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Virucidal activity of a new hand disinfectant with reduced ethanol content: comparison with other alcohol-based formulations

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Summary A new formula with reduced ethanol content (55%) in combination with 10% propan-1-ol, 5.9% propan-1,2-diol, 5.7% butan-1,3-diol and 0.7% phosphoric acid exhibited a broad spectrum of virucidal activity. In quantitative suspension tests, with and without protein load, this formulation reduced the infectivity titre of seven enveloped (influenza A and B, herpes simplex 1 and 2, bovine corona, respiratory syncytial, vaccinia, hepatitis B, bovine viral diarrhoea) and four non-enveloped (hepatitis A, polio, rota, feline calici) viruses $>10^3$ -fold within 30 s. In comparative testing, only 95% ethanol showed similar levels of activity.

In fingerpad tests, the formulation produced a \log_{10} reduction factor of the titre of poliovirus type 1 (Sabin) of 3.04 in 30 s compared with 1.32 by 60% propan-2-ol. Testing against feline calicivirus produced a \log_{10} reduction factor of 2.38 by the test formulation; in contrast, the \log_{10} reduction factors with 70% ethanol and 70% propan-1-ol were 0.68 and 0.70, respectively.

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Introduction

Many pathogenic viruses can remain viable on human hands for hours.^{1,2} This gives hands the potential to spread such infectious agents directly or indirectly³⁻⁵ in settings such as hospitals.^{6,7} Recent studies with experimentally contaminated fingertips have further substantiated the potential of hands to spread viruses.^{8,9} These observations re-emphasize the need for proper hand disinfection in the prevention and control of nosocomial outbreaks of viral infections in particular. However, hand disinfectants are often tested against vegetative bacteria only and this may not reflect on their ability to deal with viruses.¹⁰

While alcohol-based hand rubs generally have a broader and relatively rapid antimicrobial activity, they are often limited in their ability to inactivate non-enveloped viruses.⁷ Raising the ethanol content may address this issue to some degree, but increases the risk of tissue toxicity¹¹ and lowers the flash point. At present, only one formulation with broad virucidal activity exists with an ethanol content of 95 vol%. Therefore, efforts were made to reduce the ethanol content without reducing the virucidal activity to decrease the flash point and increase skin tolerance and compliance. As a result of these efforts, a synergistic combination was developed with an ethanol content of 55% (w/w) in combination with 10% (w/w) propan-1-ol, 5.9% (w/w) propan-1,2-diol, 5.7% (w/w) butan-1,3-diol and 0.7% phosphoric acid.¹² This ready-to-use formulation is registered by the US Food and Drug Administration (NDC-6673-1230-(I)-(9)). Since introduction of the evaluated product in Austrian hospitals, no relevant unwanted side-effects have been reported to date.

Materials and methods

Cells

The following cells were used: FL (amnion) cells (Stephan Angeloff Institute of Microbiology, Sofia, Bulgaria; ATCC No. CCL-62) in Dulbecco's modified Eagle medium (DMEM) (GIBCO BRL, Paisley, Scotland, UK) containing 10% heat-inactivated fetal bovine serum (GIBCO BRL, Grand Island, NY, USA) supplemented with 10 mmol/L HEPES buffer (VWR International GmbH, Darmstadt, Germany) and antibiotics (penicillin, 100 U/mL, streptomycin, 100 µg/mL); BS-C-1 (*Cercopithecus* monkey kidney) cells (ATCC No. CCL-26, USA) in DMEM; Madin-Darby canine kidney cells (ATCC No. CCL-34) in DMEM;

Hep-2 cells (No. NBIMCC-95, National Bank for Industrial Micro-organisms and Cell Cultures, Sofia, Bulgaria) in DMEM; MRC-5 (human embryo lung diploid) cells (ATCC No. X55) in DMEM; Madin-Darby bovine kidney (MDBK) cells (No. NBIMCC-1031) in DMEM; human diploid foreskin fibroblasts (National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria) in DMEM; KE-R cells (provided by Dr Riebe, Cell Bank for Cell Lines in Veterinary Medicine, Federal Research Institute for Animal Virus Diseases, Isle of Riems, Germany) in Eagle's minimum essential medium (EMEM) (Cambrex Bio Science Verviers s.p.r.l., Verviers, Belgium) containing 10% fetal calf serum (FCS) (Biochrom AG, Berlin, Germany); GMK-AH 1 (Institute of Medical Microbiology, University of Kiel, Germany) in DMEM; Vero cells (ATCC No. CCL81) in DMEM; HRT-18 (human rectal tumour) cells (provided by Dr Herbst, Institute for Animal Hygiene and Infectious Diseases, University of Giessen, Germany) in DMEM; calf trachea cells in DMEM; HepG2 cells (supplied by ATCC cell HB 8065) in DMEM; and MA-104 in EMEM.

