.05$) was found between the control group and the experimental group following a 30 s rinse. A significant difference ($p<0.05$) was found between the control group and the experimental group at 30 min. post rinse. When the controls were compared over the course of both Trials of the experiment, there were no significant changes noted from the post rinse time period to the 30 s time period as compared with baseline. However, there was a trend of a 1–2 log reductions using the sterile distilled water rinse. When the experimental subjects were compared over the course of the experiment, significant reductions were found at the $p<0.001$ between baseline and the post rinse and between baseline and the post 30 min. rinse.

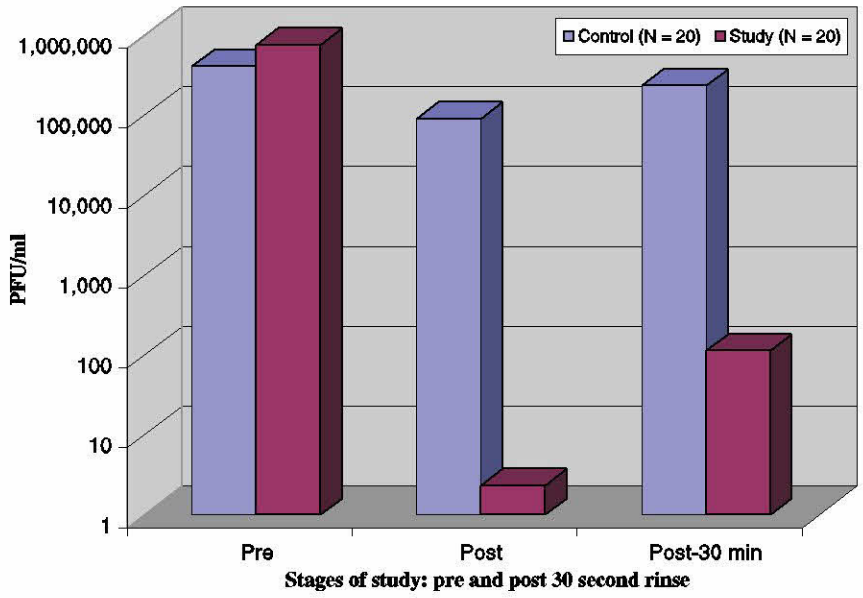

Fig. 2. Trial #1 mean recovered virions illustrating in vivo efficacy of Listerine® Antiseptic.

Conclusions

In a study investigating anti-microbial efficacy of mouthrinses, LA was found to completely kill microorganisms including methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Helicobacter pylori*, *Candida albicans*, *Streptococcus mutans*, *Actinomyces viscosus*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinobacillus actinomycetemcomitans* within 30 s when exposed to LA (Okuda et al. 1998). In the same study, LA was also reported to weakly inactivate HIV-1 viruses (Okuda et al. 1998).

Dennison et al. (1995) found chlorhexidine to completely inhibit HSV and influenza A virus for an exposure time of 30 s; whereas, rotavirus, a non-enveloped virus was only minimally inhibited (12.2%) (Dennison et al. 1995). Several other investigators also reported anti-HSV effects of LA and also found comparative ineffectiveness of LA and chlorhexidine in non-enveloped viruses (Bernstein et al. 1989, Croughan & Behbehani 1988, Park & Park 1989). In our in vitro study (Baqui et al. 2001), LA was found to possess slightly better anti-HSV effectiveness than 0.2% chlorhexidine. This work also confirmed the antiviral effectiveness of LA against HSV-1 McIntyre strain and

