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Efficacy of MANORAPID SYNERGY against the feline calicivirus (FCV) in the quantitative suspension test

INVESTIGATION REPORT

It is not possible to conduct inactivation tests for noroviruses (synonyms: Norwalk-like Viren or small round structured viruses, SRSVs) by chemical disinfectants, because no suitable replication systems are available for these viruses. Therefore the feline calicivirus (FCV) is used as a surrogate virus for these organisms in inactivation tests (1). FCV (Genus Vesivirus) is the causative agent of feline cough and like the noroviruses (Genus Vesivirus) it belongs to the family of Caliciviridae (2,3). It is a non-enveloped, single-stranded RNA virus with biochemical properties and genomic organization similar to the noroviruses (4,5).

On using surrogate viruses in virucidal investigations it is possible to obtain data on the concentrations and exposure times for chemical disinfectants used against important viral pathogens of nosocomial infections for which there are no suitable replication systems.

For this reason FCV was used as test virus. The inactivation tests were performed in accordance with the guideline of the former German Federal Health Office (BGA, now known as the Robert Koch Institute) and of the German Society for Control of Viral Diseases (DVV) governing the testing of chemical disinfectants for efficacy against viruses (6,7).

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EXPERT INVESTIGATION REPORT

The virucidal efficacy of the hand disinfectant MANORAPID SYNERGY, manufactured by Antiseptica Chemisch-pharmazeutische Produkte GmbH, against the feline calicivirus (FCV) strain F9 was investigated in accordance with the guideline of the former German Federal Health Office (BGA, now known as the Robert Koch Institute) and of the German Society for Control of Viral Diseases (DVV) governing the testing of chemical disinfectants for efficacy against viruses. FCV was used as a surrogate virus for the noroviruses viruses because there is no suitable replication system for these viruses. By testing this surrogate virus it is possible to obtain data on the use recommendations for the test disinfectant against Norwalk-like viruses.

The BGA and DVV guidelines assume that the virucidal efficacy of a product is assured if after a certain exposure time the initial virus titer has been reduced by \geq four \log_{10} levels (inactivation 99.99 %).

MANORAPID SYNERGY was tested in an undiluted state. The exposure times were 0.5, 1.0, 2.0, 3.0 and 5.0 minutes

Summarizing the tests results, it is recommended that the hand disinfectant MANORAPID SYNERGY for inactivation of FVC be used as follows:

concentrated

30 seconds

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1. Laboratory

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2. Identification of samples

Product designation	MANORAPID SYNERGY
Batch no.	00102 of 07.06.2001
Appearance and smell	Clear, colorless liquid alcoholic
pH value (glass electrode)	Undiluted: 3.34
Manufacturer	ANTISEPTICA Chemisch- pharmazeutische Produkte GmbH
Delivery date	28.11.2002
Storage conditions	Room temperature, dark
Active substance(s) and their concentration(s) in 100 g	54.1 % ethanol 10.0 % n-propanol

3. Test Conditions

Test period	02.12.2002 – 14.01.2003
Test temperature	20°C ± 1°C
Product test concentration	80.0 %
Exposure times	0.5, 1.0, 2.0, 3.0 and 5.0 minutes
Protein challenge	Serum albumin and FCS
Diluent	Not applicable
Virus strain	FCV strain F9

4. Materials and Methods

4.1 Preparation of the Virus Suspension

The feline calicivirus strain F9 was obtained from Prof. H. Schirmeier, Institute for Viral Diagnostics of the German Federal Research Center (BFA) for Viral Diseases in Animals on Riems Island. Prior to inactivation, FCV was passaged thrice in KE-R cells (full embryo, feline, Catalog No. 138). The KE-R cells were obtained from Dr. R. Riebe, cell bank for cell lines in veterinary medicine at the Federal Research Center for Viral Diseases in Animals.

To prepare the viral suspension, KE-R cells that had been cultured with Minimum Essential Medium (Eagle) and 10 % or 2 % fetal calf serum (FCS) were inoculated with the FCV in 175 cm² cell culture flasks (Nunc GmbH & Co. KG Wiesbaden). Once a cytopathogenic effect had been induced (approx. 16-20 hours), freezing and thawing was carried out thrice. The cell debris was removed by centrifugation at 770 x g for ten minutes and the supernatant was recovered as viral suspension.

4.2 Inactivation Tests

The inactivation tests were performed in accordance with the BGA and DVV guidelines. Eight volume parts of the disinfectant were mixed with one volume part viral suspension and one volume part of double-distilled water. For the tests conducted using protein challenge, one volume part of fetal calf serum (FCS, Biochrom AG Berlin) or 2 % serum albumin solution (bovine serum albumin = BSA, Cohn Fraction V, Sigma, Taufkirchen) were used instead of double-distilled water.

