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27.04.2009
Dr. St/NM

MikroLab GmbH, Norderoog 2, D-28259 Bremen

Cavex Holland BV
B.O.Pox 852

NL 2003 RW Haarlem

Efficacy against human influenza A virus of CAVEX ImpreSafe in a quantitative suspension test at 20°C following the guideline of DVV/RKI dating 01.08.2008

EXPERT OPINION

This expert opinion is based on the test report O09ML710I dating 27.04.2009.

The virus-inactivating properties of the disinfectant CAVEX ImpreSafe of Cavex Holland BV against human Influenza Virus A / Panama / 2007 / 99 (H3N2) were investigated by a quantitative suspension test following the guideline of the Deutsche Vereinigung zur Bekämpfung der Viruskrankheiten e.V. (German Association for the Control of Virus Diseases) and of the Robert Koch-Institute (RKI).

According to this suspension test, a disinfectant or a disinfectant solution at a particular concentration is considered as having virus-inactivating properties if within the recommended exposure period the titre is reduced by ≥ 4 $\log_{10}$ (inactivation ≥ 99.99 %).

CAVEX ImpreSafe was examined as 3.0 % solution at 20°C. 1, 2, 3 and 5 minutes were chosen as exposure times. After an exposure time of 3 min virus reduction exceeded 4 $\log_{10}$-steps in the assay following Lycke. Therefore, a sufficient activity is demonstrated as follows:

3.0 % 3 min

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Wirksamkeit von CAVEX ImpreSafe gegenüber dem humanen Influenza A Virus im quantitativen Suspensionsversuch bei 20°C in Anlehnung an die Leitlinie von DVV/RKI in der Fassung vom 01.08.2008

GUTACHTEN

Dieses Gutachten basiert auf dem Prüfbericht O09ML710I vom 27.04.2009.

Das Desinfektionsmittel CAVEX ImpreSafe der Cavex Holland BV wurde gemäß Auftrag auf seine virusinaktivierenden Eigenschaften gegenüber dem humanen Influenza Virus A / Panama / 2007 / 99 (H3N2) in Anlehnung an die Leitlinie der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten e.V. (DVV) und des Robert Koch-Institutes (RKI) untersucht.

In der Leitlinie der DVV und des RKI wird dann von einer Virus-Wirksamkeit eines Desinfektionsmittels ausgegangen, wenn nach einer bestimmten Einwirkzeit eine Reduktion des initialen Virustiters um ≥ 4 $\log_{10}$-Stufen (Inaktivierung ≥ 99,99 %) erfolgt ist.

Das Desinfektionsmittel CAVEX ImpreSafe wurde als 3,0 %ige Lösung bei 20°C untersucht. Die Einwirkzeiten betrugen 1, 2, 3 und 5 Minuten. Nach 3 Minuten war im Ansatz nach Lycke eine Reduktion des Virustiters in allen Ansätzen um ≥ vier $\log_{10}$-Stufen nachweisbar. Deshalb ergibt sich eine Wirksamkeit wie folgt:

3,0 % 3 Minuten

Dr. J. Steinmann

Accredited by
Zentralstelle der Länder
für Gesundheitsschutz
bei Arzneimitteln
und Medizinprodukten
ZLG-P-429.08.10

Final test report #O09ML710I
submitted to

Cavex Holland BV
B.O.Pox 852
NL 2003 RW Haarlem

Evaluation of the effectiveness of

CAVEX ImpreSafe

against

human influenza virus (H3N2)

Test method following guideline of DVV and RKI
dating 01.08.2008

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27.04.2009

Test report number #O09ML710I

1. Identification of test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

2. Identification of sample

Name of product	CAVEX ImpreSafe
Manufacturer	Cavex Holland BV
Application	disinfection of dental impressions
Lot no.	-
Expiry date	-
Active substance(s) and concentration(s) in 100 g	30 g benzalkonium chloride
Appearance and odour	clear, blue fluid Product specific
pH-value (s) (in hard water)	undiluted: 8.32 (20°C)
Conditions of storage	room temperature in the dark (area with limited access)
Date of receipt at laboratory	17.11.2008

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Cambrex Bio Science Verviers s.p.r.l., catalogue no. 12-125F)
- fetal calf serum (Biochrom AG, article no. S 0115)
- formaldehyde (Riedel-de-Häen, article no. 33220)
- Aqua bidest. (Fresenius Kabi Deutschland, article no. P2N 1636071)
- PBS (Invitrogen, article no. 18912-014)

3.2 Virus and cells

The human influenza A (H3N2) virus was obtained from the Robert Koch-Institute Berlin. The *MDCK cells* were obtained from Dr. R. Riebe, cell bank for cell lines in veterinary medicine at the Federal Research Institute for Animal Health, D-17493 Greifswald - Isle of Riems.

