ANTIMICROBIAL TEST LABORATORIES

Study Report

www.AntimicrobialTestLabs.com - Info@AntimicrobialTestLabs.com - (512) 310-8378

Study Title

Determination of the Antimicrobial Effectiveness of
KHG FiteBac Technology Test Substance Delivered via Spray Device Against
Feline Calicivirus (US EPA-Approved Human Norovirus Surrogate) and Influenza A (H1N1)

Test Method

Association of Analytical Communities Test Method 961.02
Germicidal Spray Products as Disinfectants Modified for Viruses

Study Identification Number

NG5922-A1

Study Sponsor

Kirk Kimmerling
KHG FiteBac Technology
3698 Largent Way Ste 202
Marietta, GA 30064
(770) 423-4900
kirkkimmerling@fitebac.com

Test Facility

Antimicrobial Test Laboratories
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378

History of the Laboratory

Antimicrobial Test Laboratories was launched in 2006 to provide testing services to the antimicrobial industry. The company has grown considerably since then but its focus remains the same: Test antimicrobial agents, test them well, and test them fast! Antimicrobial Test Laboratories operates a 15,000+ square foot facility near Austin, Texas, where hundreds of studies are conducted annually by a staff of friendly, knowledgeable, and experienced microbiologists and virologists.

Laboratory Qualification Statement

Antimicrobial Test Laboratories was founded by microbiologist Dr. Benjamin Tanner. The laboratory ensures consistent, reproducible results by utilizing a well-trained and educated scientific staff who work from a comprehensive system of Standard Operating Procedures, official standard methods from ASTM, AOAC, and other organizations, and custom study protocols. The laboratory provides testing services to dozens of Fortune 500 companies and has been inspected for GLP compliance by the US government.

Scientist Qualifications

Your Study was designed, conducted and reported by: Erika Guin, B.S.

Erika graduated from St. Edward's University with a Bachelors of Science in Biology.

Erika has a strong educational background in microbiology, and is a dedicated and enthusiastic professional. As a Microbiologist at Antimicrobial Test Labs she has the lead in a wide range of virology and custom studies. Her strong work ethic and interest in comprehensive and accurate testing make her an asset to the laboratory as well as a competent conductor of antimicrobial studies.

If you have any questions about your study, please don't hesitate to contact Erika at:

Erika@AntimicrobialTestLabs.com
or
(512) 310-8378

AOAC Germicidal Spray Products Test: General Information

Formerly known as the Association of Official Analytical Chemists, AOAC International is a globally recognized, third party, not-for-profit association that provides education and facilitates the development of test methods and standards for a wide range of industries. The AOAC Germicidal Spray Test method is a semi-quantitative test method designed to assess the performance of liquid spray disinfectants. The method can be modified to evaluate the virucidal efficacy of spray disinfectants. In a Modified AOAC Spray Products Test, a viral inoculum is dried onto carriers, followed by exposure to a test formulation via spray device for the specified contact time(s). The carriers are neutralized then 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 relative to control carriers. The AOAC Germicidal Spray Products method for use with spray devices and modified for viruses is recognized by regulatory agencies as an approved method for claim substantiation.

Laboratory Qualifications Specific to AOAC Germicidal Spray Products Test Method Modified for Viruses

Antimicrobial Test Laboratories has considerable experience in the proper execution of the Modified AOAC Germicidal Spray Products Test Method. The laboratory has performed many modified AOAC germicidal spray tests in order to assess the virucidal efficacy of a broad spectrum of disinfectant products. Each test is performed at Antimicrobial Test Laboratories in a manner appropriate to the test substances submitted by the Study Sponsor, while maintaining the integrity of the study.

Study Timeline

**Amended report delivered 12 MAY 2015*

Test Substance Information

The Control Substance was received on 30 JAN 2015. The Test Substance was received on 10 MAR 2015. The following pictures were taken prior to test initiation.
(note: photos depict the test substances that were analyzed in this study)

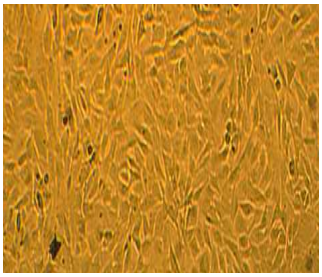
Test Substances Received:

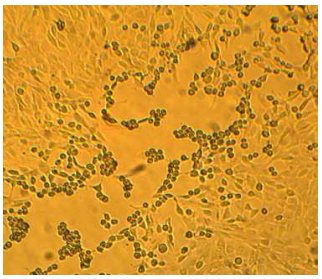
Control Solution: Ethanol, Acetone, and Water (Left)
0.025% K21 in Water, Ethanol, and Acetone (Right)

The Test and Control Substances arrived as ready to use. Test and Control Substances were not diluted prior to use in the study. Test and Control Substances were loaded into ethanol-sanitized, ATL-provided spray bottles for use within this study.

