



DR. BRILL + DR. STEINMANN
INSTITUTE FOR HYGIENE AND MICROBIOLOGY



19/12/2018

Test report L18/0650aMV.1

Evaluation of the effectiveness of **Sterisept Plus**

Test virus: modified vaccinia virus Ankara (MVA)

Method: based on EN 14476:2013+A1:2015 (dirty conditions)

quantitative suspension test for the evaluation
of virucidal activity of chemical disinfectants and
antiseptics used in human medicine

Sponsor:
Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Norderoog 2, DE - 28259 Bremen
Tel.: +49 40-557631-0, Fax: +49 40-557631-11
info@brillhygiene.com, <http://www.brillhygiene.com>

1. Identification of test laboratory

Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Norderoog 2, DE - 28259 Bremen

2. Identification of sample

Manufacturer	Chemi-Pharm AS
Name of product	Sterisept Plus
Confirmation no.	207258
Product diluent recommended by the manufacturer	-
Batch number	212040918
Application	surface disinfection
Production date	04/09/2018
Expiry date	04/09/2021
Active compound (s) (100 g)	- 12.5 % didecyl-dimethyl-ammonium chloride (DDAC) (CAS nr: 7173-51-5) - 12.5 % N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine
Appearance, odour	clear, colorless, slightly viscous liquid product specific
pH-values	undiluted: 12.50 (20 °C) 0.5 %: 10.52 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	07/09/2018

3. Materials

3.1 Culture medium and reagents

- Eagle's Minimum Essential Medium with Hank's BSS (MEM, Biozym Scientific GmbH, catalogue no. 880144)
- fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % formaldehyde solution (dilution of Roti®-Histofix 4 %, Carl Roth GmbH)
- Aqua bidest. (SG ultrapure water system, type Ultra Clear; serial no. 86996-1)
- PBS (Invitrogen, article no. 18912-014)

*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019

- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153)
- sheep erythrocytes (Fiebig Nährstofftechnik).

3.2 Virus and cells

The modified vaccinia virus Ankara (MVA) originated from Dr. Manteufel, Institut für Tierhygiene und Öffentliches Veterinärwesen, DE - 04103 Leipzig. Before inactivation assays, virus had been passaged three times in *BHK 21-cells* (Baby Hamster Kidney).

BHK 21-cells (passage 104) originated from the Friedrich-Löffler-Institut, Bundesforschungsinstitut für Tiergesundheit (formerly Bundesforschungsanstalt für Viruskrankheiten der Tiere, Isle of Riems).

The cells were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

3.3 Apparatus, glassware and small items of equipment

- CO₂ 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)
- Polystyrol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019

4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	0.5 %, 0.25 %, 0.15 %, 0.05 % and 0.025 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	5, 10 and 30 minutes
Interfering substance	3.0 g/l bovine serum albumin + 3.0 ml/l erythrocytes (dirty conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water of standardised hardness (WSH)
Stability of product in the mix with virus and interfering substance (0.5 % solution)	medium clouding, strong precipitation
Virus strain	modified vaccinia virus Ankara (MVA) (ATCC VR-1508)
Date of testing	05/10/2018 – 19/12/2018
End of testing	19/12/2018

5. Methods

5.1 Preparation of test virus suspension

For preparation of test virus suspension, *BHK 21-cells* were cultivated with MEM and 10 % or 2 % fetal calf serum. Cells were infected with a multiplicity of infection of 0.1. After cells showed a cytopathic effect, they were subjected to a freeze/thaw procedure followed by a low speed centrifugation 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 tested as 0.5 %, 0.25 %, 0.15 %, 0.05 % and 0.025 % (demonstrating of non-active range) solutions. Due to the addition of interfering substance and test virus suspension the solutions had to be prepared by the factor 1.25.

These solutions were prepared with WSH immediately before the inactivation tests.