3.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Centrifuge 5804 R (Eppendorf AG)
- Water bath (JULABO, Julabo U 3)
- Adjustable and fixed-volume pipettes (Eppendorf AG)

4. Experimental conditions

Test temperature	20°C ± 0.5°C
Concentration of test product	3.0 % and 0.3 %
Contact times	1, 2, 3 and 5 minutes
Interfering substance	not possible
Procedure to stop action of disinfectant	immediate dilution, gel filtration alone and in combination with test method according to Lycke
Diluent	water of standardised hardness
Virus strain	human influenza virus A / Panama / 2007 / 99 (H3N2)
Date of testing	17.11.2008 – 27.04.2009
End of testing	27.04.2009

5. Methods

5.1 Preparation of test virus suspension

To prepare the test virus suspension, *MDCK cells* that had been cultured with Eagle´s minimum essential medium (EMEM) and 10 % or 2 % fetal calf serum (FCS, Biochrom AG, Berlin, Germany) were inoculated with human influenza virus in 175 cm^2 cell culture flasks (Nunc GmbH & Co. KG, Wiesbaden, Germany). Once a cytopathic effect had been induced (approx. 24 hours), freezing and thawing was carried out once. The cell debris was removed by centrifugation at 3.000 rpm for ten minutes (4°C) and the supernatant was recovered as test virus suspension and stored in aliquots at -80°C.

5.2 Preparation of disinfectant (dilutions)

The test product was evaluated as 3.0 % solution. The 0.3 % concentration was chosen for the demonstration of the non-active range. Dilutions were prepared immediately before the inactivation tests with water of standardized hardness.

5.3 Inactivation assays and controls

Tests were carried out in accordance with the DVV and RKI guideline (1). Eight parts by volume of the disinfectant were mixed with one part by volume of test virus suspension and one part by volume of Aqua bidest. Tests with interfering substances are not possible since proteins like FCS have influenza virus inhibitory and trypsin-neutralizing activity.
Immediately at the end of the chosen exposure time, activity of the disinfectant was stopped by serial dilutions.

Due to a more convenient handling and due to a limited amount of test virus suspension, the volumes in the inactivation assay were 0.1 mL test virus suspension, 0.1 mL Aqua bidest. and 0.8 mL test product.

Virus controls were incorporated after the longest exposure time. One part by volume of test virus suspension was mixed with nine parts by volume of Aqua bidest. or with one part by volume of FCS and eight parts by volume of Aqua bidest.

A control was carried out with one part by volume of test virus suspension, four parts by volume of PBS (0.1 M, pH value 7.0) and five parts by volume of 1.4 % formaldehyde solution. 5, 15, 30 und 60 minutes were chosen as contact times.

For determination of cytotoxicity of the disinfectant, two parts by volume of Aqua bidest. were mixed with eight parts by volume of the disinfectant, diluted with ice-cold EMEM and inoculated onto permissive cells. Values are given as $\log_{10}CD_{50}$/mL (in analogy to $\log_{10}TCID_{50}$/mL).

Inactivation tests were carried out in sealed test tubes (Sarstedt AG & Co., D-51588 Nümbrecht, Germany) in a water bath at 20°C ± 0.5°C. Aliquots were retained after appropriate exposure times, and the residual infectivity was determined. To reduce cytotoxicity, immediately at the end of the exposure time the mixture was added to a MicroSpin™ S-400 HR column and centrifuged.

The inactivation experiments were run in two independent assays (two different days).

A control of efficiency for suppression of disinfectant activity was not included since at the end of the exposure time dilutions were done immediately.
Furthermore, a cell control was incorporated.

