Final report submitted to

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Evaluation of the effectiveness of

CHEMISEPT G

against

human rotavirus strain Wa

Test method according to guideline of BGA and DVV

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1. Introduction

As requested, the hand disinfectant CHEMISEPT G of CHEMI – PHARM AS was studied for its virus-inactivating properties against human rotavirus strain Wa. This study was carried out in accordance with the guideline for testing chemical disinfectants for effectiveness against viruses published by the Federal Office of Health (Bundesgesundheitsamt, BGA, now Robert Koch-Institute, D-13353 Berlin, Germany) and the German Association for the Control of Viruses Diseases (Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e. V., DVV) (1,2).

2. Identification of test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

3. Identification of sample

Name of product	CHEMISEPT G
Manufacturer	CHEMI – PHARM AS
Lot no.	-
Project no.	C05ML260
Application	hand disinfection
Appearance and smell of product	clear, colourless solution, product specific
pH-value	undiluted: 6.68 (20°C)
Expiry date	-
Date of receipt at laboratory	2005-12-05
Conditions of storage	room temperature in the dark (area with limited access)
Active substance(s) and concentration(s)	ethanol 75 g; blend of N-alkylbenzyldimethyl-ammonium chloride and N-alkyldimetyl-ammonium chloride 0.1 g

4. Experimental conditions

Date of examinations	2005-12-05 – 2006-01-15
Test temperature	20°C ± 1°C
Dilution of product	undiluted (80.0%)
Contact times	30, 60 and 120 s
Interfering substances	not possible
Diluent	-
Procedure to stop action of disinfectant	Immediate dilution
Test virus	rotavirus strain Wa

5. Material and methods

5.1. Preparation of test virus suspension

The human rotavirus strain Wa (serotype 1, subgroup II) was obtained from Prof. Dr. Holger Rabenau, Institute of Medical Virology of the Johann Wolfgang Goethe University of Frankfurt, D-60596 Frankfurt. Before the described tests, the virus had been passaged ten times in MA-104 cells (embryonic rhesus monkey kidney cell line).

After three washings with serum-free Eagle`s Minimum Essential Medium (EMEM; Cambrex Bio Science Verviers s.p.r.l., B-4800 Verviers, Belgium) cells were incubated with EMEM without fetal calf serum (FCS , Biochrom AG, D-12247 Berlin, Germany) for three hours to eliminate all FCS. This was followed by the addition of virus to MA-104 cells with a multiplicity of infection of 0.01-0.1 $TCID_{50}$/cell in the presence of 5.0 µg/mL 1:250 trypsin (SERVA Electrophoresis GmbH, D-69115 Heidelberg, Germany) at 37°C for 60 minutes. After this time, EMEM with 5.0 µg/mL 1:250 trypsin was added. After appearance of the cytopathic effect, cells were subjected to a rapid three-fold freeze-thawing procedure (-80°C for 20 min; 37°C for 10 min). The resulting fluid was centrifuged at 800 x g for 10 min at 4°C to eliminate cell debris. After aliquotation the supernatant was stored at –80°C.

5.2. Inactivation tests

Tests were carried out in accordance to the BGA and DVV guideline (1,2). Eight parts by volume of the disinfectant were mixed with one part by volume of virus suspension and one part by volume of double distilled water. Tests with interfering substances are not possible since proteins like FCS have rotavirus inhibitory and trypsin-neutralizing activity.

Inactivation tests were carried out in sealed test tubes (Sarstedt AG & Co., D-51588 Nümbrecht, Germany) in a water bath at 20°C ± 1°C. Aliquots were removed after appropriate times, and residual infectivity was determined. In addition, in accordance with the guideline, virus controls were carried out. Activity of disinfectant was stopped by immediate dilution.

5.3. Determination of infectivity

Infectivity was determined by means of end point dilution titration in a micro-procedure. For this, samples were diluted with ice-cold EMEM with 5 µg/mL trypsin and 100 µL of each dilution were placed after aspiration of the medium in eight wells of a sterile polystyrene flat bottom 96-well microtitre plate (Nunc A/S, DK-4000 Roskilde, Denmark) with a preformed MA-104 monolayer (28^{th}-35^{th} passage) . After one hour at 37°C, 100 µL EMEM with 5 µg/mL trypsin were added. Incubation was at 37°C in a CO_2-atmosphere (5.0% CO_2 - content). Finally, cultures were observed for cytopathic effects for ten days of inoculation. The infective dose ($TCID_{50}$) was calculated according to the method of Spearman (3) and Kärber (4) with the following formula:.

$$\log_{10}TCID_{50} = - (X_0 - 0.5 + \sum r/n)$$

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100% positive reaction

r = number of pos. determinations of lowest dilution step with 100% positive and all higher positive dilution steps

n = number of determinations for each dilution step.

5.4. Determination of cytotoxicity

For determination of cytotoxicity of the disinfectant, two parts by volume of phosphate-buffered saline (PBS) were mixed with eight parts by volume of the hand disinfectant, diluted with ice-cold EMEM with trypsin and inoculated into cell culture.

