

## Comparison of Claro to Other Dermatological Devices for Acne Treatment

### Quantitative Assessment of Light Illumination on Organism Reduction

**Test Description:** Duplicate microtiter wells containing a suspension of *Propionibacterium acnes* will be exposed to a series of devices for 3 seconds and 6 seconds. Following exposure, each well will be assayed for survivors to determine organism reduction.

**Organism:** *Propionibacterium acnes* (ATCC 11827)

**Test Temperature:** Room Temperature

**Soil:** No additional soil load required

**Exposure Times:** 3 seconds and 6 seconds

**Test Materials:**

Subculture agar: TSA + 5% Sheep's blood (Blood agar)

Organism diluent: Butterfield's buffer or 0.85% Saline

**Test Procedure:**

**Test Devices**

Each device containing rechargeable batteries will be fully charged (minimally overnight) prior to use.

**Test Organism**

*P. acnes* -- From stock cultures, inoculate sufficient Blood agar plates. Incubate for 4-7 days at 35-37°C under anaerobic conditions. Using a sterile swab, transfer sufficient inoculum to sterile diluent to generate a suspension matching a 0.5 McFarland turbidity suspension.

Mix the suspension and prepare one ten-fold serial dilution in sterile diluent to target  $1 \times 10^7$  CFU/mL. This will be used as in the inoculum for the test.

**Exposure:**

Transfer 300 µL of prepared suspension into each microtiter well. Duplicate wells, per device and exposure time, will be prepared.

A template < 1 mm in thickness constructed of cardboard covered with foil will be used as a distance guide for each device. The template will have appropriate circular cut-outs which will be used to expose a given well to the test device.

Duplicate test wells will be exposed to each device, in a similar fashion, for each exposure time. The distance between the lowest contact point on the device and the top of the well should not exceed 2 mm. The foil template will be placed over the well plate and aligned with the well(s) of interest prior to exposure. Exposure will be monitored using a calibrated timer. Placement of the activated device over the well will signify the start of the exposure period. Removal of the device from the template will signify the end of the exposure period.

**Exposure (continued):**

Each device will be used as follows:

**Claro**

Press the power button to turn the device on. Place the device over the template ensuring contact between the device and the template. A confirmatory beep will sound. Press and hold the power button which will initiate multiple flashes of light lasting approximately three seconds. Initiate sufficient cycles of light for the duration of the exposure times.

**Evis Marvel-Mini**

Plug the device into an outlet. Press the power button to turn the device on. Press the button labeled "P" to initiate the pulse mode. Place the device over the template ensuring contact between the device and the template. Expose each well for each exposure time. Duplicate wells may be exposed simultaneously for this device.

**Zeno**

Press the power button to turn the device on; an amber colored light will be illuminated. Wait for the amber light to turn green prior to initiating exposure. Place the device over the template ensuring contact between the device and the template. Press the activation button and expose each well for each exposure time.

**Tanda**

Press the power button to turn the device on. Grasp the sides of the base of the unit to initiate the treatment light. Place the device over the template ensuring contact between the device and the template. Expose each well for each exposure time. Duplicate wells may be exposed simultaneously for this device.

**Thermaclear**

Remove the protective cover. Press the power button to turn the device on. Using the small power button, adjust the device to the low-heat setting (as indicated by a single blue light bar). Place the device over the template ensuring contact between the device and the template. Expose each well for each exposure time. One treatment cycles lasts approximately two seconds. Initiate multiple cycles to ensure treatment for the duration of each exposure time.

**Subculture**

Following exposure, each well will be assayed for survivors. The well ( $10^0$ ) will be serially diluted using 0.1 mL (100  $\mu$ L) aliquots and 0.9 mL (900  $\mu$ L) aliquots of diluent. 0.1 mL (100  $\mu$ L) of  $10^0$  through  $10^{-5}$  will be spread-plated, in duplicate, onto Blood agar.

