eurofins | Product Testing

Tikkurila Oyj
Heidehofintie 2
01300 VANTAA
FINLAND

Eurofins Product Testing A/S
Smedeskovvej 38
8464 Galten
Denmark

CustomerSupport@eurofins.dk
www.eurofins.dk/dk/product-testing

TEST REPORT

04 January 2021

1 Sample Information

Sample name	Argentum 20 A-base, sample 1 / batch 20LLI01
Sample reception	21/08/2020
Sample no.	392-2020-00305201
Analysis period	15/07/2020 - 23/12/2020

2 Picture of Sample

3 Results

Please see enclosure with detailed results.

4 Conclusion

The sample **has antiviral effect** in the adopted experimental conditions tested according to ISO 21702.

Eurofins Product Testing A/S

Jeanette K. Pedersen
Analytical Service Manager

Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

Eurovir® Hygiene-Labor
Antivirale Validierung & Rabies

Testing of the antiviral equipped product

„Argentum 20 A-base“

against the *Bovine Coronavirus (BoCV)* at 25 °C

- Evaluation of the virucidal activity against the *Bovine Coronavirus (S379 Riems)* using the quantitative carrier test according to ISO 21702:2019

- Excerpt from the test report *TeR_Eur-09_181220_BoCV* -

by
PD Dr. Olaf Thraenhart and Dr. Christian Jursch

Study time: in December 2020

Principal: Eurofins Biolab S.r.l.
Via Bruno Buozzi, 2
20090 Vimodrone (MI), Italy

Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

Aim of the testing and performing the test

The antiviral equipped product ***Argentum 20 A-base*** should be tested for its ability to inactivate the ***Bovine Coronavirus***.

To test this feature, test squares (carrier) were coated directly with the original product the *Argentum 20 A-base.* Using this test specimen the test virus material, containing the *Bovine Coronavirus (S379 Riems)* were evenly distributed on the surface of the test specimen and incubated at 25 °C in a climate chamber. After 24 h of incubation the virus material was then recovered from the test carriers and the remaining amount of virus was quantified.

The underlying test was carried out according to ISO 21702:2019.

Test results

The testing of the product *Argentum 20 A-base* according to ISO 21702 and under the described test conditions using the *Bovine Coronavirus* as the test virus has shown that:

1. The conditions given in clause 8.2.2, 8.2.3 and 8.2.4 are satisfied directly. With the introduction of the *Large Volume Plating* titration method, controlled by the flanking susceptibility test an after-effect as outlined with clause 8.2.5 can be excluded. In conclusion, the present testing can be considered as valid.

2. the tested product *Argentum 20 A-base* has shown a high virus inactivating activity. After a contact time of t = 24 hours the virus reduction amounted to RF $\geq$ 5,95 ± 0,24 Log, corresponding to a virus inactivation of more than 99,99%.

Judgement

On the basis of the data obtained it can be concluded that the described antiviral effect on the *Bovine Coronavirus* can clearly be attributed to the effect of the antiviral coating product *Argentum 20 A-base*.

Luckenwalde, 18th of December 2020

Dr. Ch. Jursch
(GF and Laboratory Manager Eurovir)

Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

- Test Report -

Virucidal activity of the antiviral equipped product

„Argentum 20 A-base“

against the *Bovine Coronavirus (BoCV)* at 25 °C

- Evaluation of the virucidal activity against the *Bovine Coronavirus (S379 Riems)* using the quantitative carrier test according to ISO 21702:2019 -

by

PD Dr. Olaf Thraenhart and Dr. Christian Jursch

Study time:	in December 2020
Principal:	Eurofins Biolab S.r.l. Via Bruno Buozzi, 2 20090 Vimodrone (MI), Italy

1. Introduction

The product under test ***Argentum 20 A-base*** represents a product equipped with an antiviral active component.

2. Aim of the examination

The present examination was carried out in order to demonstrate that the tested product *Argentum 20 A-base* is specifically virucidal active against the ***Bovine Coronavirus***. This testing using specimens with the real product incorporated was performed under praxis near conditions according to ISO 21702:2019.

The present test report summarises the data obtained in the testing with the *Bovine Coronavirus (BoCV; strain: S379 Riems)* as the test virus according to the methodology of the norm ISO 21702:2019. The test virus *BoCV* (virus genus *Beta-Coronavirus*) was used as a model virus (non-infectious to humans) for evaluation of a virucidal activity vs. *SARS-CoV-1* and *SARS-CoV-2*.

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 carrier test according to the ISO 21702:2019.

