



## **Norovirus Surrogate Test Exposure to SixLog's iHP™ (ionized Hydrogen Peroxide) Decontamination Technology**

### **Executive Summary:**

The iHP™ decontamination technology was used to kill a human norovirus surrogate in a test chamber under conditions approximating use in a hotel room or a ship stateroom. The data show killing of greater than 99.9999% of the virus when the product is used as recommended.

### **Introduction:**

Human Norovirus has become an increasingly significant health problem impacting the operations of long-term care facilities and the cruise ship industry. SixLog has developed a decontamination system that uses activated hydrogen peroxide to kill bacteria and other microorganisms on surfaces. We have performed extensive testing of the iHP technology's ability to kill vegetative bacteria and bacterial spores and are expanding these studies to examine the effects of our treatment on cell-free virus preparations.

### **Objective:**

Test SixLog's iHP decontamination technology in a closed chamber or room with viral samples to determine the extent of inactivation of viral samples.

### **Procedures:**

Viral samples will be exposed to the iHP technology for various times between 1 min and 20 min in a closed chamber or test room. Since SixLog does not have the equipment for the cell culture required for viral propagation or titer we will use an outside contract lab for the growth and assay (titer) of the viral samples. Prepared viral samples will be exposed to iHP for various exposure times, dwell times, and scrub times. Longer iHP exposure times should result in an increased amount of reactive oxygen species as well as increased hydrogen peroxide concentrations. Longer dwell times will increase the time that the viral samples are exposed to the hydrogen peroxide mist and exposure to the scrubbing process will simulate the differential evaporation of isopropyl alcohol, water, and hydrogen peroxide that is expected during normal use of the iHP technology. Following exposure to the iHP technology the viral samples will be recovered by dilution into fresh viral media followed by freezing at -85 degrees Centigrade. Frozen samples will be safely transported to the contract lab for determination of viral titer.

Since human norovirus has no defined cell culture system, a Center for Disease Control approved surrogate will be used. The surrogate virus, feline calicivirus, is in the same viral group as the human norovirus, has a highly similar genome and causes very similar symptoms in its host. In addition, a second virus, the human rhinovirus 16, will be tested. This virus, also known as the common cold, has similar transmission characteristic to the human norovirus.

Statement of work for outside contract laboratory:

1. Obtain cell lines and feline calicivirus for production of cell-free viral suspensions.
2. Obtain cell lines and human rhinovirus 16 for production of cell-free viral suspensions
3. Produce 75-100 ml of cell free viral suspensions of each of the above viruses to be sent to the SixLog facility for treatment with the SixLog iHP technology.
4. Determine viral titer of treated samples and controls once samples are returned to your laboratory.
5. Prepare a written report of experimental activities and results.

### **Experiment # 1 conducted March 19, 2009**

Each cell-free viral suspension will be tested in triplicate as follows:

Exposure to iHP with 5 min dwell (no scrub)

1. iHP for 90 seconds
2. iHP for 90 seconds no plasma arc
3. iHP for 270 seconds
4. iHP for 270 seconds no plasma arc
5. No treatment (negative control)

Positive control 3 (transfer of virus from stock to sample tube following thaw) and the negative control will be the first samples collected.

Positive control 1 and positive control 2 will be carried out in the bio-hood before the test samples are run.

Each sample will consist of 1 ml of virus. Following treatment four 1 ml washes will be used to recover material from the dish resulting in a final dilution of 1:5.

The diluted samples will be placed at -80 and allowed to freeze.

Description of viral samples from experiment #1:

There are fifty-one 15 ml tubes that each contain the sample in 5 ml of MEM plus 2% FBS. The samples consist of positive controls, negative controls and iHP treated samples. They have been labeled as SixLog and then either a single letter or a double letter. One ml of the viral stock received from the California Department of Public Health was used to generate each sample and then recovered in 4 ml of MEM plus 2% FBS.