*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019

5.3 Infectivity assay

Infectivity was determined as endpoint titration according to EN 5.5 transferring 0.1 ml of each dilution into eight wells of a microtitre plate to 0.1 ml of freshly trypsinised *BHK 21-cells* ($10\text{--}15 \times 10^3$ cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO₂-atmosphere. The cytopathic effect was read by using an inverted microscope after six days. Calculation of the infective dose TCID₅₀/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

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

meaning

X_0 = log₁₀ of the lowest dilution with 100 % positive reaction

r = number of pos. determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

5.4 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. The difference is given as reduction factor (RF).

According to the EN 14476, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by 4 log₁₀ steps within the recommended exposure period. This corresponds to an inactivation of ≥ 99.99 %.

5.5 Inactivation assay (end point titration)

Determination of virucidal activity has been carried out according to EN 5.5. The test product was examined as 0.5 %, 0.25 %, 0.15 %, 0.05 % and 0.025 % (demonstration of non-active range) solutions in WSH at 20 °C based on EN 14476. 5, 10 and 30 minutes were chosen as contact times.

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10⁻⁸.

*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019

Titration of the virus control were performed at the beginning of the test and after the longest exposure time (EN 5.5.7). One part by volume of test virus suspension was mixed with one part interfering substance and eight parts by volume of WSH or Aqua bidest. (RTU products).

Furthermore, a cell control (only addition of medium) was incorporated.

Inactivation tests were carried out in sealed test tubes in a water bath at $20\text{ }^{\circ}\text{C} \pm 1.0\text{ }^{\circ}\text{C}$. Aliquots were retained after appropriate exposure times and residual infectivity was determined.

5.6 Inactivation assay following the large volume plating method (LVP)

Following the large volume plating method (4) the inactivation assays were further diluted 1:1,000 in cell culture medium. The total volume was added (without any further dilution) to the permissive cells. By introducing such a huge dilution it is possible to eliminate cytotoxicity of the test product in order to demonstrate a $4\log_{10}$ reduction of virus titre. Calculation of virus titre follows formula of Taylor or Poisson (5, 6). This method is necessary for those products which demonstrate a great cytotoxicity.

62.5 µl of the inactivation assays were added to 62.5 ml medium (total dilution of 1:1,000) and then the total volume was distributed in 6 microtitre plates (108 µl / well, 576 wells total). After 6 days of inoculation cultures were observed for cytopathic effects.

The calculation of virus titre without residual virus followed the formula of Poisson:

$$c = \ln p / -V$$

c = number of virus particles

p = the probability to find no virus. The probability to find no virus should not greater than 5 % ($p=0.05$). By doing so, the number of virus particles can be calculated with a probability of 95 %.

V = test volume (ml)

The titre to be used for calculating the reduction factor (RF) was finally calculated as followed: the determined number of virus particle is first converted with the aid of the dilution factor in the number of particle per ml. Subsequently, the numbers of particles per ml have to be converted in the tissue culture infectious dose per ml (TCID₅₀/ml) (1.0 TCID₅₀

*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019

corresponds to 0.69 infectious virus particles). The common logarithm of this value results in the virus titre ($\log_{10}$ TCID₅₀/ml) used for calculating the reduction factor (RF).

In assays with residual virus, formula according to Taylor was used for calculating the virus titre:

$$c/ml = \frac{D}{V_w} \times \left(-\ln \frac{n - n_p}{n} \right)$$

c = number of virus particles

D = dilution

V_w = volume per well

n = number of inoculated wells

n_p = number of virus-positive wells

For calculating the reduction factor using the formula according to Taylor the number of virus particles is converted to the logarithmic titre ($\log_{10}$ TCID₅₀/ml) as described above.

5.7 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.4.1.

5.8 Cell sensitivity to virus

For the control of cell sensitivity to virus two parts by volume of water were mixed with eight parts by volume of the lowest apparently non-cytotoxic dilution of the product. These mixtures or PBS as control were added to a volume of double concentrated cell suspension. After 1 h at 37 °C the cells were centrifuged and re-suspended in cell culture medium (EN 5.5.4.2b).

