MICROCHEM LABORATORY

STUDY REPORT

Study Title

Antiviral Activity and Efficacy of Global Health RX Test Substance
Using a Suspension Time-Kill Procedure Against Virus

Test Method

ASTM International Standard Test Method E1052
Assessment of Antimicrobial Agents Against Viruses in Suspension

Study Identification Number

NG9827

Study Sponsor

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Global Health RX
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Test Facility

Microchem Laboratory
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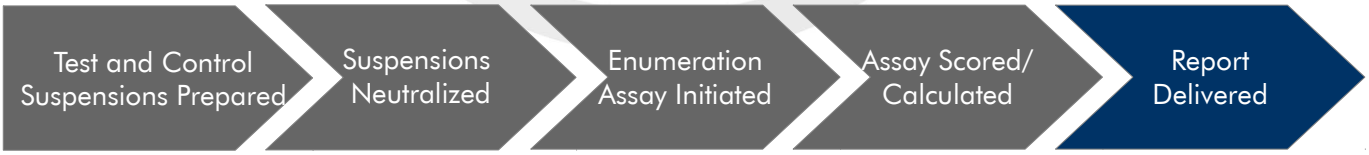
ASTM E1052: General Information

ASTM International, formerly the American Society for Testing and Materials (ASTM), is an internationally recognized organization that develops and publishes product and testing standards. The ASTM E1052 test method is used to determine the virucidal effectiveness of liquid products such as hand soaps, over-the-counter topical agents, and other skin care products. It is also ideal for the initial evaluation of liquid antimicrobial products designed for use on hard, nonporous surfaces. In an ASTM E1052 test, a suspension of virus is exposed to a test product at a ratio of 1:10 (1 part virus suspension + 9 parts prepared test product). A Control suspension is concurrently processed in the same manner, with cell culture medium employed in place of the test product. Following neutralization, the suspensions are enumerated using standard cell culture (e.g. $TCID_{50}$) or plaque assay techniques. Log_{10} and percent reduction values are calculated to determine the effectiveness of the test product suspension relative to the control suspension.

Laboratory Qualifications Specific to ASTM E1052

Microchem Laboratory has considerable experience in the proper execution of the ASTM E1052 test method. The laboratory has performed many ASTM E1052 tests in order to assess the virucidal efficacy of a broad spectrum of antiseptic and disinfectant products. In addition, the laboratory has experience modifying the method as needed to accommodate customer needs. Each ASTM E1052 test at Microchem Laboratory is performed in a manner appropriate to the test substances submitted by the Study Sponsor, while maintaining the integrity of the study.

Study Timeline



Herpes Simplex Virus 1, ATCC VR-260				
28DEC2018	28DEC2018	28DEC2018	04JAN2018	31JAN2018
Human Adenovirus 1, ATCC VR-1				
19JAN2018	19JAN2018	19JAN2018	24JAN2018	31JAN2018

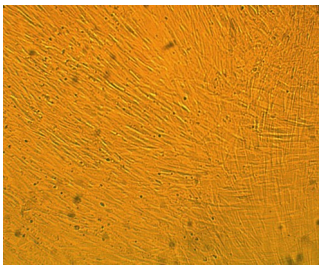
Test Substance Information

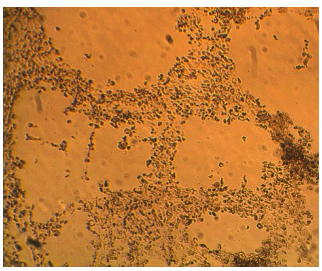
The test substance was received on 27NOV2017 and the following picture was taken.
(note: photo depicts the test substance that was analyzed in this study)

Test Substance Received: Global Health RX Hexagen Eye care Formula #LM-200-159A.
Test Substance arrived ready to use for the conduct of the Study. Test substance was not diluted prior to use in the study.

Test Microorganism Information

The test microorganism(s) selected for this test:





Human Adenovirus 1, ATCC VR-1
This virus is a relatively large, non-enveloped, double-stranded DNA member of the *Adenoviridae* family that was first isolated from human adenoid tissue. Adenovirus 1 is a highly endemic adenoviral serotype associated with respiratory diseases including pneumonia, bronchitis, and other upper respiratory tract infections. This virus is primarily spread via aerosolized respiratory droplets and infected fomites. Adenovirus 1 is understood to be highly stable and capable of surviving outside of a host on common environmental surfaces for extended periods of time. Because this virus is extremely hardy when deposited on environmental surfaces, adenovirus 1 can be relatively difficult to inactivate via disinfection.
Permissive Host Cell Line for Adenovirus 1: MRC-5 (Human Lung Fibroblast Cells), ATCC CCL-171

Test Microorganism Information (Cont.)

