

**EVALUATION OF MANORAPID SYNERGY AGAINST  
POLIOVIRUS TYPE 1 (SABIN)  
USING FINGERPADS OF ADULT VOLUNTEERS**

Test method according to ASTM Standard E 1838-96

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## Summary

The ability of the hand disinfectant MANORAPID SYNERGY to eliminate poliovirus type 1 (Sabin) from contaminated fingerpads was studied by the ASTM Standard E 1838-96 with 5 volunteers. 2-propanol (60 vol%) was chosen as a reference. In this clinical trial the virus reduction factor (RF) served as test parameter for effectiveness of the disinfectants. MANORAPID SYNERGY produced a log RF of 3.04 and 3.13 after 30 and 60 seconds, respectively. In comparison, the values were 1.32 and 1.30 log RF testing 2-propanol.

### 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 rhinoviruses 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 the virucidal efficacy 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. Consequently, it was the main objective of this study to determine the virus-eliminating effectiveness of MANORAPID SYNERGY when poliovirus contaminated fingerpads are treated with it.

### 2. Material and methods

#### 2.1. Test product and reference

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. VP-100 ) consists of 54.1 g ethanol and 10.0 g 1-propanol.

As a reference 2-propanol (60 vol%) was chosen, because this alcohol is also used according to EN 1500 (evaluation of hygienic hand disinfectants) and to the guidelines of DGHM (Deutsche Gesellschaft für Hygiene und Mikrobiologie) and ÖGHMP (Österreichische Gesellschaft für Hygiene, Mikrobiologie and Präventivmedizin) for hygienic hand disinfection.

#### 2.2. Virus and cells

The Sabin vaccine strain of poliovirus type 1 (Lsc 2ab; ATCC VR-59) was used throughout the study. The Behringwerke AG (D-6583 Liederbach, Germany) kindly provided the virus.

suspension was harvested by freeze/thawing and centrifuged at low speed to remove cell debris followed by an ultracentrifugation step at 100000 g for 2.5 hours. The sediments were suspended in Eagle's minimal essential medium (EMEM) with 5 % fetal bovine serum.

### 2.3. Volunteers

Three adult males and two adult females were recruited as volunteers. In table 1 information on sex and age of the test persons is given. The current numbers for the test persons correspond with those in the tables containing the results of the study.

A healthy skin was necessary for participation without any clinical evidence of dermatoses, open wounds, or other skin disorders. All volunteers had participated in oral polio vaccination program before this study and were seropositive for poliovirus type 1 by neutralisation test. All panelists signed a written consent and an information sheet was supplied to them. The Ethic Committee of Bremen had given its permission for this clinical study (No. 50-09-97).

### 2.4. Test procedure

The study was performed according to the ASTN standard. Briefly, 10 µl of the virus suspension is placed on a demarcated area on each fingertip and the inoculum allowed drying. The dried inoculum then is exposed to the reference (60% 2-propanol) or MANORAPID SYNERGY contained in a glass vial (order no. 9002-P, Serolab, D-94501 Aidenbach, Germany). The contact time was 30 and 60 seconds. Following a water rinse with standard hard water in a sterile 25-cm<sup>2</sup> plastic cell culture flask the virus remaining on the fingerpads was eluted with 1 ml Earle's balanced salt solution containing 1 % trytone. Afterwards, the virus titer of the eluent was determined directly by endpoint titration (virus titration on monolayers of cells on microtiter plates). The calculation of the virus titer was performed by the Spearman-Kärber method.

Controls were included to determine the amount of infectious virus in the inoculum (i), the infectious virus placed on the two thumbpads without drying (ii) and the infectious virus remaining on fingerpads after the drying of the inoculum (iii). The difference for infectious virus in the inoculum 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 the drying of the inoculum (iii) was used as the baseline to determine the extent of virus elimination after treatment with MANORAPID SYNERGY and 60% 2-propanol. We used four randomly selected fingerpads for treatment with MANORAPID SYNERGY and two for treatment with 60% 2-propanol as reference.

### 2.5. Statistical evaluation

From the difference between the individual log prevalences (the amount of infectious viruses after drying) as given in table 3 and log postvalues a log reduction factor (log RF) was established. The mean log RF's and the standard deviations were calculated from all single values of the 5 panelists.

The quick, compact two-sample test according to Tukey at the  $\alpha = 0.1$  level was used to compare the log RF between MANORAPID SYNERGY and 60% 2-propanol.

## 2.6. GCP Criteria

The evaluation was performed according to § 40 AMG. The Ethic Committee of Bremen had given its permission for this clinical study.

## 3. Results

### 3.1. Virus content of the control

The virus titer of the suspension was  $10^{7.95}$  infective dosis  $_{50}$ /ml. The values of the inoculum control (ii) (two thumbpads without drying) and the dried virus control (iii) = (two fingerpads) are demonstrated in table 2 and table 3 (mean 5.99 and 5.07  $id_{50}$ ).

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

This study allows testing the ability of hand disinfectant to reduce levels of selected infectious virus such as poliovirus from experimentally contaminated fingerpads of adult volunteers. Such reduction in the virus load may be due to a combination of virus inactivation and removal of poliovirus from the skin. MANORAPID SYNERGY and 60% 2-propanol as a reference were tested after 30 and 60 seconds.

The results are given in the tables 4 and 5 and are summarised in the table 6. Summarising the results of these tables, MANORAPID SYNERGY produced a log RG of 3.04 after 30 seconds. After 60 seconds the log RF was 3.13. In comparison with the reference agents values of 1.32 and 1.30 were obtained. Statistically MANORAPID SYNERGY was significantly more effective than 60 % 2-propanol at the  $\alpha = 0.1$  level.