5. Product sample(s)

Principal	Eurofins Biolab S.r.l. (Italy)	
Study-ID	STULV20AA4802-1	
Test items	Test squares coated with the product(s)	control test squares (uncoated)
Product description	test squares prepared from plastic material, coated with product(s)	test squares prepared from plastic material, non-coated
Product designation	*Argentum 20 A-base* *(treated samples)*	*Un-treated Control* *(untreated samples)*
Lot-ID	20LLI01	not indicated *(product samples tested as received)*
Date of manufacture	22.06.2020	not indicated
Expiry date	22.06.2022	not indicated
Characteristics	test squares prepared from plastic material, with a hard, flat surface and with a thickness of 1 mm	test squares prepared from plastic material, with a hard, flat surface and with a thickness of 1 mm
Storage conditions	at room conditions *(with restricted access)*	
Product sample received	18.09.2020 *(in visibly impeccable condition)*	
Provision of test sample(s)	The product samples were selected and provided by the principal of this testing	

6. Experimental conditions

Test virus	Bovine Coronavirus (strain: S379 Riems) [Betacoronavirus]
Detection cells	HRT-18 cells (human rectal carcinoma cells)
Test temperature(s)	T = 25 °C
Humidity	90%
Exposure time(s)	24 hours
Protein load(s)	no additional protein load *(cell culture supernatant was used as the virus source)*
Test square	4 cm x 4 cm = 16 cm²
Virus contamination	400 µL of virus suspension; evenly distributed onto a test area of 4 cm x 4 cm (16 cm²) and covered with PP-foil (110 µm)
Stop of disinfecting activity and/or detoxification method	Main samples: immediate limiting dilution, w/o detoxification Controls: immediate limiting dilution, w/o detoxification
Study period	in December 2020
Test end	17.12.2020 *(release date of the exp. testing)*

7. Material

7.1 Cell culture media and reagents

- Dulbecco's Minimum Essential Medium (DMEM, Biowest, Katalog-Nr. L0101-500)
- L-Glutamin (Biowest, Katalog-Nr. X0550-100)
- Penicillin/Streptomycin-Lsg. (Life Technologies, Katalog-Nr. 15140122)
- Foetales Kälberserum (FKS, Biowest, Katalog-Nr. S1810-500)
- PBS (Life Technologies, Katalog-Nr. 14190169)
- BSA (Carl Roth GmbH & Co. KG, Cohn-Fraction V, Katalog-Nr. T844.2)
- Trypsin (Invitrogen GmbH, Katalog-Nr. 25300054 6)

7.2 Test virus

- Test virus: Bovine Coronavirus (BoCV); strain: S379 Riems
- Origin: Virusbank der BFA f. Viruskrankheiten der Tiere; Friedrich Löffler-Institut, Insel Riems; University of Greifswald, Germany
- kindly provided by Dr. S. Reiche
- Virus material used: cell culture supernatant; virus propagation BoCV/#2 from 21.07.2020 using HRT-18 cells [virus passage: FLI +2].

7.3 Detection cells

- HRT-18 cells (human rectal carcinoma cells)
- Origin: Institut f. Hygiene und Infektionskrankheiten der Tiere; University of Giessen, Germany
- Cells used: passage [Gi +12] / + 7 / + 36 (V1). 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)
- Climate chamber KBF 115 (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)
- Petri dish (PS), d = 94 mm; Artikel-Nr. 633180 (Greiner GmbH)

8. Methods

8.1 Preparation of the test virus suspension

HRT-18 cells were cultured with DMEM/2% FCS in a 150 cm² cell culture flask. For virus propagation the medium was removed and BoCV virus material (FLI original virus [FLI +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 in order to recover the virus. The cell detritus was removed by low speed centrifugation. The virus material was aliquoted and then stored at -70 °C. This material represents the test virus suspension.

8.2 Preparation of the test inoculum

24 volumes of medium were mixed with one volume of the initial virus suspension. With the controls cell culture medium was used without addition of the virus suspension.

8.3 Preparation of the test specimen

Test samples were prepared according to ISO 21702:2019 under the running working bench at a temperature of 22 °C ± 3 °C.

Per test point (concentration/exposure time) 3 redundant test samples were prepared.

The test items with the dimensions of 5 cm x 5 cm were placed into a sterile Petri dish (D = 9,4 cm). 400 µL of virus suspension (or medium with the controls) were transferred onto the test items and were evenly distributed on a test area of 16 cm² (4 cm x 4 cm). Afterwards, a piece of PP-foil (4 cm x 4 cm) was placed onto the virus material in order to generate a homogenous virus film. After preparation the test samples were incubated for 24 h at 25 °C and 90 % r.LF with the petri dishs lid closed.

8.4 Recovery of the test virus

After the exposure time was due 10 mL of cell culture medium were added to the test specimen. Within that medium the cover-foil was washed at least 4 times as well as the test surface. Additionally, the test surface was mechanically treated using a sterile glass spatula.

8.5 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 7 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 = 4. Calculation of the virus titer as well of its 95 %-confidence interval was carried out according to the EN 14476:2019.

With the main samples low amounts of residual virus could be expected. Therefore, estimation of residual virus was performed with these samples by using the LVP-method. Immediately after the exposure was due 5 mL of the resuspension fluid was mixed with 35 mL of cell culture medium (VF = 8; for detoxification). Afterwards, 38,4 mL of the test mixture was distributed to 192 cell culture units with 0,2 mL per well (equivalent to 4,8 mL of the initial resuspension fluid).

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 Poisson-Formula. Because the prerequisite of V >> v (as specified) was not valid with the present testing a modified Poisson-Formula was used (7). With this formula „v“ as well as „V“ is taken into account. Calculation of the virtual virus titer was carried out with V = 10,2 mL and v = 4,8 mL for the single sample or with V = 30,6 mL and v = 14,4 mL when all three samples was taken into account.

