

HBA-CPC AND STANNOUS FLUORIDE TIME KILL STUDY
MICROBIOLOGY FINAL REPORT FOR THE MONOGRAPH

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Test Products : 0.05% High Bioavailable CPC Rinse Formulation
0.036% CPC Solution prepared in house
Sterile Water (to be used as negative control)
Crest Gum Care (1:4 Dentifrice Slurry)

PURPOSE:

The purpose of this experiment is to evaluate the *in vitro* antimicrobial hostility of High Bioavailable Cetylpyridinium Chloride (HBA-CPC), a standard CPC rinse in water and Crest Gum Care™ dentifrice against a battery of organisms well accepted as the representative organisms responsible for or associated with gingivitis. The battery includes *Actinomyces viscosus*, *Prevotella intermedia*, *Candida albicans*, *Lactobacillus casei*, *Fusobacterium nucleatum*, *Pseudomonas aeruginosa*, *Streptococcus sanguinis*, *Streptococcus mutans*, *Haemophilus actinomycetemcomitans*, *Eikenella corrodens*, *Campylobacter rectus*, *Porphyromonas gingivalis* and pooled saliva sample. A kill time determination or kill kinetic study was planned for this purpose. Kill kinetics have historically provided an appropriate measure of the efficacy of actives / products. The assay is based on the time taken by the test product to kill the representative microorganism. A faster kill therefore indicates greater efficacy of the test product(s).

BACKGROUND:

CPC, a quaternary ammonium chloride, is a cationic surface-active agent and has a long heritage as a broad-spectrum antimicrobial against oral bacteria. The CPC molecule has both a positively charged hydrophilic region and a hydrophobic region. The surfaces of bacteria under normal physiological conditions have a net negative charge, which associates with the positively charged CPC region. Subsequently the hydrophobic portion of CPC then inserts into the cell membrane, which causes leakage of cellular components, disruption of bacterial metabolism, inhibition of cell growth, and finally cell death. Common excipients added to commercial oral care formulations such as surfactants can diminish or even completely neutralize the antimicrobial activity of CPC^{1,2}. Evaluation of CPC-containing mouthrinses using the *In Vitro* Disk Retention Assay and *Ex Vivo* Plaque Glycolysis methods have been published by P&G in the *J. Clin. Dent.* 8: 107-113, 1997. This publication established that CPC in the experimental mouthrinse formulations prepared in-house were sufficiently available and biologically active to deliver antimicrobial benefits. The formulations studied had 0.025%, 0.05%, 0.075% and a 0.1% CPC and showed a percentage availability in the range of 72-77%. The activity of the High

Bioavailable CPC rinse formulations were compared to commercial rinses including ACT[®] Fluoride Anti-Cavity Treatment (Johnson and Johnson Products Inc., Skillman, NJ), Cepacol[®] Mouthwash/Gargle (J.B. Williams Co., Glen Rock, NJ), Scope[®] Mouthwash/Gargle (Procter and Gamble Co., Cincinnati, OH) and a placebo rinse (i.e. experimental mouthwash formulation minus CPC). The three commercial rinses showed percentage availability for CPC of 4%, 54%, and 38%, respectively. From the published analyses, it was apparent that formulations with higher available CPC are associated with greater biological activity as measured by plaque glycolysis, and suggest that these formulations would have a higher probability of showing clinical efficacy.

The FDA Plaque Subcommittee after a series of meetings (from 1993 to 1998) in which they reviewed 40 oral care active ingredients, provided their report to the FDA recommending only three Category I (Safe and Efficacious) actives: cetyl pyridinium chloride (at levels between 0.045% and 0.1%), a fixed combination of essential oils, and stannous fluoride (at 0.454%), entitling anti-gingivitis and anti-plaque claims under the monograph rule-making process. Based on the technical precedence that Warner Lambert had established with the FDA Advisory Committee in its pursuit of the new claim, "Fights Germs that Cause Gingivitis," it was reasonable for Procter & Gamble to generate microbiocidal data for both CPC and stannous fluoride³. Like Warner Lambert, the current investigation was designed to dimensionalize the antimicrobial activity of a 0.05% HBA-CPC rinse formulation and a stannous dentifrice against organisms causing gingivitis in support of the claim "Fights Germs that Cause Gingivitis" for both of these actives.

REQUIREMENTS:

TEST PRODUCTS

0.05% High Bioavailable CPC Rinse Formulation

Preparation:

0.053g CPC was dissolved per 100 mL of water (this accounts for the 95% purity of the CPC used in the making of this formulation. For the complete formulation refer to Appendix 1). Product was prepared in the Process Research Laboratory Organization (PRLO) and dispensed in approximately 500 mL volumes in amber colored bottles (Lot# HCS 723-067).

0.036% CPC Solution prepared in house

Preparation:

This solution was prepared fresh before use in each experiment. Each experiment utilized 40 mL volume of the standard CPC solution prepared by adding 0.0152g of CPC (M# 10045571, batch #11) to 40 mL sterile water (this accounted for the 95% purity of CPC used in the making of this solution).

Sterile water (was used as a negative control)

Crest Gum Care™ (1:4 Dentifrice Slurry)

Preparation:

The slurry was prepared fresh before use in each experiment. Ten (10) grams of the dentifrice was weighed out into a sample cup containing a sterile magnet. Thirty (30) mL sterile water was added to the weighed dentifrice and the contents were vortexed at high speed for 2 minutes. The slurry was subsequently placed on a magnetic stirrer at a low speed for 5-10 minutes (to help break the foam generated by vortexing the slurry).

(Product Batch # for Crest Gum Care: HCS2879-021; Master formula # HCS887-155 -- contained 0.454% SnF₂. For the formula card refer to Appendix 2).

TEST ORGANISMS

Actinomyces viscosus ATCC 19246

Propagation: This organism was re-hydrated and grown in Brain Heart Infusion Broth (BHI, Anaerobe Systems Catalog# AS-872) at 36°C in a CO₂ incubator (5% CO₂) for 24 hrs before being used for the time kill assay

Assay: The assay medium was Brucella Blood Agar (BBA – Anaerobe Systems Catalog # AS-141). After plating, the inoculated plates were incubated at 36°C in a CO₂ incubator (5% CO₂) for 48 hrs before being read in an automated colony counter.

Prevotella intermedia ATCC 25611

Propagation: This organism was re-hydrated and grown in Chopped Meat Medium (CMM, Anaerobe Systems Catalog # AS-811) at 35°C under anaerobic conditions for 48 hrs before being used for the time kill assay.

Assay: The assay medium was Brucella Blood Agar (BBA – Anaerobe Systems Catalog # AS-141). After plating, the plates were incubated anaerobically at 35°C for 48 hrs before being read in an automated colony counter

Candida albicans ATCC 10231

Propagation: This organism was re-hydrated and grown in Yeast and Mold Broth (YM, Difco 271120, 0711-17; prepared in house) at 23°C under aerobic conditions for 48 hrs before being used for the time kill assay.

Assay: The assay medium was Potato Dextrose Agar (PDA – Difco 213200;0013-07; prepared in house). After plating, the plates were incubated aerobically at 23°C for 48 hrs before being read in an automated colony counter.

Lactobacillus casei ATCC 393

Propagation: This organism was re-hydrated and grown in Lactobacilli MRS Broth (Difco 0881; prepared in-house) at 36°C in a CO₂ incubator (5% CO₂) for 24 hrs before being used for the time kill assay.

Assay: The assay medium was MRS Agar (MRSA–Difco 288210; 0882-17; prepared in-house). After plating, the plates were incubated at 36°C in a CO₂ incubator (5% CO₂) for 48 hrs before being read in an automated colony counter.

Fusobacterium nucleatum ATCC 10953

This organism was re-hydrated and grown in Brain Heart Infusion Broth (BHI, Anaerobe Systems Catalog # AS-872) at 35°C under anaerobic conditions for 24 hrs before being used for the time kill assay.

Assay: The assay medium was Brucella Blood Agar (BBA – Anaerobe Systems Catalog # AS-141). After plating, the plates were incubated anaerobically at 35°C for 48 hrs before being read in an automated colony counter.

Pseudomonas aeruginosa ATCC 27853

Propagation: This organism was re-hydrated and grown in Nutrient Broth (Difco 0003-17-8; prepared in house) aerobically at 33°C for 24 to 48 hrs before being used for the time kill assay.

Assay: The assay medium was Tryptic Soy Agar [TSA - Difco (BD) 236920; prepared in house]. After plating, the plates were incubated aerobically at 33°C for 24 to 48 hrs before being read in an automated colony counter.

Streptococcus sanguinis ATCC 10556

Propagation: This organism was re-hydrated and grown in Brain Heart Infusion Broth (BHI, Anaerobe Systems Catalog# AS-872) at 36°C in a CO₂ incubator (5% CO₂) for 24 hrs before being used for the time kill assay.

Assay: The assay medium was Tryptic Soy Agar [TSA – Difco(BD)236920; prepared in house]. After plating, the plates were incubated at 36°C in a CO₂ incubator (5% CO₂) for 48 hrs before being read in an automated colony counter.

Streptococcus mutans ATCC 35668

Propagation: This organism was re-hydrated and grown in Brain Heart Infusion broth (BHI, Anaerobe Systems Catalog# AS-872) at 36°C in a CO₂ incubator (5% CO₂) for 24 hrs before being used for the time kill assay.

Assay: The assay medium was Tryptic Soy Agar [TSA – Difco(BD)236920]. After plating, the plates were incubated at 36°C in a CO₂ incubator (5% CO₂) for 48 hrs before being read in an automated colony counter.

***Haemophilus actinomycetemcomitans* ATCC 29522**

Propagation: This organism was re-hydrated and grown in Brain Heart Infusion Broth (BHI, Anaerobe Systems Catalog# AS-872) at 36°C in a CO₂ incubator (5% CO₂) for 48 hrs before being used for the time kill assay.

Assay: The assay medium was Brain Heart Infusion Agar (BHIA, Anaerobe Systems Catalog# AS-6426) After plating the plates were incubated at 36°C in a CO₂ incubator (5% CO₂) for 48 hrs before being read in an automated colony counter

***Eikenella corrodens* ATCC 23834**

Propagation: This organism was re-hydrated and grown in Brain Heart Infusion Broth (BHI, Anaerobe Systems Catalog# AS-872) supplemented with 16.7% filter sterilized Fetal Bovine Serum at 36°C in a CO₂ incubator (5% CO₂) for 48 hrs before being used for the time kill assay.