Viruses

The virus test strains and their respective culture media were as follows: poliovirus type 1 (Mahoney/Pette, Stephan Angeloff Institute of Microbiology), cultivated in FL cells (maintenance medium DMEM without serum), virus titre 1.3×10^9 plaque-forming units/mL; human rotavirus strain Wa, cultivated in Ma-104 cells without serum, virus titre $10^{7.8}$ cell culture infective dose CCID₅₀/mL; hepatitis A virus (HAV, HM 175/18 f cell culture adapted cytopathic clone B, ATCC No. VR-1402), cultivated in BS-C-1 cells (maintenance medium DMEM plus 2% FCS), virus titre $10^{6.8}$ CCID₅₀/mL; bovine viral diarrhoea virus (BVDV) (Istituto Zooprofilattico, Perugia, Italy), cultivated in calf trachea cells (maintenance medium DMEM plus 0.5% FCS), virus titre $10^{7.0}$ CCID₅₀/mL; influenza A virus [Aichi/2/68 (H3N2), Stephan Angeloff Institute of Microbiology], cultivated in allantoic fluid of 10-day-embryonated eggs at 37 °C, virus titre $10^{7.5}$ CCID₅₀/mL; influenza B virus (Lee/40, ATCC No. VR-101), inoculated in the same manner and cultivated at 35 °C, virus titre $10^{7.5}$ CCID₅₀/mL; human rhinovirus (HRV) type 14 strain 1059, ATCC No. VR-284, cultivated in MRC-5 cells (maintenance medium DMEM plus 2% FCS), virus titre $10^{6.6}$ CCID₅₀/mL; bovine corona virus (BCV) strain L9 (BCV-L9, provided by Dr Herbst), cultivated in HRT-18, virus titre $10^{7.5}$ CCID₅₀/mL; respiratory syncytial virus (RSV) (District Centre of

Hygiene and Epidemiology, Plovdiv, Bulgaria), cultivated in Hep-2 cells [maintenance medium MEM (GIBCO BRL, Grand Island) without serum], virus titre $10^{6.5}$ CCID₅₀/mL; simian virus (SV) 40 (pml-1, ATCC No. VR-820), cultivated in BS-C-1 cells (maintenance medium DMEM without serum), virus titre $10^{7.5}$ CCID₅₀/mL; human adenovirus type 2 (adenoid 6, NBIMCC No. 515), cultivated in Hep-2 cells (maintenance medium DMEM without serum), virus titre $10^{8.5}$ CCID₅₀/mL; herpes simplex virus (HSV) type 1 (DA) and HSV type 2 (BYA, National Centre of Infectious and Parasitic Diseases), cultivated in human diploid foreskin fibroblasts (maintenance medium DMEM without serum) or in MDBK cells (maintenance medium DMEM without serum), virus titre $10^{6.0}$ CCID₅₀/mL; feline calicivirus (FCV) strain F9 (Institute for Virus Diagnostic, Federal Research Institute for Animal Virus Diseases, Isle of Riems, Germany), cultivated in KE-R cells (maintenance medium MEM with 2% FCS), virus titre $10^{7.5}$ CCID₅₀/mL; vaccinia virus strain Elstree (German Association for the Control of Virus Diseases, DVV), cultivated in Vero cells, virus titre $10^{9.5}$ CCID₅₀/mL; and hepatitis B virus (HBV) from a pool of infected plasma (Virology Laboratory, Angers, France), cultivated in a modified HepG2 cell system (virus titre $10^{5.4}$ infectious units/mL) as described previously.¹³

For poliovirus type 1, stock virus suspensions with an infectious titre $<10^8$ CCID₅₀/mL were centrifuged at low speed and then at 70 000 g (L8, Beckman Instruments, Porterville Plants, CA, USA) for 30 min at +4 °C. The sediment was resuspended in Dulbecco's phosphate-buffered saline (PBS) and centrifuged again at low speed. The clarified supernatant contained a titre of at least 10^8 CCID₅₀/mL.