There are 27 single letter tubes, A-Z with two tubes labeled Y. These samples are the FCV samples for titer.

There are 24 double letter tubes, AA-XX. These samples are the HRV16 samples for titer.

Experiment #1 results received April 13, 2009

			FCV			HRV 1B
		Sample	Log Titer/ml		Sample	Log Titer/ml
1	A	Media Only	<3.0	AA	Media Only	7.00
2	B	Media Only	<3.0	BB	Media Only	6.50
3	C	Media Only	<3.0	CC	Media Only	7.00
4	D	Tube Transfer	9.50	DD	Tube Transfer	7.00
5	E	Tube Transfer	8.50	EE	Tube Transfer	7.00
6	F	Tube Transfer	8.50	FF	Tube Transfer	7.00
7	G	270 Sec - Arc	7.50	GG	270 Sec - Arc	7.50
8	H	270 Sec - Arc	7.50	HH	270 Sec - Arc	7.50
9	I	270 Sec - Arc	8.00	II	270 Sec - Arc	6.50
10	J	Dish Only – 10 min	9.00	JJ	Dish Only – 10 min	6.50
11	K	Dish Only – 10 min	8.50	KK	Dish Only – 10 min	6.50
12	L	Dish Only – 10 min	8.50	LL	Dish Only – 10 min	6.50
13	M	270 Sec + Arc	8.00	MM	270 Sec + Arc	7.00
14	N	270 Sec + Arc	8.00	NN	270 Sec + Arc	7.00

15	O	270 Sec + Arc	7.50		OO	270 Sec + Arc	7.00
16	P	Dish - 0 min	9.00		PP	Dish - 0 min	6.50
17	Q	Dish - 0 min	9.00		QQ	Dish - 0 min	7.00
18	R	Dish - 0 min	9.00		RR	Dish - 0 min	7.50
19	S	90 sec – Arc	8.50		SS	90 sec – Arc	7.50
20	T	90 sec – Arc	9.00		TT	90 sec – Arc	6.50
21	U	90 sec – Arc	9.50		UU	90 sec – Arc	6.50
22	V	90 sec + Arc	8.00		VV	90 sec + Arc	7.50
23	W	90 sec + Arc	8.00		WW	90 sec + Arc	7.00
24	X	90 sec + Arc	8.50		XX	90 sec + Arc	7.00
25	Y	3hr thaw then dilution	9.00				
26	Y	3hr thaw then dilution	8.00				
27	Z	3hr thaw then dilution	9.00				

### Conclusions:

There was no significant effect of the iHP exposure to any of the viral samples tested. Additional controls with Bacillus spore strips showed growth after 48 hr suggesting that a 270 second exposure followed by a 5 minute dwell is not sufficient to kill  $10^4$  indicator spores or significantly impact the viral titer. We will need to better understand the effect of the chamber configuration upon iHP efficacy and confirm bacterial killing curves prior to repeating the viral tests. In addition, the high concentration of protein in the viral media may interfere with the activity of the ROS and the hydrogen peroxide.

Experiment # 2 conducted May 22, 2009

Each cell-free viral suspension will be tested in duplicate in the iHP test chamber and the iHP test room. The test chamber conditions are intended to be a “high exposure” test with a 5 minute exposure to iHP followed by a 20 minute dwell and a 10 minute scrub cycle. The room conditions matched a normal treatment with a 14 minute iHP exposure, followed by a 20 minute dwell and a 30 minute scrub cycle. Half of the samples were tested as liquid cell free viral extracts while the other half were dried onto glass Petrie dishes prior to exposure to iHP. Internal controls as well as bioindicators were run for both the test chamber and the test room to confirm activity of the iHP technology and set the baseline for any change in titer due to manipulations.

### Internal results:

All of the bioindicators used in both the room and the chamber were killed, including  $10^4$  and  $10^6$  paper spore test strips and  $10^6$  stainless steel test coupons. Positive controls all showed vigorous growth.