## 4. Discussion

The scope of the standard test method E 1838-96 is to assess the capacity of hygienic hand disinfectants to reduce virus levels on contaminated hands. According to this standardised method a variety of humanpathogenic viruses can be selected for the evaluation of hand disinfectants. In our study the resistant hydrophilic poliovirus was chosen as a representative for the enterovirus family.

Our results with artificially contaminated fingerpads clearly indicate that 60% 2-propanol with a proven efficacy against bacteria produces only a poor effectiveness against poliovirus, which is in accordance with results from the suspension assay.

This clinical trial confirms results from the group of Prof. Sattar and our group that ethanol and ethanol-based formulations were much more effective against poliovirus than preparations based upon 1-propanol and 2-propanol studying the whole-hand and the fingerpad model. MANORAPID SYNERGY was able to reduce the virus titer only after 60 seconds by 3.13 logs. This means a reduction of more than 99.9% and demonstrates the great effectiveness of this hand disinfectant against resistant poliovirus.

Table 1: sex and age of volunteers

Panelists	sex	age
1	m	19
2	m	19
3	f	46
4	m	51
5	f	58

Table 2: poliovirus ( $\log id_{50}$ ) at the artificially contaminated hand without drying (ASTM 10.6)

Panelists	Thumbpad 1	Thumbpad 2	Mean
1	6.38	6.00	6.19
2	6.38	6.25	6.32
3	6.38	6.00	6.19
4	5.50	6.38	5.94
5	5.00	5.63	5.32

Table 3: poliovirus ( $\log id_{50}$ ) at the artificially contaminated hand after drying (ASTM 10.8)

Panelists	Fingerpad 1	Fingerpad 2	Mean
1	4.38	6.25	5.32
2	4.13	5.88	5.01
3	4.13	6.00	5.07
4	5.50	4.88	5.19
5	5.13	4.38	4.76

Table 4: Virus-eliminating effectiveness of MANORAPID SYNERGY and 60% 2-propanol after 30 seconds. Data are given as log RF.

Agent		Panelists				
		1	2	3	4	5
MANORAPID SYNERGY	fingerpad 3	0.94	2.51	0.94	4.44	2.49
	fingerpad 4	4.32	3.01	2.57	4.07	3.51
	fingerpad 5	2.19	2.64	2.94	3.06	2.76
	fingerpad 6	3.82	3.88	2.19	4.57	4.01
<b>Mean</b>		2.82	3.01	2.16	4.03	3.19
60% 2-propanol	fingerpad 7	1.57	1.01	0.57	2.05	0.26
	fingerpad 8	1.70	2.26	0.57	2.32	0.88
<b>Mean</b>		1.64	1.64	0.57	2.19	0.57

Table 5: Virus-eliminating effectiveness of MANORAPID SYNERGY and 60% 2-propanol after 60 seconds. Data are given as log RF.

Agent		Panelists				
		1	2	3	4	5
MANORAPID SYNERGY	fingerpad 3	3.32	1.39	1.82	3.82	1.76
	fingerpad 4	3.32	2.26	3.82	3.69	3.38
	fingerpad 5	4.57	2.88	3.57	3.82	2.89
	fingerpad 6	3.57	4.41	1.57	4.19	2.51
<b>Mean</b>		3.70	2.74	2.70	3.88	2.64
60% 2-propanol	fingerpad 7	2.32	0.63	0.12	2.44	0.76
	fingerpad 8	2.57	0.63	0.57	2.19	0.06
<b>Mean</b>		2.44	0.63	0.69	2.32	0.41

Table 6: Summary of the results

Preparation	Application time	Number of finger-pads examined	log RF
MANORAPID SYNERGY	30 sec.	20	3.04 ± 1.05
MANORAPID SYNERGY	60 sec.	20	3.13 ± 0.96
2-propanol	30 sec.	10	1.32 ± 0.76

## Literature

- Ansari, S. A., S. A. Sattar, V. S. Springthorpe, G. A. Wells and W. Tostowaryk: Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surface.  
*J. Clin. Microbiol.* 26, 1513-1518, 1988
- Ansari, S. A., S. A. Sattar, V. S. Springthorpe, O. A. Wells and W. Tostowaryk: 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, 1989
- ASTM International (1996): Standard test method for determining the virus-eliminating effectiveness of liquid hygienic handwash agents using the fingerpads of adult volunteers.
- Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche.  
*Arch. exp. Path. Pharmacol.* 162, 480-487, 1931
- Mbithi, J. N.; V. S. Springthorpe and S. A. Sattar: Comparative in vivo efficiency of hand-washing agents against hepatitis A virus and poliovirus type 1 (Sabin).  
*Appl. Environ. Microbiol.* 59, 3463-3469, 1993
- Sachs, L.: *Angewandte Statistik*. 7. Auflage, Springer-Verlag, Berlin, Heidelberg, New York, 1992
- Sattar, S. A., H. Jacobsen, V. S. Springthorpe, T. M. Cusack and J. R. Rubino: Chemical disinfection to interrupt transfer of rhinovirus type 14 from environmental surface to hands.  
*Appl. Environ. Microbiol.* 59, 1579-1585, 1993
- Spearmen, C.: The method of 'right and wrong cases' ('constant stimuli') without Gauss's formulae.  
*Br. J. Psychol.* 2, 227-242, 1908
- Steinmann, J., R. Nehr Korn, A. Meyer and K. Becker: Two in-vivo protocols for testing virucidal efficacy of handwashing and hand disinfection.  
*Zbl. Hyg.* 196, 425-436, 1995
- Steinmann, J., R. Nehr Korn, E. Lösche, E. Sasse und B. Bogumil-Puchert: Viruswirksamkeit der hygienischen Händedesinfektion.  
*Hyg + Med.* 15, 7-14, 1990