Assay: The assay medium was GC Agar [BD 211275 (4311275)] and Brucella Blood Agar (BBA – Anaerobe Systems Catalog# AS-141). After plating, the plates were incubated at 36°C in a CO₂ incubator (5% CO₂) for 48-72 hrs before being read in an automated colony counter.

***Campylobacter rectus* ATCC 33238**

Propagation: This organism was re-hydrated and grown in Peptone Yeast Extract with Glucose (PYG), Formate and Fumarate (Anaerobe Systems Catalog# AS-858) anaerobically at 35°C for 24 hrs before being used for the time kill assay.

Assay: The assay medium was Brucella Blood Agar (BBA - Anaerobe Systems Catalog# AS-141) After plating, the plates were incubated anaerobically at 35°C for 48 hrs before being read in an automated colony counter.

***Porphyromonas gingivalis* ATCC 33277**

Propagation: This organism was re-hydrated and grown in Brain Heart Infusion Broth (BHI, Anaerobe Systems Catalog# AS-872) anaerobically at 37°C for 48 hrs before being used for the time kill assay.

Assay: The assay medium was Brucella Blood Agar (BBA - Anaerobe Systems Catalog# AS-141). After plating, the plates were incubated anaerobically at 35°C for 48 hrs before being read in an automated colony counter

Pooled saliva sample.

Stimulated saliva was collected from six subjects (10 mL per subject). Equal volumes from each were combined to produce pooled stimulated whole saliva. This was characterized for the presence of different class of organisms by plating the pooled sample on various growth media. The saliva sample was simultaneously subjected to the kill kinetics.

For characterization, the following media were used:

MRSA [Difco 288210; 0882-17]: prepared in-house: for Lactobacilli. The growth conditions included incubation at 36°C in a 5% CO₂ atmosphere for 48 hrs.

TSA [Difco (BD) 236920]: prepared in-house: for Total Aerobes. The growth conditions included incubation aerobically at 33°C for 48 hrs.

BBA [Catalog # Anaerobe Systems AS-141]: for Total Facultative Anaerobes. The growth conditions included incubation anaerobically at 35°C for 48 hrs.

Enriched Tryptic Soy Agar containing Nalidixic acid and Vancomycin [ETSA-NV; Anaerobe Systems Catalog # AS- 547]: for Total Facultative GNAs. The growth conditions included incubation anaerobically at 36°C for 48 hrs.

Mitis Salivarius Agar [MSA; Anaerobe Systems catalog #AS-544]: for Total Streptococci. The growth conditions included incubation at 35°C in a 5% CO₂ atmosphere for 48 hrs.

Potato Dextrose Agar [PDA; Difco 213200;0013-07]: (prepared in-house) for Total Yeasts. The growth conditions included incubation aerobically at 23°C for 48 hrs.

Cadmium Sulfate Fluoride Acridine Trypticase Agar [CFAT, Anaerobe Systems catalog# AS-6424]: for Corynebacterium. The growth conditions included incubation anaerobically at 35°C for 48 hrs.

For the kill kinetics, the following media were used:

TSA for Total Aerobes. The growth conditions included incubation aerobically at 33°C for 48 hrs

BBA for Total Facultative Anaerobes. The growth conditions included incubation anaerobically at 35°C for 48 hrs

ETSA-NV for Total Facultative GNAs. The growth conditions included incubation anaerobically at 35°C for 48 hrs

Tween Modified Letheen Broth (TMLB) [Catalog # 263010; 0630-17]

EQUIPMENT

1. Analytical balance- Mettler- Serial # F4A169 (HC 2211), located in DV1 404
2. Spiral platers : Spiral Biotech Autoplate 4000, plater#1, serial # AP4A260 and plater #2, serial # AP4A169, both located in DV1-614
3. Vortex Genie 2 #1 VWR Scientific Model G-560 Serial # 2-220005, located in DV1 614
4. Vortex Genie 2 #2 Fischer Scientific Model G-560 Serial # 2-138021, located in DV1 614
5. Warm Room, 33°C, 60% RH, location: DV1-407
6. Walk- In 23°C Incubator, 60% RH, location: DV1-409
7. Spiral Biotech Q Counter (serial # 00060030 located in DV1-418)
8. Incubator: VWR Scientific CO₂ Incubator, model # 2300, HC 00003150
9. VWR Multi-Tube vortexer, serial # 0375, Catalog# 58816-115
10. Stirrer/ Hot plate, Corning Model# PC-320 (LR-33491)
11. Anaerobic Chambers #1 AALC-S Serial # AC861901R, located in DV1-404
12. Anaerobic Chambers #2 AALC, Serial # AAL900402, located in DV1-614

LAB-WARE

1. Sample cups (120mL volume)
2. Calibrated Eppendorf pipettes
3. Eppendorf pipette tips catalog # 2249006-1
4. Sterile glass tubes for preparing dilutions
5. 50 mL serological pipettes (VWR 53283-712)
6. 10 mL serological pipettes (VWR 53283-708)

PROCEDURE FOR THE KILL KINETICS

1. The ATCC strains for representative organisms were used in the kill kinetic study after their second or third pass in the medium of choice. In each case, the inoculum was enumerated at the start, middle, and at the end of the assay. The dilutions plated included 10⁻³, 10⁻⁵ and 10⁻⁷. In the case of the pooled saliva sample, the same three dilutions were plated for enumeration of total organisms in the sample. This step was important since each product was tested against each organism at each of the 4 time points (15, 30, 60 and 120 seconds) in triplicate. Hence, checking the viability / bio-load of each representative organism was an essential internal control for the assay.
2. 1.0 mL of inoculum was introduced into 9.0 mL of the test product and the contents were mixed by vortexing
3. Aliquots of 1.0 mL were withdrawn and expelled at 15, 30, 60 and 120 seconds after exposure into 9.0 mL of TMLB to give a 10⁻¹ dilution.
4. Further 10⁻³ and 10⁻⁵ dilutions of the above (in step 3) were prepared in 0.9% sterile saline solution (0.1 mL of the diluted organism + 9.9 mL of saline).
5. All the three dilutions were spiral-plated on appropriate growth medium and incubated under optimum growth conditions (as outlined in the section on **Test organisms**).
6. To **characterize** the pooled whole saliva inoculum, a 10⁻¹ dilution was plated to enumerate Total Corynebacterium while 10⁻², 10⁻⁴ and 10⁻⁶ dilutions were plated to enumerate Total Aerobes, Total Facultative Anaerobes, Total

Yeasts, Total Streptococci, Total Lactobacilli, Total Facultative GNAs and Total Corynebacteria. The 10^{-1} , 10^{-3} and 10^{-5} dilutions were plated for the **time kill kinetics** of pooled saliva sample and the samples were evaluated for Total Aerobes, Total Facultative Anaerobes and Total Facultative GNAs.

7 All plates were counted using an automated colony counter and the results were reported as colony-forming units per milliliters (**cfu/mL**). For the kill kinetics, the cfu/mL for the 10^{-1} , 10^{-3} , and 10^{-5} dilutions were averaged for each experimental set, which was then used to calculate the **log cfu/mL**. The log cfu/mL value for each of the test products was next subtracted from the log cfu/mL for the Water Control to yield a **log reduction in cfu/mL** post exposure of the representative organism to the test products. A **percentage reduction in cfu** was also calculated and reported for each test product with respect to the Water Control.

References:

- 1 Jenkins S, Addy M, Wade W, Newcombe RG. The magnitude and duration of the effects of some mouthrinse products on salivary bacteria counts. *J Clin Periodontol* 21:397-401, 1994.
- 2 Pader M: Mouthwash formulation. In: *Oral Hygiene Products and Practice, Cosmetic Science and Technology Series*, Jungermann, E, Ed., Marcel Dekker, Inc., New York, 489-516, 1998.
3. Memo by Todd J. Banks on Review of Warner Lambert's Deliberations with the Plaque and Gingivitis Subcommittee, dated April 4th 2001

OBSERVATIONS / TEST RESULTS

Tabulated below in Tables 1 and 2, is the summary of the kill kinetic data after 30 and 60 seconds of exposure of the representative organism to the test product. Photographs of plates showing inhibition of organisms are attached at the end of the report.

Table # 1: KILL KINETIC STUDY DATA AFTER 30 SECONDS EXPOSURE OF THE REPRESENTATIVE ORGANISM TO THE TEST PRODUCT

Representative Organisms	0.05% HBA-CPC		0.036% CPC solution		Crest Gum Care		Water	
	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water
<i>A.viscosus</i> ATCC19246	3.6	99.97	4.07	99.81	3.6	99.97	7.22	-----
<i>C.albicans</i> ATCC 10231	4.18	98.78	3.70	99.82	3.60	99.86	6.47	-----
<i>C.rectus</i> ATCC 33238	3.6	93.08	3.6	93.08	3.6	93.08	4.84	-----
<i>F.nucleatum</i> ATCC 10953	3.6	99.99	6.72	85.48	3.6	99.99	7.65	-----
<i>H.actinomycetemcomitans</i> ATCC 29522	5.01	99.85	7.69	34.89	4.11	99.97	7.90	-----
<i>L.casei</i> ATCC 393	7.64	50.01	7.79	29.17	3.6	Approx 100	7.94	-----
<i>P.aeruginosa</i> ATCC 27853	6.02	98.85	5.70	99.5	7.55	74.6	8.29	-----
<i>P.intermedia</i> ATCC 25611	3.6	Approx 100	3.65	Approx 100	3.6	Approx 100	8.76	-----
<i>P.gingivalis</i> ATCC 33277	3.6	Approx 100	3.6	Approx 100	3.6	Approx 100	8.25	-----
<i>S.mutans</i> ATCC 35668	5.33	99.64	4.89	99.87	3.6	99.99	7.77	-----

Table # 1: KILL KINETIC STUDY DATA AFTER 30 SECONDS EXPOSURE OF THE REPRESENTATIVE ORGANISM TO THE TEST PRODUCT

Continued

Representative Organisms	0.05% HBA-CPC		0.036% CPC solution		Crest Gum Care		Water	
	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water
<i>S.sanguinis</i> ATCC 10556	3.6	99.69	3.6	99.69	3.6	99.69	6.12	-----
Stmulated Whole Saliva Total Aerobes	3.65	99.16	3.6	99.27	3.6	99.27	5.74	-----
Stmulated Whole Saliva Total Facultative Anaerobes	3.6	96.80	3.62	96.72	3.6	96.80	5.16	-----
Stmulated Whole Saliva Total GNAs	3.69	99.88	3.62	99.90	3.6	99.90	6.63	-----
<i>Eikenella corrodens</i> ATCC 23834 (GC agar)	4.95	96.98	5.32	93.95	3.63	99.89	6.82	-----
<i>Eikenella corrodens</i> ATCC 23834 (BBA agar)	5.08	96.13	5.65	84.29	3.6	99.90	6.83	-----

