REPORT

Virucidal efficacy of Germstar sanitizers against novel swine influenza virus H1N1

Sponsor:

Germstar

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TEST CONDITIONS

Challenge virus: Novel swine influenza virus H1N1 (A/California/4/2009/H1N1).

Host: Madin-Darby Canine Kidney (MDCK) cells

Products: **Sanitizer 1:** with 63% ethyl alcohol.
Sanitizer 2: with 63% ethyl alcohol, low alcohol odor.
Sanitizer 3: with 70% isopropyl alcohol.

Exposure temperature: Ambient room temperature (approx. 23ºC)

Diluent: Cell culture Maintenance medium

Growth medium: Minimum essential medium (MEM) with Earle's salt (Cell grow, Media Tech, VA, USA) containing antibiotics (150 IU/mL penicillin, 150 µg/mL streptomycin, 50 µg/mL neomycin and 1 µg/mL fungizone), 8% fetal calf serum, 0.05% trypsin, and Edamin S as additive.

Maintenance medium: The same medium as above except no fetal calf serum was added.

Protocol for the surface test: Sterilized stainless steel discs (approximately 1 cm in diameter) were placed in sterile 24-well tissue culture plates. On each disc, 20 µL of the test virus was placed separately and allowed to dry for 30 min. 1X concentration of one of the three sanitizers in 40 µL amount was placed on discs with the virus. Negative controls were maintained in which 40 µL of phosphate buffered saline was used instead of sanitizer. The amount of virus eluted from negative control wells was taken as the amount of initial virus. Virus from control and treated discs was eluted after contact times of 0.5, 1, and 2 minutes by adding 0.9 mL of 3% beef extract (pH 7.2) in each well. The beef extract solution was allowed to stay in contact with the disc for 30 minutes at room temperature in a bio-safety cabinet. The solution was then vigorously pipeted to release any remaining virus from the disc. Serial 10-fold dilutions of all eluates were prepared in the maintenance medium. All dilutions were inoculated in MDCK cells grown in 96-well tissue culture plates. The cells were observed for 5 days post infection for the appearance of virus specific CPE (cytopathic effects). The virucidal effects were expressed as $\log_{10}$ reduction in virus titers, which was calculated by the difference between virus titer of the virus-sanitizer mixture and virus titer of the control suspension. Per cent virus reduction at various time points was calculated and the results are shown in Table 1.

Table 1: Virucidal activity of Germstar against novel swine influenza virus H1N1 as determined by the surface test

	Percent reduction in virus titer After indicated contact time (minutes)		
	0.5	**1**	**2**
Sanitizer 1	99.76	99.46	99.87
Sanitizer 2	99.68	99.76	99.87
Sanitizer 3	99.76	99.76	99.87

Conclusion: The results shown are an average of 3 experiments.
All three sanitizers were found to reduce virus titer by >99.0% within 30 seconds.