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TEST REPORT
VIRUCIDAL ACTIVITY AGAINST NOROVIRUSES
(under obligatory conditions according to the EN 14476 using feline calicivirus)

a) Test laboratory

Virology Laboratory of Clinic for Birds, Reptiles, Amphibian and Fish, Justus Liebig University Giessen

b) Identification of the sample

- 1) Name of the product: DISIFIN MED
- 2) Disinfectant sample: Tablets each of ~2.78 g
- 3) Batch number: 040108
- 4) Date of production: 2008-01
- 5) Date of delivery of the product: 30/09/2008

6) Date of expiry: 2011-01

7) Manufacturer: RMP chem. –techn. Spezialprodukte GmbH & Co. KG, 72119 Ammerbuch, Germany

8) Storage conditions: At room temperature, away from humidity and day light

9) Appearance of the product: White, round tablets

10) Active substances and their concentrations: 2.50 g tosylchloramid-Na/ tablet $\pm 5 \%$

c) Experimental conditions

1) Period of testing: 15/10/2008 – 17/11/2008

2) Test temperature: $20 \pm 1 \text{ }^\circ\text{C}$

3) Product diluent recommended by the manufacturer for use: Water

4) Method of Titration: Quantal tests, dilution in \lg_{10} steps, virus titration using monolayers of cells on microtiter plates

5) Concentrations of the product tested: 0.05 %, 0.1 %, 0.25 %, 0.5 %, 1.0 %

6) Contact times: 60 min ± 10 s (obligatory)

7) Procedure to stop action of product: Dilution in ice-cold cell culture medium immediately at the end of the contact time

8) Interfering substances:

0.3 g/l bovine albumin (clean conditions), 3 g/l bovine albumin + 3 ml/l packed sheep erythrocytes in reaction mixture (dirty conditions)

9) Product diluent: Hard water

10) Test-organism: Feline calicivirus (FCV) strain F9

12) Cell culture: Crandell-Reese feline kidney (CRFK) cell line

13) Incubation temperature: $37 \text{ }^\circ\text{C}$

d) Validation of test results

1) Titre of virus suspension: 7.0-7.1 TCID₅₀/ml

2) Maximum detectable virus inactivation: $> 4 \lg$

3) Virus inactivation of the reference virus inactivation test: Not done

e) Presentation of the results

1) Description of results: In the first test, the virus titre of the control for clean conditions was 7.0 TCID₅₀/ml. The control for dirty conditions had a titre of 7.1 TCID₅₀/ml. In the second set, a titre of 7.1 TCID₅₀/ml was determined for both control.

The results of cytotoxicity tests showed that the product has not cytotoxic effect within used dilution steps (level of cytotoxicity = <2.5).

The application of the dilution step 10⁻² of 0.5 % DISIFIN MED (lowest apparently non-cytotoxic dilution of the highest test concentration) on the cells for 60 min did not affect the virus susceptibility of the cell culture.

The 0.05 % and higher concentrations of DISIFIN MED led to disinfection of feline calicivirus under clean conditions. Under dirty conditions, the concentration of 0.05 % was ineffective. 0.1 % of DISIFIN MED disinfected the test virus only in the second test. However, the concentration of 0.25 % inactivated it in both tests.

2) Tables of results

Table 1: Results of the tests under clean conditions

Product	Concentration of the active compound	Interfering substance	Level of cytotoxicity	Test Nr.	lg ID ₅₀ /ml after 60 min	>4 lg reduction after ... min
DISIFIN MED	0.05 % (w/v)	0.3 g/l BSA	<2.5	1	<2.5	60
				2	<2.5	60
	0,1 % (w/v)		<2.5	1	<2.5	60
				2	<2.5	60
	0.25 % (w/v)		<2.5	1	<2.5	60
				2	<2.5	60
	0.5 % (w/v)		<2.5	1	<2.5	60
				2	<2.5	60
Virus control	n. a.	0.3 g/l BSA	n. a.	1	7.0	n. a.
				2	7.1	n. a.
Virus control of sensibility test	10 ⁻² of 0.5 % (w/v)		n. a.	1	7.0	n. a.

