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The Effectiveness of Over the Counter Mouthwashes Containing Hydrogen Peroxide or Alcohol Against Streptococcus *mutans*

A Thesis

Presented to the Faculty of the Advanced Education in General Dentistry, Two-Year Program,

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In Partial Fulfillment of the Requirements for the Degree of

Master of Science in Oral Biology

Dr. Miles Lee Renick

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The Effectiveness of Over the Counter Mouthwashes Containing Hydrogen Peroxide or Alcohol Against Streptococcus *mutans*

A REPORT ON
Research project investigating the effect of OTC mouth rinses containing H2O2 or alcohol on the inhibition S. *mutans*

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ABSTRACT

Purpose: The purpose of this study is to 1) examine and compare effectiveness of Colgate Perioguard™ containing alcohol and Paroex™ Chlorhexidine Gluconate which contains no alcohol. 2) examine the effectiveness of chlorhexidine mouth rinses and OTC mouth rinses. 3) examine and compare the effectiveness of Listerine™ Healthy White Vibrant and Crest 3D Lux Glamorous White™ both whitening mouth rinses to Listerine™ Original and Crest Pro Health™

Methods: 6 groups of mouth rinses and one control were run through serial dilution tests. The mouth rinses were mixed with stock S. mutans bacteria for 1 min and put though 4 serial dilutions. Each of the serial dilutions was streaked on a blood agar plate and incubated for 24-48 hours. Once incubated, CFUs were counted and the original sample of bacterial CFU/ml was determined.

Results: A significant difference was found between the control and the mouth rinses tested. No significant difference was found between each mouth rinse. From this study we can hypothesize that Colgate Perioguard™ containing alcohol and Paroex™ Chlorhexidine Gluconate without alcohol have the same antimicrobial effectiveness against S. *mutans*, Colgate Perioguard™ and Paroex™ Chlorhexidine Gluconate when compared to Crest Pro-Health™ and Listerine™ Original have the same antimicrobial effectiveness against S. *mutans*. Listerine™ Healthy White Vibrant and Crest 3D White Lux Glamorous White™ chloride may have the least antimicrobial effectiveness against S. *mutans*.

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INTRODUCTION

Bacteria colonize the oral cavity within hours after birth. This colonization leads to polymicrobial interactions of various bacteria that create a dental plaque[1]. Bacteria in the biofilm of dental plaque can ferment sugars and release acidic by-products leading to tooth decay. Microbial biofilms provide resistance to chemical removal and disruption of biofilms is imperative to the prevention and management of oral diseases such as caries, periodontal disease and gingivitis[2, 14]. Mouth rinses provide a chemical means to remove bacteria, deodorize and provide prevention or relief of infection[3]. While nothing can replace proper brushing and flossing, only around 10% of patients floss which leaves interproximal plaque that tooth brushing alone cannot reach[2]. Besides providing proper oral hygiene instruction on the benefits of flossing the dental practitioner often adds mouthwash into the patient's oral hygiene regiment to potentially break up and reduce biofilms inter-proximally and on soft tissues. The dominant flora found in the oral cavity are *streptococcus* and *actinomyces* species. S. *mutans* is a facultative anaerobe, gram positive bacteria. This bacterium is cariogenic and heavy colonization of this bacteria by individuals categorizes them as high risk for caries.

Besides providing antimicrobial effects mouth rinses are now marketed for tooth whitening. Esthetics has become important in today's society. People wanting whiter teeth has led to an increase in whitening mouthwashes on the market. Staining of extrinsic origin will affect esthetics and discolor teeth[4.] To combat staining many mouth

rinses now offer hydrogen peroxide in their formulations that claim to whiten teeth[15]. Hydrogen peroxide penetrates the tooth and produces free radicals[15]. These free radicals break apart chromophore bonds which results in a color change in the tooth. These whitening mouth rinses can provide a cost-effective way to whiten teeth[4]. Although there have been many studies that show the effectiveness of whitening product to remove stain and whiten teeth, few studies show if the whitening ingredients alter the antimicrobial effect of these mouth rinses.

Alcohol is still a primary active ingredient in many OTC mouthwashes and helps dissolve the active ingredients in the mouthwash[2]. This ingredient can be problematic for many patients and has been shown to cause burning sensation, drying of mucosa and oral pain[12]. Alcohol free mouthwashes have entered the market and are advertised as being just as effective as alcohol containing mouthwash.

The main ingredients seen in common OTC mouth rinses are Cetylpyridinium Chloride, Essential oils and Chlorhexidine

Cetylpyridinium Chloride (CPC) mouth rinse has a quaternary ammonium compound that reacts with the negatively charged bacterial membrane causing increased permeability and bacterial death[5,17]. It also alters bacterial metabolism and growth[19]. CPC has a substantivity of 6 hours after use[17].

Essential oils mouth rinses contain 2 phenol related essential oils thymol and eucalyptol mixed with menthol and dissolved in alcohol[17,19]. They kill microorganisms by destroying the bacterial cell wall as well as prevent bacterial aggregation in biofilms [18, 19]. Menthol and Thymol can disrupt the lipophilic part of the bacterial membrane causes cellular leakage. It may also cause intracellular damage to components in the cytoplasm

of the bacteria. EO has also been shown to have anti-inflammatory action[18]. Essential oils have substantivity for a few hours after use and approval by the ADA for control of supragingival plaque and gingivitis[19].

Chlorhexidine is the gold standard in the dental industry and the effective method to prevent and disrupt plaque accumulation. It is a broad-spectrum antibiotic and works well against gram negative as well as gram positive bacteria[17]. CHX also has great substantivity and can stay on hard/soft tissue for up to 45 days[17]. The chemical structure is a symmetrical bisbiguanide synthetic antiseptic that disrupts the cell membrane of bacteria causing cellular leakage and cell death[1,17]. It has a dicationic nature that allows it to interact with negatively charged phospholipids in the bacterial cell membrane increasing its permeability[17]. The disruption in the bacterial cell wall cause cytoplasmic leakage and lysis of the cell. Side effects of chlorhexidine include brown discoloration of teeth and altered taste sensation[17] In the United States chlorhexidine mouth rinses are available only through prescription.

PURPOSE

The purpose of this study is to 1) examine and compare effectiveness of Colgate Perioguard™ containing alcohol and Paroex™ Chlorhexidine Gluconate which contains no alcohol. 2) examine the effectiveness of chlorhexidine mouth rinses and OTC mouth rinses. 3) examine and compare the effectiveness of Listerine™ Healthy White Vibrant and Crest 3D Lux Glamorous White™ both whitening mouth rinses to Listerine™ Original and Crest Pro Health™

The mouth rinses will be put through a series of dilutions and plated to determine CFU counts for comparison. The positive control for this study will be Peridex™ chlorhexidine .12% containing alcohol and Paroex™ chlorhexidine .12% with no alcohol. The negative control will be no mouthwash in the serial dilution. The null hypothesis is there will be no difference in the antimicrobial activity of mouth rinses containing H2O2 or alcohol. We will test 6 mouth rinses: Colgate Perioguard™, Paroex™ Chlorhexidine Gluconate, Listerine™ Original, Listerine™ Healthy White Vibrant, Crest Pro-Health™, Crest 3D White Lux Glamorous White™

HYPOTHESIS

Research questions: Will there be a significant change in effectiveness of alcohol containing chlorhexidine mouth rinses and non-alcohol containing chlorhexidine mouth rinse? Will there be a significant change in effectiveness of chlorhexidine mouth rinses and OTC mouth rinses? Will there be a significant change in effectiveness of Listerine™ Healthy White Vibrant and Crest 3D Lux Glamorous White™ both whitening mouth rinses to Listerine™ Original and Crest Pro Health™?

Null hypothesis #1: There will be no difference in effectiveness of alcohol containing chlorhexidine mouth rinses vs non-alcohol containing chlorhexidine mouth rinse.

