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Short Report

Evaluation of the substitution of poliomyelitis virus for testing virucidal activities of instrument and surface disinfection

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SUMMARY

The Global Polio Eradication initiative has the goal to eradicate poliomyelitis worldwide. This means that poliomyelitisvirus type 1 strain LSc 2ab (PV-1) can no longer be used for the evaluation of virucidal activity of chemical disinfectants. This study evaluated murine parvovirus ATCC VR 1346 (minute virus of mice) as suitable surrogate for PV-1 when testing virucidal activity of biocides in instrument and surface disinfectants. Suspension testing in different laboratories with two commercially available active biocidal substances based on glutaraldehyde (0.01–0.25%) and peracetic acid (0.005–0.1%) with an exposure time of 30 min was performed. Both pathogens showed comparable susceptibility and dose-dependent reduction of virus titres following German and European Guidelines.

Introduction

In the future, it will no longer be possible to examine the virucidal activity of chemical solutions and commercially available active biocidal substances in suspension with poliomyelitisvirus type 1 strain LSc 2ab (PV-1) due to global efforts for poliomyelitis eradication. To date, PV-1 is used in experimental assays since vaccination of the staff in testing laboratories is easily feasible and high titres in cell culture can be obtained. PV is a stable, non-enveloped, hydrophilic virus and is used to evaluate activity of strong chemical disinfectants used in human medicine.

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Minute virus of mice (MVM) is a candidate for substitution and is also known to be a stable virus; it has already been introduced in norms and guidelines where instrument disinfectants must be examined for virucidal activity in quantitative suspension tests at elevated temperatures [1,2]. Additionally, in carrier tests for surface and instrument disinfection, PV-1 has already been omitted and substituted by MVM because of high reduction of PV-1 titres during desiccation [3]. Therefore, MVM can be found in several norms and guidelines where drying of the test virus on a carrier is necessary. Considering the aforementioned, it could be appropriate to additionally incorporate this stable, non-human-pathogenic parvovirus in testing protocols for quantitative suspension tests even without elevated temperature for PV-1 substitution. To initiate the necessary comparison of the behaviour of these two viruses as a prerequisite for substitution, we used glutaraldehyde (GDA) and peracetic acid (PAA)-based disinfectants in a ring trial of four laboratories. These products demonstrate a strong virucidal activity including PV-1 inactivation [4].

Methods

Test viruses

The virucidal activities of two commercially available active biocidal substances using different concentrations with two test viruses of the European Guideline EN 14476 as phase 2/step 1 procedure (quantitative suspension assay) were examined [2]. The poliovirus type 1 LSc 2ab (Sabin original (LSc-2ab)) manufactured by Chiron–Behring, Marburg (Supply source Eurovir, Luckenwalde, Germany) (PV-1) and murine parvovirus (minute virus of mice) strain Crawford ATCC VR 1346 (MVM) were incorporated as test viruses.

Test products

Both solutions to be tested for virus inactivation were prepared from commercially available products. Lerasept spezial (5 g/100 g PAA) was delivered from Stockmeier Chemie GmbH & Co. KG (D-33609 Bielefeld). A stock solution with the factor 1.25% was prepared immediately before examination by adding 11.16 mL of Lerasept spezial to 50 mL water of standardized hardness (WSH) in all participating laboratories followed by dilution.

The product based on glutaraldehyde (GDA) Bioban GA antimicrobial (50 m/m %) originated from Dow Deutschland Anlagengesellschaft GmbH (D-65824 Schwalbach). A stock solution was produced with the factor 1.25% by adding 2.21 mL–100 mL WSH.

Both products were sent to the four participating test laboratories in Germany.

Virus propagation

PV-1 and MVM were propagated in well-established culture media including Dulbecco's minimum essential medium (DMEM) and Eagle's minimum essential medium (EMEM). Cells were frozen and thawed twice after exhibiting a cytopathic effect (PV-1 for 4–5 days, MVM for 10–14 days), followed by centrifugation at 1600 *g* for 10 min. The supernatant was aliquoted as test virus suspensions at −80 °C. The virus titres of these test suspensions ranged from 10^7 to 10^8 tissue culture infectious dose 50% ($TCID_{50}^{+}$)/mL.

