corresponds to 0.69 infectious virus particles). The common logarithm of this value results in the virus titre (log_{10} $TCID_{50}$/ml) used for calculating the reduction factor (RF).

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

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

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 ($log_{10}TCID_{50}$/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).

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 following 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 ≥ 4 $\log_{10}$ reduction (maximal virus reduction ≥ 5.47 ± 0.26, LVP)

b) The test product (80.0 %) showed cytotoxicity in the 1:1,000 dilutions thus allowing the detection of a 4 $\log_{10}$ reduction of virus titre (LVP assay).

c) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *RAW cells* showed no significant difference (< 1 $\log_{10}$; EN 5.7) of virus titre: 8.25 ± 0.44 (PBS, LVP) versus 8.50 ± 0.00 (1:10,000 dilutions of disinfectant as 80.0 % solution, LVP) $\log_{10}$ $TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant's activity (80.0 %) showed a decrease of 1.13 (7.13 ± 0.45 versus 8.25 ± 0.33 $\log_{10}$ $TCID_{50}$/ml) and failed the requirement of the EN (≤ 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 1:10,000 (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 MNV according to EN 14476 is valid.

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.

Since it was not possible to show a reduction in virus titre of 4 log_{10}-steps testing the undiluted test product due to cytotoxicity, this solution was tested using the large volume plating method. The further dilutions were examined using the end point dilution method.

Testing the product as 50.0 % solution, no residual virus could be detected after 5 minutes of exposure time (table 1). Due to cytotoxicity 4 log_{10}-steps could not be shown. The reduction factor was $\geq 3.75 \pm 0.23$ log_{10} $TCID_{50}$.

The 10.0 % solution was not active within 30 minutes of exposure time (table 2).

In parallel to the end point dilution method the large volume plating method (LVP) was introduced testing the undiluted test product with 30 seconds and 1, 3 and 5 minutes of exposure time.
The mean virus titre in the first assay was log_{10} $TCID_{50}$/ml = 8.00 ± 0.29 (table 6) and in the second assay log_{10} $TCID_{50}$/ml = 8.31 ± 0.26 (table 8).

In the second assay, the undiluted test product was active after 30 seonds of exposure time (table 9). Since residual virus was found in 37 of 576 cell culture units, the result according to the formula of Taylor was 3.95 log_{10} $TCID_{50}$. The reduction factor was therefore 4.36 ± 0.26 (8.31 ± 0.26 log_{10} $TCID_{50}$ minus 3.95 log_{10} $TCID_{50}$) after 30 seconds of exposure time. This corresponded to an inactivation of ≥ 99.99 %.

8. Conclusion

The surface disinfectant Sterisept Wipes tested undiluted demonstrated activity against MNV after an exposure time of 30 seconds under dirty conditions.

Therefore, the surface disinfectant Sterisept Wipes can be declared as active against MNV as follows:

undiluted 30 seconds dirty conditions

Bremen, 23/04/2019

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

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 (BGBI. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBI. 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.

11. Literature

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

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

4. 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 Wipes (50.0 %) tested against MNV

Table 2: Raw data for Sterisept Wipes (10.0 %) tested against MNV

Table 3: Raw data for formaldehyde solution (0.7 %) tested against MNV

Table 4: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %)

Table 5: Raw data (MNV) for cell sensitivity (80.0 %) (LVP)

Table 6: Determination of virus titre (LVP) (1st assay)

Table 7: Inactivation of MNV by Sterisept Wipes (80.0 %) (5 minutes) (LVP) (1st assay)

Table 8: Determination of virus titre (LVP) (2nd assay)

Table 9: Inactivation of MNV by Sterisept Wipes (80.0 %) (30 seconds) (LVP) (2nd assay)

Table 10: Inactivation of MNV by Sterisept Wipes (80.0 %) (1 minute) (LVP) (2nd assay)

Table 11: Inactivation of MNV by Sterisept Wipes (80.0 %) (3 minutes) (LVP) (2nd assay)

Table 12: Inactivation of MNV by Sterisept Wipes (80.0 %) (5 minutes) (LVP) (2nd assay)

Table 13 (a+b): Summary of results (end point dilution method) with Sterisept Wipes and MNV

Table 14: Summary of results (LVP) with Sterisept Wipes and MNV

Legend to the Figures

Figure 1: Virus-inactivating properties of Sterisept Wipes (80.0 %) (LVP)