Finally, a comparative titration of the test virus suspension was performed on the pre-treated (disinfectant) and non-pre-treated (PBS) cells as described above.

5.9 Control of efficacy for suppression of disinfectant's activity

Furthermore, a control of efficiency for suppression of disinfectant's activity was included (EN 5.5.5).

*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019

5.10 Reference virus inactivation test

As reference for test validation a 0.7 % formaldehyde solution according to EN 5.5.6 was included. 5, 15, 30 and 60 minutes were chosen as contact times. In addition, cytotoxicity of formaldehyde test solution was determined based on EN 5.5.6.2 with dilutions up to 10^{-5} .

6. Verification of the methodology

The following criteria as mentioned in EN 5.7 were fulfilled:

- a) The titre of the test virus suspension allowed the determination of a $\geq 4 \log_{10}$ reduction (maximal virus reduction $\geq 4.85 \pm 0.26$, LVP)
- b) The test product (0.5 %) showed cytotoxicity in the 1:100 dilutions thus allowing the detection of a $4 \log_{10}$ reduction of virus titre.
- c) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *BHK 21-cells* showed no significant difference ($< 1 \log_{10}$; EN 5.7) of virus titre: 6.63 ± 0.25 (PBS, LVP) versus 6.63 ± 0.41 (1:1,000 dilutions of disinfectant as 0.5 % solution, LVP) $\log_{10}$ TCID₅₀/ml.
- d) The control of efficacy for suppression of disinfectant's activity (0.5 %) showed a decrease of ≥ 3.13 ($\leq 3.50 \pm 0.00$ versus $6.63 \pm 0.41 \log_{10}$ TCID₅₀/ml) and failed the requirement of the EN ($\leq 0.5 \log_{10}$; EN 5.5.5.1). In these experiments at the end of the defined exposure time the test mixture was immediately diluted not 1:10 as described in the control of efficacy for suppression of disinfectant's activity but directly 1:1,000 (LVP) and the dilution transferred to the cell culture. For this reason this control is not relevant when using the LVP. Therefore, despite the insufficient control of efficacy for suppression of disinfectant's activity the assay is valid.
- e) One concentration demonstrated a $4 \log_{10}$ reduction and (at least) one concentration demonstrated a $\log_{10}$ reduction of less than 4.

Since all criteria according EN 5.7 were fulfilled, examination with MVA based on EN 14476 is valid.

*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019

7. Results

Results of examination are shown in tables 1 to 14. Tables 1 to 12 demonstrate the raw data, whereas tables 13 (a+b) and 14 give a summary of results.

Testing the 0.5 % solution using the end point dilution method, no residual virus was found after 5 minutes exposure time (table 1). The reduction factor was $\geq 2.88 \pm 0.18$. Due to cytotoxicity, a reduction of 4 $\log_{10}$ -steps could not be shown and the large volume plating method was introduced.

Testing the 0.25 % solution using the end point dilution method, no residual virus was found after 10 minutes exposure time (table 2). The reduction factor was $\geq 2.88 \pm 0.18$. Due to cytotoxicity, a reduction of 4 $\log_{10}$ -steps could not be shown and the large volume plating method was introduced.

The 0.15 % and 0.025 % solutions were not active within 10 minutes of exposure time (tables 3 and 5).

The 0.05 % solution was not active within 30 minutes of exposure time (table 4).

In parallel to the end point dilution method the large volume plating method (LVP) was introduced testing the test product as 0.5 % solution with 5 minutes of exposure time and as 0.25 % solution with 5 and 10 minutes of exposure time. The mean virus titre was $\log_{10} \text{TCID}_{50}/\text{ml} = 6.69 \pm 0.26$ (table 9).

The test product as 0.5 % solution was active after 5 minutes of exposure time (table 10). Since no residual virus was found in 576 cell culture units, the result according to the formula of Poisson was $\leq 1.84 \log_{10} \text{TCID}_{50}$. The reduction factor was therefore $\geq 4.85 \pm 0.26$ ($6.69 \pm 0.26 \log_{10} \text{TCID}_{50}$ minus $\leq 1.84 \log_{10} \text{TCID}_{50}$) after 5 minutes of exposure time. This corresponded to an inactivation of ≥ 99.99 %.

