



19.08.2015

Test report P15L0210M

Evaluation of the effectiveness of IR114G (active solution)

Test virus: murine norovirus (as surrogate of human norovirus)

Method: EN 14476:2013/FprA1 2015

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

Sponsor:
PDI International
Aber Park
GB - FLINT CH6 5EX

1. Identification of test laboratory

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

2. Identification of sample

Manufacturer	PDI International
Name of product	IR114G
Product diluent recommended by the manufacturer	-
Batch number	30.04.2015
Application	surface disinfection
Production date	-
Expiry date	-
Active compound (s) (kg)	0.45 % didecylammoniumchloride
Appearance, odour	active solution after squeezing out the wipes product specific
pH-values (in WSH)	undiluted: 9.90 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	30.04.2015

3. Materials

3.1 Culture medium and reagents

- Dulbecco's Modified Eagle's Medium (DMEM, Biozym Scientific GmbH, catalogue no. 880006)
- Fetal calf serum (Thermo Fisher, article no. CH30160.02)
- 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).

*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. 421. 27819102, Telefax +49. 421. 2760283, 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 2015

3.2 Virus and cells

Murine norovirus (MNV) was obtained from PD. Dr. E. Schreier, Head of FG15 Molecular Epidemiology of Viral Pathogens at the Robert Koch-Institute (RKI) in Berlin. Prior to inactivation, MNV was passaged three times in *RAW 264.7 cells* (a macrophage-like, Abelson leukemia virus transformed cell line derived from BALB/c mice, ATCC TIB-71). RAW 264.7 cells were cultured with Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose and fetal calf serum with low endotoxin.

Furthermore, cells (passage 22) 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)
- 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 (97.0 % and 80.0 %) and 10.0 % (demonstration of non-active range) solution
Appearance of product dilutions	no precipitation
Contact times	1, 2 and 5 minutes
Interfering substance	3.0 g/l bovine serum albumin (clean conditions, EN 14476:2013)
Procedure to stop action of disinfectant	immediate dilution
Diluent	water
Stability of product in the mix with virus and interfering substance (97.0 % and 80.0 % solutions)	medium flocculation, minor precipitation
Virus strain	murine norovirus (Berlin 06 / 06 / DE Isolate S99)
Date of testing	30.04.2015 – 19.08.2015
End of testing	19.08.2015

5. Methods

5.1 Preparation of test virus suspension

To prepare the test virus suspension, *RAW 264.7 cells* which have been cultured with Dulbecco's Modified Eagle's Medium with 4.5 g/l glucose and 10 % fetal calf serum with low endotoxin were inoculated with MNV (stock virus solution) in a 175 cm² cell culture flask. Once a cytopathic effect had been induced (approx. 1-3 days), freezing and thawing was carried out two times. The cell debris was removed by low speed centrifugation (400 g_N and 15 min) and the supernatant was recovered as test viral suspension, aliquoted and stored at -80 °C.

5.2 Preparation of disinfectant (dilutions)

The test product was tested as undiluted. Due to the addition of interfering substance and test virus suspension an 80.0 % solutions resulted. Additionally, the test product was examined as 97.0 % solutions (0.1 part test virus suspension + 0.2 part interfering substance (5-fold) + 9.7 parts disinfectant).

Furthermore, the product was evaluated as 10.0% solution (demonstrating of non-active range). This solution was prepared with water 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. 421. 27819102, Telefax +49. 421. 2760283, 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 2015

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 *RAW 264.7 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 five 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

X₀ = 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 14476:2013, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by four 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 in accordance to EN 5.5. The test product was examined undiluted (97.0 % and 80.0 %) and as 10.0 % (demonstration of non-active range) solutions in water at 20 °C according to EN 14476:2013. 1, 2 and 5 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.

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

*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. 421. 27819102, Telefax +49. 421. 2760283, 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 2015

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

5.6 Inactivation assay following the large volume plating method

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 DMEM (total dilution of 1:10,000) and then the total volume was distributed in 3 microtitre plates (216 μl / well, 288 wells total). After 5 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 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:

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

*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. 421. 27819102, Telefax +49. 421. 2760283, 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 2015

c = number of virus particles
 D = dilution
 V_w = volume per well
 n = number of inoculated wells
 n_p = number of virus-positive wells

The logarithmic titre ($\log_{10}TCID_{50}/ml$) for calculating the reduction factor using the formula according to Taylor is received 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 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:

*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. 421. 27819102, Telefax +49. 421. 2760283, 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 2015

- a) The titre of the test virus suspension allowed the determination of a $\geq 4 \log_{10}$ reduction (maximal virus reduction = 4.57 ± 0.30 , LVP).
- b) The test product (80.0 % solution) showed cytotoxicity in the 1:1,000 dilutions thus allowing the detection of a $4 \log_{10}$ reduction of virus titre in the assay following the large volume plating method.
- c) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *RAW 264.7 cells* showed no significant difference ($< 1 \log_{10}$; EN 5.7) of virus titre: 8.00 ± 0.46 (PBS) versus 8.00 ± 0.44 (1:10,000 dilutions of disinfectant as 97.0 % solution) $\log_{10}\text{TCID}_{50}/\text{ml}$.
- d) The control of efficacy for suppression of disinfectant's activity (97.0 % solution) showed no decrease ($< 0.5 \log_{10}$; EN 5.5.5.1) in virus titre (6.75 ± 0.35 versus $6.63 \pm 0.25 \log_{10}\text{TCID}_{50}/\text{ml}$).
- 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 14476:2013 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.

Testing the product undiluted with the 97 % assay no residual test virus could be detected within 1, 2 and 5 minutes (Table 1).

In addition, the undiluted test product (80.0 % solution) was able to inactivate MNV after 5 minutes under clean conditions in this quantitative suspension test (no residual test virus) (Table 2). The reduction factor was $\geq 3.50 \pm 0.31$. Due to high cytotoxicity, a reduction of $4 \log_{10}$ steps could not be shown.

Therefore, the large volume plating method (LVP) was introduced with 1 and 2 minutes exposure time testing the 97.0 % solution. The results are presented in tables 8 and 9.

*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. 421. 27819102, Telefax +49. 421. 2760283, 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 2015

The virus titres in the twofold assay were $\log_{10}\text{TCID}_{50}/\text{ml} = 6.75 \pm 0.35$ and 7.13 ± 0.49 (Table 7). The mean value was 6.94 ± 0.30 .

Since 66 of 288 cell culture units showed residual virus after 1 minute the result according to the formula of Taylor was $4.24 \log_{10}\text{TCID}_{50}$. The reduction factor was therefore $6.94 \pm 0.30 \log_{10}\text{TCID}_{50}$ minus $4.24 \log_{10}\text{TCID}_{50} = 2.70 \pm 0.30$ after 1 minute of exposure time.

After 2 minutes of incubation time, 1 of 288 cell culture units showed residual virus in the LVP assay (Table 9). The result according to the formula of Taylor was $2.37 \log_{10}\text{TCID}_{50}$. The reduction factor was 4.57 ± 0.30 . This corresponded to an inactivation of $\geq 99.99\%$.

The test product as 10.0 % solution was not able to sufficiently inactivate MNV after 5 minutes under clean conditions in this quantitative suspension test (Table 3). The reduction factor was $\geq 2.38 \pm 0.61$ at this time point.

8. Conclusion

The surface disinfectant IR114G tested as 97.0 % solution demonstrated effectiveness against MNV after an exposure time of 2 minutes under clean conditions.

Therefore, the surface disinfectant IR114G can be declared as active against MNV as follows:

undiluted 2 minutes

Bremen, 19.08.2015

- Dr. Jochen Steinmann -
Scientific Director



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 entirety, 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. 421. 27819102, Telefax +49. 421. 2760283, 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 2015



11. Literature

1. EN 14476:2013/FprA1 March 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 IR114G (97.0 %) tested against MNV
Table 2:	Raw data for IR114G (80.0 %) tested against MNV
Table 3:	Raw data for IR114G (10.0 %) tested against MNV
Table 4:	Raw data for formaldehyde solution (0.7 %) tested against MNV
Table 5:	Raw data for control of efficacy for suppression of disinfectant's activity (97.0 %)
Table 6:	Raw data (MNV) for cell sensitivity (97.0 %) (LVP)
Table 7:	Determination of virus titre (LVP)
Table 8:	Inactivation of MNV by IR114G (97.0 %) (1 min) (LVP)
Table 9:	Inactivation of MNV by IR114G (97.0 %) (2 min) (LVP)
Table 10 (a+b):	Summary of results (end point dilution) with IR114G and MNV
Table 11:	Summary of results (LVP, 1:10,000) with IR114G and MNV



Legend to the Figures

Figure 1: Virus-inactivating properties of IR114G (97.0 %) (LVP)

