

SHIC - FINAL RESEARCH GRANT REPORT

I. Project Title and SHIC project identification number: Evaluation of disinfectants against Seneca Valley virus (Project No. 15-187).

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II. Summary: This study was designed to determine the efficacy of three commonly used disinfectants against the newly emerging Seneca Valley virus (SVV) when applied to five different surfaces (cement, rubber, plastic, stainless steel, and aluminum). The disinfectants tested included bleach, Tek-Trol, and Synergize at 4⁰C and at room temperature (~25⁰C). The results showed that a 1:20 dilution of household bleach (5.25% sodium hypochlorite) was effective in killing the virus at either temperature and at any of the five surfaces tested. Tek-Trol was found not to be effective at either temperature while Synergize gave results that were between those of bleach and Tek-Trol.

III. Keywords: Disinfectants, Seneca Valley virus, surface test, virucidal efficacy, cement, rubber, plastic, stainless steel, and aluminum.

IV. Scientific Abstract: We conducted the present study to evaluate the virucidal efficacy of three commercial disinfectants against SVV by the surface test using five different surfaces and two different temperatures (~25⁰C and 4⁰C). (1) Household bleach at 1:20 dilution was able to inactivate $\geq 99.99\%$ of the virus within 10-15 minutes on aluminum, rubber, and plastic at room temperature. At this temperature, the results obtained with bleach on stainless steel and cured cement were similar (99.97 and 99.98%, respectively). At 4⁰C, bleach was found to be a little better; it killed 99.99% virus within 5-15 minutes on all surfaces except rubber (on rubber, virus inactivation was 99.91% after a contact period of 15 min). (2) Tek-Trol was the least effective. It did not kill more than 82.41% of the virus at either temperature and on either surface even after a contact time of 60 min. Even when the concentration of Tek-Trol was increased (a dilution of 1:125 v/s 1:250 as recommended by the manufacturer), the amount of virus inactivated did not increase (see Table 31) (3) Synergize gave results that were between those obtained with bleach and Tek-Trol. It was able to inactivate 93% to 99% of the virus within 60 min at both temperatures and at all surfaces. These results indicate that there is a significant amount of variation among the efficacy of various disinfectants and hence disinfectants should be tested against various pathogens before being used in the field.

V. Introduction: Seneca Valley virus (SVV) is a small RNA virus belonging to the family *Picornaviridae*. The virus has recently been isolated from cases of neonatal mortality in pigs in Brazil and the US. It also causes a vesicular disease in pigs, the lesions of which are very similar to those of foot-and-mouth disease. Hence, it is important to conduct studies on this virus including its susceptibility to routinely used disinfectants. To control virus transmission, it is essential to disinfect the farm premises. The use of disinfectants is considered as a useful option to prevent and control viral infections among swine farms. In this study we evaluated the effectiveness of three different disinfectants against SVV when applied to five different surfaces at two different temperatures. This information can provide guidance on effective disinfection procedures against this virus to the swine producers.

VI. Project Objectives – The overall objective is to evaluate the efficacy of certain disinfectants on the inactivation of Seneca Valley Virus (SVV) applied to various surfaces including cured cement, aluminum, stainless steel, plastic and rubber boots at two different temperatures (4°C and ~25°C).

Specific aims:

1. Inactivation kinetics of SVV on stainless steel, cured cement, aluminum plate, and pieces of rubber boot in the presence of 5% bleach.
2. Inactivation kinetics of SVV on stainless steel, cured cement, aluminum plate, and pieces of rubber boot in the presence of Synergize at a concentration as recommended by the manufacturer (1:256).
3. Inactivation kinetics of SVV on stainless steel, cured cement, aluminum plate, and pieces of rubber boot in the presence of a phenolic (Tek-Trol) at a concentration as recommended by the manufacturer (1:250).