For fingerpad experiments, Sabin vaccine strain of poliovirus type 1 (LSc 2ab, ATCC VR-59, kindly provided by the Behringwerke AG, Liederbach, Germany) or the abovementioned strain F9 of FCV was used.

Volunteers

Three male and four female adults participated in fingerpad tests using poliovirus type 1 and FCV (strain F9). All volunteers had received oral polio vaccination, were seropositive by neutralization tests, and confirmed in writing their informed consent to participate in this study. The Ethics Committee of Central Hospital St-Jürgen-Str (Bremen, Germany) gave permission for this clinical study (No. 50-09-97). Before each test, the hands of

the volunteers were carefully inspected to preclude dermatoses, open wounds or other skin disorders.

Hand disinfectants and chemicals

For assessing the influence of ethanol content on efficacy against poliovirus type 1, three commercial hand disinfectants were tested in diluted and undiluted forms. Product A contained only 75% (w/w) ethanol with no other active ingredients. Product B contained 80% (w/w) ethanol in combination with 0.5% non-ionic detergent. Product C contained 80% (w/w) ethanol in combination with 0.1% benzalkonium chloride, 0.025% 2-phenylphenol and 0.025% clorophen.

For developing the new formulation (hereafter called the test product), various different chemicals (Table I) were screened in combination with an ethanol concentration of 55% (w/w). The chemicals were purchased from VWR International GmbH. The final formulation, a ready-to-use preparation, contained 55% (w/w) ethanol, 10% (w/w) propan-1-ol, 5.9% (w/w) propan-1.2-diol, 5.7% (w/w) butan-1.3-diol and 0.7% phosphoric acid. For testing the undiluted solution using the quantitative suspension assay,¹⁴ a formulation with $1.2\times$ the regular concentration of the active ingredients was produced (resulting in a concentration of 96% of the test product). To test vaccinia-virus- and FCV-inactivating properties, a formulation of $1.25\times$ was used (resulting in 100% of the test product). For some in vitro experiments, the formulation to be tested was diluted with double-distilled water.

In fingerpad tests with poliovirus, the test product (100%) and the reference standard [60% (v/v) propan-2-ol] were used as recommended in EN 1500.¹⁵ For testing FCV, 70% (v/v) ethanol and 70% (v/v) propan-1-ol were included as references.

Quantitative suspension test with viruses

The guideline of the German Federal Health Office (Robert Koch-Institut, Berlin, Germany) and the DVV for testing the effectiveness of chemical disinfectants against viruses¹⁴ was strictly followed. Briefly, virus suspension, 2% serum albumin (Sigma-Aldrich, St Louis, MO, USA), FCS, double-distilled water and the hand disinfectant or the hand disinfectant dilutions were brought to 20 °C. One part of the virus suspension was mixed with either one part of double-distilled water, one part of the 2% serum albumin solution or one part of FCS. No protein load was added in the experiments with rotavirus, since FCS has rotavirus-inhibitory and trypsin-neutralizing activity. Then, eight parts of

Table I Results from screening the ethanol-based formula (quantitative suspension test with an exposure time of 1.5 min and poliovirus type 1 strain Mahoney/Pette as test virus)

Ethanol 55% (w/w) + 0.7% phosphoric acid			Reduction factor
Acetone (content in %, w/w)	Propan-1.2-diol (content in %, w/w)	Butan-1.3-diol (content in %, w/w)	
-	5	-	2.4 ± 0.24 ^a
-	10	-	2.4 ± 0.21
-	20	-	2.1 ± 0.25
-	-	5	1.5 ± 0.26
-	-	10	1.1 ± 0.21
-	-	20	0.8 ± 0.29
-	5	5	2.5 ± 0.26
-	10	10	4.8 ± 0.26
2	-	5	3.9 ± 0.25

^a 95% Confidence limits.

