University of California, Irvine
School of Medicine – Beckman Laser Institute and Medical Clinic

Study Report – PWS-OEH-VL-0822

Effects of Mouthwash Use on Oral Viral Load

Study Goal:

Double-blinded, randomized in vivo study in 30 subjects to compare the effects on oral viral load of a test vs control mouth rinse used twice daily over a period of 60 days.

Study Overview:

Baseline saliva samples were collected in thirty individuals (age 25-35) with gingivitis (GI > 2) to ensure they all carried measurable load of HSV1, CMV and EBV. Subjects were randomized to use either Lumineux Oral Essentials Clean and Fresh Mouthwash^{R} (Oral Essentials, Beverly Hills, CA 90210) (Test), or de-ionized water (Control). Subjects gargled with the allocated mouth rinse twice daily over a period of 60 days. Saliva samples were collected at baseline and Day 60 and sent for mRNA analysis using RT-PCR of viral load of HSV1, CMV and EBV. Subjects also maintained a daily health log, recording any presence, duration and severity of signs or symptoms of URTI and other unwellness.

Test group baseline means were significantly higher compared to Control group for CMV and EBV. For HSV-1, Test group baseline mean was lower than for Controls, with the Difference approaching significance. Baseline differences did not have an effect on the differences between groups in change over time. Paired differences (change Day 0 – Day 60) were significantly greater for the Test group than for the Control group for all 3 viruses. These differences remained significant after adjusting for baseline values. Subjects using the test rinse made 2 entries in the health log, whereas those using the control rinse recorded 5. However, there was no significant difference in frequency of health log entries between the 2 groups.

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1. PURPOSE

Goal of this single center, double-blinded, randomized in vivo study was to compare the effect on oral viral load in 30 subjects of twice daily use over a period of 60 days of Lumineux Oral Essentials Clean and Fresh Mouthwash^{R} (Oral Essentials, Beverly Hills, CA 90210) vs. de-ionized water. On Days 0 and 60, saliva samples were collected from all subjects according to standard technique and immediately frozen for subsequent mRNA analysis using RT-PCR of viral load of HSV1, CMV and EBV. A daily health log was used to monitor presence and severity of any signs/symptoms of URTI and any other unwellness throughout the study.

2. MATERIALS AND METHODS

2.1. Subjects

30 subjects who met inclusion/exclusion criteria were recruited, and provided written, informed consent under University of California, Irvine IRB-approved protocol # 2020-5719.

Subjects met the following inclusion and exclusion criteria:

Inclusion Criteria

1. Male or female subjects aged 25-35
2. GI >2
3. Able to provide written informed consent
4. Able to attend study visits
5. Available for follow up on the telephone.
6. Minimum of 20 teeth
7. Measurable salivary viral load for HSV1, CMV and EBV at baseline

Exclusion Criteria

1. Use of antibacterial mouth rinse within 3 months or during study
2. Systemic or topical oral antibiotic, antiviral, antifungal medications within 3 months or during study
3. Any dental treatment within 1 month or during study
4. History of significant adverse effects following use of oral hygiene products such as toothpastes and mouth rinses. Allergy to personal care/consumer products or their ingredients.
5. Presence of any condition, abnormality, or situation at Baseline that in the opinion of the Principal Investigator may preclude the volunteer’s ability to comply with study requirements, including completion of the study or the quality of the data.

2.2. Protocol

This study was performed in full compliance with University of California, Irvine IRB protocol 2020-5719, and all clinical procedures were conducted in accordance with the Helsinki Declaration of 1975, as updated in 2013. No significant changes were made in the study design after commencement of the study.

Thirty subjects were recruited as per inclusion/exclusion criteria listed above. Subjects were randomized in a 1:1 ratio (randomizer.com) to use either an OTC non-bactericidal mouthwash that targets the products of periodontopathogens (Lumineux Oral Essentials Clean and Fresh Mouthwash^{R} (Oral Essentials, Beverly Hills, CA 90210)), or de-ionized water. Mouthwash bottles were masked to conceal the rinse's identity from study participants and investigators. Subjects were contacted by telephone weekly to monitor and reinforce compliance. All subjects were asked to keep up any pre-existing handwashing and mask-wearing routine, not to change other hygienic habits, and not to take any cold remedies during the intervention period. Subjects also maintained a daily health log, recording presence, duration, as well as any signs or symptoms that deviated from full health. This log included any unwellness, including any URTI complaints such as nasal symptoms (rhinorrhoea and sneezing), pharyngeal symptoms (soreness and scratchiness), bronchial symptoms (cough and phlegm), and general symptoms (feverishness, arthralgia, malaise, and any other deviations from full health).

