

2015

## How Effective are CleanWave® UV-C Portable Toothbrush Sanitizers in Removing Streptococcus Mitis from Toothbrushes?

Rafaila Ramirez

*University of Nebraska at Kearney*

Follow this and additional works at: <https://openspaces.unk.edu/undergraduate-research-journal>

 Part of the [Biology Commons](#)

---

### Recommended Citation

Ramirez, Rafaila (2015) "How Effective are CleanWave® UV-C Portable Toothbrush Sanitizers in Removing Streptococcus Mitis from Toothbrushes?," *Undergraduate Research Journal*: Vol. 19 , Article 16.

Available at: <https://openspaces.unk.edu/undergraduate-research-journal/vol19/iss1/16>

This Article is brought to you for free and open access by the Office of Undergraduate Research & Creative Activity at OpenSPACES@UNK: Scholarship, Preservation, and Creative Endeavors. It has been accepted for inclusion in Undergraduate Research Journal by an authorized editor of OpenSPACES@UNK: Scholarship, Preservation, and Creative Endeavors. For more information, please contact [weissell@unk.edu](mailto:weissell@unk.edu).

---

# How Effective are CleanWave® UV-C Portable Toothbrush Sanitizers in Removing Streptococcus Mitis from Toothbrushes?

## **Cover Page Footnote**

I would like to thank Betty Jacques for helping prepare the saline solution and Luria Broth media, for helping set up the experiment, and proofreading the paper. Also, I thank Dr. Travis Bourret for helping proofread the paper, and Dr. Marc Albrecht for helping interpret the results. I would also like to thank Maurice Chessmore for proofreading the paper and taking time to help interpret the results. Lastly, I would like to thank Dr. Shaffer for serving as my mentor and helping me throughout the project. This work was funded by the Undergraduate Research Fellows Program.

# **How effective are CleanWave® UV-C Portable Toothbrush Sanitizers in removing Streptococcus mitis from toothbrushes?**

*Rafaila Ramirez*

## **ABSTRACT**

The oral cavity is home to a wide variety of microorganisms<sup>11</sup>. More than 700 bacterial species have been detected within the oral cavity<sup>1</sup>. Thus, it is not surprising that some of these microorganisms are transferred to a toothbrush during use. Furthermore, studies have shown that various microorganisms can grow on toothbrushes after use. More microorganism development occurs on toothbrushes stored in a moist environment such as a closed container<sup>8</sup>. Since toothbrushes can become a potential portal of entry for organisms,<sup>3</sup> the purpose of this study was to see how effective the CleanWave® UV-C Portable Toothbrush Sanitizer was in re-moving the microbe *Streptococcus mitis* from toothbrushes. Earlier studies have indicated that the sanitizer may not work if toothbrushes were not allowed to dry before use. In this study, six toothbrushes were left to air dry, while another six were stored wet directly after contamination. Twenty four toothbrushes were also used for the levels of air drying and they followed the same procedure. After treatment, 100- $\mu$ L were directly deposited on selective Mitis Salivarius Agar plates. A paired t-test for dry versus wet and an analysis of variance (ANOVA) test for the levels of air drying was performed at the 95% confidence interval<sup>2</sup>. This study demonstrated that when the toothbrush was stored wet, the CleanWave® UV-C Portable Toothbrush Sanitizer's effects were hindered by water, and for it to work effectively in removing *S. mitis* the toothbrush had to be completely dry. What is more, when the toothbrush was air dried, it too showed to have the same effect without the UV-C sanitizer, indicating that air drying alone is effective in removing *S. mitis*.

## **INTRODUCTION**

Daily tooth brushing plays an important role for personal oral hygiene and effective plaque removal. For that reason, appropriate toothbrush care and maintenance are important factors to consider for a sound oral hygiene<sup>15</sup>. When not followed properly, the oral cavity can become an entrance for bacteria that may cause disease<sup>5</sup>.