the formulation were added to each preparation. The mixtures were kept at 20 °C (in a water bath) for the exposure period. At the end of the exposure period, 10-fold serial dilutions of the control and test samples were prepared immediately in ice-cold culture medium (0.5 mL sample + 4.5 mL medium), inoculated into respective host cells in suspension, placed in 96-well microtitre plates (Costar, Cambridge, MA, USA), and incubated at 37 °C. Six or eight wells per sample dilution were inoculated. Virus control preparations contained identical protein concentrations. The cytopathic effect was recorded microscopically, and infectious virus titre in CCID₅₀ was evaluated by the endpoint dilution method of Reed and Muench.¹⁶ The log₁₀ of the reduction factor, hereafter called the reduction factor (RF), was determined by subtracting the logarithmic titre CCID₅₀ at any test point from the logarithmic titre CCID₅₀ of the virus control. The 95% confidence limits of the reduction factors were obtained according to the EMEA guideline.¹⁷ In this quantitative suspension test, a disinfectant solution was considered to have virucidal efficacy if, within the tested exposure period, the titre was reduced at least 10⁴-fold (RF=4, inactivation 99.99%). This does not mean that an RF of 4 is regarded as sufficient for practical purposes. However, in general, the virucidal efficacy can not be followed over a titre range of more than 10⁴-fold due to the cytotoxicity of the substances tested. In fingerpad methods, the efficacy of a hand disinfectant has to be considered by comparison with a selected reference.

An indirect immunofluorescence test was used for detection of HAV. Contact sample dilutions inoculated in BS-C-1 cells were incubated in 96- and 24-well plates at 37 °C. After three weeks, cell sheets were scraped by a rubber policeman and transferred

to standard microscope slides. The smears were dried at 20 °C and fixed by cold acetone for 15 min. They were then treated for 30 min at 37 °C in a humid atmosphere with 1:10 anti-HAV human hyperimmune serum (titre in Abbott enzyme-linked immunosorbent assay 1:400 anti-HAV immunoglobulin G, and 1:40 in indirect immunofluorescence test vs HAV, HM 175/18 f strain, in BS-C-1 cells), twice rinsed for 5 min with PBS and once for 5 min with distilled water. Samples were dried at 37 °C and treated for 30 min at 37 °C (placed in a humid atmosphere) with a 1:8 dilution of anti-human fluorescein isothiocyanate labelled goat serum (National Centre of Infectious and Parasitic Diseases) supplemented with a contrast solution of Evans' blue (VWR International GmbH) (final solution of 1:80 000). Samples were again rinsed twice with PBS for 5 min, washed with distilled water, dried at 37 °C, and observed under a fluorescence microscope. Virus titre was calculated according to Reed and Muench.¹⁶

In addition, a control for cytotoxicity was included in parallel with virucidal testing. Eight parts of the hand disinfectants were mixed with two parts of Dulbecco's PBS; serial dilutions were prepared and added to the respective host cell suspension. The effect of compound tested on cellular morphology was traced for overt signs of cytotoxicity until the end of the period of infectious virus assay recording.

All reported data are mean values from three repeated experiments, carried out simultaneously. For CCID₅₀ evaluation according to the routine Reed and Muench method,¹⁶ a Δlog₁₀ RF value of 1.7 (as compared to untreated controls) is considered to be statistically significant (the minimum effective concentration), and a Δlog₁₀ RF value of 1.6-1.0 is considered to be borderline.

Depending on test conditions, the threshold for demonstrating virucidal efficacy against HBV is a reduction of $\geq 10^3$ -fold.¹³

Fingerpad method

The study was performed for poliovirus type 1 according to ASTM standard E-1838-96¹⁸ and for FCV according to ASTM standard E-1838-02.¹⁹ Briefly, 10 μ L of virus suspension was placed on a demarcated area on each fingerpad and the inoculum was allowed to dry. The dried inoculum was then exposed to 1 mL of the reference [70% (v/v) ethanol, 70% (v/v) propan-1-ol and 60% (v/v) propan-2-ol] or standard hard water (SHW) or test formulation contained in a glass vial (Serolab, Aidenbach, Germany). The contact time was either 30 or 60 s. For poliovirus testing, tests without soil load were performed, whereas for FCV, a tripartite soil load according to E-1838-02 with bovine serum albumin, mucin and tryptone was included. Virus remaining on fingerpads was eluted with 1 mL Earle's balanced salt solution containing 1% tryptone (FCV without tryptone). Afterwards, virus titre in the eluate was determined directly by endpoint titration (poliovirus titration with GMK-AH 1 cells, FCV titration with KE-R cells) in microtitre plates. The calculation of virus titre for the fingerpad method was performed by the methods of Spearman²⁰ and Kärber.²¹

Three controls were included to determine: (1) the amount of infectious virus in the inoculum; (2) the infectious virus placed on the two thumbpads without drying; and (3) the infectious virus remaining on fingerpads after drying of the inoculum. The difference in infectious virus in the inoculum control and the dried virus control represents the loss in virus infectivity due to drying of the inoculum. The amount of infectious virus remaining after drying of the inoculum was used as the baseline to determine the extent of virus elimination after treatment with test product or references. We used four randomly selected fingerpads for treatment with test product and two for treatment with references.

