



# MICROBIOTEST

*The Microbiology and Virology Laboratory*

## CONFIDENTIAL FINAL REPORT

**SPONSOR:** Violight, Inc.

**SPONSOR'S REPRESENTATIVE:** Joel Pinsky

**STUDY TITLE:** VIRUCIDAL EFFICACY EVALUATION OF VIOLIGHT ULTRAVIOLET SANITIZATION DEVICE USING SWINE INFLUENZA VIRUS (H1N1)

**STUDY IDENTIFICATION:** MICROBIOTEST Project No. 700-101 (refer to signed protocol)

<u>TEST AGENT NAME</u>	<u>SERIAL NO.</u>	<u>DATE RECEIVED</u>	<u>DS NO.</u>
VIOLIGHT Toothbrush Sanitizer-Control Sample	1812297663	07/31/09	10238a
VIOLIGHT Toothbrush Sanitizer-Test Sample	1812297663	07/31/09	10238b
VIOLIGHT Toothbrush Sanitizer-Baseline Sample	1812297663	07/31/09	10238c

**ACTIVE INGREDIENT(S):** Ultraviolet light

**CELL CULTURE MEDIUM:** MEM + 1.0 µg/mL Trypsin

**EXTRACTION MEDIUM / NEUTRALIZER:** MEM + 1% HEPES

**CHALLENGE ORGANISM:** Swine Influenza Virus (H1N1), A/Swine/Iowa/15/30, ATCC VR-333

**HOST:** MDCK cells, ATCC CCL-34

**EXPOSURE TIME:** As determined by the device (set at approximately 6 minutes per sanitizing cycle)

**NUMBER OF REPLICATES:** Four wells per dilution

<b>CONTACT TEMPERATURE:</b>	Ambient room temperature (20C)
<b>INCUBATION TEMPERATURE:</b>	36±2C with 5±1% CO <sub>2</sub>
<b>INCUBATION TIME:</b>	4-6 days
<b>VIRUS APPLICATION:</b>	0.2 mL stock virus was spiked onto the toothbrush bristles.

### CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL

The 50% tissue culture infectious dose per mL (TCID<sub>50</sub>/mL) was determined using the Spearman-Kärber method using the following formula:

$$m = x_k + \left(\frac{d}{2}\right) - d \sum p_i$$

where:

- m = the logarithm of the titer relative to the test volume
- x<sub>k</sub> = the logarithm of the smallest dosage which induces infection in all cultures
- d = the logarithm of the dilution factor
- p<sub>i</sub> = the proportion of positive results at dilution i

The values were converted to TCID<sub>50</sub>/mL using a sample inoculum of 1.0 mL.

The viral titer of each sample is reported as ± the 95% confidence intervals. The standard error, σ<sub>m</sub>, was calculated using the following formula:

$$\sigma_m^2 = d_f^2 \sum \frac{p_i(1-p_i)}{(n_i-1)}$$

where:

- d<sub>f</sub> = the logarithm of the dilution factor
- p<sub>i</sub> = the proportion of positive results at dilution i
- σ<sub>m</sub> = the standard error
- n<sub>i</sub> = number of replicates at dilution i

and  $\sum$  denotes the summation over dilutions beginning at the k<sup>th</sup> dilution. The 95% confidence interval is  $m \pm 1.96\sigma_m$ .

## CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL (continued)

When a sample contains a low concentration of virus there is a discrete probability that if only a fraction of the sample is tested for virus, that fraction will test negative due to random distribution of virus throughout the total sample. The probability,  $p$ , that the sample analyzed does not contain infectious virus is expressed by:  $p = [(V-v)/V]^y$ , where  $V$  is the total volume of the container,  $v$  is the volume of the fraction being tested, and  $y$  is the absolute number of infectious viruses randomly distributed in the sample. If  $V$  is sufficiently large relative to  $v$ , the Poisson distribution can approximate  $p$ :

$$P = e^{-cv} \quad \text{or} \quad c = -[\ln(P)] / v$$

Where  $c$  is the concentration of infectious virus and  $v$  is the total sample volume.