For 60 days, after shaking the bottle thoroughly, subjects rinsed for 60s twice daily with 20ml of their allocated mouthwash, directly after morning and evening meals. They abstained from food and drink for at least 30 mins after rinsing. On Day 0, before eating and oral hygiene, before mouthwash use had begun, and at least 60 minutes after drinking, unstimulated saliva was collected. Subjects were asked to accumulate saliva in the floor of the mouth and spit it out into a graduated Zymo Collection Tube^{R} every 60 seconds for 5 minutes, then to shake it vigorously to ensure proper stabilization. Saliva was again collected in the same way on Day 60 of the study. Samples were frozen in an -80^{0}C freezer where they were stored until all the samples from all patients were acquired and were processed together.

2.3. Mouthwashes

1. Test: Lumineux Oral Essentials Clean and Fresh Mouthwash^{R} (Oral Essentials, Beverly Hills, CA 90210).

2. Control: de-ionized water (UCI storehouse)

2.4. Endpoints

1. Changes in Log Salivary Viral Load (HSV1, CMV and EBV)
    (a) Day 60 vs Day 0
    (b) Lumineux OE vs. de-ionized water

2. Presence, severity of any illness and of URTI-specific symptoms on the health log.

3. RESULTS

All subjects completed the study in full compliance with the protocol. No adverse events were reported or observed. All Viral Load Data are recorded as Log Viral Load.

3.1. Subjects

Age range: 25-33; Control group: 25-34; Test group: 25-35
Mean Age: 28.9: Control group: 28.0; Test group: 29.8
Median age: 28: Control group: 28; Test group: 29
M/F: 17/13: Control group: 9/7; Test group: 8/6

3.1.1. Descriptive Salivary Viral Load Data (HSV1, CMV and EBV)

	HSV1 Day 0	HSV1 Day 60	HSV1 DIF	CMV Day 0	CMV Day 60	CMV DIF	EBV Day 0	EBV Day 60	EBV DIF
N	15	15	15	15	15	15	15	15	15
Minimum	2.16	1.84	-1.28	2.73	1.89	-0.37	4.07	1.68	-2.2
Maximum	7.63	4.51	-3.12	8.14	7.12	-2.42	10.42	5.82	-5.27
Median	4.49	2.99	-1.53	5.21	3.84	-1.37	8.86	4.38	-4.03
MEAN	4.469	3.022	-1.447	5.294	3.933	-1.361	8.011	4.085	-3.926
S.E.	0.404	0.207	0.271	0.433	0.394	0.15	0.526	0.33	0.237
S.D.	1.563	0.802	1.051	1.677	1.527	0.583	2.037	1.279	0.92

Table 1: Descriptive Data, Control Group, HSV1, CMV, EBV at Day 0, Day 60, and Difference (Day-0 - Day-60)

	HSV1 Day 0	HSV1 Day 60	HSV1 DIF	CMV Day 0	CMV Day 60	CMV DIF	EBV Day 0	EBV Day 60	EBV DIF
N	15	15	15	15	15	15	15	15	15
Minimum	2.64	0.52	-2.01	4.1	0.31	-3.79	3.12	0.58	-2.3
Maximum	9.83	2.05	-7.82	10.33	1.41	-8.92	8.15	1.42	-6.73
Median	5.49	0.98	-4.21	7.69	0.9	-6.6	6.09	1.15	-4.94
MEAN	5.62	1.146	-4.474	7.379	0.867	-6.512	5.958	1.009	-4.949
S.E.	0.483	0.144	0.367	0.482	0.087	0.409	0.43	0.076	0.368
S.D.	1.872	0.559	1.422	1.865	0.337	1.586	1.666	0.294	1.425

Table 2: Descriptive Data, Test Group (OE), HSV1, CMV, EBV at Day 0, Day 60, and Difference (Day-0 - Day-60)

3.1.2. Comparison of Baseline Salivary Viral Load in Control vs. Test Group

Test group baseline means are significantly higher compared to Control group for CMV and EBV. For HSV-1, Test group baseline mean is lower than for Controls, Difference approaches significance.

