

FINAL STUDY REPORT

**ASSESSMENT OF THE ACTIVITY OF THREE ETHANOL-BASED HANDRUBS
AGAINST MURINE NOROVIRUS TYPE 1**

CHALLENGE VIRUS

Murine Norovirus type 1

IDENTITY OF PRODUCTS

- (1) ET_63_07194_1
- (2) ET_63_07194_2
- (3) ET_63_07194_3

PROTOCOL NUMBER

CREM-200605A-312

CREM PROJECT NUMBER

CREM-2007-08-07/SI

STUDY DIRECTOR

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STUDY COMPLETION DATE

August 07, 2007

PERFORMING LABORATORY

Centre for Research on Environmental Microbiology (CREM)
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SPONSOR

Soaptronic International, LLC
20562 Crescent Bay Drive
Lake Forest, California 92630
U.S.A.

TEST SUMMARY

Title: ASSESSMENT OF THE ACTIVITY OF THREE ETHANOL-BASED HANDRUBS AGAINST MURINE NOROVIRUS TYPE 1

Study Design: This study was performed according to the signed protocol and recording sheets issued by the Study Director.

Test Articles: ET_63_07194_1, ET_63_07194_2, and ET_63_07194_3 were shipped to us by the Sponsor in clear plastic containers and received here in good condition on July 10, 2007. According to the Sponsor, all three samples contained 63% (v/v) ethanol.

The Sponsor supplied the test articles as finished formulations and also provided us the details on the storage conditions. Once the materials for testing were received here, they were stored under lock and key in a secure place with access to authorized individuals only.

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EXPERIMENTAL

Many viruses and bacteria that can cause disease in humans may remain alive on hands for several hours, increasing the potential spread of such microbes through this route. Proper decontamination of hands with hygienic handwash and handrub agents is therefore, crucial in preventing the spread of infections. The best means to determine how well a given product would work for this purpose is by using *in vivo* methods where hands of healthy normal adults are contaminated in the laboratory and treated with the formulation under test. The fingerpad method, which is an internationally accepted standard (ASTM 2002), is particularly well suited for this purpose (Ansari et al., 1988, 1989). This method was developed at CREM and we have permission from the Ottawa Hospital Research Ethics Board (OHREB) to place a variety of viruses and bacteria on the intact skin of the hands of adults after they have provided written consent.

The work for this study was performed in accordance with the current state of the laboratory research art and best efforts were made to comply with all government regulatory requirements concerning Good Laboratory Practices (GLP) and Good Clinical Practices (GCP) as considered being appropriate for each experiment.

OBJECTIVE

The objective of this study was to determine if the formulations tested could effectively inactivate (kill) the mouse norovirus (MNV) on the hands in a contact time of 20 or 30 seconds.

Articles for use during the washout period

All human subjects selected for the study were provided with locally-bought personal care products (non-germicidal hand soap, bath soap, shampoo, under-arm deodorant and hand cream) and instructed to commence using them at least one week prior to their participation in the study. This was necessary to ensure that any anti-microbial activity seen in the tests was not due to residual effects from microbicidal ingredients in personal care products themselves.

Study Population

In accordance with the requirements of OHREB, the Principal Investigator (PI) was not permitted to seek volunteers directly for this study. A member of his staff approached eligible individuals and provided to the PI a list of only those persons who had agreed to be considered as subjects. Sufficient numbers of such subjects were then screened for their eligibility and six of those meeting the following inclusion/exclusion criteria were allowed to participate.

A. *Inclusion Criteria for Panelists*

Subjects were eligible for participation in this study if they met the following criteria:

1. A male or a female between the ages of 18 and 65 years of age, inclusive;
2. Had signed the Informed Consent Form approved by OHREB;
3. Had hands that are free of psoriasis, eczema, skin cancer, skin allergies, dermatoses, cuts, lesions, fingernail infections and other skin disorders;
4. Are in good health;
5. Were willing to refrain from using any oral or topical antibiotic medications or any antimicrobial soaps, antimicrobial cleansers, creams or lotions during the entire study period;
6. Were willing to comply with all study protocol requirements.