Bacteria are normal inhabitants of the human mouth. The vast majority of these oral species are referred to as commensal species, indicating that they do not harm the host. A small number of species, however, can cause a shift in oral health when they are able to achieve sufficiently large numbers in localized areas of the teeth and gums<sup>9</sup>. As a result, high numbers of bacteria on a toothbrush can have deleterious effects on the human body<sup>10</sup>.

When we eat, the bacteria present in the oral cavity blend with the food particles and hold to the surface of teeth, forming a sticky layer of plaque eventually converting into tartar. The gum becomes diseased when the bacteria, via tartar, multiply and damage the gum tissues. Poor oral health and untreated oral disease can have a substantial impact on the overall quality of life, as the condition of the mouth mirrors the condition of the body as a whole<sup>12</sup>.

Recent scientific literature suggests a link between intra-oral bacteria and a number of systemic diseases, including cardiovascular disease, stroke, preterm birth, diabetes, and respiratory diseases<sup>10</sup>. Recent medical research studies have also demonstrated that more than 90 percent of the diseases affecting different systems of the body are due to the bacteria in the oral cavity. These diseases can arise from the swelling of gums, dry mouth, mouth ulcers or severe gum problems. In 2009, researchers from the University of Buffalo reported a link between the amount of oral bacteria and an increased risk of heart attack<sup>4</sup>. Dr. Robert Genco presented evidence that people with periodontal disease are 2.7 times more likely to suffer a heart attack than those with healthy gingiva. That is, the link is not due to the types of bacteria, but their numbers<sup>13</sup>.

To reduce bacterial contamination, the American Dental Association recommends people practice appropriate handling of the toothbrush and replace the toothbrush approximately every three to four months or sooner if the bristles become frayed with use<sup>15</sup>. The most common method used for toothbrush storage is rinsing and air drying. However, the bathroom can be a perfect breeding ground for bacteria where they thrive in warm and moist conditions. For this reason, others choose to store their toothbrush in a toothbrush holder. However, this type of storage provides the bacteria on the toothbrush with a moist environment, increasing growth<sup>8</sup>.

To prevent bacteria from contaminating the toothbrush or growing in a moist environment, a new type of storage is being marketed: the CleanWave® UV-C Portable Toothbrush Sanitizer. One of the most effective purifiers is natural sunlight because the sun's UV-C rays inhibit the growth and reproduction of dust mites, bacteria, viruses, fungi, and molds<sup>14</sup>. Specifically, UV-C causes damage to the nucleic acids of microorganisms by forming covalent bonds between adjacent bases in the DNA<sup>7</sup>. For these reasons, the powerful UV-C light from the CleanWave® UV-C Portable Toothbrush Sanitizer safely eliminates viruses and bacteria from the toothbrush.

Previous studies have indicated that sanitization is not as effective when a toothbrush is wet. The purpose of this study was to see how effective the CleanWave® UV-C Portable Toothbrush Sanitizer was in removing the microbe *Streptococcus mitis* from toothbrushes. The hypothesis was that when allowed to air dry the CleanWave® UV-C Portable Toothbrush Sanitizer would effectively kill the microbe. However, when put inside the case wet directly after use or when stored in a toothbrush holder wet, it would not be as effective. Additionally, the levels of air-drying for toothbrushes were examined to show if air-drying alone was enough to kill the microbe.

## MATERIALS AND METHODS

Colonies of *Streptococcus mitis* (ATCC#6249) were streaked onto Luria Broth agar plates for isolation. After 72 hours of incubation at 37°C, 10-mL Luria Broth were inoculated with the microorganism and incubated for 72 hours at 37°C without agitation. A control and an experimental group were set up for each trial. The control group was not treated with the CleanWave® UV-C Portable Toothbrush Sanitizer; instead it was either held on a beaker for 120-minutes to air dry after contamination following storage in a toothbrush holder for six minutes or stored in a toothbrush holder directly after contamination for six minutes, whereas, the experimental group was stored in the CleanWave® UV-C Portable Toothbrush Sanitizer after being held in the beaker and/or the toothbrush holder. The same procedure followed for the levels of air-drying; however, there were time intervals of 30, 60, 90, and 120 minutes. Thirty-six toothbrushes were contaminated with 10-mL *S. mitis* Luria Broth for two minutes<sup>15</sup>, rinsed with 20-mL of sterile water and shaken two times to remove excess water.

