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- Test Report -

Virucidal activity of the nucleic acid preservation product

„DNA/RNA Shield™“

against the *murine parvovirus (MVM)* at 20 °C

- Evaluation of the virucidal activity against *MVM*
using the quantitative suspension test following EN 14476:2017 -

by
PD Dr. Olaf Thraenhart and Dr. Christian Jursch

Study time:	in May 2018
Manufacturer:	Zymo Research Europe GmbH Mühlhauser Str. 9 D-79110 Freiburg, Germany

1. Introduction

The product under test „DNA/RNA Shield™“ represents a multi-purpose product attributed to the DNA/RNA preparation field with a main application in the sample preparation or sample protection of nucleic acid containing specimen. The product is configured as a ready-to-use product and could be applied in a 1:1 ratio with a wide range of different samples at ambient conditions, normally close to 20 °C.

Some of the samples in question could contain disease causative agents which are infectious for humans such as viruses.

2. Aim of the examination

The present examination was carried out in order to demonstrate that the tested product ***DNA/RNA Shield™*** is specifically virucidal active against the ***murine parvovirus***.

In accordance with the requirements of the European regulation EN 14476:2017, a successful testing against a specific test virus is required when the product to be tested is to be validated for a specific virucidal claim. Following the rationale of EN 14476, the *murine parvovirus (MVM)* was selected as the test virus in order to demonstrate a *general virucidal activity*, as it is the case with linen disinfection.

The present test report summarises the data obtained in the corresponding testing.

3. Laboratory

EUROVIR® Hygiene-Labor GmbH, Im Biotechnologiepark TGZ1, D-14943 Luckenwalde, Germany

4. Test procedure

The present testing was performed using the quantitative virucidal suspension test following the EN 14476:2017 *(phase 2, step 1 test according to EN 14885)*.

5. Product sample

Manufacturer	Zymo Research (US)
Application	DNA/RNA transportation medium for any biological sample (nucleic acid preservation)
Product designation	DNA/RNA Shield™
Lot-ID	ZRC 1858 38
Date of manufacture	27.02.2018
Expiry date	02/2023
Characteristics	visual appearance: clear, colourless to yellowish liquid odour: odourless
pH-value	5,0 - 7,0 *(as specified by the manufacturer)*
Storage conditions of tested product sample	at room conditions *(with restricted access)*
Product sample received	01.11.2017

6. Experimental conditions

Test virus	Minute Virus of Mice (MVM) / synonym: Murine Parvovirus *Strain: Crawford*
Detection cells	A9 cells
Product concentration(s)	50% (v/v)
Working solution	undiluted product (ready-to-use product)
Test temperature(s)	T = 20 °C
Exposure time(s)	30 minutes
Interfering substanc(s)	1. clean conditions (cf. EN 14476) 2. dirty conditions (cf. EN 14476) and 3. whole blood *(10% in test)*
Stop of disinfecting activity and/or detoxification method	Main samples: Large Volume Plating (LVP) Controls: immediate limiting dilution, w/o detoxification
Study period	in May 2018
Test end	17.05.2018 *(release date of the exp. testing)*

7. Material

7.1 Cell culture media and reagents

- Dulbecco's Minimum Essential Medium (DMEM, Biochrom AG, Katalog-Nr. F 0435)
- L-Glutamin (Biochrom AG, Katalog-Nr. K 0283)
- Penicillin/Streptomycin-Lsg. (Biochrom AG, Katalog-Nr. A 2213)
- Foetales Kälberserum (FKS, Biochrom AG, Katalog-Nr. S 0115)
- PBS (Biochrom AG, Katalog-Nr. L 1820)
- BSA (Carl Roth GmbH & Co. KG, Cohn-Fraction V, Katalog-Nr. T844.2)
- Formaldehyde-Sol. 4% (Carl Roth GmbH & Co. KG, Katalog-Nr. 3105.1)
- Blood from Sheep, defibrinated (Acila GmbH, Art-Nr.: 2101051)
- Trypsin (Invitrogen GmbH, Katalog-Nr. 25300-096)

7.2 Test virus

- Test virus: murine parvovirus (MVM; Minute Virus of Mice), strain: Crawford
- Origin: Paul Ehrlich-Institute, Langen (cell culture supernatant v. 05.11.1997, corresponding to the 6. virus passage; kindly provided at 23.06.2006)
- Virus material used: virus propagation MVM #2 v. 02.10.2006, using A9 cells. This virus material is equivalent to the 7. virus passage ([PEI (+6)] +1). For recovery of the virus material the cell cultures were freezed and thawed twice. Afterwards the cell detritus was removed by low speed centrifugation.

7.3 Detection cells

- Cells: A9 cells
- Origin: Paul Ehrlich-Institut, Langen (cell culture dated 19.06.2006, received with passage no. 11, kindly provided at 23.06.2006.
- Cells used: passage [PEI (#11)] / + 5 / + 6. The cells were regularly checked for morphological alterations and no morphological alterations have been observed.

7.4 Equipment and consumable

- CO_2-Incubator, Model: CB 210 and CB 150 (Binder GmbH)
- Working bench, Model: Safe 2010/1800 (Heto-Holten A/S)
- Laboratory shaker, Model: MS2 Minishaker (IKA®-Werke GmbH & Co. KG)
- pH-meter, Model: inoLab Level 1 (WTW GmbH)
- Centrifuge, Model: Megafuge 1 OR (Heraeus GmbH)
- Reversed microscope, Model: Axiovert 40 C (Carl Zeiss Microscopy GmbH)
- Centrifuge, Model: L-15 (Sigma Laborzentrifugen GmbH)
- Water bath, Model: 1003 (GFL - Gesellschaft für Labortechnik mbH)
- Single- and multichannel pipettes, div. models (Gilson Inc. and Eppendorf AG)
- 96-well microtiter plates (TPP Techno Plastic Products AG)
- Cell culture flasks (TPP Techno Plastic Products AG)
- screw cab test tube 15 mL, Artikel-Nr. 60.732.001 (Sarstedt AG, Nümbrecht)
- Cool-/heating metal block, Model: HC-1V-10-60 (VLM GmbH, Bielefeld)

8. Methods

8.1 Preparation of the test virus suspension

A9 cells were cultured with DMEM/5% FCS in a 150 cm² cell culture flask. For virus propagation the medium was removed and MVM virus material (PEI original virus [PEI +0]) was added to the cell monolayer. After 1 hour at 37° C cell culture medium was supplemented. After showing a complete cytopathic effect the cells were freezed/thawed twice in order to recover the virus. The cell detritus was removed by low speed centrifugation. The virus material was aliquoted and stored at -70° C.