Table # 2: KILL KINETIC STUDY DATA AFTER 60 SECONDS EXPOSURE OF THE REPRESENTATIVE ORGANISM TO THE TEST PRODUCT

Representative Organisms	0.05% HBA-CPC		0.036% CPC solution		Crest Gum Care		Water	
	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water
A.viscosus ATCC 19246	3.6	99.98	3.62	99.97	3.6	99.98	7.24	-----
C.albicans ATCC 10231	4.01	99.12	3.6	99.86	3.6	99.86	6.47	-----
C.rectus ATCC 33238	3.6	92.26	3.6	92.26	3.6	92.26	4.80	-----
F.nucleatum ATCC 10953	3.6	99.99	3.71	99.99	3.6	99.99	7.72	-----
H.actinomycetemcomitans ATCC 29522	3.94	99.99	6.23	97.6	3.63	99.99	7.92	-----
L.casei ATCC 393	6.74	94.44	7.47	70.7	3.6	Approx 100	8.02	-----
P.aeruginosa ATCC 27853	5.80	99.40	5.58	99.74	6.77	96.41	8.28	-----
P.intermedia ATCC 25611	3.6	Approx 100	3.6	Approx 100	3.6	Approx 100	8.65	-----
P.gingivalis ATCC 33277	3.6	Approx 100	3.6	Approx 100	3.6	Approx 100	8.25	-----
S.mutans ATCC 35668	4.97	99.84	4.77	99.89	3.6	99.99	7.76	-----

Table # 2: Kill kinetic study data after 60 seconds exposure of the representative organism to the test product

Continued

Representative Organisms	0.05% HBA-CPC		0.036% CPC solution		Crest Gum Care		Water	
	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water	Log cfu/mL	% Germ kill wrt Water
S.sanguinis ATCC 10556	3.6	99.72	3.6	99.72	3.6	99.72	6.16	-----
Stmulated Whole Saliva Total Aerobes	3.6	99.24	3.6	99.24	3.6	99.24	5.73	-----
Stmulated Whole Saliva Total Facultative Anaerobes	3.6	96.65	3.6	96.65	3.6	96.65	5.12	-----
Stmulated Whole Saliva Total GNAs	3.6	99.9	3.6	99.9	3.6	99.9	6.62	-----
Eikenella corrodens ATCC 23834 (GC agar)	3.93	99.91	5.04	98.95	3.6	99.96	7.08	-----
Eikenella corrodens ATCC 23834 (BBA agar)	4.08	99.91	5.05	99.24	3.6	99.97	7.19	-----

Table # 3: LOG REDUCTION IN COLONY FORMING UNITS AFTER 30 AND 60 SECONDS EXPOSURE OF THE REPRESENTATIVE ORGANISMS TO TEST PRODUCTS

Representative Organisms	0.05% HBA-CPC		Crest Gum Care		0.036% CPC solution	
	Log reduction in cfu/mL (wrt water)					
	30 seconds exposure	60 seconds exposure	30 seconds exposure	60 seconds exposure	30 seconds exposure	60 seconds exposure
A.viscosus ATCC19246	3.62	3.64	3.62	3.64	3.15	3.62
C.albicans ATCC 10231	2.29	2.46	2.87	2.87	2.77	2.87
C.rectus ATCC 33238	1.24	1.2	1.24	1.20	1.24	1.2
F.nucleatum ATCC 10953	4.05	4.12	4.05	4.12	0.93	4.01
H.actinomycetemcomitans ATCC 29522	2.89	3.98	3.79	4.29	0.21	1.69
L.casei ATCC 393	0.30	1.28	4.34	4.42	0.15	0.55
P.aeruginosa ATCC 27853	2.27	2.48	0.74	1.51	2.59	2.7
P.intermedia ATCC 25611	5.16	5.05	5.16	5.05	5.11	5.05
P.gingivalis ATCC 33277	4.65	4.65	4.65	4.65	4.65	4.65
S.mutans ATCC 35668	2.44	2.79	4.17	4.16	2.88	2.99

The log reduction values are calculated from tables 1 and 2 above.
**Table # 3: LOG REDUCTION IN COLONY FORMING UNITS AFTER 30 AND 60 SECONDS
EXPOSURE OF THE REPRESENTATIVE ORGANISMS TO TEST PRODUCTS**
Continued

Representative Organisms	0.05% HBA-CPC		Crest Gum Care		0.036% CPC solution	
	Log reduction in cfu/mL (wrt water)					
	30 seconds	60 seconds	30 seconds	60 seconds	30 seconds	60 seconds
<i>S.sanguinis</i> ATCC 10556	2.52	2.56	2.52	2.56	2.52	2.56
Stimulated Whole Saliva Total Aerobes	2.09	2.13	2.14	2.13	2.14	2.13
Stimulated Whole Saliva Total Facultative Anaerobes	1.56	1.52	1.56	1.52	1.54	1.52
Stimulated Whole Saliva Total GNAs	2.94	3.02	3.03	3.02	3.01	3.02
<i>Eikenella corrodens</i> ATCC 23834 (GC agar)	1.87	3.15	3.19	3.48	1.50	2.04
<i>Eikenella corrodens</i> ATCC 23834 (BBA agar)	1.75	3.11	3.23	3.59	1.18	2.14

The log reduction values are calculated from Tables 1 and 2 above.

KEY LEARNINGS

Tables 1, 2, and 3 showing the percentage germ kill and the log reductions in colony forming units clearly indicate that both the High Bioavailable CPC (HBA-CPC) rinse formulation and Crest Gum Care™ dentifrice demonstrate substantial antimicrobial hostility toward the representative microorganisms implicated in gingivitis within 30 seconds of exposure to the test product. An increased kill was evident in case of some organisms after an increased exposure time of 60 seconds. *P.aeruginosa* and *L.casei* seemed to be the least sensitive of the entire battery of microorganisms selected for microbiocidal testing to the hostility of the High Bioavailable CPC and Crest Gum Care™. Neither *P.aeruginosa* and *L.casei* are directly implicated as causative agents of gingivitis. The data generated, therefore, provides compelling evidence that 0.05% HBA-CPC formulation and Crest Gum Care™ show *in vitro* antimicrobial activity against organisms causing or associated with gingivitis.

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John Barnes 8/14/02

Microbiological analyst:

John Barnes

Dated

Nivedita Ramji 08/13/02

Study Director:

Nivedita Ramji

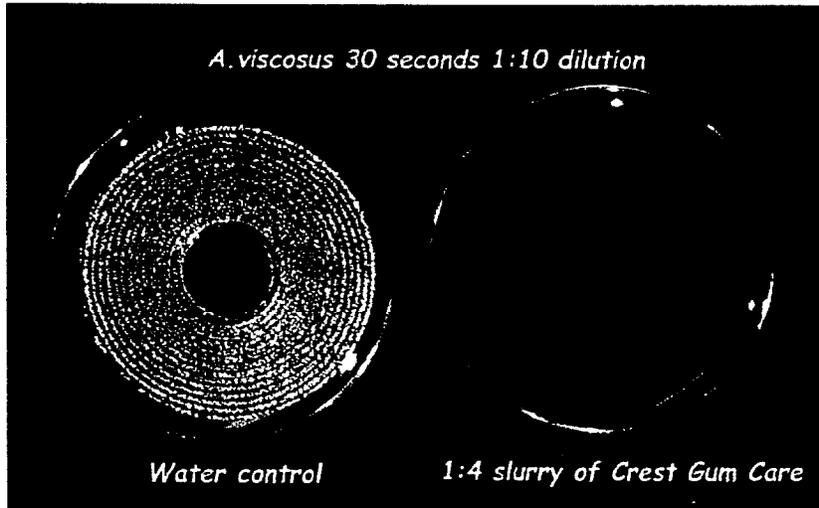
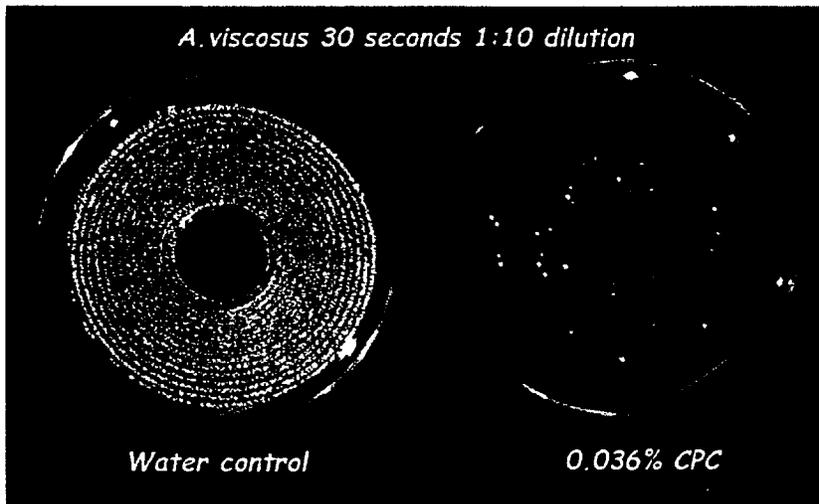
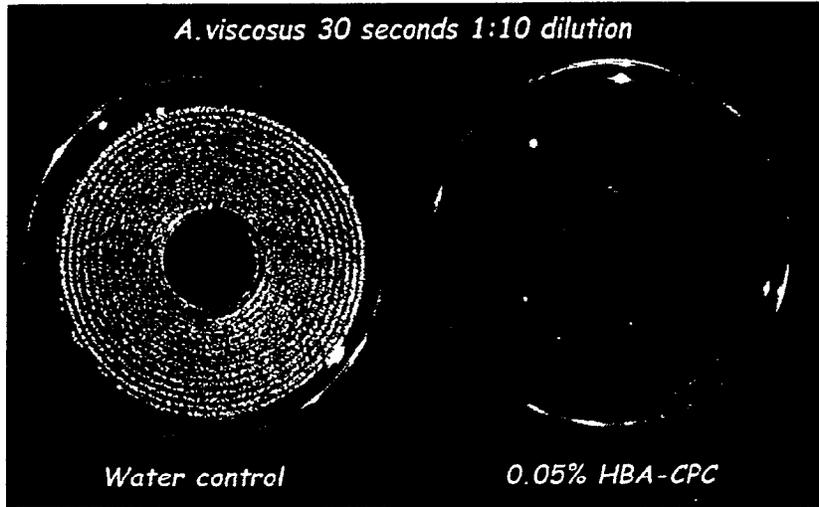
Dated

