

Comparative *In Vitro* Antimicrobial Activity of Peroxide Gels in Strip or Tray Bleaching Systems

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ABSTRACT

There are many options for at-home tooth whitening, including the carbamide peroxide tray systems which have been widely used over the past decade, or the more recently developed hydrogen peroxide whitening strip system. Comparative *in vitro* research was conducted to assess the antimicrobial effects of the new hydrogen peroxide whitening strip gel (Crest Whitestrips™, 5.3% or 6.0% H₂O₂) to marketed carbamide peroxide system gels (Opalescence®, 10% CPx or 20% CPx+F). Susceptibility of 14 supragingival, subgingival and opportunistic microorganisms was evaluated in triplicate using a Modified Zone of Inhibition Assay. All 4 gels exhibited modest hostility against a broad representation of oral microbes, including the opportunistic yeast, *Candida albicans*. Despite the perception that yeast and fungi are "holes" in the spectra for peroxides, the H₂O₂ and CPx gels exhibited mean ZOI's for *C. albicans* ranging from 11-24 mm in diameter, comparable to *Escherichia coli*. No *in vitro* hostility was observed against the microaerophilic bacteria, while the strict anaerobes, *Porphyromonas gingivalis* and *Veillonella parvula*, were highly susceptible to all four gels. The H₂O₂ and CPx gels were generally comparable with respect to antimicrobial activity. **This research demonstrates the comparable antimicrobial activity of the hydrogen peroxide whitening strip system relative to marketed carbamide peroxide systems, and further establishes the safety of these systems.**

INTRODUCTION

Crest Whitestrips is a novel system for rapid, safe delivery of a tooth whitening substance, hydrogen peroxide, in a gel matrix deposited on a flexible, disposable strip for conformable contact with the appropriate tooth surfaces. It is designed to ensure focused delivery of whitening power with minimal interference for the wearer and low exposure to their oral soft tissues. The use of hydrogen peroxide in the oral cavity has a long history for cosmetic dental whitening, and a therapeutic heritage in debriding infected tissues and killing offending microbes. In recognition of hydrogen peroxide's antimicrobial activity, it is important to understand the effects that a hydrogen peroxide whitening strip system may have on oral microflora in comparison to similar marketed products with established safety pedigrees.

MATERIALS AND METHODS

Modified Zone of Inhibition Analyses (Agar Depressions)	
Test Organisms	Growth Media
Supragingival Flora	
Gram-Positive Cocci:	Brain Heart Infusion (BHI) Broth (<i>A. viscosus</i> ,
<i>Streptococcus mutans</i> , ATCC# 25175	<i>A. naeslundii</i> , <i>V. parvula</i> , <i>P. gingivalis</i>
	<i>P. intermedia</i> , <i>C. rectus</i> and <i>F. nucleatum</i>)
Gram-Positive Cocci:	Brucella Blood (BBA) Agar (<i>A. viscosus</i> ,
<i>Streptococcus sanguis</i> , ATCC# 49296	<i>A. naeslundii</i> , <i>V. parvula</i> , <i>P. gingivalis</i> ,
	<i>P. intermedia</i> , <i>C. rectus</i> and <i>F. nucleatum</i>)
Gram-Positive Rod:	Mann, Rogosa, Sharpe (MRS)Broth/Agar
<i>Actinomyces viscosus</i> , ATCC# 15987	(<i>L. acidophilus</i>)
Gram-Positive Rod:	Trypticase Soy (TS) Broth/Agar (<i>S. mutans</i> ,
<i>Actinomyces naeslundii</i> , ATCC# 19039	<i>S. sanguis</i> , <i>S. aureus</i> , <i>E. coli</i> , and
Gram-Positive Rod:	<i>P. aeruginosa</i>)
<i>Lactobacillus acidophilus</i> , ATCC#7469	Sabouraud Dextrose (SD)Broth/Agar
Gram-Negative Cocci:	(<i>C. albicans</i>)
<i>Veillonella parvula</i> , ATCC# 17745	
Subgingival Flora	
Gram-Negative Rod:	Equipment
<i>Porphyromonas gingivalis</i> , ATCC# 33277	Micro Dissecting Curette, 3.5 mm
Gram-Negative Rod:	Sterile Swabs
<i>Prevotella intermedia</i> , ATCC# 25611	Sterile Pipette Tips 10 ml
Gram-Negative Rod:	Pipettor, 10 - 100 ml
<i>Campylobacter rectus</i> , ATCC# 33238	Electronic Calipers
Gram-Negative Rod:	Aerobic Incubator (ambient air) set at 30 - 35° C
<i>Fusobacterium nucleatum</i> , ATCC# 25586	Anaerobic Incubator (85% N, 10% CO ₂ , 5% H)
Opportunistic Pathogens	set at 35 - 37° C
Gram-Positive Cocci:	Stability Cold Room, 4° C, 60% R.H.
<i>Staphylococcus aureus</i> , ATCC# 6538	Bunsen burner and propane fuel
Gram-Negative Rod:	Autoclaves
<i>Escherichia coli</i> , ATCC# 8739	
Gram-Negative Rod:	
<i>Pseudomonas aeruginosa</i> , ATCC# 9027	
Yeast: <i>Candida albicans</i> , ATCC# 10231	