**Incubation and Evaluation**

Incubate all plates at 35-37°C for 3-7 days under anaerobic conditions. Plates may be refrigerated at 2-8°C for up to three days prior to evaluation. Following incubation, count the resultant colonies. Count plates containing 30-300 colonies where possible.

**Plates will be saved to take digital photographs.**

**Controls**

**Purity control:** Perform a streak plate for isolation using the prepared test organism. Incubate as in the test. The acceptance criterion is pure growth typical of the test organism.

**Untreated well control:** Inoculate duplicate wells as in the test procedure. Serially dilute and plate each untreated well as in the test procedure. Incubate as in the test. There is no acceptance criterion. This control is performed for calculation purposes only.

## Calculations

Determine the CFU/mL for each test or control well as follows:

$$\text{CFU/mL} = \frac{(\text{Average CFU}) \times (\text{dilution factor})}{(\text{volume plated in mL})}$$

Determine the average CFU/mL per set of wells

$$\text{Average CFU/mL} = \frac{(\text{CFU/mL for replicate 1}) + (\text{CFU/mL for replicate 2})}{2}$$

Determine the Log<sub>10</sub> Average CFU/mL

Determine the Log<sub>10</sub> reduction as follows:

$$\text{Log}_{10} \text{ reduction} = (\text{Log}_{10} \text{ Average CFU/mL for untreated wells}) - (\text{Log}_{10} \text{ Average CFU/mL for a given device/exposure period})$$

Determine the percent reduction as follows:

$$\% \text{ reduction} = [(a - b) / a] \times 100$$

where:

a = Average CFU/mL for untreated wells

b = Average CFU/mL for a given device/exposure

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## STUDY REPORT

### GENERAL STUDY INFORMATION

**Study Title:** Custom Microbiology – Quantitative Assessment of Light Illumination on Organism Reduction

**Project Number:** A06378

**TRF Number:** SPD01060908.CUST

### TEST SUBSTANCE IDENTITY

**Test Substance Name:** Claro, Zeno, Tanda, Thermaclear and Evis Marvel-Mini

### STUDY DATES

**Date Sample Received:** June 6, 2008  
**Study Initiation Date:** June 12, 2008  
**Experimental Start Date:** June 16, 2008  
**Experimental End Date:** June 19, 2008  
**Study Completion Date:** June 23, 2008

Test Organism	ATCC #	Growth Medium
<i>Propionibacterium acnes</i>	11827	Tryptic Soy Agar with 5% Sheep Blood (BAP)

The microorganism used in this study was obtained from the American Type Culture Collection, Manassas, Virginia.

### **Recovery Media**

**Agar Plate Medium:** Tryptic Soy Agar with 5% Sheep Blood (BAP)

**Test Exposure:** 3 seconds and 6 seconds  
**Exposure Temperature:** Room temperature (23°C)  
**Soil Load Description:** No organic soil load required

### EXPERIMENTAL DESIGN

Duplicate suspensions of bacterial cells, contained within micro-titer wells, were exposed to each test substance (device) for each exposure time. After exposure, each treated suspension was assayed for survivors. Appropriate purity and untreated population controls were performed. Percent and log reductions were determined as compared to the untreated population control.

## STUDY RESULTS

**TABLE 1: CONTROL RESULTS**

The following results from controls confirmed study validity:

Type of Control	Results
	<i>Propionibacterium acnes</i> (ATCC 11827)
Purity Control	Pure

**TABLE 2: UNTREATED POPULATION CONTROL RESULTS**

DILUTION	Test Organism: <i>Propionibacterium acnes</i>	
	Replicate Well #1	Replicate Well #2
	Number of Survivors	
$10^0$	TNTC,TNTC	TNTC,TNTC
$10^{-1}$	TNTC,TNTC	TNTC,TNTC
$10^{-2}$	TNTC,TNTC	TNTC,TNTC
$10^{-3}$	TNTC,TNTC	TNTC,TNTC
$10^{-4}$	181,157	TNTC,TNTC
$10^{-5}$	22,9	31,30

