


Brief Report

Virucidal Efficacy of Household Dishwashers

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Abstract: Not only since SARS-CoV-2, have transmission routes of viruses been of interest. Noroviruses e.g., can be transmitted via smear infection, are relatively stable in the environment and very resistant to chemical disinfection. Some studies determined the virucidal efficacy of laundering processes, but few studies focused on the virucidal efficacy of dishwashing processes. Here, especially consumer related conditions are of interest. Households for example are a hotspot of norovirus infection and thus a sufficient reduction of these and other viruses from dishes must be insured to avoid an infection via this route. The likelihood of such an event should not be underestimated, since it was shown that the washing machine can be a reservoir for the transmission of extended spectrum beta-lactamase producing bacteria in newborns. Although viruses do not replicate in these devices a transmission via contaminated cutlery e.g., cannot be excluded. Using a consumer related approach to determine the virucidal efficacy of dishwashers, we found a combination of a bleach containing dishwasher detergent, a cleaning temperature of 45 °C for 45 min and a rinsing temperature of 50 °C, to be sufficient to reduce viral titer of bovine corona virus, murine norovirus and modified vaccinia virus by 4.8, 4.2 and 3.8 logarithmic stages respectively.

Keywords: norovirus; dishwasher; virucidal efficacy; corona virus; vaccinia virus



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1. Introduction

The persistence of viruses in the environment and their resistance to biocidal methods depends on their morphology and in general non-enveloped viruses are more stable than enveloped viruses. Transmission routes of viruses are diverse and have been studied intensely. While respiratory viruses like SARS-CoV-2 spread mainly via person to person contact by droplet and aerosol transmission [1], human noroviruses that can cause severe diarrhea are transmitted primarily through the fecal-oral route, either by direct person-to-person spread or fecally contaminated food or water [2]. For noroviruses the transmission through contaminated surfaces via smear infection is also possible. Noroviruses, as well as enveloped viruses such as SARS-CoV-2 can be relatively stable in the environment and can survive on surfaces for days, although viral titer decrease over time by several logarithmic stages [3,4]. Additionally, noroviruses are known to be very resistant to chemical disinfection [5].

Although there are some studies on the virucidal efficacy of laundering processes and Heinzl et al. [6] found a logarithmic reduction of >4 at 40 °C, using a standard laundry detergent for Poliovirus in situ, only few studies [7,8] focused on the virucidal efficacy of dishwashing processes. But this was done rather in terms of the disinfection of medical equipment than with respect to consumer related usage and conditions.

However, especially consumer related conditions are of interest since households have been found to be a hotspot of human norovirus infection for example [9] and thus a sufficient reduction of these viruses from dishes must be insured. Furthermore, nursery homes, hospitals and gastronomy use these devices and have to ensure a sufficient reduction to avoid further spread of viral infection via this route.

The likelihood of such an event should not be underestimated, since Schmithausen RM et al. [10] showed that the washing machine can be a reservoir for the transmission of extended spectrum beta-lactamase (CTX-M-15)-producing *Klebsiella oxytoca* in newborns and although viruses do not replicate in these devices a transmission via contaminated cutlery e.g., cannot be excluded.

In this study we employed a consumer related approach to determine the virucidal efficacy of household dishwashers and used modified vaccinia virus Ankara (MVA), but especially bovine corona virus (BCV) and murine norovirus (MNV) as viral surrogates for SARS CoV-2 and human noroviruses, respectively. BCV and MNV are closely related to the human pathogens SARS CoV-2 and human norovirus. BCV represents an enveloped and MNV a non-enveloped RNA-virus, to account for the fact that in general non-enveloped viruses are more resistant than enveloped viruses. MVA is an attenuated enveloped DNA-virus and was included to account for these types of viruses. All these viruses are recommended surrogates for virucidal tests by DIN EN 14,476 and DVV/RKI [11,12] and thus ensure a good comparison to other virucidal studies in this field. Furthermore, bovine coronavirus was also chosen as a surrogate for SARS-CoV-2 with regards to the actual pandemics since it is not common to use biosafety-level-3 strains (such as SARS-CoV-2) for these tests. See also Table 1 for comparison of viral strains.

Table 1. Surrogate viruses used in the current study.

