STUDY TITLE

Assessment of the Activity of an Anatomical Embalming Fluid against Human Pathogenic Viruses using ASTM Protocol #E1053-20:
Testing with Hepatitis A Virus (HAV) as a representative Non-enveloped Virus[1]

TEST ORGANISM

Hepatitis A virus; HM175/18f (ATCC VR-1402)

TEST ITEM/SUBSTANCE IDENTITY

GreenMBalm Anatomical Embalming Fluid
Lot # C0223-008

TEST STANDARD

ASTM E1053-20

AUTHOR/STUDY DIRECTOR

Bahram Zargar, PhD

STUDY COMPLETION DATE

Jan/12/24

TEST FACILITY

CREM Co. Labs. Units 1-2, 3403 American Dr., Mississauga, Ontario, Canada L4V 1T4

SPONSOR

Green Solutions Group
2085 Gold Knob Road
Salisbury, NC 28146

STUDY NUMBER

GSG-231116-HAV

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2. STUDY PERSONNEL

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STUDY REPORT

3. GENERAL STUDY INFORMATION

Study Title: Assessment of the Activity of an Anatomical Embalming Fluid against Human Pathogenic Viruses using ASTM Protocol #E1053-20: Testing with Hepatitis A Virus (HAV) as a representative Non-enveloped Virus

Study Number: GSG-231116-HAV

Protocol Number: GSG-231116-HAV

Sponsor Green Solutions Group
2085 Gold Knob Road, Salisbury, NC 28146

Testing Facility CREM Co Labs
Unit 1-2, 3403 American Drive, Mississauga, ON, Canada L4V 1T4

4. TEST SUBSTANCE IDENTITY

Test Substance Name: GreenMBalm Anatomical Embalming Fluid
Lot/Batch(s): Lot # C0223-008

5. STUDY DATES

Date Sample Received: Dec/04/23
Study Initiation Date: Dec/01/23
Experimental Start Date: Dec/06/23
Experimental End Date: Dec/25/23
Study Completion Date: Jan/12/24

6. OBJECTIVE AND RATIONALE

The objective of this study was to evaluate the virucidal activity of one lot (B23-675 and B23-676) of one test sample provided by the Sponsor against HAV as a representative, non-enveloped healthcare-associated pathogen. The test procedure was to simulate the way in which the product is intended to be used. This method complies with the requirements of Health Canada and U.S. Environmental Protection Agency (EPA).

HAV was chosen as a test virus because it may be present in the tissues to be preserved and also because of its relatively high resistance to chemical disinfection. HAV is also represents other hepatitis viruses such as Hepatitis B and C. Hepatitis B and C are also enveloped viruses therefore would be expected to be less resistant to disinfectant as compared to HAV.

7. SUMMARY OF RESULTS

Test Substance: GreenMBalm Anatomical Embalming Fluid
Lot No.: C0223-008

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Test Carriers	100 mm diameter Plastic Petri dishes • Number of carriers for control: 3 • Number of carriers tested per lot of test substance: 3
Dilution:	Ready to Use (RTU)
Test Organism	Hepatitis A virus (HAV) ATCC VR-1402
Contact Time:	10 minutes
Neutralizer:	Dey-Engley Broth
Exposure Temperature:	23±2ºC
Soil Load:	5% Fetal bovine serum (FBS)
Efficacy Result:	The test substance (GreenMBalm Anatomical Embalming Fluid, Lot #: C0223-008) demonstrated 3.29±0.38 $\log_{10}$ reduction (99.93±0.059 Percent reduction) in 10-minute contact time against Hepatitis A virus (HAV) and passed the performance criteria.
Neutralization and Cytotoxicity Result:	Neutralization validation test was also performed on test substance GreenMBalm Anatomical Embalming Fluid using Dey-Engley Broth and Sephadex column LH-20. The used neutralizer was suitable for neutralizing activity of GreenMBalm Anatomical Embalming Fluid without showing any cytotoxicity.