Testing

Testing was performed in four laboratories located in Germany (lab 1–4). Tests according to DIN EN 14476 were run with PV-1 and MVM in clean conditions with a fixed exposure time of 30 min at 20 °C. Hard water was used as control and cytotoxicity was additionally determined. Infectivity was stopped by immediate serial dilution with ice-cold medium. One hundred microlitres of each dilution were placed in eight wells of a sterile polystyrene flat-bottomed 96-well microtitre plate containing 100 µL cell suspension. Cultures were observed for cytopathic effects (CPE) after 4–18 days of inoculation depending on the cell culture system. Differences in numbers of replicates between the labs are the result of additional assays performed in some labs (GDA: lab 1, $N = 1$; lab 2, $N = 3$; lab 3, $N = 3$; lab 4, $N = 1$; PAA: lab 1, $N = 2$; lab 2, $N = 3$; lab 3, $N = 3$; lab 4, $N = 1$).

The virus titres were determined using the method of Spearman and Kaerber and expressed as $TCID_{50}$/mL. Titre reduction is presented as the difference between the $\log_{10}$ virus titre after 30 min contact time with the water control and both active biocidal substances. The difference is expressed as reduction factor (RF). A reduction of infectivity of $\geq$4 $\log_{10}$ steps (inactivation $\geq$99.99%) is regarded in the DIN EN 14476 as evidence of sufficient virucidal activity [2].

All calculations were performed using GraphPad Prism v9.2.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results

The virucidal activity of GDA and PAA against PV-1 or MVM given as reduction factors with a fixed exposure time of 30 min is given in Figure 1. A dose-dependent reduction of both viruses was observed in all four laboratories with increasing concentrations of GDA (Figure 1A) as well as PAA (Figure 1B). The susceptibility of PV-1 and MVM to the biocidal substances was highly comparable and next evaluated in a correlation analysis. A strong correlation of PV-1 and MVM reduction factors for GDA (0.8794) (Figure 2A) and PAA (0.9545) (Figure 2B) indicated a comparable stability and inactivation profile for both viruses.

Discussion

The PV-1 served for a long time as test virus in Europe for testing virus inactivation by all types of chemical disinfectants in humane medicine. The introduction of this virus was based on profound knowledge of this type of viral inactivation, studied mainly by using formaldehyde as disinfectant [5]. In Germany, the first guideline even started with the highly pathogenic wild strain which was later replaced by the vaccine