The operating instructions for the CleanWave® UV-C Portable Toothbrush Sanitizer were followed after storage. The steps were to open the lid of the sanitizer and insert the toothbrush with the neck in the clip (although the unit has air circulation holes, it is recommended that excess water be shaken off prior to placing the toothbrush in the sanitizer). The lid was closed turning on the UV-C light to illuminate the toothbrush. The built-in timer provided a complete 6-minute sanitizing cycle before automatically turning itself off.

Next, the toothbrushes were placed head first into 10-mL saline tubes and vortexed for 1-minute. This was done for both the control and the experimental groups. This procedure quantified and washed off any remaining bacteria. Selective media were used to see if any *S. mitis* had remained on the toothbrush. The medium used was Mitis Salivarius Agar (MSA). Bacto Chap-man potassium 1% Tellurite solution was added allowing the selective medium to show the isolation of *S. mitis*. This medium is indigo in color. The colonies that grow are very small and “gum drop” in appearance. For each toothbrush used, 100- $\mu$ L of the 10-mL saline solution were placed onto three separate MSA plates and incubated for 72 hours at 37°C. Three days later the colonies were identified and counted. The data for air drying versus wet was analyzed using a paired t-test ( $P < 0.050$ ), and an ANOVA test ( $P < 0.050$ ) was performed on the levels of air drying<sup>2</sup>.

## RESULTS

The following hypotheses ( $H_0$ =null,  $H_a$ =alternative) were tested using the paired t-test ( $\mu$ ) at the 5% significance level<sup>2</sup> to determine if the CleanWave® UV-C Portable Toothbrush Sanitizer was the effective in killing *S. mitis* directly after use.

1. Effect of wet storage: toothbrush holder versus UV-sanitizer

$H_0$ :  $\mu_{\text{toothbrush holder}} = \mu_{\text{UV}}$  versus  $H_a$ :  $\mu_{\text{toothbrush holder}} \neq \mu_{\text{UV}}$

2. Effect of 2-hour air drying + dry storage: toothbrush holder versus UV-C sanitizer

$H_0$ :  $\mu_{\text{toothbrush holder}} = \mu_{\text{UV}}$  versus  $H_a$ :  $\mu_{\text{toothbrush holder}} \neq \mu_{\text{UV}}$

The paired t-test suggests that the CleanWave® UV-C Portable Toothbrush Sanitizer was not effective in completely killing *S. mitis* from toothbrushes that were stored wet since the null

hypothesis for the effect of wet storage (paired t-test hypothesis 1) was not rejected (p-value 0.7765). Additionally, this test suggested that the toothbrush holder and the UV-C sanitizer did not differ in *S. mitis* concentrations from wet toothbrushes. With the null hypothesis being in favor of the alternative hypothesis, it is assumed that when stored wet, there will be a high number of bacterial growth, regardless if one stores their toothbrush in a toothbrush holder or UV sanitizes.

The null hypothesis for the effect of 2-hour air drying + dry storage (paired t-test hypothesis 2) was also not rejected (p-value 0.6656). Statistically there was no significance ( $\alpha=0.05$ ) demonstrating that the UV-C sanitizer was not more effective in killing *S. mitis* from toothbrushes when compared to the toothbrush holder. With the null hypothesis being in favor of the alternative hypothesis at the 5% significance level, it is established that whether one uses a toothbrush holder or UV-C sanitizer to store their toothbrush one will get approximately the same effects as long as the toothbrush is dry (See table 1).