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





Table 1: Raw data for IR11G (97.0 %) tested against MNV at 20 °C (quantal test; 8 wells) (#3911)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)									
				1	2	3	4	5	6	7	8	9	
test product	97.0%	clean conditions	1	tttt	tttt	tttt	tttt	0000	0000	0000	0000	n.d.	n.d.
			2	tttt	tttt	tttt	tttt	0000	0000	0000	0000	n.d.	n.d.
			5	tttt	tttt	tttt	tttt	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.	n.d.
			n.a.	tttt	tttt	tttt	tttt	0000	0000	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	97.0%	clean conditions	n.a.	tttt	tttt	tttt	tttt	0000	0000	n.d.	n.d.	n.d.	
virus control	n.a.	clean 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	0004	4000	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)

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norddeog 2, DE - 28259 Bremen, Germany, Telephone +49, 421, 27819102, Telefax +49, 421, 2760283, 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 2015





Table 2: Raw data for IR11G (80.0 %) tested against MNV at 20 °C (quantal test; 8 wells) (#4028)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)										
				1	2	3	4	5	6	7	8	9		
test product	80.0%	clean conditions	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2	ttt	ttt	ttt	4440	0000	0000	0000	0000	n.d.	n.d.	
			5	ttt	ttt	ttt	0404	0000	0000	0000	0000	n.d.	n.d.	
			30	n.d.	n.d.	n.d.	0000	0000	0000	0000	n.d.	n.d.	n.d.	
			n.a.	ttt	ttt	ttt	0000	0000	n.d.	n.d.	n.d.	n.d.		
			0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
test product cytotoxicity	80.0%	clean conditions	n.a.	ttt	ttt	ttt	0000	0000	n.d.	n.d.	n.d.	n.d.		
virus control	n.a.	clean conditions	60	4444	4444	4444	4444	4444	4444	4044	0000	0040	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)

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderroog 2, DE – 28259 Bremen, Germany, telephone +49, 421, 27819102, Telefax +49, 421, 2760283, 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 2015





Table 3: Raw data for ID 213 (10.0 %) tested against MNV at 20 °C (quantal test; 8 wells) (#4028)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)										
				1	2	3	4	5	6	7	8	9		
test product	10.0%	clean conditions	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2	n.d.	n.d.	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.	n.d.	n.d.
test product cytotoxicity	10.0%	clean conditions	30	tttt	tttt	4444	4044	0000	0000	0000	0000	0000	n.d.	n.d.
			n.a.	tttt	tttt	0000	0000	0000	0000	n.d.	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	clean conditions	0	n.d.	n.d.	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	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)

* 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, 421, 27819102, Telefax +49, 421, 2760283, 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 2015



Deutscher
Akkreditationsdienst
D-PL 13412 01-02



Anerkannt und/oder
Zentralinstitut für Labor- und
Medizinische Diagnostik
ZILG-AP-306, 10.31



Table 4: Raw data for formaldehyde solution (0.7 %) tested against MNV at 20 °C (quantal test; 8 wells) (#4028)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log ₁₀)									
				1	2	3	4	5	6	7	8	9	
formaldehyde (0.7% (m/V))	0.7%	PBS	5	tttt	tttt	tttt	4444	4444	4444	3000	n.d.	n.d.	
				tttt	tttt	tttt	4444	4444	4444	0000	n.d.	n.d.	
			15	tttt	tttt	tttt	4444	4444	3303	0000	n.d.	n.d.	
				tttt	tttt	tttt	4444	4444	0300	0000	n.d.	n.d.	
			30	tttt	tttt	4444	4404	0040	0000	0000	n.d.	n.d.	
				tttt	tttt	4444	4444	0400	0000	0000	n.d.	n.d.	
60	tttt	tttt	4444	0340	0000	0000	0000	n.d.	n.d.				
	tttt	tttt	4444	0300	0000	0000	0000	n.d.	n.d.				
formaldehyde cytotoxicity (0.7% (m/V))	0.7%	PBS	n.a.	tttt	tttt	tttt	0000	0000	0000	0000	n.d.	n.d.	
				tttt	tttt	tttt	0000	0000	0000	0000	n.d.	n.d.	
virus control	n.a.	PBS	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0044	0000	0000	
				4444	4444	4444	4444	4444	4444	4444	4444	0004	0000
virus control	n.a.	PBS	60	4444	4444	4444	4444	4444	4444	4444	4444	0000	0000
				4444	4444	4444	4444	4444	4444	4444	4444	0004	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)

