Objective: To assess the effect of commonly used contact lens disinfectants against severe acute respiratory syndrome corona virus 2 (SARS-CoV-2).

Methods: The efficacy of five disinfectant solutions against SARS-CoV-2 was tested in the presence and absence of contact lenses (CLs). Three types of unused CLs (hard gas permeable, soft hydrogel, and soft silicone hydrogel) and worn silicone hydrogel CLs were tested. Contact lenses were infected with SARS-CoV-2 and disinfected at various times, with and without rubbing and rinsing, as per manufacturer’s instructions. Reverse-transcriptase polymerase chain reaction (RT-PCR) and viability polymerase chain reaction (PCR) were applied to detect SARS-CoV-2 RNA and viral infectivity of SARS-CoV-2, respectively.

Results: In the presence of SARS-CoV-2–infected CLs, no SARS-CoV-2 RNA could be detected when disinfectant solutions were used according to the manufacturer’s instructions. When SARS-CoV-2–infected CLs were disinfected without the rub-and-rinse step, SARS-CoV-2 RNA was detected at almost each time interval with each disinfecting solution tested for both disinfected without the rub-and-rinse step, SARS-CoV-2 was detected with all disinfectant solutions except Menicon Procent at all time points.

Conclusions: Disinfectant solutions effectively disinfect CLs from SARS-CoV-2 if manufacturer’s instructions are followed. The rub-and-rinse regimen is mainly responsible for disinfection. The viability PCR may be useful to indicate potential infectiousness.

Key Words: SARS-CoV-2—Contact lenses—Contact lens disinfectant solutions—Efficiency.

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An estimated 150 million people use contact lenses (CLs) worldwide.1 Contact lens wearers have a higher risk of microbial keratitis,2–5 and viruses have been involved in severe corneal infections.6–8 Although the prevalence of CL-related viral keratitis is lower than keratitis caused by other pathogens, outcomes are often poor and may require corneal transplantation or lead to blindness in severe cases.6,9,10

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19), which has rapidly become a global health issue since COVID-19 was first identified in Wuhan, China in December 2019.11 The World Health Organization (WHO) declared COVID-19 a pandemic on March 11, 2020. Human-to-human transmission of SARS-CoV-2 is believed to occur mainly through respiratory droplets, but because SARS-CoV-2 has been detected in several body fluids, other routes are under investigation.12 Severe acute respiratory syndrome corona virus 2 has also been found in tear fluid and conjunctival secretions of COVID-19 patients.13 In a recent observational multicenter study, including 243 symptomatic laboratory-confirmed COVID-19 patients, RNA of SARS-CoV-2 has been detected in conjunctival swabs of 17 COVID-19 patients (7.0%).14 If SARS-CoV-2 detected in tear fluid and conjunctival secretions contains intact viral particles remains uncertain. The presence of infectious virus can be determined by the capability of SARS-CoV-2 to replicate in a particular cell line. This virus culture provides an indication of potential infectiousness. However, viral culturing of SARS-CoV-2 is time-consuming, labor-intensive, requires a biosafety level 3 laboratory, and lacks sensitivity compared with reverse-transcriptase polymerase chain reaction (RT-PCR).

Insufficient CL care hygiene may lead to prevalence of microbial keratitis among wearers. This behavior includes failure of hand washing before handling CLs, inappropriate cleaning, disinfection, or replacement of CLs and lens case, exposure of CL or lens case to tap water, and removal of the rub-and-rinse step that is required in certain multipurpose solutions.15–17 Contact lens solutions are designed for cleaning, disinfecting, and storing CLs to reduce the risk of eye infections.
Recently, Yasir et al.\textsuperscript{18} evaluated the antiviral effect of multipurpose contact lens disinfecting solutions against mouse hepatitis virus (MHV), a surrogate for human SARS-CoV-2. However, to the best of our knowledge, no study has yet tested the effectiveness of CL solutions against SARS-CoV-2. The main purpose of this study was to assess the effect of commonly used CL disinfecting solutions against SARS-CoV-2, in particular the effect of the rub-and-rinse step in disinfection efficacy. To determine the viability of SARS-CoV-2 particles, we used a recently introduced viability polymerase chain reaction (PCR).\textsuperscript{19}

**METHODS**

**Study Design**

The efficacy of five disinfecting solutions was tested in the presence and absence of CLs. Contact lenses were infected with SARS-CoV-2 and were disinfected according to the manufacturer’s instructions, with and without rubbing and rinsing step, at different times. Viral infectivity of SARS-CoV-2 and SARS-CoV-2 RNA were determined using viability PCR and RT-PCR, respectively. Both detected viral RNA and infectious virus were defined as disinfection efficacy.