Null hypothesis #2: There will be no difference in effectiveness of chlorhexidine mouth rinses and OTC mouth rinses.

Null hypothesis #3: There will be no difference in the effectiveness of Listerine™ Healthy White Vibrant and Crest 3D Lux Glamorous White™ both whitening mouth rinses to Listerine™ Original and Crest Pro Health™.

MATERIALS AND METHODS

This study was designed to determine the antimicrobial effectiveness of OTC mouth rinses containing hydrogen peroxide or alcohol against *S. mutans.* The experiment was an in vitro serial dilution using S. mutans stock bacteria, Blood agar media and 6 commonly used mouth rinses. Two serial dilution runs were completed and the results were documented for analysis. The study was conducted at San Antonio, TX after IRB approval.

Bacterial Preparation:

A S. *mutans* inoculated loop was streaked on a blood agar (SBA) petri dish and allowed to incubate for 18-48 hours at 37 degrees C in 5% CO2 with no agitation.

Reagents:

The four over the counter mouth rinses were purchase at a local drug store. The two prescription mouth rinses were ordered through the Fort Hood Billy Johnson Dental Clinic supply.

Serial Dilutions:

A new batch of bacterial suspension was made using phosphate buffer solution (PBS) and previously plated S. *mutans* on a blood agar plate.

This 5ml sample of S. *mutans* bacterial suspension in PBS at 0.281 A_{600} in a 10ml glass tube was used for the serial dilution and plating. The tube was labeled Tube-

0. An OD600 at Time=0 at 0.281 A_{600} was around 5X10^8 CFU/ml. 900µl of each test mouth rinses were added to a new sterile micro-vial labeled T-tube (testing tube). Using a sterile micropipette, 100µl from the bacterial suspension was used to create a 10^7 CFU/ml, a 1/10^{th} dilution. The tube was vigorously swished for one min to simulate intraoral swishing. For the negative control 100µl from Tube 0 was used to create a 10^7 CFU/ml, a 1/10^{th} dilution with no added mouth rinse. 100µl of solution was then transferred from each T-tube containing the 6 mouth rinses to a new tube with 900µl of fresh PBS labeled Tube 1 now containing 10^6 CFU/ml (1/10^{th}) and vortexed for 5 sec. 100µl of solution was transferred from Tube 1 to a new tube with 900µl of fresh PBS labeled Tube 2 containing 10^5 CFU/ml (1/10^{th}) and vortexed for 5 sec. 100µl of solution was transferred from the Tube 2 to a new tube with 900µl of fresh PBS labeled Tube 3 containing 10^4 CFU/ml (1/10^{th}) and vortexed for 5 sec. 100µl of solution was transferred from the Tube 3 to a new tube with 900µl of fresh PBS labeled Tube 4 now containing 10^3 CFU/ml (1/10^{th}) and vortexed for 5 sec.

50µl of solution from Tube 1, 2, 3, 4 was spread on a blood agar plate using a sterile T shaped spreader and incubated for 18-48 hours. Once incubated, the CFUs from each plate was counted for the 4 dilutions plated. Each of the 6 mouth rinses and a negative control were put through 5 serial dilutions.

Figure 1

SERIAL DILUTION FORMAT

Run Dulution	**Tube 0**	**T-tube**	**Tube 1**	**Tube 2**	**Tube 3**	**Tube 4**
Control	S. *mutans* in PBS at 0.281 A_{600} 10^8 CFU/ml	.100 Tube-0 .900 new PBS 10^7 CFU/ml	.100 T-tube .900 new PBS 10^6 CFU/ml	.100 Tube-1 .900 new PBS 10^5 CFU/ml	.100 Tube-2 .900 new PBS 10^4 CFU/ml	.100 Tube-3 .900 new PBS 10^3 CFU/ml
Perioguard	S. *mutans* in PBS at 0.281 A_{600} 10^8 CFU/ml	.100 Tube-0 .900 Perioguard 10^7 CFU/ml	.100 T-tube .900 new PBS 10^6 CFU/ml	.100 Tube-1 .900 new PBS 10^5 CFU/ml	.100 Tube-2 .900 new PBS 10^4 CFU/ml	.100 Tube-3 .900 new PBS 10^3 CFU/ml
Paroex	S. *mutans* in PBS at 0.281 A_{600} 10^8 CFU/ml	.100 Tube-0 .900 Paroex 10^7 CFU/ml	.100 T-tube .900 new PBS 10^6 CFU/ml	.100 Tube-1 .900 new PBS 10^5 CFU/ml	.100 Tube-2 .900 new PBS 10^4 CFU/ml	.100 Tube-3 .900 new PBS 10^3 CFU/ml
Listerine	S. *mutans* in PBS at 0.281 A_{600} 10^8 CFU/ml	.100 Tube-0 .900 Listerine 10^7 CFU/ml	.100 T-tube .900 new PBS 10^6 CFU/ml	.100 Tube-1 .900 new PBS 10^5 CFU/ml	.100 Tube-2 .900 new PBS 10^4 CFU/ml	.100 Tube-3 .900 new PBS 10^3 CFU/ml
Crest	S. *mutans* in PBS at 0.281 A_{600} 10^8 CFU/ml	.100 Tube-0 .900 Crest 10^7 CFU/ml	.100 T-tube .900 new PBS 10^6 CFU/ml	.100 Tube-1 .900 new PBS 10^5 CFU/ml	.100 Tube-2 .900 new PBS 10^4 CFU/ml	.100 Tube-3 .900 new PBS 10^3 CFU/ml
Listerine Whitening	S. *mutans* in PBS at 0.281 A_{600} 10^8 CFU/ml	.100 Tube-0 .900 Listerine Whitening 10^7 CFU/ml	.100 T-tube .900 new PBS 10^6 CFU/ml	.100 Tube-1 .900 new PBS 10^5 CFU/ml	.100 Tube-2 .900 new PBS 10^4 CFU/ml	.100 Tube-3 .900 new PBS 10^3 CFU/ml

Crest Whitening	S. *mutans* in PBS at 0.281 A_{600} 10^8 CFU/ml	.100 Tube-0 .900 Crest Whitening 10^7 CFU/ml	.100 T-tube .900 new PBS 10^6 CFU/ml	.100 Tube-1 .900 new PBS 10^5 CFU/ml	.100 Tube-2 .900 new PBS 10^4 CFU/ml	.100 Tube-3 .900 new PBS 10^3 CFU/ml

RESULTS

The results were obtained by running two serial dilution tests for the 6 mouth rinses and 1 control. A bacterial count was obtained on blood agar plates from 4 dilutions to determine the antimicrobial effects of these mouth rinses against S. *mutans.* The control in the study was S. *mutans* with no mouth rinse.

On figure 1 we can see that Colgate Perioguard™ and Paroex™ Chlorhexidine Gluconate both obtained similar bactericidal results with TFTC or no bacterial growth on the blood agar plates. Colgate Perioguard™, Paroex™ Chlorhexidine Gluconate, Listerine™ Original and Crest Pro-Health™ had similar bactericidal results with TFTC or no bacterial growth on each blood agar plate. Listerine™ Healthy White Vibrant and Crest 3D White Lux Glamorous White™ had the least bactericidal effect. Listerine™ Healthy White Vibrant had TNTC colonies for plate 1 showing minimal bactericidal effect compared with the control plate. Plate 2 showed an CFU count of 55 on run 2 or $1.1X10^3$ CFU/ml. Crest 3D White Lux Glamorous White™ had the least bactericidal effect of the 6 mouth rinses with TNTC CFU on plates 1 and 2. Plate 3 had an average of 26.2 CFU for both runs or $5.3X10^2$ CFU/ml.

