MICROCHEM LABORATORY

STUDY REPORT

## Study Title

Antimicrobial Efficacy of KHG fiteBac Test Substance Using a Suspension Time-Kill Procedure Against Influenza A (H1N1) and Feline Calicivirus

## Test Method

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

## Study Identification Number

NG6878

## Study Sponsor

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## Test Facility

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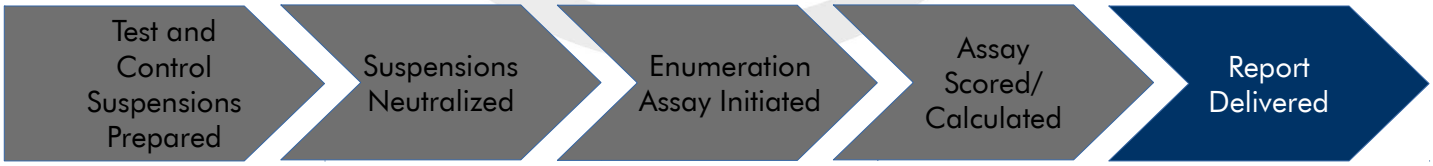
## ASTM E1052: General Information

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




| | | | | |
|---|---|---|---|---|
| **Feline calicivirus** | | | | |
| 15 FEB 2016 | 15 FEB 2016 | 15 FEB 2016 | 22 FEB 2016 | |
| **Influenza A (H1N1)** | | | | 24 FEB 2016 |
| 16 FEB 2016 | 16 FEB 2016 | 16 FEB 2016 | 23 FEB 2016 | |

# Test Substance Information

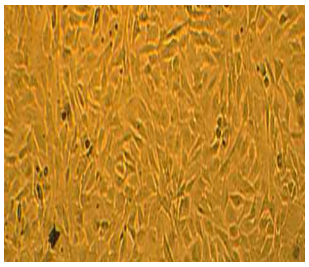
The test substances were received on 29 JAN 2016 and 15 FEB 2016 and the following pictures were taken prior to use in testing.

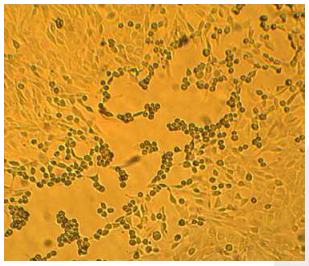

Test Substances Received: (LEFT) fiteBac Germicidal Hand Softening Gel (exp. 09/2018)
(RIGHT) fiteBac Skin Care LLC, Version #1 (Placebo – Vehicle Control)

Test Substances arrived ready to use for the conduct of the Study. Test substances were not diluted for the Study.

# Test Microorganism Information

The test microorganism(s) selected for this test:

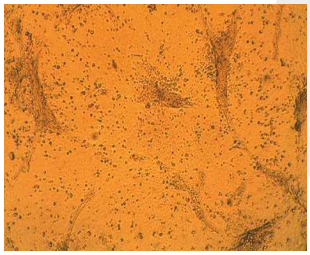


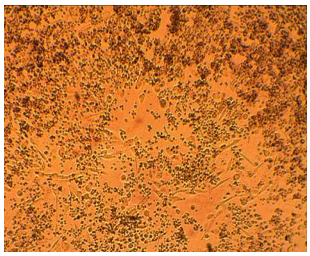


**Feline calicivirus (FCV), ATCC VR-782**

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



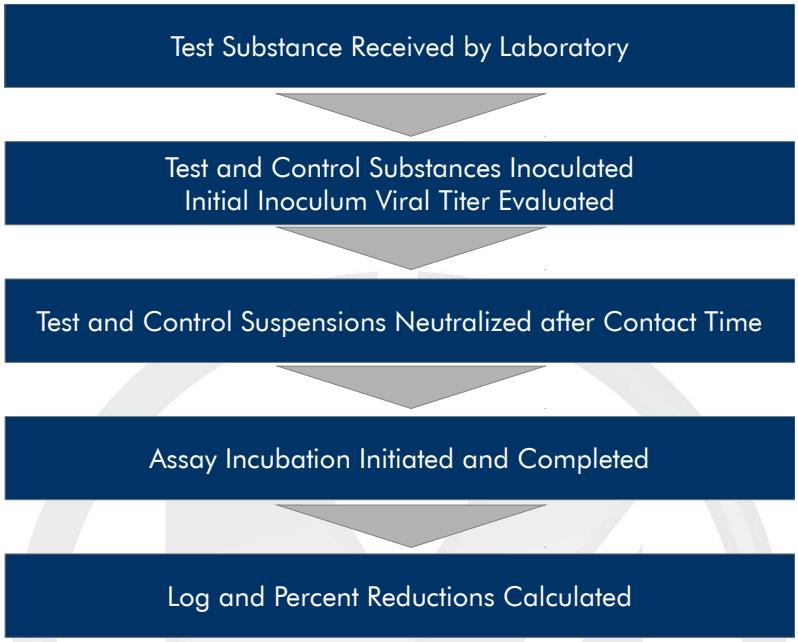


**Influenza A (H1N1)**

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.
- 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