8.2 Preparation of the product working solution

The product under test *DNA/RNA Shield™* is designed as a ready-to-use product. According to manufacturer the product was introduced into test with a concentration of 50% (v/v).

8.3 Preparation of the test mixture

All reagents were tempered at 20 °C. 1 volume of the interfering substance and 1 volume of the virus suspension were mixed with 3 volumes of medium. Then, 5 volumes of neat product was added, starting virus inactivation. Afterward the samples were incubated for 30 min. at 20 °C.

8.4 Virus recovery

After the exposure time was due the main samples were diluted immediately with VF = 100 with cell culture medium. Afterwards, a 80.000-fold dilution was prepared with 2 subsequent dilution steps. Virus titration was performed using the Large Volume Plating methodology (LVP). Parallel assays were also run in which the virus titer was estimated by the limiting dilution method and without detoxification.

8.5 Controls

In addition to the virus inactivation tests the following control tests were conducted (according to EN 14476):

1. Virus control(s): test mixture as with the inactivation tests but with A. bidest instead of the product solution. This sample represents the reference point (virus input) for estimation of the virus reduction.

2. Cytotoxicity control(s): test mixture as with the inactivation tests but with medium instead of test virus suspension.

3. After-effect control test: test mixture as with the cytotoxicity controls. After incubation, 10 µL of the virus suspension was added to the first dilution step (lg -0,7) of the dilution series used for virus titration (limiting dilution method). After the average handling time (1 min.) was due the corresponding virus titration was completed.

4. Interference control/susceptibility test: test mixture as with the cytotoxicity controls (medium instead of virus). These samples were then diluted in cell culture medium (VF = 80.000) and distributed to detection cells as with the main virus inactivation samples (cf. point 5.5.4.3; Large Volume Plating [LVP]). Subsequently, a comparing virus titration was performed a) on the cells treated with product and b) in parallel on untreated cells.

5. Cell control: with the titrations a cell control (cells with medium only) was conducted.

6. Reference test: test mixture as with the inactivation tests but with 0,7% Formaldehyde instead of the product solution and PBS as the interfering substance.

8.6 Virus titration and calculation of the virus titer

Virus titration was performed on the basis of virus infectivity or the virus induced cytopathic effect (CPE), respectively. The detection cells served as the substrate for the virus. The cell cultures were held on 37° C and 5 % CO_2 for 14 days. Afterwards, the virus positive cell culture units were identified visually by CPE using an inverted microscope.

With the control samples as well as with the additional main samples virus titration was performed by quantal limiting dilution technique (end-point titration). The sample volume was 0,1 mL and the dilution factor VF = 5. Calculation of the virus titer as well of its 95 %-confidence interval was carried out according to the EN 14476:2017.

With the main samples a high product-associated cytotoxicity could be expected in combination with low amounts of residual virus. Therefore, estimation of residual virus was performed with these samples by using the LVP-method (cf. point 5.5.4.3). Immediately after exposure a test sample of the test mixture was diluted with cell culture medium (cf. point 8.4). The resulting test sample was then completely distributed to cell culture units with 0,2 mL per well.

In the case that residual virus was detectable the virus titer was calculated using the Taylor-Formula. In the case that no residual virus could be detected in the test sample the virtual virus titer was calculated by using the (modified) Poisson-Formula (7). Calculation of the virtual virus titer was carried out with V = 0,3 mL and v = 0,0012 mL (single sample) or V = 0,6 mL and v = 0,0024 mL (both samples).

8.7 Calculation of the virus reduction factor

Calculation of the virus reduction factor (RF) as well as of its 95 %-confidence interval was performed according to the EN 14476:2017.

8.8 Scale of testing

For assessment of the virucidal efficacy of the procedure under test one complete test run was performed, containing either the virus inactivation samples and all controls as outline above.

8.9 Judgement of the test results

The judgement of the virucidal efficacy as well as the assessment of the validity of the test run was conducted according to the acceptance criteria of the EN 14476:2017.

9. Test results - Section 1: control of test validity

9.1 pH of test samples

The pH of the product was not measured because the used pH electrode was designed for measurement of aqueous solutions only and is affected by high concentration of surface-active substances (e.g. as quaternary ammonium compounds).

9.2 Visible product associated cytotoxicity (without detoxification)

With the cytotoxicity test it was shown that the main test samples were accompanied with a very high product associated cytotoxicity. Cytotoxicity was titrated to lg TD_{50} = 6,95/mL with 50% of product (cf. Tab. 2).

9.3 Influence of the disinfectant procedure on the susceptibility of the detection cells

With the main samples virus titration was performed using the LVP method. The corresponding detection cells which were inoculated with the different test mixtures containing the tested product remained completely susceptible for the test virus. The threshold value Δ lg $ID_{50} \leq 1,0$ as specified with the EN 14476:2017 was fulfilled with all tested product concentrations and protein loads (cf. Tab 3).

9.4 Test of an after-effect

This test was relevant only for the virus titration using the limiting dilution technique. With the after-effect test the test virus was added into the first dilution step (lg -0,7) of the dilution series used for virus titration. After addition of the inactivation mixture the sample was incubated at room temperature for another 60 sec. (maximum handling time). Afterwards, virus titration was completed.

Even with the neat product no ongoing disinfecting activity was recorded (Δ lg $ID_{50} \leq 0,5$). With the two control dilutions the same result was obtained (cf. Tab 4; cf. section 11).

9.5 Virus-input (virus control)

With 10% whole blood the titer of the input virus control amounted to lg ID_{50} = 7,65 ± 0,32/mL. Under dirty- or clean conditions the titer of input virus amounted to lg ID_{50} = 7,39 ± 0,29/mL or to lg ID_{50} = 7,22 ± 0,29/mL, respectively (cf. Tab. 5).

9.6 Maximum detectable virus reduction factor

Virus reduction is calculated by input amount of virus (titer of virus control) minus residual amount of virus. When no viable virus is remaining the (virtual) virus titer is given by the lower detection limit of detection system.

9.6.1 Virus titration by limiting dilution (end-point titration)

When the limiting dilution method was used for virus titration the maximum detectable virus reduction amounted to $RF_{max} < 1$ due to the extraordinary high product associated cytotoxicity of lg TD_{50} = 6,95/mL (cf. Tab. 6).