The inactivation tests were conducted in sealed glass tubes in a water bath at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$. After expiry of the different exposure times, partial quantities were withdrawn and residual infectivity ascertained. In addition, control tests were performed to determine the virus titer (double-distilled water instead of the disinfectant).

4.3 Ascertainment of Infectivity

Infectivity was ascertained by means of final dilution titration using the microtiter process. To that effect, 100 μl aliquots of the samples, which had been diluted with ice-cold Minimum Essential Medium (Eagle) in integral orders of magnitude after withdrawal, were transferred to eight cups of a sterile polystyrol plate with a flat bottom (Nunc GmbH & Co. KG, Wiesbaden). Already on the previous day 100 μl aliquots of a freshly trypsinized KE-R cell culture with approx. 2.7×10^4 cells had been placed in each cup (preformed monolayer). Incubation took place at 37°C in a CO_2 incubator (5 % CO_2 content) for five to seven days. Results were read with a reversed microscope and the infectious dose LD_{50}/ml was calculated as per the Karber (7) and Spearman (8) method.

4.4 Ascertainment of Cytotoxicity

To ascertain the cytotoxicity, two volume parts PBS were mixed with eight volume parts of the disinfectant and, following serial dilution as outlined in 4.3, transferred to the microtiter plate with the preformed monolayer. The cytotoxic dose was calculated as $\log_{10} \text{CD}_{50} / \text{ml}$ (analogous to the LD_{50} value).

4.5 Determining Virucidal Efficacy

Virucidal efficacy of the test disinfectant was determined by calculating the titer reduction compared with the respective control titrations containing no disinfectant.

5. Results

The cytotoxicity of MANORAPID SYNERGY (80.0 %) was measured in parallel to the inactivation tests. The investigations have demonstrated that for the hand disinfectant tested a cytotoxicity of 1 : 10 dilution was evidenced (table 1). Mathematically, this yields a \log_{10} CD_{50} /ml of 2.5. Cytotoxicity testing is indispensable in order to determine the lower detection threshold for non-inactivated FCV.

The results of the inactivation tests are given in table 2. MANORAPID SYNERGY was used in a non-diluted state. The exposure times were 0.5, [SIC] 1.0, 2.0, 3.0 and 5.0 minutes.

Table 2 shows that the test hand disinfectant MANORAPID SYNERGY is endowed with excellent virucidal activity against the test virus. In no test, not even in that containing serum albumin and FCS, was it possible to detect FCV after a 30-minute exposure time. Accordingly, logarithmic reduction factors of $\geq 5.30 \log_{10}$ levels (tests with and without FCS challenge) and $\geq 5.13 \log_{10}$ levels (test with BSA) were obtained. This corresponds to an inactivation level of 99.999 % and attests to virucidal efficacy. As well known, the BGA and DVV guidelines assume that virucidal efficacy is assured if evidence can be produced for a titer reduction equal to or greater than four \log_{10} levels (inactivation ≥ 99.99 %).

(signature)
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Table 1: Cytotoxicity of MANORAPID SYNERGY

Concentration	Challenge	Dilution Stages				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
80.0%	none	+	-	-	-	-
80.0%	0.2%	+	-	-	-	-
80.0%	BSA	+	-	-	-	-
	10.0%	+	-	-	-	-
	FCS					

Table 2: FCV inactivation by MANORAPID SYNERGY (80.0%) in the quantitative suspension test at 20°C

Product	Conc.	Challenge	log ₁₀ LD ₅₀ /ml after					≥ 4 log ₁₀ reduction after
			0.5 min	1.0 min	2.0 min	3.0 min	5.0 min	
MANORAPID SYNERGY	80.0%	none	< 2.50	< 2.50	< 2.50	< 2.50	< 2.50	0.5 min
MANORAPID SYNERGY	80.0%	0.2% BSA	< 2.50	< 2.50	< 2.50	< 2.50	< 2.50	0.5 min
MANORAPID SYNERGY	80.0%	10.0% FCS	< 2.50	< 2.50	< 2.50	< 2.50	< 2.50	0.5 min
virus control sample	n.a.	none	n.c.	n.c.	n.c.	n.c.	7.88	n.a.
virus control sample	n.a.	0.2% BSA	n.c.	n.c.	n.c.	n.c.	7.63	n.a.
virus control sample	n.a.	10.0% FCS	n.c.	n.c.	n.c.	n.c.	7.88	n.a.

n.c. = not conducted
n.a. = not applicable