

**EVALUATION OF THE EFFECTIVENESS OF  
MANORAPID SYNERGY AGAINST FELINE  
CALICIVIRUS (SURROGATE OF NOROVIRUS)  
USING FINGERPADS OF ADULT VOLUNTEERS**

Test method according to ASTM Standard E 1838-02

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## Summary

The ability of the hand disinfectant MANORAPID SYNERGY to eliminate the feline calicivirus (FCV) from contaminated fingerpads was examined by the ASTM Standard E 1838-02 with four volunteers. FCV was tested as a surrogate of norovirus, since these agents can not be cultivated in cell culture. Ethanol (70 vol%), 1-propanol (70 vol%) and Standard Hard Water were chosen as references. In this clinical trial the lg reduction factor (lg RF) served as a test parameter for effectiveness of the disinfectants. MANORAPID SYNERGY produced a lg RF of  $\geq 2.38$  after an exposure time of 30 seconds. In comparison, the lg RF's were 0.68, 0.70 and 1.39 testing ethanol, 1-propanol and Standard Hard Water. Therefore, the product is well suited for hand disinfection in daily routine as well as for outbreak situations with norovirus.

## 1. Introduction

In laboratory experiments, it has been shown that viruses can survive for prolonged periods on artificially contaminated hands and that viruses like hepatitis A virus can be transferred from one hand to another and from environmental surfaces to hand.

The role of contaminated hands in the spread of viral diseases has been recognized mainly by observations of persons with experimentally acquired cold. The hygienic hand disinfection or hand washing with antimicrobially effective preparations is one of the most effective and most frequently performed measures for infection prophylaxis. Testing virucidal effectiveness of hand disinfectants with a suspension method is only of limited value and is often not predictive of their behaviour on human skin. Therefore, it is necessary to evaluate the virus-eliminating potential of hand disinfectant on human skin.

Since 1996, a standardised method exists from the American Society for Testing and Materials (ASTM) using the fingerpads of adult volunteers (revised in 2002). Consequently, it was the main objective of this study to determine the virus-eliminating effectiveness of MANORAPID SYNERGY testing feline calicivirus contaminated fingerpads after treatment with the hand disinfectant.

## 2. Material and methods

### 2.1 Test product and references

MANORAPID SYNERGY is manufactured by ANTISEPTICA (D-50259 Pulheim, Germany; Fax +49-2234-984660) and licenced for hygienic hand disinfection. It is an alcoholic rub not requiring any subsequent rinsing.

MANORAPID SYNERGY (lot no. 00102) consists of 54.1 g ethanol, 10.0 g 1-propanol, 5.9 g 1.2-propandiol, 5.7 g butandiol and 0.7 g phosphoric acid.

As references ethanol (70 vol%) and 1-propanol (70 vol%) were chosen. For ethanol and 1-propanol it was shown that for both a concentration of 70 vol% was most effective in comparison to other concentrations [Gehrke et al., in press]. For differentiating the mechanical effect of virus removal from the virus-inactivating effectiveness of MANORAPID SYNERGY Standard Hard Water (standard hardness of 200 ppm as calcium carbonate) in accordance with AOAC 960.09 *E* and *F* was additionally chosen for reference.

## 2.2 Virus and cells

The feline calicivirus strain F9 was cultivated and titrated in Crandell's feline kidney (CRFK) cell line. These cells and the virus were kindly provided by Prof. Dr. H. Schirrmeyer, Bundesforschungsanstalt für Viruskrankheiten der Tiere [Federal Research Centre for Virus Diseases of Animals], Insel Riems, Germany.

The cells were grown in Eagle's minimal essential medium (EMEM) with 10 % fetal bovine serum (FBS).

For production of the virus pool FCV was added to a monolayer of CRFK cells with a multiplicity of infection of about 10, and following an adsorption period of two hours EMEM (without FBS) was added to the culture. After 16 to 24 hours a cytopathic effect had developed in the cell culture, and the virus was harvested by freeze-thawing three times followed by removal of cell debris by centrifugation. This FCV suspension was employed for all inactivation experiments.

## 2.3 Soil load

A tripartite soil load according to 7.4.2 of E 1838-02 was chosen, because FBS increased the virus-eliminating properties of alcoholic hand disinfectants [Gehrke et al., in press]. FCV was therefore suspended in a tripartite soil load. 25 µl of a 5 % bovine serum albumin, 35 µl of 5 % tryptone and 100 µl of 0.4 % mucin were added to 340 µl of the virus suspension (ASTM E 1838-02; 7.4.2). This mixture is approximately equivalent to the protein content of a 5 % solution of fetal bovine serum.