A statistical significance was found between the groups of mouth rinses tested and the control using a one-way ANOVA test seen below:

One-way ANOVA of your k=7 independent treatments:

source	sum of squares SS	degrees of freedom ν	mean square MS	F statistic	p-value
treatment	549,813,075,653,765.8750	6	91,635,512,608,960.9844	2.9146	0.0246
error	880,328,764,568,676.1250	28	31,440,313,020,309.8633		
total	1,430,141,840,222,442.0000	34			

Data that had TNTC on every dilution was given a number of 200 CFU which was the cutoff for TNTC and a dilution of 1/10,000. Data that had TFTC for every dilution was given a number of 1 CFU and a dilution of 1/10.

The Bonferroni and Holm multiple comparison test determined there was no statistical difference between each individual mouth rinse but a statistical difference was found between the control and mouth rinses.

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
A vs B	4.2505	0.0722981	insignificant
A vs C	4.2498	0.0723671	insignificant
A vs D	4.2505	0.0722981	insignificant
A vs E	4.2505	0.0722981	insignificant
A vs F	4.2505	0.0722981	insignificant
A vs G	1.8572	0.8200095	insignificant
B vs C	0.0006	0.8999947	insignificant
B vs D	0.0000	0.8999947	insignificant
B vs E	0.0000	0.8999947	insignificant
B vs F	0.0000	0.8999947	insignificant
B vs G	2.3933	0.6110920	insignificant

C vs D	0.0006	0.8999947	insignificant
C vs E	0.0006	0.8999947	insignificant
C vs F	0.0006	0.8999947	insignificant
C vs G	2.3926	0.6113455	insignificant
D vs E	0.0000	0.8999947	insignificant
D vs F	0.0000	0.8999947	insignificant
D vs G	2.3933	0.6110920	insignificant
E vs F	0.0000	0.8999947	insignificant
E vs G	2.3933	0.6110920	insignificant
F vs G	2.3933	0.6110920	insignificant

treatments pair	Bonferroni and Holm T-statistic	Bonferroni p-value	Bonferroni inferfence	Holm p-value	Holm inferfence
A vs B	3.0055	0.0332453	insignificant	0.0332453	* p<0.05
A vs C	3.0051	0.0332831	insignificant	0.0110944	* p<0.05
A vs D	3.0055	0.0332453	insignificant	0.0277045	* p<0.05
A vs E	3.0055	0.0332453	insignificant	0.0221636	* p<0.05
A vs F	3.0055	0.0332453	insignificant	0.0166227	* p<0.05
A vs G	1.3132	1.1986075	insignificant	0.1997679	insignificant

Figure 2

Plating Counts

RUN 1	Plate 1	Plate 2	Plate 3	Plate 4
Control	TNTC	TNTC	130	35
Perioguard	TFTC	0	TFTC	0
Paroex	0	TFTC	TFTC	TFTC
Listerine	0	0	TFTC	0
Crest	TFTC	TFTC	TFTC	TFTC
Listerine Whitening	28	TFTC	TFTC	TFTC
Crest Whitening	TNTC	TNTC	24	12

RUN 2	Plate 1	Plate 2	Plate 3	Plate 4
Control	TNTC	TNTC	126	22
Perioguard	0	0	TFTC	0
Paroex	0	0	TFTC	0
Listerine	TFTC	0	0	TFTC
Crest	0	0	TFTC	0
Listerine Whitening	TNTC	55	19	TFTC
Crest Whitening	TNTC	TNTC	29	12

RUN 3	Plate 1	Plate 2	Plate 3	Plate 4
Control	TNTC	TNTC	TNTC	TNTC
Perioguard	TFTC	TFTC	TFTC	TFTC
Paroex	0	5	1	1
Listerine	TFTC	TFTC	TFTC	TFTC
Crest	TFTC	TFTC	TFTC	TFTC
Listerine Whitening	TNTC	TNTC	147	17
Crest Whitening	TNTC	TNTC	148	18

RUN 4	Plate 1	Plate 2	Plate 3	Plate 4
Control	TNTC	TNTC	TNTC	48
Perioguard	TFTC	TFTC	TFTC	TFTC
Paroex	TFTC	TFTC	TFTC	TFTC
Listerine	TFTC	TFTC	TFTC	TFTC
Crest	TFTC	TFTC	TFTC	TFTC
Listerine Whitening	TNTC	TNTC	TNTC	28
Crest Whitening	TNTC	TNTC	TNTC	66

RUN 5	Plate 1	Plate 2	Plate 3	Plate 4
Control	TNTC	TNTC	TNTC	46
Perioguard	TFTC	TFTC	TFTC	TFTC
Paroex	84	31	7	TFTC
Listerine	TFTC	TFTC	TFTC	TFTC
Crest	TFTC	TFTC	TFTC	TFTC
Listerine Whitening	TNTC	TNTC	TNTC	24
Crest Whitening	TNTC	TNTC	TNTC	96

Series 1 bacterial plate counts

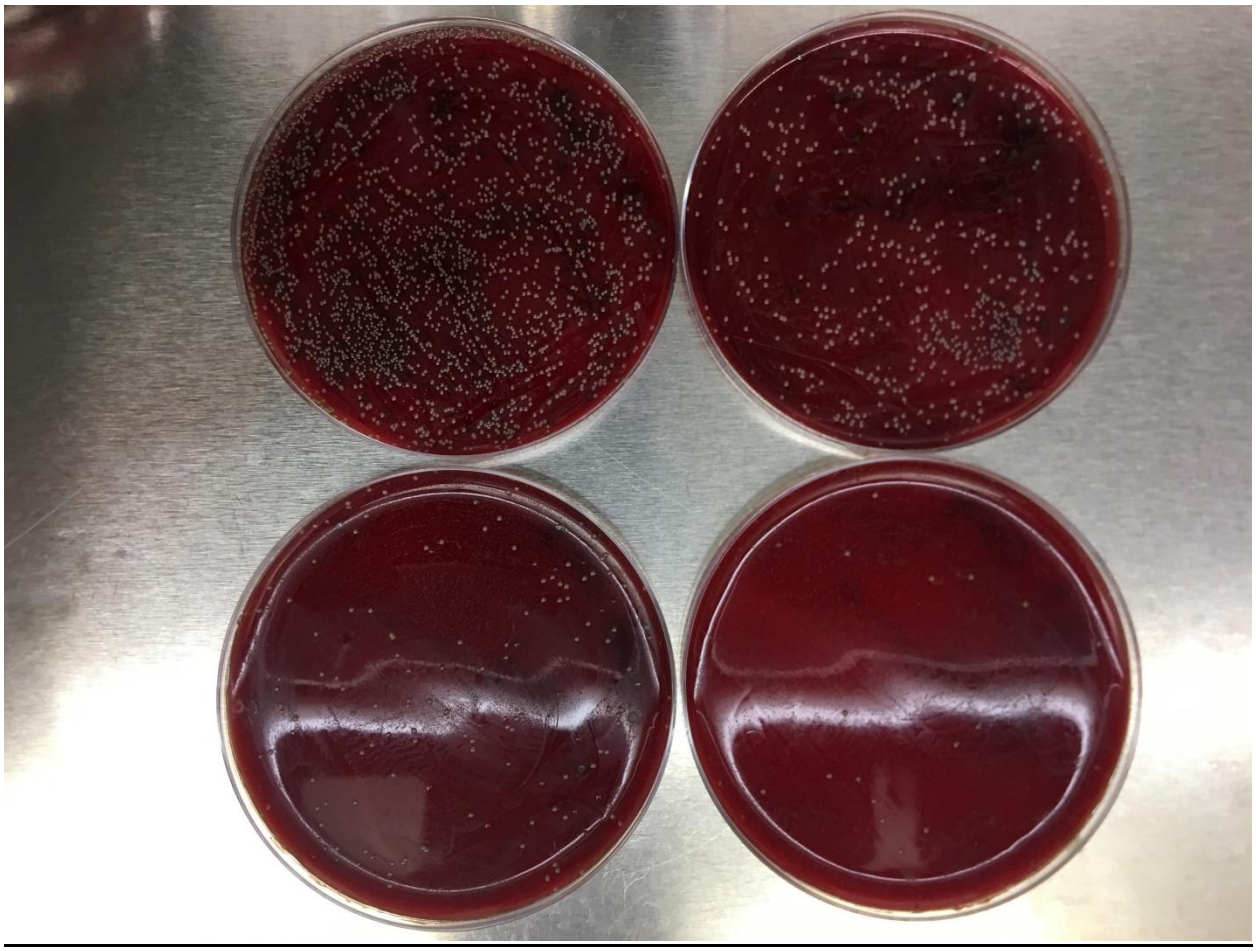
Control:

UL plate 1-TNTC

UR plate 2-TNTC

LL plate 3-130 CFU

LR plate 4-35 CFU



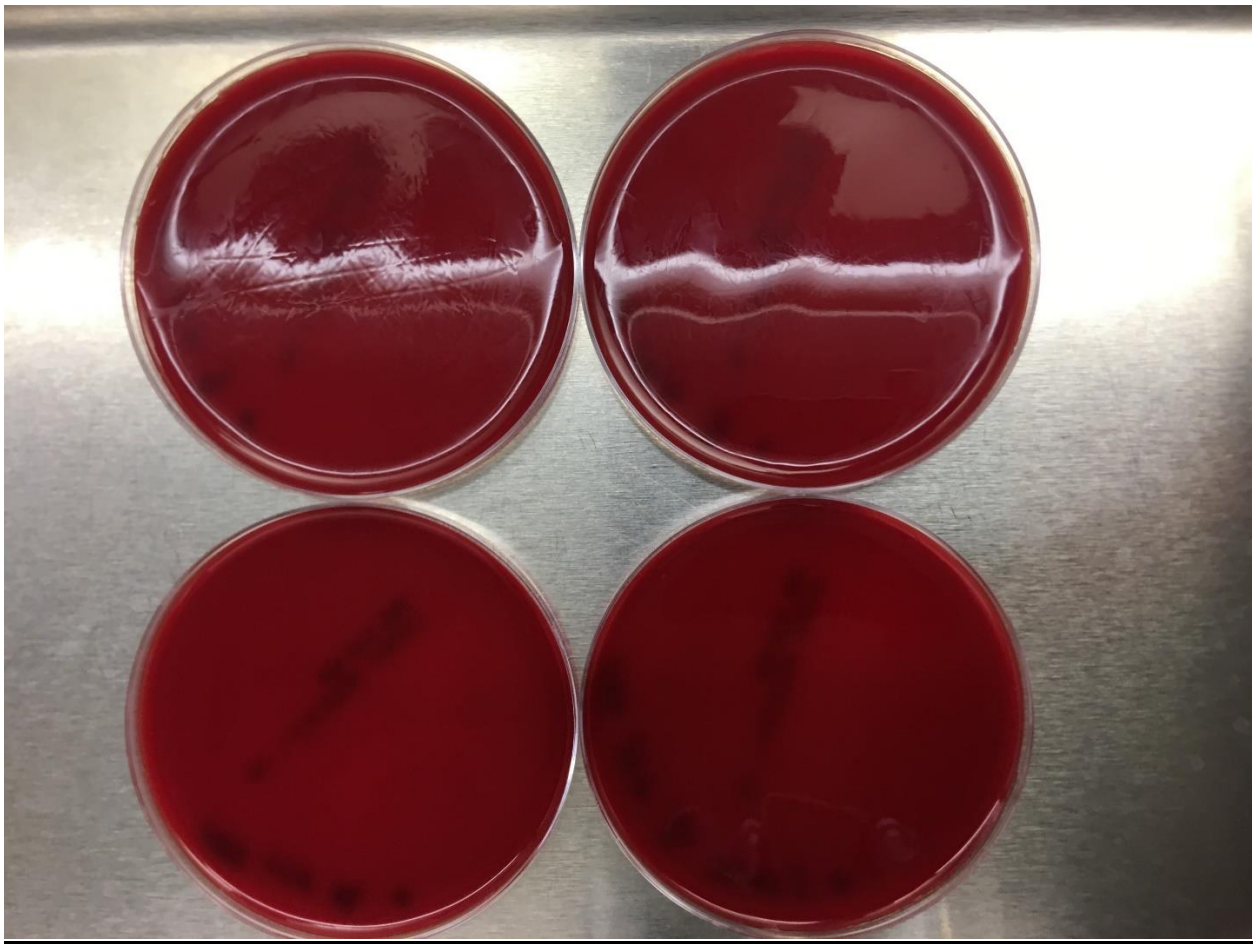
Perioguard:

UL plate TFTC

UR plate 2-0 CFU/ml

LL plate 3-TFTC

LR plate 4-0 CFU/ml



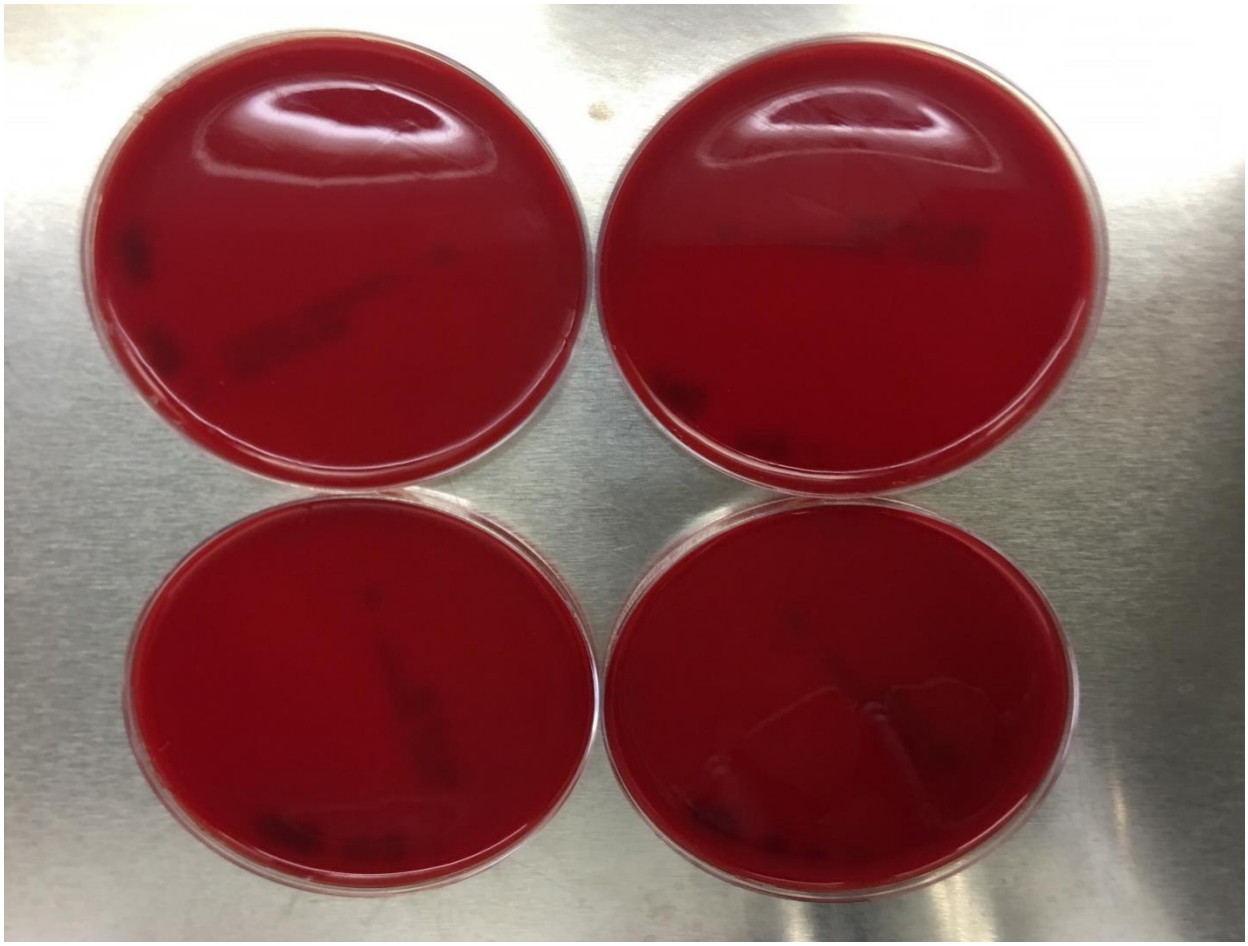
Paroex

UL plate 1-0

UR plate 2-TFTC

LL plate 3-TFTC

LR plate 4-TFTC



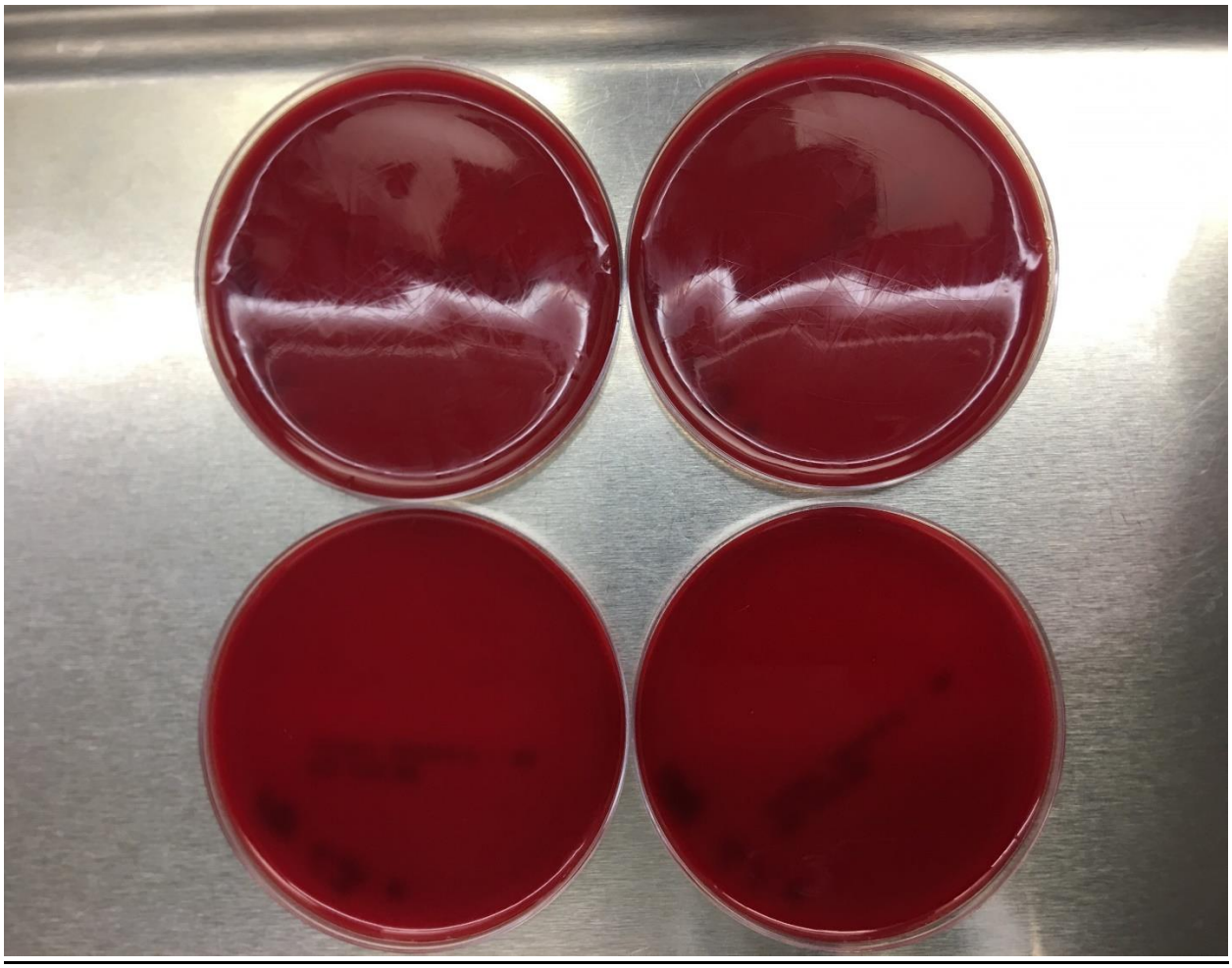
Listerine

UL plate 1-0 CFU/ml

UR plate 2-0 CFU/ml

LL plate 3-TFTC

LR plate 4-0 CFU/ml



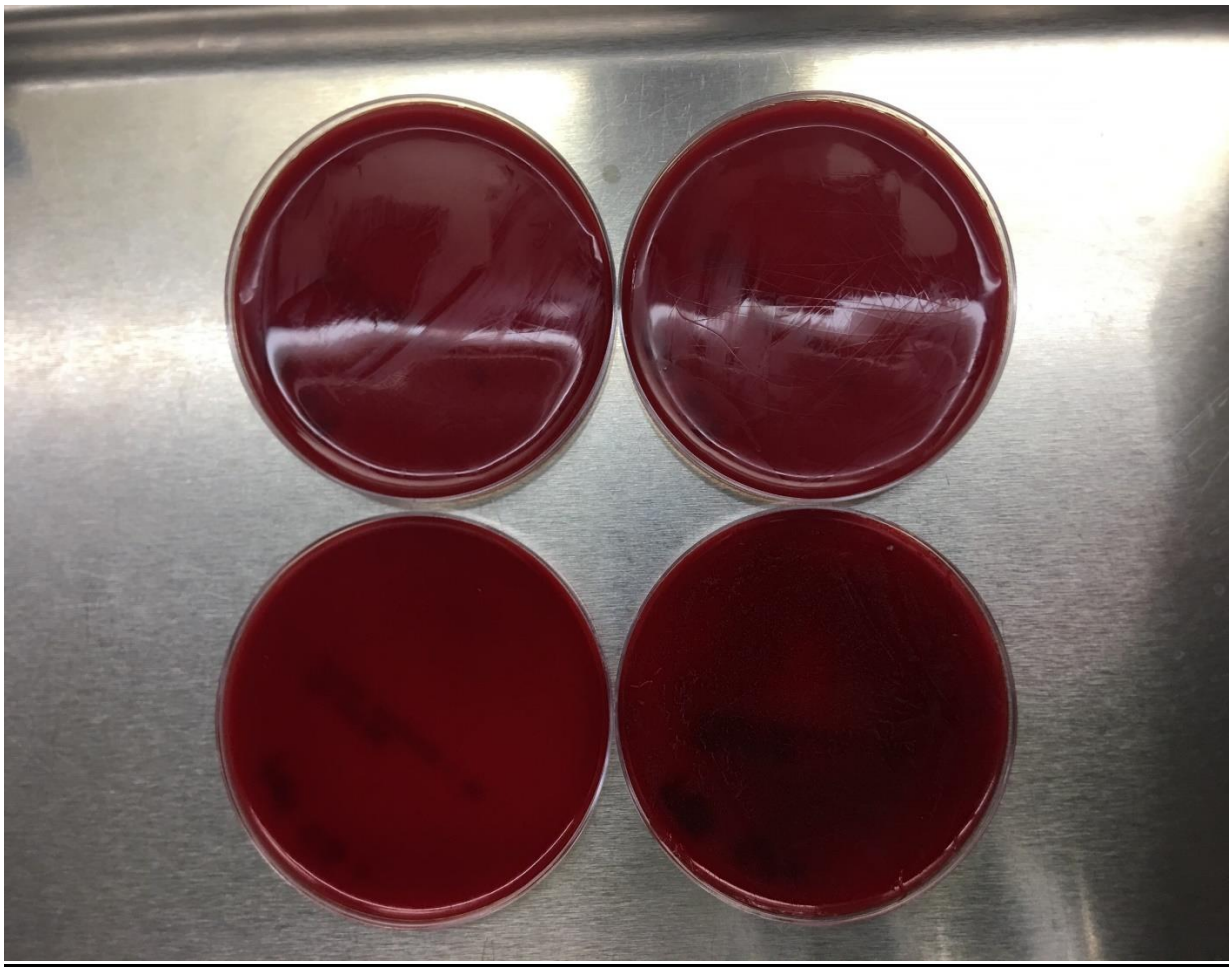
Crest

UL plate 1-TFTC

UR plate 2-TFTC

LL plate 3-TFTC

LR plate 4-TFTC



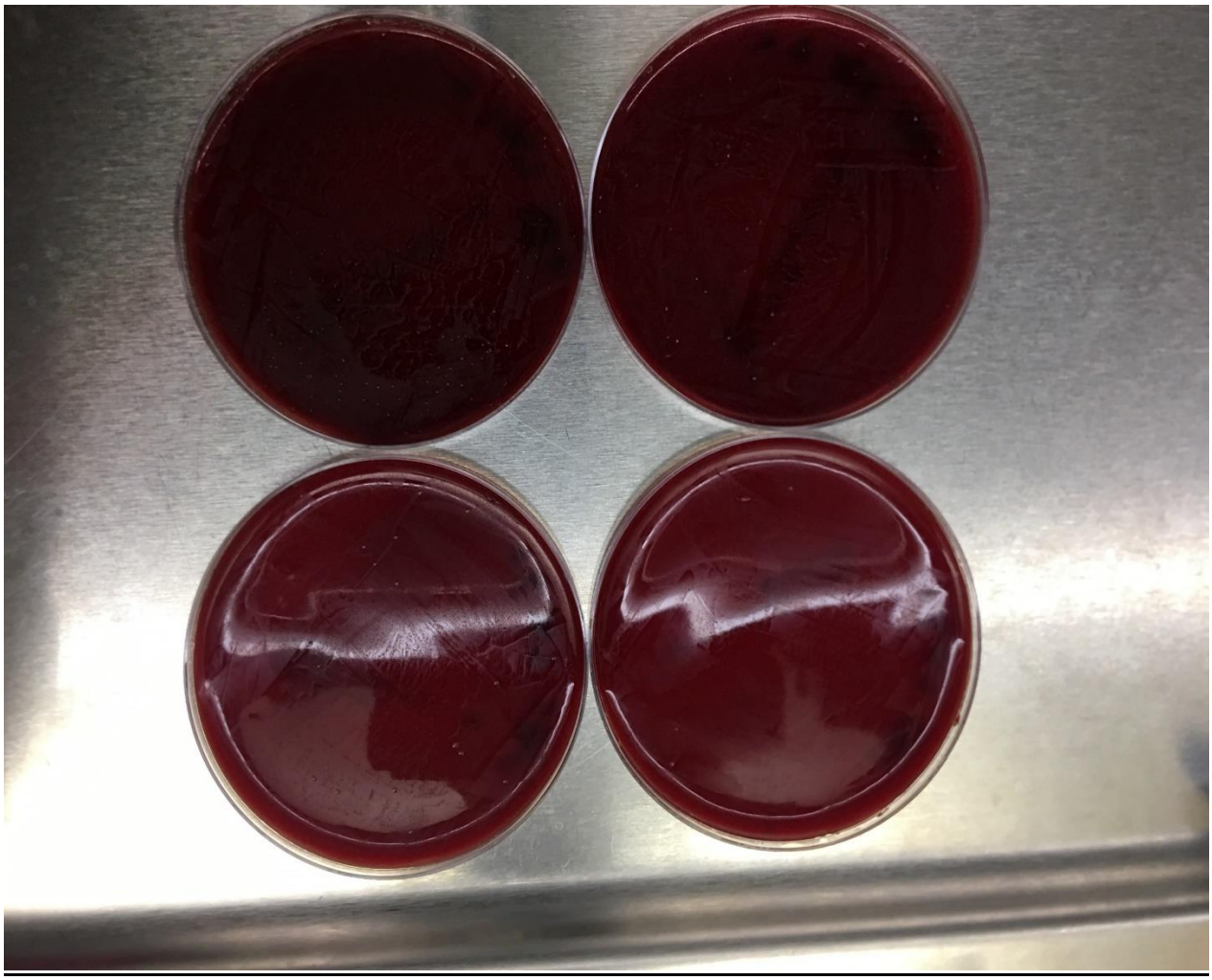
Listerine Whitening

UL plate 1-28 CFU/ml

UR plate 2-TFTC

LL plate 3-TFTC

LR plate 4-TFTC



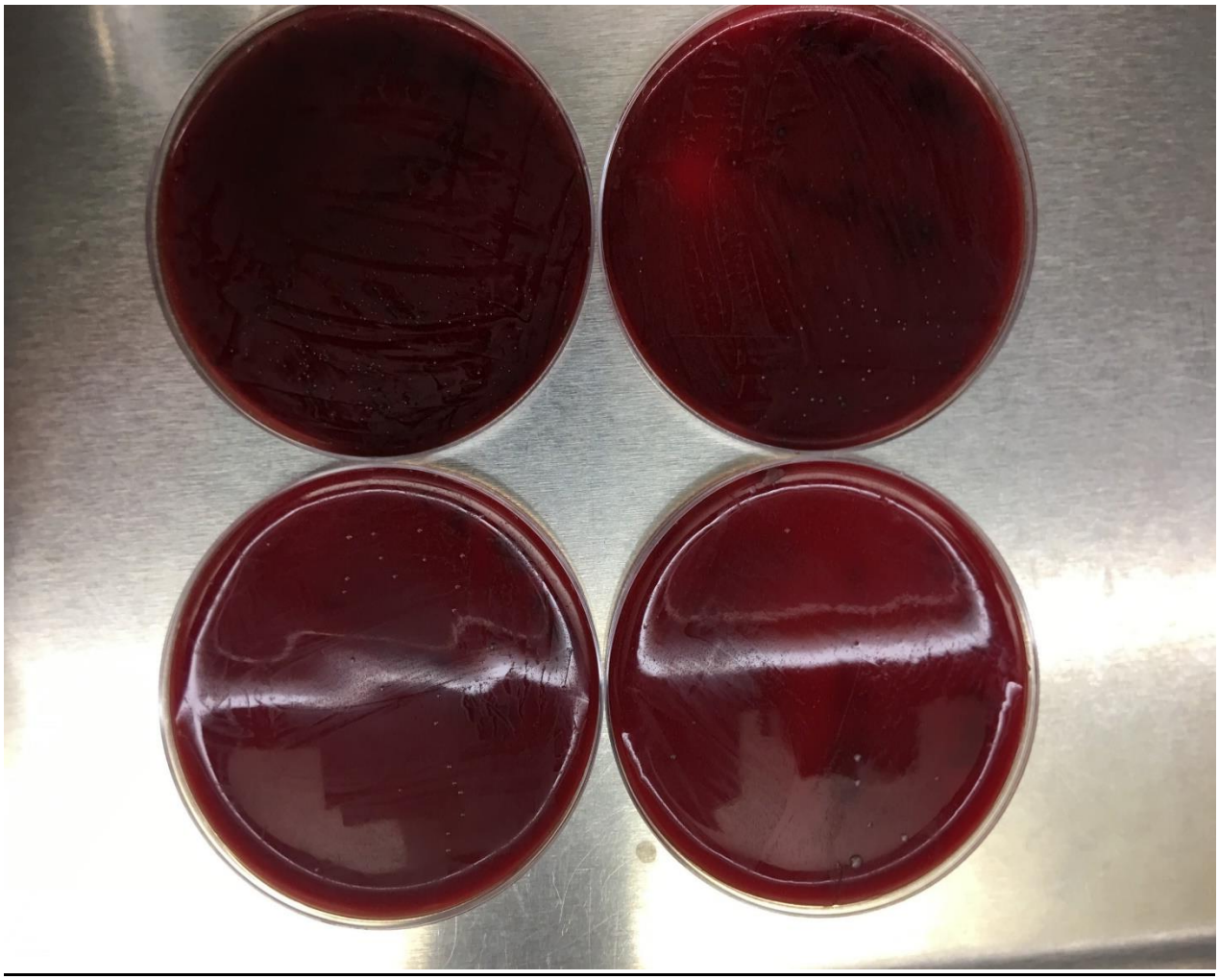
Crest Whitening

UL plate 1-0 TNTC

UR plate 2-0 TNTC

LL plate 3-24 CFU/ml

LR plate 4-12 CFU/ml



DISCUSSION:

Previous studies researching Chlorhexidine mouth rinses with and without alcohol have concluded that both have the same effectiveness in reducing gingival bleeding and plaque compared to brushing alone.

Jose et. al. conducted a randomized, examiner blind, parallel group study consisting of three hundred and nineteen subjects with mild to moderate gingivitis. A baseline gingival severity index (GSI), gingival index (GI) and plaque index (PI) was measured. The group was given a baseline prophy and split into three groups. One group was instructed to brush and use CHX with alcohol, one group was instructed to brush and use CHX without alcohol and the control group was instructed to brush only. After 6 weeks, new GSI, GI and PI were taken. The groups that were using CHX with and without alcohol had similar results and significantly reduced GSI, GI and PI compared to brushing alone.

SANTOS et. al. conducted a randomized, double blind control study consisting of thirty-five dental students from Rio Grande do Sul. The students had three testing periods lasting 4 days each. The students were randomly assigned to three groups. Group A rinsed with CHX with alcohol; Group B CHX without alcohol and Group C rinsed with a placebo. The teeth were evaluated for biofilm accumulation at 24, 48, 72 and 96 hours. Afterward patient reset with a 10-day washout period and switched mouth rinses or placebo. Compared with the placebo, CHX with and without alcohol had similar effects had a higher rate of plaque free zones.

Our study showed similar results when comparing CHX with alcohol vs non-alcohol. Both were just as effective at inhibiting bacterial growth showing no growth or TFTC on blood agar plates (figure 2).