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

Table 1: Raw data for Sterisept Wipes (50.0 %) tested against MNV at 20 °C (quantal test; 8 wells) (#5996)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	50.0 %	dirty conditions	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	50.0 %	dirty conditions	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0044 4440	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4044	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 Wipes (10.0 %) tested against MNV at 20 °C (quantal test; 8 wells) (#5996)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0 %	dirty conditions	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	4444 4444	4444 4440	4400 0000	0000 0000	0000 0000	n.d.	n.d.
test product cytotoxicity	10.0 %	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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0044 4440	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4044	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 formaldehyde solution (0.7 %) tested against MNV at 20 °C (quantal test; 8 wells) (#5996)

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 tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0000 0040	0000 0000	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	4444 4444	0400 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	0400 0044	0000 0400	0000 0000	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	4444 4444	0440 4000	0000 0000	0000 0000	0000 0000	n.d.
formaldehyde cytotoxicity	0.7 % (m/V)	PBS	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	PBS	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	4444 4444	4444 4444	4444 4444	4040 0444	0000 0400	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 control of efficacy for suppression of disinfectant's activity (80.0 %) (#5996)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	dirty conditions	tttt tttt	tttt tttt	tttt tttt	4444 4444	4444 4444	0404 4004	0040 0000	0000 0000	n.d.
corresponding virus control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4044	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 5: Raw data (MNV) for cell sensitivity (80.0 % solution) (#5996) (LVP)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4340 4400	0000 0300	n.d.
test product	1:10,000	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4434 4444	0000 0000	n.d.

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: Determination of virus titre (LVP) at 20 °C (#5961) (1st assay)

Virus titration	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
1st control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0004 4400	0000 0000	n.d.
2nd control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0440 0440	0040 0000	n.d.

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

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

Table 7: Inactivation of MNV by Sterisept Wipes (80.0 %) at 20 °C (5 minutes) (LVP, 1:10,000) (#5961) (1st assay)

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 8: Determination of virus titre (LVP) at 20 °C (#5996) (2nd assay)

Virus titration	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
1st control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4440 4044	0000 0000	n.d.
2nd control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4404 4044	0040 0000	n.d.

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

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

Table 9: Inactivation of MNV by Sterisept Wipes (80.0 %) at 20 °C (30 seconds) (LVP, 1:10,000) (#5996) (2nd assay)

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	4000 0000	0000 0000	0040 0000	0000 0000	0000 4000	0000 0000	0000 0000	0400 0000	0044 0000
	plate 2/6	0000 0000	0000 0000	0000 0000	0000 0400	0440 0004	0000 0040	0000 0000	0004 0000	0004 0000	0000 0000	0000 0000	0000 0000
	plate 3/6	0000 0000	0000 0000	0000 0040	0000 0000	0000 0000	0000 0000	0400 0000	0000 0000	0000 0000	0000 0000	0400 0000	0000 0000
	plate 4/6	0000 0000	0000 0000	0000 0400	0000 0400	0000 0300	0000 0004	0004 0000	0000 0000	0000 0000	0000 0000	0000 0000	4000 0000
	plate 5/6	0000 0000	0000 0000	0040 0000	0000 0000	0000 4000	0000 0040	0000 0000	0000 0000	0000 0000	0000 0300	0000 0004	0000 0000
	plate 6/6	0000 3000	0003 0000	0000 0000	0000 0000	0004 0040	0000 0000	0000 0000	0400 0000	0004 4000	0000 0400	0400 0004	0000 0000

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 MNV by Sterisept Wipes (80.0 %) at 20 °C (1 minute) (LVP, 1:10,000) (#5996) (2nd assay)

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 0320	0000 0000	0000 3033	0000 0303	0000 2000	0000 0330	0000 2000	0000 0000	0000 2230	0000 0000
	plate 2/6	0000 0000	0000 0000	0000 1000	0030 3000	0000 0003	0033 3000	0000 0000	0000 0030	0000 0000	0000 0020	0000 0300	0000 0031
	plate 3/6	0000 0202	0000 0003	0320 0000	0000 0000	0000 0000	0000 0030	0000 0003	0003 0000	0000 0030	0000 0000	0030 3030	0000 0000
	plate 4/6	0000 0000	0000 3300	0000 0000	0000 0030	0030 0023	0000 0000	3000 2000	0000 0022	0003 0000	0000 0000	0000 0030	0000 3200
	plate 5/6	0000 0000	0000 0200	3000 0000	0000 0000	0001 0000	0003 0000	0000 0020	0000 0330	0000 0000	0004 0000	0000 3000	0000 0000
	plate 6/6	0000 0000	0000 3000	0000 0000	0003 0000	0003 0030	0000 0003	0200 0020	0000 0340	0000 0300	0000 0000	0000 0030	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 MNV by Sterisept Wipes (80.0 %) at 20 °C (3 minutes) (LVP, 1:10,000) (#5996) (2nd assay)

