Project number: C05ML260

Final report submitted to

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Evaluation of the effectiveness of

CHEMISEPT G

against Herpes Simplex Virus type 1

Test method according to guideline of BGA and DVV

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2006-02-23

Introduction

As requested, the hand disinfectant CHEMISEPT G was evaluated for its virus-inactivating properties against Herpes Simplex Virus (HSV) type 1. The testing was carried out in accordance with the guideline on testing chemical disinfectants for effectiveness against viruses published by the Federal Health Office (Bundesgesundheitsamt, BGA, now Robert Koch-Institute, Berlin) and the German Association for the Control of Virus Diseases (Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e. V., DVV) (1,2).

1. Identification of test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

2. Identification of sample

Name of product	CHEMISEPT G
Manufacturer	CHEMI – PHARM AS
Lot no.	-
Application	hand disinfection
Appearance and smell of product	clear, colourless solution, product specific
pH-value(s)	undiluted: 6.68 (20°C)
Expiry date	-
Date of receipt at laboratory	2005-12-05
Storage conditions	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

3. Experimental conditions

Period of analysis	2006-01-18 – 2006-02-23
Test temperature	20°C ± 1°C
Concentration of test product	undiluted (80.0%)
Contact times	0.5, 1.0, 2.0 and 5.0 minutes
Interfering substances	2.0% solution of bovine serum albumin (BSA) fetal calf serum (FCS)
Diluent of product	-
Procedure to stop action of disinfectant	immediate dilution
Test virus	Herpes Simplex Virus type 1 strain MacIntyre (ATCC No. VR-539)

4. Material and methods

4.1 Preparation of virus suspension

HSV type 1 (MacIntyre; ATCC No. VR-539) was obtained from Prof. Dr. K. Schneeweis, Institute of Medical Microbiology and Immunology at the University of Bonn, D-53127 Bonn, Germany. Before the described tests, virus had been passaged ten times in Vero cells (cells from Cercopithecus aethiops = African green monkey, ATCC CCL81). For preparation of stock solution, virus was grown in BGM cells (buffalo green monkey, permanent monkey kidney cells) in Eagle`s Minimum Essential Medium (EMEM) supplemented with L-glutamine according to the method described by Roizman and Spear (3). After appearance of the cytopathic effect, cells were subjected to a rapid freeze-thawing procedure. This was followed by low-speed centrifugation (10 min and 1000 x g) in order to sediment cell debris. After aliquotation, virus suspension was stored at –80°C.

4.2 Inactivation tests

Tests were carried out in accordance to BGA and DVV guideline. 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. In tests with interfering substances, instead of double distilled water, one part by volume of fetal calf serum or of a 2% serum albumin solution

(bovine serum albumin, BSA, Cohn fraction V, Sigma-Aldrich Chemie GmbH, D-82018 Taufkirchen, Germany) was added.
Inactivation tests were carried out in sealed glass test-tubes in a water bath at 20°C ± 1°C. Aliquots were removed after appropriate times, and residual infectivity was determined.
A control was one part by volume of virus suspension, four parts by volume of PBS and five parts by volume of 1.4% formaldehyde. The concentration of formaldehyde was determined by the hydroxylammonium chloride method.
In addition, in accordance with the guideline, virus controls were carried out after the longest exposure time.

4.3 Determination of infectivity

Infectivity was determined by means of end point dilution titration in 96-well microtitre plates. For this, samples were diluted at the end of the exposure time with ice-cold EMEM with 10% FCS and 100 µL of each dilution were placed in 8 wells of a sterile polystyrene flat bottomed 96-well microtitre plate (Nunc A/S, DK-4000 Roskilde, Denmark). 100 µL of a fresh trypsinized BGM cell culture were added. The suspension was adjusted to reach approximately 10-15 x 10^3 cells per well. Incubation was at 37°C in a CO_2-atmosphere (5.0% CO_2 - content). Finally, cultures were observed for cytopathic effects for five days of inoculation. The infective dose ($TCID_{50}$/mL) was calculated according to the method of Spearman (4) and Kärber (5) 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.

4.4 Determination of cytotoxicity

For determination of cytotoxicity of the disinfectant, two parts by volume of PBS were mixed with eight parts by volume of the disinfectant, diluted with ice-cold DMEM and inoculated into cell culture. These tests were also performed with interfering substances.