Results

Screening with various ethanol-based combinations for selection of the new formulation

An ethanol concentration below 80% alone (Product A) as well as in combination with detergent

(Product B) or with phenolics and quaternary ammonium compounds (Product C) was not sufficiently effective ($RF < 4$) against poliovirus within 1 min (Table II). Therefore, combinations with longer-chain alcohols were tested. The selected basic formulation, ethanol 55% (w/w) + 0.7% phosphoric acid, was most effective in combination with propan-1.2-diol and butan-1.3-diol (Table I). Due to the replacement of propan-1.2-diol by acetone, a lower efficacy was measured. Addition of a mineral acid was important for efficacy, whereas there was no difference between the influences of phosphoric acid and HCl (Table III). In previous tests, the formulation with lactic acid was not stable for more than three months (data not shown). The content of 10% (w/w) propan-1-ol is essential to get the defined *in vivo* efficacy for surgical disinfection (data not shown).

Virucidal efficacy of the test product *in vitro* against a wide variety of viruses

As expected, influenza viruses A and B, HSV types 1 and 2, RSV, vaccinia virus, HRV and BVDV (surrogate of hepatitis C virus) proved to be quite sensitive to the action of the formulation tested, and these viruses were inactivated within 30 s even in the presence of protein load (Table IV). No replication of BCV (surrogate of severe acute respiratory syndrome corona virus, SARS-CoV) could be determined after 30 s. The test product (even 80%) was able to inactivate FCV (surrogate of norovirus) after a contact time of 30 s, even in the presence of protein load. HAV was inactivated by the test product with 96% use concentration within 30 s, but poliovirus was only inactivated after 1 min. HBV was also inactivated within 30 s with an $RF = 3.4$. SV 40 was clearly more resistant; 3 min was needed to inactivate the virus in the presence of protein load and 1 min without protein. Adenovirus strain type 2 was the most resistant test virus, resulting in efficacy of the hand disinfectant without protein load within 2 min and with protein addition within 3 min.

Virucidal activity of the test product on fingerpads

After 30 s, the test product was significantly more effective ($RF = 3.04$) against poliovirus in the fingerpad test than 60% (v/v) propan-2-ol. After 60 s, the RF was 3.13 (Table V). In comparison, the values with the reference solution were only 1.32 and 1.23, respectively (significance level for both exposure times $P = 0.001$).

Table II Reduction factors in relation to ethanol content of antiseptics against poliovirus type 1 strain Mahoney/Pette in the quantitative suspension test according to the guideline of the German Federal Health Office and the German Association for the Control of Virus Diseases¹⁵

Commercial preparation	Ethanol content (%w/w)	Reduction factor	
		1 min	2 min
Product A	75 ^a	3.8 ± 0.26 ^b	4.2 ± 0.26
	70	2.3 ± 0.28	3.3 ± 0.28
Product B	80 ^a	4.2 ± 0.22	4.1 ± 0.26
	75	3.5 ± 0.27	4.4 ± 0.27
	70	3.2 ± 0.28	4.5 ± 0.25
Product C	60	2.8 ± 0.24	3.5 ± 0.25
	80 ^a	3.3 ± 0.27	3.3 ± 0.27

^a Content in the use concentration.

^b 95% Confidence limits.

Results with FCV are shown in Table VI. The overall RF of the test product was 2.38 ± 1.24 with a tripartite soil load compared with 0.68 ± 0.58 [70% (v/v) ethanol], 0.70 ± 0.42 [70% (v/v) propan-1-ol] and 1.39 ± 0.18 (SHW) after an exposure time of 30 s (*P* values 0.0004, 0.0005 and 0.03, respectively).

Discussion

In the present study, the virucidal activity of this formulation against poliovirus was evaluated during development in comparison with three other alcohol-based hand disinfectants. Furthermore, a broad spectrum of human pathogenic viruses causing nosocomial infections or their surrogates was included later in the study design.