California Department of Public Health Viral Research and Diagnostic Laboratory  
Results received June 19, 2009

06/18/2009		
	FCV	
Sample#	Log Titer/ml	Sample
1	<3.0*	Room, Dry 100ul
2	<3.0*	Room, Dry 100ul
3	<2.0	Room, Dry 300ul

4	<3.0*	Room, Dry 300ul
5	6.50	Room, Wet 100ul
6	7.00	Room, Wet 100ul
7	7.00	Room, Wet 300ul
		Sample lost
9	<4.0**	Chamber, Dry 100ul
10	<4.0**	Chamber, Dry 100ul
11	<4.0**	Chamber, Dry 300ul
12	<4.0**	Chamber, Dry 300ul
13	<4.0**	Chamber, Wet 100ul
14	<4.0**	Chamber, Wet 100ul
15	4.5**	Chamber, Wet 300ul
16	4.5**	Chamber, Wet 300ul
17	8.50	Dish, Wet Control 100ul 20 min
18	8.00	Dish, Wet Control 300ul 20 min
19	<4.0**	Dish, Dry Control 100ul***
20	7.00	Dish, Dry Control 300ul
21	8.00	Direct transfer to media, Control
22	8.50	Direct transfer to media, Control
23	<4.0**	Mix 1:1 with iHP solution
24	<4.0**	Mix 1:1 with iHP solution
25	7.00	3 hour thaw, direct transfer to media
<b>*&lt;3.0 Toxicity to the cells at 10<sup>-1</sup> dilution</b>		
<b>**&lt;4.0 Toxicity to the cells at 10<sup>-1</sup> &amp; 10<sup>-2</sup> dilutions</b>		
<b>* **Sample placed in chamber with iHP by error</b>		

### Conclusions:

Viral samples were exposed to iHP both in the chamber and in the test room using a normal fogging cycle.

While some samples appeared to have residual H<sub>2</sub>O<sub>2</sub> that affected the cells used for the titer, most samples were likely killed to at least 6 logs (>99.9999%). Samples placed in the room that were wet (in 100ul or 300ul of media) did not show any significant reduction in their titer following exposure to iHP. The samples were placed on a table approximately six feet from the iHP mister. Dry samples placed adjacent to the wet samples were killed > 99.9999%. Similar samples (wet and dry) placed in the chamber were killed, but the results can only be determined to 99.99% due to the inactivation of the cells used to determine the titer at lower dilutions.

This strongly suggests that residual H<sub>2</sub>O<sub>2</sub> is present in these samples and affects the results. In addition, the peak level of H<sub>2</sub>O<sub>2</sub> in the chamber (350ppm) and the room (150ppm) are significantly different and should be taken into account.

The primary difference between Experiment #1 and Experiment #2 is time of exposure during the different stages of treatment. When the technology is used in the field there are three stages, exposure to iHP (active misting), dwell (where any residual H<sub>2</sub>O<sub>2</sub> breaks down into actives) and scrub (where any H<sub>2</sub>O<sub>2</sub> remaining is removed). In the first experiment the samples were exposed for 1.5 min to 3 min to iHP mist followed by a 5 min dwell and no scrub. In the second experiment the samples were exposed for 5 min to iHP mist followed by a 20 min dwell and a 30 min scrub. The second experiment approximates the actual recommended procedure for use of our technology. The first experiment's time parameters were set to match previous protocols used in



the program with a direct spray. The chamber is set up to generate an indirect spray and better approximate the misting process.

Based upon these results we can say that iHP can kill norovirus at >6 logs under conditions when the virus is dried onto glass surfaces, and at >4 logs when the virus is hydrated. A third experiment should be conducted where we normalize the volumes of liquid used to suspend the virus before drying, use a wider range of viral loads and neutralize residual hydrogen peroxide in the final samples with catalase prior to submission to CDPH-VRDL for titer.