## 2.4 Volunteers

A healthy skin was necessary for participation without any clinical evidence of dermatoses, open wounds, or other skin disorders.

The Ethic Committee of Bremen had given its permission for this clinical study (No. 50-09-97).

## 2.5 Test procedure

The experiments with artificially FCV contaminated fingertips of adult panelists were performed according to the standard test method from the American Society for Testing and Materials (ASTM Standard E 1838-02) for determining the virus-eliminating effectiveness of liquid handwash agents.

Briefly, the washed and disinfected fingertips were contaminated in a marked area with 10 µl FCV suspension, which allowed to become visibly dry. Afterwards, the dried inoculum is exposed to 1 ml of MANORAPID SYNERGY or the different references in a plastic vial. The plastic vial on the contaminated area was then shaken for 30 seconds with 30 inversions.

Determination of virus elimination was performed using a plastic vial containing 1 ml of an eluent (Earle's balanced salt solution, EBSS) that was placed on the same area and shaken for 20 seconds with 20 inversions.

Two virus controls were performed. For the virus controls, 10 µl FCV suspension was eluted directly from two thumbpads without previous drying (input control) and 10 µl FCV suspension was eluted from two fingerpads after drying. The difference between infectious virus in the input control and the dried virus control represents the loss in virus infectivity due to the drying of the inoculum. The amount of infectious virus remaining after drying of the inoculum was used as the baseline to determine the extent of virus elimination after treatment with MANORAPID SYNERGY and the references.

For one panelist each experiment was performed three times. Each experiment included the virus controls with drying (two fingertips), the inactivation procedure for MANORAPID SYNERGY (four fingertips) and a reference (two fingertips). In the first experiment ethanol was chosen as reference, in the second and third performance 1-propanol and Standard Hard Water were used. The virus input controls without previous drying were performed only once in the first experiment.

The eluted virus suspensions from the inactivation tests and the virus controls were kept on ice and the FCV titres were determined by endpoint titration in 96-well microtitre plates with a preformed monolayer of CRFK cells. The calculation of the virus titres was performed by the Spearman-Kärber method.

## **2.6 Statistical evaluation**

From the difference between the individual lg values of the virus controls after drying as given in table 1 and the values after treatment with MANORAPID SYNERGY or the references as given in table 2 a lg reduction factor (lg RF) was established. The mean lg RF's and the standard deviations were calculated from all single values of the four volunteers.

## **3. Results**

### **3.1 Virus content of the control**

The virus titre of the suspension was  $10^{7.5}$  TCID<sub>50</sub>/ml (data not shown in table). The values of the inoculum controls with (six fingerpads of each panelist) and without drying (two fingerpads of each panelist) are given in table 1 (mean values  $10^{5.38}$  and  $10^{6.10}$ ).

### **3.2 Virus-eliminating effectiveness of MANORAPID SYNERGY**

This study allows testing the ability of hand disinfectant to reduce levels of FCV from experimentally contaminated fingerpads of adult volunteers. Such reduction in virus load may be due to a combination of virus inactivation and removal of FCV from skin. MANORAPID SYNERGY and 70 % ethanol, 70 % 1-propanol and Standard Hard Water as references were tested after 30 seconds.

The results of the inactivation tests with MANORAPID SYNERGY and the references are given in table 2. The results of the lg RF's given as difference between the virus titres of virus controls after drying and FCV titres after treatment with MANORAPID SYNERGY and the references are demonstrated for each panelist in table 3 and are summarised in table 4. Summarising the results of these tables, MANORAPID SYNERGY produced a lg RF of  $\geq 2.38$ . In comparison with the references lg RF's of 0.68, 0.70 and 1.39 were obtained testing 70 % ethanol, 70 % 1-propanol and Standard Hard Water.

#### **4. Discussion**

The scope of the standard test method E 1838-02 is to assess the capacity of hygienic hand disinfectants to reduce virus levels on contaminated hand. According to this standardised method a variety of humanpathogenic viruses can be selected for the evaluation of hand disinfectants. In this study the feline calicivirus was chosen as a surrogate for noroviruses.

The results with artificially contaminated fingerpads indicate that MANORAPID SYNERGY show a high effectiveness against FCV in comparison to 70 % ethanol and 70 % 1-propanol. Therefore, the product is well suited for hand disinfection in daily routine as well as for outbreak situations with norovirus.