8.6 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.

8.7 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.

8.8 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 ISO 21702:2019.

9. Test results

The test results described in the following sections refer exclusively to the product samples under investigation.

9.1 Test section 1 - control of test validity

9.1.1 Test items

A short description of the test items and the test area used is given in Tab. 1.

9.1.2 Visible product associated cytotoxicity (without detoxification)

With the cytotoxicity test it was shown that the test samples were accompanied with no product associated cytotoxicity. Cytotoxicity was titrated to lg $TD_{50} \leq 1{,}30$/mL for all test samples of both products (cf. Tab. 2).

9.1.3 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 resuspension fluid of all test specimens (from the non-treated controls and the treated test squares as well) remained completely susceptible for the test virus. The threshold value Δ lg $ID_{50} \leq 1{,}0$ as specified with the EN 14476 was fulfilled with all test samples (cf. Tab 3).

9.1.4 Control test of an after-effect

With the after-effect test the test virus was added directly into the resuspension medium. The corresponding test mixture was then incubated at T = 25 °C for another 30 min.

With all three test samples of the untreated control (N-4 to N-6) no ongoing disinfecting activity was recorded (average Δ lg $ID_{50} \leq 0{,}5$; cf. Tab. 4).

The same result (no ongoing disinfecting activity; Δ lg $ID_{50} \leq 0{,}5$) was obtained with 2 out of 3 test samples (N-1 and N-2). With the remaining test sample (N-3) Δ lg ID_{50} amounted to 1,2 ± 0,37. With all three test samples taken together Δ lg ID_{50} amounted to 0,65 ± 0,37 (cf. Tab. 4).

9.1.5 Titer of the virus suspension

When 400 µL of the virus suspension was added to 10 mL of the (virus free) medium (used for resuspension) the virus titer of the resulting mixture amounted to lg ID_{50} = 5,55 ± 0,22/mL (average from three test samples, designated Aus-1 to -3; cf. Tab. 5).

9.1.6 Titer of the virus material recovered from the control test item after t = 0

When the virus material from the untreated controls test items was resuspended at t = 0 min. with 10 mL of (virus free) medium the titer of the resulting mixture amounted to lg ID_{50} = 5,55 ± 0,28/mL (average from three test samples; cf. Tab. 5). With relation to the inoculated test area (16 cm²), this corresponds to a virus quantity of 5,35 Log/cm².

When compared with the titer of the initial virus suspension the virus loss due to the handling of the test specimens amounted to RF = 0,0 ± 0,36.

9.1.7 Titer of the virus material recovered from the control test item after t = 24 h

When the virus material from the untreated controls test items was resuspended after t = 24 hours with 10 mL of (virus free) medium the titer of the resulting mixture amounted to lg ID_{50} = 5,30 ± 0,24/mL (average from three test samples; cf. Tab. 5), corresponding to 5,10 Log/cm².

When compared with the amount of virus recovered at t = 0 (cf. clause 9.1.6) the virus loss which can be ascribed to the incubation at 25 °C over 24 hours amounted to RF = 0,25 ± 0,38.

9.1.8 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.

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

When the limiting dilution technique was used for virus titration the maximum detectable virus reduction amounted to RF_{max} = 4,0 with t = 24 h (cf. Tab. 6).

9.1.8.2 Virus titration by Large Volume Plating

With the *Large Volume Plating* method applied the maximum detectable virus reduction amounted to RF_{max} = 5,47 with t = 24 h (cf. Tab. 6).

9.2. Test section 2 - Virus inactivation by the product under test

9.2.1 Virus titration with the limiting dilution technique

After t = 24 h and with all three product specimens (In-1 to In-3) no residual test virus could be detected with each of the test samples.

With In-1 the residual virus amounted to lg $ID_{50} \leq 1{,}3$/mL (average from two titrations; cf. Tab. 7), corresponding to RF $\geq 4{,}0 \pm 0{,}24$ (cf. Tab. 9).

With In-2 the residual virus amounted to lg $ID_{50} \leq 1{,}3$/mL (average from two titrations; cf. Tab. 7), corresponding to RF $\geq 4{,}0 \pm 0{,}24$ (cf. Tab. 9).

With In-3 the residual virus amounted to lg $ID_{50} \leq 1{,}3$/mL (average from two titrations; cf. Tab. 7), corresponding to RF $\geq 4{,}0 \pm 0{,}24$ (cf. Tab. 9).

When the average amount of residual test virus is calculated from all three redundant test samples the (average) virus titer amounted as well to lg $ID_{50} \leq 1{,}3$/mL (cf. Tab. 7). This result was equivalent to the virus reduction of RF $\geq 4{,}0 \pm 0{,}24$ (cf. Tab. 9).

9.2.2 Virus titration by Large Volume Plating

When the LVP method was applied for virus titration none of the cell culture units inoculated with the resuspension fluid became virus positive. This was true for all three redundant test samples (In- to In-3).