9.6.2 Virus titration by Large Volume Plating

When the Large Volume Plating method was applied the maximum detectable virus reduction amounted to RF_{max} = 4,4 (10% blood), to RF_{max} = 4,1 (dirty conditions) and to RF_{max} = 4,0 (clean conditions) (cf. Tab. 6).

It could be summarised that with the introduction of the LVP titration method into the present testing the determination of virus reduction of 4 log could be achieved in the experiments.

9.7 Virus inactivation with the reference procedure

When 0,7% Formaldehyde was introduced into test the virus reduction at T = 20 °C was quantified after t = 30 min. to RF = 0,52, after t = 60 min. to RF = 0,52 and after t = 120 min. to RF = 1,05 (cf. Tab. 7).

10. Test results - Section 2: Virus inactivation by the product(s) under test

10.1 Virus titration with the limiting dilution technique

When the limiting dilution titration method was applied virus detection was almost completely inhibited by the extraordinary high product associated cytotoxicity. For calculation of the virus reduction a given susceptibility of the cells with the first dilution step was assumed where the cells were not-visibly associated with cytotoxicity. However, this is not necessarily given actually.

10% whole blood: With 50% of product and after 30 min. of exposure no residual virus was detected with both test samples above the toxicity level. The virtual virus titer equals the detection limit and amounted to lg $ID_{50} \leq$ 6,95/mL (cf. Tab. 8). This result was equivalent to RF $\geq$ 0,7 ± 0,32 (cf. Tab. 10).

dirty conditions: Under dirty conditions no residual virus was detected above the toxicity level as well ($ID_{50} \leq$ 6,95/mL; cf. Tab. 8). The corresponding virus reduction was determined to RF $\geq$ 0,4 ± 0,29 (cf. Tab. 10).

clean conditions: And under clean conditions the same result was obtained. With no residual virus above the cytotoxicity level ($ID_{50} \leq$ 6,95/mL; cf. Tab. 8) The corresponding virus reduction amounted to RF $\geq$ 0,3 ± 0,29 (cf. Tab. 10).

10.2 Virus titration by Large Volume Plating

10% whole blood: When 50% of product was introduced to the test no residual test virus was detected in the inoculated cell cultures with both redundant test samples. With 0 virus positive cell culture units out of 960 cell culture unites inoculated with the 80.000-fold diluted test sample the virtual virus titer was calculated to lg $ID_{50} \leq$ 3,26/mL when the modified Poisson-formula was applied (cf. Tab. 9). This was equivalent to the virus reduction RF $\geq$ 4,4 ± 0,32 (cf. Tab. 11).

dirty conditions: Under dirty conditions and with 50% of product 0 cell culture units became virus positive out of 960 cell culture units inoculated with the 80.000-fold diluted test sample. Using the modified Poisson-formula the virus titer amounted to lg $ID_{50} \leq$ 3,26/mL (cf. Tab. 9). Correspondingly, the virus reduction was equivalent to RF $\geq$ 4,1 ± 0,29 (cf. Tab. 11).

clean conditions: Under clean conditions and with 50% of product again 0 cell culture units became virus positive out of 960 cell culture units inoculated with the 80.000-fold diluted test sample. Using the modified Poisson-formula the virus titer amounted also to lg $ID_{50} \leq 3{,}26$/mL (cf. Tab. 9) and correspondingly, the virus reduction was equivalent to RF $\geq 4{,}0 \pm 0{,}29$ (cf. Tab. 11).

11. Validity of the testing (according to the EN 14476:2017)

The test is considered to be valid when the following criteria are met:

a) The titer of the test virus enables the detection of a virus reduction of at least 4 Log.

⇒ With the limiting dilution method the maximum detectable virus reduction amounted to $RF_{max} = 0{,}7$ when the neat product was introduced with a 1:1 ratio in the test (cf. Tab. 6).

⇒ When the LVP method was applied the maximum detectable virus reduction amounted to $RF_{max} = 4{,}4$ to 4,0 (cf. Tab. 6). In conclusion, a virus reduction over at least 4 Log could be determined with the LVP virus titration method.

b) The inactivation of the actually used test virus by the reference substance (0,7% Formaldehyde) at 20 °C was not different from the default value(s) as specified in the EN 14476:2017.

⇒ Tenacity of the actually used test virus is compatible with the default values as specified with EN 14476:2017. In addition, the data are compatible with the results obtained in a series of comparable tests (cf. Tab. 7).

c) The product associated cytotoxicity did not influence cell morphology and/or cell growth. Or these influences are correspondingly small to enable detection of a reduction of 4 Log.

⇒ limiting dilution method w/o detoxification: the extraordinary high product associated cytotoxicity has significantly influenced cell morphology and cell growth (lg $TD_{50} = 6{,}95$/mL; cf. Tab. 2). As a consequence, a 4 Log reduction could not be demonstrated when the limiting dilution method was applied.

⇒ Large Volume Plating (detoxification by dilution; VF = 80.000): cell morphology and cell growth was not influenced (cf. Tab. 9) and could be assumed as maintained.

d) The susceptibility test exhibits no decrease of susceptibility of the detection cells for the used test virus (Δ lg $ID_{50} \leq 1{,}0$).

⇒ Large Volume Plating: with the susceptibility test it was shown that the susceptibility of the cells was maintained with all tested product concentrations (cf. Tab. 3).

e) A negligible / non-existent after-effect of the disinfectant can be assumed if Δ lg $ID_{50} \leq 0{,}5$.

⇒ limiting dilution method and w/o detoxification: with the after-effect test it has shown that no ongoing disinfecting activity was evident with the 5-fold dilution (lg -0,7) of the initial test mixture (cf. Tab. 4).

⇒ Large Volume Plating: with the LVP method the initial test mixture was immediately diluted 80.000-fold (lg -4,9) after the exposure time was due. With this dilution unprotected cells were not affected. Therefore, any after-effect could be excluded with LVP titration.

Accordingly, the acceptance criteria of the EN 14476:2017 are met. Thus, the present examination can be considered as valid.

12. Summary of the test results

The product under test „DNA/RNA Shield™“ represents a multi-purpose product attributed to the DNA/RNA preparation field with a main application in the sample preparation or sample protection of nucleic acid containing specimen. Some of these samples could contain viruses which are infectious for humans.

The present examination using the *murine parvovirus* as the test virus was carried out following the EN 14476:2017 in order to demonstrate that the tested product ***DNA/RNA Shield™*** is specifically virucidal active against the ***parvoviruses***.

12.1 Validation experiments

With the present testing the product associated cytotoxicity was reduced only by the dilution technique. Other methods of detoxification were not applied.

