CONFIDENTIAL FINAL REPORT

SPONSOR: GWELLKOREA.CO.LTD

SPONSOR'S REPRESENTATIVE: Warren Kim

STUDY TITLE: EVALUATION OF INACTIVATION OF AIRBORNE VIRUS BY AN AIR DISINFECTION DEVICE – Human Coronavirus (Similar to MERS-Coronavirus and SARS-Coronavirus)

STUDY IDENTIFICATION: MicroBioTest Project No. 916-101 (refer to signed protocol)

TEST AGENT NAME	LOT NO.	DATE RECEIVED	DS NO.
UPI Device (LUVA Air Purifier)	N/A	03/09/16	G122

ACTIVE INGREDIENT(S): Positive/negative ions

CHALLENGE ORGANISM: Human Coronavirus, strain 229E; ATCC VR-740

HOST CELL LINE: MRC-5 cells, ATCC CCL-171

DILUTION MEDIUM: 1X Minimum Essential Medium (MEM) + 2% Fetal Bovine Serum (FBS)

SEMI-SOLID COLLECTION MEDIUM : 1X MEM + 5% Gelatin + 1% FBS + 1% HEPES + 10 µg/mL Gentamicin + 1% $NaHCO_3$ + 1% Penicillin-Streptomycin + 2.5 µg/mL Amphotericin B

FLUSH MEDIUM : 1X MEM + 1% FBS + 1% HEPES + 10 µg/mL Gentamicin + 1% $NaHCO_3$ + 1% Penicillin-Streptomycin + 2.5 µg/mL Amphotericin B

AEROSOL MEDIUM: 0.1X MEM

VIRUS AEROSOL TIME: 25 minutes

EXPOSURE (CONTACT) TIME: 10 minutes and 30 minutes

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EXPOSURE TEMPERATURE: Ambient temperature

NUMBER OF REPLICATES: Two replicates per contact time at four wells per dilution

INCUBATION TEMPERATURE: 33±2°C with 5±1% CO_2

INCUBATION TIME: 5 – 7 days (7 days actual)

DEVICE CONNECTION: The UPI Device (LUVA Air Purifier) was connected to the adapter and plugged into a power supply to operate.

CALCULATION OF TITER:

The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) was determined using the Spearman-Karber method using the following formula:

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

where:

m = the logarithm of the dilution at which half of the wells are infected relative to the test volume
x_k = the logarithm of the smallest dosage which induces infection in all cultures
d = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i
$\sum p_i$ = the sum of p_i (starting with the highest dilution producing 100% infection)

The values were converted to $TCID_{50}$/mL using a sample inoculum of 1.0 mL.

RESULTS:

Results are presented in Tables 1-2.

The Viral load was determined in the following manner:

Viral Load (Log_{10} $TCID_{50}$) = Titer (Log_{10} $TCID_{50}$/mL) + Log_{10}[Volume (mL)]

The log_{10} Reduction Factor (LRF) was calculated in the following manner:

Log_{10} Reduction Factor = Initial viral load (Log_{10} $TCID_{50}$) – Output viral load (Log_{10} $TCID_{50}$)

RESULTS (Continued):

The Mean Viral Log_{10} Reduction from n replicates was determined as follows:

$$Log_{10}\left[\frac{10^{(Log_{10}Load_1)} + 10^{(Log_{10}Load_2)} + \ldots + 10^{(Log_{10}Load_n)}}{n}\right]$$

The Percentage of Virus Inactivation was calculated in the following manner:

[1-Output Viral Load / Initial Viral Load] x 100 = 1-10^ (-Log_{10} Reduction Factor) x 100

Table 1
Titer Results

Sample	Atomizer time	Replicates	Titer ($Log_{10}TCID_{50}$/mL)	Volume (mL)	Viral Load ($Log_{10}TCID_{50}$)
Cell viability/media sterility control	N/A	N/A	no virus detected, cells viable; media sterile		
Volume application evaluation			average volume of challenge per run: 10.1 mL		
Virus Stock Titer Control			7.00	-	-
Virus Input Load Control	N/A	1	4.75	10	5.75
		2	5.00	10	6.00
	Average				5.89
UPI Device (LUVA Air Purifier)*	10 minutes	1	2.75	10	3.75
		2	3.00	10	4.00
	30 minutes	1	1.50	10	2.50
		2	1.75	10	2.75

*Cytotoxicity observed at the undiluted dilution
NA = Not Applicable

RESULTS (Continued):

Table 2
Viral Reduction

Test Agent(s)	Atomizer Time	Replicate Number	Initial Viral Load* ($Log_{10}TCID_{50}$)	Output Viral Load ($Log_{10}TCID_{50}$)	Log_{10} Reduction	Percentage Log_{10} Reduction
UPI Device (LUVA Air Purifier)	10 minutes	1	5.89	3.75	2.14	99.1
		2		4.00	1.89	
	Mean Reduction ± 95% Confidence Interval				**2.04 ± 0.25**	
	30 minutes	1	5.89	2.50	3.39	99.9
		2		2.75	3.14	
	Mean Reduction ± 95% Confidence Interval				**3.29 ± 0.25**	

* Results represent the average of two replicates.

CONCLUSION:

GWELLKOREA.CO.LTD UPI Device (LUVA Air Purifier) was evaluated for its ability to inactivate Human Coronavirus (Similar to MERS-Coronavirus and SARS-Coronavirus). MicroBioTest personnel performed the inactivation procedure using Human Coronavirus, Strain: 229E. Samples were titrated by 50% tissue culture infectious dose ($TCID_{50}$) endpoint assay using MRC-5 cells.

The viral reductions for the GWELLKOREA.CO.LTD UPI Device (LUVA Air Purifier) are presented in Table 2. All of the controls met the criteria for a valid test.

Study Director:  06/08/2016

S. Steve Zhou, Ph.D. Date