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## Short Report

# Virucidal efficacy of different formulations for hand and surface disinfection targeting SARS CoV-2

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## ARTICLE INFO

**Article history:**

Received 6 November 2020

Accepted 1 March 2021

Available online 23 March 2021

**Keywords:**

SARS-CoV-2

Disinfection

Virucidal activity

Vaccinia

EN 14476



## SUMMARY

In the ongoing SARS CoV-2 pandemic, effective disinfection measures are needed, and guidance based on the methodological framework of the European Committee for Standardization (CEN) may enable the choice of effective disinfectants on an immediate basis. This study aimed to elucidate whether disinfectants claiming 'virucidal activity against enveloped viruses' as specified in the European Standard EN 14476 as well as in the German Association for the Control of Viral Diseases/Robert Koch Institute (DVV/RKI) guideline are effectively inactivating SARS-CoV-2. Two commercially available formulations for surface disinfection and one formulation for hand disinfection were studied regarding their virucidal activity. Based on the data of this study the enveloped SARS-CoV-2 is at least equally susceptible compared to the standard test virus vaccinia used in the EN 14476 and DVV/RKI guidelines. Thus, chemical disinfectants claiming 'virucidal activity against enveloped viruses' based on the EN 14476 and DVV/RKI guidelines will be an effective choice to target enveloped SARS-CoV-2 as a preventive measure.

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## Introduction

On January 30<sup>th</sup>, 2020, the World Health Organization (WHO) declared the outbreak of a novel coronavirus, designated SARS-CoV-2, a public health emergency of international concern (PHEIC), being WHO's highest level of alarm [1]. Throughout the ongoing SARS-CoV-2 pandemic from December 2019 to

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October 11<sup>th</sup>, 2020, more than 37 million cases of COVID-19 and one million deaths have been reported on a global basis. Recently, within only one week (October 5<sup>th</sup> to 11<sup>th</sup>, 2020) more than 2.2 million new cases of SARS-CoV-2 infections and 39,000 deaths associated with COVID-19 were reported, being the highest number of cases so far in the ongoing SARS-CoV-2 pandemic [2].

In order to prevent further spreading of SARS-CoV-2, the WHO recommended hygiene measures such as the use of 70% ethanol [3]. To enable the use of other suitable disinfectants, the Robert Koch Institute (RKI) in Germany has been recommending the use of disinfectants claiming at least 'virucidal activity against enveloped viruses' in the context of the SARS-CoV-2 outbreak [4].

This recommendation is based on the methodological framework of the European Committee for Standardization (CEN): CEN has specified a set of test organisms, which are representative for certain groups of micro-organisms. A proven efficacy against these representative test organisms allows efficacy claims for the respective group of organisms, e.g. bactericidal, yeasticidal, fungicidal, or virucidal efficacy [5]. For the claim 'virucidal activity against enveloped viruses', vaccinia virus has been specified as the relevant test organism.

As such, disinfectants and antiseptics claiming 'virucidal activity against enveloped viruses', based on the methodological framework of CEN, can be claimed to be effective against all enveloped viruses including coronaviruses such as SARS-CoV-2 [6]. This also holds true for disinfectants claiming 'virucidal activity against enveloped viruses' based on the German Association for the Control of Viral Diseases (DVV)/RKI guideline [7].

Despite this guidance throughout the ongoing SARS-CoV-2 pandemic, the question has repeatedly arisen as to whether a certain formulation has proven efficacy against SARS-CoV-2.

Therefore, this study aimed to investigate the efficacy of three different typical formulations used for hand or surface disinfection against SARS-CoV-2 using the European Standard EN 14476 protocol. Efficacy data using SARS-CoV-2 were compared to data obtained with the test virus vaccinia as specified in EN 14476 and the comparable German DVV/RKI guideline, respectively.