V. Materials & Methods:

1. Viruses and cells: We used a contemporary strain of SVV for these experiments. The virus was propagated and titrated in ST (swine testicular) cells. The cells were grown in Minimum Essential Medium (MEM) with Eagle's salts supplemented with L-glutamine, 8% fetal bovine serum, 50 µg/mL gentamicin, 150 µg/mL neomycin sulfate, 1.5 µg/mL fungizone, and 455 µg/mL streptomycin. The maintenance medium included MEM with antibiotics and 4% donor horse serum. The cells were washed three times with phosphate buffered saline (PBS, pH 7.2) before virus inoculation. After virus inoculation, the cells were incubated at 37°C for 1-2 h for virus adsorption using appropriate maintenance medium. Inoculated cells were incubated at 37°C under 5% CO₂ and were observed for the appearance of virus-induced cytopathic effects (CPE). The CPE appeared at 3-4 days post-infection. The cells were subjected to three freeze-thaw cycles (-80°C/25°C) followed by centrifugation at 2500×g for 15 min at 4°C. The supernatant (virus stock) was collected and aliquoted into 50 mL centrifuge tubes (Corning Corp., Life Sciences, Tewksbury, MA) followed by storage at -80°C until use.

2. Virus titration: Serial 10-fold dilutions of all samples were prepared in MEM followed by inoculation of cell culture monolayers grown in 96-well microtiter plates. Each dilution was inoculated in three wells. The highest dilution showing cytopathic effects in inoculated cell cultures after an appropriate incubation period (up to 4 days post inoculation) was considered the end point. Virus titers were calculated by the method of Karber (1931) and expressed as 50% tissue culture infective dose (TCID₅₀/mL). The amount of surviving virus was compared with the virus titer in negative control to determine the amount of virus inactivated.

3. Disinfectants: Three disinfectants; Household bleach, Synergize, and Tek-Trol were evaluated. Dilutions of disinfectants were prepared in sterile distilled water. The household bleach is a solution of 5.25% sodium hypochlorite. This solution was used at a 1:20 dilution in these experiments. For the other two disinfectants we used dilutions as recommended by their manufacturers e.g., Synergize (1:256) and Tek-Trol (1:250).

4. Repeatability: All experiments were done in triplicate and the values were averaged.

5. Temperatures: All experiments were done at room temperature (approximately 25°C) and at 4°C.

6. Test procedure: We used the surface test to evaluate these disinfectants. In this test, the virus is applied to a surface followed by treatment with a certain concentration of a disinfectant being evaluated. We tested five different surfaces e.g., stainless steel, cured cement, aluminum plate, plastic, and pieces of rubber boot. Briefly, the stock virus was applied to coupons of a surface contained in 24-well microtiter plates. The amount of virus applied per coupon was 40 µl. The plate containing coupons was placed in a

biosafety cabinet and the virus was allowed to air dry following which 50 µl of the disinfectant solution was applied making sure that the disinfectant covered the whole area where virus had been applied. The negative controls consisted of virus-applied coupons and 50 µl of maintenance media (rather than the disinfectant). After 1, 3, 5, 10, and 15 min at room temperature (~25°C) or at 4°C, any surviving virus was eluted using 400 µl of elution buffer (3% beef extract-0.05M glycine, pH 7.2). Serial ten-fold dilutions of elutes were prepared for virus titration. Virus titers from maintenance media-treated and disinfectant-treated eluates were compared to determine the efficacy of a given disinfectant. In certain cases, the times were increased to 10, 15, 30, and 60 min because there was no appreciable decrease in virus titer after treatment for 15 min. In one experiment, higher concentration (lower dilution) of Tek-Trol was used. The results are shown below in tables 1-31.

VI. Results:

The SVV is a non-enveloped virus. However, its resistance to disinfectants was different as can be seen in Tables 1-31.

VII. Discussion:

The SVV is a very important virus because the lesions produced by this virus can be confused with those of FMDV, which might result in a false alarm. Hence, research on this virus is necessary so that we can be proactive in case it becomes a major problem. This virus is non-enveloped as compared to enteric coronaviruses (such as TGEV, PEDV, and PDCoV). In general non-enveloped viruses are less susceptible to the action of disinfectants than enveloped viruses. As can be seen from the results of this study, there is a lot of variation between the efficacy of the three disinfectants studied. It is important, therefore, to evaluate various disinfectants used in the field to ensure that they serve their intended purpose.