Test Microorganism Information

The test microorganism(s) selected for this test:

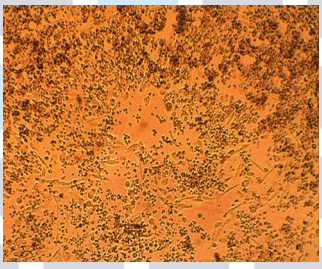




Feline calicivirus (FCV), ATCC VR-782

This virus is a non-enveloped, positive-stranded RNA member of the genus *Vesivirus*, and a common cause of respiratory infections in cats. Symptoms of infection in felines include nasal discharge and mouth ulcers. As a member of the *Caliciviridae* viral family, FCV is closely related to human noroviruses, which cause acute gastroenteritis marked by nausea, vomiting, and diarrhea. Unlike human norovirus, however, a simple cell culture assay system is available for FCV. Therefore, feline calicivirus is the US EPA-approved surrogate microorganism for human norovirus label claims. Both FCV and human norovirus are able to remain viable on environmental surfaces for extended periods of time and are resistant to a number of disinfectant actives.

Permissive Host Cell Line Selected for FCV: CRFK (Crandell-Rees Feline Kidney Cells), ATCC CCL-94



Influenza A (H1N1)

Influenza A virus is an enveloped, minus-stranded member of the family *Orthomyxoviridae*, and causative agent of the illness influenza (which is more widely recognized by the term 'flu'). Influenza is more serious than other seasonal mild, respiratory tract infections (e.g. the common cold) with symptoms that can last for upwards of several weeks. Young children and the elderly are particularly susceptible to severe illness and death due to infection. Influenza is readily transmitted via infective aerosols direct contact with infective respiratory secretions. Potential transmission by contaminated environmental surfaces (fomites) has increasingly become of interest, and Influenza virus is highly vulnerable to inactivation by drying and exposure to variety of disinfectant actives.

Permissive Host Cell Line Selected for Influenza A (H1N1): MDCK (Madin Darby Canine Kidney Cells), ATCC CCL-34

Diagram of the Procedure

Summary of the Procedure

- Stock virus is thawed and may be supplemented with an organic soil load, if requested.
- Sterile glass Petri dish carriers (100 x 15 mm) are inoculated with a volume of virus suspension containing an adequate titer to recover a minimum of 4-Log_{10} infectious viruses per carrier. A sufficient number of test and control carriers are prepared.
- Inoculated carriers are dried at room temperature under laminar flow conditions.
- The test substance is prepared according to the Study Sponsor's instructions as requested, and applied to the test carriers using a spray device. The distance, angle, and number of sprays applied are recorded.
- The treated carriers are held for the predetermined contact time(s), and then neutralized in a manner appropriate for the test substance (e.g. dilution and/or gel filtration).
- The control carrier is harvested using an equivalent volume cell culture medium or other suitable buffer.

Summary of the Procedure

- Following neutralization of test and control carriers, 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 viral films exposed to the test product relative to the titer obtained for the study control carrier(s), and reported to the Study Sponsor.

Criteria for Scientific Defensibility of a Germicidal Spray Study Modified for Viruses

For Antimicrobial Test Laboratories to consider a Germicidal Spray Products Test Modified for Viruses 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 carrier.
2. Viral cytopathic effects are distinguishable from cytotoxic effects caused by test substance exposure.
3. Neutralization effectiveness is demonstrated by recovery of comparable levels of infectious viruses from control (e.g. PBS), neutralizer (where applicable), and neutralized test substance.
4. Assay wells designated as sterility controls are absent of infectivity, contamination, and cytotoxicity.

Passing Criteria

AOAC International has defined the passing criteria for the Germicidal Spray test for viruses as:

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

Test Substance Diluent:	N/A (Ready to use)	Carrier Type:	Glass Petri Dish
Carriers per Test:	See Study Notes	Number of Sprays:	4
Spray Distance:	6-8 inches	Spray Angle:	45°
Neutralization:	0.3% Lecithin, 1% Tween in 0% FBS EMEM (2.0ml) + Sephacryl Column		

Viral Inoculum Volume:	0.200 ml per Carrier	Carrier Inoculation Area:	10-in^2
Carrier Dry Time:	See Study Notes	Carrier Dry Conditions:	See Study Notes
Contact Time:	10 Minutes	Contact Conditions:	27.2°C,52% RH
Host Cell Line:	MDCK ; CRFK	Cell Passage Number:	p.99 ; p.209
Assay Medium:	See Study Notes	Soil Load:	None
Incubation Period	7 Days	Incubation Conditions:	See Study Notes

Study Modifications

No further modifications were made to the method for this study.

