MICROBIOTEST

CONFIDENTIAL FINAL REPORT

SPONSOR: Global Future Solutions

SPONSOR'S REPRESENTIVE: Brian Rhoades

STUDY TITLE: Virucidal Efficacy Suspension Test – Swine Influenza Virus (H1N1)

STUDY IDENTIFICATION: MICROBIOTEST Project No. 694-102 (refer to signed protocol)

TEST AGENT NAME	LOT NO.	DATE RECEIVED	DS NO.
GFS BioProtect™ Surface Spray Disinfectant Hospital Grade	20-7-09	07/22/09	10227

ACTIVE INGREDIENT(S): 3-(trimethoxysilyl) propydimethyloctadecyl, ammonium chloride, polyhexamethylene bioguanide, benzalkonium chloride

NEUTRALIZER: Fetal bovine serum + 0.5% Lecithin

CELL CULTURE MEDIUM: MEM + 1.0 μg/mL Trypsin

CHALLENGE ORGANISM: Swine Influenza Virus (H1N1), A/Swine/1976/31, ATCC VR-99

HOST: MDCK cells, ATCC CCL-34

EXPOSURE TIME: 60 Minutes

NUMBER OF REPLICATES: Four wells per dilution

CONTACT TEMPERATURE: Ambient Temperature (20C)

VIRUS APPLICATION: Direct mixing – 0.3 mL stock virus was spiked with 2.7 mL test agent and mixed by vortexing.

CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL

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 titer 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

The values were converted to $TCID_{50}$/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_m, was calculated using the following formula:

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

where:

d_f = the logarithm of the dilution factor
p_i = the proportion of positive results at dilution i
σ_m = the standard error
n_i = number of replicates at dilution i

and $\sum$ denotes the summation over dilutions beginning at the k^{th} dilution. The 95% confidence interval is $m \pm 1.96\sigma_m$.

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:

CALCULATION OF TITER AND 95% CONFIDENCE INTERVAL (continued)

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

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

$$c = -[\text{Ln}(0.05)] / v = 3 / v$$

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:

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

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.

Viral Load ($\log_{10}$) = Virus Titer ($\log_{10}$/mL) + $\log_{10}$ [volume (mL) x volume correction (mL)]

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

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

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_{output} in calculating the 95% confidence interval of the log reduction factor.

RESULTS (continued):

Table 1 – Titer Results

Sample	Titer ± 95% CI ($log_{10}TCID_{50}$/mL)	Volume (mL)	Volume Correction[a]	Viral Load ($log_{10}TCID_{50}$)
Virus Stock Titer Control	≥ 7.50 ± 0.00	-	-	-
Cell Viability Control	No virus was detected, cell remained viable; media were sterile			
GFS BioProtect™ Surface Spray Disinfectant Hospital Grade[b]	≤ 2.83 *	3	2	≤ 3.61
Virus Recovery Control	7.25 ± 0.25	3	2	8.03 ± 0.25
Column Titer Control	≤ 7.50 ± 0.00	3	2	±

[a] volume correction accounts for the neutralization of the sample post contact time.
[b] Cytotoxicity was observed at 10^-2
* No Virus was detected. The theoretical titer was determined based on Poisson distribution

Table 2 – Neutralizer Effectiveness and Cytotoxicity Related Controls

Dilution of the Test Agent/Neutralizer Mixture	Neutralizer Effectiveness Control	Cytotoxicity Control
10^-2	Cytotoxicity observed	Cytotoxicity observed
10^-3	Virus detected in all inoculated wells	No cytotoxicity observed
10^-4	Virus detected in all inoculated wells	No cytotoxicity observed

Table 3 – Reduction Factor(s)

Test Agent	Initial Load ($log_{10}TCID_{50}$)	Output Load ($log_{10}TCID_{50}$)	log_{10}Reduction	Reduction (%)
GFS BioProtect™ Surface Spray Disinfectant Hospital Grade	8.03 ± 0.25	≤ 3.61	≥ 4.42 ± 0.25	≥ 99.996

CONCLUSION

The viral reduction 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: _____ 08/20/2009
S. Steve Zhou, Ph.D. Date

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