B. *Exclusion Criteria for Panelists*

No one was allowed to participate in the study if he/she:

1. Was currently participating in another clinical study at this or any other facility;
2. Have cuts, scratches, fingernail infections, artificial fingernails, or a rash on their hands;
3. Had a history of soap, detergent, antimicrobial cleanser and/or perfume allergy;
4. Had used oral or topical antibiotic medications or any antimicrobial soaps/cleansers, creams, or lotions seven days prior to the start of the testing;
5. Had any other medical condition, which in the opinion of the Principal Investigator precluded the individual's participation.
6. Was pregnant or lactating.

Panelist Withdrawal

After admission to the study, a panelist could withdraw from it at anytime for any reason but was expected to report the reason(s) for the withdrawal fairly and accurately. **In this investigation, no one withdrew after the initial recruitment.**

TEST CONDITIONS

Soil Load:

The soil load in the tests was heat-inactivated bovine serum at the final concentration of 5%. This was added to the virus suspension to simulate the presence of any residual body fluids that could interfere with the activity of the handrub agent(s).

BASIC CONTROLS:

Treatment of Virus Suspension:

The virus was mixed with the soil load and the suspension was then used for testing. **The serum used as the soil load did not prove to be harmful to the test virus.**

Cell Culture Controls:

In each plaque assay, two wells received 100 µL each of EBSS only to act as cell (negative) controls. The rest of the procedure was the same as for the wells used to determine the plaque forming units (PFU) in control and test samples.

Cytotoxicity Controls:

At the start of the study, the test formulations were assessed for their potential to cause any apparent damage (cytotoxicity) in the host cell line used for plaque assays. This was done by first exposing a clean and uncontaminated fingerpad for 10 seconds to one mL of the test formulation in a 2 mL cryovial. The fingerpad was gently scraped on the inside wall of the vial. The skin was then eluted with 1 mL of the eluent to recover any of the formulation that may have remained on the fingerpad. The eluate (100 µL) was introduced into at least three host cell monolayers; control wells received an equivalent volume of EBSS. The cultures were held for 30 minutes at room temperature and examined under an inverted microscope for any obvious signs of cell damage. **The formulations tested did not show any obvious signs of cytotoxicity.**

Neutralization Validation:

The initial steps in the procedure for neutralization validation were the same as described above for cytotoxicity controls, except that the eluates received countable PFU of test virus. The inoculated vials were held at room temperature for 30 minutes before plaque assay. Neutralization was validated if the number of PFU in the test and control eluates was nearly the same.

The neutralization procedure employed here was effective as indicated by the lack of any virus inactivation after exposure to the eluate.

Virus Susceptibility Control:

In each plaque assay plate, one well received an undiluted virus suspension to act as infectivity (positive) control. The presence of virus-induced cytopathic effects (CPE) was used as an indicator of the presence of infectious virus in the inocula and the susceptibility of the host cells to the virus.

All such controls in this investigation showed virus-induced CPE, thus confirming that the host cells remained susceptible to the test virus.

Plaque Interference Test:

Non-cytotoxic residues of the formulations in the eluates could interfere with the plaque-forming ability of the test virus resulting in either a lower or a higher number of PFU in the host cell monolayers. A lower than expected number could be due to either virus inactivation or damage/blocking of virus receptors on host cells. Any deaggregation of virus clumps or an enhanced unraveling/modification of host cell receptors could lead to a higher than expected number of PFU.

For this control, host cell monolayers are first exposed for 30 minutes to a non-cytotoxic level of the test formulation. This is followed by exposure of the cells to countable numbers of virus PFU and a further incubation to allow for virus adsorption. Control monolayers receive EBSS instead of the formulation but otherwise treated in an identical manner.

The formulations used in this study did not show any interference with the plaque-forming ability of the virus tested.

STUDY DATES AND FACILITIES

The laboratory phase of the test was performed at CREM, Faculty of Medicine, University of Ottawa, 451 Smyth Rd., Ottawa, ON K1H 8M5. Experimental testing was initiated on July 13, 2007, and concluded on July 31, 2007.

RECORDS TO BE MAINTAINED

CREM will store (electronic and hard copies) all test data, protocols used, test material records, the final report, and correspondence between the Study Director and the Sponsor for one year from the date of submission of this report.

DATA ANALYSIS

All raw data was subjected to preliminary statistical analyses using Microsoft Excel and made available to the Sponsor for any further review and analyses.