With the Poisson-formular applied the (virtual) virus titer for the single samples amounted to lg $ID_{50} \leq -0{,}17$/mL (cf. Tab. 8). This result is correspondent to the virus reduction factor of RF $\geq 5{,}47 \pm 0{,}24$ (cf. Tab. 10).

When all three test samples were taken together (with v = 14,4 mL from V = 30,6 mL transferred to the detection cells) the (virtual) virus titer amounted to lg $ID_{50} \leq -0{,}65$/mL (cf. Tab. 8), corresponding to the virus reduction factor of RF $\geq 5{,}95 \pm 0{,}24$ (cf. Tab. 10).

10. Validity of the testing (according to ISO 21702:2019)

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

Clause 8.2.1: When the conditions given in 8.2.2, 8.2.3, 8.2.4 and 8.2.5 are satisfied, the test is deemed valid. If all conditions are not met, the test is considered as invalid and the specimens shall be retested.

Clause 8.2.2: The virus titer recovered immediately after inoculation from the untreated test specimens shall satisfy the requirement of Formula (5): $(L_{max} - L_{min}) / (L_{mean}) \leq 0{,}2$.

⇒ With $(L_{max} - L_{min}) / (L_{mean}) = 0{,}13$ the corresponding condition was satisfied (cf. Tab. 5).

Clause 8.2.3: The average amount of virus recovered immediately after inoculation from the untreated test specimens shall be within the range of lg $ID_{50} = 5{,}40/cm^2$ to lg $ID_{50} = 6{,}08/cm^2$.

⇒ With lg $ID_{50} = 5{,}35/cm^2$ the amount of virus recovered immediately after inoculation from the untreated test specimens (t = 0) complies with the threshold value (cf. Tab. 5).

Clause 8.2.4: The amount of virus recovered from each untreated test specimen after contacting for 24 h shall not be less than lg $ID_{50} = 2{,}8/cm^2$

⇒ With lg $ID_{50} = 5{,}10/cm^2$ the amount of virus recovered after 24 h from the untreated test specimens complies with the threshold value (cf. Tab. 5).

Clause 8.2.5: The suppressive efficiency of the agent's activity described in 6.6 is to be confirmed.

Clause 6.6.2: With the three untreated test specimens as well as with the three treated test specimens no cytotoxic effect on the detection is visible.

⇒ With the corresponding test samples *(untreated test specimens: T-4 to T-6; treated test specimens: T-1 to T-3)* no cytotoxic effect was visible (cf. Tab. 2).

Clause 6.6.3: The virus titer of the negative control is not different from the virus titer of the untreated test specimens or the treated test specimens:

⇒ With the untreated test specimens: $S_n - S_u = 0{,}0 \pm 0{,}33$

In that test sample group the conditions of Δ lg $ID_{50} \leq 0{,}5$ has been fulfilled (cf. Tab. 4).

⇒ With the treated test specimens: $S_n - S_t = 0{,}65 \pm 0{,}37$

In that test sample group the conditions of Δ lg $ID_{50} \leq 0{,}5$ has not been fulfilled completely. With two out of three samples this condition was fulfilled whereas with the remaining test sample it was not (cf. Tab. 4).

But with the Large Volume Plating method applied an after-effect is decreased significantly. After resuspension of the test samples (duration: max. 2 minutes [instead of 30 min. of contact applied with the after-effect test]) the resuspension fluid was diluted immediately (8-fold) to a dilution level that is tolerated by the (naked) detection cells (cf. susceptibility test; Tab. 3). It can be concluded, therefore, that compared to the conditions which are present in the after effect test as specified in the norm, an after-effect can be excluded with the LVP-methodology applied.

According to *Clause 8.2.1* the test is deemed valid when the conditions given in 8.2.2, 8.2.3, 8.2.4 and 8.2.5 are satisfied. If all conditions are not met, the test is considered as invalid.

⇒ The conditions given in 8.2.2, 8.2.3 and 8.2.4 are satisfied directly. With the introduction of the LVP-method, controlled by the susceptibility test an after-effect as outlined with clause 8.2.5 can be excluded.

In conclusion, the present testing can be considered as valid.

11. Summary of the test results

The product/coating under test *Argentum 20 A-base* is intended to be applied as a functional surface which is equipped with an antiviral component.

With the present examination according to ISO 21702:2019 using the *Bovine Coronavirus* as the test virus the antiviral activity of the two products should be tested.

11.1 Validation experiments

It was shown from the control experiments that the validity of the test system was ensured (cf. point 10).

11.2 Virus inactivation

After t = 24 h and with all three product specimens (In-1 to In-3) no residual test virus could be detected with each of the test samples. With an average virus titer of lg $ID_{50} \leq$ -0,65/mL (cf. Tab. 8) the corresponding virus reduction amounted to RF $\geq$ 5,95 ± 0,24 (cf. Tab. 10).

In conclusion, a certain virus inactivating activity of *Argentum 20 A-base* vs. the *Bovine Coronavirus* was demonstrated with the present testing amounting to a virus reduction of more than 99,99%.

End of the test report.

The test results described refer exclusively to the examined product sample.