**Severe Acute Respiratory Syndrome Corona Virus 2**

Severe acute respiratory syndrome corona virus 2 was obtained from anonymous positive residual material from the diagnostics and diluted in virus transport medium (VTM), a medium used to collect and transfer viruses, to a final cycle threshold (Ct) value of approximately 28. This Ct value was based on a viral load one log higher than the Ct values of 31 and 37 that were described in recent literature.\textsuperscript{20,21} RT-PCR was performed to verify the Ct value as described below. At the same time, as explained underneath, viability PCR was carried out to discriminate between infectious and noninfectious virus. Only samples containing viable SARS-CoV-2 in VTM were used to contaminate CLs.

**Contact Lenses**

Hard gas permeable, soft hydrogel, and soft silicone hydrogel CLs were used. These lenses were new and unused before testing. To determine whether there were differences between new CLs and worn CLs, worn silicone hydrogel CLs were also tested. The worn CLs were worn for 3 weeks to 1 month by five subjects who visited their eye care professional for a regular check-up. The CLs would be tested for viability of SARS-CoV-2. Contact lenses were cleaned per manufacturer’s recommendations and stored in a new, clean lens case containing fresh CL disinfecting solution. Rubbing of CLs before rinsing and soaking overnight is required for some multipurpose disinfecting solutions. The effect of rub-and-rinse on disinfection efficacy was examined for solutions that require a rub-and-rinse step according to the manufacturer’s instructions. An overview of the experiments performed in the presence of CLs is shown in Figure 1A.

**Experimental Setup**

Contact lenses were placed in a 24-well plate (Corning, Inc., Corning, NY) and soaked in 1 mL VTM containing SARS-CoV-2 Ct 28 in an incubator at 35°C for 16 hr. After incubation (T0), 90 μL of VTM was tested for detection of SARS-CoV-2 RNA and 200 μL was tested for viability of SARS-CoV-2. Contact lenses were cleaned per manufacturer’s recommendations and stored in a new, clean lens case containing fresh CL disinfecting solution. Rubbing of CLs before rinsing and soaking overnight is required for some multipurpose disinfecting solutions. The effect of rub-and-rinse on disinfection efficacy was examined for solutions that require a rub-and-rinse step according to the manufacturer’s instructions. At time intervals of 1, 8, and 24 hr, 90 μL of disinfecting solution was tested for detection of SARS-CoV-2 RNA, except for Menicon Progent intensive cleaner, which was only tested after 30 min according to the manufacturer’s instructions. An overview of the experiments performed in the absence of CLs is shown in Figure 1B.

**Rub-and-Rinse**

Multipurpose disinfecting solution (MPDS) manufacturers recommend performing a rub-and-rinse step to achieve disinfection of CLs. To determine if this step is necessary, we tested the disinfection efficacy of CLs with and without the rub-and-rinse step. After 16 hr of incubation, 11 CLs were rubbed for 20 sec using a sterile hand glove, rinsed with CL disinfecting solution for 5 sec, and stored in a new, clean lens case containing fresh CL disinfecting solution. Twelve CLs were immediately stored in a new, clean lens case containing fresh CL disinfecting solution.

**Viability-Polymerase Chain Reaction**

Viability polymerase chain reaction of SARS-CoV-2 was performed to discriminate between infectious virus and noninfectious virus. In viability PCR, samples are pretreated with a photoreactive dye such as propidium monoazide (PMA) that intercalates covalently into nucleic acids after photoactivation. Propidium monoazide xx solution (20 mM in H2O; Biotium, Inc., Hayward, CA) was used as an intercalating dye and added to 200 μL of sample to achieve a final Ct value of approximately 28. At each time point, 90 μL of CL disinfecting solution was tested for detection of SARS-CoV-2 RNA and after 1, 8, and 24 hr, 200 μL of disinfecting solution was tested for viability of SARS-CoV-2. An overview of the experiments performed in the absence of CLs is shown in Figure 1B.
Reverse-Transcriptase Polymerase Chain Reaction Analysis

