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ZLG-P-429.08.10

TEST REPORT

10.03.2011

Test report no. J10ML1137-2B

Evaluation of the effectiveness of

Divodes FG VT 29

Testvirus:	Bovine Viral Diarrhea Virus (BVDV) (Surrogate of HCV)
Method:	according to the guideline of DVV and RKI (dating 01.08.2008)

Sponsor:
Diversey Polska Sp. z.o.o.
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1. Identification of test laboratory

MikroLab GmbH, Norderoog 2, D-28259 Bremen

2. Identification of sample

Name of product	Divodes FG VT 29
Manufacturer	Diversey Polska Sp. z.o.o.
Application	surface disinfection
Lot no.	ENS 344306
Expiry date	-
Date of production	-
substance(s) and concentration(s) in 100 g	52.5 g propan-1-ol 17.5 g propan-2-ol
Appearance and odour	clear, colourless liquid; product specific
pH-value (s) (in hard water)	undiluted: 8.19 (20°C)
Conditions of storage	room temperature in the dark (area with limited access)
Date of receipt at laboratory	01.11.2010

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Lonza Group Ltd., catalogue no. BE12-125F)
- Fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % Formaldehyde solution (Chemisch-technologisches Laboratorium Dr. Melzer, D-28199 Bremen)
- Aqua bidest. (Fresenius Kabi Deutschland, article no. P2N 1636071)
- PBS (Invitrogen, article no. 18912-014)

3.2 Virus and cells

BVDV strain NADL (VR-534) was obtained from Dr. Stephanie Bendtfeld, Institute of Virology at the School of Veterinary Medicine Hannover (Tierärztliche Hochschule, D-30559

Hannover). Prior to inactivation assays, the virus was passaged once in *primary bovine kidney cells* and five times in *KOP-R cells* (primary cells from bovine oropharyngeal tissue). *KOP-R cells* originated from the Friedrich-Löffler-Institut, Bundesforschungsinstitut für Tiergesundheit (formerly Bundesforschungsanstalt für Viruskrankheiten der Tiere, isle of Riems) (Dr. R. Riebe, catalogue no. RIE 244). In the inactivation assays *ekl cells* (embryonal cells from bovine lung tissue) were used. These cells originated from Mrs. A. Kyas (Henkel KGaA, D-40191 Düsseldorf).

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)
- Transferpettor® (Brand GmbH & Co. KG, Wertheim, Germany)
- Polysterol 96-well microtitre plates (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Cell culture flasks (Nunc GmbH & Co. KG, Wiesbaden, Germany)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht, Germany)
- MicroSpin™ S-400 HR columns (GE Healthcare, Freiburg, Germany)

4. Experimental conditions

Test temperature	20°C ± 0.5°C
Concentration of test product	undiluted (80.0 %) and as 10.0 % solution (non-active range)
Contact times	5 and 15 minutes
Interfering substance	fetal calf serum (FCS)
Procedure to stop action of disinfectant	immediate dilution and gel filtration
Diluent	water of standardised hardness (10.0 % solution)
Virus strain	BVDV strain NADL
Date of testing	01.11.2010 – 10.03.2011
End of testing	10.03.2011

5. Methods

5.1 Preparation of test virus suspension

For the preparation of the test virus suspension, *KOP-R cells*, which were cultivated with Eagle's Minimum Essential Medium (EMEM) supplemented with L-glutamine, sodium pyruvate and 10 % or 2 % fetal calf serum (FCS), were infected with BVDV (stock virus suspension). As soon as cells showed a constant cytopathic effect, they were subjected to a rapid freeze/thawing procedure. This was followed by low-speed centrifugation (10 min and 1000 x g) in order to sediment cell debris. After aliquotation, test virus suspension was stored at –80°C.

5.2 Preparation of disinfectant (dilutions)

The test product was evaluated undiluted. Due to the addition of test virus suspension and interfering substance an 80.0 % solution resulted. The product was additionally tested as 10.0 % solution (demonstration of non-active range).

The 10.0 % solution was prepared with water of standardised hardness immediately before the inactivation tests.