Chlorhexidine has been the gold standard in dentistry and is prescribed by many specialties for post-surgical treatment of soft tissues. Brushing and flossing alone only account for 25% of the oral mucosal surface area. CHX bathes the tooth pellicle, tongue and oral mucosa and inhibits supra and sub gingival plaque[5]. CHX is a positively charged molecule that can attach to the negatively charged inner membrane of bacterial and many fungal organisms. Once bound it disrupts the membrane and causes cytoplasmic leakage and eventually cell death. It is a broad-spectrum antibiotic and kills around 100% of gram positive and negative bacteria. CHX also has been shown to inhibit biofilm growth making it great for treatment after periodontal and oral surgeries to help in would healing. These two mechanisms of action make CHX bactericidal as well as bacteriostatic. It also has great substantivity and stays on the oral mucosal surfaces for at least 12-24 hours. The substantivity is long because the molecule is incorporated into the bacteria and/or pellicle making it unavailable for disposal.

CHX in long term use has its disadvantages. Some side effects of chlorhexidine are tooth/tongue staining, increased tarter formation, dysgeusia and dry mouth. Possible mechanisms for staining are the accumulation of dietary chromogens especially with coffee and tea. Dysgeusia is causes by reduction of paracellular ion channels and mainly effects salty foods.

Over the counter mouth rinses can be purchased anywhere without a prescription from a dentist. The active ingredients of each mouth rinse are different and have different formulations.

Listerine uses essential oils such as eucalyptol 0.092%, thymol 0.064%, methyl salicylate 0.060% and menthol 0.042%. These essential oils are antimicrobial and able to penetrate biofilms effectively. Essential oils are concentrated natural products produced by aromatic plants that can inhibit or slow the growth of bacteria, yeast and mold. The mechanism of action of essential oils is directly related to its hydrophobic nature. Essential oils can permeate the hydrophobic cell wall of bacteria and disrupt the cell wall permeability. This causes cell membrane leakage. Once in the bacterial cell wall it reduces ATP synthesis which prevents essential proteins from being produced. Essential oils have more of an effect on gram positive bacteria than gram negative due to the nature of the membrane. Gram negative bacterial have an outer membrane which is more difficult to penetrate[21].

In our study, Listerine™ Original with essential oils was very effective in preventing S. *mutans* growth on blood agar plate with no growth or TFTC. The result is not surprising since S. *mutans* is gram positive and more susceptible to essential oils when compared to gram negative bacteria. When compared with both CHX mouth rinses it inhibits bacteria just as effectively.