8. TEST SYSTEM

9.1 Test Microorganism

Hepatitis A virus (HAV) is a small, non-enveloped hepatotropic virus classified in the genus *Hepatovirus* within the family Picornaviridae. It causes an acute infection of the liver. HAV can be transmitted through contaminated water, food and via the faecal–oral route among close contacts (e.g. household contacts, sexual contacts, healthcare settings, day-care centers or schools). HAV is relatively resistant to environmental stressors and also to food preservatives and disinfectants.

9.2 Host Cell Line

FRhK-4 cells were used as the host for replication of HAV. It is a rapidly growing fetal rhesus monkey kidney cell line.

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The cells were seeded into 12-well multi-well cell culture plates in a modified Eagle's medium (1XMEM) supplemented with fetal bovine serum (FBS), non-essential amino acids and maintained at 36±1°C in a humidified atmosphere of 5% CO2. Efficacy tests were performed when the cell monolayer reached >90% confluency.

9.3 Preparation of Test Inocula

To prepare the test inocula, a stock culture of virus stored in labelled cryovials at -80°C was grown in monolayers of FRhK-4 cells at 36±1ºC. FRhK-4 cells was used for plaque assay of the virus.

9. TEST METHOD

10.1 Preparation of Test Substances

The efficacy tests were performed on RTU samples as specified by the Sponsor.

10.2 Test Procedure

ASTM International's method E1053-20 was used to test the environmental surface decontamination process. Each test carrier (flat glass 100 mm Petri dish) was inoculated with 200 µL of the test virus suspension with 5% FBS as a soil load. All inoculated carriers were left under an operating biological safety cabinet (BSC) for 60±5 minutes or until visibly dry. 2mL of test substance was placed over the dried film. Control carriers received an equivalent volume of Earl's balanced salt solution (EBSS) harmless to the test virus and its host cells. Immediately at the end of the contact time (10 minute), 2 mL of the neutralizer was added to each test and control carrier prior to assaying for viral infectivity in host cell monolayers.

A 10-fold dilution series was prepared for each test and control eluate using EBSS. The appropriate dilutions were inoculated onto monolayers of FRhK-4 cells and incubated at 33±1°C for 60 minutes for virus adsorption.

After virus adsorption, an overlay medium was added to each well and the plates left in a BSC for the overlay to solidify. They were then incubated at 36±1°C in an incubator with 5% CO_2. The monolayers were fixed and stained after 7 days of incubation and the plaques on them counted and recorded to determine $\log_{10}$ reductions in the viability of the virus.

10.3 Experimental Design

a) Input

The stock virus utilized in the testing was titrated by a 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control were for informational purposes only.

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b) Cytotoxicity Control

The cytotoxicity control test was performed on the test substance and, temperature and humidity were recorded. The cytotoxicity test included the following steps:

- 2mL of the test substance was placed in a sterile petri dish and then 2 mL of the neutralizer (Dey-Engley) was added and mixed properly. 2 mL of the mixture was collected and immediately passed through a Sephadex gel column (20%). 100 µL of the filtrate obtained (D0) was added to the first row of the monolayer of host cells in the labelled cytotoxicity plate.

- 2 mL of the neutralizer was passed from the separate sephadex column, and 100 µL the collected filtrate was added in each well of the second row of the cytotoxicity plate.

- 2 mL of EBSS was also passed through a separate Sephadex column, and 100 µL the collected filtrate was added in each well of the third row of the cytotoxicity plate.

- The plate was incubated at 36±1ºC for an hour and examined under a microscope for any cytotoxicity.

- In case no cytotoxicity was observed, plate was washed with EBSS and then 100 µL of 10^{-4} dilution of virus was added to all the wells of the cytotoxicity test plate and the plate incubated for a further 60 minutes at 36±1ºC.

c) Neutralization Verification

The neutralization control test was performed for the test substance. Contact time, temperature and humidity were recorded. The neutralization control test included the following steps:

- 2 mL of the test substance was placed in a sterile petri dish and then 2 mL of the neutralizer (Dey-Engley Broth) was added and mixed properly. 2 mL of the mixture was collected and immediately passed through a Sephadex gel column (20%). The filtrate obtained was called D0. 100 mL of 10^{-3} dilution of the virus was added to 1 mL of the filtrate (D0) and was left for the contact time (10 minutes) and then 100 mL of the mixture was added to the first row of the monolayer of host cells in the labelled neutralization plate.