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. In tests with interfering substance, instead of Aqua bidest., one part by volume of fetal calf serum was added. 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 interfering substance (FCS) 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.

Since the cytotoxicity did not allow following the reduction of residual infectivity titre over the range of four log_{10}-steps, ready to use MicroSpin™ S-400 HR columns were used in order to remove the cytotoxic agents according to the instructions of the manufacturer. Virus controls with and without MicroSpin™ S-400 HR columns were included.

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 and 30 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).

For the control of cell sensitivity two parts by volume Aqua bidest. or one part by volume of FCS and one part by volume Aqua bidest were mixed with eight parts by volume of the lowest apparently non-cytotoxic dilution of the product or PBS. This mixture was added to the permissive cell culture. After 1 h at 37°C the mixture was discharged and a comparative titration of the test virus suspension was performed on the pre-treated and non pre-treated (PBS) cells as described above.

Inactivation tests were carried out in sealed test tubes in a water bath at 20°C ± 0.5°C. Aliquots were retained after appropriate exposure times, and the residual infectivity was determined.
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 titration in a micro-procedure. For this, samples were diluted with ice-cold EMEM and 100 µl of each dilution were placed in 8 wells of a sterile polystyrene flat bottomed microtitre plate. 100 µl of *ekl cells* were added into the plates one day earlier. Suspension was adjusted to reach approximately 10-15 x 10^3 cells per well. Incubation was at 37°C in a CO_2-atmosphere (5.0 % CO_2 - content). Finally, cultures were observed for cytopathic effects for ten days of inoculation. The infective dose ($TCID_{50}$) (with 95 % level of confidence) was calculated according to 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 positive 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.

6. Results

6.1 Determination of cytotoxicity

In parallel with the inactivation tests, the cytotoxicity of Divodes FG VT 29 (80.0 % and 10.0 %) and 0.7 % formaldehyde was measured.

The formaldehyde solution was toxic for the *ekl 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 the surface disinfectant Divodes FG VT 29 achieved a $log_{10}CD_{50}$/ml of 2.50 (80.0 %) and 1.50 (10.0 %), respectively (Table 1). After treatment with the columns, the undiluted product showed a cytotoxicity of 1.50.

These tests to measure cytotoxicity are imperative, because in this manner the lower detection threshold for non-inactivated BVDV could be determined.

6.2 Virus-inactivating properties of formaldehyde control

Formaldehyde (0.7 %) reduced the BVDV titre after five and 15 minutes by ≥ 1.63 ± 0.51 and ≥ 2.13 ± 0.25 log_{10} steps. After 30 and 60 minutes a reduction factor of ≥ 2.13 ± 0.25 was measured (Table 3).

6.3 Virus-inactivating properties of disinfectant

Results of inactivation assays are demonstrated in tables 2 to 5 (raw data see appendix).

The surface disinfectant Divodes FG VT 29 was examined undiluted (80.0 %) and as 10.0 % solution. 5 and 15 minutes were chosen as exposure times in these experiments.

Divodes FG VT 29 was active against BVDV undiluted after 5 minutes of exposure time. The reduction factors were ≥ 4.13 ± 0.43 and ≥ 4.13 ± 0.25 (assays without soil load) and ≥ 4.00 ± 0.60 and ≥ 4.00 ± 0.46 (assays with soil load), respectively (Tables 2 and 3). After introduction of the columns, the reduction factors after 5 minutes incubation time were ≥ 4.88 ± 0.25 (assay without soil load) and ≥ 5.13 ± 0.41 (assay with soil load), respectively (Table 4). This corresponded to an inactivation of ≥ 99.999 %

Additionally, the product was examined as 10.0 % solution in the presence of FCS for demonstrating the non-active range. After 15 minutes no sufficient reduction of virus titre was detectable. The reduction factor was 0.25 ± 0.57 at that time point (Table 5).