Crest Pro-Health™ contains Cetylpyridinium chloride, a quaternary ammonium compound, that disrupts the cell membrane of bacteria and also inhibits many bacterial functions. Diane Osso et. al. conducted a systematic review comparing 0.12% chlorhexidine gluconate, essential oils, 0.7% cetylpyridinium chloride and

20% aloe vera gel mouth rinses and found 3 studies showing no difference between essential oils and cetylpyridinium, 1 study favored essential oils over cetylpyridinium and 1 found both essential oils and chlorhexidine better than cetylpyridinium in reducing plaque and gingival inflammation. Our study showed no difference in anti-microbial effect of Crest Pro-Health™ with Cetylpyridinium chloride when comparing with CHX and essential oils mouth rinses.

Mouth rinses that advertise secondary benefits such as tooth whitening can make the decision in choosing a mouth rinse difficult for many consumers. Will whitening mouth rinses still provide the antimicrobial benefit of their non-whitening counterparts? To our knowledge no studies have researched the antimicrobial effectiveness of whitening mouth rinses. Many studies conducted with whitening brands have focused efforts to determine how effective they are on whitening compared to other products or methods of whitening. The brands of whitening mouth rinses included in this study were Listerine™ Healthy White Vibrant and Crest 3D White Lux Glamorous White™. Our study focused on the microbial effect of whitening mouth rinses instead of its whitening ability.

The main ingredient in most whitening mouth rinses is sodium fluoride or fluoride ion. In vitro studies have shown that fluoride has some antimicrobial activity against Streptococcus and Lactobacillus species. HF can be transported into the bacterial cell. Once in the cell it disassociates into H+ and F- and acidifies the bacterial cytoplasm. This acidification disrupts the ATP machinery preventing production of vital proteins for the bacteria. Another possible benefit of fluoride is the inhibition of bacteria adhesion to the tooth surface[23].

Ann Thomas et al compared the antimicrobial efficacy of chlorhexidine (0.2%), sodium fluoride (0.05%), fluoride with essential oils (0.05%), alum (0.02 M), green tea, and garlic with lime mouth rinses against S*. mutans*, lactobacilli, and *Candida albicans* in children with severe childhood caries. The study found that CHX and essential oils had more antimicrobial effect when compared to sodium fluoride.

Our study had similar results with whitening mouth rinses showing minimal to no antimicrobial effectiveness against S. *mutans* when compared to CHX, Crest Pro-Health™ and Listerine™ Original. This may have an impact on what recommendations dentists should make based on antimicrobial effects for patients prone to caries.