Jamie Fitzgerald 08/13/02

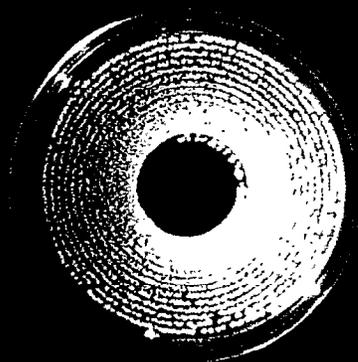
Reviewed by:

Jamie Fitzgerald

Dated



C. albicans, 30 seconds, 1:10 dilution

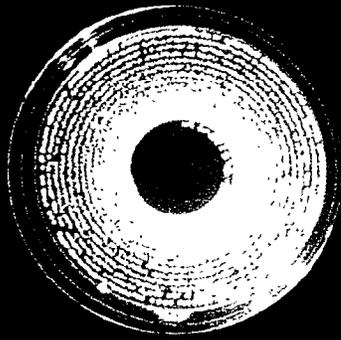


Water control



0.05% HBA-CPC

C. albicans, 30 seconds, 1:10 dilution

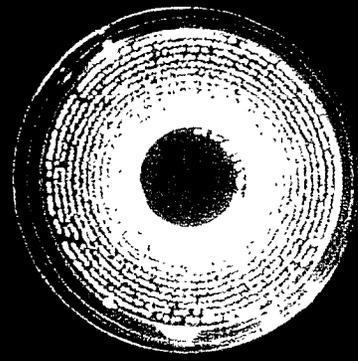


Water control

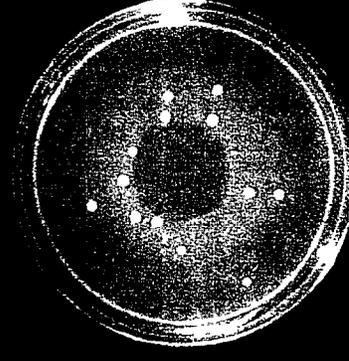


0.036% CPC

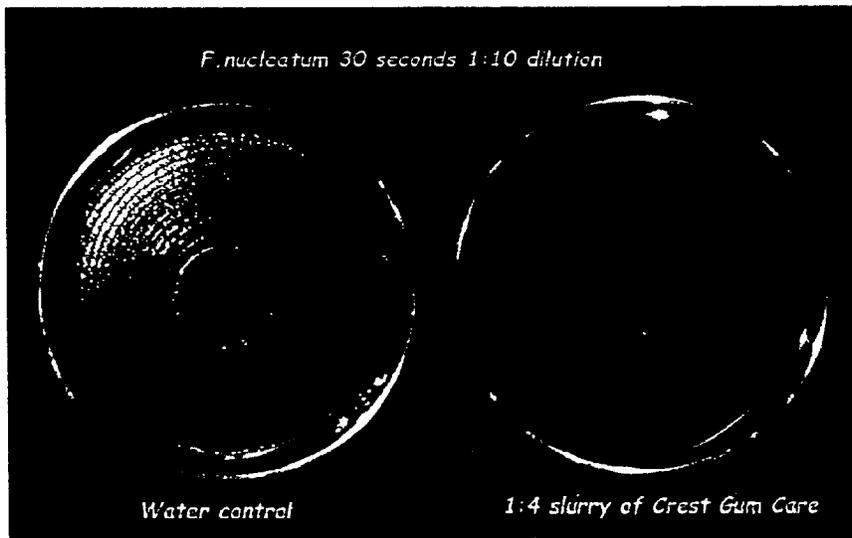
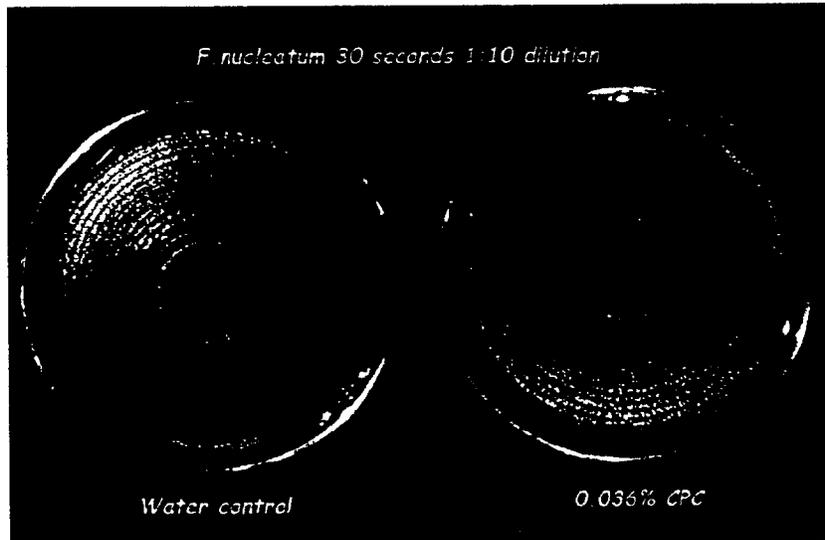
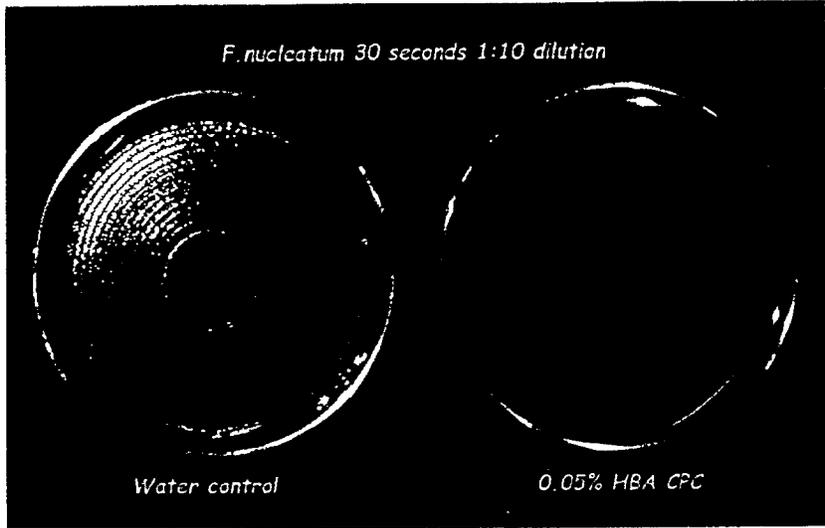
C. albicans, 30 seconds, 1:10 dilution



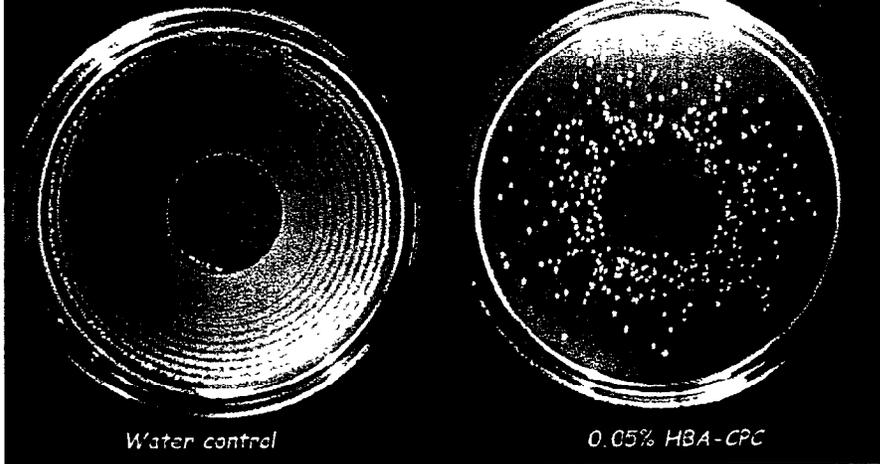
Water control



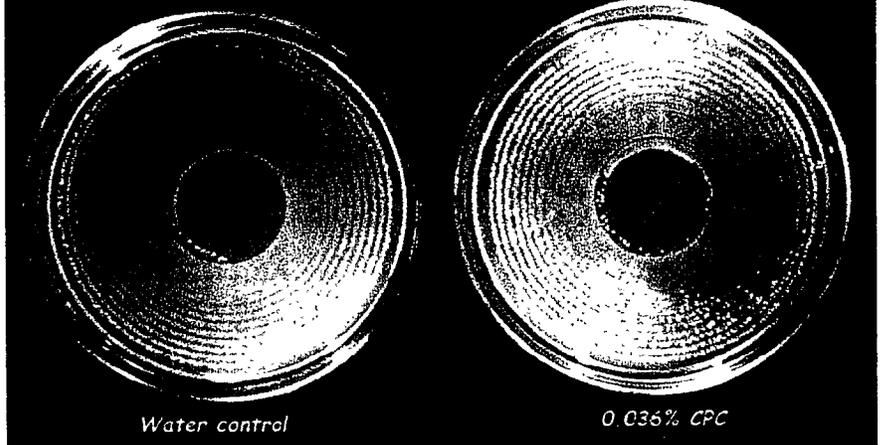
Crest Gum Care



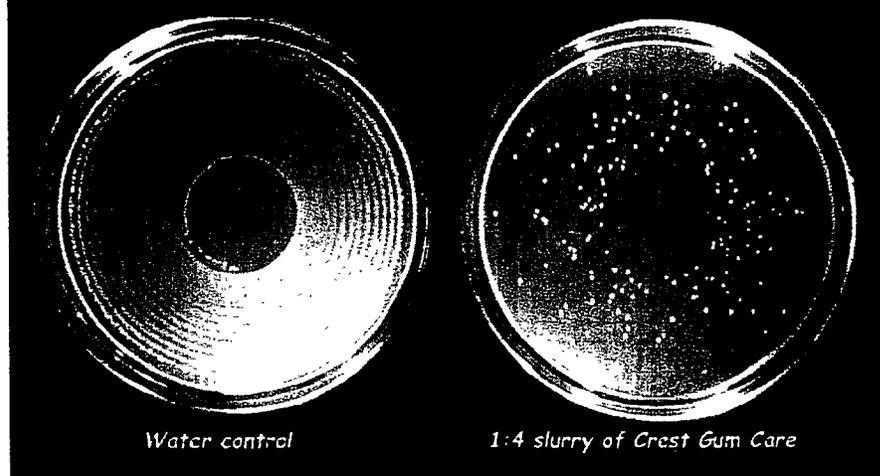
H. actinomycescomitans, 1:10 dilution, 30 seconds