THE FINGERPAD PROTOCOL

Study Design and Standard Procedures

A. *Preparation of the Virus Inoculum*

Pools of the test virus were prepared in the RAW cell line of mouse macrophages and the number of plaque forming units (PFU) in them determined using a plaque assay method in the same host cells. A soil load was added to the test virus suspension to simulate the presence of body fluids. This inoculum contained no less than 10^4 PFU/10 μ L.

B. *The Fingerpad Method*

The testing followed the procedures described below.

1. Each person who had agreed to participate in the study visited the test facility to meet with the Principal Investigator or his designate to discuss the study and, if fully satisfied with the information provided, signed the Consent Form.
2. Just prior to the experimental contamination of the fingerpads the panelist thoroughly washed and dried his/her hands using a non-medicated liquid soap to remove any dirt and oil from hands.
3. Approximately 3.0 mL of 75% (v/v) ethanol was dispensed into the palms of the panelist's hands. He/she rubbed the alcohol over the entire surface of both hands until the alcohol had completely evaporated.
4. To determine the amount of the infectious virus that was placed onto each fingerpad, the panelist performed the 'zero time' control on the right and left thumbpads using the procedure described below in section C.
5. To determine the amount of infectious virus that survived the drying of the inoculum, the panelist performed the 'baseline drying control' on two randomly selected (one on the right and the other on the left hand) fingerpads using the procedure described below in section D.
6. To determine the amount of infectious virus that was eliminated by the test formulation, the panelists performed the procedure described below in section E.

C. *Zero Time Control*

For this control, the panelist pressed the mouth of an empty 2.0 mL cryovial, with an inside diameter of 8 mm against the right thumbpad to demarcate a circular target area for inoculation. The technician then dispensed 10 μ L of the virus suspension in the center of the demarcated area. Immediately following the inoculation, the panelists eluted the virus off the thumb by inverting an open vial containing 1.0 mL of the eluent over the inoculated area. The following procedure was used for elution of the virus.

1. The panelist inverted the vial over the right thumbpad and allowed the solution to remain in contact with the skin for 10 seconds.
2. Following the 10-second contact, the panelist performed 20 full inversions of the vial while keeping the thumbpad over the mouth of the vial.
3. The panelist repeated steps 1 and 2.

4. The panelist turned the vial upright and scraped the thumbpad against the inside rim of the vial to remove as much of the remaining fluid from the skin as possible.
5. The panelist decontaminated the thumbpad by pressing it against a paper towel soaked in either 75% (v/v) ethanol for 3 minutes.
6. The panelist repeated the above procedures with the left thumbpad.

D. *Baseline Drying Control*

All eight fingerpads were demarcated at this stage and each one received 10 μ L of the test virus suspension and the inocula dried for 20-25 minutes under ambient conditions.

The baseline drying control was included to determine the amount of infectious virus that remained after the drying of the inoculum on the skin. For this control, two randomly selected fingerpads (one on each hand) were used. The panelist eluted the virus off one finger using the procedure described above in section C above immediately at the end of the inoculum drying period. The second finger was eluted at the end of the entire test. The two values were averaged to represent the difference in the amount of infectious virus in the zero time control and the dried virus control represented the loss in viral infectivity due to the drying of the inoculum. In the drying control, the amount of infectious virus on each fingerpad was not less than 10^4 PFU.

E. *Virus Elimination by the Test Formation*

1. The panelist simultaneously but separately placed one fingerpad each with the dried inoculum over the mouth of two vials containing 1.0 mL of the test formulation.
2. The panelist inverted the vials over the fingerpads and allowed the fluid to remain in contact with the skin for 20-30 seconds.
3. The panelist turned the vials upright and let the remaining formulation stay on the fingerpads.
4. The virus from the two fingerpads was then eluted at the same time using the procedure described in section C.
5. The panelist decontaminated the fingerpads by pressing them against a paper towel soaked in either 75% (v/v) ethanol.
6. The panelist repeated the above procedures using the standard hard water (see below) or any positive controls or predicate products included in the study.

The treatment/formulation applied to each fingerpad was recorded on the data sheet.

F. *Hard Water Rinse Control*

The hard water rinse was included to control for the mechanical removal of the virus without any inactivation. For this control, the panelist exposed the dried inoculum on two randomly selected fingerpads separately to 1.0 mL of standard hard water as described above for 30 seconds. The virus was eluted off the fingers using the procedure described in section C. The standard hardness of the water was 200 ppm in the form of CaCO_3 .