Herpes Simplex Virus 1 (HSV-1), ATCC VR-260

This virus is an enveloped, double-stranded DNA virus of the genus *Simplexvirus*. Clinical signs of infection include small, fluid filled blisters on the lips or mouth (cold sores), fever, a sore throat, and swollen lymph nodes. HSV-1 infection is less stigmatized than HSV-2 (the main cause of genital herpes), but both viral strains can cause genital herpes and be spread by those without active symptoms. Although there are multiple treatments for symptoms such as cold sores, there is currently no cure for HSV-1 and carriers of the virus will continue to have symptomatic outbreaks for the rest of their lives. HSV-1 is common world-wide, and it is estimated that the majority of United States citizens are exposed to or infected by HSV-1 by the time they reach adolescence.

Permissive Host Cell Line Selected for HSV 1: Vero (African Green Monkey Kidney Cells), ATCC CCL-81

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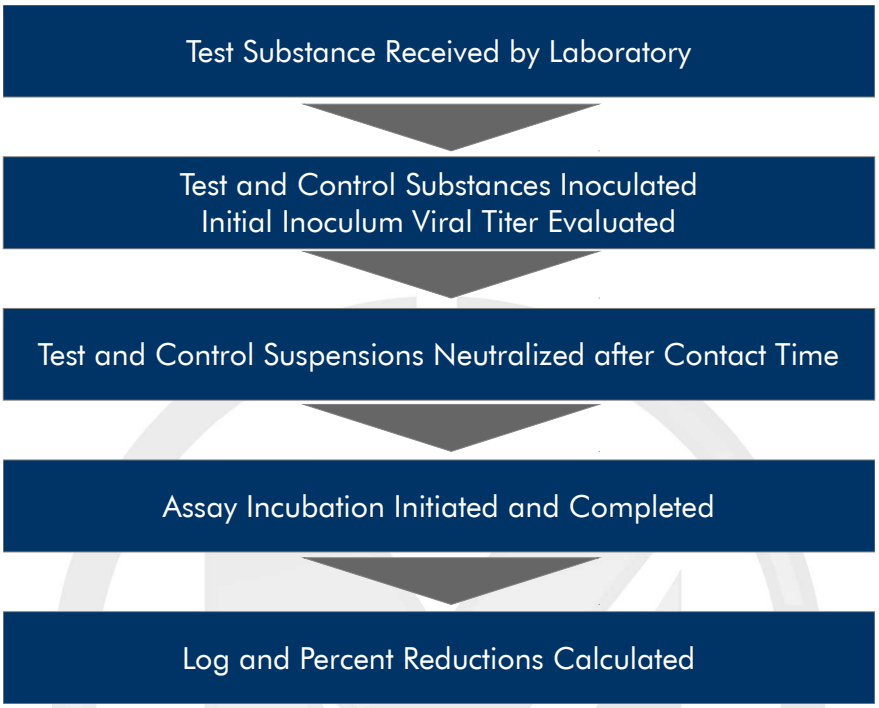
Diagram of the Procedure



Summary of the Procedure

- Stock virus is thawed and may be supplemented with an organic soil load, if requested.
- Test and control substances are dispensed in 9-part equivalent volumes into sterile vessels.
- Test and control substances are each inoculated with 1-part equivalent volumes of the test virus.
- The test suspensions are held for the contact time(s) specified by the Study Sponsor, and then neutralized by ten-fold serial dilutions into the appropriate solution. Gel filtration is employed for neutralization as suitable.
- The control suspension is neutralized in the same manner as the test suspensions.
- Following neutralization, the viral suspensions are quantified to determine the levels of infectious virus using standard cell culture (e.g. $TCID_{50}$) or plaque assay techniques.
- Assay trays/plates are incubated for the period most suitable for the virus-host cell system (e.g. 7 days).
- After the incubation period, the assay is scored for the presence/absence of test virus and cytotoxic effects. The appropriate calculations are performed (e.g. Spearman-Karber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- Log_{10} and percent reductions are computed for test suspensions relative to the control suspensions, and reported to the Study Sponsor.