All samples were analyzed at the Department of Medical Microbiology, Maastricht University Medical Center, Maastricht, the Netherlands. Viral RNA was extracted using the MagNA Pure 96 system (Roche Diagnostics GmbH, Mannheim, Germany). Extraction was performed using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Diagnostics GmbH) and the Pathogen Universal 200 Protocol (MagNA Pure 96 system, Roche Diagnostics). A 90 μL sample was extracted and eluted in 50 μL elution buffer and diluted with 50 μL water for molecular biology (VWR). RT-PCR was carried out on a Quantstudio 5 system (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA) using a validated multiplex in-house developed assay that targeted the E and N1 gene. The forward and reverse primer sequences for the E gene were 5'-ACAGGTACGTATATTGTAATAGCGT-3' and 5'-ATATTGCAGCAGTACGCACACA-3', respectively. The probe sequence was 5'-6-FAM ACCTAGCCATCCCT-TACTGGCCTTCC-BHQ-1-3'. For the N1 gene, the forward and reverse primer sequences were 5'-GACCCAAAATCAGC-GAAAT-3' and 5'-TCTGTTACTGCCAGTTGAATCTG-3', respectively, and the probe sequence was 5'-ABY-ACCCCCGACAT-TACGGTGTGGGACC-BHQ-2-3'. Final reaction volume was 20 μL and contained 5 μL 4x Taqpath 1-step RT-qPCR MasterMix (Applied Biosystems, Thermo Fisher Scientific), 5 μL primer/probe mix, and 10 μL sample. Cycling conditions consisted of uracil-N-glycosylase (UNG) incubation at 25°C for 2 min, RT incubation at 50°C for 30 min, enzyme activation at 95°C for 2 min, and 42 cycles of denaturation at 94°C for 3 sec and annealing/extension at 60°C for 30 sec. The Ct values were considered as undetermined (UD).

Statistical Analysis

Statistical analyses were carried out with IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY). Mean Ct values and standard deviations are reported as descriptive statistics. Concentrations were calculated from Ct values to allow statistical analyses. Concentrations from samples with no detectable Ct value were set at 0 copies/mL (c/mL). A Wilcoxon signed rank test was used to determine differences between rub-and-rinse and no rub-and-rinse regimen. A Mann–Whitney U test was used to determine differences between VTM and contact lens disinfecting solutions and between worn and new contact lenses. Friedman’s ANOVA for repeated measures was performed to determine difference between the time intervals.

RESULTS

Disinfecting Efficacy in the Presence of Contact Lenses

Contact Lenses Cleared According to the Manufacturer’s Instructions

When MPDSs were used according to the manufacturer’s instructions (with rub-and-rinse regimen for MeniCare Plus, OPTI-FREE PureMoist, and ReNu MPS sensitive eyes), no SARS-CoV-2 RNA could be identified after testing at time intervals of 1 hr, 8 hr, and 24 hr. Comparable results were found for VTM, which was expected to maintain SARS-CoV-2 RNA. Figure 2A shows the results for new CLs, where the mean SARS-CoV-2 RNA concentration at time interval 0 was 4.11±0.26 (range 3.87–4.38) log c/mL. Figure 2B shows the results for worn CLs, where the mean SARS-CoV-2 RNA concentration at time interval 0 was 4.31±0.09 (range 4.17–4.43) log c/mL.
Contact Lenses Cleaned Without Rub-and-Rinse Step

The no rub-and-rinse regimen of appropriate MPDSs was tested because of the suspected high noncompliance rate of CL wearers and to test whether the rub-and-rinse step is necessary. When these MPDSs were tested without the rub-and-rinse principle, SARS-CoV-2 RNA was determined at almost all time intervals with every disinfecting solution tested for both new CLs and worn Biofinity silicone hydrogel lenses (Fig. 2C,D). The mean SARS-CoV-2 RNA concentration found at time interval 0 for new CLs was $4.02 \pm 0.20$ (range 3.90–4.58) log c/mL, whereas the mean detectable concentrations found at time intervals 1, 8, and 24 were $1.27 \pm 1.02$ (range 0–2.38), $1.07 \pm 0.82$ (range 0–2.03), and $0.91 \pm 1.00$ (range 0–2.19) log c/mL, respectively. These results correspond to mean log removals of 2.60, 2.66, and 2.91 c/mL. For worn Biofinity silicone hydrogel lenses, the mean SARS-CoV-2 RNA concentration found at time interval 0 was $4.02 \pm 0.20$ (range 3.90–4.58) log c/mL, whereas the mean detectable concentrations found at time intervals 1, 8, and 24 were $1.27 \pm 1.02$ (range 0–2.38), $1.07 \pm 0.82$ (range 0–2.03), and $0.91 \pm 1.00$ (range 0–2.19) log c/mL, respectively. These results correspond to mean log removals of 2.60, 2.66, and 2.91 c/mL.