In the quantitative suspension test without and with protein load, all tested enveloped viruses were inactivated after an exposure time of 30 s, resulting in an RF of 4.

The test product was also able to inactivate the more resistant non-enveloped viruses. Ethanol 55% (w/w) in combination with long-chain alcohols reached a high virucidal efficacy against poliovirus after an exposure time of 1 min. In another study, only 90% ethanol was able to inactivate echovirus 11, which has a comparable tenacity as echovirus 11 and poliovirus both belong to the enteroviruses.²² Lower concentrations of ethanol failed to inactivate echovirus 11 after an exposure time of 1 min. Additionally, another product with a high ethanol content of 80% in combination with 0.2% peracetic acid displayed poliovirus-inactivating properties.²³ The most remarkable effect of the test product was demonstrated when testing HAV. Against HAV, inactivation was found after an exposure time of 30 s. In another study, an alcohol mixture with a high content of ethanol (80% ethanol and 5% isopropanol) was not able to reduce the virus titre by an RF of 4 (RF = 2.2 after 1 min).²⁴ Furthermore, two other products with a high concentration of

Table III Influence of mineral acids on the virucidal efficacy of the test product (quantitative suspension test with poliovirus type 1 strain Mahoney/Pette)

Combination of the four alcohols (ethanol, propan-1-ol, propan-1.2-diol, butan-1.3-diol)	Reduction factor I		Reduction factor II	
	1 min	2 min	1 min	2 min
Without mineral acid	2.9 ± 0.25 ^a	3.3 ± 0.28	3.4 ± 0.25	3.9 ± 0.26
With phosphoric acid				
0.70%	4.8 ± 0.26	5.9 ± 0.25	5.8 ± 0.23	5.8 ± 0.26
0.45%	3.6 ± 0.27	5.4 ± 0.24	> 5.5	> 5.5
With HCl				
0.30%	> 5.8	> 5.8	> 5.8	> 5.8
0.10%	> 5.8	> 5.8	4.1 ± 0.27	> 5.8

I, 8 parts of the formulation + 1 part of virus suspension + 1 part of fetal calf serum; II, 8 parts of the formulation + 1 part of virus suspension + 1 part of distilled water.

^a 95% Confidence limits.

Table IV Dilution of test product demonstrating virucidal efficacy log₁₀ reduction factor (RF) > 4 without and with protein load (bovine serum albumin or fetal calf serum)

Virus	Contact time (min)	Dilution (%) of use concentration		
		I	II	III
Influenza virus A	0.5	96 ^a	96 ^a	96 ^a
	1.0	20	20	20
Influenza virus B	0.5	96 ^a	96 ^a	96 ^a
	1.0	80	80	80
Human rhinovirus 14	0.5	96 ^a	96 ^a	96 ^a
Herpes simplex virus 1	0.5	96 ^a	96 ^a	96 ^a
	1.0	10	20	40
Herpes simplex virus 2	0.5	96 ^a	96 ^a	96 ^a
	1.0	20	40	40
Bovine corona virus	0.5	80	80	80
Respiratory syncytial virus	0.5	96 ^a	96 ^a	96 ^a
	1.0	10	50	50
Vaccinia virus	0.5	100	100	100
Hepatitis B virus	0.5	100 ^b	100 ^b	100 ^b
Bovine viral diarrhoea virus	0.5	96 ^a	96 ^a	96 ^a
Feline calicivirus	0.5	80	80	80
Rotavirus	0.5	80	n.d.	n.d.
Hepatitis A virus	0.5	96	96	96
	1.0	96	80	80
Poliovirus type 1	0.5	96 ^c	96 ^c	96
	1.0	80	80	80
SV 40	1.0	96	96 ^c	96 ^c
	3.0	96	96	96
Adenovirus type 2	2.0	96	96 ^c	96 ^c
	3.0	96	96	96

I, 8 parts antiseptic + 1 part of virus suspension + 1 part of double-distilled water; II, instead of double-distilled water, 1 part of 2% bovine serum albumin; III, instead of double-distilled water, 1 part of fetal calf serum.

^a No other dilution tested.

^b Depending on the test conditions, the threshold for virucidal efficacy is RF ≥ 3.