Table 1. Inactivation of SVV by bleach (5%) on aluminum at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
1	2.83	5.50	99.78	99.70
	2.83		99.78	
	3.16		99.54	
3	< 1	5.16	99.99	99.50
	3.16		99.00	
	2.83		99.53	
5	4.16	5.16	90.00	96.43
	2.83		99.53	
	2.50		99.78	
10	< 1	5.50	≥ 99.99	≥ 99.99
	< 1		≥ 99.99	
	< 1		≥ 99.99	
15	< 1	5.50	≥ 99.99	≥ 99.99
	< 1		≥ 99.99	
	< 1		≥ 99.99	

Table 2. Inactivation of SVV by bleach (5%) on stainless steel at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
1	4.16	5.16	90.00	96.52
	2.50		99.78	
	2.50		99.78	
3	2.50	5.83	99.95	97.71
	4.50		95.32	
	4.16		97.86	
5	3.50	6.50	99.90	99.96
	2.50		99.99	
	2.50		99.99	
10	2.83	5.83	99.90	99.93
	2.50		99.95	
	2.50		99.95	
15	< 1	5.83	≥ 99.99	99.97
	2.50		99.95	
	1.83		99.99	

Table 3. Inactivation of SVV by bleach (5%) on rubber at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
1	1.83	4.50	99.78	99.77
	2.16		99.54	
	< 1		≥ 99.99	
3	2.16	5.83	99.97	99.76
	2.83		99.90	
	3.16		99.41	
5	3.83	6.50	99.78	99.74
	4.16		99.54	
	3.5		99.90	
10	3.16	5.50	99.54	99.62
	2.83		99.78	
	3.16		99.54	
15	< 1	4.50	≥ 99.99	≥ 99.99
	< 1		≥ 99.99	
	< 1		≥ 99.99	

Table 4. Inactivation of SVV by bleach (5%) on cured cement at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
1	2.16	3.83	97.86	76.56
	3.16		78.62	
	3.50		53.22	
3	3.83	5.16	95.32	94.95
	4.16		90.00	
	2.83		99.53	
5	< 1	4.5	≥ 99.99	92.53
	2.5		99.00	
	3.83		78.62	
10	2.83	5.16	99.53	97.95
	3.16		99.00	
	3.83		95.32	
15	< 1	5.83	≥ 99.99	99.98
	2.16		99.97	
	2.16		99.97	

Table 5. Inactivation of SVV by bleach (5%) on plastic at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
1	2.16	5.50	99.95	99.96
	1.83		99.97	
	1.83		99.97	
3	1.83	5.50	99.97	99.98
	< 1		≥ 99.99	
	1.83		99.97	
5	1.83	5.50	99.97	99.98
	< 1		≥ 99.99	
	< 1		≥ 99.99	
10	< 1	5.50	≥ 99.99	≥ 99.99
	< 1		≥ 99.99	
	< 1		≥ 99.99	
15	< 1	4.50	≥ 99.99	≥ 99.99
	< 1		≥ 99.99	
	< 1		≥ 99.99	

Table 6. Inactivation of SVV by bleach (5%) on aluminum at 4 °C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
1	2.50	4.83	99.53	99.53
	2.50		99.53	
	2.50		99.53	
3	2.50	5.50	99.90	99.90
	2.50		99.90	
	2.50		99.90	
5	2.50	5.83	99.95	99.95
	2.50		99.95	
	2.50		99.95	
10	< 1	4.83	≥ 99.99	99.83
	< 1		≥ 99.99	
	2.50		99.53	
15	< 1	4.50	≥ 99.99	≥ 99.99
	< 1		≥ 99.99	
	< 1		≥ 99.99	

