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CHEMI – PHARM AS

Põllu 132

10917 Tallinn

(<http://www.chemi-pharm.com>)

Evaluation of the effectiveness of

CHEMISEPT G

against Adenovirus type 5

Test method according to guideline of BGA and DVV

Dr. Jochen Steinmann

MikroLab GmbH

Norderoog 2

D-28259 Bremen

phone: +49 (0) 421-27819102

fax: +49 (0) 421-2760283

E-mail: Mikrolab.GmbH@t-online.de

<http://www.mikrolab-gmbh.de>

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Introduction

As requested, the hand disinfectant CHEMISEPT G was evaluated for its virus-inactivating properties against adenovirus type 5. 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 Virus-krankheiten e. V., DVV) (1,2).

1. Identification of test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

2. 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-alkylbenzyltrimethylammonium chloride and N-alkyldimethylammonium chloride 0.1 g



3. Experimental conditions

Period of analysis	2006-01-14 – 2006-03-12
Test temperature	20°C \pm 1°C
Concentration of test product	undiluted (80.0%)
Contact times	0.5, 1.0, 2.0 and 3.0 minutes
Interfering substances (soil load)	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	Adenovirus type 5, strain Adenoid 75 (ATCC VR-5)

4. Material and methods

4.1 Preparation of virus suspension

The adenovirus type 5 strain adenoid 75 was obtained from PD Dr. A. Heim, Institute of Medical Virology, Hannover Medical School, Hannover, Germany. Before the inactivation assays, the virus had been passaged 3 times in A549 cells (human lung epithelial carcinoma cells).

The A549 cells also originated from the Institute of Medical Virology, Hannover Medical School.

For preparation of virus suspension, A549, which were cultivated with Dulbecco's modified Eagle's medium (DMEM, Cambrex Bio Science Verviers s.p.r.l., B-4800 Verviers, Belgium) in the presence of L-glutamine, HEPES buffer and sodium pyruvate and 10% or 2% fetal calf serum (FCS, Biochrom AG, D-12247 Berlin, Germany) in 175 cm² flasks (Nunc GmbH & Co. KG, D-65203 Wiesbaden, Germany), were infected with 5 mL of an adenovirus type 5 suspension. The flask was kept at 37°C for 60 minutes to allow for virus adsorption. Supplemented DMEM with 2% FCS was added to the inoculated monolayer. After the cells showed a constant cytopathic effect, they were mechanically shaken off the surface of the plastic flask following by a low-speed centrifugation at 800 x g for 10 minutes. The supernatant was discharged, and the sediment was taken up in 2 mL phosphate buffered saline (PBS) (cell pack procedure). After three rapid freeze/thaw cycles, low-speed



centrifugation was again performed in order to sediment cell debris. The supernatant was dispensed in aliquots of 1 mL and 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 $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$. 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 a micro-procedure. For this, samples were diluted with ice-cold DMEM with 1% FCS and 100 μL of each dilution were placed in eight wells of a sterile polystyrene flat bottomed microtitre plate (Nunc GmbH & Co. KG). 100 μL of fresh trypsinized A549 cells were added. The suspension was adjusted so that there were approx. $10\text{--}15 \times 10^3$ cells per well. Incubation was at 37°C in a CO_2 -atmosphere (5.0% CO_2). After four days, 100 μL DMEM with 5% FCS were added. 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:

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

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 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).

5. Results

In parallel with the inactivation tests, cytotoxicity of the 0.7% formaldehyde solution and of the hand disinfectant CHEMISEPT G (80.0%) was measured (Table 1). The formaldehyde solution was toxic for the A549 cells in the 1:1000 dilutions. This corresponded 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 3.50 (cytotoxicity in the 1:100 dilutions; table1).

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

Results of inactivation tests are found in table 2 (raw data see appendix).

Formaldehyde (0.7%) reduced the adenovirus titre after 5 and 15 minutes by 1.00 and 2.12 \log_{10} steps. After 30 and 60 minutes, reduction factors were ≥ 3.62 and ≥ 4.00 (Table 2).