- 2 mL of the neutralizer was passed from the separate sephadex column, the filtrate was collected. 100 mL of 10^{-3} dilution of the virus was added to 1 mL of the filtrate and was left for the contact time (10 minutes) and then 100 mL of the mixture was added to the second row of the neutralization test plate.

- 2 mL of EBSS was also passed through a separate Sephadex column and filtrate was collected. 100 mL of 10^{-3} dilution of the virus was added to 1 mL

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of the filtrate and was left for the contact time (10 minutes) and then 100 mL of the mixture was added to the third row of the neutralization test plate.

- The neutralization plate was incubated for a further 60 minutes at 36±1ºC and 5% CO_2.

d) Efficacy Test on BioProtect DP Concentrate

The test was initiated with processing one control carrier before processing the test carriers and one control carrier at the end after processing the test carriers. This was done to consider the changes in the input level of the test organisms during the experiment. The efficacy test included the following steps:

- Each carrier received 200 µL of virus inoculum.
- The carriers were left under a BSC for 60±5 minutes (or until visible dry) with the lids open.
- At the end of drying time, the carriers received 2 mL of test substance. The control carriers received 2 mL of EBSS in lieu of the test substance.
- The contact time was calculated from the time of putting the product on the dried film using a calibrated timer. Immediately, at the end of the contact time (10 minutes), 2 mL of the neutralizer was added and the mixture was scraped with a sterile scrapper. The mixture was collected and 2 mL of it was passed immediately through a Sephadex gel column (20%). The filtrate obtained was considered as dilution zero (D0). For the control, EBSS was used instead of the test substance.
- 100 µL of D0 was added to 900 µL of EBSS to prepare D1.
- A 10-fold dilution was made by diluting D1 using EBSS as a diluent (100 µL of D1 was added to 900 µL of EBSS to make 1 mL of D2).
- After washing plates with EBSS, 12-well cell culture plates (containing monolayer of FRhK-4 cells) were inoculated with 100 µL of the dilutions (each well) prepared from test and control samples. Uninfected indicator cell cultures (cell controls) were inoculated with 100 µL EBSS alone. The plates were incubated for 60±5 min at 36±1°C and 5% CO_2.
- After 60-minute incubation, the plates were washed with EBSS, 2 mL of an agar overlay was added to each well and each plate was left inside a BSC for 10 minutes for the agar overlay to solidify.
- The plates were incubated at 36±1°C in a humidified atmosphere of 5% CO_2 for 4 days before fixing and staining them for counting the plaque-forming units (PFU).

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e) Sterility controls

2 mL of EBSS was added to each of the two sterile, uninoculated carriers. 2 mL of the neutralizer was also added into a separate sterile, uninoculated carrier and both two carriers were kept for the contact time. 100 µl of EBSS from the first carrier was taken and added into the each well of first row of the vero cells in a 12-well plate. 2 mL of EBSS from the second carrier was taken and passed through the Sephadex column (20%). Then 100 µl of the filtrate was added to each well of the second row of the plate. 100 µl of the neutralizer from the third carrier was taken and added into the each well of third row of the vero cells in a 12-well plate.

Plates were incubated for 4 days at 36± 1°C, 5%CO_2 before examination. No bacterial contamination or cytopathic effects (CPE) must be visible for the test to be valid.

f) Initial Titer of Virus

Hepatitis A virus (HAV) stock was serially diluted in EBSS (diluent). Six ten-fold dilutions were prepared and used to inoculate the host cells. This helped to confirm if the host cells were susceptible to the virus and to determine if the titre of the stock virus was appropriate for the test.

