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Outline

The protocol TP016 was executed to evaluate the effectiveness of the client's product, my-shield Hand Sanitizer, on Murine Norovirus 1 (MNV-1), on fresh, washed, shaved, and sterilized porcine skin to simulate human skin contact. The virus stock is prepared using the following materials, MNV-1 animal CW1, ATCC PTA-5935 hosted in RAW 264.7 Murine cells, ATCC T1B71. Once murine cell culture reached confluence, they were infected with MNV-1. These infected cells were incubated until full lysis of the cell monolayer occurs with virus stocks being tested on a set of porcine skin, M121015D, pads (3" x 6" pads) without the hand sanitizer (control) to ensure this can be used as a suitable substrate for challenging the clients' product. On all three sets of pig skin pads, the MNV-1 is applied, dried, and treated with my-shield Hand Sanitizer. The pads are sampled and evaluated for viability of MNV-1. The second and third sets of pads are repeatedly washed with a mild non-antimicrobial soap to simulate hand washing. The second set of pads are washed 5x and the third set of pads are washed 9x. Each set of pads are sampled to evaluate viability of MNV-1. Following the 5x and 9x washings, each set of pads are contaminated again with MNV-1, dried, and sampled to determine long-term protection of the initial application of the product. To assay virus viability and potential effectiveness of my-shield hand sanitizer, samples from skin pads were inoculated with murine cell (ATCC T1B71) monolayers and after 72 hours of incubation, were examined for cytopathogenic effects (CPE) and graded using a 10 point scale (0= no CPE and 10 = 100% murine cell destruction).

Reference Procedure

- TP016 testing protocol, dated September 14th, 2012, version 0
- ASTM E2897-12, Standard Guide for Evaluation of the Effectiveness of Hand Hygiene Topical Antimicrobial Products using Ex Vivo Porcine Skin.
- ASTM WK36911 – New Guide for Measuring the Inactivation of Persistent Activity of a topical antimicrobial Product Using Ex Vivo Porcine Skin.

Planned Changes to Protocol

Several changes were made in relation to the sampling procedure. Sterile swabs and gauze were determined to be problematic with respect to the virus. The procedure was changed to a rinsing exercise to eliminate the use of the sterile swabs and gauze.

An additional control was added to ensure no interference from the mild non-antimicrobial soap washings of the porcine skin pads on the procedure.


Results

The results are reported in Table A, titled "Data for the Evaluation of my-shield Hand Sanitizer and its effects on Murine Norovirus (MNV-1) using porcine skin to simulate human skin".

Interpretation of results

Following some changes to the protocol, a practice was developed to eliminate any type of interference from the activities outlined in the protocol. In addition, the mild non-antimicrobial was confirmed to have no effect on the viability of the healthy and infected cells. The healthy and infected cells are visually distinguishable.

Following the modifications, the controls performed as expected and were supportive for the duration of the testing.

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The fresh, washed, and shaved porcine skin was sectioned into 3" x 6" pads and sterilized prior to use. These pads are used on latex gloves in pairs and rubbed together to simulate hand washing during all application of stock virus, client's product, and mild non-antimicrobial soap to the porcine pads.

The first evaluation is of the client's product, my-shield Hand Sanitizer, applied directly, approx. 2-3ml, on the virus supported on the porcine skin. The client's product has an immediate positive effect on the MNV-1 to be totally inactivated following application.

The second evaluation is to evaluate the effectiveness of hand washing on the durability of the hand sanitizer. Following the contamination of the second pair of pads with MNV-1 and treating with the client's product, the pads are used in a hand washing simulation for five times with a mild non-antimicrobial soap followed by a re-contamination of stock virus and evaluated. There are three samples drawn from this step and evaluated by plaque assay for evaluation. The client's product is confirmed to be effective from the first sample. The second sample demonstrates a clean surface prior to recontamination. The recontamination shows that the client's product does have some protective qualities, showing a reduction of 40-50% of infected cells.

The third evaluation is to evaluate the effectiveness of hand washing on the durability of the hand sanitizer after nine washings. Following the contamination of the third pair of pads with MNV-1 and treating with the client's product, the pads are used in a hand washing simulation for nine times with a mild non-antimicrobial soap followed by a re-contamination of stock virus and evaluated. There are three samples drawn from this and evaluated by plaque assay for evaluation. The client's product is confirmed to be effective from the first sample. The second sample demonstrates a clean surface after the nine washings prior to recontamination. The recontamination shows that the client's product does not have any effect on the infected cells, showing a 100% viability of infected cells. This last sample was identical to the any of the untreated samples that were seen in this protocol.

Verifications

All testing was completed by qualified and knowledgeable personnel with the appropriate level of supervision. The raw data from testing protocol TP016 has been reviewed and approved for inclusion in this report.

Approved:



 Doug Drabble

May 7th, 2013

 Date



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TABLE A

Data for the Evaluation of my-shield Hand Sanitizer and its effects on Murine Norovirus (MNV-1) using porcine skin to simulate human skin

Sample ID	Description	Application	Virus Assay Results (CPE*)	Comments @ 72 hours post infection and incubation
Control	Stock of virus applied and dried on porcine skin followed by rinsing	None	10	CPE assay performed; all murine cells infected
Control	Porcine pads are washed 9x followed by contamination of porcine pads with virus stock	Use of mild antimicrobial soap	10	CPE assay performed; all murine cells infected
Product applied	Product is applied to contaminated porcine pads and rubbed to dryness	Hand Sanitizer applied	0	No CPE = MNV-1 is fully inactivated
		No Soap washing		
Product applied, 5x washing, recontamination with stock virus	Product is applied to contaminated porcine pads and rubbed to dryness	Hand Sanitizer applied	0	No CPE = MNV-1 is fully inactivated
	Porcine pads washed 5x	Use of mild antimicrobial soap	0	No CPE = MNV-1 is fully inactivated
	Stock of virus applied and dried on porcine skin followed by rinsing after 5 minutes	None	5-6	Virus moderately viable, but some level of protection following 5 washings.
Product applied, 9x washing, recontamination with stock virus	Product is applied to contaminated porcine pads and rubbed to dryness	Hand Sanitizer applied	0	No CPE = MNV-1 is fully inactivated
	Porcine pads washed 9x	Use of mild antimicrobial soap	0	No CPE = MNV-1 is fully inactivated
	Stock of virus applied and dried on porcine skin followed by rinsing after 5 minutes	None	10	MNV-1 is fully viable. No level of protection following 9 washings.

* Cytopathogenic Effect (CPE) is a measurement of cell damage and destruction on a 0 to 10 scale (10 being 100% infected or destroyed and 0 being not infected and no CPE).