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**Table 1:** FCV titres with or without drying

panelist	experimental serie	FCV-titre (lg TCID <sub>50</sub> /ml) on contaminated thumbpads without drying (input-control)			FCV titre (lg TCID <sub>50</sub> /ml) on contaminated fingerpads after drying		
		thumb 1	thumb 2	mean	finger 1	finger 2	mean
1	1	5.50	5.88	5.73	5.13	4.88	5.02
	2	-	-	-	4.63	4.63	4.63
	3	-	-	-	5.38	4.75	5.17
2	1	6.50	5.88	6.29	5.50	5.38	5.44
	2	-	-	-	5.00	4.88	4.94
	3	-	-	-	5.38	5.00	5.23
3	1	6.50	5.75	6.27	5.25	5.25	5.25
	2	-	-	-	4.75	3.88	4.50
	3	-	-	-	5.13	5.00	5.07
4	1	5.75	5.88	5.82	5.50	5.38	5.44
	2	-	-	-	5.63	6.25	6.04
	3	-	-	-	5.63	5.50	5.57

**Table 2:** FCV titres on contaminated fingerpads after treatment with Manorapid Synergy or references A: 70 % ethanol, B: 70 % n-propanol and C: Standard Hard Water for 30 seconds. Soil load: 7.4.2 (mucin, BSA and tryptone).

panelist	experimental serie and reference	FCV titre (lg TCID <sub>50</sub> /ml) after treatment with Manorapid Synergy					FCV titre (lg TCID <sub>50</sub> /ml) after treatment with references		
		finger 1	finger 2	finger 3	finger 4	mean	finger 1	finger 2	mean
1	1 and A	2.38	≤1.50	2.88	2.38	≤2.50	5.00	5.00	5.00
	2 and B	≤1.25	3.13	≤1.25	0.00	2.54	3.63	3.38	3.52
	3 and C	4.38	3.63	3.50	2.50	3.90	4.00	3.75	3.89
2	1 and A	4.13	3.63	4.00	3.63	3.90	5.13	5.00	5.07
	2 and B	3.63	2.88	3.25	3.38	3.36	4.63	4.38	4.52
	3 and C	2.75	3.63	2.75	3.25	3.25	3.75	4.13	3.98
3	1 and A	3.00	3.75	3.63	3.5	3.55	4.50	4.50	4.50
	2 and B	4.00	3.13	2.88	3.38	3.56	4.00	4.50	4.32
	3 and C	3.63	3.88	3.75	3.63	3.74	3.63	3.75	3.69
4	1 and A	≤1.56	5.25	1.75	2.13	≤4.65	4.00	3.75	3.89
	2 and B	1.63	0.00	2.38	1.63	1.91	5.38	4.75	5.17
	3 and C	1.88	≤1.50	≤1.38	2.13	≤1.82	4.25	4.00	4.14

**Table 3:** Reduction of the FCV titres after treatment with Manorapid Synergy in comparison to 70 % ethanol, 70 % n-propanol and SHW for 30 seconds. Reduction of titres ( $\Delta$  lg TCID<sub>50</sub>) is calculated based on the mean values of FCV titres after drying (see table 1).

substance	experimental serie	panelist 1		panelist 2		panelist 3		panelist 4	
		value	mean	value	mean	value	mean	value	mean
<b>Manorapid Synergy</b>	1	2.64	$\geq 2.74$	1.31	1.61	2.25	1.78	$\geq 3.88$	$\geq 2.77$
		$\geq 3.52$		1.81		1.50		0.19	
		2.14		1.44		1.62		3.69	
		2.64		1.88		1.75		3.31	
	2	$\geq 3.38$	$\geq 3.22$	1.31	1.66	0.50	1.15	4.41	4.63
		1.50		2.06		1.37		6.04	
		$\geq 3.38$		1.69		1.62		3.66	
		$\geq 4.63$		1.56		1.12		4.41	
	3	0.79	1.67	2.48	2.14	1.44	1.35	3.69	$\geq 3.85$
		1.54		1.60		1.19		$\geq 4.07$	
		1.67		2.48		1.32		$\geq 4.19$	
		2.67		1.98		1.44		3.44	
<b>70 % ethanol</b>	1	0.02	0.02	0.31	0.38	0.75	0.75	1.44	1.57
		0.02		0.44		0.75		1.69	
<b>70 % n-propanol</b>	2	1.00	1.13	0.31	0.44	0.50	0.25	0.66	0.98
		1.25		0.56		0.00		1.29	
<b>SHW</b>	3	1.17	1.42	1.48	1.29	1.44	1.38	1.32	1.45
		1.66		1.10		1.32		1.57	

**Table 4:** Summary of the results

substance	number of fingerpads examined	lg RF
<b>MANORAPID SYNERGY</b>	48	2.38 $\pm$ 1.03
<b>70 % ethanol</b>	6	0.68 $\pm$ 0.58
<b>70 % n-propanol</b>	6	0.70 $\pm$ 0.37
<b>SHW</b>	6	1.39 $\pm$ 0.06