5.4 Determination of infectivity

Infectivity was determined by means of end point dilution method using the microtitre process. For this, 100 µl aliquots of the samples, which had been serially diluted with ice-cold EMEM, were transferred to eight cups of a sterile polystyrol 96-well microtitre plate with a flat bottom (Nunc GmbH & Co. KG, Wiesbaden, Germany). Already on the previous day 100 µl aliquots of a freshly trypsinized *MDCK cells* (approx. 2.9×10^4 cells) had been placed in each well (preformed monolayer). Incubation took place at 37°C in a CO_2 incubator (5 % CO_2 content) for five days. Finally, cultures were observed for cytopathic effects with a reversed microscope and the infective dose $TCID_{50}$/mL was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

$$\log_{10} TCID_{50} = X_0 - 0.5 + \sum r/n$$

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.

5.5 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant (virus control). The difference is given as reduction factor (RF).

According to the guideline (Leitlinie) of DVV/RKI, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if within the recommended exposure period the titre is reduced at least by four $\log_{10}$ steps.

5.6 Inactivation assay following the method of Lycke in combination with the columns

Due to the high cytotoxicity of the test product gel filtration was following by a modified procedure as described by Lycke (4). After usage of the columns, the test mixture was further diluted 1:1000 in EMEM and then the total volume was added (without any further dilution) to the permissive cells. By introducing such a huge dilution it is possible to eliminate cytotoxicity of the test product in order to demonstrate a 4 $\log_{10}$-reduction of virus titre. This method is necessary for those products which demonstrate a great cytotoxicity.

100 µL of the test virus suspension and 100 µL Aqua bidest. were mixed with 800 µL disinfectant (3.0 %) at 20°C. At the end of the exposure time (3 and 5 min) 100 µl was laid onto the columns. Finally 62.5 µL of the eluate was immediately added to 62.5 mL EMEM (1:1000 dilutions) and then the total volume was distributed in 6 microtitre plates (108 µL / well). After 10 days of inoculation cultures were observed for cytopathic effects. The calculation of virus titre followed the formula of Lycke:

$$-\log_{10} = [1.4 \times \ln (1-p)]$$

p is meaning the relation between the positive wells with virus detection in comparison to the total number of wells.

For the control of cell sensitivity 200 µL Aqua bidest. were mixed with 800 µL disinfectant (PBS as control). After the gel filtration the eluate was diluted 1:1000 and 108 µL of this dilution were added to the wells of the microtitre plates. After three days, a comparative titration was performed on the cells treated in such a manner or treated with PBS only.

Determination of the initial virus titre was performed in a quantitative suspension tests by a fivefold assay (see 5.3). The virus-inactivating properties of the test product were calculated by subtracting the virus titre in the test mixture from the virus control.

6. Results

6.1 Determination of cytotoxicity

In parallel with the inactivation tests, the cytotoxicity of CAVEX ImpreSafe (3.0 % and 0.3 %) and 0.7 % formaldehyde was measured. The formaldehyde solution was toxic for the *MDCK cells* in the 1:1,000 dilutions. This corresponded to a $\log_{10}CD_{50}$/mL of 4.50 (Table 1).
Examinations also showed that disinfectant CAVEX ImpreSafe tested as 3.0 % solution achieved a $\log_{10}CD_{50}$/mL of 4.50 (cytotoxicity in the 1:1,000-dilutions). The 0.3 % solution was cytotoxic in the 1:100-dilutions (Table 1).
These tests to measure cytotoxicity are imperative, because in this manner the lower detection threshold for non-inactivated influenza virus could be determined.

6.2 Virus-inactivating properties of formaldehyde control

Results of inactivation tests are found in table 2. Formaldehyde (0.7 %) reduced the influenza virus titre after five minutes by ≥ 1.38 ± 0.37 $\log_{10}$ steps. After 15 and 30 minutes identical reduction factors of ≥ 1.38 ± 0.37 were measured (Table 3).

6.3 Virus-inactivating properties of disinfectant (suspension test)

Results of inactivation assays (quantitative suspension test) are demonstrated in tables 2 to 4. The disinfectant CAVEX ImpreSafe was examined as 3.0 % and as 0.3 % % solutions. 1, 2, 3 and 5 minutes were chosen as exposure time in these experiments. No sufficient reduction was demonstrated due to the high cytotoxicity of the product.

6.4 Virus-inactivating properties of the disinfectant following the method of Lycke

As mentioned above, a 4 $\log_{10}$-reduction could not be measured in the quantitative suspension test due to high cytotoxicity. Therefore, the method of Lycke in combination with columns was introduced. The results are given in tables 5 and 6.