Table 7. Inactivation of SVV by bleach (5%) on stainless steel at 4 °C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
1	3.50	5.16	97.81	98.86
	2.50		99.78	
	3.16		99.00	
3	3.50	5.16	97.81	99.20
	2.16		99.90	
	2.16		99.90	
5	2.50	6.16	99.99	99.99
	2.83		99.99	
	2.50		99.99	
10	2.50	6.16	99.99	99.99
	2.50		99.99	
	2.50		99.99	
15	< 1	4.50	≥ 99.99	≥ 99.99
	< 1		≥ 99.99	
	< 1		≥ 99.99	

Table 8. Inactivation of SVV by bleach (5%) on rubber at 4 °C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
1	2.50	4.83	99.53	99.53
	2.50		99.53	
	2.50		99.53	
3	2.50	4.16	97.81	97.81
	2.50		97.81	
	2.50		97.81	
5	2.50	4.50	99.00	99.18
	2.16		99.54	
	2.50		99.00	
10	2.50	5.16	99.78	99.78
	2.50		99.78	
	2.50		99.78	
15	2.16	5.16	99.90	99.91
	1.83		99.95	
	2.16		99.90	

Table 9. Inactivation of SVV by bleach (5%) on cured cement at 4 °C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
1	2.16	3.83	97.86	90.60
	3.16		78.62	
	2.50		95.32	
3	2.83	4.16	95.32	94.37
	3.16		90.00	
	2.50		97.81	
5	< 1	4.50	99.99	99.33
	2.50		99.00	
	2.50		99.00	
10	1.83	4.16	99.53	97.55
	2.50		97.81	
	2.83		95.32	
15	< 1	3.83	≥ 99.99	≥ 99.99
	< 1		≥ 99.99	
	< 1		≥ 99.99	

Table 10. Inactivation of SVV by bleach (5%) on plastic at 4 °C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
1	1.83	4.50	99.78	99.85
	< 1		≥ 99.99	
	1.83		99.78	
3	1.83	4.50	99.78	99.62
	2.16		99.54	
	2.16		99.54	
5	< 1	4.80	≥ 99.99	99.92
	2.16		99.78	
	< 1		≥ 99.99	
10	< 1	4.50	≥ 99.99	≥ 99.99
	< 1		≥ 99.99	
	< 1		≥ 99.99	
15	< 1	4.50	≥ 99.99	≥ 99.99
	< 1		≥ 99.99	
	< 1		≥ 99.99	

Table 11. Inactivation of SVV by Tek-Trol (1:250) on aluminum at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	4.50	5.16	78.12	52.08
	5.16		00.00	
	4.50		78.12	
15	5.16	5.50	54.29	36.19
	5.50		00.00	
	5.16		54.29	
30	5.16	5.16	00.00	00.00
	5.50		00.00	
	5.16		00.00	
60	4.16	5.16	90.00	77.74
	4.16		90.00	
	4.83		53.22	

Table 12. Inactivation of SVV by Tek-Trol (1:250) on stainless steel at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.50	4.50	90.00	74.30
	4.16		54.29	
	3.83		78.62	
15	4.16	4.50	54.29	18.10
	4.50		00.00	
	4.50		00.00	
30	4.50	4.83	53.22	61.68
	4.16		78.62	
	4.50		53.22	
60	3.83	4.16	53.22	61.52
	3.83		53.22	
	3.50		78.12	

Table 13. Inactivation of SVV by Tek-Trol (1:250) on rubber at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	5.50	5.50	00.00	60.00
	4.50		90.00	
	4.50		90.00	
15	5.16	5.50	54.29	36.19
	5.50		00.00	
	5.16		54.29	
30	4.50	5.50	90.00	78.10
	4.50		90.00	
	5.16		54.29	
60	4.50	5.50	90.00	56.20
	4.83		78.62	
	5.83		00.00	

Table 14. Inactivation of SVV by Tek-Trol (1:250) on cured cement at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	5.50	5.50	00.00	26.20
	4.83		78.62	
	5.50		00.00	
15	5.50	5.16	00.00	26.20
	4.50		78.12	
	5.16		00.00	
30	5.50	5.50	00.00	26.20
	4.83		78.02	
	5.50		00.00	
60	5.16	5.50	54.29	18.10
	5.50		0.000	
	5.50		0.000	