The test product as 0.25 % solution was active after 10 minutes of exposure time (table 12). Since residual virus was found in 20 of 576 cell culture units, the result according to the formula of Taylor was $2.68 \log_{10} \text{TCID}_{50}$. The reduction factor was therefore 4.01 ± 0.26 ($6.69 \pm 0.26 \log_{10} \text{TCID}_{50}$ minus $2.68 \log_{10} \text{TCID}_{50}$) after 10 minutes of exposure time. This corresponded to an inactivation of ≥ 99.99 %.

*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019

8. Conclusion

The surface disinfectant Sterisept Plus tested as 0.5 % solution demonstrated activity against MVA after an exposure time of 5 minutes under dirty conditions. The 0.25 % solution was active after 10 minutes exposure time.

Therefore, the surface disinfectant Sterisept Plus can be declared as active against MVA as follows:

0.5 %	5 minutes	dirty conditions
0.25 %	10 minutes	dirty conditions

Bremen, 19/12/2018



- Dr. Britta Becker -
Head of Laboratory



- Dr. Dajana Paulmann -
Scientific Project Manager



*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019

9. 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.

10. Records to be maintained

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

The use of the Dr. Brill + Partner GmbH name, logo or any other representation of Dr. Brill + Partner GmbH, other than distribution of this report in it's entirely, without the written approval of Dr. Brill + Partner GmbH is prohibited. In addition, Dr. Brill + Partner GmbH may not be referred to in any form of promotional materials, press releases, advertising or similar materials (whether by print, broadcast, communication or electronic means) without the express permission of Dr. Brill + Partner GmbH.

The test results in this test report relate only to the items examined.

*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019

11. Literature

1. EN 14476:2013+A1:2015: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemicals disinfectants and antiseptics in human medicine test - Test method and requirements (phase 2, step 1)
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 Pharmac; 162, 1931, 480-487
4. Rabenau HF., Schwebke I., Blümel J., Eggers M., Glebe D., Rapp I., Sauerbrei A., Steinmann E., Steinmann, J., Willkommen H. Wutzler P.: Leitlinie der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) e.V. und des Robert Koch-Instituts (RKI) zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren in der Humanmedizin (Fassung vom 1. Dezember 2014). Bundesgesundheitsbl; 58, 2015, 493–504
5. Bekanntmachung über die Zulassung von Arzneimitteln, Anforderungen an Validierungsstudien zum Nachweis der Virussicherheit von Arzneimitteln aus menschlichem Blut oder Plasma vom 20. Dezember 1993/21. Januar 1994. Bundesanzeiger Nr. 84: 4740-4744 bzw. CPMP/BWP/268/95: Note for Guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses.
<http://www.ema.europa.eu>
6. Taylor JR.: An Introduction to Error Analysis: The study of Uncertainties in Physical Measurements. 2nd ed. University Science Books, 1997, 327 pp

Appendix:

Legend to the Tables

Table 1:	Raw data for Sterisept Plus (0.5 %) tested against MVA
Table 2:	Raw data for Sterisept Plus (0.25 %) tested against MVA
Table 3:	Raw data for Sterisept Plus (0.15 %) tested against MVA
Table 4:	Raw data for Sterisept Plus (0.05 %) tested against MVA
Table 5:	Raw data for Sterisept Plus (0.025 %) tested against MVA
Table 6:	Raw data for formaldehyde solution (0.7 %) tested against MVA
Table 7:	Raw data for control of efficacy for suppression of disinfectant's activity (0.5 %)
Table 8:	Raw data (MVA) for cell sensitivity (0.5 %) (LVP)
Table 9:	Determination of virus titre (LVP)
Table 10:	Inactivation of MVA by Sterisept Plus (0.5 %) (5 minutes) (LVP)
Table 11:	Inactivation of MVA by Sterisept Plus (0.25 %) (5 minutes) (LVP)
Table 12:	Inactivation of MVA by Sterisept Plus (0.25 %) (10 minutes) (LVP)
Table 13 (a+b):	Summary of results (end point dilution method) with Sterisept Plus and MVA