TNTC = Too Numerous To Count = > 300 colonies

**TABLE 3: TEST RESULTS**

Test Substance: Claro

DILUTION	Test Organism: <i>Propionibacterium acnes</i>			
	EXPOSURE TIME: 3 seconds		EXPOSURE TIME: 6 seconds	
	Replicate Well #1	Replicate Well #2	Replicate Well #1	Replicate Well #2
	Number of Survivors			
$10^0$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-1}$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-2}$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-3}$	TNTC,TNTC	TNTC,TNTC	117,140	131,110
$10^{-4}$	67,43	30,59	22,21	18,19
$10^{-5}$	18,6	6,7	0,0	0,0

Test Substance: Evis Marvel-Mini

DILUTION	Test Organism: <i>Propionibacterium acnes</i>			
	EXPOSURE TIME: 3 seconds		EXPOSURE TIME: 6 seconds	
	Replicate Well #1	Replicate Well #2	Replicate Well #1	Replicate Well #2
	Number of Survivors			
$10^0$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-1}$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-2}$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-3}$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-4}$	102,104	92,90	62,68	84,88
$10^{-5}$	24,24	10,12	9,7	12,12

TNTC = Too Numerous To Count = > 300 colonies

TABLE 3: TEST RESULTS (continued)

Test Substance: Tanda

DILUTION	Test Organism: <i>Propionibacterium acnes</i>			
	EXPOSURE TIME: 3 seconds		EXPOSURE TIME: 6 seconds	
	Replicate Well #1	Replicate Well #2	Replicate Well #1	Replicate Well #2
	Number of Survivors			
10 <sup>0</sup>	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
10 <sup>-1</sup>	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
10 <sup>-2</sup>	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
10 <sup>-3</sup>	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
10 <sup>-4</sup>	170,152	150,80	88,84	94,96
10 <sup>-5</sup>	10,19	20,24	20,16	10,20

Test Substance: Zeno

DILUTION	Test Organism: <i>Propionibacterium acnes</i>			
	EXPOSURE TIME: 3 seconds		EXPOSURE TIME: 6 seconds	
	Replicate Well #1	Replicate Well #2	Replicate Well #1	Replicate Well #2
	Number of Survivors			
10 <sup>0</sup>	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
10 <sup>-1</sup>	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
10 <sup>-2</sup>	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
10 <sup>-3</sup>	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
10 <sup>-4</sup>	62,143	130,108	115,78	130,72
10 <sup>-5</sup>	12,19	7,14	10,10	16,12

TNTC = Too Numerous To Count = > 300 colonies

**TABLE 3: TEST RESULTS (continued)**

Test Substance: Thermaclear

DILUTION	Test Organism: <i>Propionibacterium acnes</i>			
	EXPOSURE TIME: 3 seconds		EXPOSURE TIME: 6 seconds	
	Replicate Well #1	Replicate Well #2	Replicate Well #1	Replicate Well #2
	Number of Survivors			
$10^0$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-1}$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-2}$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-3}$	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC	TNTC,TNTC
$10^{-4}$	91,92	94,90	88,88	74,96
$10^{-5}$	13,13	14,20	20,20	12,12

TNTC = Too Numerous To Count = > 300 colonies



TABLE 4: CALCULATED DATA

Test Substance: Claro

Test Organism	Exposure Time	Average Untreated Population Control CFU/mL* (Average Log <sub>10</sub> )	Average Number of Survivors (CFU/mL)*	Log <sub>10</sub> Number of Survivors	Percent Reduction	Log <sub>10</sub> Reduction
<i>Propionibacterium acnes</i>	3 seconds	2.4 x 10 <sup>7</sup>	5.0 x 10 <sup>6</sup>	6.70	79.2 %	0.68
	6 seconds	(7.38)	1.25 x 10 <sup>6</sup>	6.097	94.8 %	1.28