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 12: Inactivation of MNV by Sterisept Wipes (80.0 %) at 20 °C (5 minutes) (LVP, 1:10,000) (#5996) (2nd assay)

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 13a: Summary of results (end point dilution method) with Sterisept Wipes and MNV

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after …min
				1	5	10	30	60	
test product	50.0 %	dirty conditions	4.50	n.d.	≤ 4.50±0.00	n.d.	n.d.	n.d.	≥ 5 (RF ≥ 3.75±0.23)
test product	10.0 %	dirty conditions	3.50	n.d.	n.d.	n.d.	5.63±0.41	n.d.	> 30 (RF = 2.63±0.53)

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

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

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after … min
				0	5	15	30	60	
formaldehyde	0.7 % (w/v)	PBS	4.50	n.d.	7.63±0.25	7.63±0.25	7.00±0.44	5.88±0.37	> 60 (RF = 2.38±0.57)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	8.25±0.44	n.a.
virus control	n.a.	dirty conditions	n.a.	8.13±0.45	n.d.	n.d.	n.d.	8.13±0.45	n.a.
suppression control	80.0 %	dirty conditions	4.50	n.d.	n.d.	n.d.	7.13±0.45	n.d.	n.a.

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

Table 14: Summary of results (LVP) with Sterisept Wipes and MNV

Product*	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ...min
				0.5	1	3	5	30	
test product (1)	80.0 %	dirty conditions	n.a.	n.d.	n.d.	n.d.	≤ 2.84	n.d.	5 (RF ≥ 5.16±0.29)
test product (2)	80.0 %	dirty conditions	n.a.	3.95	4.25	≤ 2.84	≤ 2.84	n.d.	0.5 (RF = 4.36±0.26)
virus control (1)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.88±0.37 8.13±0.45 (Ø8.00±0.29)	n.a.
virus control (2)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	8.25±0.33 8.38±0.41 (Ø8.31±0.26)	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	8.25±0.44	n.a.
sens. product	80.0 % → 1:10,000	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	8.50±0.00	n.a.

*the number in the brakets 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 Wipes (80.0 %) (LVP)