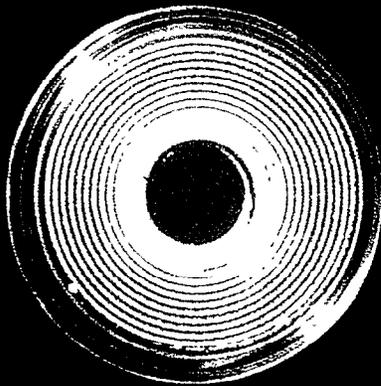
H. actinomycescomitans, 1:10 dilution, 30 seconds



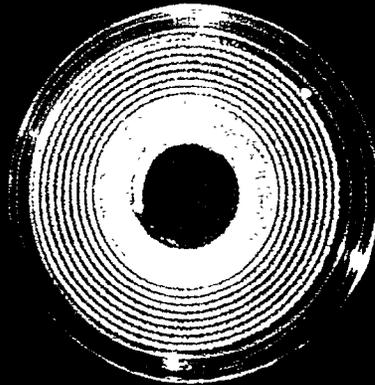
H. actinomycescomitans, 1:10 dilution, 30 seconds



Lactobacillus casei 30 seconds 1:10 dilution

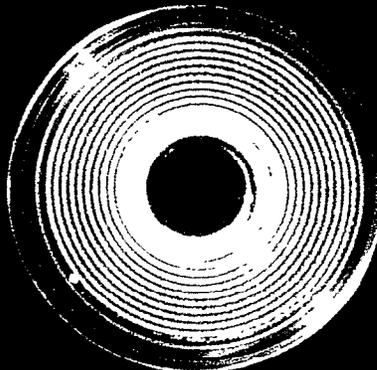


Water control



0.05% HBA-CPC

Lactobacillus casei 30 seconds 1:10 dilution



Water control

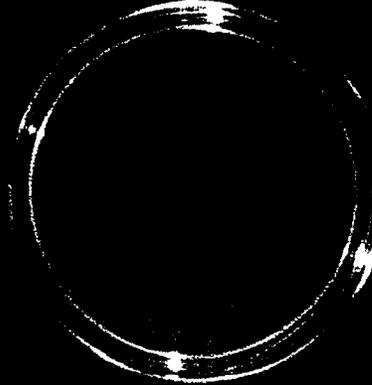


0.036% CPC

Lactobacillus casei 30 seconds 1:10 dilution

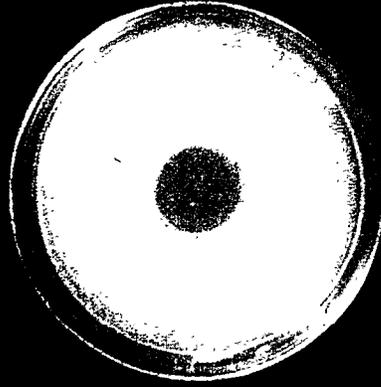


Water control

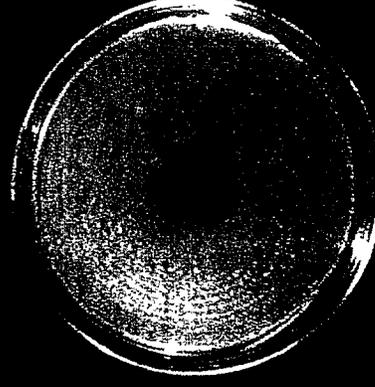


1:4 slurry of Crest Gum Care

Pseudomonas aeruginosa 30 seconds 1:10 dilution

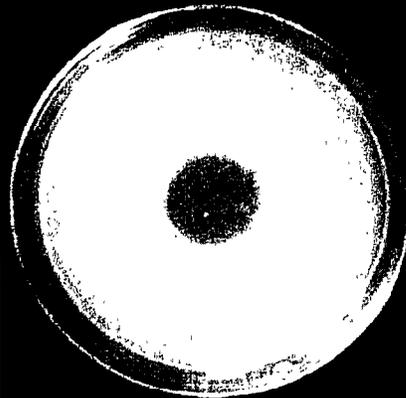


Water control

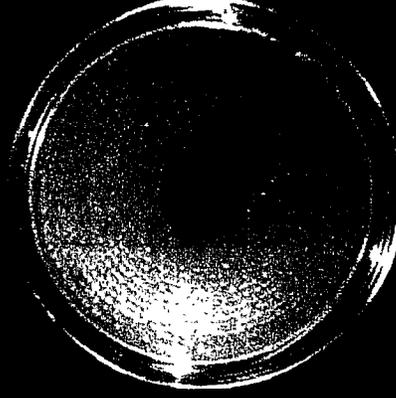


0.05% HBA-CPC

Pseudomonas aeruginosa 30 seconds 1:10 dilution

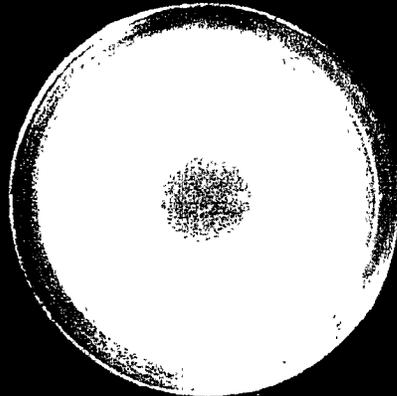


Water control

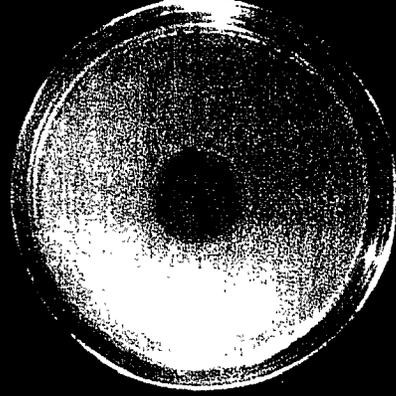


0.036% CPC

Pseudomonas aeruginosa 30 seconds 1:10 dilution