n. a. not applicable
n. d. not done

Table 2: Results of the tests under dirty conditions

Product	Concentration of the active compound	Interfering substance	Level of cytotoxicity	Test Nr.	lg ID ₅₀ /ml after 60 min	>4 lg reduction after ... min	
DISIFIN MED	0.05 % (w/v)	3 g/l BSA + 3 ml/l erythrocytes	<2.5	1	5.9	-	
				2	5.1	-	
	0.1 % (w/v)		<2.5	1	3.4	-	
				2	<2.5	60	
	0.25 % (w/v)		<2.5	1	<2.5	60	
				2	<2.5	60	
	0.5 % (w/v)		<2.5	1	<2.5	60	
				2	<2.5	60	
	Virus control		n. a.	n. a.	1	7.1	n. a.
					2	7.1	n. a.
Virus control of sensibility test	10 ⁻² of 0.5 % (v/v)	n. a.	n. a.	1	6.9	n. a.	
n. a. not applicable							
n. d. not done							

Table 3: Raw data of the product DISIFIN MED with 0.3 g/l BSA tested against feline calicivirus strain L9 (quantal test; 8 wells)

Product	Concentration	Interfering substance	Contact time	Test Nr.	Dilutions (lg) ^a							
					2	3	4	5	6	7	8	
DISIFIN MED	0.05 % (w/v)	0.3 g/l BSA	60 min	1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
				2	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.	
	0.1 % (w/v)			1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	
				2	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.	
	0.25 % (w/v)			1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	
				2	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.	
	0.5 % (w/v)			1	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	
				2	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.	
DISIFIN MED Cytotoxicity test	0.05 % (w/v)	1	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.			
	0.1 % (w/v)	1	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.			
	0.25 % (w/v)	1	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.			
	0.5 % (w/v)	1	0000 0000	0000 0000	0000 0000	0000 0000	n. d.	n. d.	n. d.			
Virus control	n. a.	1	4444 4444	4444 4444	4444 4444	4444 4444	0400 0444	0000 0000	0000 0000			
		2	4444 4444	4444 4444	4444 4444	4444 4444	4400 4440	0000 0000	0000 0000			
a) 1 to 4 virus present, degree of CPE in 8 cell culture units (in wells of microtiter plates) 0 no virus present n. a. not applicable n. d. not done												

Table 4: Raw data of the product DISIFIN MED with 3 g/l BSA + 3 ml/l erythrocytes tested against feline calicivirus strain L9 (quantal test; 8 wells)

Product	Concentration	Interfering substance	Contact time	Test Nr.	Dilutions (lg) ^a							
					2	3	4	5	6	7	8	
DISIFIN MED	0.05 % (w/v)	3 g/l BSA + 3 ml/l erythrocytes	60 min	1	4444	4444	4444	0440	0000	0000	0000	
					4444	4444	4444	0000	0400	0000	0000	
	2			4444	4444	0000	4000	0000	0000	0000		
				4444	4444	4344	0000	0400	0000	0000		
	0,1 % (w/v)			1	0444	0000	0000	0000	0000	0000	0000	
					4444	0000	0000	0000	0000	0000	0000	
	2			0000	0000	0000	0000	n. d.	n. d.	n. d.		
				0000	0000	0000	0000	n. d.	n. d.	n. d.		
	0.25 % (w/v)			1	0000	0000	0000	0000	0000	0000		
					0000	0000	0000	0000	0000	0000	0000	
	2			0000	0000	0000	0000	n. d.	n. d.	n. d.		
				0000	0000	0000	0000	n. d.	n. d.	n. d.		
0.5 % (w/v)	1	0000	0000	0000	0000	0000	0000					
		0000	0000	0000	0000	0000	0000	0000				
2	0000	0000	0000	0000	n. d.	n. d.	n. d.					
	0000	0000	0000	0000	n. d.	n. d.	n. d.					
DISIFIN MED Cytotoxicity test	0.05 % (w/v)	1	0000	0000	0000	0000	n. d.	n. d.	n. d.			
	0.1 % (w/v)	1	0000	0000	0000	0000	n. d.	n. d.	n. d.			
	0.25 % (w/v)	1	0000	0000	0000	0000	n. d.	n. d.	n. d.			
	0.5 % (w/v)	1	0000	0000	0000	0000	n. d.	n. d.	n. d.			
Virus control	n. a.	1	4444	4444	4444	4444	4044	0000	0000			
			4444	4444	4444	4444	0404	0000	0000			
2	4444	4444	4444	4444	4440	0000	0000					
	4444	4444	4444	4444	0404	0000	0000					
a) 1 to 4 virus present, degree of CPE in 8 cell culture units (in wells of microtiter plates) 0 no virus present n. a. not applicable n. d. not done												

