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## Biological Research/Product Safety

### Expert Opinion

#### on the Virucidal Efficacy of Cryofect

#### against

#### Polio Virus, Simian Virus 40, Herpes simplex Virus and Vaccinia Virus

#### at -20°C

### 1. Summary

The product Cryofect is a surface disinfectant containing ethanol as active ingredient. It was tested for virucidal efficacy against Polio Virus, Simian Virus 40 (SV 40), Herpes simplex Virus and Vaccinia Virus at a temperature of -20°C according to the guideline of the „Deutsche Vereinigung zur Bekämpfung von Viruskrankheiten“ (DVV, German Association for the Control of Virus Diseases, Zbl. Hyg. 1990: 189, 554-562).

The concentrated product showed virucidal efficacy (log reduction factor >4 or >3.5 respectively) within 10 to 15 minutes and can therefore be recommended for the virucidal disinfection of equipment at low temperatures down to -20°C.

### 2. Method

The virus suspensions used for the test had titers of  $10^9$ - $10^{10}$  ID<sub>50</sub>/ml. The virus titration and the cytotoxicity test were carried out on RD cells (for Polio Virus), CV1 cells (for SV 40) and vero cells (for Vaccinia Virus and Herpes simplex Virus type 1). To determine the efficacy, the product was tested in a virus suspension test at -20°C with exposure times of 5, 10 and 15 minutes with and without protein soiling (0.2% bovine serum albumin, BSA or 10% fetal bovine serum, FBS). Since it is, due to methodological reasons, impossible to test a concentrated product in this test, a 1.25 fold concentrated product sample was prepared and tested at a concentration of 80%, so that the concentration in the test corresponded to the application of the undiluted product. After the individual reaction time the test suspension was diluted immediately according to the progression 1:10 / 100 / 1000 etc. The virus content was determined by means of a microtitration system. In addition to cell, virus and water controls, the cytotoxicity of the product was controlled as well. The test was performed under laboratory number LIMS 96.00293.

#### 4. Results

The results are summarized in table 1.


The 80% solution of the 1.25 fold concentrated product sample of Cryofect inactivated all test viruses at -20°C within 10 to 15 minutes. Due to the cytotoxicity of the product the maximum detectable virus titer reduction in the tests was a log reduction factor of 4.5 for Polio Virus, 3.5 for SV 40 and 4.0 for Vaccinia Virus and Herpes simplex Virus. A protein soiling of 0.2% bovine serum albumin or 10% fetal bovine serum did not lead to a considerable loss of efficacy under the application conditions tested.

#### 5. Evaluation and Recommended Application

Cryofect showed good virucidal efficacy in the virus suspension test according to the DVV guidelines against Polio Virus, Simian Virus 40, Herpes simplex Virus type 1 and Vaccinia Virus in a concentration corresponding to the application of the concentrated product within 10 to 15 minutes. For the inactivation of these viruses we recommend the application of undiluted Cryofect with an exposure time of 10 to 15 minutes.

Düsseldorf, July 18th 1996

  
Prof. Dr. M. Heinzel

  
Dr. B. Meyer

**Table 1: Efficacy of 1.25 fold concentrated Cryofect in the virus suspension test according to the DVV guideline against Polio Virus, Simian Virus 40, Herpes simplex Virus and Vaccinia Virus at -20°C**

The table shows the log<sub>10</sub> reduction factors in dependence on the exposure time at a concentration of 80% for the product and the titer of the water control.

Product	Concentration	Soiling	Virus	Exposure time in minutes		
				5	10	15
Cryofect	80%	no	Polio	4.4	4.4	4.4
		FBS		4.0	4.0	4.0
		BSA		3.9	≥4.5	4.4
Water control		no				8
		FBS				8
		BSA				8
Cryofect	80%	no	SV 40	≥3.5	≥3.5	≥3.5
		FBS		≥3.5	≥3.5	≥3.5
		BSA		≥3.5	≥3.5	≥3.5
Water control		no				7
		FBS				7
		BSA				7
Cryofect	80%	no	Herpes simplex	≥4	≥4	≥4
		FBS		≥4	≥4	≥4
		BSA		≥4	≥4	≥4
Water control		no				7.5
		FBS				7.5
		BSA				7.5
Cryofect	80%	no	Vaccinia	≥4	≥4	≥4
		FBS		≥4	≥4	≥4
		BSA		≥4	≥4	≥4
Water control		no				7.5
		FBS				7.5
		BSA				7.5

no = no additional protein soiling  
 FBS = addition of fetal bovine serum  
 BSA = addition of bovine serum albumin