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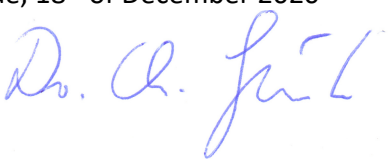
Luckenwalde, 18th of December 2020



Dr. Christian Jursch
(Managing Director of Eurovir)

12. Literature

1. ISO 21702:2019

2. DIN EN 14476/A2:2019

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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

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Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

- Attachment: tables and figures -

Tab. 1: Examined product(s)

Test item [Function]	Product designation	Description	Test surface inoculated and covered by PP-foil
Test squares 5 cm x 5 cm *(equipped with the active component(s)*	*Argentum 20 A-base*	test squares prepared from plastic material, with a hard, flat surface and with a thickness of 1 mm; coated with product	4 cm x 4 cm = 16 cm²
Test squares 5 cm x 5 cm *(non-equipped control)*	*Un-treated Control*	test squares prepared from plastic material, with a hard, flat surface and with a thickness of 1 mm; non-coated	4 cm x 4 cm = 16 cm²

Tab. 2: Visible cytotoxic effect of the product(s) on the detection cells / cytotoxicity without detoxification [cf. 6.6.2] *(Titration by limiting dilution)*

Product(s)	Exposure	Sample ID	Dilution factor (lg) / VF = 4							Titer per 100 µL (lg TD_{50})	Titer per 1 mL (lg TD_{50})	Cytotoxic effect visible
			-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2			
Argentum 20 A-base (treated samples)	24 h at 25 °C and 90% humidity	T-1	0/4 [1]							≤ 0,30	≤ 1,30	no
		T-2	0/4							≤ 0,30	≤ 1,30	
		T-3	0/4							≤ 0,30	≤ 1,30	
Un-treated Control (untreated samples)		T-7	0/4							≤ 0,30	≤ 1,30	no
		T-8	0/4							≤ 0,30	≤ 1,30	
		T-9	0/4							≤ 0,30	≤ 1,30	

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

Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

Tab. 3: Virus susceptibility of the detection cells when the virus titration by Large Volume Plating was used [cf. 6.6.3] *(Titration by limiting dilution)*

Test sample(s)	Sample ID	Dilution factor [1]	Dilution (lg) / VF = 4										Titer per 100 µL (lg ID_{50})	Δ Titer [3] (lg ID_{50})	Cells susceptible [4]
			-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2	-4,8	-5,4	-6	-6,6			
untreated cells	VK/E-1	n.a.	4/4 [1]	4/4	4/4	4/4	4/4	4/4	4/4	2/4	0/4			4,8 ± 0,3	-	-
	VK/E-2		4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4			4,95 ± 0,26	-	-
	VK/E-3		4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	1/4	0/4	0/4	5,25 ± 0,26	-	-
Average virus titer			12/12	12/12	12/12	12/12	12/12	12/12	12/12	9/12	1/12	0/12		5,0 ± 0,19	-	-
Argentum 20 A-base (treated cells)	E-1	VF = 8	4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	2/4	0/4		5,1 ± 0,42	-0,1 ± 0,46	yes
	E-2		4/4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	1/4	0/4	5,1 ± 0,47	-0,1 ± 0,51	yes
	E-3		4/4	4/4	4/4	4/4	4/4	4/4	4/4	1/4	0/4			4,65 ± 0,26	0,35 ± 0,32	yes
Average virus titer			12/12	12/12	12/12	12/12	12/12	12/12	12/12	5/12	3/12	1/12	0/12	4,95 ± 0,26	0,05 ± 0,32	-
Un-treated Control (treated cells)	E-4	VF = 8	4/4	4/4	4/4	4/4	4/4	4/4	3/4	3/4	1/4	0/4		4,95 ± 0,45	0,05 ± 0,49	yes
	E-5		4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4			4,95 ± 0,26	0,05 ± 0,32	yes
	E-6		4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4			4,95 ± 0,26	0,05 ± 0,32	yes
Average virus titer			12/12	12/12	12/12	12/12	12/12	12/12	11/12	9/12	1/12	0/12		4,95 ± 0,21	0,05 ± 0,28	-

[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 a virus susceptibility of the detection cells applies as given when Δ Titer is ≤ lg 1,0

Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

Tab. 4: Control test - stop of the residual disinfecting activity [1] (after-effect test [cf. 6.6.3]) *(Titration by limiting dilution)*