It was shown from the control experiments that the validity of the test system was ensured (cf. point 11).

The selected dilution used for virus titration (LVP) did not induce a cytotoxic effect to the detection cells and the cells remained fully susceptible for an infection with the test virus (cf. Tab. 3).

In addition, by using the LVP method an ongoing disinfecting activity of the products could be excluded (cf. point 11).

With respect to tenacity the actual used test virus complies with expectations (cf. Tab. 7).

The virucidal efficacy of the product under test could not be demonstrated with the quantal endpoint titration method (cf. Tab. 2 and 6). But with the LVP titration method the extraordinary high product associated cytotoxicity was counteracted and a virus reduction factor of 4 Log could be demonstrated (cf. Tab. 6).

12.2 Virus inactivation

Under the test conditions specified above the tested product was virucidal active against the *murine parvovirus* at 20 °C within an exposure time of t = 30 min. with 50% of product.

With 10% whole blood as the interfering substance no residual virus was detected and the virus reduction amounted to RF $\geq$ 4,4 ± 0,32 (cf. Tab. 11) demonstrating a virucidal activity.

Under „dirty conditions“ a virucidal activity was demonstrated as well (RF $\geq$ 4,1 ± 0,29 [cf. Tab. 11]).

And also under „clean conditions“ a virucidal activity was demonstrated (RF $\geq$ 4,0 ± 0,29 [cf. Tab. 11]).

13. Assessment of the test results

According to the results obtained with the present examination a virucidal activity against the ***murine parvovirus*** could be demonstrated for the product under test the ***DNA/RNA Shield™***. This virucidal activity has been demonstrated within 30 minutes at 20 °C with 10% of whole blood as well as under „dirty conditions“ and under „clean conditions“.

Therefore, the criteria for claiming a specific virucidal activity against the *murine parvovirus* following the EN 14476:2017 are fulfilled.

The relevant parameters for inactivation of the *murine parvovirus* are as follows:

Concentration(s):	*DNA/RNA Shield™* (neat product in a 1:1 ratio to specimen)
Exposure time:	30 minutes
Temperature:	20 °C

Luckenwalde, 1st of June 2018

Dr. Christian Jursch
(Managing Director of Eurovir)

14. Literature

1. DIN EN 14885

2. DIN EN 14476/A2:2017-01 (Version 2017-01)
   (simplified in the text as: EN 14476:2017)

3. Leitlinie der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) e.V. und des Robert Koch-Instituts (RKI): Prüfung of chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren in der Humanmedizin. Fassung vom 1. Dezember 2014. Bundesgesundheitsblatt (2015); 58: 493-504

4. Spearman, C.: The method of “right and wrong cases” (“constant stimuli”) without Gauss’s formulae. British Journal of Psychology (1908); 2: 227-242

5. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche [A contribution to the collective treatment of a pharmacological experimental series]. Archiv für experimentelle Pathologie und Pharmakologie (1931); 162: 480-483

6. Note for Guidance on quality of biotechnological product: viral safety evaluation of biotechnology product derived from cell lines of human or animal origin - CPMP/ICH/295/95; Appendix 3: statistical considerations for assessing virus assays; http:// www.ema.europa.eu

7. BioPharm international.com (2013): Process Development and Spiking Studies for Virus Filtration of r-hFSH; http://www.biopharminternational.com/process-development-and-spiking-studies- virus-filtration-r-hfsh

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- Attachment: tables and figures -

Tab. 1: Examined product and its pH in the working solution

Product(s)	Interfering Substance	Dosage	Dosage in test sample	pH of working solution
DNA/RNA Shield™	Sheep Blood	1 vol. of interfering substance + 1 vol. of virus suspension 3 vol. of medium plus 5 vol. of neat product (ready-to-use-product)	50%	n.d.
	dirty conditions *(acc. to EN 14476)*		50%	n.d.
	clean conditions *(acc. to EN 14476)*		50%	n.d.

Tab. 2: Visible cytotoxic effect of the product(s) on the detection cells / cytotoxicity without detoxification *(Titration by limiting dilution)*

Product(s)	Product conc. *(in test)*	Exposure time	Interfering Substance	Test-run	Dilution factor (lg) / VF = 5										Titer per 100 µL (lg TD_{50})	Titer per 1 mL (lg TD_{50})
					-0,7	-1,4	-2,1	-2,8	-3,5	-4,2	-4,9	-5,6	-6,3	-7,0		
DNA/RNA Shield™	50%	30 min.	Sheep Blood	V1	4/4 [1]	4/4	4/4	4/4	4/4	4/4	4/4	4/4	mV	0/4	5,95	6,95
	50%	30 min.	dirty conditions	V1	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	mV	0/4	5,95	6,95
	50%	30 min.	clean conditions	V1	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	mV	0/4	5,95	6,95

[1] = first number: number of cell cultures with a visible cytotoxic alteration; second number: total number of cell cultures

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Tab. 3: Virus susceptibility of the detection cells when the virus titration by Large Volume Plating was used *(Titration by limiting dilution)*

Test sample(s)	Interfering Substance	Product conc. *(in test)*	Dilution factor [1]	Dilution (lg) / VF = 5											Titer per 100 µL ($lg\ ID_{50}$)	Δ Titer [3] ($lg\ ID_{50}$)	Cells susceptible [4]
				-0,7	-1,4	-2,1	-2,8	-3,5	-4,2	-4,9	-5,6	-6,3	-7,0	-7,7			
1st test run (V1)																	
untreated cells	n.a.	w/o	n.a.	16/16	16/16	16/16	16/16	16/16	16/16	7/16	3/16	0/16			4,99 ± 0,23	-	-
DNA/RNA Shield™ (treated cells)	Sheep Blood	50%	VF = 80.000	8/8 [2]	8/8	8/8	8/8	8/8	8/8	6/8	1/8	1/8	0/8		5,25 ± 0,34	-0,26 ± 0,41	yes
	dirty conditions	50%	VF = 80.000	8/8	8/8	8/8	8/8	8/8	8/8	4/8	0/8				4,90 ± 0,26	0,09 ± 0,35	yes
	clean conditions	50%	VF = 80.000	8/8	8/8	8/8	8/8	8/8	8/8	5/8	0/8				4,99 ± 0,26	0,0 ± 0,34	yes