Table 5: Results of the tests carried out for determination of cell sensitivity to virus.

Product	Concentration	Interfering substance	Dilution step (10 ⁷)	Dilutions (lg) ^a							
				2	3	4	5	6	7	8	
DISIFIN MED Cytotoxicity test	0.5 % (w/v)	0,3 g/l BSA	2	4444	4444	4444	4444	0004	0000	0000	
				4444	4444	4444	4444	0444	0000	0000	
		3 g/l BSA + 3 ml/l erythrocytes		4444	4444	4444	4444	0440	0000	0000	
				4444	4444	4444	4444	0040	0000	0000	
a) 1 to 4 virus present, degree of CPE in 8 cell culture units (in wells of microtiter plates) 0 no virus present n. d. not done											

3) Graphic presentation of all results

Figure 1: Graphic presentation of results (clean conditions, see table 1)

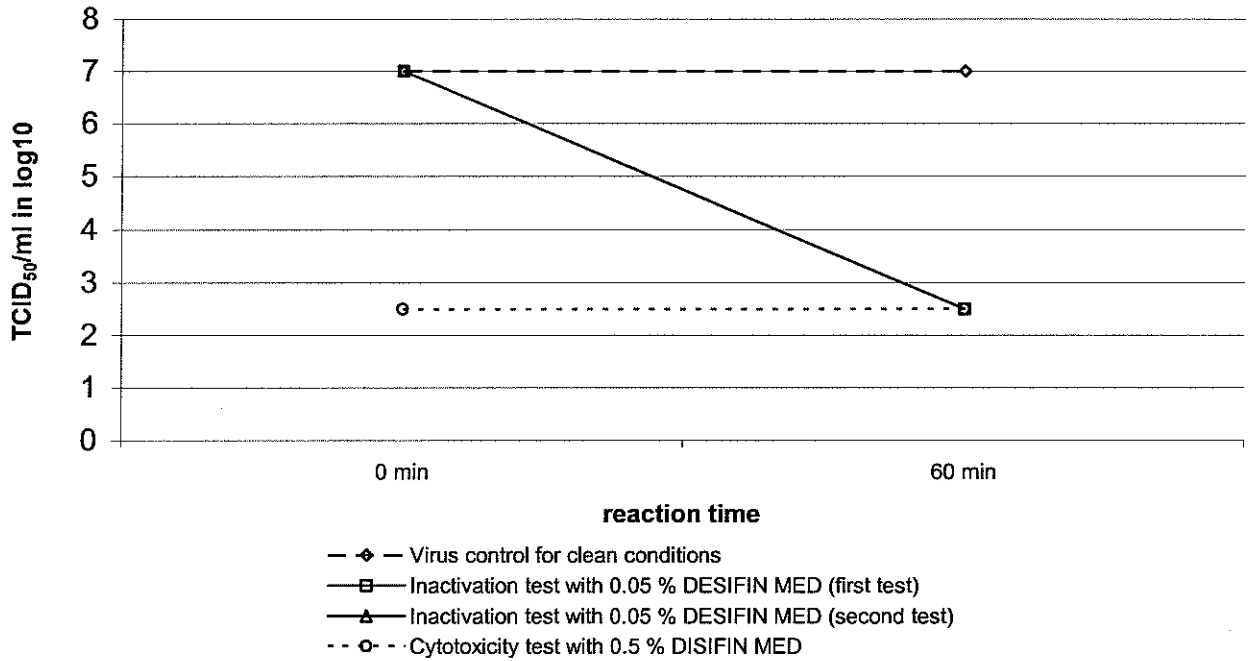
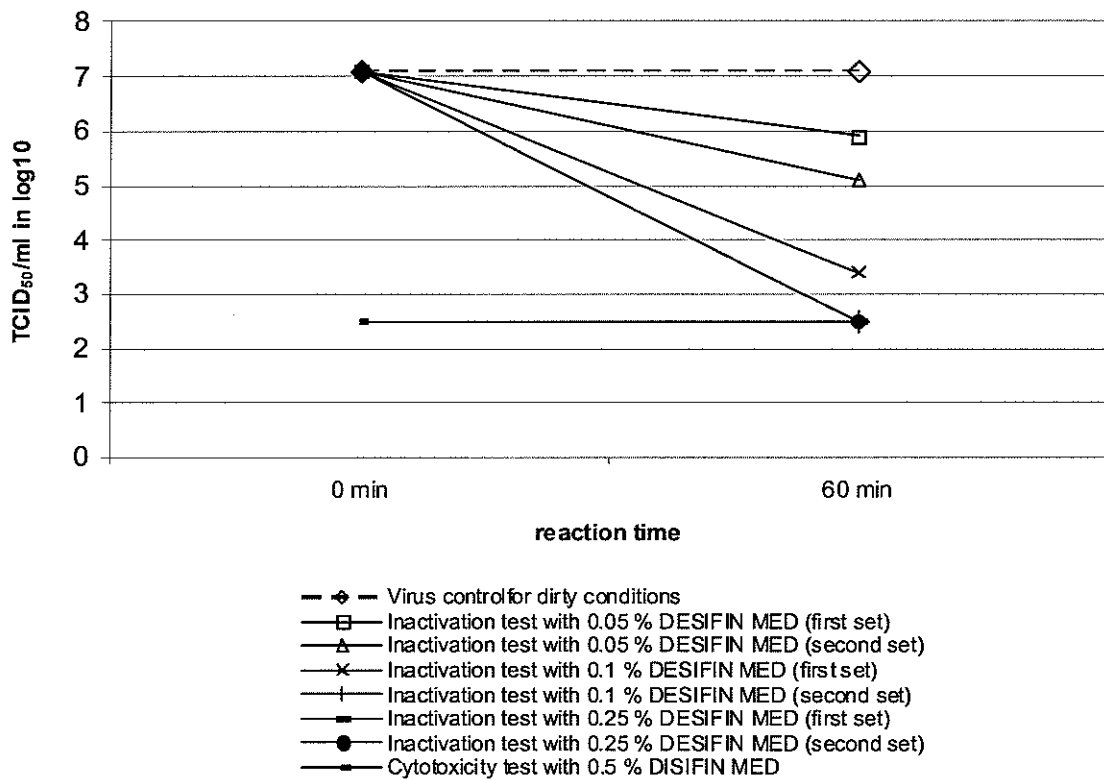


Figure 2: Graphic presentation of results (dirty conditions, see table 2)



f) Conclusion: For the product DISIFIN MED, the virucidal concentration against feline calicivirus determined according to the EN 14476 (Chemical disinfectants and antiseptics - Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine – Test method and requirements (phase 2, step 1). Date February 2007) at obligatory conditions (test temperature: 20 ± 1 °C; contact time: 60 min) is:

0.05 % or higher under clean conditions

0.25 % or higher under dirty conditions

Because the structure of viruses within a virus family is very similar it is assumed that the results of these examinations are also applicable for disinfections of other caliciviruses including member of the genus Norovirus (Norwalk-like viruses).

17/11/2008



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