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## In Vitro Efficacy Testing of Breathess Mouthwash vs. Mouth organisms

Project No. MB96-19,21

Prepared for:  
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10 Ponderosa Drive  
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September 9, 1996

In Vitro Efficacy Testing of Breathless Mouth Wash vs Bacterial Challenge Of  
*Klebsiella pneumoniae*

**Sponsor:** Arco Research Inc.  
10 Ponderosa Drive  
Melville, NY 11747

**Contact:** Valerie Alliger

**Study Director:** Stephen Spiegelman

**Study Date:** Initiation: 7/18/96 completion: 7/19/96 Lab. notebook reference no. 212,058

**Test Solutions:** 1. Breathless Mouth Wash:  
Part A - Lot MW12062096 - 1A ; Part B -Lot MW 13062096-2B  
- Lot MW 22062096 -3A  
(As per sponsor's request, parts A and B were mixed 1:1 and aged 30 secs  
prior testing)

2. Peridex Mouth Rinse (P&G) Lot 111 (Active 0.12% Chlorhexidine  
gluconate)

**Test Method:** In -Vitro Germicidal Suspension Test

**Test Organisms:** *Klebsiella pneumoniae*, ATCC13883 (Revived from Cultiloop)

**Purpose:** To determine the germicidal effectiveness of Breathless mouth rinse formulation

**Media:** A) Culture - Brain Heart Infusion Broth, Tryptic Soy Agar (TSA)  
B) Neutralizing medium - D/E Neutralizing Broth

**Germicidal Contact:** 0.5, 1, 2 and 4 minutes

**Procedure:**

1. The challenge organism was revived and cultured on TSA slants then put through a single passage in BHI broth and incubated for 24 hours at 32°C. The challenge culture was grown overnight in BHI broth at 32°C, harvested by centrifugation, washed and resuspended in phosphate buffer.
2. A 0.1 ml aliquot of the challenge culture was transferred to 9.9 ml of each test product. Peridex mouth rinse served as positive control.
3. Arco's Breathless mouth rinse was prepared by combining equal volumes of the two-part

formulation and aged 30 seconds prior to exposure to the challenge.

4. At 0.5, 1, 2 and 4 minutes, 1ml of the reaction mixture was withdrawn and mixed with 99 ml neutralizing medium, D/E broth.
5. Surviving bacteria were determined by plating serial dilutions of the recovery mixtures.
6. Control titer was determined by inoculating 9.9 ml phosphate buffer blank with 0.1 ml challenge culture, then diluting 1ml of the suspension in 99 ml D/E broth and serially diluting and plating in TSA.

**Neutralization:**

1. Add 1 ml of each test formula to 99ml D/E. Vortex, then inoculate "neutralized" mixture with 1 ml of challenge culture (*K. pneumoniae*).
2. Mix thoroughly and allow to stand for approximately 4 minutes. Serially dilute and plate.
3. Pour plates with Tryptic Soy Agar medium.
4. Incubate plates at 37°C for 24 hours

**Test Results**

**Mouth Rinse Suspension Test**

Challenge Organism : *K. pneumoniae* ATCC 13883

Recovery (cfu/ml) Over 4 Minutes Contact Time

Test Solutions	0.5 min	1 min	2 min	4 min
Breathless mouth wash Activator Lot MW 12062096-1A Base Lot MW 13062096-2B	< 100	<100	<100	<100
Breathless mouth wash Activator Lot MW 22062096 -3A Base Lot MW 13062096-2B	< 100	<100	<100	<100
Peridex Oral Rinse (Chlorhexidine gluconate, 0.12%) Lot 111, Exp. 9/94	1.3 X 10 <sup>5</sup>	<100	<100	<100

**Control Titer:** 2.6 x 10<sup>8</sup> cfu/ml (at time zero)

**Neutralization: Challenge Organism, *K. pneumoniae* ATCC 13883**

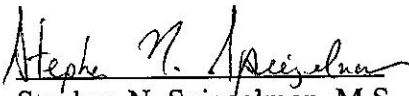
Test Formulations	Count/Plate 1 ml "neutralized" mixture	Count/Plate 0.1 ml "neutralized" mixture
Breathless Mouth Wash # 1	336	35
Breathless Mouth Wash # 2	308	41
Peridex	304	32
Control Titer	360	29


**Conclusion:**

The results indicate that both of the Arco mouth rinse formulations were effective in reducing the challenge population from 8 logs to less than 2 logs after 30 seconds contact. This represents a greater efficacy than the Peridex control which showed recovery of 5.11 logs after 30 seconds under the same test conditions.