A major drawback to this study was the limited data set. Only five runs using the mouth rinses and bacterial could be achieved. This pilot run could be used as a basis for a more thorough experiment which could achieve this number of experimental runs in the future. Another drawback to this study was it involved mostly planktonic bacteria. Bacteria on tooth surfaces form a biofilm which allows for added antimicrobial protection. The possibility of growing an artificial biofilm for a future experiment will allow a better real-life scenario. Additional studies with modifications to the bacterial environment to include the creation of a biofilm to test against the mouthwashes has the potential to determine the true antimicrobial effectiveness of whitening mouth rinses. However, this study indicates there is potential for use in high caries risk individuals but further studies may indicate if other supplemental whitening and a more effective mouth rinse should be used.

CONCLUSION:

We cannot reject any of the null hypotheses
From this pilot study we can infer that:

1. Colgate Perioguard™ containing alcohol and Paroex™ Chlorhexidine Gluconate without alcohol have the same antimicrobial effectiveness against S. *mutans*.
2. Colgate Perioguard™ and Paroex™ Chlorhexidine Gluconate when compared to Crest Pro-Health™ and Listerine™ Original have the same antimicrobial effectiveness against S. *mutans*.
3. Listerine™ Healthy White Vibrant and Crest 3D White Lux Glamorous White™ chloride may have the least antimicrobial effectiveness against S. *mutans*.

BIBLIOGRAPHY:

1. Haerian-Ardakani, Ahmad; Rezaei, Mahsa; Ardakani, Mohammadreza Talebi; Valian, Nasrin Keshavarz; Amid, Reza; Meimandi, Mansoor; Esmailnejad, Azadeh; Ariankia, Azarnoosh. "Comparison of Antimicrobial Effects of Three Different Mouthwashes." *"Comparison of Antimicrobial Effects of Three Different Mouthwashes; Iranian Journal of Public Health, Vol. 44, Issue 7, July 1, 2015* | *Online Research Library: Questia*. N.p., n.d. Web. 11 Feb. 2017.

2. Marchetti, Enrico, Stefano Mummolo, Jonathan Di Mattia, Fabio Casalena, Salvatore Di Martino, Antonella Mattei, and Giuseppe Marzo. "Efficacy of Essential Oil Mouthwash with and without Alcohol: A 3-Day Plaque Accumulation Model." *Trials* 12.1 (2011): n. page. Web.

3. Akande, OO, et al. “Efficacy of Different Brands of Mouth Rinses on Oral Bacterial Load Count in Healthy Adults.” *African Journal of Biomedical Research*,

Bioline for the Ibadan Biomedical Communications Group, www.ajol.info/index.php/ajbr/article/view/54160.

4. Jose, A., A. Butler, D. Payne, R. Maclure, P. Rimmer, and M. L. Bosma. "A Randomised Clinical Study to Evaluate the Efficacy of Alcohol-free or Alcohol-containing Mouthrinses with Chlorhexidine on Gingival Bleeding." *Bdj* 219.3 (2015): 125-30. Web.

5. Oliveira, Jbs, Rs Sarlo, E. Bresciani, and Tmf Caneppele. "Whitening Efficacy of Whitening Mouth Rinses Used Alone or in Conjunction With Carbamide Peroxide Home Whitening." *Operative Dentistry* (2017): n. page. Web.

6. Radford, J.r., D. Beighton, Z. Nugent, and R.j. Jackson. "Effect of Use of 0.05% Cetylpyridinium Chloride Mouthwash on Normal Oral Flora." *Journal of Dentistry* 25.1 (1997): 35-40. Web.

7. Haas, Alex Nogueira, Tassiane Panta Wagner, Francisco Wilker Mustafa Gomes Muniz, Tiago Fiorini, Juliano Cavagni, and Roger Keller Celeste. "Essential Oils-containing Mouthwashes for Gingivitis and Plaque: Meta-analyses and Meta-regression." *Journal of Dentistry* 55 (2016): 7-15. Web.

8. Dabholkar, Charuta Sadanand. "Comparative Evaluation of Antimicrobial Activity of Pomegranate-Containing Mouthwash Against Oral-Biofilm Forming

Organisms: An Invitro Microbial Study." *Journal Of Clinical And Diagnostic Research* (2016): n. pag. Web.

9. Tombes, Mary Beth, and Betty Gallucci. "The Effects of Hydrogen Peroxide Rinses on the Normal Oral Mucosa." *Nursing Research* 42.6 (1993): n. pag. Web.

10. Balouiri, Mounyr, Moulay Sadiki, and Saad Koraichi Ibnsouda. "Methods for in Vitro Evaluating Antimicrobial Activity: A Review." *Journal of Pharmaceutical Analysis* 6.2 (2016): 71-79. Web.

11. Aneja KR, Joshi R, Sharma C (2010). The antimicrobial potential of ten often used mouthwashes against four dental caries pathogens. Jundishapur J Microbiol, 3(1): 15-2

12. Tomas I, Cousido MC, Tomas MJL, Caballero L, Diz P (2008). In vivo bactericidal effect of 0.2% chlorhexidine but not 0.12% on salivary obligate anaerobes. Arch Oral Biol, 53(3):1186-1191.

13. Mathew, Shibu Thomas. "Alcohol-containing Mouthwash and Oral Cancer Risk: AÂ Systematic Review." *International Journal of Dental and Medical Specialty* 2.3 (2015): 21. Web.

14. Brown, A.E. 2005. *Benson's Microbiological Applications*, eighth ed. McGraw Hill, New York, NY.

15. Chandki, Rita, et al. “Biofilms: A Microbial Home.” *Journal of Indian Society of Periodontology*, Medknow Publications, 2011, www.ncbi.nlm.nih.gov/pmc/articles/PMC3183659/.

16. Karadas, Muhammet, and Omer Hatipoglu. “Efficacy of Mouthwashes Containing Hydrogen Peroxide on Tooth Whitening.” *The Scientific World Journal*, Hindawi Publishing Corporation, 2015, www.ncbi.nlm.nih.gov/pmc/articles/PMC4534617/.

17. Parashar, Amit. “Mouthwashes and Their Use in Different Oral Conditions.” *Scribd*, Scribd, 2015.

18. Marchetti, Enrico, et al. “Efficacy of Essential Oil Mouthwash with and without Alcohol: a 3-Day Plaque Accumulation Model.” *SpringerLink*, BioMed Central, 15 Dec. 2011, link.springer.com/article/10.1186/1745-6215-12-262.

19. Osso, Diane. “Antiseptic Mouth Rinses: an Update on Comparative Effectiveness, Risks and Recommendations.” *AromaticScience LLC*, 21 Aug. 2014, www.aromaticscience.com/antiseptic-mouth-rinses-an-update-on-comparative-effectiveness-risks-and-recommendations/.

20. Gabriella Otero dos SANTOS. “Chlorhexidine with or without alcohol against biofilm formation: efficacy, adverse events and taste preference” Original Research Periodontology, Braz. Oral Res. 2017;31: e32.

21. Nazzaro, Filomena, et al. “Effect of Essential Oils on Pathogenic Bacteria.” *MDPI*, Multidisciplinary Digital Publishing Institute, 25 Nov. 2013, www.mdpi.com/1424-8247/6/12/1451.

22. Thakur, Sneha, et al. “Comparison of the Antimicrobial Efficacy of Chlorhexidine, Sodium Fluoride, Fluoride with Essential Oils, Alum, Green Tea, and Garlic with Lime Mouth Rinses on Cariogenic Microbes.” *Journal of International Society of Preventive and Community Dentistry*, vol. 5, no. 4, 2015, p. 302., doi:10.4103/2231-0762.161759.

23. Lussi, Adrian, and Thiago Saads Carvalho. “The Future of Fluorides and Other Protective Agents in Erosion Prevention.” *Caries Research*, vol. 49, no. 1, 2015, pp. 18–29., doi:10.1159/000380886.