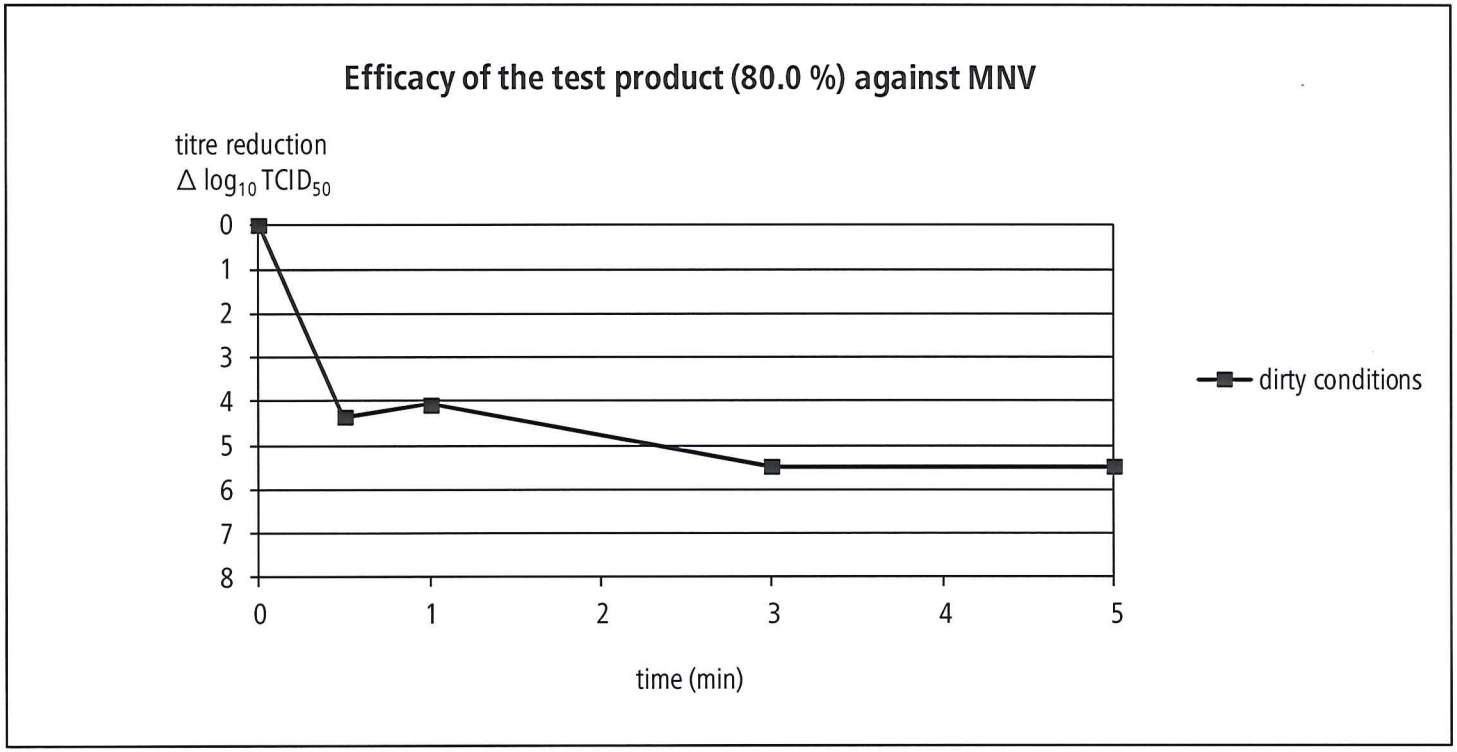
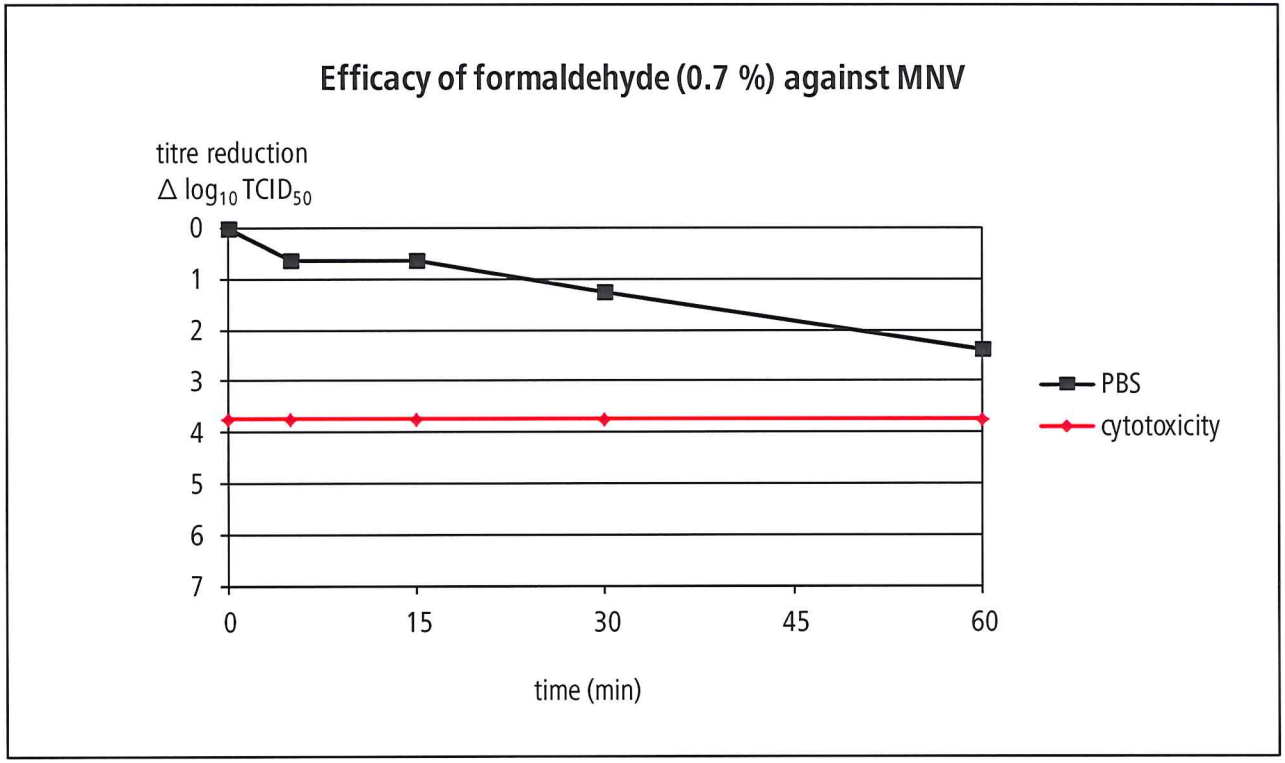


Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)



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Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 23/04/2019

Expert opinion

Activity of Sterisept Wipes against human rotavirus strain Wa in a quantitative suspension test based on EN 14476:2013+A1:2015 under dirty conditions

This expert opinion is based on the test report L18/0650eR.3.U dating 23/04/2019.

The virus-inactivating properties of the surface disinfectant Sterisept Wipes of Chemi-Pharm AS against human rotavirus strain Wa were investigated by a quantitative suspension test based on EN 14476 under dirty conditions.

According to EN 14476, a disinfectant or a disinfectant solution at a particular concentration is considered as having virus-inactivating properties if within the recommended exposure period the titre is reduced by ≥ 4 $\log_{10}$ (inactivation ≥ 99.99 %).

The surface disinfectant Sterisept Wipes was examined undiluted at 20 °C. 30 seconds were chosen as exposure time. In summary, a virucidal activity against human rotavirus strain Wa was measured as follows:

undiluted **30 seconds** **dirty conditions (3.0 g/l BSA + 3.0 ml/l erythrocytes)**

Dr. J[illegible]n

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

ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01 D-PL-13412-01-02
Anerkannt durch/Recogaized by Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten www.zlg.de
ZLG-AP-216.11.02

23/04/2019

Test report L18/0650eR.3.U

Evaluation of the effectiveness of

Sterisept Wipes

Test virus: human rotavirus strain Wa

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

TEST REPORT

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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 Wipes
Confirmation no.	208639
Product diluent recommended by the manufacturer	-
Batch number	14300818
Application	surface disinfection
Production date	30/08/2018
Expiry date	30/08/2021
Active compound (s) (100 g)	- 0.45 % didecyl-dimethyl-ammonium chloride (DDAC) (CAS nr: 7173-51-5) - 0.45 % N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine
Appearance, odour	clear, colorless, slightly viscous liquid product specific
pH-values	undiluted: 10.83 (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 Earle`s BSS (EMEM, Biozym Scientific GmbH, catalogue no. 880121)
- 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)

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

3.2 Virus and cells

The human rotavirus strain Wa (serotype 1, subgroup II) was obtained by Prof. Dr. Holger Rabenau, Institute of Medical Virology of the Johann Wolfgang Goethe University of Frankfurt, DE - 60596 Frankfurt. Before the described tests, the virus had been passaged in *MA-104 cells* (embryonic rhesus monkey kidney cell line).

The cells (passage 44) 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

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Centrifuge 5804 R (Eppendorf AG)
- Water bath (JULABO, Julabo U 3)
- Adjustable and fixed-volume pipettes (Eppendorf AG)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	30 seconds 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	Aqua bidest.
Stability of product in the mix with virus and interfering substance (80.0 % solution)	strong clouding, strong precipitation
Virus strain	human rotavirus strain Wa
Date of testing	22/03/2019 – 23/04/2019
End of testing	23/04/2019

5. Methods

5.1 Preparation of test virus suspension

After washing with serum-free Eagle`s Minimum Essential Medium twice, cells were incubated with EMEM without fetal calf serum for three hours to eliminate all FCS. This was followed by the addition of virus (stock virus suspension) to *MA-104 cells* in the presence of trypsin for two hours ± 10 minutes at 37 °C. After this time, medium with trypsin was added. If 90 % of the cells showed a cytopathic effect, cells were subjected to a rapid two-fold freeze-thawing procedure followed by a centrifugation at 1.620 g for 30 minutes at 4 °C in order to sediment cell debris. After aliquotation the supernatant was stored as test virus suspension at –80 °C.

5.2 Preparation of disinfectant (dilutions)

The test product was tested undiluted. Due to the addition of interfering substance and test virus suspension an 80.0 % solution resulted.

Furthermore, the product was evaluated as 50.0 % and 10.0 % solutions (demonstrating of non-active range). These solutions were prepared with Aqua bidest. immediately before the inactivation tests.