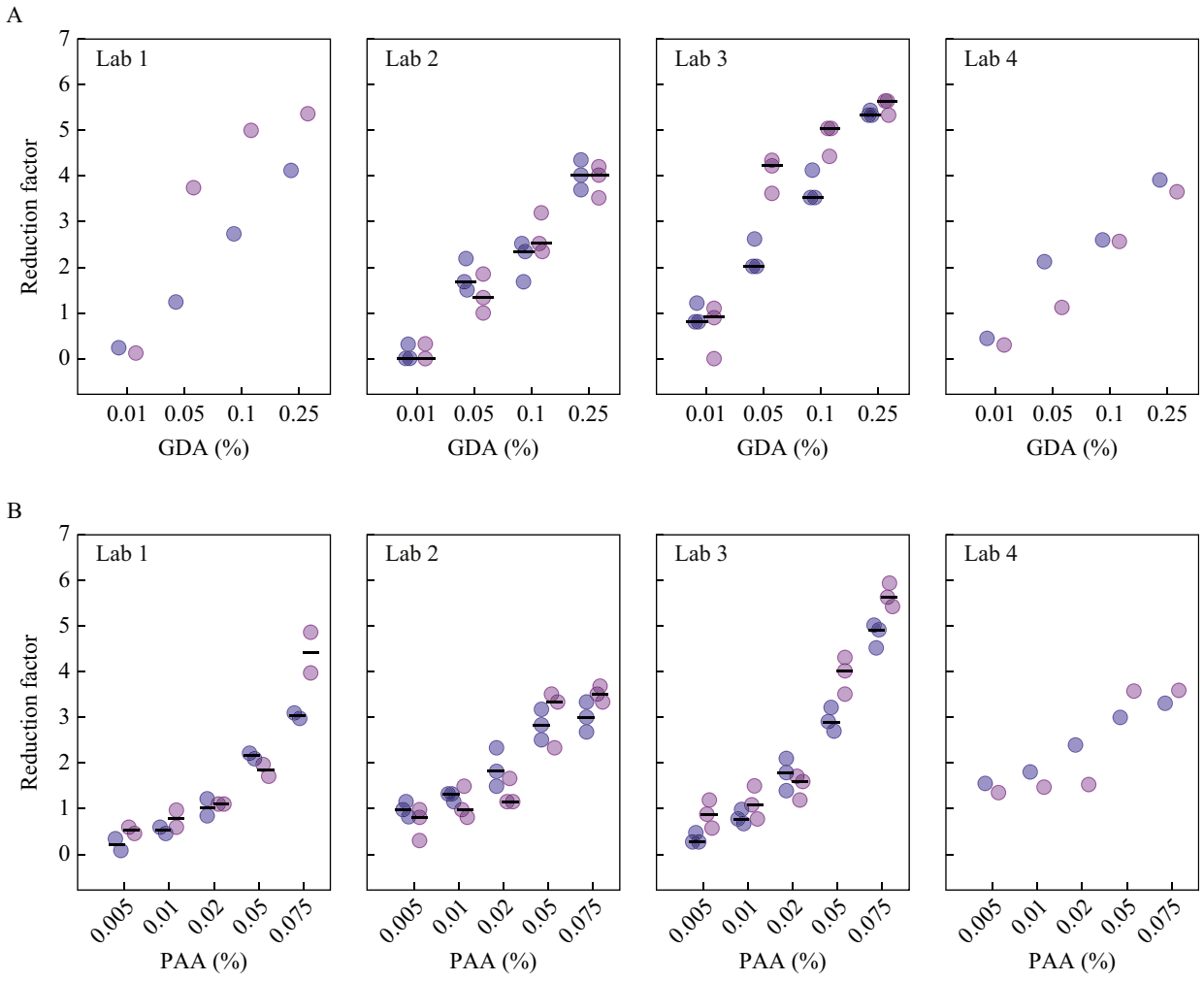

Figure 1. Viral reduction factors of poliomyelitisvirus type 1 (PV-1) and murine parvovirus ATCC VR 1346 (MVM) by glutaraldehyde (A) and peracetic acid (B). Inactivation of PV-1 and MVM was tested at different concentrations of glutaraldehyde (GDA) (A) and peracetic acid (PAA) (B) with a fixed exposure time of 30 min by four laboratories (labs 1–4). Individual replicates per lab and virus (PV: purple dots; MVM: blue dots) and their respective medians (black line) are shown in comparison (GDA: lab 1, $N = 1$; lab 2, $N = 3$; lab 3, $N = 3$; lab 4, $N = 1$; PAA: lab 1, $N = 2$; lab 2, $N = 3$; lab 3, $N = 3$; lab 4, $N = 1$).

strain being also used in France [6]. High titres in cell culture, a high stability towards disinfectants and a sufficient protection of the staff via vaccination account for the successful use of this virus in the past. Recently, the poor stability of this virus upon desiccation on surfaces and finally the global polio eradication programme, which was initiated by the World Health Organization (WHO) in 1988 and specified by the WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses required a replacement [3,7]. Therefore, different animal parvoviruses have been studied and compared to other test viruses in suspension and in a quantitative carrier test for surface disinfectants in order to identify an alternative model virus. In this study, we chose PAA- and GDA-based biocides used in surface and instrument disinfection because of their high virucidal activity [8,9]. Our data demonstrate a high correlation of PV-1 and MVM reduction factors with scores between 0.87 and 0.95.