10. PROTOCOL/STUDY PLAN CHANGE

N/A

11. DATA ANALYSIS

For calculation of the weighted mean count, the following formula was used:

$$\frac{PFU}{Carrier} = \frac{\sum(C_1 + C_2 + \cdots C_n)}{(n_1 + n_2 \times V_2 + \cdots n_n \times V_n) \times d} \times \frac{V_{tot}}{t}$$

Where,

t is the test volume that was added per dilution step to a plate;

V_{tot} is the total virus suspension recovered from each carrier;

c_1 is the PFU number of all plates of the first dilution step (lowest dilution step) with nonconfluent plaques;

c_2 is the PFU number of all plates of the second dilution step with nonconfluent plaques;

c_n is the PFU of all plates of the last dilution (highest dilution step);

n_1 is the number of all plates of the first dilution step (lowest dilution step) with nonconfluent plaques to which c_1 corresponds;

n_2 is the number of all plates of the second dilution step with nonconfluent

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plaques (c_2);

v_2 is the dilution factor between n_1/ n_2 (e.g. $n_1 = 10^{-3}$ and $n_2 = 10^{-4}$, then $v_2 = 0{,}1$);

n_n is the number of all plates of the last dilution for which PFUs were counted (c_n);

v_n is the dilution factor between n_1/ n_n (e.g. $n_1 = 10^{-3}$ and $n_n = 10^{-6}$, then $v_n = 0.001$)

d is the dilution step of c_1.

NOTE: PFU/Carrier = Density/Carrier

Calculation of Mean Log_{10} Density

The log_{10} density (LD) for each carrier was calculated by taking the log_{10} of the density (per carrier).

Calculation of Log_{10} Reduction

Log_{10} Reduction = (Log_{10} of average PFU from control carriers) – (Log_{10} of average PFU from the test carriers)

Calculation of % Reduction

In this study, because no plaque was observed in the samples treated with the test substance, the following formula was used to calculate minimum percentage reduction:

Minimum Percentage Reduction = $(1–10^{-X}) \times 100$

Where, X = Average log_{10} Reduction

12. STUDY PERFORMANCE CONTROL

- Host Cell Purity Control: For a valid test, the host cells were required to show no bacterial, fungal, or cytopathogenic viral contamination.
- All sterility controls were also to be negative for a valid test.

13. THE TEST ITEM/SUBSTANCE PERFORMANCE CRITERIA

- The test formulation was to show a >3 log_{10} in the viability titer of the test organism in the tested contact time to meet the product performance criterion.

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14.TEST RESULTS

a. Input

The stock virus utilized in the testing was titrated by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are summarized in Table 1

Table 1: PFU in each dilution virus stock for the three tests on GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008)

Dilution	PFU		
	Test #1	Test #2	Test #2
10^{-6}	14,5,8	10,6,8	10,16,13
10^{-7}	4,3,5	0,0,0	5,3,4
PFU/mL	$2.2x10^{8}$	$8.0x10^{7}$	$2.7x10^{8}$

TNTC: Too numerous to count

b. Validation of Absence of Cytotoxicity

Three cytotoxicity validation test were performed on the test substance GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008) as was explained in Section 10.3.b. The results are summarised in Tables 2, 3 and 4, for the three tests on the test substance. No cytotoxicity was observed after one hour of incubation of cytotoxicity test plates at 33±1°C. The average of PFU's in control and neutralizer toxicity in the three tests performed on the test substance were not significantly different (p-value=0.423, 0.385 and 0.693) confirming that the neutralizer was not toxic for the coronavirus and did not reduce the infectivity of the virus. The average of PFU's in control and neutralized test substance were not significantly different in the three tests (p-value=0.478, 1.000 and 0.585) confirming that the neutralized disinfectant was not toxic to the host cell and neither the susceptibility of the cell line nor the infectivity of the virus was reduced by neutralized test substance.