Table 14: Summary of results (LVP) with Sterisept Plus and MVA

Legend to the Figures

Figure 1: Virus-inactivating properties of Sterisept Plus (0.5 %) (LVP)

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)

*Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE – 28259 Bremen, Germany, Telephone +49. 40. 557631-0, Telefax +49. 40. 557631-11, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2019



Table 1: Raw data for Sterisept Plus (0.5 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5732)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)								
				1	2	3	4	5	6	7	8	9
test product	0.5 %	dirty conditions	5	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			10	tttt tttt	tttt tttt	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.
test product cytotoxicity	0.5 %	dirty conditions	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	2343 4433	1313 1031	0000 0000	0000 0000	0000 0000	0000 0000

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

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)



Table 2: Raw data for Sterisept Plus (0.25 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5732)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)										
				1	2	3	4	5	6	7	8	9		
test product	0.25 %	dirty conditions	5	tttt	tttt	0030	0000	0000	0000	0000	0000	n.d.	n.d.	
				tttt	tttt	0320	0000	0000	0000	0000	0000	n.d.	n.d.	
			10	tttt	tttt	0000	0000	0000	0000	0000	0000	0000	n.d.	n.d.
				tttt	tttt	0000	0000	0000	0000	0000	0000	n.d.	n.d.	
test product cytotoxicity	0.25 %	dirty conditions	15	n.d.	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.	n.d.	
			n.a.	tttt	tttt	0000	0000	0000	0000	n.d.	n.d.	n.d.	n.d.	
virus control	n.a.	dirty conditions	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
			60	4444	4444	4444	2343	1313	0000	0000	0000	0000	0000	

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

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)



Table 3: Raw data for Sterisept Plus (0.15 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5827)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)								
				1	2	3	4	5	6	7	8	9
test product	0.15 %	dirty conditions	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			10	tttt tttt	tttt tttt	4224 4444	0000 0320	2000 0003	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.
test product cytotoxicity	0.15 %	dirty conditions	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	2233 2332	0020 2030	0000 0000	0000 0000	
			60	4444 4444	4444 4444	4444 4444	4444 4444	4303 3222	2000 0002	0000 0000	0000 0000	

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

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)



Table 4: Raw data for Sterisept Plus (0.05 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5827)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)								
				1	2	3	4	5	6	7	8	9
test product	0.05 %	dirty conditions	5	tttt	4444	4444	4444	2002	0000	0000	n.d.	n.d.
			10	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.
test product cytotoxicity	0.05 %	dirty conditions	30	tttt	4444	4444	4444	3000	0000	0000	n.d.	n.d.
			n.a.	tttt	0000	0000	0000	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444	4444	4444	4444	2233	0020	0000	0000	0000
			60	4444	4444	4444	4444	2332	2030	0000	0000	0000

n.a. = not applicable

0 = no virus present; t = cytotoxic

n.d. = not done

1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)



Table 5: Raw data for Sterisept Plus (0.025 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5827)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)								
				1	2	3	4	5	6	7	8	9
test product	0.025 %	dirty conditions	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			10	tttt	4444	4444	4444	3230	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.
test product cytotoxicity	0.025 %	dirty conditions	n.a.	tttt	0000	0000	0000	0000	n.d.	n.d.	n.d.	
virus control	n.a.	dirty conditions	0	4444	4444	4444	4444	2233	0020	0000	0000	0000
				4444	4444	4444	4444	2332	2030	0000	0000	0000
			60	4444	4444	4444	4444	4303	2000	0000	0000	0000
				4444	4444	4444	4444	3222	0002	0000	0000	

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

0 = no virus present; t = cytotoxic

1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)