Virus	Acronym	Strain	Structure	Source	Reference
Murine Norovirus	MNV	S99	non-enveloped ss-RNA	EVAg	[11,12]
Bovine Coronavirus	BCV	S378 Riems	enveloped ss-RNA	EVAg	[11,12]
Modified Vaccinia Virus	MVA	Ankara	enveloped ds-DNA	Dr. Brill + Partner	[11,12]

2. Materials and Methods

2.1. Preparation of Viral Stocks

Culturing of murine macrophage cells (RAW 264.7) for MNV expansion was done according to Hwang et al. [13]. Large cell debris was pelleted by centrifugation in a tabletop tissue culture centrifuge at $2000 \times g$ for 20 min at 4 °C. Cell culture supernatant was collected and pooled. After application of 2.25 mL of sterile filtered 30% sucrose solution to the bottom of an ultra-clear centrifuge tubes (16 mL, Oak Ridge Style 3138, Nalgene, New York, NY, USA), 12.75 mL of the cell culture supernatant were carefully overlaid onto the sucrose cushion. After centrifugation in a Sorvall LYNX 6000 (fixed angle rotor) centrifuge at $50,000 \times g$ for 3 h at 4 °C, the supernatant was discarded, leaving 1.5 mL of 30% sucrose. After addition of 1 mL of cell culture PBS the pellet was re-suspended and 200 µL aliquots were prepared and stored at –80 °C. Concentration procedure was the same for BCV and MVA, however culturing was done in human rectum adenocarcinoma cells (HRT-18) for BCV [14] and in Syrian hamster kidney fibroblast cells (BHK-21) cells for MVA [15].

2.2. Preparation of Soil Matrix and Bio Carriers

20 g of oatmeal (Peter Kölln GmbH & Co. KGaA, Elmshorn, Germany) were suspended in 200 mL of sterile tap water (hardness = 6.64 °dH (degree german hardness)) and heated to 95 °C for 10 min. After cooling down to 40 °C under constant stirring, the oatmeal slime was mixed with the concentrated virus suspension and 400 µL of the mix were immediately applied to a sterile stainless-steel carrier. These bio carriers were dried for 1.5 h at 37 °C (20% humidity) under sterile conditions [16,17].

2.3. Dishwasher Process

Two bio carrier were placed into a Miele GSL (G1222 SC) dishwasher (Miele, Gütersloh, Germany) and the test run, using the respective program and cleansing agent, was started. For each virus type a separate dishwasher run was performed and two test runs were done

per each respective program. For validation purposes, the temperature profiles of each run were recorded. For this, two data loggers (TELID[®]311; Microsensys GmbH, Erfurt, Germany) per test run were used in the upper and lower chamber of the machine. Ballast soil [18] was applied to the upper chamber of the dishwasher before each test run and the dishwasher was filled with rinse aid (Somat Klarspüler, Henkel AG & Co KGaA, Düsseldorf, Germany) and dishwasher salt (Somat Salz, Henkel AG & Co KGaA, Düsseldorf, Germany). Two dishwasher detergents were employed, the commercial product Somat Tabs classic (Henkel AG & Co KGaA, Düsseldorf, Germany) and the reference detergent IEC 60436 (WFK—Cleaning Technology Institute e.V., Krefeld, Germany) of DIN EN 50242 [18]. The reference detergent was used with and without bleach.

The carriers positions (Figure 1) resembled a worst-case scenario; thus, distribution is not even and not according to the standard loading of DIN EN 50242 [18]. This ensured reasonable amounts of residual virus load, for the calculation of logarithmic reductions of ≥ 5 .