Examining the 3.0 % solution with 5 minutes exposure times, the following results were obtained: the virus titre (mean value) in the fivefold assay was $\log_{10}$ $TCID_{50}$ / mL = 6.87.
This value corresponded to the virus amount in the assay of 4.67 $\log_{10}$ $TCID_{50}$.
Since in 6 cell culture units (total 576 wells) residual virus could be detected the result according to the formula of Lycke is $\log_{10}$ = 0.0146. The reduction factor is therefore $\log_{10}$ 4.67 minus $\log_{10}$ 0.0146 = 4.66 (Table 5).

In a second step, the same concentration was tested with 3 minutes exposure time.
The virus titre (mean value) in the fivefold assay was $\log_{10}$ $TCID_{50}$ / mL = 6.81.
This value corresponded to the virus amount in the assay of 4.61 $\log_{10}$ $TCID_{50}$.
Since in 37 cell culture units (total 576 wells) residual virus could be detected the result according to the formula of Lycke is $\log_{10}$ = 0.09. The reduction factor is therefore $\log_{10}$ 4.61 minus $\log_{10}$ 0.09 = 4.52 (Table 6).

- Dr. J. Steinmann -

7. Quality control

The Quality Assurance of the results was maintained by performing the determination of the virus-inactivating properties of the disinfectant in accordance with Good Laboratory Practice regulations:

1) Chemicals Act of Germany, Appendix 1, dating of 01.08 1994 (BGBl. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBl. I, 1997, page 1060).
2) OECD Principles of Good Laboratory Practice (revised 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998.

The plausibility of the results was additionally confirmed by controls incorporated in the inactivation assays.

8. Recorders to be maintained

All testing data, protocol, protocol modifications, the final report, and correspondence between MikroLab GmbH and the sponsor will be stored in the archives at MikroLab GmbH.

9. Literature

1. Leitlinie der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) e.V. und des Robert Koch-Institutes (RKI) zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren in der Humanmedizin (in der Fassung vom 1. August 2008)
   Bundesgesundheitsbl., 51, 2008, 936-445

2. Spearman, C.: The method of `right or wrong cases` (constant stimuli) without Gauss's formulae.
   Brit J Psychol; 2 1908, 227-242

3. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche.
   Arch Exp Path Pharmak; 162, 1931, 480-487

4. Lycke, E.: Studies of the Inactivation of Poliomyelitis Virus by Formaldehyde.
   Arch Ges Virusforsch; 7, 1957: 483-493

table 1: cytotoxicity of CAVEX ImpreSafe and 0.7% formaldehyde

			dilutions				
	conc.	**soil load**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
product	0.3%	Aqua bidest.	+	+	-	-	-
product	0.3%	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.d.
product	3.0%	Aqua bidest.	+	+	+	-	-
product	3.0%	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde	0.7%	PBS	+	+	+	-	-

table 2 : inactivation of influenza virus by CAVEX ImpreSafe (3.0 %) and 0.7 % formaldehyde in a quantitative suspension test at 20°C without columns

product	conc.	soil load	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			30 s	60 s	120 s	180 s	30 s	60 s	120 s	180 s	
test product	3.0 %	Aqua bid.	n.d.	≤4.88±0.36	≤4.63±0.25	≤4.50±0.00	n.a.	≥1.00±0.52	≥1.25±.41	≥1.38±0.33	> 180 s
test product	3.0 %	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
controls	**conc.**	**soil load**	**$log_{10}TCID_{50}$/mL with 95% level of confidence after**				**reduction factor with 95% level of confidence after**				
			5 min	**15 min**	**30 min**	**60 min**	**5 min**	**15 min**	**30 min**	**60 min**	
formaldehyde	0.7%	PBS	≤4.50±0.44	≤4.50±0.00	≤4.50±0.00	n.d.	≥1.38±0.37	≥1.38±0.37	≥1.38±0.37	n.a.	≥ 5 min
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	5.88±0.37	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

table 3 : inactivation of influenza virus by CAVEX ImpreSafe (3.0 %) in a quantitative suspension test at 20°C with columns

product	conc.	soil load	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			30 s	60 s	120 s	180 s	30 s	60 s	120 s	180 s	
test product	3.0 %	Aqua bid.	n.d.	4.00±0.38	4.00±0.38	3.50±0.00	n.a.	1.50±0.52	1.50±0.38	2.00±0.35	> 180 s
test product	3.0 %	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
controls	**conc.**	**soil load**	**$log_{10}TCID_{50}$/mL with 95% level of confidence after**				**reduction factor with 95% level of confidence after**				**≥ 4 log_{10} reduction after**
			5 min	**15 min**	**30 min**	**60 min**	**5 min**	**15 min**	**30 min**	**60 min**	
formaldehyde	0.7%	PBS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	5.50±0.35	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	6.38±0.25	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	5.88±0.37	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	6.13±0.37	n.a.	n.a.	n.a.	n.a.	n.a.