Table 6: Raw data for formaldehyde solution (0.7 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5827)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)								
				1	2	3	4	5	6	7	8	9
formaldehyde	0.7 % (m/V)	PBS	5	tttt	tttt	tttt	0000	0000	0000	0000	0000	n.d.
			15	tttt	tttt	tttt	0000	0000	0000	0000	0000	n.d.
			30	tttt	tttt	tttt	0000	0000	0000	0000	0000	n.d.
			60	tttt	tttt	tttt	0000	0000	0000	0000	0000	n.d.
formaldehyde cytotoxicity	0.7 % (m/V)	PBS	n.a.	tttt	tttt	tttt	0000	0000	n.d.	n.d.	n.d.	
virus control	n.a.	PBS	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	
			60	4444	4444	4444	4444	4333	0320	0000	0000	
				4444	4444	4444	4444	3223	4020	0000	0000	

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

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)



Table 7: Raw data for control of efficacy for suppression of disinfectant's activity (0.5 %) (#5827)

Product	Interfering substance	dilutions (log ₁₀)								
		1	2	3	4	5	6	7	8	9
test product	dirty conditions	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
corresponding virus control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4303 3222	2000 0002	0000 0000	0000 0000	0000 0000

n.a. = not applicable

0 = no virus present; t = cytotoxic

n.d. = not done

1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)



Table 8: Raw data (MVA) for cell sensitivity (0.5 % solution) (#5827) (LVP)

Product	Dilution	Dilutions (log ₁₀)								
		1	2	3	4	5	6	7	8	9
PBS	-	4444	4444	4444	4444	4442	0000	0000	0000	n.d.
		4444	4444	4444	4444	3244	0003	0000	0000	
test product	1:1,000	4444	4444	4444	4444	4404	0000	0000	0000	n.d.
		4444	4444	4444	4444	4443	0303	0000	0000	

n.a. = not applicable

0 = no virus present; t = cytotoxic

n.d. = not done

1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)



Table 9: Determination of virus titre (LVP) at 20 °C (#5827)

Virus titration	Interfering substance	dilutions (log ₁₀)								
		1	2	3	4	5	6	7	8	9
1 st control	dirty conditions	4444	4444	4444	4444	4303	2000	0000	0000	n.d.
		4444	4444	4444	4444	3222	0002	0000	0000	
2 nd control	dirty conditions	4444	4444	4444	4444	3424	0000	0000	0000	n.d.
		4444	4444	4444	4444	3234	4004	0000	0000	

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)



Table 10: Inactivation of MVA by Sterisept Plus (0.5 %) at 20 °C (5 minutes) (LVP, 1:1,000) (#5827)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
dirty conditions	plate 1/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 2/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 3/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 4/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 5/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 6/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

t = cytotoxic 0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)



Table 11: Inactivation of MVA by Sterisept Plus (0.25 %) at 20 °C (5 minutes) (LVP, 1:1,000) (#5827)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
dirty conditions	plate 1/6	4444 4444	4434 2134	4204 4444	4244 4434	4244 3440	4444 4444	4444 4444	4444 1342	4234 4434	4044 4444	4444 3442	4440 4444
	plate 2/6	4442 3440	4404 4244	4444 3444	4444 4344	3244 4444	4444 4344	4444 4444	4444 1344	4444 4444	4443 2444	4444 4444	4444 4434
	plate 3/6	0444 3044	3444 4444	4144 3444	4444 4442	4444 4144	1043 4344	0440 4444	4244 4344	4244 4404	4402 3444	0444 4443	4444 4404
	plate 4/6	4444 4444	4424 4444	4444 4444	4404 4344	4444 4404	4342 4444	4443 4344	4430 4434	4232 4344	4444 4444	4044 4234	4444 4444
	plate 5/6	4444 4444	4244 4443	4444 2444	4404 4444	4444 4434	4443 4444	4444 4444	4244 4443	4444 0444	4044 4444	4444 4424	3444 4444
	plate 6/6	4442 4444	4044 4444	3444 4404	4404 2443	4444 4344	4244 4444	4434 0444	4044 4440	4444 4444	4424 0424	4044 4444	4443 4444

t = cytotoxic

0 = no virus detectable

1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)