* 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, 421, 27819102, Telefax +49, 421, 2760283, 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 2015



DAKKS
Deutsche
Zertifizierungsstelle
D-Pl. 13112-01-02



Accredited/Institutszertifiziert by
Zertifizierungsstelle für
Arztzentrifugation und
Mikrobiologie
ZLG-AP-306-10.31



Table 5: Raw data for control of efficacy for suppression of disinfectant's activity (97.0 %) (#4028)

Product	Interfering substance	dilutions (log ₁₀)								
		1	2	3	4	5	6	7	8	9
test product	PBS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	clean conditions	tttt	tttt	tttt	4444	4444	0000	0000	0000	n.d.
test product	dirty conditions	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.a. = not applicable

n.d. = not done

0 = no virus present; t = cytotoxic

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

* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norddeog 2, DE – 28259 Bremen, Germany, Telephone +49, 421, 27819102, Telefax +49, 421, 2760283, 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 2015





Table 6: Raw data (MNV) for cell sensitivity (97.0 %) (#4028)

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	3000 0030	0440 0000	n.d.
test product	1:100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	1:1,000	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product	1:10,000	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0400 4040	0040 0000	n.d.

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)

* 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, 421, 27819102, Telefax +49, 421, 2760283, 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 2015



Deutsche
Akkreditierungsstelle
D-PL 33412 01 02



Akreditiert/Recognized by
Zentralstelle der Länder
für Arzneimittel und
Medizinprodukte
ZLG-AP-306.10.31



Table 7: Determination of virus titre (LVP) (#4028)

Virus titration	Interfering substance	dilutions (log ₁₀)								
		1	2	3	4	5	6	7	8	9
1 st assay	clean conditions	4444	4444	4444	4444	4444	0030	0000	0000	0000
		4444	4444	4444	4444	4444	0000	0040	0000	0000
2 nd assay	clean conditions	4444	4444	4444	4444	4444	3000	0000	0000	0000
		4444	4444	4444	4444	4444	4004	3300	0000	0000

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)

* 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, 421, 27819102, Telefax +49, 421, 2760283, 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 2015



DAKKS
DIN EN ISO/IEC 17025
D-PL-13417-01-02



Amplikon Deutschland
Zentrum für
Mikrobiologie und
Molekularbiologie
ZLG-AP-306/10.31



Table 8: Inactivation of MNV by IR114G (97.0 %) (LVP, 1:10,000) (1 min) (#4028)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
		plate 1/3	0000 0004	0040 0000	0000 4000	0000 0000	0044 0000	0000 4004	4000 0000	0000 0000	0040 0000	0040 0000	0404 0000
clean conditions	plate 2/3	0400 0044	0000 4000	4040 0400	4000 0000	0040 0004	4004 0000	0404 0004	0400 0000	0044 0044	0000 4040	4400 0000	0404 0404
	plate 3/3	0404 0404	0440 0040	4000 0000	0040 4000	0004 0000	0004 0040	2000 0440	0000 0000	0040 4004	0040 0000	0000 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 9: Inactivation of MNV by IR114G (97.0 %) (LVP, 1:10,000) (2 min) (#4028)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
		plate 1/3	0000 0000										
clean conditions	plate 2/3	0000 0000											
	plate 3/3	0000 0000	0000 0400	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)

* 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, 421, 27819102, Telefax +49, 421, 2760283, 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 2015



DAKKS
Deutscher
Akkreditierungsausschuss
D.N. 13412:01:02



ZLG-AP
Zentralstelle für Labor- & Testverfahren
Mittelstraße 1
D-30658 Hannover
Tel: +49 51 3100-1
Fax: +49 51 3100-2
E-Mail: zlg@zlg.de
www.zlg.de
ZLG-AP-306.10.31



Table 10a: Summary of results (end point dilution method) with IR114G and MNV

Product	Con- centration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ /ml after ...min						> 4 log ₁₀ reduction after ... min
				1	2	5	30	60		
test product	97.0%	clean conditions	4.50	≤4.50±0.00	≤4.50±0.00	≤4.50±0.00	n.d.	n.d.	≥1 (RF ≥2.25±0.35)	
test product	80.0%	clean conditions	4.50	n.d.	≤5.13±0.37	≤4.50±0.00	n.d.	n.d.	≥5 (RF ≥3.50±0.31)	
test product	10.0%	clean conditions	3.50	n.d.	n.d.	n.d.	5.63 ±0.41	n.d.	> 30	