## Methods

### Tests strains and cultivation

Test virus suspensions were prepared by infecting susceptible cells with different multiplicities of infection. For modified vaccinia virus Ankara (provided from the Institute of Animal Hygiene and Veterinary Public Health of the University Leipzig), BHK-21 cells were used (provided by Friedrich Löffler institute); for vaccinia virus Elstree (kindly provided by Prof. Sauerbrei, University of Jena, Jena, Germany), CV1 cells (kindly provided by Prof. Sauerbrei) were used. SARS-CoV-2 (strain Essen) was propagated under biosafety level 3 on Vero E6 cells as previously described [8].

### Quantitative suspension tests according to EN 14476 or DVV/RKI guideline

Quantitative suspension tests were carried out as described in EN 14476 or in the DVV/RKI guideline [6,7]. For EN 14476 a

protein load of 0.03% bovine serum albumin (BSA) was used. These data were compared with data based on the DVV/RKI guideline with organic soiling (10% fetal calf serum (FCS)). Ten percent FCS is slightly higher soiling than 0.03% BSA; however, the influence with the type of disinfectants tested, which are based on alcohol and quaternary ammonium compounds, is negligible in our experience.

In the DVV/RKI guideline for ready-to-use products, the highest test concentration is specified as 90%, which was used for the ready-to-use product A in our study. The respective test protocol used is indicated for each data set. Briefly, efficacy of three commercially available disinfectants was studied against vaccinia virus (strain modified vaccinia virus Ankara (MVA) ATCC VR-1508 or vaccinia virus, strain Elstree, of which either strain may be used according to EN 14476 and DVV/RKI guideline) and SARS-CoV-2 [6,7].

The virus suspension was added to the product test solution and the interfering substance. A virus control mixture was also assessed using distilled water in place of the test product. After the specified contact time indicated in Table 1, virucidal activity of the solution was immediately suppressed by dilution with nine volumes of ice-cold medium (minimum essential medium + 2.0% FCS) and serially diluted 10-fold. Due to the immediate titration, no after-effect of the test product could occur. For each test suspension, six wells of a microtitre plate containing a confluent monolayer of the respective host cells were inoculated with 100 µL of test suspension, and the cells were incubated at 37°C in a humidified atmosphere under 5% CO<sub>2</sub>.

After incubation, the cells were examined microscopically for infectivity and cytopathic effects (CPE). The virus titres were expressed as tissue culture infectious dose 50% (TCID<sub>50</sub>/mL). The virucidal activity was determined as the difference between the logarithmic titre of the virus control minus the logarithmic titre of the test virus (log<sub>10</sub> TCID<sub>50</sub>/mL). This difference was expressed as logarithmic reduction factor (RF) including its 95% confidence interval. A reduction in virus titre of ≥4 log<sub>10</sub> (corresponding to an inactivation of ≥99.99%) was regarded as evidence of sufficient virucidal activity. The calculation was performed according to EN14476 or DVV/RKI guideline, respectively [6,7]. In certain cases, where cytotoxicity of the test formulation was impacting sensitivity, large volume plating (LVP) as described in EN 14476 was used to enlarge the detectability threshold [6].

A ready-to-use alcohol-based surface disinfectant designated formulation A (MikroZid® universal; 100 g contains: 17.4 g propan-2-ol, 12.6 g ethanol (94%); Schülke & Mayr GmbH, Germany) was used as one test formulation. In addition, a quaternary ammonium compound (QAC)-based formulation for surface disinfection was used, designated formulation B (MikroZid® sensitive; 100 g contains: 0.26 g alkyl(C12-16) dimethylbenzyl ammonium chloride (ADBAC/BKC (C12–16)); 0.26 g didecylidimethyl ammonium chloride (DDAC), 0.26 g alkyl(C12–14)ethylbenzyl ammonium chloride (ADEBAC (C12–14))). As a third formulation, an alcoholic hand disinfectant based on propan-2-ol was used (Desmanol® pure), designated formulation C (100 g contains: 75 g propan-2-ol). Disinfectant concentrations and contact times used throughout this study were based on the existing 'virucidal efficacy against enveloped viruses' efficacy claims for the three disinfectants. Experiments were carried out under conditions of low organic soiling (0.3 g/L BSA; 'clean conditions') as specified in EN 14476