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 a preformed *MA-104* monolayer (10-15 x 10^3 cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope after six days. Calculation of the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

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

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100 % positive reaction

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

n = number of determinations for each dilution step.

5.4 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 $\log_{10}$ 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 undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions in Aqua bidest. at 20 °C based on EN 14476. 30 seconds 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}.

Titrations 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 °C ± 1.0 °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 1:10,000 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.

6.25 µl of the inactivation assays were added to 62.5 ml medium (total dilution of 1:10,000) 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 ($TCID_{50}$/ml) (1.0 $TCID_{50}$

corresponds to 0.69 infectious virus particles). The common logarithm of this value results in the virus titre (log_{10} $TCID_{50}$/ml) used for calculating the reduction factor (RF).

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

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

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 ($log_{10}TCID_{50}$/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. This mixture or PBS as control was added to the wells of the microtitre plates with a preformed monolayer of *MA-104 cells*.

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).

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 ≥ 4 $\log_{10}$ reduction (maximal virus reduction $\geq 4.66 \pm 0.24$, LVP)

b) The test product (80.0 %) showed cytotoxicity in the 1:1,000 dilutions thus allowing the detection of a 4 $\log_{10}$ reduction of virus titre (LVP assay).

c) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *MA-104 cells* showed no significant difference (< 1 $\log_{10}$; EN 5.7) of virus titre: 7.75 ± 0.44 (PBS, LVP) versus 7.38 ± 0.41 (1:10,000 dilutions of disinfectant as 80.0 % solution, LVP) $\log_{10}$ $TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant's activity (80.0 %) showed a decrease of 0.75 (6.63 ± 0.41 versus 7.38 ± 0.25 $\log_{10}$ $TCID_{50}$/ml) and failed the requirement of the EN (≤ 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 1:10,000 (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 human rotavirus based on EN 14476 is valid.

7. Results

Results of examination are shown in tables 1 to 11. Tables 1 to 9 demonstrate the raw data, whereas tables 10 (a+b) and 11 give a summary of results.

Since it was not possible to show a reduction in virus titre of 4 log_{10}-steps testing the undiluted test product due to cytotoxicity, this solution was tested using the large volume plating method. The further dilutions were examined using the end point dilution method.

Testing the product as 50.0 % solution, no residual virus could be detected after 5 minutes of exposure time (table 1). Due to cytotoxicity 4 log_{10}-steps could not be shown. The reduction factor was $\geq 2.88 \pm 0.18$ log_{10} $TCID_{50}$.

The 10.0 % solution was not active within 30 minutes of exposure time (table 2).

In parallel to the end point dilution method the large volume plating method (LVP) was introduced testing the undiluted test product with 30 seconds of exposure time.
The mean virus titre in the first assay was log_{10} $TCID_{50}$/ml $= 7.50 \pm 0.24$ (table 6) and in the second assay log_{10} $TCID_{50}$/ml $= 7.44 \pm 0.13$ (table 8).

In both assays, the undiluted test product was active after 30 seconds of exposure time (tables 7 and 9). Since no residual virus was found in 576 cell culture units in both assays, the result according to the formula of Poisson was ≤ 2.84 log_{10} $TCID_{50}$. The reduction factors were therefore $\geq 4.66 \pm 0.24$ in the first assay (7.50 ± 0.24 log_{10} $TCID_{50}$ minus ≤ 2.84 log_{10} $TCID_{50}$) and $\geq 4.60 \pm 0.13$ in the second assay (7.44 ± 0.13 log_{10} $TCID_{50}$ minus ≤ 2.84 log_{10} $TCID_{50}$) after 30 seconds of exposure time. The mean reduction ´factor was $\geq 4.63 \pm 0.14$. This corresponded to an inactivation of ≥ 99.99 %.

8. Conclusion

The surface disinfectant Sterisept Wipes tested undiluted demonstrated activity against human rotavirus after an exposure time of 30 seconds under dirty conditions.

Therefore, the surface disinfectant Sterisept Wipes can be declared as active against human rotavirus as follows:

undiluted 30 seconds dirty conditions

Bremen, 23/04/2019

- Dr. Britta Becker -
Head of Laboratory

- Dr. Dajana Paulmann -
Scientific Project Manager

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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 (BGBI. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBI. 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.