\* colony forming units per mL of test mixture

Test Substance: Zeno

Test Organism	Exposure Time	Average Untreated Population Control CFU/mL* (Average Log <sub>10</sub> )	Average Number of Survivors (CFU/mL)*	Log <sub>10</sub> Number of Survivors	Percent Reduction	Log <sub>10</sub> Reduction
<i>Propionibacterium acnes</i>	3 seconds	2.4 x 10 <sup>7</sup>	1.11 x 10 <sup>7</sup>	7.045	53.8 %	0.34
	6 seconds	(7.38)	9.9 x 10 <sup>6</sup>	7.00	58.8 %	0.38

\* colony forming units per mL of test mixture

Test Substance: Tanda

Test Organism	Exposure Time	Average Untreated Population Control CFU/mL* (Average Log <sub>10</sub> )	Average Number of Survivors (CFU/mL)*	Log <sub>10</sub> Number of Survivors	Percent Reduction	Log <sub>10</sub> Reduction
<i>Propionibacterium acnes</i>	3 seconds	2.4 x 10 <sup>7</sup>	1.38 x 10 <sup>7</sup>	7.140	42.5 %	0.24
	6 seconds	(7.38)	9.1 x 10 <sup>6</sup>	6.96	62.1 %	0.42

\* colony forming units per mL of test mixture

TABLE 4: CALCULATED DATA (continued)

Test Substance: Thermaclear

Test Organism	Exposure Time	Average Untreated Population Control CFU/mL* (Average Log <sub>10</sub> )	Average Number of Survivors (CFU/mL)*	Log <sub>10</sub> Number of Survivors	Percent Reduction	Log <sub>10</sub> Reduction
<i>Propionibacterium acnes</i>	3 seconds	2.4 x 10 <sup>7</sup>	9.2 x 10 <sup>6</sup>	6.96	61.7 %	0.42
	6 seconds	(7.38)	8.7 x 10 <sup>6</sup>	6.94	63.8 %	0.44

\* colony forming units per mL of test mixture

Test Substance: Evis Marvel-Mini

Test Organism	Exposure Time	Average Untreated Population Control CFU/mL* (Average Log <sub>10</sub> )	Average Number of Survivors (CFU/mL)*	Log <sub>10</sub> Number of Survivors	Percent Reduction	Log <sub>10</sub> Reduction
<i>Propionibacterium acnes</i>	3 seconds	2.4 x 10 <sup>7</sup>	9.7 x 10 <sup>6</sup>	6.99	59.6 %	0.39
	6 seconds	(7.38)	7.6 x 10 <sup>6</sup>	6.88	68.3 %	0.50

\* colony forming units per mL of test mixture

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## **ANALYSIS**

Under the conditions of this study, Claro, demonstrated a 79.2% (0.68 log<sub>10</sub>) reduction of *Propionibacterium acnes* survivors after a 3 second exposure, and a 94.8% (1.28 log<sub>10</sub>) reduction of *Propionibacterium acnes* survivors after a 6 second exposure when tested at room temperature (23°C).

Under the conditions of this study, Zeno, demonstrated a 53.8% (0.34 log<sub>10</sub>) reduction of *Propionibacterium acnes* survivors after a 3 second exposure, and a 58.8% (0.38 log<sub>10</sub>) reduction of *Propionibacterium acnes* survivors after a 6 second exposure when tested at room temperature (23°C).

Under the conditions of this study, Tanda, demonstrated a 42.5% (0.24 log<sub>10</sub>) reduction of *Propionibacterium acnes* survivors after a 3 second exposure, and a 62.1% (0.42 log<sub>10</sub>) reduction of *Propionibacterium acnes* survivors after a 6 second exposure when tested at room temperature (23°C).

Under the conditions of this study, Thermaclear, demonstrated a 61.7% (0.42 log<sub>10</sub>) reduction of *Propionibacterium acnes* survivors after a 3 second exposure, and a 63.8% (0.44 log<sub>10</sub>) reduction of *Propionibacterium acnes* survivors after a 6 second exposure when tested at room temperature (23°C).

