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Evaluation of the effectiveness of

CHEMISEPT G

against Polyomavirus SV 40

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 tested for its virucidal properties against polyomavirus (formerly papovavirus) SV 40 strain 777. Tests were 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 e. V. (Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e. V., DVV) (1,2).

2. Identification of test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen, Germany

3. Identification of sample

Name of the product	CHEMISEPT G
Manufacturer	CHEMI – PHARM AS
Lot no.	
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

Period of analysis	2006-01-14 – 2005-03-09
Test temperature	20°C ± 1.0°C
Concentration of test product	undiluted (80.0%)
Contact times	3, 5, 15 and 20 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	polyomavirus SV 40 strain 777

5. Material and methods

5.1 Preparation of virus suspension

Polyomavirus (formerly papovavirus) SV 40 strain 777 was obtained from PD Dr. A. Sauerbrei, Institute of Virology and Antiviral Chemotherapy at the Friedrich Schiller University of Jena. Before inactivation assays, the virus had been passaged two times in CV-1 cells (kidney cells of African green monkey).

For preparation of virus suspension, CV-1 cells, which were cultivated with Eagle's Minimum Essential Medium with Earle` salts (EMEM; Cambrex Bio Science Verviers s.p.r.l., B-4800 Verviers, Belgium) supplemented with L-glutamine, sodium pyruvate and 10% or 2% fetal calf serum (FCS, Biochrom AG, D-12247 Berlin, Germany), were infected with SV 40 with a MOI (multiplicity of infection) of 0.1. After cells showed a constant cytopathic effect, they were subjected to a rapid three-fold freeze/thawing procedure. This was followed by low-speed centrifugation (10 min and 1000 x g) in order to sediment cell debris. After aliquotation, test virus suspension was stored at –80°C.

5.2 Inactivation tests

Tests were carried out in accordance to the 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 a 2.0% solution of bovine serum albumin (BSA, Sigma-Aldrich Chemie GmbH, D-82018 Taufkirchen) or one part by volume of fetal calf serum was added.

Inactivation tests were carried out in sealed test tubes (Sarstedt AG & Co., D-51588 Nümbrecht, Germany) in a water bath at 20°C ± 1.0°C. Aliquots were removed after the appropriate exposure 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 hydroxyl-ammonium chloride method.

In addition, in accordance with the guideline, virus controls were carried out.

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 and 100 µL of each dilution were placed in 8 wells of a sterile polystyrene flat bottomed plate (Nunc A/S, DK-4000 Roskilde, Denmark). 100 µL of a fresh trypsinized CV-1 cell culture (passage 81^{st}-85^{th}) were added. This 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). Finally, cultures were observed for cytopathic effects for three weeks of inoculation. The infective dose ($TCID_{50}$) was calculated according to the method of Spearman (3) and Kärber (4).

5.4 Determination of cytotoxicity

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

5.5 Calculation of the virucidal effectiveness

The virucidal effectiveness 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).

6. Results

In parallel with the inactivation tests, cytotoxicity of CHEMISEPT G (80.0%) and formaldehyde (0.7%) was measured. The formaldehyde solution was toxic for the CV-1 cells in the 1:1000 dilutions. This corresponds to a $\log_{10}CD_{50}$/mL of 4.50 (Table 1).
Examinations showed that the hand disinfectant CHEMISEPT G had a $\log_{10}CD_{50}$/mL of 2.50 (cytotoxicity in the 1:10 dilution; Table 1).
These tests to measure cytotoxicity are imperative, because in this way the lower detection threshold for non-inactivated SV 40 is determined.

Results of inactivation tests are found in table 2. Formaldehyde (0.7%) reduced the SV 40 titre after 5 and 15 minutes by 0.13 $\log_{10}$ steps. After 30 and 60 minutes RF were 0.63 and 1.25 (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 of the inactivation experiments were 3, 5, 15 and 20 minutes.

Testing CHEMISEPT G undiluted against SV 40, an efficacy was measured after an exposure time of five minutes in all assays without and with interfering substances (Table 2). The RF were ≥ 5.13 (assay without protein load), ≥ 4.88 (assay with BSA) and ≥ 5.00 (assay with FCS), respectively. These RF correspond to an inactivation of ≥ 99.999% (assay with BSA ≥ 99.99%) indicating virus-inactivating properties of the test product.
Due to the lack of guidelines simulating practical conditions, results of this quantitative suspension test lead to the recommendation to use the hand disinfectant CHEMISEPT G for inactivation of SV 40 as follows:

undiluted **5 min**

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

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 SV 40 by CHEMISEPT G (80.0%) und formaldehyde (0.7%) in a quantitative suspension test at 20°C ± 1°C

product	conc.	soil load	$log_{10}TCID_{50}$/mL after				≥ 4 log_{10} reduction after
			3 min	5 min	15 min	20 min	
test product	80.0%	without	3.50	≤ 2.75	≤ 2.50	≤ 2.50	3 min
test product	80.0%	0.2% BSA	3.50	≤ 3.25	≤ 2.50	≤ 2.50	3 min
test product	80.0%	10.0% FCS	4.25	≤ 3.00	≤ 2.50	≤ 2.50	5 min
			5 min	**15 min**	**30 min**	**60 min**	
formaldehyde	0.7%	without	7.75	7.75	7.25	6.63	> 60 min
virus control	n.a.	without	n.d.	n.d.	n.d.	7.88	n.a.
virus control	n.a.	0.2% BSA	n.d.	n.d.	n.d.	8.13	n.a.
virus control	n.a.	10.0% FCS	n.d.	n.d.	n.d.	8.00	n.a.

n.d. = not done

n.a. = not applicable

Appendix table 1: raw data (SV 40) 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.	3	tttt tttt	4444 3444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			5	tttt tttt	0000 3004	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			20	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
		0.2% BSA	3	tttt tttt	4444 4444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			5	tttt tttt	3220 3020	0012 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			20	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
		10.0% FCS	3	tttt tttt	4444 4444	2344 0033	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			5	tttt tttt	3220 0003	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			20	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.	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4443	0000 0234	0000 0000	0000 0000
		0.2% BSA	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4402 2120	0000 0000	0000 0000
		10.0% FCS	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0004 4030	0003 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 (SV 40) 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	4444 4444	4444 4444	4344 4444	0003 0040	0000 0000	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	4344 3444	0220 0040	0000 0000	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	4230 3340	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	4444 4444	3033 3333	2200 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)