| | | | |
|---|---|---|---|
| Test Substance Conc.: | RTU | Test Substance Volume: | 1.00 ± 0.05 g |
| Test Substance Diluent: | N/A | Replicates: | One |
| Control Substance: | PBS | Control Substance Volume: | 1.00 ml |
| Neutralization Method: | 2.0 ml of 0.1% sodium thiosulfate + column filtration | | |

| | | | |
|---|---|---|---|
| Viral Inoculum Volume: | 0.100 ml | Target Inoculum: | ≥4.00 $log_{10}$ per aliquot |
| Contact Time(s): | 90 seconds | Contact Conditions: | Ambient |
| Host Cell Line: | See Study Notes | Cell Passage Number: | See Study Notes |
| Assay Medium: | See Study Notes | Soil Load: | None requested |
| Incubation Period: | 7 days | Incubation Conditions: | See Study Notes |





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## Study Modifications

The received test substance was observed to be highly viscous. To control for potential viral retention within the matrix of the substance a vehicle control was included within the study design. Viral reductions are evaluated relative to the vehicle control.

To facilitate study performance and to conform with previous bacteriological testing the test and control substances were evaluated on a per weight basis. 1.0 gram of the test or control substance was weighed directly into a sterile 50-ml conical tube and centrifuged at approximately 1000 x g for 2-3 minutes to collect.

## Study Notes

**Host Cell Lines and Passage Numbers:**

Feline calicivirus: CRFK cell line (ATCC CCL-94), passage number 227
Influenza A (H1N1): MDCK cell line (ATCC CCL-34), passage number 151

**Assay Medium:**

Feline calicivirus: 2% FBS EMEM
Influenza A (H1N1): Influenza Infection Medium

**Incubation Conditions:**

Feline calicivirus: 37 ± 2°C, 5 ± 1% $CO_2$
Influenza A (H1N1): 34 ± 2°C, 5 ± 1% $CO_2$

The inoculated test substances were mixed for the duration of the contact time to ensure maximum contact between the viral inoculum and the test and control substances.





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## Control Results

| | | | |
|---|---|---|---|
| Sterility: | Confirmed | Virus Control Titer | See study results |
| Cytotoxicity Titer: | No cytotoxicity observed | | |
| Neutralization: | Neutralization method efficacy confirmed | | |

## 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 = $\text{Log}_{10}$ of Virus Control Carrier
C = $\text{Log}_{10}$ of Virus Test Carrier