- Dr. J. Steinmann -

Wiss. Techn. Leiter der MikroLab GmbH

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. Records 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

Table 1: Cytotoxicity of Divodes FG VT 29 and 0.7 % formaldehyde with and without treatment with MicroSpin™ S-400 HR-columns

without treatment	Conc.	Interfering substance	dilutions 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
product	80.0%	Aqua bidest.	t	-	-	-	-
product	80.0%	10.0% FCS	t	-	-	-	-
product	10.0%	Aqua bidest.	n.d.	n.d.	n.d.	n.d.	n.d.
product	10.0%	10.0% FCS	-	-	-	-	-
formaldehyde	0.7 %	PBS	t	t	t	-	-
with treatment	**Conc.**	**Interfering substance**	**dilutions 10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
product	80.0%	Aqua bidest.	-	-	-	-	-
product	80.0%	10.0% FCS	-	-	-	-	-

t = cytotoxic n.d. = not done

Table 2: Inactivation of BVDV by Divodes FG VT 29 (80.0 %) and formaldehyde in a quantitative suspension test at 20°C without columns (1st assay)

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/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	
test product	80.0%	Aqua bid.	≤2.50±0.00	≤2.50±0.00	n.d.	n.d.	≥4.13±0.43	≥4.13±0.43	n.a.	n.a.	5 min
test product	80.0%	10.0% FCS	≤2.50±0.00	≤2.50±0.00	n.d.	n.d.	≥4.00±0.60	≥4.00±0.60	n.a.	n.a.	5 min
Controls	**Conc.**	**Interfering substance**	$Log_{10}TCID_{50}$/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.	6.63±0.43	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	6.50±0.60	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 BVDV by Divodes FG VT 29 (80.0 %) and formaldehyde in a quantitative suspension test at 20°C without columns (2nd assay)

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/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	
test product	80.0%	Aqua bid.	≤2.50±0.00	≤2.50±0.00	n.d	n.d.	≥4.13±0.25	≥4.13±0.25	n.a.	n.a.	5 min
test product	80.0%	10.0% FCS	≤2.50±0.00	≤2.50±0.00	n.d	n.d.	≥4.00±0.46	≥4.00±0.46	n.a.	n.a.	5 min
Controls	**Conc.**	**Interfering substance**	$Log_{10}TCID_{50}$/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	≤5.00±0.44	≤4.50±0.00	≤4.50±0.00	≤4.50±0.00	≥1.63±0.51	≥2.13±0.25	≥2.13±0.25	≥2.13±0.25	≥ 15 min
virus control	n.a.	Aqua bid.	n.d	n.d.	n.d.	6.63±0.25	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	6.50±0.46	n.a.	n.a.	n.a.	n.a.	n.a.
interference control PBS	n.a.	-	n.d.	n.d.	n.d.	6.38±0.49	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	Aqua bid.	n.d.	n.d.	n.d.	6.25±0.33	n.a.	n.a.	n.a.	n.a.	n.a.
interference control disinf.	n.a.	10.0% FCS	n.d.	n.d.	n.d.	6.00±0.44	n.a.	n.a.	n.a.	n.a.	n.a.

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

Table 4: Inactivation of BVDV by Divodes FG VT 29 (80.0 %) and formaldehyde in a quantitative suspension test at 20°C with columns

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/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	
test product	80.0%	Aqua bid.	≤1.50±0,00	n.d	n.d	n.d.	≥4.88±0.25	n.a.	n.a.	n.a.	5 min
test product	80.0%	10.0% FCS	≤1.50±0,00	n.d	n.d	n.d.	≥5.13±0.41	n.a.	n.a.	n.a.	5 min
Controls	**Conc.**	**Interfering substance**	$Log_{10}TCID_{50}$/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.	6.38±0.25	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	6.63±0.41	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 BVDV by Divodes FG VT 29 (10.0%) and formaldehyde in a quantitative suspension test at 20°C

Product	Conc.	Interfering substance	$Log_{10}TCID_{50}$/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	
test product	10.0 %	Aqua bid.	n.d	n.d.	n.d	n.d.	n.a.	n.a.	n.a.	n.a.	n.d.
test product	10.0 %	10.0% FCS	6.38±0.25	6.25±0.33	n.d.	n.d.	0.13±0.53	0.25±0.57	n.a.	n.a.	> 15 min
Controls	**Conc.**	**Interering substance**	$Log_{10}TCID_{50}$/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	
frmaldehyde	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.	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.
virus control	n.a.	FCS	n.d.	n.d.	n.d.	6.50±0.46	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

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BVDV efficacy of Divodes FG VT 29 in a quantitative suspension test at 20°C according to the guideline of DVV/RKI dating 01.08.2008

EXPERT OPINION

This expert opinion is based on the test report J10ML1137-2B dating 10.03.2011.