Under the conditions of this study, Evis Marvel-mini, demonstrated a 59.6% (0.39 log<sub>10</sub>) reduction of *Propionibacterium acnes* survivors after a 3 second exposure, and a 68.3% (0.50 log<sub>10</sub>) reduction of *Propionibacterium acnes* survivors after a 6 second exposure when tested at room temperature (23°C).





UNTREATED  
POPULATION CONTROL



3 SECONDS

CLARO



6 SECONDS





UNTREATED  
POPULATION CONTROL



EVIS MARVEL-MINI

3 SECONDS



6 SECONDS

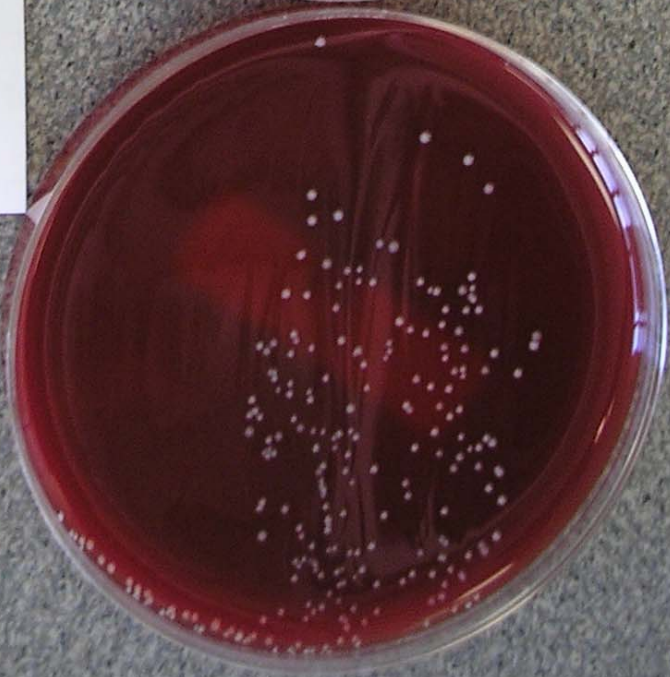




UNTREATED  
POPULATION CONTROL



3 SECONDS



6 SECONDS

TANDA





UNTREATED  
POPULATION CONTROL



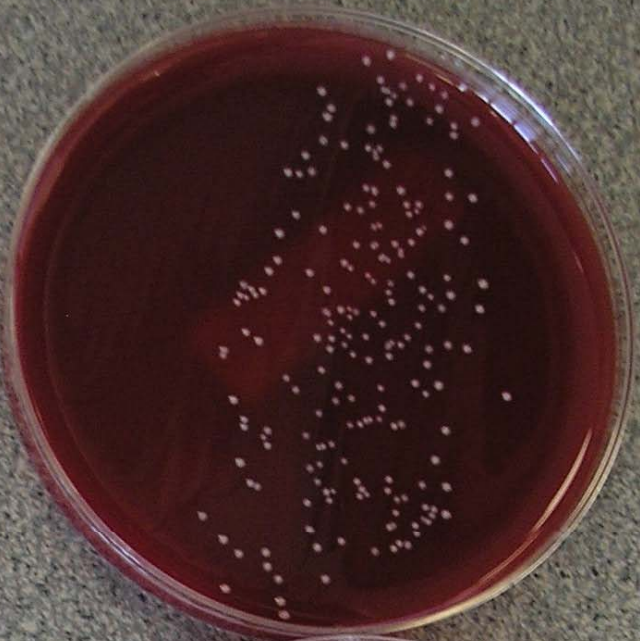
THERMACLEAR

3 SECONDS



6 SECONDS





UNTREATED  
POPULATION CONTROL



ZENO



3 SECONDS

6 SECONDS