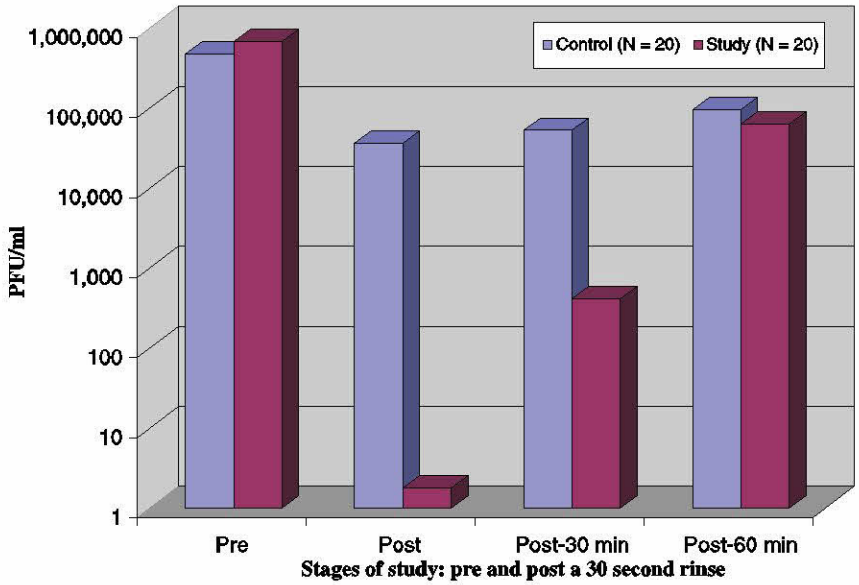

Fig. 3. Trial #2 mean recovered virions illustrating in vivo efficacy of Listerine® Antiseptic.

Table 3. Trial #2 in vivo efficacy of LA against *Herpes simplex* virus (PFU/ml)

Pt #	Sex	Age	Stage	Pre	Post	30 min. post	60 min. post	DIF
1	F	29	2	5.5 × 10 (6)	0	0	8.6 × 10 (3)	HSV-1
2	M	22	2	4.6 × 10 (5)	0	0	4.6 × 10 (1)	HSV-1
3	M	40	2	3.1 × 10 (4)	1.0 × 10 (1)	0	7.4 × 10 (3)	HSV-1
4	F	22	1	6.1 × 10 (5)	0	0	3.7 × 10 (3)	HSV-1
5	F	20	2	1.8 × 10 (5)	0	1.4 × 10 (1)	2.6 × 10 (4)	HSV1
6	M	34	2	3.7 × 10 (4)	0	6.7 × 10 (3)	6.1 × 10 (4)	HSV- 1
7	F	31	2	2.6 × 10 (3)	0	0	9.8 × 10 (3)	HSV-1
8	M	43	3	2.4 × 10 (4)	0	8.9 × 10 (2)	4.6 × 10 (5)	HSV-1
9	M	28	3	7.4 × 10 (4)	0	0	2.2 × 10 (4)	HSV-1
10	F	19	2	4.9 × 10 (3)	0	5.7 × 10 (2)	5.4 × 10 (5)	HSV-1
11	M	38	2	8.8 × 10 (4)	0	0	4.6 × 10 (3)	HSV-1/2
12	M	33	2	5.0 × 10 (5)	2.6 × 10 (1)	0	2.0 × 10 (4)	HSV-1
13	F	29	2	9.1 × 10 (4)	0	0	9.1 × 10 (4)	HSV-1
14	F	38	2	4.2 × 10 (4)	0	1.9 × 10 (1)	5.4 × 10 (3)	HSV-1
15	M	29	3	8.9 × 10 (5)	0	9.4 × 10 (1)	5.7 × 10 (2)	HSV-1
16	M	21	2	6.4 × 10 (4)	0	0	1.0 × 10 (3)	HSV-1
17	F	28	1	5.4 × 10 (4)	0	2.6 × 10 (1)	2.2 × 10 (3)	HSV-1
18	F	24	2	3.8 × 10 (5)	0	1.6 × 10 (1)	7.4 × 10 (3)	HSV-1
19	F	36	2	1.6 × 10 (3)	0	0	9.4 × 10 (2)	HSV-1
20	M	25	3	7.4 × 10 (4)	0	0	3.7 × 10 (3)	HSV-1
21	F	19	3	5.8 × 10 (6)	1.7 × 10 (5)	1.0 × 10 (5)	2.4 × 10 (5)	HSV-1
22	F	25	2	6.1 × 10 (5)	2.9 × 10 (5)	5.0 × 10 (5)	6.4 × 10 (5)	HSV-1/2
23	M	26	2	2.6 × 10 (4)	7.7 × 10 (3)	5.0 × 10 (3)	6.1 × 10 (4)	HSV-1
24	M	18	1	7.9 × 10 (4)	2.0 × 10 (5)	9.5 × 10 (4)	6.6 × 10 (4)	HSV-1
25	F	23	2	6.1 × 10 (5)	8.4 × 10 (4)	5.0 × 10 (4)	6.4 × 10 (4)	HSV-1
26	M	37	3	1.8 × 10 (4)	3.9 × 10 (3)	9.8 × 10 (3)	6.1 × 10 (3)	HSV-1
27	F	30	2	2.7 × 10 (4)	2.0 × 10 (2)	5.8 × 10 (3)	6.0 × 10 (3)	HSV-1
28	F	26	2	6.6 × 10 (5)	4.7 × 10 (4)	3.9 × 10 (3)	4.7 × 10 (4)	HSV-1
29	M	26	2	1.8 × 10 (5)	3.4 × 10 (4)	3.0 × 10 (4)	1.9 × 10 (5)	HSV-1
30	M	21	2	7.9 × 10 (5)	1.3 × 10 (3)	8.9 × 10 (3)	6.4 × 10 (4)	HSV-1
31	F	31	2	2.7 × 10 (4)	1.0 × 10 (4)	2.2 × 10 (3)	1.6 × 10 (4)	HSV-1
32	M	25	2	1.0 × 10 (5)	7.4 × 10 (3)	6.1 × 10 (4)	9.8 × 10 (3)	HSV-1
33	M	20	2	1.4 × 10 (4)	3.4 × 10 (4)	1.9 × 10 (3)	1.2 × 10 (4)	HSV-1
34	M	38	2	6.7 × 10 (4)	5.7 × 10 (3)	9.7 × 10 (3)	4.1 × 10 (1)	HSV-1
35	M	33	3	9.8 × 10 (3)	8.7 × 10 (3)	7.2 × 10 (4)	5.5 × 10 (3)	HSV-1
36	M	25	2	3.4 × 10 (5)	2.4 × 10 (4)	1.9 × 10 (4)	3.3 × 10 (5)	HSV-1
37	F	44	3	4.7 × 10 (4)	6.7 × 10 (3)	5.6 × 10 (4)	1.9 × 10 (4)	HSV-1
38	M	23	2	7.6 × 10 (3)	5.0 × 10 (3)	1.7 × 10 (4)	2.0 × 10 (4)	HSV-1
39	F	30	2	1.8 × 10 (4)	3.1 × 10 (3)	6.6 × 10 (3)	7.1 × 10 (4)	HSV-1
40	F	23	2	3.1 × 10 (4)	9.1 × 10 (3)	4.9 × 10 (3)	2.9 × 10 (4)	HSV-1

