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Clinic for Birds, Reptiles, Amphibia and Fish
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TEST REPORT

The virucidal activity of hand antiseptic

MANORAPID SYNERGY

product of the company Antiseptica Chemisch-Pharmazeutische Produkte GmbH, Carl-Friedrich-Gauss Straße 7, D-50259, Pulheim/Brauweiler. The above mentioned preparation was obtained from Mr Molitor at 27 January 2004 for the examination of virucidal activity on avian influenza A viruses.

Avian influenza also known as "bird flu" is a serious threat to commercial birds and causes dramatically economic losses. After nearly 20 years without serious influenza A epidemics, two outbreaks, one in Italy and one in Netherlands, resulted in death or culling of more than 40 million domestic birds. The recent outbreak in Far East caused by subtype H5N1 of influenza virus A (IVA) takes a similar course. Normally avian IVA subtype H5N1 circulate among wild birds.

However, they can also infect domestic birds and men, and lead to heavy, even lethal infections.

Efficient disinfection has a major importance for prevention of virus spread via indirect transmission. To test disinfectant activity of MANORAPID SYNERGY against avian IVA was used a highly pathogenic influenza A virus (HPAI) (A/Carduelis/Germany/72, H7N1) as surrogate virus, which yields a relative high titre in embryonated SPF (specific pathogen free) chicken eggs and induces light-microscopic easily detectable virus specific cytopathic effects (CPE) in chicken embryo fibroblast (CEF) cultures after a relatively short replication time.

The tests were carried out according to the guideline of the Department of Federal Public Health (Bundes Gesundheitsamt, BGA, now Robert-Koch-Institute) and the German Union to the Fight of the Virus Diseases e.V., (Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten, DVV), to evaluate virucidal activity of chemical disinfectants.

The virus was propagated in the allantoic cavity of 10-day-old embryonated specific pathogen free chicken eggs and harvested after 48 h incubation at 37 °C. Although the first appearance of the virus replication was already visible on the second day after the inoculation using the light microscope, the plates were read at the 5 d.p.i.

Preparation for virucidal testing

To evaluate the virucidal activity, the experiments were carried out in accordance with the guidelines of the BGA and the DVV. Eight volume parts of the disinfectant were mixed with one volume part of virus suspension and one volume part of aqua bidest. In the tests with interfering substance, instead of aqua bidest. one volume part foetal calf serum (FCS, Seromed, Bio-Chrome, Berlin)

as well as 2% bovine serum albumin (BSA, Cohn fraction V, Serva, Heidelberg) were used.

The inactivation tests were carried out in sealed test tubes placed in a silicon oil bath at $20 \pm 1^\circ\text{C}$. After exposition times of 15 and 30 seconds samples were taken and examined for residual infectivity. To determine the initial titres the virus was mixed with double distilled water instead of the disinfectant. The results were confirmed by one repetition. Decisively for the definition "virucidal" against the test virus tested was the log-reduction, which determined by subtracting the logarithmic titre of the reaction mixture with disinfectant from the logarithmic titre of the virus control.

Determination of the infectivity

The infectivity of the test virus was determined by end point dilution method. The samples were diluted in ice-cold BME (Basal Medium Eagle with Earle's salts, Seromed, Biochrom, Berlin) in ten fold steps up to 10^{-4} . 0.1 ml of each virus dilution was transferred into 8 cell culture units (CEF) in 96-well flat bottom cell culture plates. The final reading and recording of results were performed at the fifth day after inoculation. The 50% tissue culture infecting dose per ml ($\text{TCID}_{50}/\text{ml}$) were calculated by the Spearman and Kaerber method.

Determination of the cytotoxicity

The cytotoxicity of the disinfectant was determined by the micro titration method using 96-well flat bottom cell culture plates. Two volume parts of PBS were mixed with eight volume parts of the disinfectant and diluted in tenfold steps until 10^{-5} . Four wells of the test system (CEF) were inoculated with 0.1 ml of each di-

lution. The cytotoxic dose was calculated in analogy to the $TCID_{50}/ml$ as $\log_{10} CD_{50}/ml$.

Results

The virus titres of the negative controls in the first set were 7.5 $TCID_{50}/ml$ (without protein load), 7.6 $TCID_{50}/ml$ (with FCS), and 7.5 $TCID_{50}/ml$ (with 0,2 % BSA). In the second set the titres of controls were 7.5 $TCID_{50}/ml$ (without protein load), 7.5 $TCID_{50}/ml$ (with FCS), and 7.6 $TCID_{50}/ml$ (with BSA). The results of the toxicity tests show that the cytotoxic dose of the MANORAPID SYNERGY was 1.5 CD_{50}/ml . Therefore, the cultures which were inoculated with the dilution step 10^{-1} were not assessed.