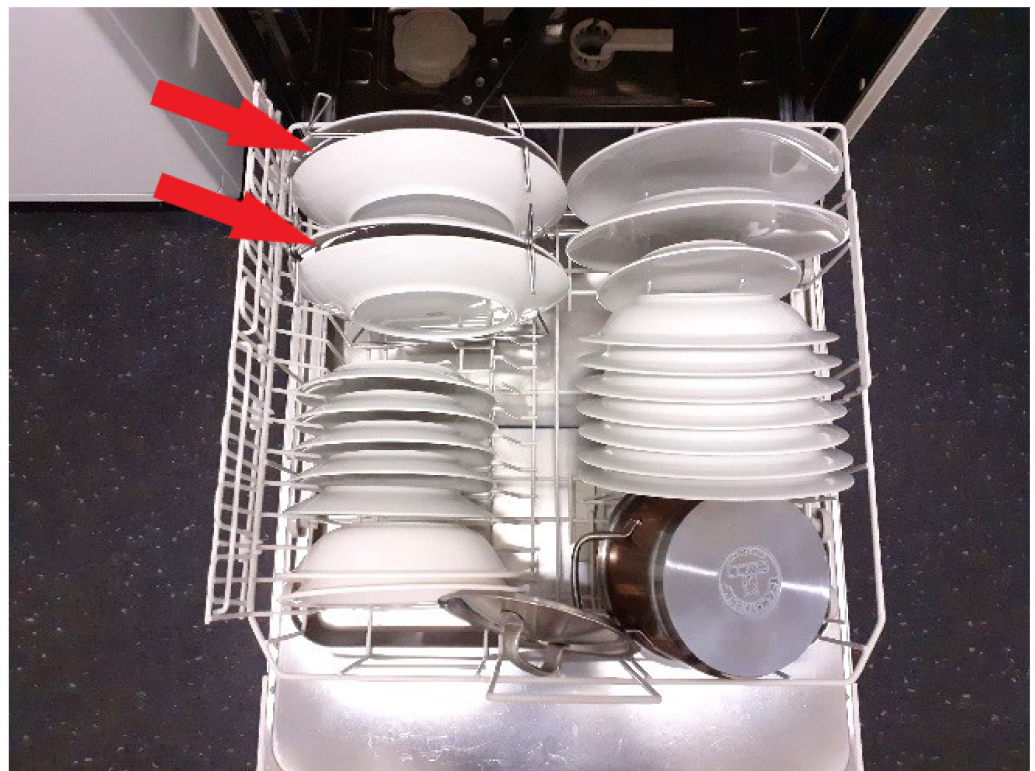


Figure 1. Position of bio carriers in the dishwasher. The standard loading [18] of the machine was abstracted (arrows) and bio carriers were placed in-between two plates, to resemble a worst-case scenario.

A third carrier served as a positive control, to determine the initial viral load on the bio carrier (is not treated in dishwasher). A fourth carrier, without any virus, served as the negative control (no viral load and not treated in dishwasher).

2.4. Extraction of Viral RNA/DNA after Treatment with Propidium Monoazide

We used a method described by Quijada et al. [19] for the determination of live viruses by PMA (propidium monoazide) coupled qPCR or RT qPCR, for DNA or RNA viruses, respectively. Although the plaque assay is the gold standard for the detection of live viral particles, PMA coupled qPCR or RT qPCR is an excellent supplementation method for the assessment because it is less time consuming and easy to handle. Furthermore, Quijada et al. [19] proved an excellent correlation to PFU (plaque forming units) with R^2 value between 0.92 and 0.99.

After treatment of the bio carriers in a dishwasher according to the programs depicted in Table 2, the bio carriers were removed and submerged in 4 mL of PBS pH 7.2 in a reaction tube (WITEG Labortechnik GmbH, Wertheim, Germany) for 10 min. Following this incubation, the residual soil matrix was scraped off the carrier, into the PBS solution, by use of a disposable 10 µL inoculation loop (Sarstedt AG & Co. KG, Nümbrecht, Germany). Suspensions of carriers 1 and 2 were pooled prior two RNA/DNA extraction.

Table 2. Dishwasher programs and cleansing agents.

Cleaning Temp. (°C)	Cleaning Time (min)	Rinsing Temp. (°C)	Cleansing Agent
45	45	50	water control
45	45	50	commercial product
45	45	50	reference w/o bleach
45	45	50	reference with bleach
45	45	30	commercial product
45	45	70	commercial product

500 µL of this suspension were treated with 150 µM propidium monoazide (incubated for 10 min at RT (room temperature) in the dark and then 3 min at RT under a 600 W halogen lamp at a distance of 20 cm. Propidium monoazide is a photo reactive dye that penetrates damaged or disrupted viral envelopes and/or nucleocapsids and binds covalently to RNA or DNA when exposed to light. This leaves the RNA or DNA blocked for amplification in qPCR or RT qPCR and ensures an amplification of RNA or DNA of vital viruses only [19]. After treatment with propidium monoazide, the viral RNA or DNA was extracted by PureLink™ Viral RNA/DNA Mini Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions.