Table 15. Inactivation of SVV by Tek-Trol (1:250) on plastic at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.50	4.16	78.12	73.78
	3.83		53.22	
	3.16		90.00	
15	3.50	4.16	78.12	52.08
	4.16		00.00	
	3.50		78.12	
30	3.16	4.16	90.00	73.78
	3.83		53.22	
	3.16		78.12	
60	2.83	3.83	90.00	47.74
	3.83		00.00	
	3.50		53.22	

Table 16. Inactivation of SVV by Tek-Trol (1:250) on aluminum at 4°C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.83	4.16	53.22	43.78
	3.50		78.12	
	4.16		00.00	
15	3.16	4.16	90.00	82.08
	3.50		78.12	
	3.50		78.12	
30	4.16	4.50	54.29	62.40
	3.83		78.62	
	4.16		54.29	
60	4.16	4.16	00.00	26.04
	3.50		78.12	
	4.16		00.00	

Table 17. Inactivation of SVV by Tek-Trol (1:250) on stainless steel at 4°C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.50	3.83	53.22	47.74
	2.83		90.00	
	3.83		00.00	
15	3.83	3.83	00.00	00.00
	3.83		00.00	
	3.83		00.00	
30	3.16	4.16	90.00	60.00
	4.16		00.00	
	3.16		90.00	
60	3.50	4.50	90.00	82.41
	3.83		78.62	
	3.83		78.62	

Table 18. Inactivation of SVV by Tek-Trol (1:250) on rubber at 4°C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.50	3.83	53.22	35.48
	3.50		53.22	
	3.83		00.00	
15	4.16	4.16	00.00	17.74
	3.83		53.22	
	4.16		00.00	
30	4.16	4.16	00.00	35.48
	3.83		53.22	
	3.83		53.22	
60	3.50	4.50	90.00	79.90
	4.16		54.29	
	3.16		95.42	

Table 19. Inactivation of SVV by Tek-Trol (1:250) on cured cement at 4°C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.83	4.50	78.62	70.51
	3.83		78.62	
	4.16		54.29	
15	4.50	4.16	00.00	43.78
	3.83		53.22	
	3.50		78.12	
30	4.50	4.16	00.00	00.00
	4.16		00.00	
	4.16		00.00	
60	4.16	4.16	00.00	17.74
	3.83		53.22	
	4.16		00.00	

Table 20. Inactivation of SVV by Tek-Trol (1:250) on plastic at 4°C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.83	4.16	53.22	17.74
	4.16		00.00	
	4.16		00.00	
15	3.50	4.50	90.00	56.20
	3.83		78.62	
	4.50		00.00	
30	4.16	4.50	54.29	18.09
	4.83		00.00	
	4.50		00.00	
60	4.16	4.50	54.29	18.09
	4.50		00.00	
	4.50		00.00	

Table 21. Inactivation of SVV by Synergize (1:256) on aluminum at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	2.50	3.50	90.00	90.00
	2.50		90.00	
	2.50		90.00	
15	2.83	4.16	95.32	96.15
	2.50		97.81	
	2.83		95.32	
30	2.50	4.50	99.00	98.62
	2.83		97.86	
	2.50		99.00	
60	2.50	4.16	97.81	96.98
	2.50		97.81	
	2.83		95.32	

Table 22. Inactivation of SVV by Synergize (1:256) on stainless steel at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	2.50	3.50	90.00	86.20
	2.83		78.62	
	2.50		90.00	
15	2.50	4.16	97.81	96.15
	2.83		95.32	
	2.83		95.32	
30	2.50	4.16	97.81	97.81
	2.50		97.81	
	2.50		97.81	
60	2.50	3.83	95.32	95.32
	2.50		95.32	
	2.50		95.32	

Table 23. Inactivation of SVV by Synergize (1:256) on rubber at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	2.50	3.50	90.00	90.00
	2.50		90.00	
	2.50		90.00	
15	3.16	3.83	78.62	86.20
	2.83		90.00	
	2.83		90.00	
30	2.83	4.50	97.86	92.62
	3.50		90.00	
	3.50		90.00	
60	2.50	4.83	99.53	99.53
	2.50		99.53	
	2.50		99.53	