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

table 4 : inactivation of influenza virus by CAVEX ImpreSafe (0.3 %) and 0.7 % formaldehyde in a quantitative suspension test at 20°C without columns

product	conc.	soil load	$log_{10}TCID_{50}$/mL with 95% level of confidence after				reduction factor with 95% level of confidence after				≥ 4 log_{10} reduction after
			30 s	60 s	120 s	180 s	30 s	60 s	120 s	180 s	
test product	0.3 %	Aqua bid.	n.d.	4.75±0.41	n.d.	n.d.	n.a.	1.13±0.49	n.a.	n.a.	> 60 s
test product	0.3 %	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
controls	**conc.**	**soil load**	**$log_{10}TCID_{50}$/mL with 95% level of confidence after**				**reduction factor with 95% level of confidence after**				**≥ 4 log_{10} reduction after**
			5 min	**15 min**	**30 min**	**60 min**	**5 min**	**15 min**	**30 min**	**60 min**	
formaldehyde	0.7%	PBS	n.d	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	5.88±0.37	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.

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

table 5 : inactivation of influenza virus by CAVEX ImpreSafe (3.0 %) in the assay following the method of Lycke (5 minutes of exposure time)

Soil load	Row	1	2	3	4	5	6	7	8	9	10	11	12
Aqua bid.	plate 1/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 2/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0004	0000 0000	0000 0000	0000 0000
	plate 3/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 4/6	0000 0000	0000 0000	0000 0000	0200 0000	0000 0000	0400 0000	0200 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 5/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0002	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 6/6	0000 0040	0000 0000	0000 0000	0000 0000	0400 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

t = cytotoxic
0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in eight wells of a microtitre plate)

table 6: inactivation of influenza virus by CAVEX ImpreSafe (3.0 %) in the assay following the method of Lycke (3 minutes of exposure time)

Soil load	Row	1	2	3	4	5	6	7	8	9	10	11	12
Aqua bid.	plate 1/6	0000 0000	0000 0400	0000 0000	0000 0000	0000 0000	4000 0000	0000 0000	0040 0000	0400 0000	0000 0000	0000 0000	0000 0000
	plate 2/6	0000 0000	0000 0000	0004 0400	0000 0000	0000 0200	0400 0000	0003 0002	0000 0000	0000 0000	0000 0020	0000 0000	0000 0000
	plate 3/6	0000 0002	0000 0000	0000 4000	0000 0000	0000 0000	0000 0000	0040 0000	2000 0000	0000 0000	0000 0400	0000 0000	0400 0000
	plate 4/6	0040 0400	0000 0000	1000 0000	0440 0000	0000 0004	0000 0000	0000 0000	0000 0000	0000 0000	0200 0000	0000 0000	0000 0000
	plate 5/6	0000 0000	0200 0043	0000 0000	0000 0400	0000 0000	0000 4000	0000 0000	0000 0000	0000 0000	0000 0000	4000 0004	0000 0000
	plate 6/6	0000 0000	0000 0000	0000 0000	0000 0400	0000 0000	0000 0000	0000 0000	0020 0000	0000 2200	0000 0000	2000 0040	0000 0000

t = cytotoxic
0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in eight wells of a microtitre plate)

Appendix Table 1: Raw data (influenza virus) of CAVEX ImpreSafe without columns (1687)

product	concentration	interfering substance	exposure time (sec.)	dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	3.0 %	Aqua bidest.	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	0004 4001	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			120	tttt tttt	tttt tttt	tttt tttt	0010 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			180	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
		10.0% FCS	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			120	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			180	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	3.0 %	Aqua bidest.	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control with columns	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4014 3434	0000 0400	0000 0000	0000 0000	0000 0000	n.d.
	n.a.	10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control without columns	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4244 4344	4000 1200	0000 0000	0000 0000	0000 0000	n.d.
	n.a.	10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	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 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Appendix Table 2: Raw data (influenza virus) of CAVEX ImpreSafe with columns (1687)