2.5. RT qPCR/qPCR

RT qPCR was done using Kappa Sybr Fast 1-Step Kit (Merck KGaA, Darmstadt, Germany) on LightCycler 480 (Roche Diagnostics GmbH, Mannheim, Germany) by application of 1 µL of sample to 10 µL of master mix. The master mix contained 0.075 µM of forward and reverse primer. RT qPCR program was set at: (i) reverse transcription step (42 °C for 5 min), (ii) an initial denaturation step (95 °C for 3 min), (iii) 40 cycles of (95 °C for 10 s; 54 °C for 20 s and 72 °C for 10 s).

qPCR was done using FastStart Essential DNA Green Master (Roche Diagnostics GmbH, Mannheim, Germany) on Light Cycler 480 (Roche Diagnostics GmbH, Mannheim, Germany) by application of 1 µL of sample to 10 µL of master mix. The master mix contained 0.1 µM of F and R primer. qPCR program was set at: (i) initial denaturation step (95 °C for 10 min), (ii) 40 cycles of (95 °C for 15 s; 54 °C for 15 s and 72 °C for 15 s).

For both, RT qPCR and qPCR, calibration curves were established for each virus and incorporated into each analysis to calculate the copies mL⁻¹ of RNA or DNA, in duplicate determination. Assuming one copy of RNA or DNA per virus this corresponds to viral particles mL⁻¹.

2.6. Primer Pairs for RT qPCR and qPCR

Primer pairs for RT qPCR and qPCR respectively are depicted in Table 3.

Table 3. Primer pairs for qPCR and RT qPCR.

Virus	Primer	Sequence 5'-3'	Reference	Supplier
BCV	forward	TGG ATC AAG ATT AGA GTT GGC	[20]	Eurofins Genomics
BCV	reverse	CCT TGT CCA TTC TTC TGA CC	[20]	Eurofins Genomics
MNV	forward	ATG GTR GTC CCA CGC CAC	[21]	Eurofins Genomics
MNV	reverse	TGC GCC ATC ACT CAT CC	[21]	Eurofins Genomics
MVA	forward	CGG CTA AGA GTT GCA CAT CCA	[22]	Eurofins Genomics
MVA	reverse	CTC TGC TCC ATT TAG TAC CGA TTC T	[22]	Eurofins Genomics

2.7. Calculations of Log Reduction

Log reduction of viral titer was calculated as follows:

$$LR = \log_{10} \left(\text{initial viral load in copies mL}^{-1} \right) - 10 \left(\text{remaining viral load in copies mL}^{-1} \right) \quad (1)$$

2.8. Statistical Analysis

LR results, based on RT qPCR/qPCR experiments, are shown as the mean values of two independent tests. Mean value, standard deviation and Tukey's multiple comparison test results, for determination of significance, were calculated using Prism 6.07 for Windows (2015).

3. Results

3.1. Temperature Profiles of Dishwasher Programs

For validation purposes the temperature profile of each dishwasher run was recorded in duplicate determination. All programs reached the designated max. temperature of the respective cleaning cycle and rinsing cycle. Only slight temperature profile differences were observed (data not shown) between test runs of the same program. Test runs are thus comparable with respect to their virucidal efficacy.

3.2. Logarithmic Reduction of Viruses under Specific Conditions

As shown in Figure 2, dishwasher program with a cleaning temperature of 45 °C, a cleaning time of 45 min and a rinsing temperature of 50 °C, achieves a mean logarithmic reduction (LR) of 1.6 for BCV, 0.9 for MNV and 1.0 for MVA, when no dishwasher detergent is used (water control). Upon the addition of a commercial product, in this case Somat Tabs classic, or the reference detergent IEC 60,436 plus bleach, LRs of ≥ 4 are reached with the exception that the commercial product reaches a mean LR of 3.8 for MVA here. Using the reference detergent IEC 60,436 without bleach, mean LRs are reduced to 3.0 for BCV, 2.3 for MNV and 2.8 for MVA, emphasizing that bleach is a critical factor in the reduction of vital virus particles.