Table 12: Inactivation of MVA by Sterisept Plus (0.25 %) at 20 °C (10 minutes) (LVP, 1:1,000) (#5827)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
dirty conditions	plate 1/6	0000 0000	0000 0000	0000 2000	0000 0000	0000 0000	0000 0000	0000 0003	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 2/6	0000 0000	0000 0000	3000 0000	0002 0000	0000 0004	0000 0000	0000 0000	0000 0000	0000 0000	0203 0000	0000 0000	0000 0400
	plate 3/6	0000 0000	0000 0002	0000 0000	0000 0000	0000 0000	0000 0000	0000 0003	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 4/6	0000 0000	0000 0000	0020 0000	0000 0000	0000 0000	0000 0000	0000 3000	0000 0000	0000 0000	0200 0000	0000 0000	0000 0000
	plate 5/6	0000 0000	0000 0000	0040 0000	0000 0000	0000 0000	0000 0000	0000 0300	0000 0000	0000 0000	0400 0000	0000 0020	0000 0000
	plate 6/6	0000 0000	0200 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 2000	0000 0000	0000 0000	0300 0000	0000 0000	0000 0000

t = cytotoxic

0 = no virus detectable

1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)



Table 13a: Summary of results (end point dilution method) with Sterisept Plus and MVA

Product*	Con- centration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ /ml aftermin					> 4 log ₁₀ reduction after ...min
				1	5	10	30	60	
test product (1)	0.5 %	dirty conditions	3.50	n.d.	≤ 3.50±0.00	≤ 3.50±0.00	n.d.	n.d.	≥ 5 (RF = 2.88±0.18)
test product (1)	0.25 %	dirty conditions	3.50	n.d.	≤ 3.88±0.37	≤ 3.50±0.00	n.d.	n.d.	≥ 10 (RF = 2.88±0.18)
test product (2)	0.15 %	dirty conditions	3.50	n.d.	n.d.	5.00±0.46	n.d.	n.d.	> 10 (RF = 1.63±0.62)
test product (2)	0.05 %	dirty conditions	2.50	n.d.	6.25±0.33	n.d.	5.88±0.37	n.d.	> 30 (RF = 0.75±0.55)
test product (2)	0.025 %	dirty conditions	2.50	n.d.	n.d.	6.38±0.41	n.d.	n.d.	> 10 (RF = 0.25±0.58)

*The number in brackets gives the number of the corresponding virus control, see table 13b

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



Table 13b: Summary of results (end point dilution method) with Sterisept Plus and MVA

Product	Con- centration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ /ml aftermin					> 4 log ₁₀ reduction after ... min
				0	5	15	30	60	
formaldehyde	0.7 % (w/v)	PBS	4.50	n.d.	≤ 4.63±0.25	≤ 4.50±0.00	≤ 4.50±0.00	≤ 4.50±0.00	≥ 15 (RF ≥ 2.50±0.27)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.00±0.38	n.a.
virus control (1)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.38±0.25	n.a.
virus control (2) (+ suppression)	n.a.	dirty conditions	n.a.	6.88±0.37	n.d.	n.d.	n.d.	6.63±0.41	n.a.
suppression control	0.5 %	dirty conditions	3.50	n.d.	n.d.	n.d.	≤ 3.50±0.00	n.d.	n.a.

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



Table 14: Summary of results (LVP, 1:1,000) with Sterisept Plus and MVA

Product	Con- centration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ /ml aftermin					> 4 log ₁₀ reduction aftermin
				1	5	10	30	60	
test product	0.5 %	dirty conditions	n.a.	n.d.	≤ 1.84	n.d.	n.d.	n.d.	5 (RF ≥ 4.85±0.26)
test product	0.25 %	dirty conditions	n.a.	n.d.	4.30	2.68	n.d.	n.d.	10 (RF = 4.01±0.26)
virus control	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.63±0.41 6.75±0.33 (Ø6.69±0.26)	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.63±0.25	n.a.
sens. product	0.5 % → 1:1,000	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.63±0.41	n.a.