	Toothbrush Holder	UV-C Sanitizer	P-value
Wet Storage	285.3 (+41.1)	279.6 (+43.5)	0.7756
2-hour Air Dry	1.1 (+1.4)	0.8 (+1.0)	0.6656

*Table 1. For two separate experiments the average number of colony counts detected under wet and dry conditions using a toothbrush holder and UV-C sanitizer. These were tested using a student paired t-test<sup>2</sup>.*

After air drying for 2-hours, both the toothbrush holder and UV-C sanitizer showed approximately the same results (Table 1). Due to a lack of significant difference between the storage methods when air dried, the levels of air drying were examined to test for a significant difference between the different levels of air drying with the UV-C sanitizer and the toothbrush holder over time. The following hypotheses were analyzed using the ANOVA test ( $\beta$ ) at the 5% significance level<sup>2</sup>.

3. Effect of storage: UV versus toothbrush holder

Ho:  $\beta_{\text{toothbrush holder}} = \beta_{\text{UV}}$  versus Ha:  $\beta_{\text{toothbrush holder}} \neq \beta_{\text{UV}}$

4. Effect of air drying over a period of time

Ho:  $\beta_{\text{time}} = \beta_{\text{UV}}$  versus Ha:  $\beta_{\text{toothbrush holder}} \neq \beta_{\text{UV}}$

Table 2 demonstrates how over time the numbers of bacteria were reduced regardless of the storage method. For the effect of storage (ANOVA hypothesis 3), the null hypothesis was not rejected in favor of the alternative hypothesis (p-value 0.3049), but the null hypothesis for the effect of air drying (ANOVA hypothesis 4) over a period of time was rejected in favor of the alternative hypothesis (p-value <0.0001). With the null hypothesis being in favor of the alternative in the effect of storage, it is assumed that both storage methods will give approximately the same results when air dried over time. However, with the rejection of the null hypothesis in favor of the alternative in the effect of air drying over time, it is suggested that as time goes by the number of bacteria decrease significantly (p<0.0001), that is the air itself is effective in killing the microbe (See Figure 1).

Minutes	Number of Colonies Detected			
	30	60	90	120
Toothbrush Holder	251.2 (+57.1)	193.9 (+57.5)	121.6 (+36.6)	4.2 (+3.1)
UV-C Sanitizer	232.3 (+45.2)	177.6 (+37.3)	98.3 (+31.3)	3.6 (+3.0)

Table 2. For two separate experiments the average number of colonies detected as time went by for the control and non-control groups using a toothbrush holder and UV-C sanitizer. These were tested using an ANOVA test<sup>2</sup>.

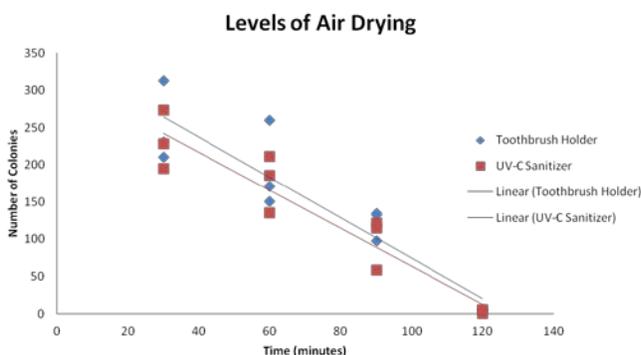


Figure 1. The scatter plot shows a significant linear decrease ( $p < 0.0001$ )<sup>2</sup> in *S. mitis* colonies as the toothbrushes were air dried over time. The two storage methods had no effect in reducing the number of colonies detected which can be shown by the closeness of the two approximate parallel linear lines.

## DISCUSSION

Our study showed that the CleanWave® UV-C Portable Toothbrush Sanitizer was not effective in completely removing *S. mitis* since there was no significant difference when it was used to store the toothbrushes wet or dry. Thus, this experiment demonstrated that there was no need for the toothbrushes to have undergone UV-C treatment. Even though there was a greater decrease in the number of colonies detected at the 90-minute interval for the UV storage method when compared to the control group (see Table 2), a paired t-test at the 5% significance level showed that the difference between the means of the populations were equal to 0. Therefore, the null hypothesis (p-value 0.1670) was not rejected. This decrease was seen throughout the levels of air drying, but they, too, showed no significant difference when a t-test was performed.