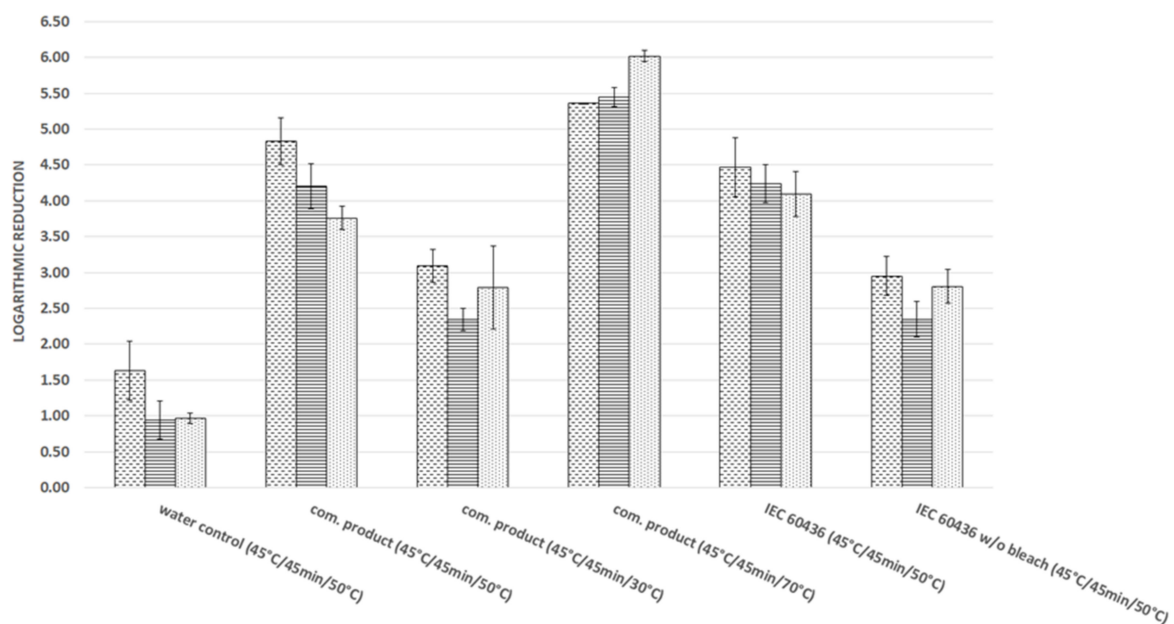


Figure 2. Log reduction of BCV (dashed lines), MNV (continuous lines) and MVA (dots) after treatment in a dishwasher at different conditions with the reference detergent (IEC 60436) and the commercial product (com. product). Differences in log reductions at different conditions are statistically significant except commercial product and IEC 60436 detergent at 45 °C/45 min/50 °C and the commercial product at 45 °C/45 min/30 °C and IEC 60436 detergent without bleach at 45 °C/45 min/50 °C (see Appendix A—Table A1).

The rinsing temperature also has a strong effect on the virucidal efficacy. Using the same conditions as above with varying rinsing temperatures, LRs between 3.8 and 4.8 are found at a rinsing temperature of 50 °C. If a rinsing temperature of 70 °C is used the logarithmic reduction increases to ≥ 5 for BCV and MNV and ≥ 6 for MVA. However, a rinsing temperature of 30 °C gives LRs of 3.1 for BCV, 2.3 for MNV and 2.8 for MVA only.

Tukey's multiple comparison test reveals that different log reductions at different conditions are statistically significant, with the exception of the commercial product and IEC 60436 detergent at 45 °C/45 min/50 °C and the commercial product at 45 °C/45 min/30 °C and IEC 60436 detergent w/o bleach at 45 °C/45 min/50 °C (see Appendix A—Table A1).

A logarithmic reduction of ≥ 5 for BVC and MNV and ≥ 6 for MVA is equal or higher than the max. logarithmic reduction. The max. logarithmic reduction measurable for BCV and MNV is 5 and 6 for MVA, caused by the initial virus titer of 2.4×10^8 , 5.1×10^9 and 5.6×10^8 copies mL⁻¹ respectively and a detection limit of RT qPCR and qPCR of 10^3 copies mL⁻¹.

4. Discussion

In this study, the virucidal efficacy of the dishwashing process under consumer related conditions was assessed. We simulated a worst-case scenario with a thick and very sticky soil matrix (dried oatmeal slime) which contained a very high titer of the respective viruses. Furthermore, a sub-optimal position of the bio carrier in the dishwasher chamber with respect to the accessibility of the carrier by the spraying heads of the machine (reduced mechanical influence of water beam on the matrix) was used. The very high initial viral load of 10^8 – 10^9 copies mL⁻¹ cannot be expected to be found in a dishwasher under normal circumstances but ensured a reasonable residual viral load after the dishwashing process for the calculation of a logarithmic reduction of 4–5 log stages. This is the minimum requirement for the approval of virucidal products according to DIN EN 14476 [11].