Test sample(s)	Sample ID	Dilution (lg) / VF = 4											Titer per 100 µL (lg ID_{50})	Δ Titer [3] (lg ID_{50})	After-effect present [4]	Verification acc. clause 6.6 passed [5]
		-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2	-4,8	-5,4	-6	-6,6				
Cell culture medium (negative control)	VN-1	4/4 [1]	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4			4,35 ± 0,40	-	-	n.a.
	VN-2	4/4	4/4	4/4	4/4	4/4	4/4	0/4					3,9 ± 0,0	-	-	
	VN-3	4/4	4/4	4/4	4/4	4/4	4/4	1/4	2/4	0/4			4,35 ± 0,4	-	-	
Average virus titer (equivalent to S_n)		12/12	12/12	12/12	12/12	12/12	12/12	3/12	3/12	0/12			4,20 ± 0,22	-		
Argentum 20 A-base (treated test specimens)	N-1	4/4	4/4	4/4	4/4	3/4	2/4	2/4	1/4	0/4			3,9 ± 0,56	0,3 ± 0,6	no	yes *(with two out of tree samples)*
	N-2	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4				3,75 ± 0,4	0,45 ± 0,45	no	
	N-3	4/4	4/4	4/4	4/4	2/4	0/4						3,0 ± 0,3	1,2 ± 0,37	yes	
Average virus titer (equivalent to S_t)		12/12	12/12	12/12	12/12	9/12	4/12	3/12	1/12	0/12			3,55 ± 0,30	S_n - S_t = 0,65 ± 0,37		
Un-treated Control (untreated test specimens)	N-4	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4			4,35 ± 0,40	-0,15 ± 0,45	no	yes
	N-5	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4			4,35 ± 0,4	-0,15 ± 0,45	no	
	N-6	4/4	4/4	4/4	4/4	4/4	3/4	1/4	0/4				3,9 ± 0,37	0,3 ± 0,43	no	
Average virus titer (equivalent to S_u)		12/12	12/12	12/12	12/12	12/12	11/12	5/12	2/12	0/12			4,20 ± 0,24	S_n - S_u = 0,0 ± 0,33		

[1] = the test virus was added directly into the test sample as resuspended from the test item. Incubation was t = 30 min. at 25 °C.
[2] = first number = number of virus positive cells cultures, second number = total number of cell cultures
[3] = virus titer A (negative control [VN]) minus virus titer B (treated test specimen [N-1 to N-3]) or minus virus titer c (untreated test specimen [N-4 to N-6])
[4] = according to the EN 14476 an ongoing residual disinfecting activity (after effect) of the product(s) applies as not given when Δ Titer is ≤ lg 0,5
[5] = verification acc. ISO 21702, clause 6.6.3.3 is passed when Δ Titer is ≤ lg 0,5

Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

Tab. 5: Titration of the virus suspension and the virus material recovered from the test specimen *(Titration by limiting dilution)*

Test sample(s)	Sample ID	Dilution (lg) / VF = 4											Titer per 100 µL (lg ID_{50})	Ø Titer per 1 mL (lg ID_{50})	Verification passed [2]
		-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2	-4,8	-5,4	-6	-6,6			
Virus suspension when added directly to the resuspension medium	Aus-1	4/4 [1]	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4		0/4	4,95 ± 0,46	5,55 ± 0,22	-
	Aus-2	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4				0/4	4,35 ± 0,30		
	Aus-3	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4				0/4	4,35 ± 0,30		
Average virus titer		12/12	12/12	12/12	12/12	12/12	12/12	10/12	2/12	1/12	0/12		0/12	4,55 ± 0,22		
Virus material as recovered from the non-coated control test item after t = 0 min.	VK-1	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4			0/4	4,35 ± 0,46	5,55 ± 0,28 *(equivalent to 5,35/cm²)*	yes *(L_{max} - L_{min})/(L_{mean}) = 0,132*
	VK-2	4/4	4/4	4/4	4/4	4/4	4/4	2/4	1/4	0/4			0/4	4,35 ± 0,46		
	VK-3	4/4	4/4	4/4	4/4	4/4	4/4	3/4	1/4	3/4	0/4		0/4	4,95 ± 0,52		
Average virus titer (equivalent to U_0)		12/12	12/12	12/12	12/12	12/12	12/12	7/12	3/12	3/12	0/12		0/12	4,55 ± 0,28		
Virus material as recovered from the non-coated control test item after t = 24 hours	VK-4	4/4	4/4	4/4	4/4	4/4	4/4	3/4	0/4	1/4	0/4		0/4	4,5 ± 0,37	**5,30 ± 0,24 (U_t)** *(equivalent to 5,10/cm²)*	yes *(5,40/cm² > 2,8/cm²)*
	VK-5	4/4	4/4	4/4	4/4	4/4	4/4	1/4	0/4				0/4	4,05 ± 0,26		
	VK-6	4/4	4/4	4/4	4/4	4/4	4/4	1/4	1/4	1/4	0/4		0/4	4,35 ± 0,45		
Average virus titer (equivalent to U_t)		12/12	12/12	12/12	12/12	12/12	12/12	5/12	1/12	2/12	0/12		0/12	4,30 ± 0,24		

[1] = first number = number of virus positive cells cultures, second number = total number of cell cultures
[2] = verification acc. ISO 21702, clause 8.2.2 is passed when $(L_{max} - L_{min})/(L_{mean})$ is $\leq$ lg 0,2

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

Test virus	Dilution [1] factor	Incubation time (h)	Virus titer per 1 mL [1] *[lg ID_{50} ± $KI_{95\%}$]*	detection limit *[lg ID_{50} / mL]*	max. detectable virus reduction *(RF_{max})* [2]
Bovine Coronavirus *(S 379 Riems)*	Virus titration using the limiting dilution method (Spearman & Kärber)				
	VF = 4	24	5,30 ± 0,24	lg ID_{50} = 1,30	4,0
	Virus titration by Large Volume Plating (LVP) inoculating 192 cell cultures [3]				
	VF = 8	24	5,30 ± 0,24	lg ID_{50} = -0,17	5,47

[1] = 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] = when 192 cell culture units were inoculated; V = 10,2 mL and v = 4,8 mL.

Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

Tab. 7: Inactivation of the *Bovine Coronavirus* by *Argentum 20 A-base* at T = 25 °C - virus titration by limiting dilution [S&K]

Test sample(s)	Sample ID	Dilution (lg) / VF = 4										Titer per 100 µL (lg ID_{50})	Ø Titer per 1 mL (lg ID_{50})
		-0,6	-1,2	-1,8	-2,4	-3,0	-3,6	-4,2	-4,8	-5,4	-6	-6,6	
1. test specimen (In-1)	In-1a	0/4											≤ 0,3	≤ 1,3
	In-1b	0/4											≤ 0,3	
Average virus titer In-1		0/8											≤ 0,3	
2. test specimen (In-2)	In-2a	0/4											≤ 0,3	≤ 1,3
	In-2b	0/4											≤ 0,3	
Average virus titer In-2		0/8											≤ 0,3	
3. test specimen (In-3)	In-3a	0/4											≤ 0,3	≤ 1,3
	In-3b	0/4											≤ 0,3	
Average virus titer In-3		0/8											≤ 0,3	
Average virus titer In-1 to In-3		0/24											≤ 0,3	≤ 1,3

[1] = first number = number of virus positive cells cultures, second number = total number of cell cultures.

Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

Tab. 8: Inactivation of the *Bovine Coronavirus* at T = 25 °C - virus titration by LVP [1] (cf. EN 14476:2019)

Product(s)	Sample ID	Incubation time	Dilution 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]
Argentum 20 A-base (treated test specimens)	In-1	24 h	VF = 8	no	v = 4,8 mL	192	0 *(out of 192)*	≤ -0,17
	In-2		VF = 8	no	v = 4,8 mL	192	0 *(out of 192)*	≤ -0,17
	In-3		VF = 8	no	v = 4,8 mL	192	0 *(out of 192)*	≤ -0,17
	Sum 1-3		VF = 8	no	v = 14,4 mL	576	0 *(out of 576)*	≤ -0,65

[1] = 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 using V = 10,2 mL and v = 4,8 mL or V = 30,6 and v = 14,4 mL and the number of cell culture units as specified.

Tab. 9: Inactivation of the *Bovine Coronavirus* / virus titration by limiting dilution - Estimation of the virus reduction factor (RF)

Product(s)	Sample ID	Incubation time	lg ID_{50}/mL [lg ID_{50} ± $KI_{95\%}$]		Reduction factor [± $KI_{95\%}$]		**Virucidal activity [4] vs. BoCV**
			Virus input [1] [Ut]	Residual virus [2] [At]	Virus reduction [3]	Average [3]	
Argentum 20 A-base (treated test specimens	In-1	24 h	5,60 ± 0,23	≤ 1,3	≥ 4,0 ± 0,24	≥ 4,0 ± 0,24	**high virucidal activity**
	In-2			≤ 1,3	≥ 4,0 ± 0,24		
	In-3			≤ 1,3	≥ 4,0 ± 0,24		

[1] = amount of input virus (virus control; cf. Tab. 5)
[2] = amount of residual virus with respect to the cytotoxicity titer (cf. Tab. 2)
[3] = titer of input virus (lg ID_{50}) after t = 24 h (U_t) minus titer of residual virus (lg ID_{50}) after t = 24 h (A_t) [$R = U_t - A_t$]
[4] = according to the EN 16777 a virucidal activity against the test virus applies as given when RF ≥ lg 4,0

Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

Tab. 10: Inactivation of the *Bovine Coronavirus* / virus titration by LVP [1] - Estimation of the virus reduction factor (RF)

Product(s)	Sample ID	Incubation time	lg ID_{50}/mL [lg ID_{50} ± $KI_{95\%}$]		Reduction factor [± $KI_{95\%}$]		**Virucidal activity [5] vs. BoCV**
			Virus input [2] [Ut]	Residual virus [3] [At]	Virus reduction [4]	Average [4]	
Argentum 20 A-base (treated test specimens	In-1	24 h	5,60 ± 0,23	≤ -0,17	≥ 5,47 ± 0,24	≥ 5,47 ± 0,24	**high virucidal activity**
	In-2			≤ -0,17	≥ 5,47 ± 0,24		
	In-3			≤ -0,17	≥ 5,47 ± 0,24		
	Sum 1-3			≤ -0,65	≥ 5,95 ± 0,24		

[1] = cf. EN 14476:2019 or the DVV/RKI-guideline (2015)
[2] = amount of input virus (virus control) per 1 mL (cf. Tab. 5)
[3] = amount of residual virus (cf. Tab. 8)
[4] = titer of input virus (lg ID_{50}) after t = 24 h (U_t) minus titer of residual virus (lg ID_{50}) after t = 24 h (A_t) [$R = U_t - A_t$]
[5] = according to the EN 16777 a virucidal activity against the test virus applies as given when RF ≥ lg 4,0