Table 2: PFU in the cytotoxicity controls of the first test on GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008)

Sample ID	Control (C)		Neutralized Test Substance (NT)			Neutralizer (NE)	
Replicate No.	1	2	1	2	3	1	2
No. of PFU	30	26	27	31	32	30	30
Average	28.0		30.0			30.0	
Index			NT/C=107.1%			NE/C=107.1%	
p-value			0.475			0.423	
Cytotoxicity:			Non-toxic			Non-toxic	

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Table 3: PFU in the cytotoxicity controls of the second test on GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008)

Sample ID	Control (C)		Neutralized Test Substance (NT)			Neutralizer (NE)		
Replicate No.	1	2	1	2	3	1	2	3
No. of PFU	7	11	9	9	9	9	6	6
Average	9.0		9.0			7.0		
Index			NT/C=100.0%			NE/C=77.8%		
p-value			1.000			0.385		
Cytotoxicity:			Non-toxic			Non-toxic		

Table 4: PFU in the cytotoxicity controls of the third test on GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008)

Sample ID	Control (C)		Neutralized Test Substance (NT)			Neutralizer (NE)		
Replicate No.	1	2	1	2	3	1	2	3
No. of PFU	9	6	8	4	7	5	6	9
Average	7.5		6.3			6.7		
Index			NT/C=84.4%			NE/C=88.9%		
p-value			0.585			0.693		
Cytotoxicity:			Non-toxic			Non-toxic		

c. Neutralization Validation

Three neutralization validation tests were performed on the test substance GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008) with a contact time of 10 minutes as was explained in Section 10.3.c. The results are summarised in Table 5, 6 and 7. The average of PFU's in control and neutralizer toxicity were not significantly different (p-value=0.184, 0.768 and 0.658 for the first, second and third test, respectively) confirm that the neutralizer is not toxic for the coronavirus and did not reduce the infectivity. The average of PFU's in neutralization effect and neutralizer toxicity were not significantly different (p-value=0.285, 1.000 and 0.411 for the first, second and third test, respectively) confirm that the neutralizer could effectively neutralize the substance and the neutralized test substance did not reduce the infectivity.

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Table 5: PFU in the neutralization controls of the first test on GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008)

Sample ID	Control		Neutralization Effect NE				Neutralizer Toxicity NT	
Replicate No.	1	2	1	2	3		1	2
No. of PFU	23	21	15	16	25		24	24
Average	22.0		18.7				24.0	
Index			NE/NT=77.8%				NT/C=109.1%	
p-value			0.285				0.184	
Neutralization Effective:			Neutralized				Non-toxic	

Table 6: PFU in the neutralization controls of the second test on GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008)

Sample ID	Control		Neutralization Effect NE			Neutralizer Toxicity NT		
Replicate No.	1	2	1	2	3	1	2	3
No. of PFU	7	4	3	5	6	4	2	8
Average	5.5		4.7			4.7		
Index			NE/NT=100.0%			NT/C=84.4%		
p-value			1.000			0.764		
Neutralization Effective:			Neutralized			Non-toxic		

Table 7: PFU in the neutralization controls of the third test on GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008)

Sample ID	Control		Neutralization Effect NE			Neutralizer Toxicity NT		
Replicate No.	1	2	1	2	3	1	2	3
No. of PFU	7	5	4	2	6	7	5	4
Average	6.0		4.0			5.3		
Index			NE/NT=75.0%			NT/C=88.9%		
p-value			0.411			0.658		
Neutralization Effective:			Neutralized			Non-toxic		

d. Efficacy Test

The numbers of PFU in each dilution of control and test substance GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008) are summarized in Tables 8, 9 and 10, respectively. The $\log_{10}$ reductions and percentage reductions of each sample were calculated using a validated Excel sheet and summarized in Table 11. The $\log_{10}$ reduction for the three tests on GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008) were

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3.38, 3.62 and 2.88, respectively. In average the test substance demonstrated 3.29±0.38 log_{10} reduction (99.93±0.059 Percent reduction).

Based on the acceptance criterion of $\geq$3 log_{10} in the PFU for virucidal activity, the test substance passed.