Based on these assumptions, we found that the usage of a dishwashing detergent with bleach is a critical factor, as is the usage of a rinsing temperature of ≥ 50 °C in reducing the viral load sufficiently. We thus recommend the use of a commercial dishwasher detergent plus bleach, a cleaning temperature of at least 45 °C (for full bleach activation) for 45 min and a rinsing temperature of at least 50 °C, for sufficient reduction of viral contaminations by household dishwashers.

Although we used the indirect method of PMA RT qPCR/PMA qPCR rather than plaque assay, which is the golden standard for the detection of live viruses, we consider our findings solid and secure, since Quijada et al. and also Parshionikar et al. [19,23] proved an excellent correlation of this method to PFU (plaque forming units) with R² value between 0.92 and 0.99 [19]. If at all, our method is rather prone to give lower log reduction values in our tests than reduction value which are too high. However, further studies using different commercial products (of different dosage formulations for example) are mandatory to ensure the same performance and reduction of viruses by these formulations.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Differences in log reductions at different conditions are statistically significant except the commercial product and IEC 60436 detergent at 45 °C/45 min/50 °C and the commercial product at 45 °C/45 min/30 °C and IEC 60436 detergent without bleach at 45 °C/45 min/50 °C. Four stars (****) correspond to $p \leq 0.0001$, (ns) corresponds to not significant.

Turkey's Multiple Comparison Test	Mean. Diff.	95% CI of Diff.	Significant?	Summary
water control (45 °C/45 min/50 °C) vs. com. Product (45 °C/45 min/50 °C)	−3.087	−3.737 to −2.436	Yes	****
water control (45 °C/45 min/50 °C) vs. (45 °C/45 min/30 °C)	−1.56	−2.211 to −0.9093	Yes	****
water control (45 °C/45 min/50 °C) vs. (45 °C/45 min/70 °C)	−4.427	−5.077 to −3.776	Yes	****
water control (45 °C/45 min/50 °C) vs. IEC 60436 (45 °C/45 min/50 °C)	−3.087	−3.737 to −2.436	Yes	****
water control (45 °C/45 min/50 °C) vs. IEC 60436 w/o bleach (45 °C/45 min/50 °C)	−1.518	−2.169 to −0.8677	Yes	****
com. Product (45 °C/45 min/50 °C) vs. com. Product (45 °C/45 min/30 °C)	1.527	0.8760 to 2.177	Yes	****
com. Product (45 °C/45 min/50 °C) vs. com. Product (45 °C/45 min/70 °C)	−1.34	−1.991 to −0.6893	Yes	****
com. Product (45 °C/45 min/50 °C) vs. IEC 60436 (45 °C/45 min/50 °C)	3.974E−08	−0.6507 to 0.6507	No	ns
com. Product (45 °C/45 min/50 °C) vs. IEC 60436 w/o bleach (45 °C/45 min/50 °C)	1.568	0.9177 to 2.219	Yes	****
com. Product (45 °C/45 min/30 °C) vs. com. Product (45 °C/45 min/70 °C)	−2.867	−3.517 to −2.216	Yes	****
com. Product (45 °C/45 min/30 °C) vs. IEC 60436 (45 °C/45 min/50 °C)	−1.527	−2.177 to −0.8760	Yes	****
com. Product (45 °C/45 min/30 °C) vs. IEC 60436 w/o bleach (45 °C/45 min/50 °C)	0.04167	−0.6090 to 0.6923	No	ns
com. Product (45 °C/45 min/70 °C) vs. IEC 60436 (45 °C/45 min/50 °C)	1.34	0.6893 to 1.991	Yes	****
com. Product (45 °C/45 min/70 °C) vs. IEC 60436 w/o bleach (45 °C/45 min/50 °C)	2.908	2.258 to 3.559	Yes	****
IEC 60436 (45 °C/45 min/50 °C) vs. IEC 60436 w/o bleach (45 °C/45 min/50 °C)	1.568	0.9177 to 2.219	Yes	****

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