

# In vitro efficacy of topical ophthalmic antiseptics against SARS-CoV-2

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Shedding of SARS-CoV-2 in tears of patients with COVID-19 has been reported,<sup>1,2</sup> which could serve as a source of infection for healthy individuals, including healthcare providers. The current standard antiseptic solutions used in ophthalmology in the setting of inoffice procedures and operating rooms include povidone-iodine (PVI) 5% and chlorhexidine gluconate (CHX) 0.1% or 0.05%, which are at concentrations that are lower than those used in other surgical specialties. Although laboratory and clinical studies to date have aimed to evaluate the virucidal benefits of routine PVI use for ophthalmic surgeries,<sup>3</sup> currently there are no established guidelines regarding the optimal contact time and efficacy of varying dilutions as well as comparisons with other formulations such as CHX. Rigorous evaluation of the efficacy of virucidal agents for disinfecting ocular surface of potentially infected patients with SARS-CoV-2 is critical in mitigating the risk of transmission.

In the current study, we evaluated the virucidal efficacy and contact times for commonly used ophthalmic concentrations of PVI and CHX against SARS-CoV-2 using Vero E6 cells as indicator cell lines for residual viable virus based on previously established methodologies (online supplemental appendix).<sup>4-6</sup> PVI (5% weight per volume, w/v) and CHX (0.05% and 0.1% w/v) were tested at full strength. Fifty microlitres of ophthalmic formulations were added to 10 µL of a SARS-CoV-2 suspension (viral transport media) and incubated at room temperature for varying contact times. A total of three experiments were conducted for PVI and CHX, each including three biological replicates per time point. Individual viral titres for each biological replicate were calculated based on five replicate wells per dilution. Viable residual SARS-CoV-2 was quantified by the Reed-Muench median tissue culture

infectious dose (TCID<sub>50</sub>) procedure in Vero E6 cells.<sup>7</sup> Additional efficacy testing using 1:4 and 1:16 dilutions in phosphate buffered saline were performed in order to mimic clinical settings where dilution of the formulations occurs as a result of mixing with patients' ocular secretions. Cytotoxicity of residual PVI and CHX was predetermined at all test concentrations using uninfected Vero E6 cells.

No SARS-CoV-2 was detected with PVI at full strength and 1:4 dilution after 60 s, 5 min and 10 min of contact time (figure 1). The 1:16 PVI dilution substantially decreased viral titres after 60 s of contact time (4.45, SD 0.44 vs 0.12, SD 0.24 log<sub>10</sub> TCID<sub>50</sub>/mL, 95% CI of difference 3.53 to 5.13, p<0.001). No virus was recovered from the inoculated suspensions after 5 and 10 min of contact time with the 1:16 PVI dilution. Full strength CHX 0.1% (3.99, SD 0.08 vs 3.74, SD 0.10 log<sub>10</sub> TCID<sub>50</sub>/mL, 95% CI 0.04 to 0.46, p=0.03) and CHX 0.05% (4.3, SD 0.5 vs 4.53, SD 0.44 log<sub>10</sub> TCID<sub>50</sub>/mL, 95% CI -1.30 to 0.84, p=0.58) concentrations did not result in SARS-CoV-2 inactivation even after 30 min of contact time.

The findings from this in vitro study demonstrate that PVI at commonly used ophthalmic concentration of 5% has greater virucidal activity than CHX against SARS-CoV-2 in inoculated suspensions, with CHX proving to be ineffective at full concentration of 0.1% even after 30 min of contact time. The virucidal benefits of routine PVI in reducing patients' ocular surface viral load may be effective at 1:16 of the initial concentration with only 60 s of contact time. It is important to note that CHX 0.1% w/v is at the upper limit of the concentration commonly used in ophthalmic procedure settings. These findings are in keeping with previous findings which have shown the efficacy of PVI in managing upper respiratory