On the other hand, the slight decrease in colonies could have been because ultraviolet (UV) light kills microorganisms by damaging the DNA. Specifically, UV radiation disrupts the chemical bonds that hold the atoms of DNA together in the microorganisms. If the damage is severe enough, the bacteria cannot repair the damaged DNA and die<sup>7</sup>. However, because the toothbrush was not air dried completely, the UV-C light may not have been able to penetrate through the water. Therefore, the UV-C sanitizer may not be the best method to reduce the number of microorganisms on a toothbrush with just one sanitation cycle. Furthermore, toothbrushes that do not dry are more susceptible to bacterial contamination and biofilm formation, and when bacteria levels become excessively present it can become a health issue<sup>6</sup>; for this reason, air-drying alone is the favored storage method. For future studies, we could try

testing the CleanWave® UV-C Portable Toothbrush Sanitizer on viruses and on different microorganisms.

### **ACKNOWLEDGEMENTS**

I would like to thank Betty Jacques for helping prepare the saline solution and Luria Broth media, for helping set up the experiment, and proofreading the paper. Also, I thank Dr. Travis Bourret for helping proofread the paper, and Dr. Marc Albrecht for helping interpret the results. I would also like to thank Maurice Chessmore for proofreading the paper and taking time to help interpret the results. Lastly, I would like to thank Dr. Shaffer for serving as my mentor and helping me throughout the project. This work was funded by the Undergraduate Research Fellows Program.

## REFERENCES

- 1Aas, J.A. et al. (2005). Defining the Normal Bacterial Flora of the Oral Cavity. *Journal of Clinical Microbiology* 43 (11): 5721-732.
- 2Analyse-it 3.02 (2012). Analyse-it Software Ltd. Leeds, England.
- 3Ankola, A.V. et al. (2009). How clean is the toothbrush that cleans your tooth? *International Journal of Dental Hygiene* 7(4): 237-40.
- 4Baker, L., (2009), The more oral bacteria, the higher the risk of heart attack, UB study shows. University at Buffalo New Center, web.
- 5Barnett, M.L. (2006). The oral-systemic disease connection: An update for the practicing dentist. *J Am Dent Assoc* 137, 5S-6S.
- 6Bhat, P.K., Badiyani. B.K., Sarkar, S., Chengappa, S., Bhaskar, N.N. (2012). Effectiveness of antimicrobial solutions on *Streptococcus mutans* in used toothbrushes. *World Journal of Dentistry* 3(1), 6-10.
- 7Boylan, R., Li, Y., Simeonova, L., Sherwin, G., Kreismann, J., Craig, R.G., Ship, J.A., and McCutcheon, J.A. (2008). Reduction in bacterial contamination of toothbrushes using the Violight ultraviolet light activated toothbrush sanitizer. *Am J Dent* 21(5), 313-317.
- 8Glass, R.T., Lare, M.M. (1986). Toothbrush contamination: A potential health risk? *Quintessence Int* 17(1), 39-42.
- 9Hillman, J.D., Socransky, S.S. (1989). The theory and application of bacterial interference to oral diseases. In *New Biotechnology in Oral Research*, 1-17.
- 10Jaksha, S. (2011). The role of a toothbrush in toothbrushing, intra-oral bacteria. *The American Academy for Oral Systemic Health Newsletter* 17, 1-11.
- 11Kazor, C.E. et al. (2003). Diversity of bacterial populations on the tongue dorsa of patients with halitosis and healthy patients. *J Clin Microbiol* 41 (2): 558-63.
- 12Khan, S.E. (2007). Orally yours. *Dawn Sci-tech World*, web.
- 13Loesche, W.J. (1997). Association of the oral flora with important medical diseases. *Current opinion in periodontology* 4, 21-28.
- 14Nebot Sanz, E., Salcedo Davila, I., Andrade Balao, J.A., and Quiroga Alomso, J.M. (2007). Modelling of reactivation after UV disinfection: Effect of UV-C dose on subsequent photo reactivation and dark repair. *Water Research* 41, 3141-3151.
- 15Statement on toothbrush care: Cleaning, storage, and replacement. American Dental Association (2013).