RESULTS

Test Procedure

A micro dissecting curette with curved aperture was heated and pressed to the surface of the nutrient media (Brucella Blood Agar, Mann, Rogosa, Sharpe Agar, Sabouraud Dextrose Agar and Trypticase Soy Agar), creating a circular reservoir below the surface of the agar. The media was allowed to re-solidify and a 24-hour bacterial cell suspension of each test organism was swabbed onto the surfaces of fresh plates of nutrient media. Ten micrograms (10 mg) of the test products were placed into the center of the agar depression for each media plate. Test products were plated in triplicate, with plates incubated upright for 24-48 hours at 33-37°C under aerobic conditions and/or anaerobic conditions, depending upon the test organism. Plates were observed for zones of inhibition (no growth), indicative of antimicrobial hostility of the test material. The zones were measured using electronic calipers to the nearest millimeter and evaluated based on a direct correlation between the size of the diameter and the susceptibility of the organisms tested; that is, the larger the zone, the greater the organism's sensitivity.

PURPOSE

To comparatively assess the in vitro antimicrobial activity of the new hydrogen peroxide whitening strip gel (Crest Whitestrips, 5.3% or 6.0% H₂O₂) to marketed carbamide peroxide system gels (Opalescence, 10% CPx or 20% CPx+F) via a modified Zone of Inhibition (mZOI) Assay against a full battery of representative oral microflora, including supragingival, subgingival, and opportunistic microbes.

TABLE 1. ZONES OF INHIBITION VERSUS SUPRAGINGIVAL FLORA

TEST PRODUCT	S. MUTANS ATCC# 25175	S. SANGUIS ATCC# 49296	A. VISCOUSUS ATCC# 13987	A. NAEGLUNDII ATCC# 18039	L. ACIDOPHILUS ATCC# 3489	V. PARVULA ATCC# 17745
CREST WHITESTRIPS (5.3%)	36	>80	0	0	34	>100
CREST WHITESTRIPS (6%)	51	>80	0	0	32	>100
OPALESCENCE (10%)	21	42	0	0	24	>100
OPALESCENCE (20%)	34	46	0	0	32	>100

TABLE 2. ZONES OF INHIBITION VERSUS SUBGINGIVAL FLORA

TEST PRODUCTS	P. GINGIVALIS ATCC# 33277	P. INTERMEDIA ATCC# 25611	C. RECTUS ATCC#33238	F. NUCLEATUM ATCC# 25586
CREST WHITESTRIPS (5.3%)	>100	30	12	20
CREST WHITESTRIPS (6%)	>100	24	14	13
OPALESCENCE (10%)	>100	25	12	11
OPALESCENCE (20%)	>100	29	16	36

TABLE 3. ZONES OF INHIBITION VERSUS OPPORTUNISTIC FLORA

TEST PRODUCTS	S. AUREUS ATCC# 6538	E. COLI ATCC# 8739	P. AERUGINOSA ATCC# 9027	C. ALBICANS ATCC# 10231
CREST WHITESTRIPS (5.3%)	24	20	19	19
CREST WHITESTRIPS (6%)	30	20	26	24
OPALESCENCE (10%)	17	15	17	11
OPALESCENCE (20%)	23	17	18	15

CONCLUSION

-The *in vitro* antimicrobial activity of the hydrogen peroxide whitening strip system was generally comparable to the marketed carbamide peroxide systems, as measured by zones of inhibition.

-All 4 peroxide gels exhibited modest hostility against a broad representation of oral microbes, including the opportunistic yeast, *Candida albicans*. Despite the perception that yeast and fungi are "holes" in the spectra for peroxides, the H₂O₂ and CPx gels exhibited mean ZOI's for *C. albicans* ranging from 11-24 mm in diameter, comparable to the activity observed against *Escherichia coli*.

-No *in vitro* hostility was observed against the microaerophilic bacteria (*Actinomyces viscosus* & *Actinomyces naeslundii*), while the strict anaerobes, *Porphyromonas gingivalis* and *Veillonella parvula*, were highly susceptible to all four peroxide gels.

-This research demonstrates the comparable antimicrobial activity of the hydrogen peroxide whitening strip system relative to marketed carbamide peroxide systems, and further establishes the safety of these systems.