5.5. Calculation of virucidal efficacy

The virus-inactivating efficacy of the disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. Difference is given as reduction factor (RF).

6. Results

In parallel with inactivation tests, cytotoxicity of CHEMISEPT G (80.0%) was measured. Examinations showed that the hand disinfectant tested undiluted exhibited a cytotoxic effect at the dilution of 1:10. This means a $\log_{10}CD_{50}$/mL value (analogous to the $\log_{10}TCID_{50}$ value) of 2.50 (Table 1).

These tests to measure the cytotoxicity are imperative, because in this way the lower detection threshold for non-inactivated rotavirus is determined.

Results of inactivation tests are found in table 2. CHEMISEPT G was tested undiluted. Due to the addition of virus suspension and interfering substances a test concentration of 80.0% resulted. Exposure times were 30, 60 and 120 seconds.

The hand disinfectant CHEMISEPT G tested undiluted exhibited strong virus-inactivating properties against the test virus. After an exposure time of 30 s no rotavirus virus could be detected any longer. The virus titre reduction was ≥ 4.38 $\log_{10}$-steps. This reduction corresponds to an inactivation of ≥ 99.99% and demonstrates a rotavirus efficacy. According to the guideline of BGA and DVV and also to EN 14476:2005 (5), a disinfectant is considered as having virucidal efficacy if within the recommended exposure time the titre is reduced by four $\log_{10}$-steps.

Due to the lack of guidelines simulating practical conditions, results of the quantitative suspension test lead to the recommendation to use the hand disinfectant CHEMISEPT G for inactivation of rotavirus as follows:

undiluted 30 s

Bremen, 2006-01-15

- Dr. J. Steinmann -

Literature

1. Richtlinie des Bundesgesundheitsamtes und der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten e.V. zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren. Bundesgesundheitsblatt 1982; 25: 397-398

2. Kommentar zur Richtlinie des Bundesgesundheitsamtes und der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten e.V. zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren. Bundesgesundheitsblatt 1982; 25: 397-398

3. Spearman, C.: The method of ´right or wrong cases` (constant stimuli) without Gauss's formulae. Brit J Psychol 1908; 2: 227-242

4. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Path Pharmak 1931; 162: 480-487

5. EN 14476:2005: Chemical disinfectant and antiseptics – virucidal quantitative suspension test – Test method and requirements (phase 2, step 1).

Appendix table 1: raw data (Rotavirus) of CHEMISEPT G (BGA/DVV)

product	concentration	interfering substances	exposure time (sec)	dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Chemisept G	80.0%	Aqua bidest.	30	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			60	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			120	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			240	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		0.2% BSA	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			120	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			240	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			120	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			240	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Chemisept G cytotoxicity	80.0%	PBS	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
		0.2% BSA	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0030 0404	0000 0000	0000 0000	0000 0000
		0.2% BSA	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.a. = not applicable
n.d. = not done

t = cytotoxic

0 = no virus detectable
1 to 4 = detection of virus (degree of CPE in 8 wells of a microtitre plate)

Table 1: Cytotoxicity of CHEMISEPT G (80.0%) and 0.7% formaldehyde before and after treatment with MicroSpin™ S-400 HR columns

before treatment	conc.	soil load	dilutions				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
product	80.0%	without	+	-	-	-	-
product	80.0%	0.2% BSA	n.d.	n.d.	n.d.	n.d.	n.d.
product	80.0%	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7%	without	n.d.	n.d.	n.d.	n.d.	n.d.
after treatment	**conc.**	**soil load**	**dilutions**				
			10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
product	80.0%	without	n.d.	n.d.	n.d.	n.d.	n.d.
product	80.0%	0.2% BSA	n.d.	n.d.	n.d.	n.d.	n.d.
product	80.0%	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7%	without	n.d.	n.d.	n.d.	n.d.	n.d.

n.d = not done

Table 2: inactivation of rotavirus by CHEMISEPT G (80.0%) und formaldehyde (0.7%) in quantitative suspension test at 20°C after treatment with MicroSpin™ S-400 HR columns.

product	conc.	soil load	$log_{10}TCID_{50}$/mL after 30 s	60 s	120 s	240 s	≥ 4 log_{10} reduction after
test product	80.0%	without	≤ 2.50	≤ 2.50	≤ 2.50	n.d.	30 s
test product	80.0%	0.2% BSA	n.d.	n.d.	n.d.	n.d.	n.d.
test product	80.0%	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.d.
controls	**conc.**	**soil load**	**$log_{10}TCID_{50}$/mL after 5 min.**	**15 min.**	**30 min.**	**60 min.**	**≥ 4 log_{10} reduction after**
formaldehyde	0.7%	without	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	without	n.d.	n.d.	n.d.	6.88	n.a.
virus control	n.a.	0.2% BSA	n.d.	n.d.	n.d.	n.d.	n.a.
virus control	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.

n.d. = not done

n.a. = not applicable