Table 24. Inactivation of SVV by Synergize (1:256) on cured cement at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	4.16	4.83	78.62	78.62
	4.16		78.62	
	4.16		78.62	
15	3.83	5.83	99.00	98.62
	4.16		97.86	
	3.83		99.00	
30	3.50	5.16	97.81	90.41
	4.50		78.12	
	3.83		95.32	
60	3.50	4.83	99.53	99.81
	2.50		99.95	
	2.50		99.95	

Table 25. Inactivation of SVV by Synergize (1:256) on plastic at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.83	4.50	78.62	86.20
	3.50		90.00	
	3.50		90.00	
15	3.50	4.83	95.32	95.32
	3.50		95.32	
	3.50		95.32	
30	3.50	5.16	97.81	97.81
	3.50		97.81	
	3.50		97.81	
60	3.16	5.50	99.54	99.54
	3.16		99.54	
	3.16		99.54	

Table 26. Inactivation of SVV by Synergize (1:256) on aluminum at 4 °C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.83	4.83	90.00	91.77
	3.83		90.00	
	3.50		95.32	
15	3.50	4.50	90.00	90.00
	3.50		90.00	
	3.50		90.00	
30	3.50	4.83	95.32	95.32
	3.50		95.32	
	3.50		95.32	
60	3.50	4.83	95.32	95.32
	3.50		95.32	
	3.50		95.32	

Table 27. Inactivation of SVV by Synergize (1:256) on stainless steel at 4 °C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	2.83	4.16	95.32	96.15
	2.50		95.32	
	2.83		97.81	
15	3.50	4.50	90.00	86.20
	3.50		90.00	
	3.83		78.62	
30	2.50	5.16	99.78	97.80
	3.83		95.32	
	3.83		95.32	
60	3.50	4.83	95.32	93.54
	3.83		90.00	
	3.50		95.32	

Table 28. Inactivation of SVV by Synergize (1:256) on rubber at 4 °C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.83	4.50	78.62	78.62
	3.83		78.62	
	3.83		78.62	
15	3.50	4.50	90.00	90.00
	3.50		90.00	
	3.50		90.00	
30	3.83	4.50	78.62	82.41
	3.83		78.62	
	3.50		90.00	
60	3.83	5.16	95.32	96.98
	3.50		97.81	
	3.50		97.81	

Table 29. Inactivation of SVV by Synergize (1:256) on cured cement at 4 °C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.16	4.50	95.42	96.61
	3.16		95.42	
	2.50		99.00	
15	3.50	4.50	90.00	92.62
	3.50		90.00	
	2.83		97.86	
30	2.83	4.83	99.00	99.17
	2.83		99.00	
	2.50		99.53	
60	2.83	4.16	95.32	96.98
	2.50		97.81	
	2.50		97.81	

Table 30. Inactivation of SVV by Synergize (1:256) on plastic at 4 °C

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	3.50	4.16	78.12	78.12
	3.50		78.12	
	3.50		78.12	
15	3.50	4.16	78.12	78.12
	3.50		78.12	
	3.50		78.12	
30	3.16	5.16	99.00	97.37
	3.50		97.81	
	3.83		95.32	
60	2.83	4.83	99.00	94.77
	3.50		95.32	
	3.83		90.00	

Table 31. Inactivation of SVV by Trek-Trol (1:125) on plastic at room temperature

Time (Min)	Virus titer (TCID ₅₀ / ml)		Percent reduction in triplicate experiments	Average percent reduction
	With disinfectant	With MEM (Negative control)		
10	2.83	3.50	78.62	70.51
	3.16		54.29	
	2.83		78.62	
15	4.50	4.50	00.00	00.00
	5.16		00.00	
	4.50		00.00	
30	5.16	4.50	00.00	26.07
	4.83		00.00	
	3.83		78.22	
60	2.83	3.16	53.22	53..22
	2.83		53.22	
	2.83		53.22	