Our data clearly illustrate that, for testing instrument and surface disinfectants, PV-1 can be replaced by MVM in the future without affecting existing virucidal claims of the tested disinfectants. Unfortunately, for hand disinfectant this replacement cannot be performed as the active compound in hand disinfectants — ethanol — is only active in high concentrations against PV-1 but not at all against members of the parvovirus family when testing porcine parvovirus and MVM [10]. Therefore, for determination of virucidal activity of hand disinfectants a different solution has to be found.

In conclusion, to support the WHO Global Action Plan to minimize poliovirus-facilitated risk after type-specific

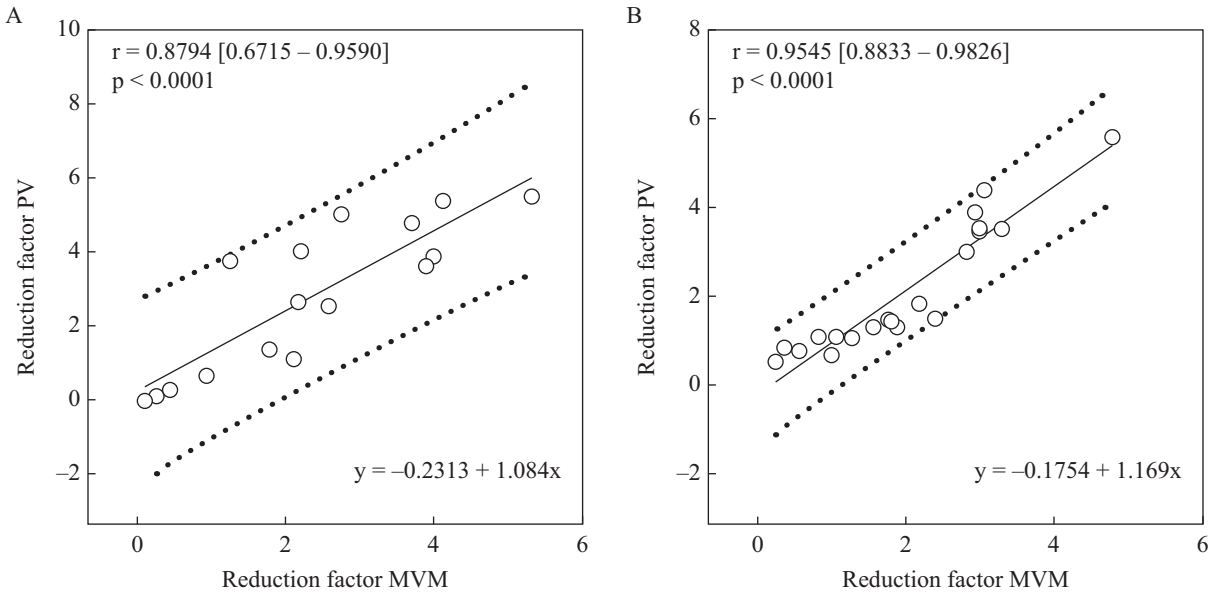

Figure 2. Correlation of reduction factors of poliomyelitisvirus type 1 (PV-1) and murine parvovirus ATCC VR 1346 (MVM) by glutaraldehyde (A) and peracetic acid (B). For each concentration of glutaraldehyde (A) and peracetic acid (B), corresponding reduction factors of each replicate for PV-1 (*y*-axes) and MVM (*x*-axes) are plotted (open circles) and Spearman *r* [95% confidence interval], *P*-value and equation for linear regression (solid line; 95% confidence interval as dotted line) shown in the respective plots.

eradiation of wild-type poliovirus, and to further increase workplace safety in testing laboratories, we confidently support the replacement of PV-1 with the non-human-pathogenic MVM in quantitative suspension tests for the evaluation of virucidal activity of surface and instrument disinfectants in the medical setting.

Conflict of interest statement
None declared.

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