product	concentration	interfering substance	exposure time (sec.)	dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	3.0 %	Aqua bidest.	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	tttt tttt	4444 4444	4444 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			120	tttt tttt	4444 4444	4040 4400	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			180	tttt tttt	4444 4444	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
		10.0% FCS	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			120	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			180	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	3.0 %	Aqua bidest.	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control with columns	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4014 3434	0000 0400	0000 0000	0000 0000	0000 0000	n.d.
	n.a.	10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control without columns	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4244 4344	4000 1200	0000 0000	0000 0000	0000 0000	n.d.
	n.a.	10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	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 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Appendix Table 3: Raw data (influenza virus) of CAVEX ImpreSafe without columns (1687)

product	concentration	interfering substance	exposure time (sec.)	dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	0.3 %	Aqua bidest.	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	tttt tttt	tttt tttt	4444 4444	4002 0000	0000 0000	0000 0000	0000 0000	tttt tttt	n.d.
			120	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			180	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			120	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			180	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product cytotoxicity	0.3 %	Aqua bidest.	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control with columns	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4014 3434	0000 0400	0000 0000	0000 0000	0000 0000	n.d.
	n.a.	10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
virus control without columns	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4244 4344	4000 1200	0000 0000	0000 0000	0000 0000	n.d.
	n.a.	10.0% FCS	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	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 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Appendix Table 4: Raw data (influenza virus) of formaldehyde control (20°C) (1764)

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 tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
formaldehyde cytotoxicity	0.7% (m/V)	PBS	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	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 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Appendix Table 5 : raw data for cell sensitivity (1764)

product	interfering substance	dilutions	dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
PBS	-	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4200 3301	0000 0003	0000 0000	0000 0000	n.d.
product	Aqua bidest.	1:10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:100	4444 4444	4444 4444	4444 4444	4444 4444	0000 4024	0000 0000	0000 0000	0000 0000	n.d.
		1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product	FCS	1:10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	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)

Appendix Table 6: determination of virus titre (assay following method of Lycke) (1764)

Virus titration	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
1st assay	Aqua bidest.	4444 4444	4444 4444	4444 4444	4444 2444	3000 0000	0000 0000	0000 0000	n.d.	n.d.
	10.0 % FCS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2nd assay	Aqua bidest.	4444 4444	4444 4444	4444 4444	4444 4443	0404 0420	0000 0000	0000 0000	n.d.	n.d.
	10.0 % FCS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3rd assay	Aqua bidest.	4444 4444	4444 4444	4444 4444	4444 4404	0210 1300	0000 0000	0000 0000	n.d.	n.d.
	10.0 % FCS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4th assay	Aqua bidest.	4444 4444	4444 4444	4444 4444	2321 0404	0000 0140	0000 0000	0000 0000	n.d.	n.d.
	10.0 % FCS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5th assay	Aqua bidest.	4444 4444	4444 4444	4444 4444	4444 4444	2030 4020	0000 0000	0000 0000	n.d.	n.d.
	10.0 % FCS	n.d.	n.d.	n.d.	n.d.	n.d.	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 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Appendix Table 7 : determination of virus titre (assay following method of Lycke) (2nd assay) (1821)

Virus titration	Interfering substance	MicroSpin columns	dilutions (log_{10})								
			1	2	3	4	5	6	7	8	9
1st assay	PBS	yes	4444 4444	4444 4444	4444 4444	4444 4444	0440 0000	0000 0000	0000 0000	n.d.	n.d.
	PBS	no	4444 4444	4444 4444	4444 4444	4444 4444	0000 0440	0000 0000	0000 0000	n.d.	n.d.
2nd assay	PBS	yes	4444 4444	4444 4444	4444 4444	4444 4442	4000 0040	0000 0000	0000 0000	n.d.	n.d.
	PBS	no	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3rd assay	PBS	yes	4444 4444	4444 4444	4444 4444	4444 4444	0240 0000	0000 0000	0000 0000	n.d.	n.d.
	PBS	no	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4th assay	PBS	yes	4444 4444	4444 4444	4444 4444	2444 4344	0000 0302	0000 0000	0000 0000	n.d.	n.d.
	PBS	no	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5th assay	PBS	yes	4444 4444	4444 4444	4444 4444	4043 4444	0044 0232	0000 0000	0000 0000	n.d.	n.d.
	PBS	no	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.