DIF, direct immunofluorescence assay; LA, Listerine® Antiseptic; HSV, *Herpes simplex* virus; M, male; F, female; PFU, plaque forming units.

extended the effectiveness to another enveloped virus, the HIV-1.

In our in vitro study, we developed a semi-quantitative tissue culture assay for measuring inactivation of HIV-1 in the presence of different dilutions of the reagents used. This utilized a sensitive CPE, which is a relatively rapid, inexpensive and simple method for determining infectivity. For in vitro and in vivo estimation of anti-HSV-1 effect, we have utilized a standard plaque formation assay on monolayers of Vero cells.

In this current in vivo study, the effects of rinsing with an essential oil containing mouthrinse (Cool Mint LA) resulted in effectively zero recoverable virions at 30 s post rinse and this reduction in viral presence in saliva remained at a significant reduction for approximately 30 min. for all experimental patients and a continued reduction at 60 min. post rinse in Trial 2. Rinsing with the control (sterile distilled water) resulted in a statistically non-significant reduction in viral presence in saliva at 30 s and no change from baseline at 30 min. These results are consistent with the previous finding of effects of LA on enveloped viruses reported by Dennison et al. (1995) and other investigators (Wood & Payne 1998). The implication of these studies is that when coupled with our in vitro studies previously published (Baqui et al. 2001) there is clinical efficacy in utilizing an oral rinse with an antimicrobial agent such as LA to reduce viral contamination of saliva.

The clinical significance may be that reduction in infectious virus levels at the level demonstrated in these experiments significantly reduces, but may not eliminate, the risk of cross contamination. The necessary level for infectivity in saliva has not been determined and may be the subject of future studies. The additional clinical implication is that the effects of antimicrobial rinses on enveloped viruses have been consistent in the literature and that other viruses such as the coronavirus recently identified in SARS (McIntosh 2002; Mufson 2000) may also be affected by rinsing with the antimicrobial agent LA. The societal value of such a rinse cannot be accurately assessed; however, any reduction in infectious virus levels should at least theoretically reduce the risk of transmission to uninfected individuals.

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Address:
Dr. Timothy F. Meiller
University of Maryland Dental School
Department of Diagnostic Sciences and Pathology
666 W. Baltimore St., Rm. 4G-03
Baltimore MD 21201
USA
Fax: 410 706 0519
E-mail: TFM001@dental.umaryland.edu