Disinfecting Efficacy in the Absence of Contact Lenses

The tests were repeated in the absence of CLs to determine the efficacy of disinfectant solutions themselves. As shown in Figure 3, Menicon Progent was the only disinfecting solution that effectively eliminated SARS-CoV-2 RNA. At T1, T8, and T24, significant differences were found between the rub-and-rinse and no rub-and-rinse regimen with $p$-values of 0.028, 0.028, and 0.018 sequentially. No significant differences were found at T1, T8, and T24 between VTM and disinfecting solutions without the rub-and-rinse principle ($p > 0.05$). No significant differences could be determined between time intervals T1, T8, and T24 ($p > 0.05$), but significant differences were determined between time intervals T0 and T1, T0 and T8, and T0 and T24 ($p < 0.05$). No significant differences were observed between new and worn CLs with and without rub-and-rinse principle ($p > 0.05$).

FIG. 1. Study design of the experiments performed. (A) Disinfection efficacy of CL solutions in the presence of CLs. (B) Disinfection efficacy of CL solutions in the absence of CLs. CL, contact lenses; Ct, cycle threshold; HP, hydrogen peroxide; VTM, virus transport medium.
No significant differences could be determined between time intervals ($P>0.05$), except between T0 and T24 ($P=0.028$), nor were any significant differences observed between VTM and AO-SEPT PLUS, MeniCare Plus, OPTI-FREE PureMoist, and ReNu MPS Sensitive Eyes ($P>0.05$).

**Viability Polymerase Chain Reaction Disinfecting Solutions**

To discriminate between infectious and noninfectious virus, viability PCR was performed for the disinfecting solutions in the absence of CLs. Figure 4 shows the SARS-CoV-2 RNA concentrations in log c/mL of samples with and without treatment with PMAxx. Mean differences in log SARS-CoV-2 RNA concentrations at time intervals T1, T8, and T24 were $-0.06$, $-0.10$, and $-0.01$, respectively. This means that viable SARS-CoV-2 was found at all time points.

**DISCUSSION**

This study evaluated the efficacy of CL disinfecting solutions against SARS-CoV-2. We tested the effect of the commonly used CLs and their corresponding disinfecting solutions against SARS-CoV-2.

The results of current study show that CL disinfecting solutions are effective against SARS-CoV-2 in the presence of infected CLs when the manufacturer’s instructions are followed. No differences could be seen between new and worn CLs. For MeniCare Plus, OPTI-FREE PureMoist, and ReNu Sensitive Eyes, the rub-and-rinse principle is required. Comparing these disinfecting solutions to VTM, a medium used to collect and transfer viruses, shows that the rub-and-rinse step is crucial, independent of the solution used. Moreover, disinfection of CLs without the rubbing step was not achieved, demonstrating the importance of providing proper education and adhering to this measure in preventing infections. In particular because we recently demonstrated that SARS-CoV-2 RNA is detectable in conjunctival samples from COVID-19 patients.

Most published studies report the disinfecting efficacy of CL solutions against bacteria, yeasts, molds, and *Acanthamoeba* spp. Only few studies examined the antiviral efficacy of CL disinfectants. Notably, none of the mentioned studies used PCR to determine the presence of microorganisms. Yasir et al. recently tested disinfection efficacy against MIV, a surrogate of the human SARS-CoV-2, and found that applying rub-and-rinse principle is essential for disinfection. A second study compared several disinfection methods for CLs infected with adenovirus type 8 (AV-8) and adenovirus type 19 (AV-19) and found significant reductions in viral titer of both serotypes, but only heat disinfection was found to eliminate the virus from soft CLs. Another study tested the disinfection efficacy of CL solutions in *Acanthamoeba* spp, herpes simplex virus 1 (HSV-1), adenovirus type 8 (AV-8), and poliovirus type 2 (PV-2). The disinfecting
The efficacy of the viruses examined in this study was good with both rub-and-rinse and no rub-and-rinse regimen. However, several studies reported low compliance rates among CL wearers with basic hygiene practices, including washing hands before handling lenses, and following rub-and-rinse practices. Nonetheless, Ramamoorthy et al. also found low compliance with rinsing CLs before storage.