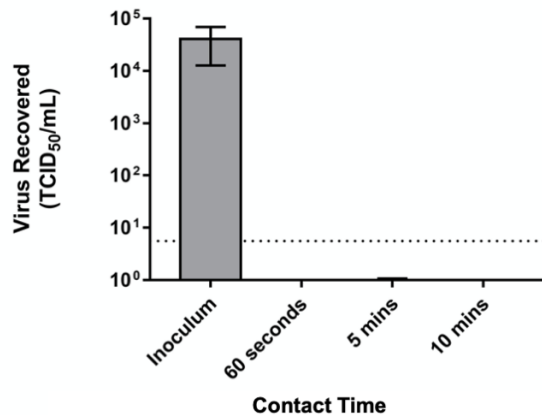
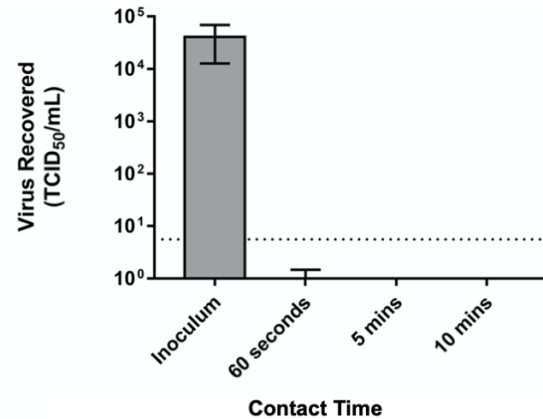
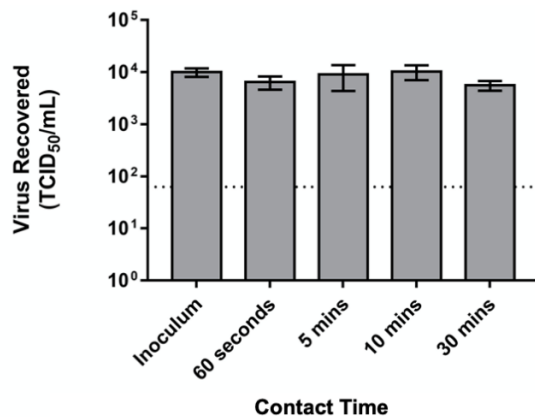
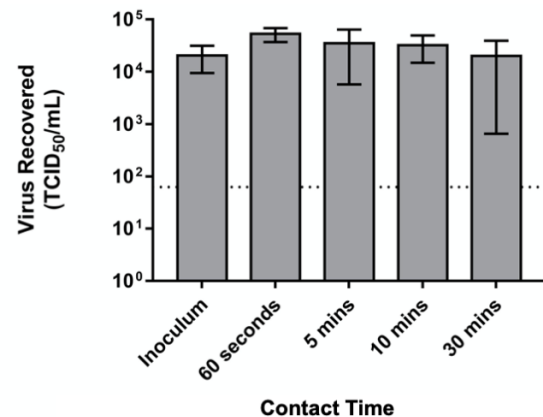


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**A Povidone-Iodine 5% w/v, 1:4 dilution**

**B Povidone-Iodine 5% w/v, 1:16 dilution**

**C Chlorhexidine Gluconate 0.1% w/v**

**D Chlorhexidine Gluconate 0.05% w/v**


**Figure 1** Virucidal efficacy of ophthalmic formulations of povidone-iodine (PVI) and chlorhexidine gluconate (CHX) against SARS-CoV-2. (A and B) Mean ( $\pm$ SD) titre of the positive control (inoculum, 0 min contact time) and the postneutralisation samples (60s, 5 min and 10 min contact time) with PVI 5% at 1:4 and 1:16 dilutions, respectively. (C and D) Mean ( $\pm$ SD) titre of the positive control (inoculum, 0 min contact time) and the postneutralisation samples (60s, 5 min, 10 min and 30 min of contact time) with CHX 0.1% and 0.05%, respectively. The horizontal dashed lines indicate the limit of detection (LOD) of the assay. Note that the LOD is higher for CHX ( $1.8 \log_{10}$  TCID<sub>50</sub>/mL) than PVI ( $0.8 \log_{10}$  TCID<sub>50</sub>/mL) due to its cytotoxicity on Vero E6 cells. TCID<sub>50</sub>, tissue culture infectious dose; w/v, weight per volume.

tract infections and suggested the use of PVI on the sinonasal and oral mucosa against the transmission of SARS-CoV-2.<sup>8</sup> Overall, this study has important implications for clinicians when selecting an ophthalmic solution for routine procedures that reduces transmissibility of SARS-CoV-2 via ocular secretions among patients and healthcare providers. The adoption of guidelines for ophthalmic surgeries such as lacrimal surgeries using PVI may be useful in decreasing viral burden in the setting of the COVID-19 pandemic and other viral infections.

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## Methods and Materials

### Cell Culture and Virus

African green monkey Vero E6 cells (ATCC CRL 1586; American Type Culture Collection, Manassas, VA, United States) were maintained at 37°C+5% CO<sub>2</sub> in Cell Culture Medium (CCM) consisting of Dulbecco's modified Eagle cell culture medium (DMEM; Hyclone SH3024302) supplemented with 10% Fetal Bovine serum (FBS; Gibco Grand Island, NY, United States) and 10 units per ml of Penicillin/Streptomycin (Gibco Grand Island, NY, United States). Medium utilized for experimental infections, denoted as virus culture medium (VCM), consisted of DMEM supplemented with 2% Fetal Bovine serum and 10 units per ml of Penicillin/Streptomycin. Low passage P2 SARS CoV-2 (*hCoV-19/Canada/ON-VIDO-01/2020*, GISAID accession# *EPI\_ISL\_425177*) was used to prepare working stocks and concentrated stock collections.