The virus-inactivating properties of the surface disinfectant Divodes FG VT 29 of Diversey Polska Sp. z.o.o. against bovine viral diarrhea virus (BVDV) strain NADL were investigated by a quantitative suspension test according to 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).
BVDV was chosen as a surrogate of hepatitis C virus (HCV) since there is no animal model or cell culture system for growing this virus. Testing this surrogate virus the possibility is created to give recommendations for the inactivation of HCV by the disinfectant.
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 %).
Divodes FG VT 29 was examined undiluted (80.0 %) at 20°C. 5 and 15 minutes were chosen as exposure time. After 5 minutes exposure time virus reduction exceeded 4 $\log_{10}$-steps. Therefore, a virucidal activity against BVDV was measured as follows:

undiluted 5 minutes

Dr. J. Steinmann

Appendix Table 1: Raw data (BVDV) of Divodes FG VT 29 (without columns) (2436) (1st assay)

Product	Concentration	Interfering substance	Exposure time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	80.0%	Aqua bidest.	5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			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.
		10.0% FCS	5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			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.
product cytotoxicity	80.0%	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.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control with columns	n.a.	Aqua bidest.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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	4444 4444	4443 0444	0000 0002	0000 0400	0000 0000	n.d.
	n.a.	10.0% FCS	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4040 0440	0400 0400	4000 4000	0000 0000	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 (BVDV) of Divodes FG VT 29 (without columns) (2nd assay) (2514)

Product	Concentration	Interfering substance	Exposure time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	80.0%	Aqua bidest.	5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			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.
		10.0% FCS	5	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			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.
product cytotoxicity	80.0%	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.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control with columns	n.a.	Aqua bidest.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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	4444 4444	4444 4444	0004 0000	0000 0000	0000 0000	n.d.
	n.a.	10.0% FCS	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4400 4444	0000 4400	0000 0000	0000 0000	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 (BVDV) of Divodes FG VT 29 (with columns) (2436)

Product	Concentration	Interfering substance	Exposure time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	80.0%	Aqua bidest.	5	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			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.
		10.0% FCS	5	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			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.
product cytotoxicity	80.0%	Aqua bidest.	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control with columns	n.a.	Aqua bidest.	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4404 3444	0000 0000	0000 0000	0000 0000	n.d.
	n.a.	10.0% FCS	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4044 4444	0400 0040	0000 0000	0000 0000	n.d.
virus control without columns	n.a.	Aqua bidest.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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 (BVDV) of Divodes FG VT 29 (2514)

Product	Concentration	Interfering substance	Exposure time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
product	10.0%	Aqua bidest.	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			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.
		10.0% FCS	5	4444 4444	4444 4444	4444 4444	4444 4444	4444 4440	0000 0000	0000 0000	n.d.	n.d.
			15	4444 4444	4444 4444	4444 4444	4444 4444	4440 0444	0000 0000	0000 0000	n.d.	n.d.
			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.
product cytotoxicity	10.0%	Aqua bidest.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
		10.0% FCS	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control with columns	n.a.	Aqua bidest.	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.a.	10.0% FCS	n.a.	4444 4444	4444 4444	4444 4444	4444 4444	4400 4444	0000 4400	0000 0000	0000 0000	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 (BVDV) of formaldehyde control (20°C) (2514)

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	0440 4000	0040 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	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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 6: Raw data for cell sensitivity (without columns) (2514)

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	4444 4000	0440 0000	0000 0000	0000 0000	0000 0000
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	4444 0044	0000 0000	0000 0000	0000 0000	0000 0000
		1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
product	10.0% FCS	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	0010 4004	0004 0000	0000 0000	0000 0000	0000 0000
		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)