**Table I**Log<sub>10</sub> reduction of SARS-CoV-2 titres by three different formulations used for either surface or hand disinfection

Formulation	Concentration (% v/v)	Contact time (s)	Soiling	Test method	Titre of the SARS-CoV-2 control (log <sub>10</sub> TCID <sub>50</sub> /mL)	Logarithmic reduction factor
A	20	15	0.03% BSA	EN 14476	6.22	≥4.02
	80	15	0.03% BSA	EN 14476	6.22	≥4.02
B	20	15	0.03% BSA	EN 14476	6.22	≥4.02
	20	60	0.03% BSA	EN 14476	6.37	≥3.17 <sup>a</sup>
	80	15	0.03% BSA	EN 14476	6.22	≥4.38 <sup>b</sup>
	80	30	0.03% BSA	EN 14476	6.37	≥4.38 <sup>b</sup>
	80	60	0.03% BSA	EN 14476	6.37	≥2.17 <sup>a</sup>
C	20	15	0.03% BSA	EN 14476	6.22	≥4.02
	20	30	0.03% BSA	EN 14476	6.22	≥3.02 <sup>a</sup>
	80	15	0.03% BSA	EN 14476	6.22	≥2.02 <sup>a</sup>
	80	30	0.03% BSA	EN 14476	6.22	≥4.38 <sup>b</sup>

TCID<sub>50</sub>, tissue culture infectious dose 50%; BSA, bovine serum albumin.<sup>a</sup> Pre-screening experiments: data are based on *N* = 1; due to cytotoxicity of the tested substance the detection limit did not allow detection of higher virus reduction.<sup>b</sup> Reduction factor value was acquired by large volume plating.

or in the presence of 10% FCS as specified in the DVV/RKI guideline [6,7].

All experiments were carried out as independent experiments and data presented are based on at least two experiments. Validation controls as specified in the test protocols (EN 14476 or DVV/RKI guideline) were found to be effective in all experiments, indicating validity of presented data.

## Results and discussion

Throughout the ongoing SARS-CoV-2 pandemic, effective disinfection protocols are needed to support prevention strategies worldwide. Thus, we investigated three different disinfectant formulations in regard to their effectiveness against SARS-CoV-2, including two formulations for surface disinfection and one hand disinfectant.

Formulations were based on either alcohol or QACs with known efficacy against the enveloped vaccinia virus (strain modified vaccinia virus Ankara or strain Elstree, respectively) as established in EN 14476 and the similar German DVV/RKI guideline [6,7]. In both test protocols a reduction of the test virus by ≥4 log<sub>10</sub> is required to claim 'virucidal activity against enveloped viruses'. Data obtained for SARS CoV-2 by using the

EN 14476 test protocol in comparison with data obtained for vaccinia virus using either the DVV/RKI or the similar EN 14476 test protocol are summarized in Tables I and II. Ten percent FCS was used as soiling in the DVV/RKI guideline, which equates to clean conditions (i.e. 0.3 g/L BSA) used in the EN 14476 test (VAH (Verbund für Angewandte Hygiene e. V.), Germany, personal communication). In preliminary screening experiments using SARS-CoV-2 the limit of detection did not enable verification of the 4 log<sub>10</sub> requirement of EN 14476 due to the cytotoxicity of the tested substance. Thus, further experiments were carried out with either lower concentrations and/or the use of large volume plating to enlarge the detectability threshold.

Formulation A (alcoholic surface disinfectant) effectively inactivated SARS-CoV-2 by ≥4.02 log<sub>10</sub> within 15 s at a 20% (v/v) dilution. In comparison, formulation A was not found to be effective under these conditions when using the test virus MVA. Here, RF ≥4.25 log<sub>10</sub> was obtained, when using the higher test concentration of 90% (v/v), indicating a higher stability of MVA to formulation A compared to SARS-CoV-2.

Formulation B was also found to be effective against SARS-CoV-2 under the chosen test parameters, indicated by ≥4 log<sub>10</sub> RF within 15 s at a concentration of both 20% and 80% (v/v).