### Stock Virus Preparation

Low passage SARS-CoV-2 (P3) was produced by infection of Vero E6 cells at a multiplicity of infection 0.01. By 3-4 days post infection, cytopathic effect (CPE) became evident where over 90% of the cell monolayer had lifted in infected flasks. At this point, supernatants from infected flasks were aspirated, pooled, and clarified by low-speed centrifugation (4500 xg) for 10 minutes. Resulting clarified supernatants were aliquoted and stored at -70°C. Fresh vials of stock virus were utilized for each experimental replicate to avoid potential effects of freeze-thaw cycles.

### Disinfectant Preparation

Povidone-iodine (PVI) 5% w/v (Bausch & Lomb, INNOVA Medical Ophthalmics, SKU: DR2CB10944CA) and chlorhexidine gluconate (CHX) 0.1% and 0.05% w/v (Aved Compounded Products, Lot: 10082020@6) were used at full concentrations as provided by manufacturer and hereby referred to as neat dilution. Commercial products requiring further dilution for efficacy testing were two-fold serially diluted in phosphate buffered saline (PBS) to achieve testing dilutions of 1:4, and 1:16 in addition to the neat preparation. Diluted products were prepared fresh on day of assay and used with 10 minutes of preparation.

### Biocide Neutralization Assay

Prior to determining the efficacy of the biocides against SARS-CoV-2, a neutralization assay, as described by Cutts et al 2019<sup>1</sup>, was conducted to determine the impact of the reagents on the virus and the health of the indicator host reporter cell line.

To test the effect of the neutralized biocides on the health of the cells, 50µl of the neat concretions of PVI and CHX were added to 950µl neutralizer (VCM). Solutions were mixed by pipetting and ten-fold serially diluted. In replicates of five per dilution series, 50µl of diluted solution was added to Vero E6 cells at 80% confluence overlaid containing 150µl of fresh VCM.

To test the effect of the neutralized biocides on the viability of the virus, 50µl of the neat concretions of PVI and CHX were added to 940µl VCM and mixed. Ten microliters of stock virus were then added to the neutralized solution, mixed well by pipetting and subsequently ten-fold serially diluted.

A corresponding positive control, consisting of 10µl of stock virus added to 990µl VCM, was included for comparison. Negative controls, consisting of cells overlaid with VCM only, were additionally included.

In replicates of five per dilution series, 50µl of the diluted test solutions were overlaid onto Vero E6 cells at 80% confluence overlaid containing 150µl of fresh VCM. Cells were monitored daily for evidence of cytotoxicity and/or cytopathic effect as appropriate for a period of 5 days.

#### Efficacy Testing

ASTM 1052<sup>2</sup> was used as a guide for this procedure with the only difference amounting to the amount of biocide being applied to the virus. ASTM 1052 indicates that a 10µl of virus should be used to 90µl of biocide, we used 50µl of biocide to 10µl of virus to increase the stringency of the assay and reflect real world condition and applications.

Inoculum preparation consisted of mixing the stock virus with a tripartite 'soil load', used to increase the bioburden against biocides and provide an additional layer of challenge to surfaces and materials needing to be decontaminated. Briefly, a tripartite soil load (12.5µl of 5% BSA, 17.5µl 5% Tryptone, and 0.4% Mucin) was added to 170µl of stock virus as per ASTM 1052<sup>2</sup>. Using a positive displacement pipette, 10µl of virus-soil load mixture was deposited on the bottom of a sterile Sarstedt cryotube. Prepared disinfectants in 50µl aliquots were added to prepared virus, mixed twice and left for the incubation times of 60 seconds, 5 minutes, and 10 minutes, with an additional 30-minute incubation time used solely for the CHX biocide assay. After set time was reached, virus-biocide mixtures were neutralized with 940µl VCM, mixed by pipetting and immediately assessed for viable virus by quantitative (50% tissue culture infective dose (TCID<sub>50</sub>) assay) and qualitative (safety flask) means. Three independent experiments consisting of three biological replicates per contact time were conducted for each of the PVI and CHX test solutions, with the exception of 0.1% CHX, which was assessed in a single experiment.

#### Quantification of residual infectivity

Vero E6 cells were seeded 96-well tissue culture plates to attain 80% confluence on the day of the assay. Quantification of viable virus from neutralized biocidal test solutions and positive controls was achieved through ten-fold dilution series in VCM. Cell culture medium was aspirated from each plate, replaced with 150µl of fresh VCM per well, and a volume of 50µl of neutralized virus-biocide solution, in replicates of five per dilution series, was added to each well and incubated for 5 days and monitored for CPE. Remaining virus titers were determined by the Reed Muench TCID<sub>50</sub> procedure.<sup>3</sup> Residual volumes of neutralized solutions were added to a single well of a six well plate of Vero E6 cells. This served as a safety flask test to qualitatively determine if any amount of virus was present that could have been missed by the smaller amounts used in the TCID<sub>50</sub> procedure.

#### Statistical Analysis

Means for continuous variables were compared using independent-group t-test. A 2-sided  $\alpha$  of less than 0.01 was considered statistically significant. All statistical analysis was performed using SPSS v24.0.

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