[1] = dilution (or dilution factor) of the test sample(s) distributed to the detection cells when the *Large Volume Plating* (LVP) method was used
[2] = first number = number of virus positive cells cultures, second number = total number of cell cultures
[3] = virus titer A (virus titration on untreated cells) minus virus titer B (virus titration on treated cells)
[4] = according to EN 14476:2017 a virus susceptibility of the detection cells applies as given when Δ Titer is $\leq$ lg 1,0

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Tab. 4: Control test - stop of the residual disinfecting activity (after-effect test) *(Titration by limiting dilution)*

Test sample(s)	Interfering Substance	Product conc. *(in test)*	Dilution factor [1]	Dilution (lg) / VF = 5											Titer per 100 µL (lg ID_{50})	Δ Titer [3] (lg ID_{50})	After-effect present [4]
				-0,7	-1,4	-2,1	-2,8	-3,5	-4,2	-4,9	-5,6	-6,3	-7,0	-7,7			
1st test run (V1)																	
untreated cells	n.a.	w/o	n.a.	16/16	16/16	16/16	16/16	16/16	16/16	16/16	10/16	3/16	2/16	0/16	5,92 ± 0,25	-	-
DNA/RNA Shield™ (treated cells)	Sheep Blood	50%	VF = 5	tox	tox	tox	tox	tox	tox	tox	6/8	3/8	0/8		6,04 ± 0,34	-0,13 ± 0,43	no
	dirty conditions	50%	VF = 5	tox	tox	tox	tox	tox	tox	tox	6/8	1/8	0/8		5,87 ± 0,29	0,05 ± 0,38	no
	clean conditions	50%	VF = 5	8/8	tox	tox	tox	tox	tox	tox	7/8	2/8	1/8	0/8	6,13 ± 0,34	-0,21 ± 0,42	no

[1] = the test virus was added into the first dilution (lg -0,7) step of the corresponding dilution series immediately before addition of the test mixture. This is corresponding to VF = 5; in contrast, with the virus titration using the LVP method the test samples were diluted with VF = 80.000.
[2] = first number = number of virus positive cells cultures, second number = total number of cell cultures
[3] = virus titer A (sample with product) minus virus titer B (sample without product)
[4] = according to the EN 14476:2017 an ongoing residual disinfecting activity (after effect) of the product(s) applies as not given when Δ Titer is ≤ lg 0,5

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Tab. 5: Titration of the virus input (virus control) [1] *(Titration by limiting dilution)*

Test sample(s)	Interfering Substance	Dilution (lg) / VF = 5											Titer per 100 µL (lg ID_{50})	Ø Titer per 1 mL (lg ID_{50})
		-0,7	-1,4	-2,1	-2,8	-3,5	-4,2	-4,9	-5,6	-6,3	-7,0	-7,7		
1st test run (V1)														
Virus control	Sheep Blood	4/4 [1]	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	2/4	0/4	6,83 ± 0,53	7,65 ± 0,32
		4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4		6,48 ± 0,35	
Average		8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	6/8	2/8	0/8	6,65 ± 0,32	
Virus control	dirty conditions	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	3/4	0/4		6,30 ± 0,49	7,39 ± 0,29
		4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4		6,48 ± 0,35	
Average		8/8	8/8	8/8	8/8	8/8	8/8	8/8	7/8	6/8	8/8		6,39 ± 0,29	
Virus control	clean conditions	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4	1/4	0/4	6,13 ± 0,35	7,22 ± 0,29
		4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	0/4		6,30 ± 0,40	
Average		8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	2/8	1/8	0/8	6,22 ± 0,29	

[1] = test sample with 8 VT A. bidest instead of product(s). The corresponding amount of virus represents the reference point for determining of the virus reduction (virus input).
[2] = first number = number of virus positive cells cultures, second number = total number of cell cultures

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Tab. 6: Titer of virus control and maximum detectable virus reduction

Test virus	Interfering Substance	Product conc. *(in test)*	Dilution [1] factor	Virus titer per 1 mL [1] *[lg ID_{50} ± $KI_{95\%}$]*	detection limit *[lg ID_{50} / mL]*		max. detectable virus reduction *(RF_{max})* [2]	
Minute Virus of Mice (MVM)	Virus titration using the limiting dilution method (Spearman & Kärber)							
	Sheep Blood	50%	VF = 5	7,65 ± 0,32	lg ID_{50} = 6,95		0,7	
	dirty conditions	50%	VF = 5	7,39 ± 0,29	lg ID_{50} = 6,95		0,44	
	clean conditions	50%	VF = 5	7,22 ± 0,29	lg ID_{50} = 6,95		0,27	
	Virus titration by Large Volume Plating (LVP) inoculating:				480 cell cultures [3]	960 cell cultures [4]	480 cell cultures [3]	960 cell cultures [4]
	Sheep Blood	50%	VF = 80.000	7,65 ± 0,32	lg ID_{50} = 3,56	lg ID_{50} = 3,26	4,09	4,4
	dirty conditions	50%	VF = 80.000	7,39 ± 0,29	lg ID_{50} = 3,56	lg ID_{50} = 3,26	3,83	4,1
	clean conditions	50%	VF = 80.000	7,22 ± 0,29	lg ID_{50} = 3,56	lg ID_{50} = 3,26	3,66	4,0

[1] = dilution (or dilution factor) of the test sample(s) distributed to the detection cells when the *Large Volume Plating* (LVP) method was used
[2] = input virus (virus control), cf. Tab. 5
[2] = maximum detectable virus reduction (RF_{max}) when no residual virus was detectable. With LVP the detection limit was calculated with the modified Poisson-Formula (cf. Ref 7).
[3] = Per single test sample: 480 cell culture units were inoculated; V = 0,3 mL and v = 0,0012 mL.
[4] = With both redundant test samples: 960 cell culture units were inoculated; V = 0,6 mL and v = 0,0024 mL.