**Table II**Log<sub>10</sub> reduction of vaccinia virus titres by three different formulations used for either surface or hand disinfection

Formulation	Concentration (% v/v)	Contact time (s)	Soiling	Test method	Titre of the vaccinia virus control (log <sub>10</sub> TCID <sub>50</sub> /mL)	Logarithmic reduction factor vaccinia virus
A	20	15	10% FCS	DVV/RKI guideline	7.44 <sup>b</sup> ± 0.41	0 <sup>b</sup> ± 0.40
	90 <sup>a</sup>	15	10% FCS	DVV/RKI guideline	7.44 <sup>b</sup> ± 0.41	≥4.25 <sup>b</sup> ± 0.28
B	80	30	10% FCS	DVV/RKI guideline	7.82 <sup>c</sup> ± 0.37	≥4.32 <sup>c</sup> ± 0.26
	80	60	10% FCS	DVV/RKI guideline	7.82 <sup>c</sup> ± 0.37	≥4.51 <sup>c</sup> ± 0.31
C	20	15	0.03% BSA	EN 14476	7.67 <sup>b</sup> ± 0.33	0.17 <sup>b</sup> ± 0.58
	80	15	0.03% BSA	EN 14476	7.67 <sup>b</sup> ± 0.33	≥4.19 <sup>b</sup> ± 0.33

TCID<sub>50</sub>, tissue culture infectious dose 50%; FCS, fetal calf serum; DVV/RKI, German Association for the Control of Viral Diseases/Robert Koch Institute; BSA, bovine serum albumin.

All experiments were carried out as independent experiments and data presented are based on at least two experiments.

<sup>a</sup> Highest test concentration for ready-to-use formulations as specified in the DVV/RKI guideline.<sup>b</sup> Test virus used: vaccinia virus strain modified virus Ankara (MVA) ATCC VR-1508.<sup>c</sup> Test virus used: vaccinia virus strain Elstree.

When using the test virus vaccinia strain Elstree, formulation B was found to be effective within 30 s contact time. However, further data are needed to evaluate whether this formulation would also meet the 4 log<sub>10</sub> requirement against vaccinia strain Elstree within 15 s.

Formulation C was found to yield  $\geq 4.02 \log_{10}$  RF within 15 s at 20% (v/v) when using SARS-CoV-2 as a test virus. For MVA only the 80% (v/v) concentration was found to result in a  $\geq 4.19 \log_{10}$  RF, whereas the 20% (v/v) concentration was not found to inactivate MVA to the same extent, indicated by 0.17 log<sub>10</sub> RF.

The data presented in this study indicate that the enveloped SARS-CoV-2 is more susceptible to the tested alcoholic biocidal formulations (A and C) compared to the enveloped MVA, which has been established as a standard test virus in European and German test protocols. For QAC-based formulation B, our data also indicate that SARS-CoV-2 is at least equally susceptible compared to the standard test virus vaccinia strain Elstree. This finding is in good agreement with recently published data indicating a good efficacy of QAC-based formulations against three different SARS-CoV-2 strains within 30 s contact time [9].

In conclusion, data from our study reinforce the validity of the test strain concept as established by national and international institutions such as the DVV in Germany and the CEN. This is in good alignment with earlier published data investigating the chemical susceptibility of the human pathogen *Candida auris* compared to the test organism *Candida albicans* [10]. In the present study, as well as in the abovementioned earlier study, the test virus and the surrogate test yeast were both found to be more resistant to the applied chemical disinfectants than was the targeted outbreak organism.

Thus, based on the use of test viruses representative of wider groups, chemical disinfectants claiming ‘virucidal activity against enveloped viruses’ will be an effective choice to target enveloped SARS-CoV-2 as a preventive measure.

#### Conflict of interest statement

K.S. and L.P. are employees of Schülke & Mayr GmbH, Norderstedt, Germany.

#### Funding sources

This study was funded by Schülke & Mayr GmbH, Norderstedt, Germany.

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