Dr. Christian Jursch
Eurovir Hygiene-Labor GmbH
Im Biotechnologiepark TGZ I
D-14943 Luckenwalde

Inactivation of the *Bovine Coronavirus* by *Argentum 20 A-base* at *T* = 25 °C
- Antiviral validation using the quantitative carrier test (acc. to ISO 21702:2019) -

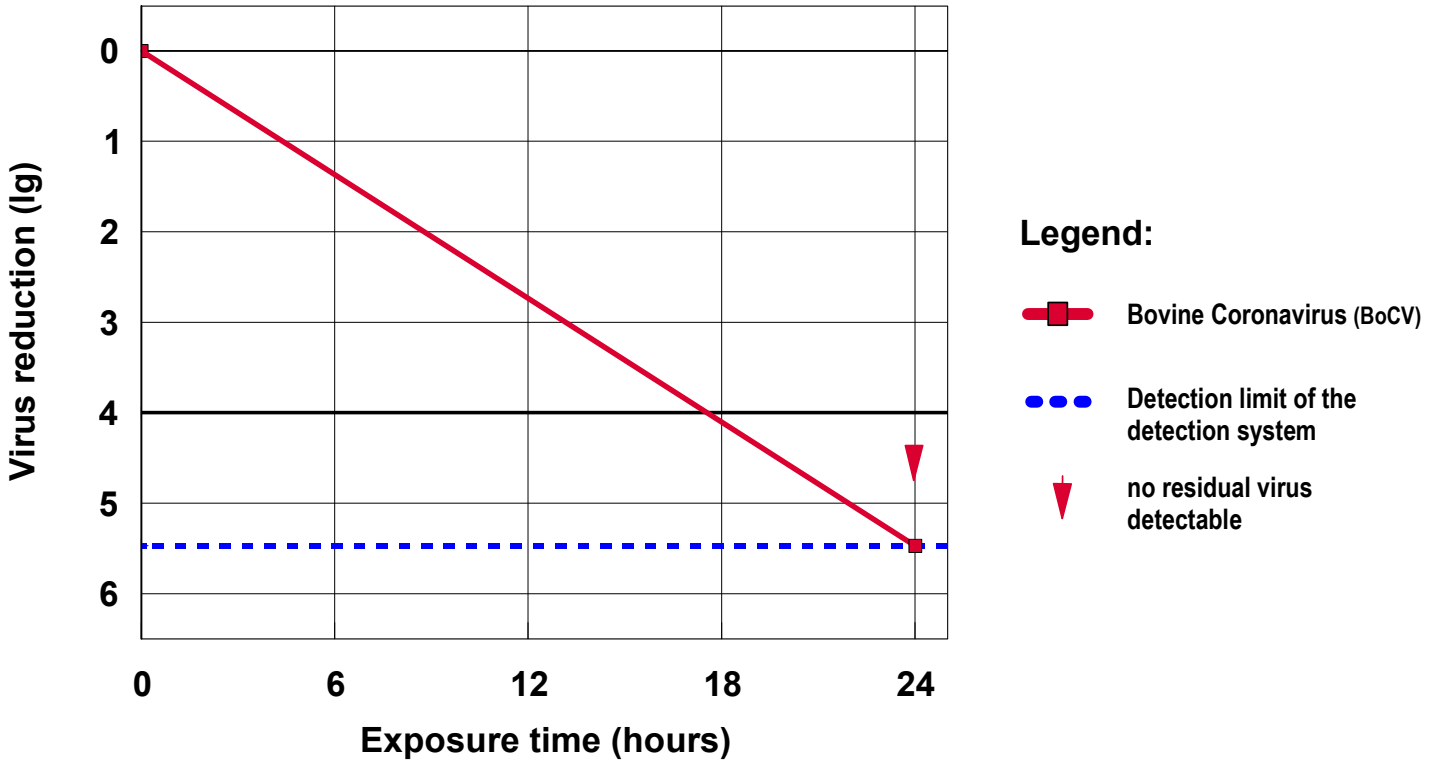

Fig. 1

Explanation of figures:

to Fig. 1: Inactivation of the Bovine Coronavirus (BoCV) at T = 25 °C

In Fig. 1, the virus reduction (lg ID_{50}) is presented in relation to exposure time (hours). 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 at least 4,0 (or 5,47 Log with LVP). The mandatory amount for certification of 4 Log is highlighted by bold-printing.

With the tested product *Argentum 20 A-base* and after an exposure of **24 hours** at **T = 25 °C** no residual test virus could be detected. With a (virtual) virus titer of lg $ID_{50} \leq$ -0,17/mL (with relation to the single sample) the corresponding virus inactivation factor (red line) amounted to **RF ≥ 5,47 ± 0,24** with the (LVP)-Poisson-formula applied.

When all three test samples are taken together the (virtual) virus titer amounted to lg $ID_{50} \leq$ -0,65/mL, corresponding to **RF ≥ 5,95 ± 0,24** (equivalent to a virus inactivation of more than 99,99%).