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

* 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, 421. 27819102, Telefax +49, 421. 2760283, 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 2015





Table 10b: Summary of results (end point dilution methode) with IR114G 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	7.00±0.38	6.63±0.41	5.88±0.37	> 60	
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	8.38±0.41	n.a.	
virus control	80% assay	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	8.00±0.44	n.a.	
virus control	97% assay	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.75±0.35	n.a.	
suppression control	97.0%	clean conditions	3.50	n.d.	n.d.	n.d.	6.63±0.25	n.d.	n.a.	
sens. control PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	8.00±0.46	n.a.	
sens. control test product	97.0% → 1:10,000	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	8.00±0.44	n.a.	

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

* 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, 421. 27819102, Telefax +49, 421. 2760283, 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 2015





Table 11: Summary of results (LVP, 1:10,000) with IR114G and MNV

Product	Con- centration	Interfering substance	Level of cytotoxicity	log ₁₀ TCID ₅₀ /ml aftermin					> 4 log ₁₀ reduction after ... min
				0	1	2	30	60	
product	97.0%	clean conditions	n.a.	n.d.	4.24	2.37	n.d.	n.d.	2 (RF = 4.57±0.30)
virus control	n.a.	clean conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.75±0.35 7.13±0.49	n.a.

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

* 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, 421, 27819102, Telefax +49, 421, 2760283, 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 2015



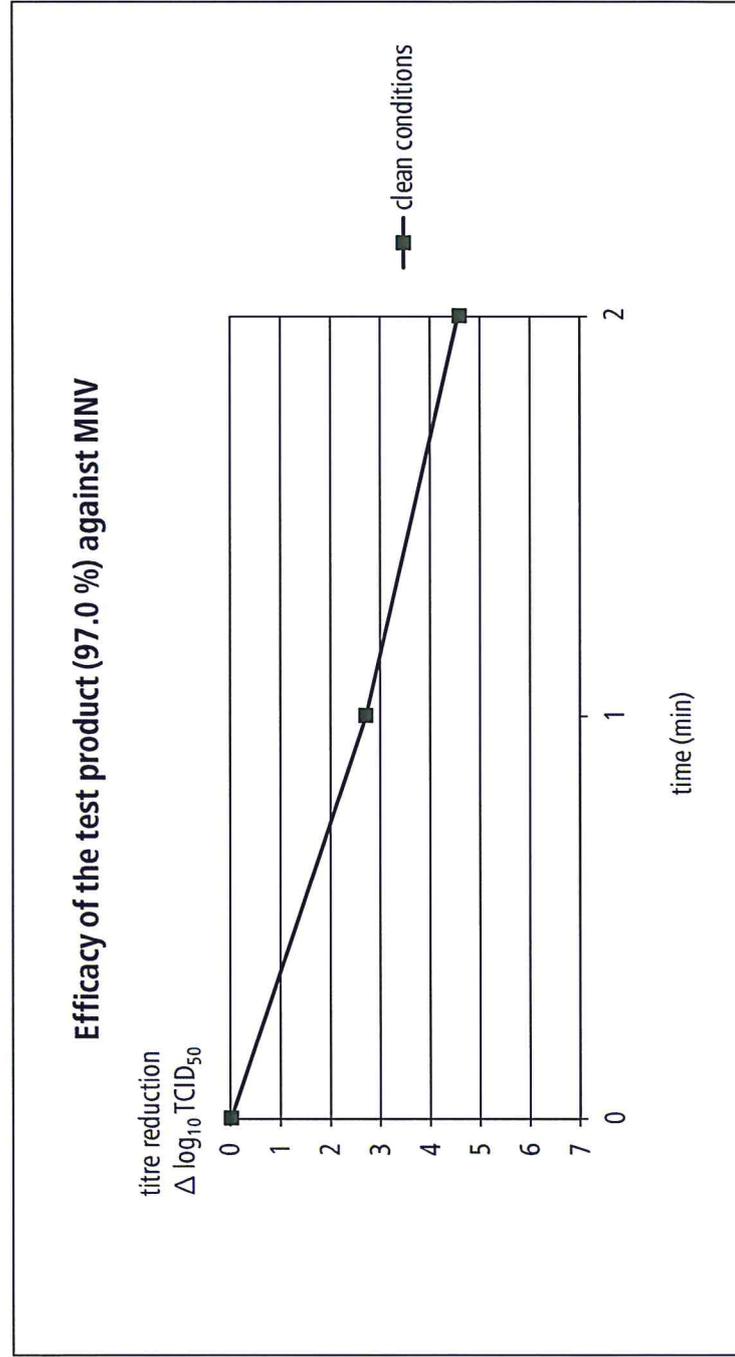
DAkkS
Deutsche
Angewandte
Zertifizierung
D.N. 13412:01.02