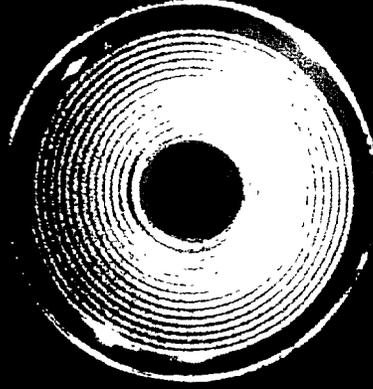


Water control

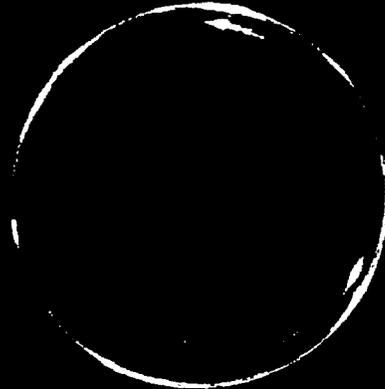


1:4 slurry of Crest Gum Care

Prevotella intermedia, 1:10 dilution, 30 seconds

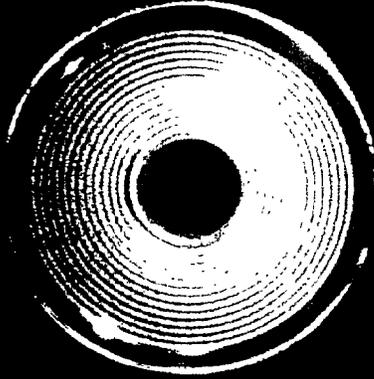


Water control

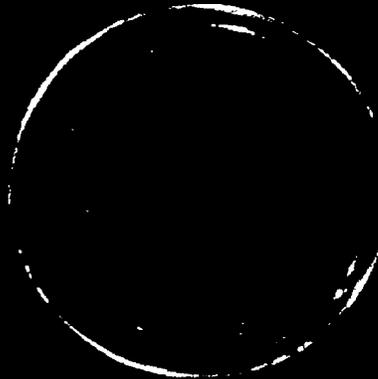


0.05% HBA-CPC

Prevotella intermedia, 1:10 dilution, 30 seconds

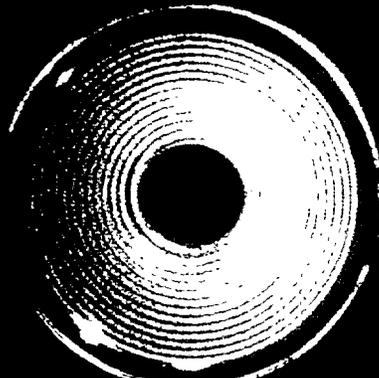


Water control



0.036% CPC

Prevotella intermedia, 1:10 dilution, 30 seconds

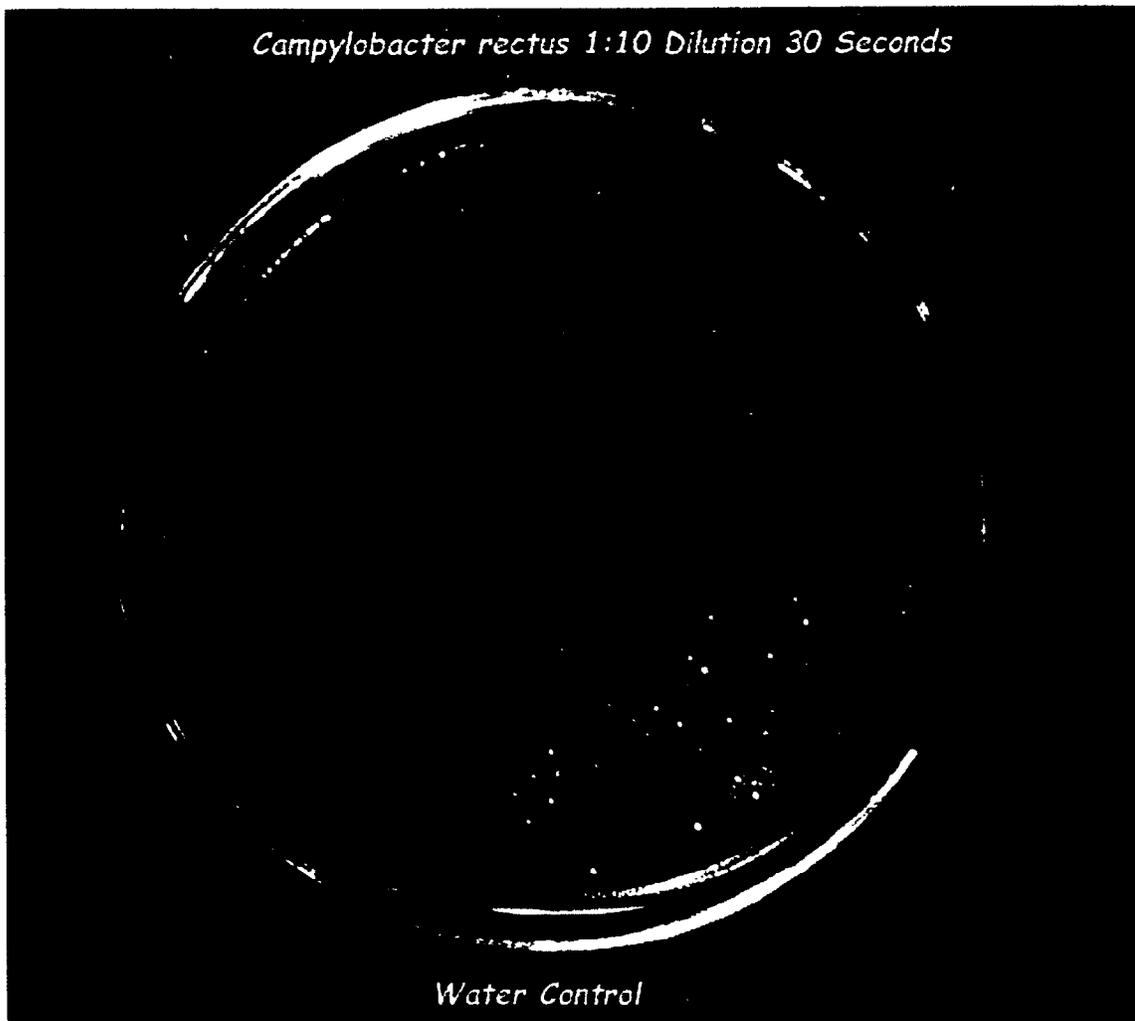


Water control



1:4 slurry of Crest Gum Care

Campylobacter rectus 1:10 Dilution 30 Seconds



Water Control

Needed to magnify the image since colonies were difficult to see

→ points to a Colony

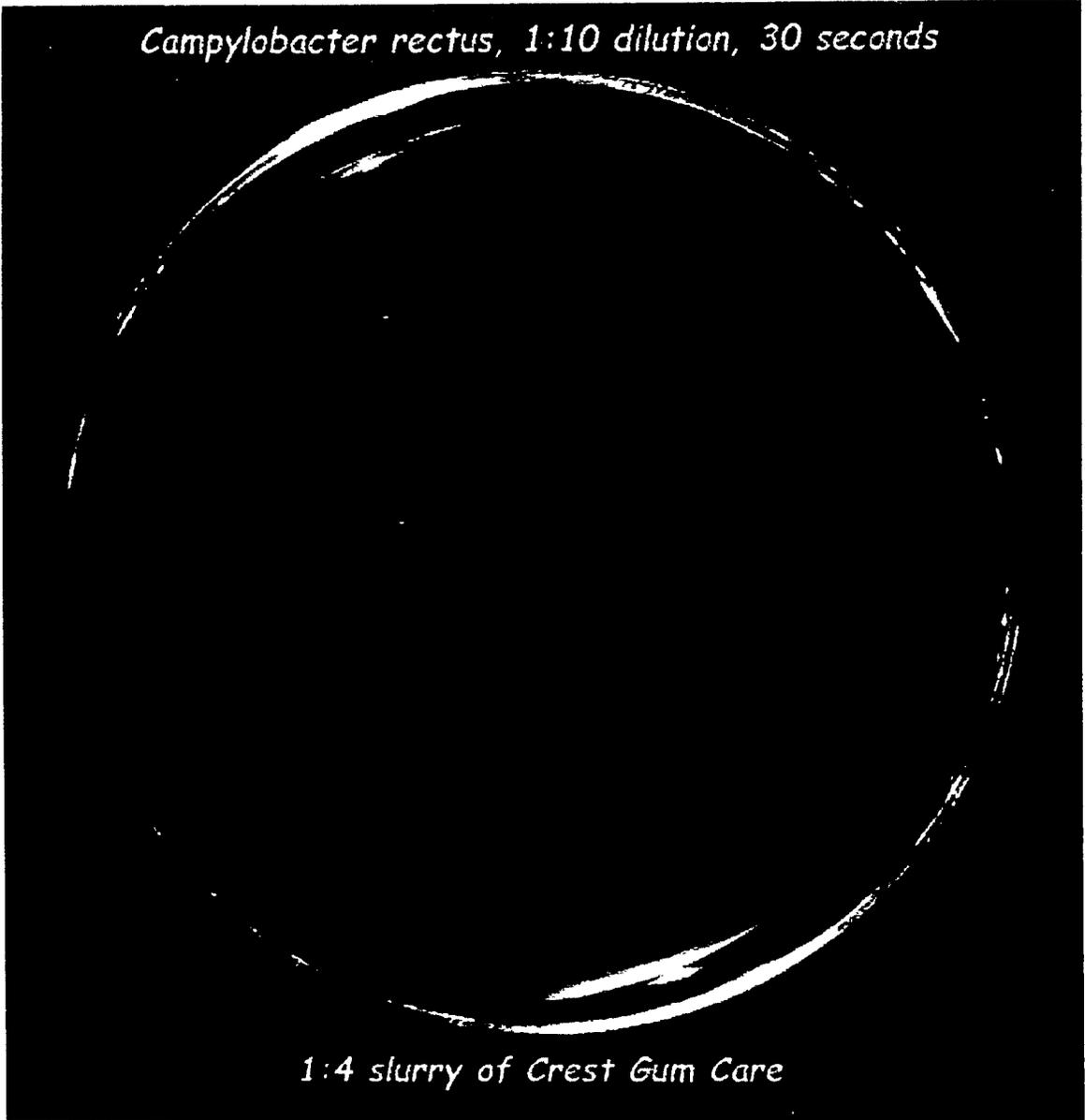
Campylobacter rectus 1:10 Dilution 30 Seconds

0.05% HBA-CPC

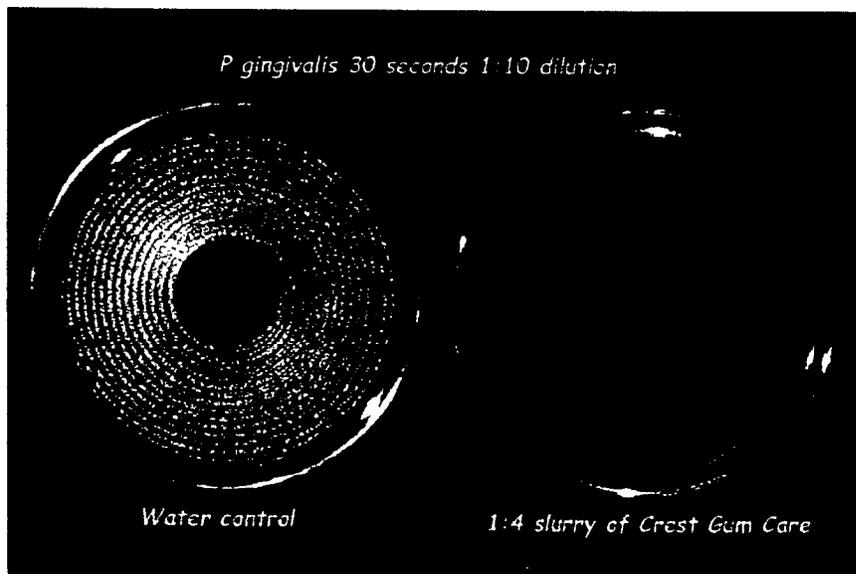
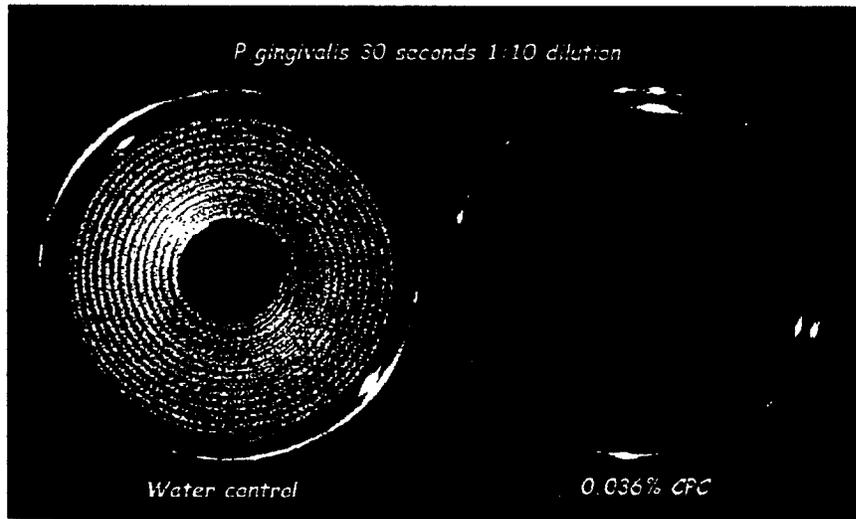
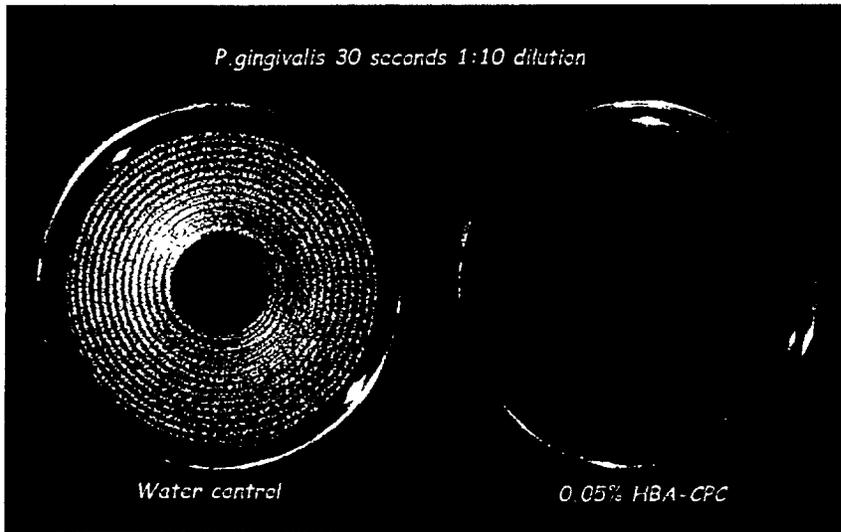
Campylobacter rectus 1:10 Dilution 30 Seconds