^c Borderline (RF 3.7-3.9).

ethanol were not effective against HAV.²⁵ Without addition of protein, the RFs were 1.8 (disinfectant 1) and 3.2 (disinfectant 2) after an exposure time of 2 min.

In the past, the fingerpad test method according to E-1838-96 has been shown to produce results comparable with the whole-hand model.²⁶ In tests of a commercially available product based upon ethanol (73.5% m/m) and propan-2-ol (10% m/m)

against poliovirus type 1 strain Sabin, similar RFs were found in both test systems (fingerpad test 2.26, whole-hand model 2.38). Therefore, this comparison demonstrates that the fingerpad method is valuable for establishing recommendations for use of hand disinfectants.

In the fingerpad test with poliovirus and an exposure time of 1 min, the low percentage ethanol formulation (55% v/v) in combination with

Table V Poliovirus type 1 (Sabin strain) eliminating efficacy of the test product in comparison to 60% (v/v) propan-2-ol in the fingerpad test

Substance	Exposure time (min)	Fingerpads examined (N)	Reduction factor	
			x	SD
Test product	0.5	20	3.04	1.05
	1.0	20	3.13	0.96
Propan-2-ol	0.5	10	1.32	0.76
	1.0	10	1.23	1.02

x, average of three replicates; SD, standard deviation.

Table VI Reduction of feline calicivirus (FCV) titres after treatment with test product in comparison with two alcohols [70% ethanol (v/v), 70% propan-1-ol (v/v)] or standard hard water (SHW) for 30 s [\log_{10} reduction factor (RF) was calculated based on the mean RF values of FCV titres after drying]

Substance	Experimental assay	Volunteer 1 \log_{10} RF		Volunteer 2 \log_{10} RF		Volunteer 3 \log_{10} RF		Volunteer 4 \log_{10} RF		Volunteers 1-4 \log_{10} RF
		Value	Mean	Value	Mean	Value	Mean	Value	Mean	Mean
Test product	1	2.64	≥ 2.74	1.31	1.61	2.25	1.78	≥ 3.88	≥ 2.77	2.38 ± 1.24
		≥ 3.52		1.81		1.50		0.19		
		2.14		1.44		1.62		3.69		
		2.64		1.88		1.75		3.31		
		≥ 3.38	≥ 3.22	1.31	1.66	0.50	1.15	4.41	4.63	
		1.50		2.06		1.37		6.04		
	2	≥ 3.38		1.69		1.62		3.66		
		≥ 4.63		1.56		1.12		4.41		
		0.79	1.67	2.48	2.14	1.44	1.35	3.69	≥ 3.85	
		1.54		1.60		1.19		≥ 4.07		
		1.67		2.48		1.32		≥ 4.19		
		2.67		1.98		1.44		3.44		
70% ethanol	1	0.02	0.02	0.31	0.38	0.75	0.75	1.44	1.57	0.68 ± 0.58
		0.02		0.44		0.75		1.69		
70% propan-1-ol	2	1.00	1.13	0.31	0.44	0.50	0.25	0.66	0.98	0.74 ± 0.42
		1.25		0.56		0.00		1.29		
SHW	3	1.17	1.42	1.48	1.29	1.44	1.38	1.32	1.45	1.39 ± 0.18
		1.66		1.10		1.32		1.57		

long-chain alcohols was considerably more effective (RF=3.13) than the reference 60% (v/v) propan-2-ol and 80% (v/v) ethanol.

With FCV as a surrogate of norovirus, a virus reduction was measured showing that the test product was more effective than 70% ethanol and propan-1-ol. The RF of the test product was 2.38 with a tripartite soil load after 30 s. Interestingly, both alcohols at a concentration of 70% v/v have been described previously as having the highest virucidal activity against FCV in tests under practical conditions in comparison with 90% concentrations.²⁷ In contrast, testing two commercial available products based on 95% ethanol and 78.2% ethanol with a 5% faecal suspension as an organic load, the product with a higher alcohol concentration was significantly more effective than the formulation with the lower content.²⁸ The RFs were 2.17 and 1.07, respectively.

The data derived from quantitative suspension assays and fingerpad tests demonstrate that as well as the other advantages, the test product fills a clinically relevant gap of virucidal hand disinfectants, demonstrating efficient, safe and fast-acting inactivation of many human pathogenic viruses and their surrogates causing nosocomial infections.

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