**Neutralization was demonstrated** with the use of *Klebsiella pneumoniae*. Arco's mouth rinse formulations and Peridex all showed comparable recovery to the test control.

Respectfully submitted:

  
Stephen N. Spiegelman, M.S.  
Manager Microbiology

  
Ray Lindsay, B.S.  
Senior Microbiologist

In Vitro Efficacy Testing of Breathless Mouth Wash vs Bacterial Challenge Of  
*Fusobacterium nucleatum*

**Sponsor:** Arco Research Inc.  
10 Ponderosa Drive  
Melville, NY 11747

**Contact:** Valerie Alliger

**Study Director:** Stephen Spiegelman

**Study Date:** Initiation: 8/28/96 completion: 9/11/96 Lab. notebook reference no. 212, 079-080

**Test Solutions:** 1. Breathless Mouth Wash:  
Part A - Lot MW12062096 - 1A ; Part B -Lot MW 13062096-2B  
- Lot MW 22062096 -3A  
(As per sponsor's request, parts A and B were mixed 1:1 and aged 30 secs. prior testing)

2. Peridex Mouth Rinse (P&G) Lot 111 (Active 0.12% Chlorhexidine gluconate)

**Test Method:** In -Vitro Germicidal Suspension Test

**Test Organisms:** *Fusobacterium nucleatum*, ATCC 25586 (Revived from Cultiloop)

**Purpose:** To determine the germicidal effectiveness of Breathless mouth rinse formulation

**Media:** A) Culture - Brain Heart Infusion (BHI) Broth and BHI agar (Supplemented<sup>1</sup>)  
B) Neutralizing medium - D/E Neutralizing Broth

**Germicidal Contact:** 0.5, 1, 2 and 4 minutes

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<sup>1</sup> Brain Heart Infusion media supplemented with the following additives to further reduce the oxygen tension and enhance growth of obligate and fastidious anaerobes like *Fusobacterium nucleatum*.

Yeast Extract  
Cysteine HCl.H<sub>2</sub>O  
Hemin  
Vitamin k<sub>1</sub>

**Procedure:**

1. The challenge organism was revived and cultured in BHI broth and incubated for 24 hours at 37°C. The challenge culture was grown grown in BHI broth (Supplemented) at 37°C under anaerobic conditions for 48 hours. The culture was harvested by centrifugation, washed and resuspended in phosphate buffer. The test is run on the same day of culture harvest. The challenge suspension is refrigerated until time of test.
2. A 0.1 ml aliquot of the challenge culture was transferred to 9.9 ml of each test product. Peridex mouth rinse served as positive control.
3. Arco's Breathless mouth rinse was prepared by combining equal volumes of the two-part formulation and aged 30 seconds prior to exposure to the challenge.
4. At 0.5, 1, 2 and 4 minutes, 1ml of the reaction mixture was withdrawn and mixed with 99 ml neutralizing medium, D/E broth.
5. Surviving bacteria were determined by plating serial dilutions of the recovery mixtures.
6. Control titer was determined by inoculating 9.9 ml phosphate buffer blank with 0.1 ml challenge culture, then diluting 1ml of the suspension in 99 ml D/E broth and serially diluting and plating using BHI agar (Supplemented).

**Test Results**

**Mouth Rinse Suspension Test**

Challenge Organism : *F. nucleatum* ATCC 25586

Recovery (cfu/ml) Over 4 Minutes Contact Time

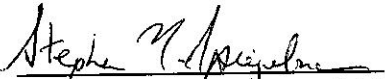
Test Solutions	0.5 min	1 min	2 min	4 min
Breathless mouth wash # 1 Activator Lot MW 12062096-1A Base Lot MW 13062096-2B	< 100	<100	<100	<100
Breathless mouth wash # 2 Activator Lot MW 22062096 -3A Base Lot MW 13062096-2B	< 100	<100	<100	<100
Peridex Oral Rinse (Chlorhexidine gluconate, 0.12%) Lot 111, Exp. 9/94	<100	<100	<100	<100

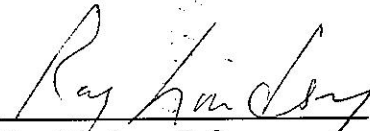
**Control Titer:**  $1.1 \times 10^5$  cfu/ml (at time zero)

**Conclusion:**

The results indicate that both of the Arco mouth rinse formulations were effective in reducing then challenge population from >5 logs to < 2 logs after 30 seconds contact (> 3 logs reduction). These results are comparable to Peridex under the same test conditions.

Respectfully submitted:

  
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