After a reaction time of 15 seconds, no replication of avian IVA (H7N1) in CEF cultures could be determined, which were inoculated with samples from reaction mixtures, which contain FCS, BSA, and deprived from protein. Identical results were observed after exposure time of 30 seconds.

According to the guidelines of the BGA and the DWV, a virucidal activity is always assumed when the disinfectant causes a titre reduction of four \log_{10} (equivalent to reduction 99.99%) or more.

The comparisons of the initial titres and titres of the remaining viruses (≤ 2.5 $TCID_{50}/ml$ after 15 seconds exposition in all sets) show that 15 second reaction of the product MANORAPID SYNERGY in 80 % concentration resulted in a titre reduction of $\geq 5.0 \log_{10}$ of the avian influenza A virus tested. This proves that the tested product is virucidal against avian influenza A virus.

According to the results shown by the above described experiment, the hand antiseptic MANORAPID SYNERGY can be used to inactivate members of the virus family Orthomyxoviridae, including the avian influenza A viruses, at 20 °C like follows:

concentrate 15 seconds



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Attachment: Tabular description of the tests

Manufacturer	Antiseptica Chemisch-pharmazeutische Produkte GmbH
Name of the product	Manorapid® Synergy
Charge No	03007
Date of delivery	2004-01-27
Durability	until March 2005
Properties of package	150 ml plastic bottle
Amount of sample	1X150 ml
Active substances	54.1% Ethanol, 10.0% n-Propanol
Appearance and smell	clear, colourless, liquid with lower viscosity, alcoholic smell
pH-Value	undiluted: 2.5
Storage conditions	in the original pack, at the room temperature
Identification of the testing laboratory	Virology laboratory of Clinic for Birds, Reptiles, Amphibia, and Fisch, Justus-Liebig-University Giessen
Period of analysis	2004-01-28 to 2004-02-07
Test temperature	20 +/-1 °C
Test concentration	undiluted
Contact times	15, 30 seconds
Number of test sets	2
Interfering substance	FCS (foetal calf serum), 2% BSA (bovine serum albumin)
Product diluent	none
Test virus	Avian Influenza A Virus (A/Carduelis/Germany/72, H7N1)
Replication system	Embryonated SPF chicken eggs
Detection system	Chicken embryo fibroblast cultures (CEF)

Table 1: Cytotoxicity of MANORAPID SYNERGY on CEF cultures

Concentration	Interfering substance	Dilution steps			Cytotoxic Dose ₅₀ /ml in log ₁₀ (CD ₅₀ /ml)
		10 ⁻¹	CD ₅₀ /ml	10 ⁻³	
80.0%	none	+	-	-	1.5
80.0%	0.2% BSA	+	-	-	1.5
80.0%	10.0% FCS	+	-	-	1.5

+ cytotoxic effect - no cytotoxic effect

Table 2: Inactivation of avian influenza A virus (A/Carduelis/Germany/72, H7N1) with MANORAPID SYNERGY in quantitative suspension test

Set no	Product	Disinfectant concentration (%)	Interfering substance	Residual infectivity in log ₁₀ TCID ₅₀ /ml after		≥ 4 log ₁₀ Reduction after
				15 Seconds	30 Seconds	
I	MANORAPID SYNERGY	80.0	none	≤2.50	≤2.50	15 seconds
	MANORAPID SYNERGY	80.0	0.2% BSA	≤2.50	≤2.50	15 seconds
	MANORAPID SYNERGY	80.0	10.0% FCS	≤2.50	≤2.50	15 seconds
II	Virus control	0.0	none	n. d.	7.5	-
	Virus control	0.0	0.2% BSA	n. d.	7.6	-
	Virus control	0.0	10.0% FCS	n. d.	7.5	-
	MANORAPID SYNERGY	80.0	none	≤2.50	≤2.50	15 seconds
	MANORAPID SYNERGY	80.0	0.2% BSA	≤2.50	≤2.50	15 seconds
	MANORAPID SYNERGY	80.0	10.0% FCS	≤2.50	≤2.50	15 seconds
	Virus control	0.0	none	n. d.	7.5	-
	Virus control	0.0	0.2% BSA	n. d.	7.5	-
	Virus control	0.0	10.0% FCS	n. d.	7.6	-

n.d. not determined