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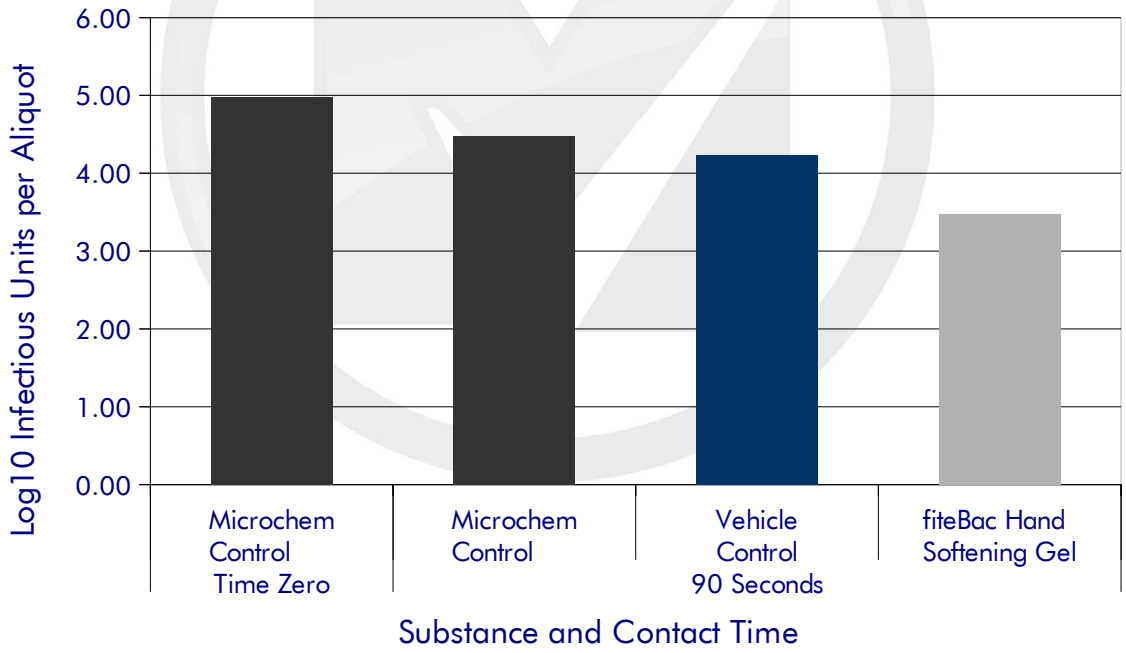
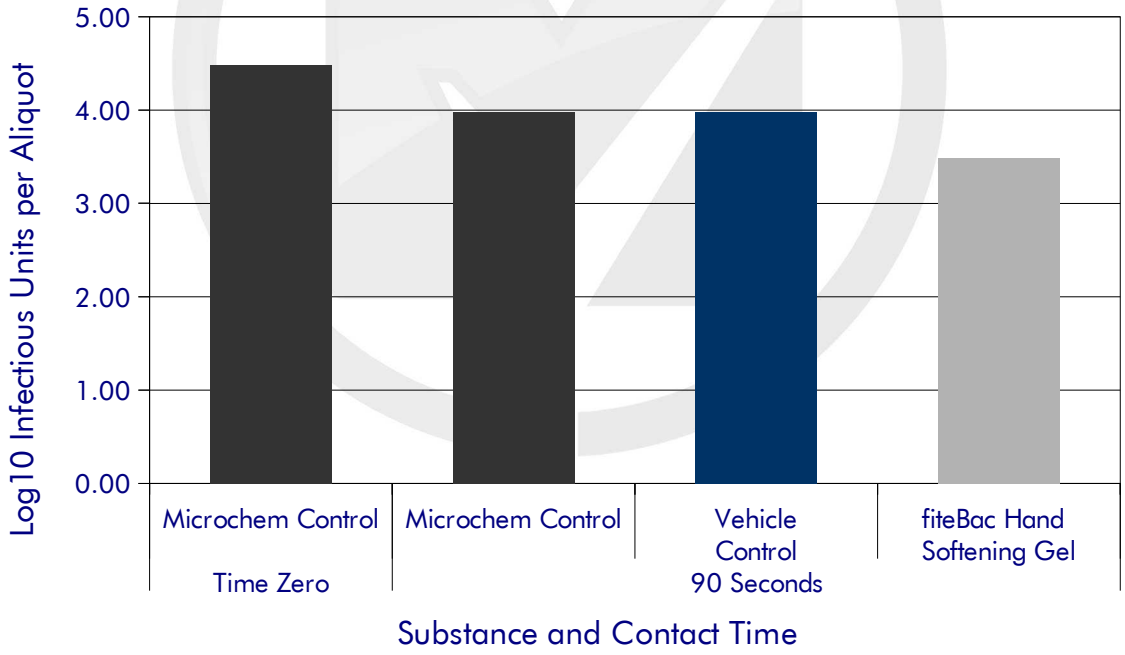
# Results of the Study

| Test Microorganism | Contact Time | Test Substance | $Log_{10}$ $TCID_{50}$/ Aliquot | Percent Reduction Compared to Control at Contact Time | $Log_{10}$ Reduction Compared to Control at Contact Time |
|---|---|---|---|---|---|
| Influenza A (H1N1) ATCC VR-1762 | Time Zero | Microchem Control | 4.98 | N/A | |
| | 90 Seconds | Microchem Control | 4.48 | | |
| | | Vehicle Control | 4.23 | | |
| | | fiteBac Hand Softening Gel | 3.48 | 82.22% | 0.75 |



# Results of the Study (cont.)

| Test Microorganism | Contact Time | Test Substance | $Log_{10}$ $TCID_{50}$/ Aliquot | Percent Reduction Compared to Control at Contact Time | $Log_{10}$ Reduction Compared to Control at Contact Time |
|---|---|---|---|---|---|
| Feline Calicivirus (US EPA-Approved Human Norovirus Surrogate) ATCC VR-782 | Time Zero | Microchem Control | 4.48 | N/A | |
| | 90 Seconds | Microchem Control | 3.98 | | |
| | | Vehicle Control | 3.98 | | |
| | | fiteBac Hand Softening Gel | 3.48 | 68.38% | 0.50 |




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