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Tab. 7: Virus reduction with the reference procedure: 0,7 % Formaldehyde at T = 20° C *(Titration by limiting dilution)*

Test sample(s)	Exposure time	Dilution (lg) / VF = 5										Titer / 100 µL [2] (lg ID_{50})	Reduction factor (RF) [3]	Comparable data MVM [4] (n = 11)	Pre set value (EN) [5]
		-0,7	-1,4	-2,1	-2,8	-3,5	-4,2	-4,9	-5,6	-6,3	-7,0	-7,7				
Virus inactivation Murine Parvovirus	30 min.	4/4	4/4	4/4	4/4	4/4	4/4	2/4	2/4	0/4			5,25 ± 0,57	0,52 ± 0,67	0,48 ± 0,35 Range: 0,0 - 1,05	0 - 2,0
	60 min.	4/4	4/4	4/4	4/4	4/4	4/4	4/4	0/4				5,25 ± 0,0	0,52 ± 0,35	0,73 ± 0,21 Range: 0,35 - 1,05	0,5 - 2,5
	120 min.	4/4	4/4	4/4	4/4	4/4	4/4	0/4	1/4	0/4			4,73 ± 0,35	1,05 ± 0,49	1,56 ± 0,34 Range: 0,88 - 2,1	-

[1] = first number = number of virus positive cells cultures, second number = total number of cell cultures.
[2] = virus titer per 100 µL, used for calculation of virus reduction
[3] = titer of virus control minus titer of test sample
[4] = Comparable data obtained with the same virus material (Eurovir tests; n = 11)
[5] = pre set value, as given by the EN 14476

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Tab. 8: Inactivation of *MVM* by *DNA/RNA Shield™* at T = 20 °C - virus titration by limiting dilution [S&K] and without detoxification

Product(s)	Test run	Interfering Substance	Exposure time	Product conc. *(in test)*	Dilution (lg) / VF = 5										Titer per 100 µL (lg ID_{50})	Ø Titer per 1 mL (lg ID_{50})
					-0,7	-1,4	-2,1	-2,8	-3,5	-4,2	-4,9	-5,6	-6,3	-7,0	-7,7	
DNA/RNA Shield™	V1	Sheep Blood	30 min.	50%	Tox	Tox	Tox	Tox	Tox	Tox	Tox	mV	0/4			≤ 5,95	≤ 6,95 [3]
					Tox	Tox	Tox	Tox	Tox	Tox	Tox	mV	0/4			≤ 5,95	
		dirty conditions	30 min.	50%	Tox	Tox	Tox	Tox	Tox	Tox	Tox	mV	0/4			≤ 5,95	≤ 6,95 [3]
					Tox	Tox	Tox	Tox	Tox	Tox	Tox	mV	0/4			≤ 5,95	
		clean conditions	30 min.	50%	Tox	Tox	Tox	Tox	Tox	Tox	Tox	mV	0/4			≤ 5,95	≤ 6,95 [3]
					Tox	Tox	Tox	Tox	Tox	Tox	Tox	mV	0/4			≤ 5,95	

[1] = cytotoxic reaction of the detection cells. Therefore, a sensitive virus detection was inhibited completely or at least not ensured.
[2] = first number = number of virus positive cells cultures, second number = total number of cell cultures.
[3] = for estimation of the virus titer a given susceptibility of the cells was assumed with the first dilution step where the cells were not-visibly associated with cytotoxicity. However, this is not necessarily actually given.

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Tab. 9: Inactivation of *MVM* by *DNA/RNA Shield™* at T = 20 °C - virus titration by LVP (cf. EN 14476:2017)

Product(s)	Test run	Interfering Substance	Exposure time	Product conc. *(in test)*	Dilution [1] factor	Morphologic alteration with the non-virus-positive cell cultures	Analysed test sample			
							analysed sample volume	cell cultures inoculated	virus positive cell cultures	Residual virus [per 1 mL] [2]
DNA/RNA Shield™	V1	Sheep Blood	30 min.	50%	VF = 80.000	no	v = 0,0024 mL	960	0 *(of 960)*	lg $ID_{50} \leq 3,26$
		dirty conditions	30 min.	50%	VF = 80.000	no	v = 0,0024 mL	960	0 *(of 960)*	lg $ID_{50} \leq 3,26$
		clean conditions	30 min.	50%	VF = 80.000	no	v = 0,0024 mL	960	0 *(of 960)*	lg $ID_{50} \leq 3,26$

[1] = dilution (or dilution factor) of the test sample(s) distributed to the detection cells when the *Large Volume Plating* (LVP) method was used

[2] = amount of residual test virus of both test samples either calculated using the Taylor-Formula (residual virus detected) or the Poisson-Formula (no residual virus detectable). Calculations were performed according to reference 7 with the following test volumes:
with the undiluted product (50% in test): 960 cell culture units were inoculated; V = 0,6 mL and v = 0,0024 mL.

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Tab. 10: Inactivation of *MVM* by *DNA/RNA Shield™* / virus titration by limiting dilution w/o detoxification - Estimation of the virus reduction factor (RF)

Product(s)	Test run	Exposure-time	Interfering Substance	Product conc. *(in test)*	lg ID_{50}/mL [lg ID_{50} ± $KI_{95\%}$]		Reduction factor [± $KI_{95\%}$]	**Virucidal activity [5] vs. rotavirus**
					Virus input [1]	Residual virus [2]	Virus reduction [4]	
DNA/RNA Shield™	V1	30 min.	Sheep Blood	50%	7,65 ± 0,32	≤ 6,95	≥ 0,7 ± 0,32	**not demonstrated**
	V1	30 min.	dirty conditions	50%	7,39 ± 0,29	≤ 6,95	≥ 0,4 ± 0,29	**not demonstrated**
	V1	30 min.	clean conditions	50%	7,22 ± 0,29	≤ 6,95	≥ 0,3 ± 0,29	**not demonstrated**

[1] = amount of input virus (virus control; cf. Tab. 5)
[2] = amount of residual virus with respect to the cytotoxicity titer (cf. Tab. 8)
[3] = input virus (lg ID_{50}) minus residual virus (lg ID_{50})
[4] = according to the EN 14476:2017 a sufficient virucidal activity applies as given when RF ≥ lg 4,0
[5] = virus reduction factor may be overestimated since virus susceptibility of the detection cells was not tested.

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Tab. 11: Inactivation of *MVM* by *DNA/RNA Shield™* / virus titration by Large Volume Plating (LVP) [1] - Estimation of the virus reduction factor (RF)

Product(s)	Test run	Exposure-time	Interfering Substance	Product conc. *(in test)*	lg ID_{50}/mL [lg ID_{50} ± $KI_{95\%}$]		Reduction factor [± $KI_{95\%}$]	**Virucidal activity [5] vs. MVM**
					Virus input [2]	Residual virus [3]	Virus reduction [4]	
DNA/RNA Shield™	V1	30 min.	Sheep Blood	50%	7,65 ± 0,32	≤ 3,26	≥ 4,4 ± 0,32	**yes**
	V1	30 min.	dirty conditions	50%	7,39 ± 0,29	≤ 3,26	≥ 4,1 ± 0,29	**yes**
	V1	30 min.	clean conditions	50%	7,22 ± 0,29	≤ 3,26	≥ 4,0 ± 0,29	**yes**