Other Study Documentation and Requirements

A. *Adverse Events*

An adverse effect (AE) was “any change, undesired, noxious or pathological event in a panelist illustrated by signs, symptoms and/or laboratory changes that occur during a clinical trial, whether

or not considered drug/treatment related.” A serious/severe AE was where the event (a) is life threatening or fatal; (b) requires or prolongs hospitalization; (c) disables the subject. A non-serious AE was an event other than the ones described above.

There was a system in place through which the PI or his designate would be immediately notified in case of any AE. He would then determine whether: a) the adverse event was likely to be associated with the product treatment or the study procedures; b) the reaction warranted termination of participation of the affected panelist; and c) to refer the case to the Medical Consultant for the study without any undue delay. The PI would also notify the Sponsor’s representative. In addition, all information pertaining to the presenting signs, assessment of the relationship of the adverse event to the product treatment, any prescribed treatment, and all the follow-up visits, including final resolution, would be documented in writing.

RESULTS

As per the agreed protocol, six individual fingerpad tests were conducted using the method E-1838 of ASTM International (ASTM 2002).

The test formulations were assessed for any cytotoxic effects and were found to be non-cytotoxic for the host cells used in this study. They also did not produce any interference with the ability of the tested virus to form plaques in the host cell monolayers.

No panelist withdrew from the study once recruited. No AE were observed in any of them.

Figures 1 and 2 represent summary data for the fingerpad testing with the two formulations. Figure 1 represents the \log_0 reduction of the virus in relation to the baseline value whereas Figure 2 illustrates the reduction in terms of percent values in comparison to the baseline. Lots ET_63_07194_1 and ET_63_07194_2 are shown along with the positive control comprising of 75% (v/v) ethanol. Both lots reduced virus infectivity by at least 3 \log_{10} after 30 seconds of contact. This represents a minimum 99.9% reduction in the virus infectivity titre. The same result was observed for ET_63_07194_2 with only 20 seconds contact time. Yet, in the case of ET_63_07194_1, the reduction at 20 seconds was slightly lower than 3 \log_{10} . This result was due to a single experiment and may not be indicative of the true reduction potential of this lot.

CONCLUSIONS

With the exception of one result from ET_63_07194_1, the formulations reduced the viral load of MNV on human fingerpads by 3 \log_{10} PFU (99.9%) at both 20 seconds and 30 seconds of contact time.

LITERATURE CITED

- Ansari *et al.* (1989). In vivo Protocol for testing the efficacy of hand-washing agents against viruses and bacteria: experiments with human rotavirus and *Escherichia coli*. Appl. Environ. Microbiol. 55: 3113-3118.
- Ansari, S.A. (1988). Rotavirus survival on human hands & transfer of infectious virus to animate & non-porous inanimate surfaces. J. Clin. Microbiol. 26:1513-1518.
- ASTM (2002). Standard test method to determine the virus-eliminating effectiveness of liquid hygienic handwash agents using the fingerpads of adult volunteers. Designation: E-1838. ASTM, West Conshohocken, PA, U.S.A.