The amount of virus which would have to be present in the total sample in order to achieve a positive result with 95% confidence ( $p = 0.05$ ) is calculated as

$$c = -[\ln(0.05)] / v = 3 / v$$

If all  $n$  dishes are negative, the virus titer after the process is considered to be less than or equal to this value. The total volume of sample assayed is  $v = v'nd$ , where  $v'$  is the test volume in a dish,  $n$  is the number of dishes per sample, and  $d$  is the sample dilution.

**RESULTS:** Results are presented in Tables 1-3. All controls met the criteria for a valid test.

The formula for determining the log reduction factor (LRF) for each step is:

$$\text{LRF} = \text{Log}_{10} \left[ \frac{\text{Input Titer} \times \text{Input Volume}}{\text{Output Titer} \times \text{Output Volume}} \right]$$

When a sample is diluted and/or neutralized prior to being assayed, a volume correction factor should be included in the calculation of the viral load.

$$\text{Viral Load (log}_{10}) = \text{Virus Titer (log}_{10}/\text{mL)} + \text{log}_{10} [\text{volume (mL)}]$$

**RESULTS (continued):**

The 95% Confidence Interval (CI) for the LRF are calculated as follows:

$$(CI_{LRF})^2 = (CI_{input})^2 + (CI_{output})^2$$

The theoretical titer was determined in the following manner:

$$\text{Viral Load (log}_{10} \text{TCID}_{50}) = \text{Titer (log}_{10} \text{TCID}_{50}/\text{mL}) + \text{Log}_{10}[\text{Volume (mL)}]$$

In the case when all negatives are observed, simply replace the output load by c x Output Volume for calculating the log reduction, where c is taken from the Poisson 95% confidence interval discussed above, and substitute 0 for CI<sub>output</sub> in calculating the 95% confidence interval of the log reduction factor.

**Table 1  
Titer Results**

Sample	Titer ± 95% CI (Log <sub>10</sub> TCID <sub>50</sub> /mL)	Volume (mL)	Viral Load (Log <sub>10</sub> TCID <sub>50</sub> )
Cell Viability Control (negative control)	no virus detected, cells viable; media sterile		
Volume application evaluation	0.2 mL		
Virus Stock Titer Control	6.50 ± 0.00	-	-
Theoretical load <sup>a</sup>			5.80 ± 0.00
Baseline (Untreated) Control	5.50 ± 0.00	10	6.50 ± 0.00
VIOLIGHT toothbrush sanitizer	≤ -0.17 *	10	≤ 0.83

a Calculated based on the titer of the stock virus applied to the test sample (0.2 mL).

\* No virus was detected; the theoretical titer was determined based on the Poisson distribution.

**Table 2  
Neutralizer Effectiveness and Cytotoxicity Related Controls**

Dilutions	Neutralizer Effectiveness/Viral Interference Control	Cytotoxicity Control
Undilute	virus detected in all inoculated wells	no cytotoxicity observed
10 <sup>-1</sup>	virus detected in all inoculated wells	no cytotoxicity observed
10 <sup>-2</sup>	virus detected in all inoculated wells	no cytotoxicity observed

**Table 3  
Reduction Factor(s)**

	Initial Load (Log <sub>10</sub> TCID <sub>50</sub> )	Output Load (Log <sub>10</sub> TCID <sub>50</sub> )	Log <sub>10</sub> Reduction	Reduction (%)
VIOLIGHT toothbrush sanitizer	6.50 ± 0.00	≤ 0.83	≥ 5.67 ± 0.00	≥ 99.9998

## CONCLUSION

The viral reduction factor for the test agent is presented in Table 3. All of the controls met the criteria for a valid test. These conclusions are based on observed data.

Study director:   
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Tien V. Mai

08/20/09  
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Date