

**NON-GLP STUDY REPORT**

STUDY TITLE

Evaluation of Antimicrobial Effectiveness of SixLog's ionized Hydrogen Peroxide Application Unit

**Virus: Adenovirus type 2**

PRODUCT IDENTITY

iHP

AUTHOR

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STUDY COMPLETION DATE

May 5, 2011

PERFORMING LABORATORY

ATS Labs  
1285 Corporate Center Drive, Suite 110  
Eagan, MN 55121

SPONSOR

SixLog Corporation  
126 E. Dryer Road, Suite C  
Santa Ana, CA 92707

PROJECT NUMBER

A11312

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## STUDY REPORT

### GENERAL STUDY INFORMATION

**Study Title:** Evaluation of Antimicrobial Effectiveness of SixLog's ionized Hydrogen Peroxide Application Unit

**Project Number:** A11312

**TRF Number:** SLC01040511.ADV

### TEST SUBSTANCE IDENTITY

**Test Substance Name:** iHP

### STUDY DATES

**Date Sample Received:** April 15, 2011  
**Study Initiation Date:** April 13, 2011  
**Experimental Start Date:** April 21, 2011  
**Experimental End Date:** April 28, 2011  
**Study Completion Date:** May 5, 2011

### TEST PARAMETERS

**Dilution:** Test substance unit and solution was prepared by Sponsor representative

**Virus:** Adenovirus type 2, ATCC VR-846

**Exposure Times:** ~30 minute run time and ~30 minute dwell time

**Exposure Temperature:** Room temperature (20.0°C)

**Testing Room Size:** ATS Labs Testing Room, approximately 3600 square feet

**Organic Soil Load:** 5% fetal bovine serum

**Test Medium:** Eagles-MEM supplemented with 5% heat-inactivated fetal bovine serum, 100 units/mL penicillin, 10 µg/mL gentamicin, 2.5 µg/mL Fungizone and 10 mM Hepes

**Indicator Cell Cultures:** A-549 (Human Lung Carcinoma) cells

## EXPERIMENTAL DESIGN

Two films of virus, dried on individual glass surfaces, were exposed to the test substance that was applied using the Sponsor provided iHP test unit for an approximate 30 minute run time and an approximate 30 minute dwell time at room temperature (20.0°C). Following the exposure time, the room was allowed a vent time of 100 minutes, then the plates were removed from the testing room (160 minutes total time that plates were in testing room). A 2.00 mL aliquot of test media was added to each plate and the plates were scraped with a plastic cell scraper then the virucidal and cytotoxic activities were removed from the virus-test substance mixtures utilizing a Sephadex gel column. The mixtures were assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls were run concurrently. A 2.00 mL aliquot of test media was added to the dried virus control carriers and the carriers were held for 160 minutes at room temperature (20.0°C). Per Sponsor's direction, the study was not required to be conducted under US EPA 40 CFR Part 160 or US FDA 21 CFR Part 58.

## CONCLUSION

Under these test conditions, iHP **demonstrated complete inactivation** of Adenovirus type 2 when applied with the Sponsor provided iHP spray unit, following an approximate 30 minute run time and approximate 30 minute dwell time. Taking the cytotoxicity and neutralization control results into consideration, a  $\geq 5.89 \log_{10}$  average reduction in viral titer was demonstrated as compared to the average titer of the corresponding virus control.

In the opinion of the Author, there were no circumstances that may have affected the quality or integrity of the data.

**STUDY RESULTS**

**TABLE 1: Virus Control Results**

Dilution	Input Virus Control	Dried Virus Control 160 minutes at room temperature	
		Replicate #1	Replicate #2
Cell Control	0 0	0 0 0 0	0 0 0 0
10 <sup>-1</sup>	++	++++	++++
10 <sup>-2</sup>	++	++++	++++
10 <sup>-3</sup>	++	++++	++++
10 <sup>-4</sup>	++	++++	++++
10 <sup>-5</sup>	++	++++	++++
10 <sup>-6</sup>	++	++++	+ 0 ++
10 <sup>-7</sup>	++	0 0 0 0	0 0 0 0
10 <sup>-8</sup>	++	0 0 0 0	0 0 0 0
10 <sup>-9</sup>	0 0	NT	NT
TCID <sub>50</sub> /0.1 mL	10 <sup>8.50</sup>	10 <sup>6.50</sup>	10 <sup>6.25</sup>
Average TCID <sub>50</sub> /0.1 mL	NA	10 <sup>6.39</sup>	

(+) = Positive for the presence of test virus  
 (0) = No test virus recovered  
 (NA) = Not applicable  
 (NT) = Not tested

**TABLE 2: Effects of iHP Following a ~30 Minute Run Time and ~30 Minute Dwell Time Exposure to Adenovirus type 2 Dried on an Inanimate Surface**

Dilution	iHP + Adenovirus type 2 ~30 Minute Run Time + ~30 Minute Dwell Time	
	Replicate #1	Replicate #2
Cell Control	0 0 0 0	0 0 0 0
10 <sup>-1</sup>	0 0 0 0	0 0 0 0
10 <sup>-2</sup>	0 0 0 0	0 0 0 0
10 <sup>-3</sup>	0 0 0 0	0 0 0 0
10 <sup>-4</sup>	0 0 0 0	0 0 0 0
10 <sup>-5</sup>	0 0 0 0	0 0 0 0
10 <sup>-6</sup>	0 0 0 0	0 0 0 0
10 <sup>-7</sup>	0 0 0 0	0 0 0 0
10 <sup>-8</sup>	0 0 0 0	0 0 0 0
TCID <sub>50</sub> /0.1 mL	≤10 <sup>0.50</sup>	≤10 <sup>0.50</sup>
Average TCID <sub>50</sub> /0.1 mL	≤10 <sup>0.50</sup>	
Average Log Reduction	≥5.89 Log <sub>10</sub>	

(0) = No test virus recovered and/or no cytotoxicity present

**TABLE 3: Cytotoxicity Control and Neutralization Control**

Dilution	Cytotoxicity Control iHP	Neutralization Control iHP
Cell Control	0 0 0 0	0 0 0 0
10 <sup>-1</sup>	0 0 0 0	++++
10 <sup>-2</sup>	0 0 0 0	++++
10 <sup>-3</sup>	0 0 0 0	++++
10 <sup>-4</sup>	0 0 0 0	++++
TCD <sub>50</sub> /0.1 mL	≤10 <sup>0.50</sup>	See below

(+) = Positive for the presence of test virus


(0) = No test virus recovered and/or no cytotoxicity present

Results of the non-virucidal level control (neutralization control) indicate that the test substance was neutralized at a TCID<sub>50</sub>/0.1 mL of ≤0.50 log<sub>10</sub>.

**PROFESSIONAL PERSONNEL INVOLVED:**

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