Angewandt durch/accredited by
Zertifizierungsgesellschaft
für
Laboratorien
ZLG-AP-306.10.31



Figure 1: Virus-inactivating properties of IR114G (97.0 %) (LVP)

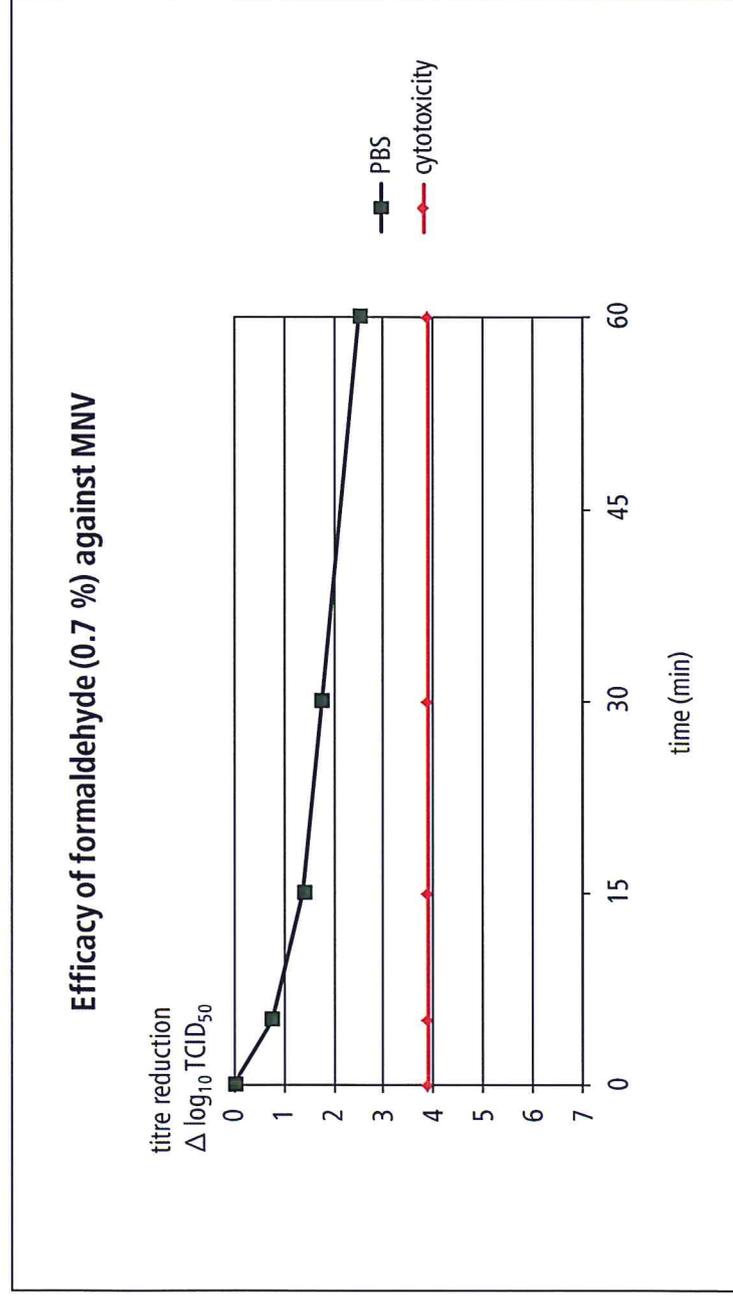


* Test procedure accredited according to DIN EN ISO/IEC 17025. Test report issued by Dr. Brill + Partner GmbH, Norderoog 2, DE - 28259 Bremen, Germany. Telephone +49. 421. 27819102, Telefax +49. 421. 2760283, www.brillhygiene.com. No copying or transmission, in whole or in part, of this test report without the explicit prior written permission. The test results exclusively apply to the tested samples. Information on measurement uncertainty on request. © Dr. Brill + Partner GmbH 2015





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



* 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. 421. 27819102, Telefax +49. 421. 2760283, 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 2015