Table 8: PFU in each dilution of control and GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008), Test #1

Dilution	Test			Control		
	T1	**T2**	T3	T1	**T2**	**T3**
10^0	12,15,17	16,125,17	22,18,26	N/A	N/A	N/A
10^{-1}	1,0,4	5,7,4	1,8,5	TNTC	TNTC	TNTC
10^{-2}	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-3}	N/A	N/A	N/A	23,24,25	27,30,21	36,36,42
10^{-4}	N/A	N/A	N/A	9,8,6,	7,8,9	21,14,16
PFU/mL	$1.11x10^1$	$1.45x10^1$	$1.82x10^1$	$2.16x10^4$	$2.32x10^4$	$3.75x10^4$
PFU/Carrier	$4.45x10^2$	$5.82x10^2$	$7.27x10^2$	$8.64x10^5$	$9.27x10^5$	$1.50x10^6$
Log PFU/Carrier	2.65	2.76	2.86	5.94	5.97	6.18
Average Log PFU/Carrier	2.76			6.03		
Log_{10} reduction	3.38					

Test: Test Substance (Lot# B23-675), TOX: Toxic, TNTC: Too numerous to count, '0': No plaques

Table 9: PFU in each dilution of control and GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008), Test #2

Dilution	Test			Control		
	T1	**T2**	T3	T1	**T2**	**T3**
10^0	5,4,4	8,7,4	3,2,1	N/A	N/A	N/A
10^{-1}	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-2}	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-3}	N/A	N/A	N/A	19,14,19	18,19,22	14,6,11
10^{-4}	N/A	N/A	N/A	1,2,2	4,2,5	4,2,3
PFU/mL	2.95	4.32	1.36	$1.30x10^4$	$1.59x10^4$	$9.09x10^4$
PFU/Carrier	$1.18x10^2$	$1.73x10^2$	$5.45x10^1$	$5.18x10^5$	$6.36x10^5$	$3.64x10^5$
Log PFU/Carrier	2.07	2.24	1.74	5.71	5.80	5.56
Average Log PFU/Carrier	2.02			5.69		
Log_{10} reduction	3.62					

Test: Test Substance (Lot# B23-676), TOX: Toxic, TNTC: Too numerous to count, '0': No plaques

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Table 10: PFU in each dilution of control and GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008), Test #3

Dilution	Test			Control		
	T1	**T2**	T3	T1	**T2**	**T3**
10^0	19,17,16	17,11,18	20,19,19	N/A	N/A	N/A
10^{-1}	0,0,3	3,1,1	2,2,7	TNTC	TNTC	TNTC
10^{-2}	0,0,0	0,0,0	0,0,0	TNTC	TNTC	TNTC
10^{-3}	N/A	N/A	N/A	19,7,15	9,8,8	18,15,19
10^{-4}	N/A	N/A	N/A	1,2,1	0,0,1	2,2,4
PFU/mL	$1.25x10^1$	$1.16x10^1$	$1.57x10^1$	$2.02x10^4$	$5.91x10^3$	$1.36x10^4$
PFU/Carrier	$5.00x10^2$	$4.64x10^2$	$6.27x10^2$	$4.09x10^5$	$2.36x10^5$	$5.45x10^5$
Log PFU/Carrier	2.70	2.67	2.80	5.61	5.37	5.74
Average Log PFU/Carrier	2.72			5.57		
Log_{10} reduction	2.88					

Test: Test Substance (Lot# B23-676), TOX: Toxic, TNTC: Too numerous to count, '0': No plaques

Table 11: Average of PFU/Control carriers, PFU/Test carriers Log_{10} reductions and % reductions in the virus titer for the three tests on GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008)

Sample ID	Test No.	Log_{10} Reduction	Percent Reduction	Average Log_{10} Reduction	Average Percent Reduction
GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008)	Test #1	>3.38	99.96	3.29±0.38	99.93±0.057
	Test #2	>3.62	99.98		
	Test #3	>2.88	99.87		

15. CONCLUSION

The test substance GreenMBalm Anatomical Embalming Fluid (Lot # C0223-008) demonstrated 3.29±0.38 log_{10} reduction (99.93±0.059 Percent reduction) against Hepatitis A virus (HAV) and passed the performance criteria.

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21. REFERENCES

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2. Environmental Protection Agency Title 40-Part 160, Good Laboratory Practice Standards, Revised as of July1, 2015.
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