*The number in brackets gives the number of the corresponding virus control

n.a. = not applicable n.d. = not done sens. = sensitivity n.c. = not calculable



Figure 1: Virus-inactivating properties of Sterisept Plus (0.5 %)

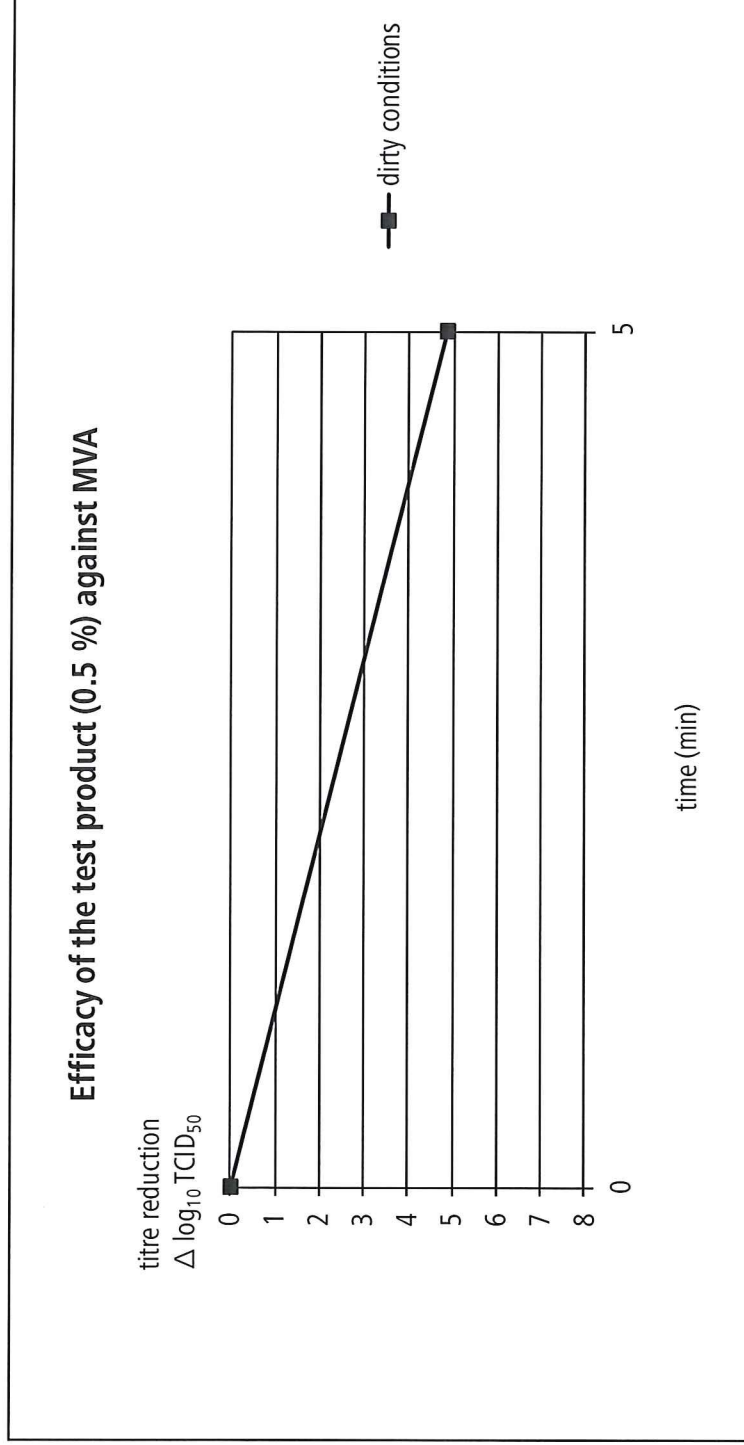




Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)

