REPORT

Virucidal efficacy of powder Stalosan F against PEDV (porcine epidemic diarrhea virus)

Study Director:

Sagar M. Goyal, Professor of Virology,
Department of Veterinary Population Medicine,
University of Minnesota, 1333 Gortner Ave,
Saint Paul, MN 55108, USA.

Tests conducted by:

Dr. Azad Singh

Date:

August 25, 2015

TEST CONDITIONS

Challenge Virus: PEDV

Product: Stalosan F powder.
Exposure temperature: Ambient room temperature (approx. 25ºC).
Virus growth medium: Minimum Essential Medium (MEM) with Earle's Salts supplemented with L-glutamine, antibiotics, 10% TPB (tryptone phosphate broth) and 3.0 µg/mL trypsin
Virus titration on: Vero-81 cells (African green monkey kidney cells).
Specific Aim: To determine the virucidal efficacy of 3 doses of powder Stalosan F against PEDV in a surface test for contact times of 5 min, 10 min, 60 min, and 6 hrs.

Test procedure:

1. A suspension of PEDV will be applied to wells of 24-well microtiter plates @100 µL of PEDV/well.
2. After the virus inoculum is dry, a dose of powder Stalosan F will be sprinkled on four virus-containing wells making sure to cover all of the virus inoculum with Stalosan F. Two other doses will be applied to eight other virus-containing wells.
3. Beef extract powder will be applied to 12 wells to serve as negative control; four wells each will be used to mimic the three doses of Stalosan F.
4. Surviving virus from pairs of wells (one experimental and one control) will be eluted in 300µL of eluent (3% beef extract solution in 0.05M glycine, pH 7.2) after 5 min, 10 min, 60 min, and 6 hrs of contact time.
5. The eluted samples will be tested for any surviving virus by preparing serial 10-fold dilutions of all eluates and inoculating them in Vero cells contained in 96-well microtiter plates.Three wells will be used per dilution.
6. The inoculated cells will be incubated at 37°C and 5% CO_2 for up to eight days. The plates will be examined daily under an inverted microscope for the appearance of virus-induced cytopathic effects (CPE).
7. The highest dilution of the sample showing positive CPE will be considered as the end point.
8. Virus titers in various samples will be calculated by the Karber method (1931).
9. Comparison of virus titers in control and experimental wells will indicate the amount and per cent of virus inactivated after a given contact time.

Results:

1. An experiment was conducted with 10 mg, 50 mg and 100 mg of Stalosan F powder using the above protocol. All samples from various doses of Stalosan and time points were cytotoxic to Vero cells while the virus titers in the control wells ranged from $10^{4.83}$ to $10^{5.16}$ per ml. The 10 mg sample was cytotoxic at 10^{-1} dilution while 50 mg and 100 mg samples were cytotoxic at up to 10^{-2} dilution. Due to cytotoxicity, no CPE could be detected in Stalosan samples and hence this experiment was abandoned.
2. We then tried the suspension test by mixing 5, 10, and 15 mg of Stalosan with 100 µL aliquots of the virus itself. Dilutions were made after a contact time of 5 minutes. All samples were highly toxic to cells. So, this approach was also abandoned.

3. Finally, surface test was tried once more using 10 mg and 20 mg of Stalosan powder with four different contact times (5, 10, and 60 minutes and 6 hours). *The difference in this experiment was that, after 90 minutes of incubation with different dilutions of the samples, the inoculated cells were washed twice with PBS (phosphate buffered saline) followed by incubation at 37C.* This approach was successful; no CPE was visible in any of the sample. This experiment was done in triplicate and the results are shown in Tables 1-3.

Table 1. Effect of Stalosan F powder on PEDV (Experiment 1)

Sanitizer	Time of Contact	Virus Titer (log_{10} $TCID_{50}$) in:		Percent virus reduction
		Control	Stalosan F	
A	5 min	4.17	<1	≥ 99.93
	10 min	4.50	<1	≥ 99.96
	60 min	4.17	<1	≥ 99.93
	6 hour	4.83	<1	≥ 99.99
B	5 min	4.50	<1	≥ 99.96
	10 min	4.50	<1	≥ 99.96
	60 min	4.17	<1	≥ 99.93
	6 hour	4.83	<1	≥ 99.99

A: 10 mg Stalosan F powder Control A: 10 mg beef extract powder
B: 20 mg Stalosan F powder Control B: 20 mg beef extract powder
$TCID_{50}$: 50% tissue culture infective dose of virus

Table 2. Effect of Stalosan F powder on PEDV (Experiment 2)

Sanitizer	Time of Contact	Virus Titer (log_{10} $TCID_{50}$) in:		Percent virus reduction
		Control	Stalosan F	
A	5 min	4.83	<1	≥ 99.99
	10 min	4.50	<1	≥ 99.96
	60 min	4.50	<1	≥ 99.96
	6 hour	4.17	<1	≥ 99.93
B	5 min	5.17	<1	≥ 99.99
	10 min	4.50	<1	≥ 99.96
	60 min	4.17	<1	≥ 99.93
	6 hour	4.50	<1	≥ 99.96

A: 10 mg Stalosan F powder Control A: 10 mg beef extract powder
B: 20 mg Stalosan F powder Control B: 20 mg beef extract powder
$TCID_{50}$: 50% tissue culture infective dose of virus

Table 3. Effect of Stalosan F powder on PEDV (Experiment 3)

Sanitizer	Time of Contact	Virus Titer (log_{10} $TCID_{50}$) in:		Percent virus reduction
		Control	Stalosan F	
A	5 min	4.83	<1	≥ 99.99
	10 min	4.83	<1	≥ 99.99
	60 min	4.50	<1	≥ 99.96
	6 hour	4.50	<1	≥ 99.96
B	5 min	4.83	<1	≥ 99.99
	10 min	4.50	<1	≥ 99.96
	60 min	5.17	<1	≥ 99.99
	6 hour	4.50	<1	≥ 99.96

A: 10 mg Stalosan F powder
B: 20 mg Stalosan F powder
Control A: 10 mg beef extract powder
Control B: 20 mg beef extract powder
$TCID_{50}$: 50% tissue culture infective dose of virus

Conclusion: Stalosan F powder, when applied at 10mg/2cm^2 and 20mg/2cm^2 surface area, was able to inactivate ≥99.97% of PEDV within 5 minutes.

Statistics: Conducting test between the mean value of control and Stalosan F at different time points revealed significant decline in the virus titer due to Stalosan F 10mg and 20mg application **(P<0.05)** .