The hand disinfectant CHEMISEPT G was 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 3.0 minutes.

Testing CHEMISEPT G undiluted, after an exposure time of 30 s a reduction of the virus titre was measured (table 2). The reduction factors were ≥ 5.00 (assay without soil load), ≥ 5.00 (assay with BSA) and ≥ 5.13 (assay with FCS). These values correspond to an inactivation of $\geq 99.999\%$ meaning virucidal efficacy. According to the guideline of BGA/DVV, a disinfectant or a disinfectant solution at a particular concentration is having virucidal efficacy 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, results of this quantitative suspension test lead to the recommendation to use the hand disinfectant CHEMISEPT G for inactivation of adenovirus as follows:

undiluted

30 s

Dr. J. Steinmann



Literature

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Bundesgesundheitsblatt 1983; 26: 413-414
3. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche.
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4. Spearman, C.: The method of 'right or wrong cases' (constant stimuli) without Gauss's formulae.
Brit J Psychol 1908; 2: 227-242

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
test product	80.0%	without	+	+	-	-	-
test product	80.0%	0.2% BSA	+	+	-	-	-
test 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
test product	80.0%	without	n.d.	n.d.	n.d.	n.d.	n.d.
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.
Formaldehyd	80.0%	without	n.d.	n.d.	n.d.	n.d.	n.d.

n.d. = not done

Table 2: Inactivation of adenovirus type 5 by Chemisept G (80.0%) und formaldehyde (0.7%) in a quantitative suspension assay at 20°C ± 1.0°C

product	conc.	soil load	log ₁₀ TCID ₅₀ /mL after				≥ 4 log ₁₀ reduction after
			0.5 min	1.0 min	2.0 min	3.0 min	
test product	80.0%	without	≤ 3.50	≤ 3.50	≤ 3.50	≤ 3.50	0.5 min
test product	80.0%	0.2% BSA	≤ 3.50	≤ 3.50	≤ 3.50	≤ 3.50	0.5 min
test product	80.0%	10.0% FCS	≤ 3.50	≤ 3.50	≤ 3.50	≤ 3.50	0.5 min
			5 min	15 min	30 min	60 min	
formaldehyde	0.7%	without	7.50	6.38	≤ 4.88	≤ 4.50	60 min
virus control	n.a.	without	n.d.	n.d.	n.d.	8.50	n.a.
virus control	n.a.	0.2% BSA	n.d.	n.d.	n.d.	8.50	n.a.
virus control	n.a.	10.0% FCS	n.d.	n.d.	n.d.	8.63	n.a.

n.d. = not done

n.a. = not applicable

Appendix table 1: raw data (adenovirus) 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	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.	
			1.0	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
			2.0	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
			3.0	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
		0.2% BSA	0.5	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
			1.0	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
			2.0	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
			3.0	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
		10.0% FCS	0.5	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
			1.0	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
			2.0	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
			3.0	ttt	ttt	0000	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
Chemisept G cytotoxicity	80.0%	PBS	n.a.	ttt	ttt	0000	0000	0000	0000	n.d.	n.d.	n.d.	n.d.		
		0.2% BSA	n.a.	ttt	ttt	0000	0000	0000	0000	n.d.	n.d.	n.d.	n.d.	n.d.	
		10.0% FCS	n.a.	ttt	ttt	0000	0000	0000	0000	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	3104	0430	0000	0000	
		0.2% BSA	n.a.	4444	4444	4444	4444	4444	4444	4444	4444	3033	0000	0000	0000
		10.0% FCS	n.a.	4444	4444	4444	4444	4444	4444	4444	4444	4333	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 (adenovirus) 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	4444	4444	4404	0200	0000	n.d.
			15	tttt	tttt	tttt	4444	4444	3434	0000	0000	n.d.
			30	tttt	tttt	tttt	4444	4203	0000	0000	0000	n.d.
			60	tttt	tttt	tttt	0100	0000	0000	0000	0000	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	tttt	tttt	tttt	0000	0000	0000	0000	0000	n.d.
				tttt	tttt	tttt	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)