[1] = cf. EN 14476:2017
[2] = amount of input virus (virus control) per 1 mL (cf. Tab. 5)
[3] = amount of residual virus (cf. Tab. 9)
[4] = input virus (lg ID_{50}) minus residual virus (lg ID_{50})
[5] = according to the EN 14476:2017 a sufficient virucidal activity applies as given when RF ≥ lg 4,0

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Inactivation of *Murine Parvovirus* by ***DNA/RNA Shield*** - 50% of product (in test)

- Antiviral validation using the quantitative suspension test (EN 14476) -

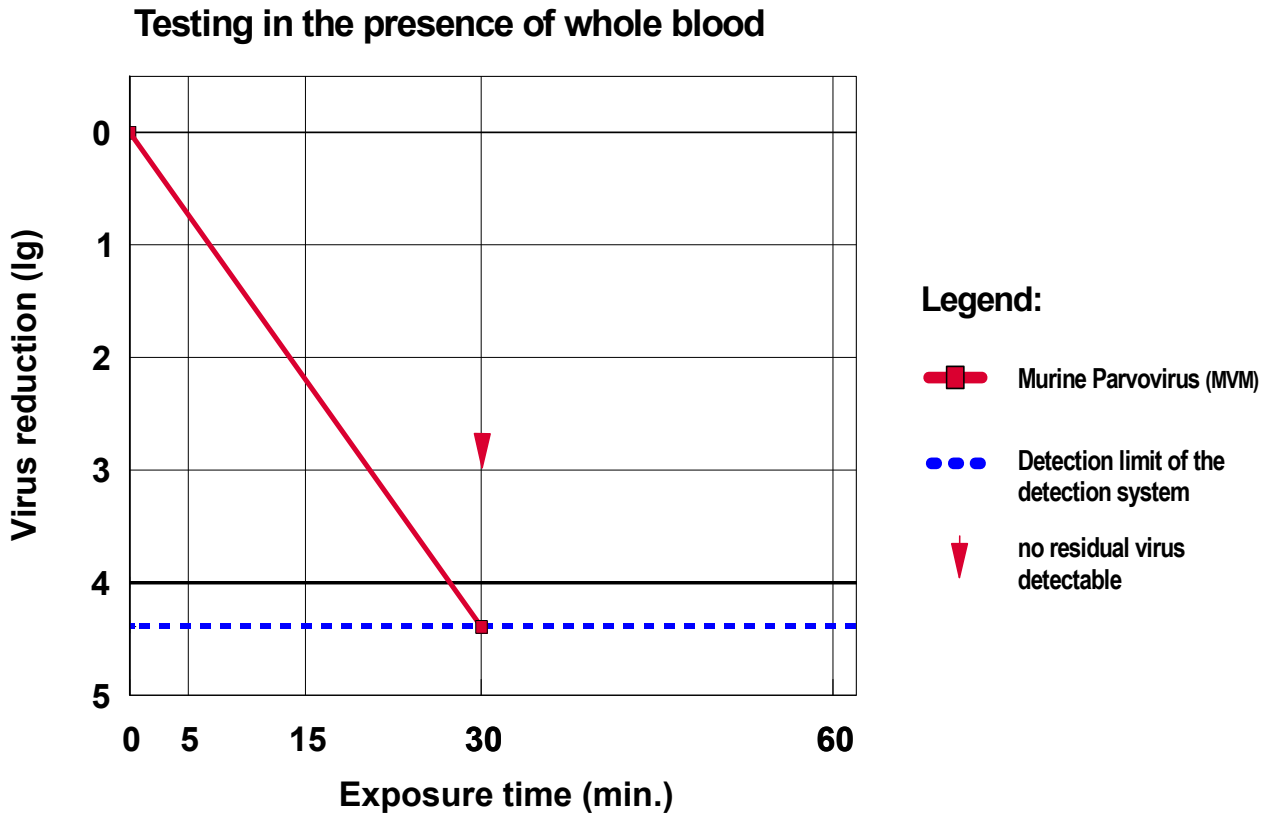

Fig. 1

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Inactivation of *Murine Parvovirus* by *DNA/RNA Shield* - 50% of product (in test)