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 Pharmak; 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 Wipes (50.0 %) tested against human rotavirus

Table 2: Raw data for Sterisept Wipes (10.0 %) tested against human rotavirus

Table 3: Raw data for formaldehyde solution (0.7 %) tested against human rotavirus

Table 4: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %)

Table 5: Raw data (human rotavirus) for cell sensitivity (80.0 %) (LVP)

Table 6: Determination of virus titre (LVP) (1st assay)

Table 7: Inactivation of human rotavirus by Sterisept Wipes (80.0 %) (30 seconds) (LVP) (1st assay)

Table 8: Determination of virus titre (LVP) (2nd assay)

Table 9: Inactivation of human rotavirus by Sterisept Wipes (80.0 %) (30 seconds) (LVP) (2nd assay)

Table 10 (a+b): Summary of results (end point dilution method) with Sterisept Wipes and human rotavirus

Table 11: Summary of results (LVP) with Sterisept Wipes and human rotavirus

Legend to the Figures

Figure 1: Virus-inactivating properties of Sterisept Wipes (80.0 %) (LVP)

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

Table 1: Raw data for Sterisept Wipes (50.0 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#6000)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	50.0 %	dirty conditions	0.5	n.d.	n.d.	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	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	50.0 %	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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4144	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4243 3220	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 Wipes (10.0 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#6000)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0 %	dirty conditions	0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	4444 4444	4444 4444	4343 2224	0000 0040	0000 0000	n.d.	n.d.
test product cytotoxicity	10.0 %	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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4144	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4243 3220	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 3: Raw data for formaldehyde solution (0.7 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#6000)

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 tttt	4444 4444	4433 4332	4424 4344	0434 0433	3000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	4444 4444	4444 4444	2434 2321	2200 3030	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	4444 4444	4333 3344	3430 0004	0020 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	4444 4444	0432 0000	0000 0003	0000 0000	0000 0000	0000 0000	n.d.
formaldehyde cytotoxicity	0.7 % (m/V)	PBS	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	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.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4221 2444	0000 0020	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 control of efficacy for suppression of disinfectant's activity (80.0 %) (#6000)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	dirty conditions	tttt tttt	tttt tttt	tttt tttt	4444 4444	2303 3344	4030 0000	0000 0000	0000 0000	n.d.
corresponding virus control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4243 3220	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 5: Raw data (human rotavirus) for cell sensitivity (80.0 % solution) (#6000) (LVP)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0444 4444	0440 0400	0000 0000	n.d.
test product	1:10,000	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 0044	0000 0400	0000 0000	n.d.

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: Determination of virus titre (LVP) at 20 °C (#5966) (1st assay)

Virus titration	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
1st control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4344 4044	0000 2004	0000 0000	n.d.
2nd control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3444 4034	0000 0000	0000 0000	n.d.

n.a. = not applicable t = cytotoxic 0 = no virus detectable
n.d. = not done 1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Table 7: Inactivation of human rotavirus by Sterisept Wipes (80.0 %) at 20 °C (30 seconds) (LVP, 1:10,000) (#5966) (1st assay)

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 8: Determination of virus titre (LVP) at 20 °C (#6000) (2nd assay)

Virus titration	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
1st control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4243 3220	0000 0000	0000 0000	n.d.
2nd control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4334 4444	4332 2223	0000 0000	0000 0000	n.d.

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

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

Table 9: Inactivation of human rotavirus by Sterisept Wipes (80.0 %) at 20 °C (30 seconds) (LVP, 1:10,000) (#6000) (2nd assay)

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 10a: Summary of results (end point dilution method) with Sterisept Wipes and human rotavirus

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after …min
				0.5	1	5	30	60	
test product	50.0 %	dirty conditions	3.50	≤ 4.50±0.00	≤ 4.50±0.00	n.d.	n.d.	n.d.	≥ 0.5 (RF ≥ 2.88±0.18)
test product	10.0 %	dirty conditions	3.50	n.d.	n.d.	n.d.	6.63±0.25	n.d.	> 30 (RF = 0.75±0.35)

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

Table 10b: Summary of results (end point dilution method) with Sterisept Wipes and human rotavirus

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after … min
				0	5	15	30	60	
formaldehyde	0.7 % (w/v)	PBS	3.50	n.d.	7.38±0.41	7.00±0.38	6.13±0.45	5.00±0.44	> 60 (RF = 2.63±0.51)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.25	n.a.
virus control	n.a.	dirty conditions	n.a.	7.50±0.00	n.d.	n.d.	n.d.	7.38±0.25	n.a.
suppression control	80.0 %	dirty conditions	4.50	n.d.	n.d.	n.d.	6.63±0.41	n.d.	n.a.