	Control			Test								
	N	Mean	SD	N	Mean	SD	Mean Difference	Lower CI	Upper CI	t	df	p-Value
HSV1 Day 0	15	4.469	1.563	15	5.62	1.872	-1.151	-2.441	0.139	-1.828	28	0.078
CMV Day 0	15	5.294	1.677	15	7.379	1.865	-2.085	-3.411	-0.758	-3.219	28	0.003
EBV Day 0	15	8.011	2.037	15	5.958	1.666	-2.053	0.661	3.445	3.022	28	0.005

Table 3: Comparison of Baseline Salivary Load in Control and Test (Two-Group t-Test)

3.1.3. Change in Salivary Viral Load from Baseline to Day 60: Control vs. Test Group

Paired differences (change Day 0 – Day 60) are significantly greater for Test group than for Control group for all 3 viruses. The first analysis shown in Table 4 ignores any possible influence of differences due to baseline values and merely analyzes change.

	Control			Test								
	N	Mean Change (Day 0-Day 60)	SD	N	Mean Change (Day 0-Day 60)	SD	Mean Difference (CO chg - TST chg)	Lower CI	Upper CI	t	df	p-Value
HSV1 DIF	15	1.447	1.051	15	4.474	1.422	-3.027	-3.963	-2.092	-6.629	28	<0.001
CMV DIF	15	1.361	0.583	15	6.512	1.586	-5.151	-6.045	-4.258	-11.809	28	<0.001
EBV DIF	15	3.926	0.92	15	4.949	1.425	-1.023	-1.92	-0.126	-2.337	28	0.027

Table 4: Comparison of Change in Salivary Viral Load from Baseline to Day 60: Control vs. Test Group (Two-Group t-Test for Difference in Paired Change Values)

After adjusting for baseline differences (Table 5), the significance of differences between groups in change over time increases, with all p-values <0.001. Adjusted values for mean differences are slightly smaller than the unadjusted differences for HSV and CMV (blue highlighted box), but larger for EBV. Baseline differences do not have an effect on the differences between groups in change over time. These differences remain significant after adjusting for baseline values.

Table 5: Comparison of Change in Salivary Viral Load from Baseline to Day 60 between

	Control					Test						
		Day 0		Day 60			Day 0		Day 60		Mean Difference	F-test*
	N	Adjusted Mean	SE	Adjusted Mean	SE	N	Adjusted Mean	SE	Adjusted Mean	SE		p-value
HSV-1	15	5.044	0.000	3.205	0.114	15	5.044	0.000	0.963	0.114	-2.242	p<0.001
CMV	15	6.336	0.000	4.421	0.209	15	6.336	0.000	0.379	0.209	-4.042	p<0.001
EBV	15	6.985	0.000	3.657	0.145	15	6.985	0.000	1.437	0.145	-2.220	p<0.001

Control and Test AFTER Adjusting for Baseline Value Which Differs between Groups (Repeated Measures ANOVA Adjusting for Baseline (Day-0) Value). *For difference between groups in change over time.

3.1.4. Health Log: Testing for Group Difference (Control vs Test)

In the control group, subjects recorded 5 health events: moderate URTI week 2; moderate cough week 4; COVID-19 week 5; COVID-19 week 6; COVID-19 week 7.

In the test group, subjects recorded 2 health events: Moderate food poisoning week 4; COVID-19 week 6.

3.1.4.1. Difference in frequency of health log entries

Using the chi-square test to evaluate for difference in frequency of health log entries, there was no significant difference in frequency of health log entries between the 2 groups (p=0.195) (Table 6).

Health Log Entries	Control		Test		Total	
	N	%	N	%	N	%
No	10	66.7	13	86.7	23	76.7
Yes	5	33.3	2	13.3	7	23.3
Total	15	100	15	100	30	100

Table 6: Difference between Groups in Frequency of Symptoms/Diagnoses