0.036% CPC

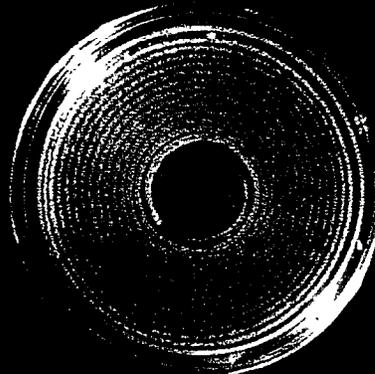
Campylobacter rectus, 1:10 dilution, 30 seconds



1:4 slurry of Crest Gum Care



S. mutans 30 seconds 1:10 dilution

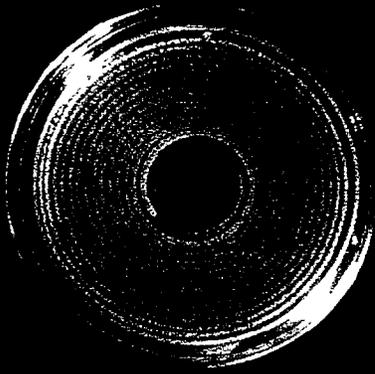


Water control

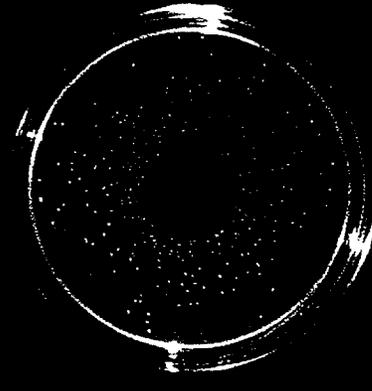


0.05% CPC

S. mutans 30 seconds 1:10 dilution

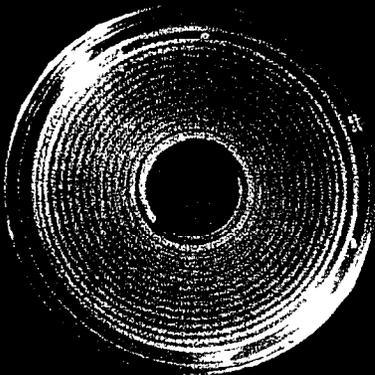


Water control

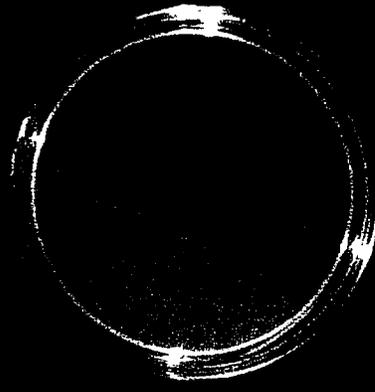


0.036% CPC

S. mutans 30 seconds 1:10 dilution

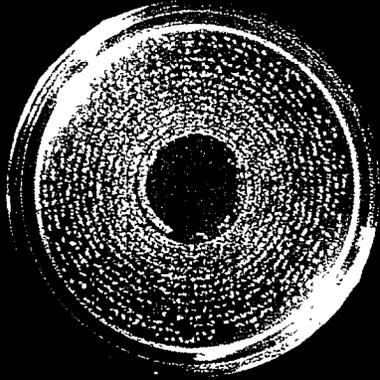


Water control

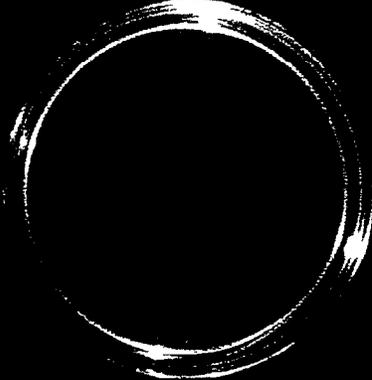


1:4 slurry of Crest Gum Care

S. sanguinis 30 seconds 1:10 dilution

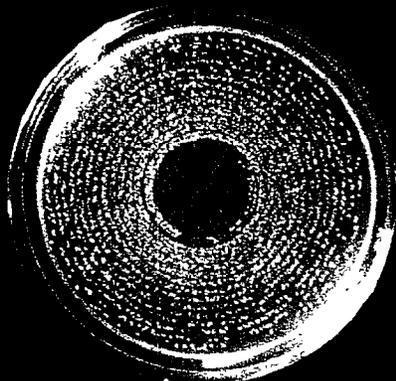


Water control

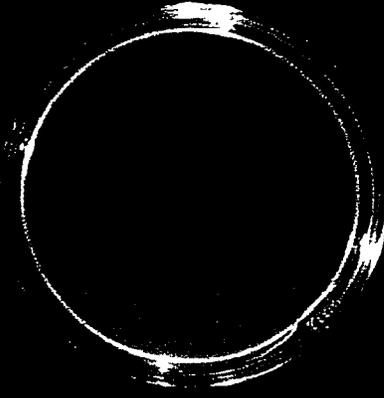


0.05% HBA-CPC

S. sanguinis 30 seconds 1:10 dilution

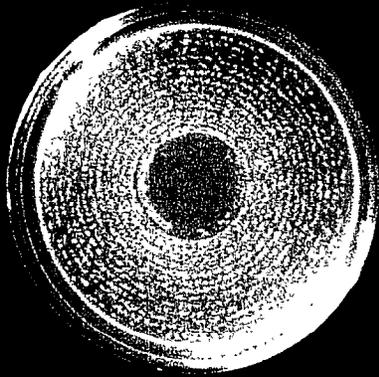


Water control

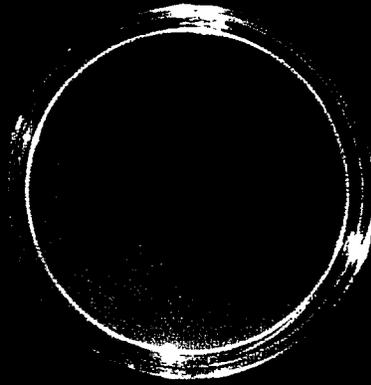


0.036% CPC

S. sanguinis 30 seconds 1:10 dilution

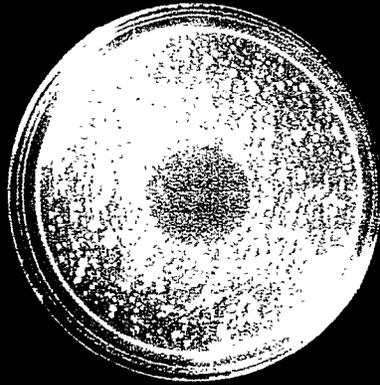


Water control



1:4 slurry of Crest Gum Care

Whole Pooled Saliva Sample, 1:10 dilution, 30 seconds

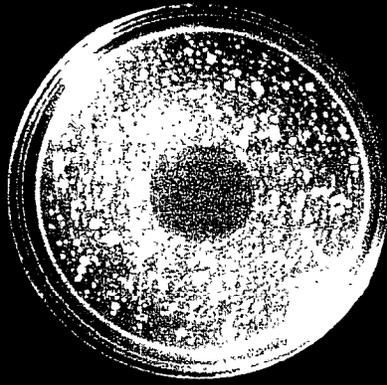


Water control

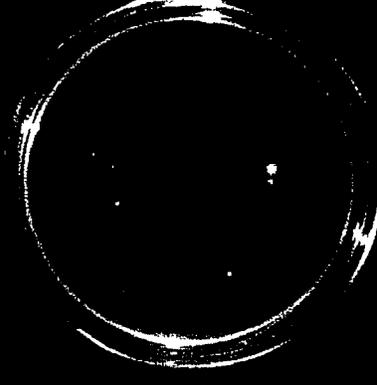


0.05% HBA-CPC

Whole Saliva Sample, 1:10 dilution, 30 seconds

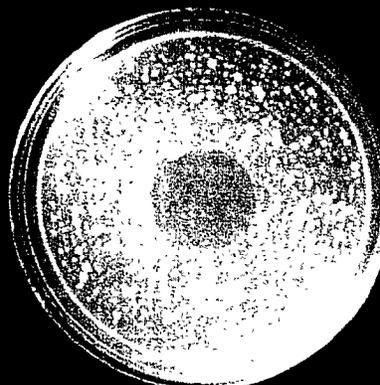


Water control



0.036% CPC

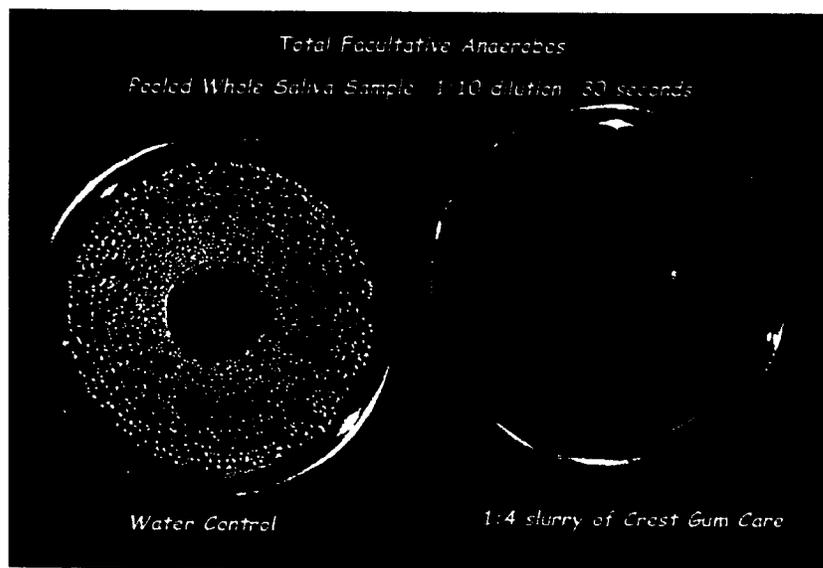
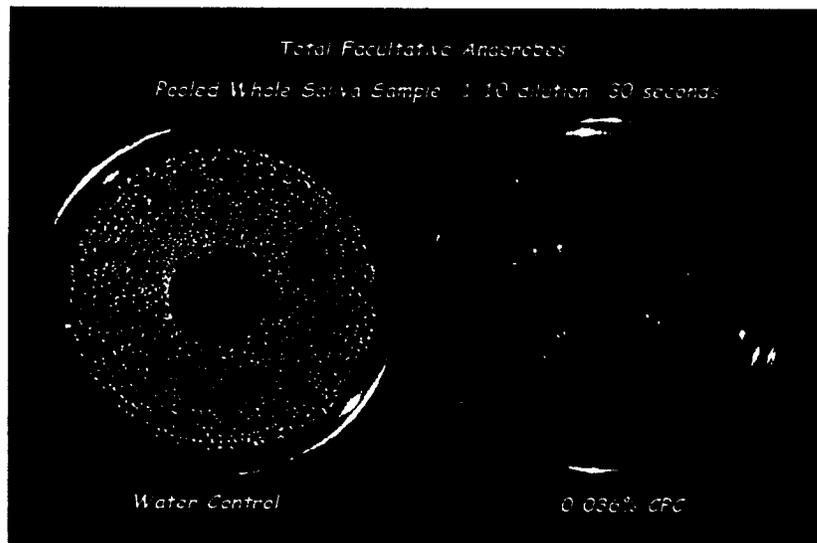
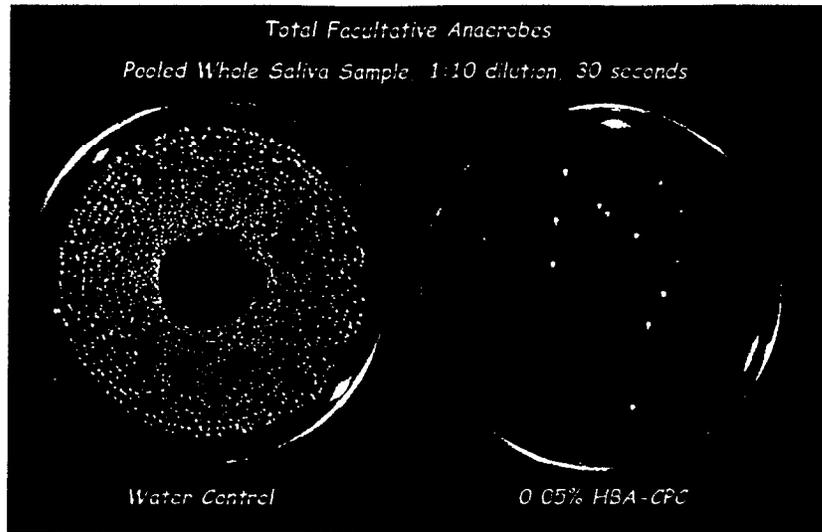
Whole Pooled Saliva, 1:10 dilution, 30 seconds