4.5 Calculation of the virucidal activity

The virucidal effect of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

5. Results

In parallel with the inactivation tests, cytotoxicity of the 0.7% formaldehyde solution and of the hand disinfectant CHEMISEPT G was measured. The formaldehyde solution was toxic for the BGM cells in the 1:1000 dilutions. This corresponds to a $\log_{10}CD_{50}$/mL of 4.50. Examinations showed that the hand disinfectant (80.0%) had a $\log_{10}CD_{50}$/mL of 2.50 (cytotoxicity in the 1:10 dilutions).

These tests to measure cytotoxicity are imperative, because in this way the lower detection threshold for non-inactivated HSV type 1 is determined.

Results of inactivation tests are found in table 2 (raw data see appendix). There is no graphic presentation of the results since no kinetics of inactivation is visible.

Formaldehyde (0.7%) reduced the HSV titre after five and 15 minutes by ≥ 2.38 and ≥ 2.63 $\log_{10}$ steps. A reduction factor of ≥ 2.63 was measured after 30 and 60 minutes contact time (table 2).

The hand disinfectant CHEMISEPT G was examined undiluted. Due to the addition of virus suspension and interfering substances a test concentration of 80.0% resulted. Exposure times were 0.5, 1.0, 2.0 and 5.0 minutes.
Testing CHEMISEPT G undiluted (80.0%), after an exposure time of 30 seconds a reduction of the virus titre was measured (table 2). The reduction factors were ≥ 4.63 (assay without soil load), ≥ 4.25 (assay with BSA) and ≥ 4.38 (assay with FCS). At this exposure time, no HSV could be detected in all assays. These values correspond to an inactivation of ≥ 99.99% meaning virus-inactivating properties. According to the guideline of BGA/DVV (1,2) and EN 14476:2005 (6), a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating properties if within the recommended exposure period the titre is reduced at least by four $\log_{10}$.

Due to the lack of guidelines simulating practical conditions, the results of the quantitative suspension test lead to the recommendation to use the hand disinfectant CHEMISEPT G for the inactivation of Herpes Simplex Virus type 1 as follows:

undiluted	**30 s**

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.
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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 1983; 26: 413-414

3. Roizman, B., and P.G. Spear: Preparation of Herpes simplex Virus of high titer.
J Virol 2, 1968, 83-84

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

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

6. EN 14476:2005: Chemical disinfectants and antiseptics- virucidal quantitative suspension test – Test method and requirements (phase 2, step1).

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	+	-	-	-	-
product	80.0%	10.0% FCS	+	-	-	-	-
formaldehyde	0.7%	without	+	+	+	-	-
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 HSV type 1 by CHEMISEPT G (80.0%) and formaldehyde (0.7%) in quantitative suspension test at 20°C.

product	conc.	soil load	$log_{10}TCID_{50}$/mL after 0.5 min.	1.0 min.	2.0 min.	5.0 min.	≥ 4 log_{10} reduction after
test product	80.0%	without	2.50	2.50	2.50	2.50	0.5 min.
test product	80.0%	0.2% BSA	2.50	2.50	2.50	2.50	0.5 min.
test product	80.0%	10.0% FCS	2.50	2.50	2.50	2.50	0.5 min.
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	≤ 4.75	≤ 4.50	≤ 4.50	≤ 4.50	≥ 15 min.
virus control	n.a.	without	n.d.	n.d.	n.d.	7.13	n.a.
virus control	n.a.	0.2% BSA	n.d.	n.d.	n.d.	6.75	n.a.
virus control	n.a.	10.0% FCS	n.d.	n.d.	n.d.	6.88	n.a.

n.d. = not done

n.a. = not applicable

Appendix table 1: raw data (HSV type 1) of Chemisept G (BGA/DVV)

product	concentration	interfering substances	exposure time (min)	dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
Chemisept G	80.0%	Aqua bidest.	0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			2.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			5.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
		0.2% BSA	0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			2.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			5.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
		10.0% FCS	0.5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			2.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			5.0	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	Aqua bidest.		4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3340 4400	0000 0000	0000 0000	0000 0000
		0.2% BSA		4444 4444	4444 4444	4444 4444	4444 4444	4444 3443	0044 0000	0000 0000	0000 0000	0000 0000
		10.0% FCS		4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3400 3000	0000 0000	0000 0000	0000 0000

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)

Appendix Table 2: raw data (HSV type 1) of formaldehyde control (20°C)

product	concentration	interfering substance	exposure time (min)	dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
formaldehyde	0.7% (m/V)	PBS	5	tttt tttt	tttt tttt	tttt tttt	3003 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	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)