The viability PCR is an innovative technology that has not been used for ophthalmic purposes before. The viability PCR allows selective PCR amplification from infectious virus. Because of the high Ct values (i.e., low viral loads) found with the SARS-CoV-2 PCR, we were unable to perform viability PCR on the samples involving CLs because of the lower sensitivity of the viability PCR. Therefore, we cannot conclude whether low viral loads are viable and infectious. In the absence of CLs, none of the abovementioned CL disinfecting solutions showed antiviral activity against the novel coronavirus. Results were comparable with those obtained with VTM. Our results are in accordance with a study that tested the disinfecting efficacy of a multipurpose solution in the absence of CLs. In this study, only low reductions in viability of viruses by culturing were found: 1.4 log for HSV-1 and less than 1 log for both AV-8 and PV-2. Another study that recently examined the antiviral effect of five MPDSs against MHV by standard plaque-forming assay found that oxidative MPDSs, including AO SEPT PLUS, were antiviral, but three other MPDSs were unable to kill the surrogate coronavirus. This is in contrast to our finding that AO SEPT PLUS was not antiviral. There is little information in the literature on the antiviral efficacy of CL disinfecting solutions. This is likely because of regulatory requirements. For example, compliance with ISO 14729 requires antibacterial properties but not antiviral properties. Although the recommended disinfection time for the tested disinfecting solutions is between 5 min and 6 hr, the 8-hr interval was chosen from the perspective of the CL wearer, assuming that most CL wearers store their CLs overnight in the CL disinfecting solution. Time intervals of 1 hr and 24 hr were chosen as extremes. Remarkably, Ct values did not differ significantly at different time intervals, indicating disinfection time did not affect the results. An exception is Menicon Progent, which is a weekly protein remover, disinfectant, and intensive cleaner. In none of the experiments, in the presence or in the absence of CLs,

FIG. 3. SARS-CoV-2 RNA loads detected after infecting various CL disinfecting solutions at different time intervals in the absence of CLs compared with control solutions (3% HP and VTM). *Results inhibited. CLs, contact lenses; C/ml, copies per mL; HP, hydrogen peroxide; VTM, virus transport medium.

FIG. 4. SARS-CoV-2 RNA concentrations detected in samples with and without PMAxx treatment. C/ml, copies per mL; HP, hydrogen peroxide; PMA, propidium monoazide; VTM, virus transport medium. *Results inhibited.
RNA of SARS-CoV-2 could be detected after a disinfection time of 30 min. Our findings suggest to use this easy-to-use cleaner weekly when disinfecting CLs in addition to daily cleaning. However, this is also influenced by a broader set of factors. A disadvantage of Menicon Progent is that this disinfecting solution is only compatible with gas-permeable CLs. When disinfecting CLs weekly with Menicon Progent, CL wearers should remember to rinse their CLs with daily CL disinfecting solution before wearing.

Subsequent studies performed by Gijs et al. and Güemes-Villalho et al. describe two patients with Ct values of 23 and 25, respectively, in conjunctival swabs. These viral loads are one to two logs higher than tested in this study. Although we would not expect a one to two log higher viral load to alter the findings in this study because of the high effectiveness of the rub-and-rinse step, we cannot conclusively predict the results at a higher viral load.

Viral culturing of SARS-CoV-2 is currently the gold standard to assess viability. However, this technique requires a biosafety level 3 laboratory, making culturing of SARS-CoV-2 impossible in most clinical settings. Instead, we developed a viability PCR, which does not require culturing of SARS-CoV-2. Although further optimization and validation are needed, this article provides an interesting direction for future research not only for SARS-CoV-2 but also for other pathogens that are involved in corneal infections.

In conclusion, MPDSs show poor antiviral activity against SARS-CoV-2, but CLs can be disinfected effectively from SARS-CoV-2 if the manufacturer’s instructions are followed. However, this effect is mainly achieved through rubbing and rinsing CLs before disinfection.

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