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

Table 11: Summary of results (LVP) with Sterisept Wipes and human rotavirus

Product*	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after …min
				0.5	1	3	5	30	
test product (1)	80.0 %	dirty conditions	n.a.	≤ 2.84	n.d.	n.d.	n.d.	n.d.	0.5 (RF ≥ 4.66±0.24)
test product (2)	80.0 %	dirty conditions	n.a.	≤ 2.84	n.d.	n.d.	n.d.	n.d.	0.5 (RF ≥ 4.60±0.13)
virus control (1)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.63±0.41 7.38±0.25 (Ø7.50±0.24)	n.a.
virus control (2)	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	7.38±0.25 7.50±0.00 (Ø7.44±0.13)	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.75±0.44	n.a.
sens. product	80.0 % → 1:10,000	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.38±0.41	n.a.

*the number in the brakets 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 Wipes (80.0 %) (LVP)

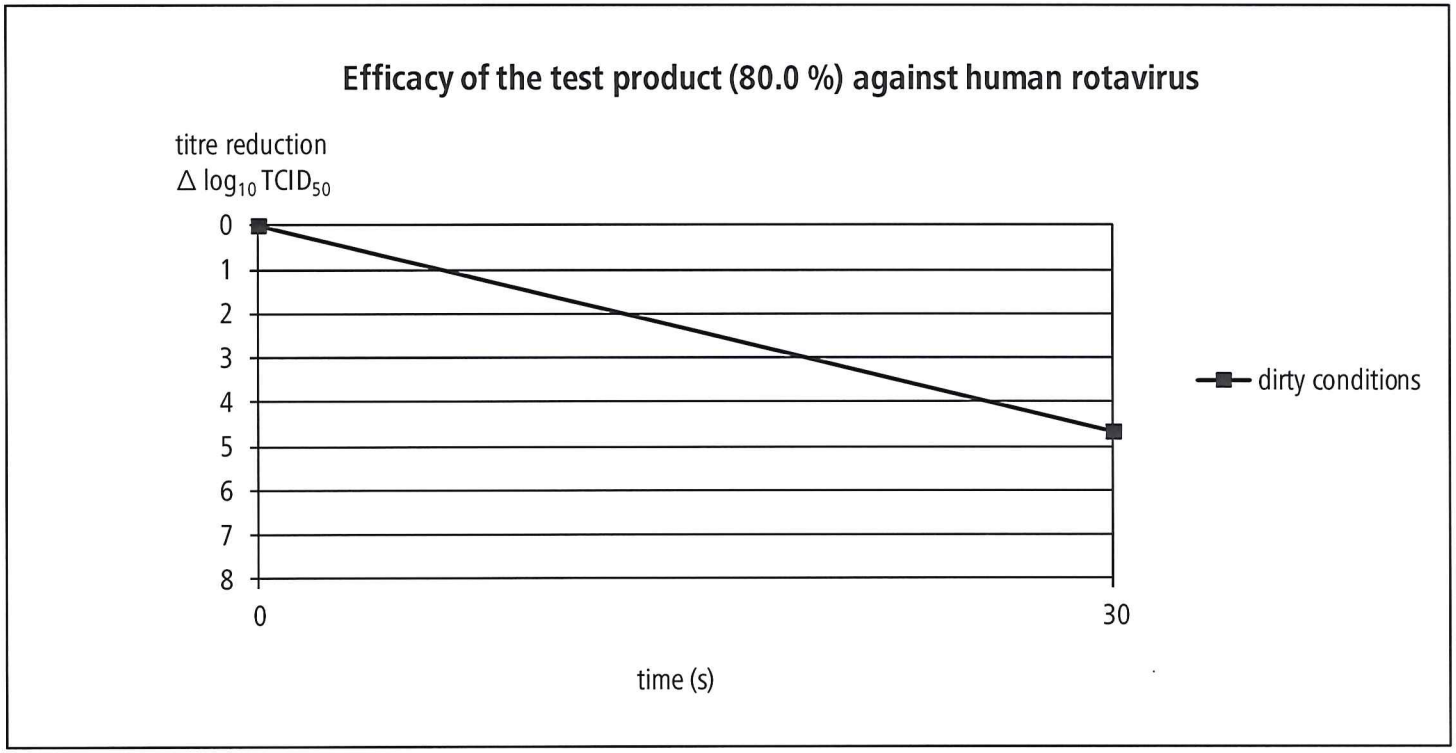

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)