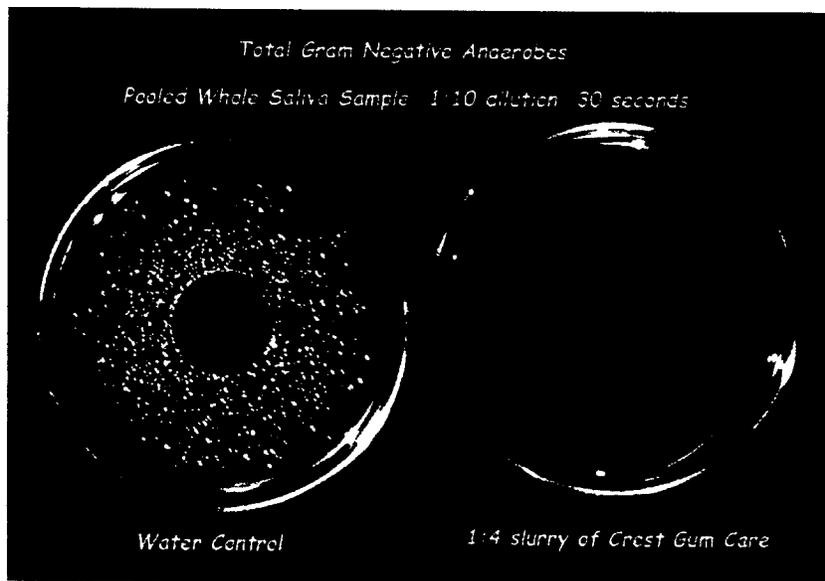
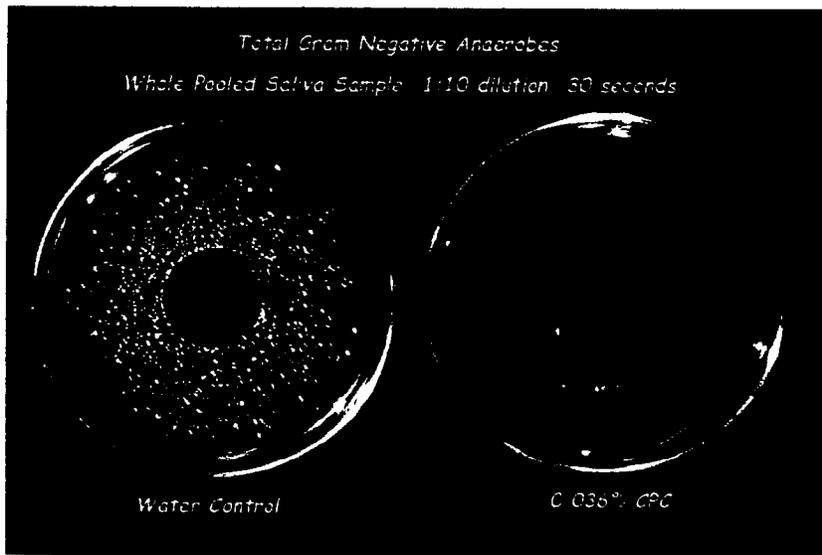
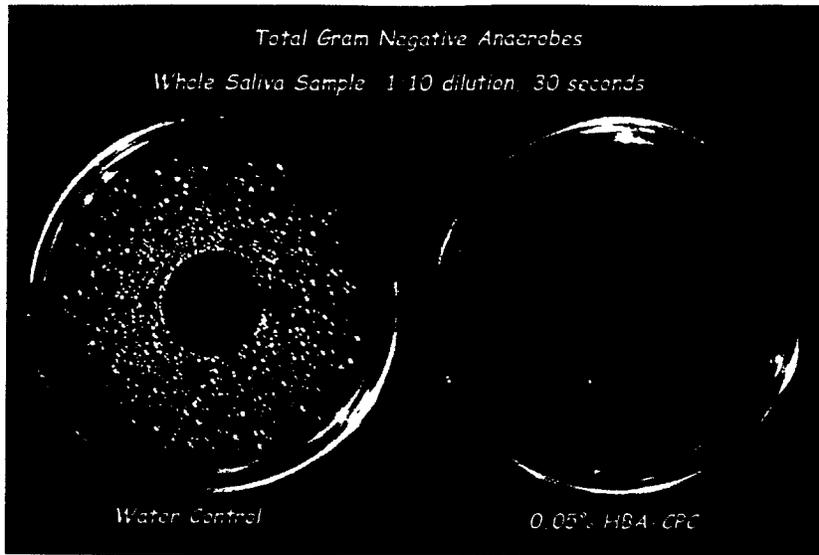


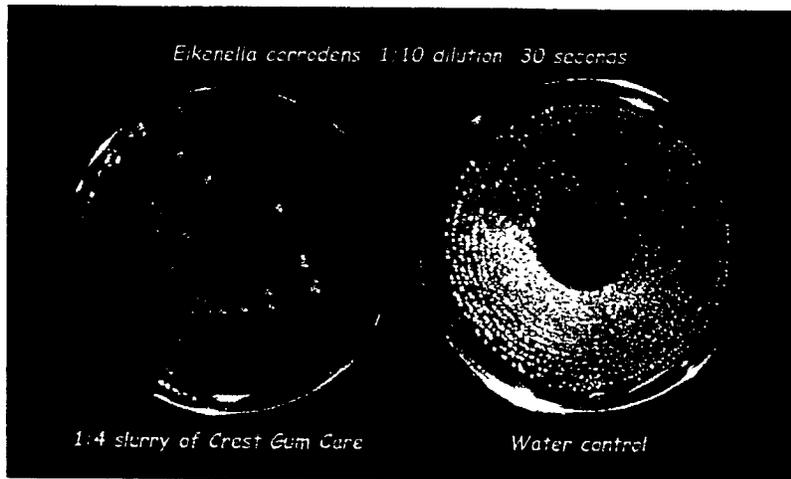
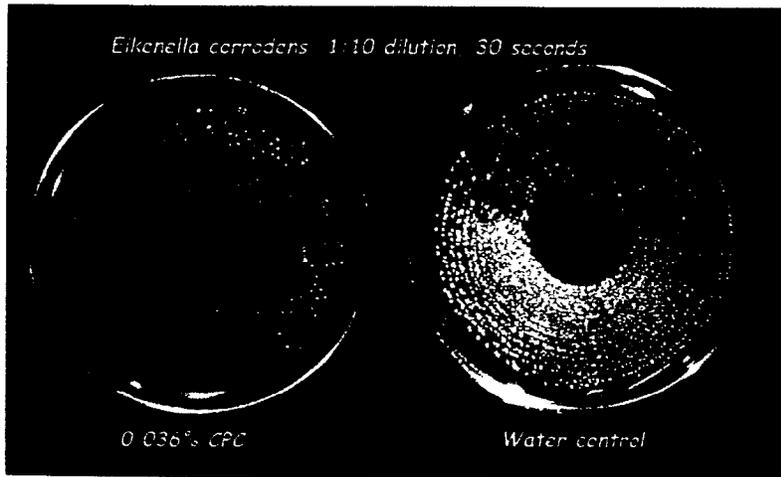
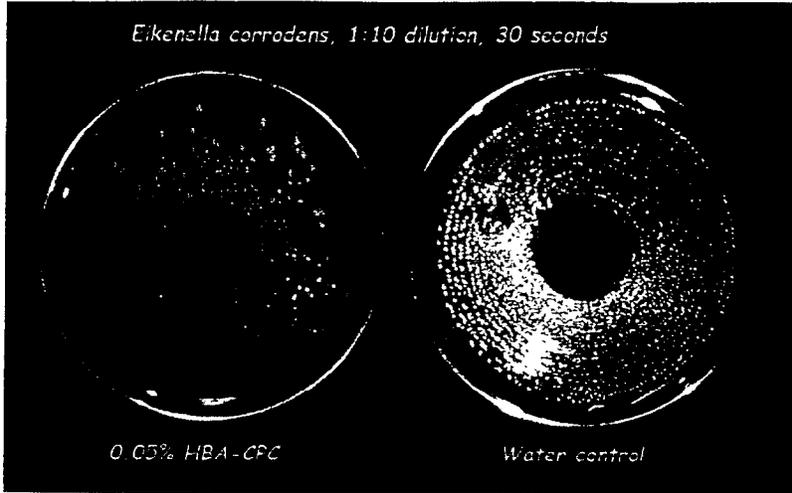
Water control



1:4 slurry of Crest Gum Care







Test products

Water control