- Antiviral validation using the quantitative suspension test (EN 14476) -

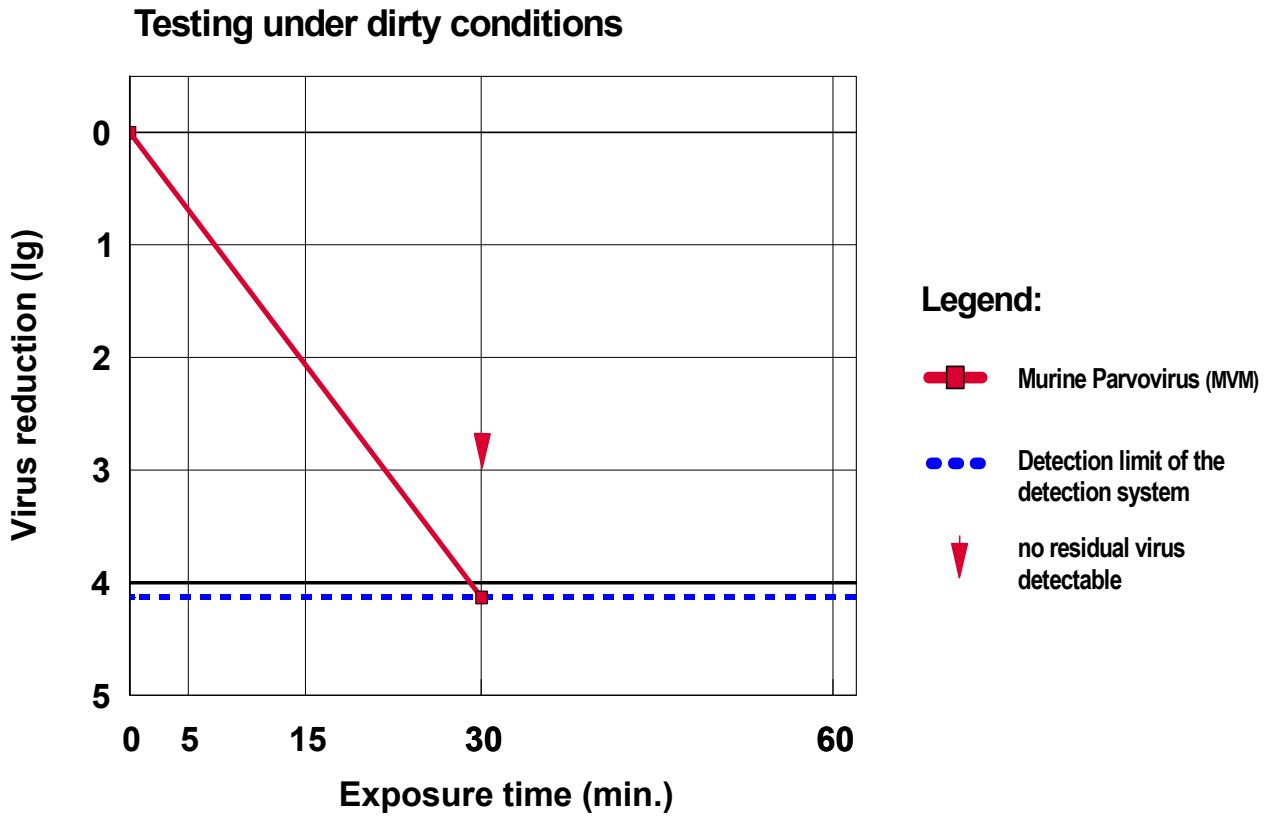

Fig. 2

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Inactivation of *Murine Parvovirus* by ***DNA/RNA Shield*** - 50% of product (in test)

- Antiviral validation using the quantitative suspension test (EN 14476) -

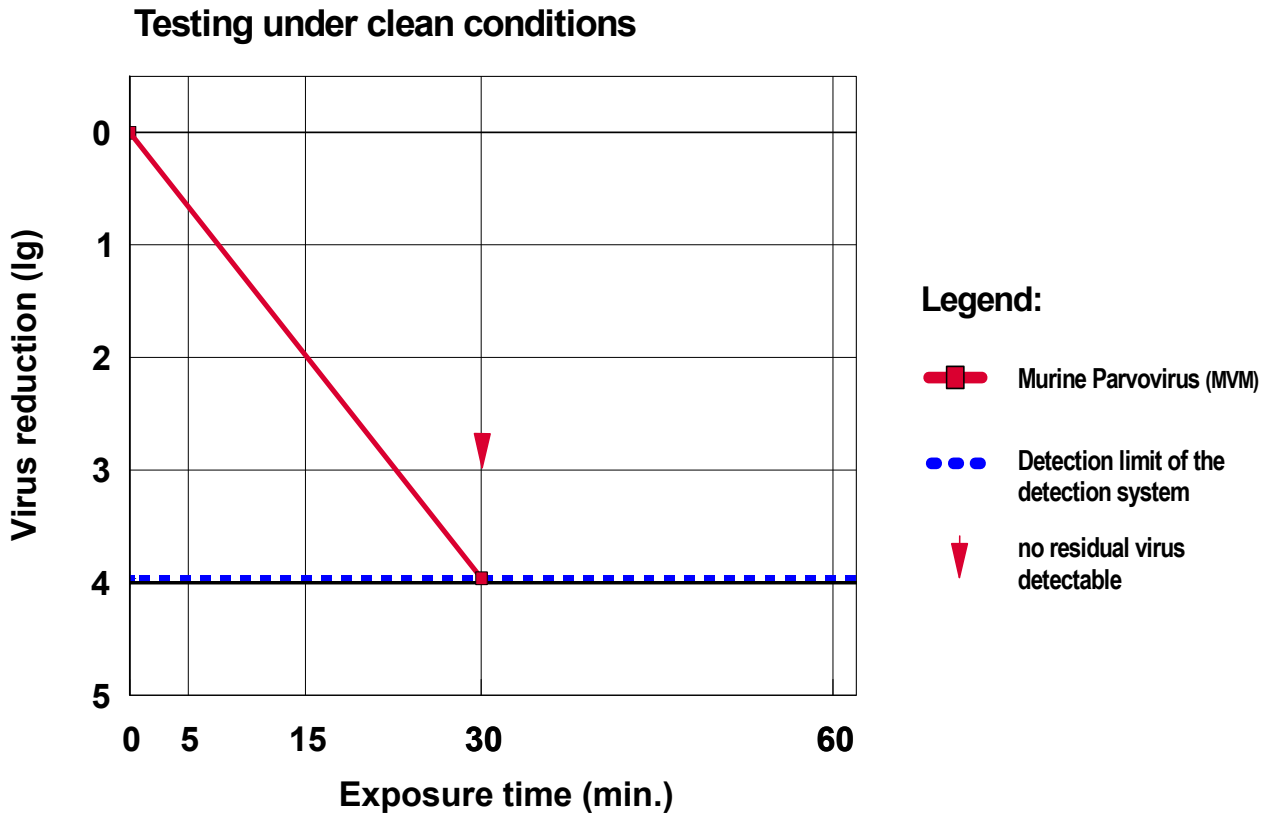

Fig. 3

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Explanation of figures:

to Fig. 1: Inactivation of Murine Parvovirus by DNA/RNA Shield™ at T = 20°C - testing of 50% of product *(in test sample)* ***according to EN 14476:2017 in the presence of whole blood***

In Fig. 1, the virus reduction (lg ID_{50}) is presented in relation to exposure time (sec.). The virus reduction is shown as the red line. The capability of the detection system is represented by the broken blue line. Virus reduction was measurable over a range of 4 Log. The mandatory amount for certification of 4 Log is highlighted by bold-printing.

After an exposure time of **30 min.** at **T = 20 °C no residual virus** could be detected. The correspondent virus inactivation factor (red line) amounted to **RF ≥ 4,4 ± 0,32** when tested in the presence of **whole blood** (10% in the final test sample).

to Fig. 2: Inactivation of Murine Parvovirus by DNA/RNA Shield™ at T = 20°C - testing of 50% of product *(in test sample)* ***acc. to EN 14476:2017 under dirty conditions***

The presentation in Fig. 2 is identical to Fig. 1.

When tested under **dirty conditions** and after an exposure time of **30 min.** at **T = 20 °C no residual virus** could be detected. The correspondent virus inactivation factor (red line) amounted to **≥ 4,1 ± 0,29**.

to Fig. 3: Inactivation of Murine Parvovirus by DNA/RNA Shield™ at T = 20°C - testing of 50% of product *(in test sample)* ***acc. to EN 14476:2017 under clean conditions***

The presentation in Fig. 3 is identical to Fig. 1.

When tested under **clean conditions** and after an exposure time of **30 min.** at **T = 20 °C no residual virus** could be detected as well. The correspondent virus inactivation factor (red line) amounted to **≥ 4,0 ± 0,29**.