Criteria for Scientific Defensibility of an ASTM E1052 Study

For Microchem Laboratory to consider a Suspension Time Kill study to be scientifically defensible, the following criteria must be met:

1. A minimum of 4-Log_{10} infectious viruses are recovered from the virus control suspension.
2. Viral cytopathic effects are distinguishable from cytotoxic effects caused by test substance exposure.
3. Effectiveness of the neutralization method (dilution and/or gel filtration) is demonstrated.
4. Assay wells designated as sterility controls are absent of infectivity, contamination, and cytotoxicity.

Passing Criteria

ASTM has defined the passing criteria for a virus suspension time-kill test to be:

1. Complete inactivation of the test virus at all dilutions.
2. If cytotoxicity is observed, a ≥3-Log_{10} reduction in viral titer is observed past the level of cytotoxicity relative to the virus control.

Testing Parameters used in this Study

Human Adenovirus 1, ATCC VR-1

Test Substance Conc.:	N/A	Test Substance Volume:	0.900 g
Test Substance Diluent:	Undiluted	Replicates:	One
Control Substance:	PBS	Control Substance Volume:	0.900 mL
Neutralization Method:	9mL 2% FBS EMEM		

Viral Inoculum Volume:	0.100 mL	Target Inoculum:	~6 $\log_{10}$ $TCID_{50}$ / ml
Contact Time(s):	1, 2, & 4 hours	Contact Conditions:	Ambient
Host Cell Line:	MRC-5	Cell Passage Number:	25
Assay Medium:	2% FBS EMEM	Soil Load:	N/A
Incubation Period:	5-7 days	Incubation Conditions:	37±1°C 5.0% CO_2

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Testing Parameters used in this Study (Cont.)

Herpes Simplex Virus 1, ATCC VR-260

Test Substance Conc.:	N/A	Test Substance Volume:	0.900 g
Test Substance Diluent:	Undiluted	Replicates:	One
Control Substance:	PBS	Control Substance Volume:	0.900 ml
Neutralization Method:	9mL 2% FBS EMEM		

Viral Inoculum Volume:	0.100 mL	Target Inoculum:	~6 $\log_{10}$ $TCID_{50}$
Contact Time(s):	1, 2, & 4 hours	Contact Conditions:	Ambient
Host Cell Line:	VERO	Cell Passage Number:	203
Assay Medium:	2% FBS EMEM	Soil Load:	N/A
Incubation Period:	5-7 days	Incubation Conditions:	37±1°C 5.0% CO_2

Study Modifications

No modifications were made to this study.

Study Notes

No additional observations or notations were made for this study.

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Control Results

Herpes Simplex Virus 1, ATCC VR-260

Sterility:	Verified	Virus Control Titer: See Results
Cytotoxicity Titer:	None Observed	
Neutralization:	Verified	

Human Adenovirus 1, ATCC VR-1

Sterility:	Verified	Virus Control Titer: See Results
Cytotoxicity Titer:	None Observed	
Neutralization:	Verified	

Calculations

Viral and cytotoxicity titers ($TCID_{50}$/$TCLD_{50}$ and $TCCD_{50}$, respectively) were determined according to the method developed my Spearman-Karber:

$$-\text{Log}_{10} \text{ of 1st Dilution} - \left(\frac{\text{sum of \% mortality at each dilution}}{100}\right) - 0.5$$

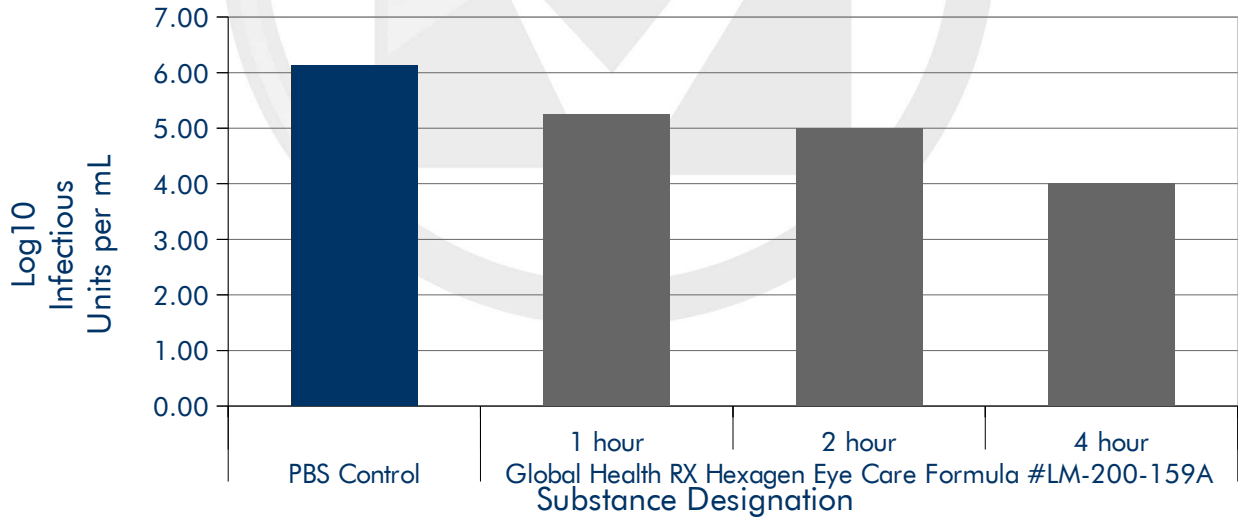
Percent Reduction of Virus is determined according to the following formula:

$$\text{Percent Reduction} = 1 - \left(\frac{C}{B}\right) * 100$$

Where:
C = Log_{10} of Virus Test Carrier
B = Log_{10} of Virus Control Carrier

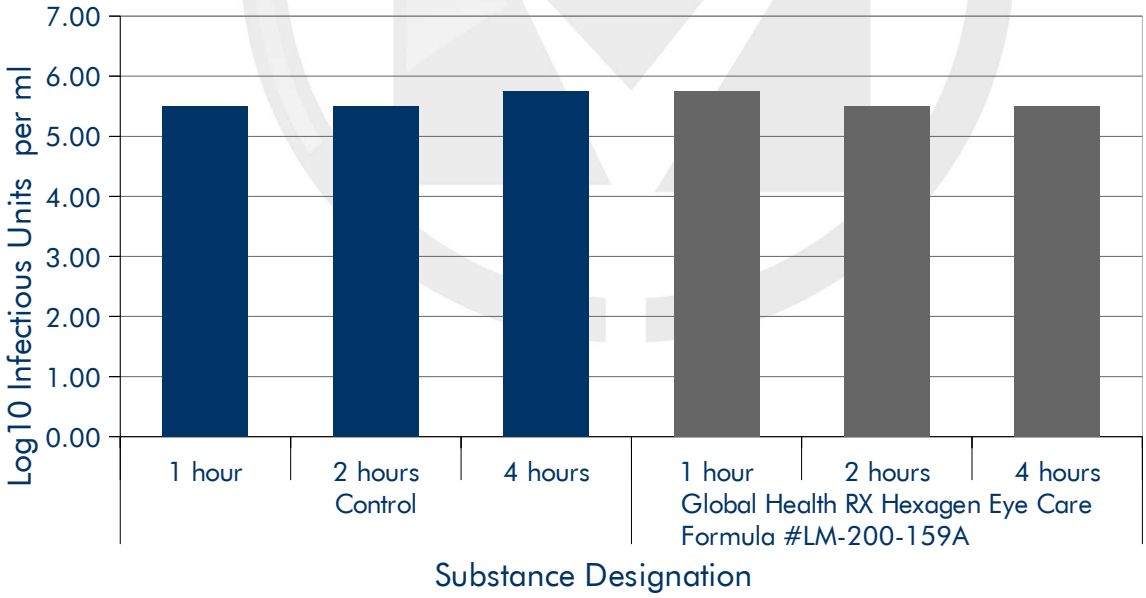
Results of the Study

Test Microorganism	Substance Designation	Contact Time	Log_{10} Infectious Units per mL	Log_{10} Reduction Relative to Control	Percent Reduction Relative to Control
Herpes Simplex 1, ATCC VR-260	PBS Control		6.13	N/A	
	Global Health RX Hexagen Eye Care Formula #LM-200-159A	1 hour	5.25	0.88	86.82%
		2 hour	5.00	1.13	92.59%
		4 hour	4.00	2.13	99.26%



Results of the Study(Cont.)

Test Microorganism	Substance Designation	Contact Time	Log_{10} $TCID_{50}$ Per 1.0 ml	Log_{10} Reduction Relative to Control	Percent Reduction Relative to Control
Human Adenovirus 1, ATCC VR-1	Control	1 hour	5.50	N/A	
		2 hours	5.50		
		4 hours	5.75		
	Global Health RX Hexagen Eye Care Formula #LM-200-159A	1 hour	5.75	No Reduction	No Reduction
		2 hours	5.50	No Reduction	No Reduction
		4 hours	5.50	0.25	43.77%



The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.

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