Study Notes

Carriers Per Test

Testing against Feline calicivirus was performed in double replicate, with two carriers prepared per lot of test substance. The viral inoculum was supplemented with 0.1% Triton X-100 to facilitate spreading across the surface of the carrier.

Testing against Influenza A (H1N1) was performed in single replicate, with one carrier prepared per lot of test substance. The viral inoculum was not supplemented.

Carrier Dry Times and Conditions

Feline calicivirus:
- Dry Time – 14 Minutes
- Dry Conditions – 26.3°C, 32% RH

Influenza A (H1N1):
- Dry Time – 41 Minutes
- Dry Conditions – 6.0°C, 92% RH

Assay Medium and Incubation Conditions

Feline calicivirus:
- Assay Medium - 2% FBS EMEM
- Incubation Conditions – 37 ± 2°C, 5% CO_2

Influenza A (H1N1):
- Assay Medium – Influenza Infection Medium
- Incubation Conditions – 34 ± 2°C, 5% CO_2

Study Notes (cont.)

Cytotoxicity

Cytotoxicity data communicated within this report is generated with the purpose of defining the impact of neutralized test substance on the health of host cell monolayers, and may be used to establish the limit of detection for the assay performed. Observed cytotoxicity may be the result of the test substance, solvents used, the chemical neutralization method, or the by-product of test substance-neutralizer interaction. Cytotoxicity may present itself in several ways including disturbance of the host cell monolayer and altered cell morphology, in all cases being distinct from viral cytopathic effects (CPE) and typical healthy monolayer appearance.

Cytotoxicity within this study was observed throughout the 10^{-1} dilution and the 10^{-2} dilution, resulting in a cytotoxicity titer of 2.50 $\log_{10}$ per 0.1 ml, or 4.10 $\log_{10}$ per carrier.

Cytotoxicity data generated from this study is particular to the given formulation of test substance as well as the specific host cell lines used (MDCK, CRFK). Different cell lines may demonstrate different levels of sensitivity to neutralization methods and test substances. Cytotoxicity data communicated in this report should not be extrapolated to the impact of the test substance on other cell lines or other organisms. Any extrapolation of findings to related materials or uses is the responsibility of the Study Sponsor.

Study Photographs

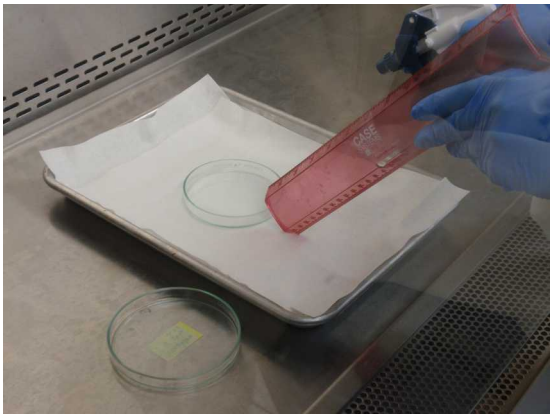


Photo 1. Application of 0.025% K21 Test Substance to an inoculuated carrier. Approximate application distance of 7 inches at a 45° angle.

Control Results

Virus Control Titer:	See Study Results	Cytotoxicity Titer:	2.50 $\log_{10}$/0.100 ml
Sterility Controls:	No Contamination Observed	Neutralization Efficacy:	Validated

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

Results of the Study

Table 1. Germicidal Spray Test Modified for Viruses Determining the Antiviral Efficacy of 0.025% K21 Against Influenza A (H1N1)					
Test Microorganism	Contact Time	Substance Designation	Log_{10} Infectious Units per Carrier	Log_{10} Reduction Relative to Control	Percent Reduction Relative to Control
Influenza A (H1N1) ATCC VR-1736	Plate Recovery Control		5.80	Not Applicable	
	10 Minutes	0.025% K21	≤ 4.10	≥ 1.70	≥ 98.00%
		Control	5.60	0.20	36.90%

** "≤" indicates a viral titer at or below the limit of detection for this assay*

Table 2. Germicidal Spray Test Modified for Viruses Determining the Antiviral Efficacy of 0.025% K21 Against Feline Calicivirus (US EPA-Approved Human Norovirus Surrogate)						
Test Microorganism	Contact Time	Substance Designation	Log_{10} Infectious Units per Carrier	Mean Log_{10} Infectious Units per Carrier	Log_{10} Reduction Relative to Control	Percent Reduction Relative to Control
Feline Calicivirus ATCC VR-782	Plate Recovery Control		5.55	5.55	Not Applicable	
	10 Minutes	0.025% K21	5.60	5.23	0.33	52.68%
			4.85			
		Control	5.35	5.48	0.08	15.86%
			5.60			

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