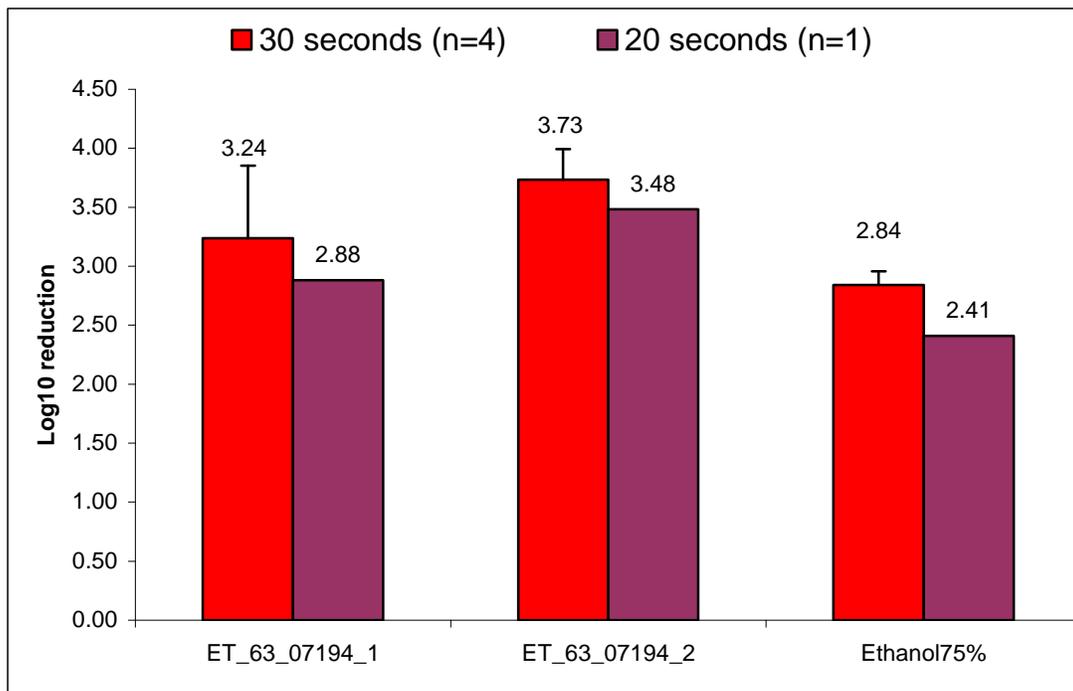


FIGURE 1: Log₁₀ reduction of mouse norovirus (MNV-1) as determined by the fingerpad method (ASTM, 2002)

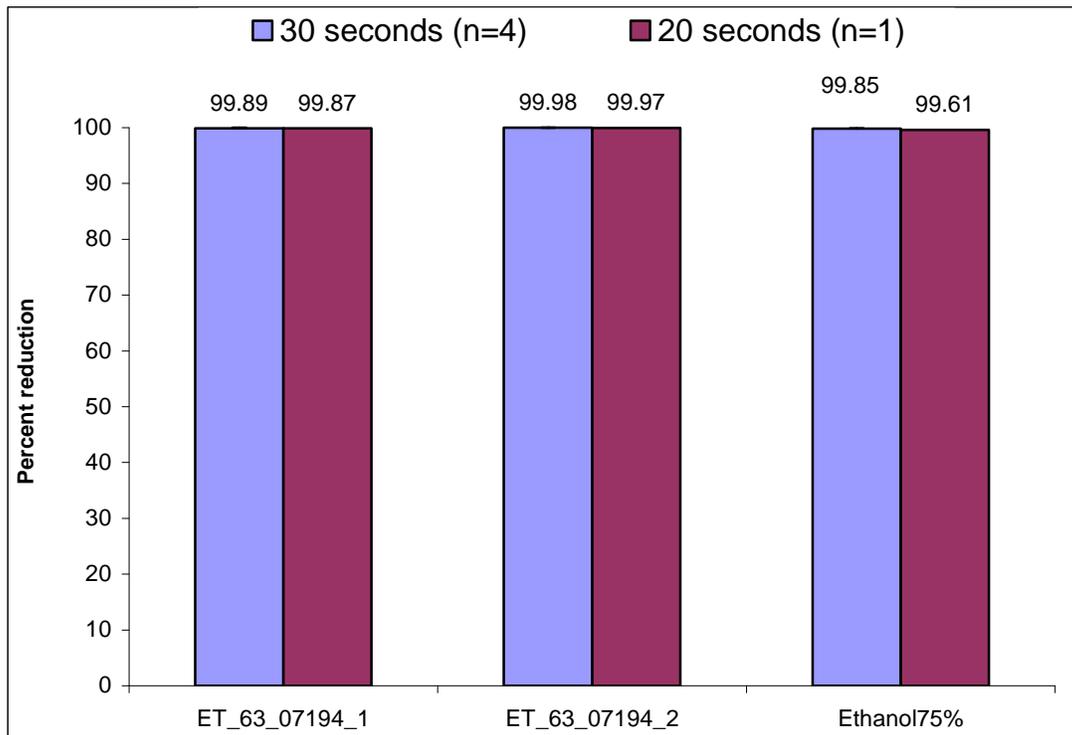


FIGURE 2: Percent reduction of mouse norovirus (MNV-1) as determined by the fingerpad method (ASTM, 2002)