

Methods to disinfect and decontaminate SARS-CoV-2: a systematic review of *in vitro* studies

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Ther Adv Infectious Dis
2021, Vol. 8: 1–12
DOI: 10.1177/
2049936121998548
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Abstract

Background: Cleaning is a major control component for outbreaks of infection. However, for the SARS-CoV-2 pandemic, there is limited specific guidance regarding the proper disinfection methods that should be used.

Methods: We conducted a systematic review of the literature on cleaning, disinfection or decontamination methods in the prevention of SARS-CoV-2.

Results: A total of 27 studies were included, reporting a variety of methods with which the effectiveness of interventions were assessed. Virus was inoculated onto different types of material including masks, nasopharyngeal swabs, serum, laboratory plates and simulated saliva, tears or nasal fluid and then interventions were applied in an attempt to eliminate the virus including chemical, ultraviolet (UV) light irradiation, and heat and humidity. At body temperature (37°C) there is evidence that the virus will not be detectable after 2 days but this can be reduced to non-detection at 30 min at 56°C, 15 min at 65°C and 2 min at 98°C. Different experimental methods testing UV light have shown that it can inactivate the virus. Light of 254–365 nm has been used, including simulated sunlight. Many chemical agents including bleach, hand sanitiser, hand wash, soap, ethanol, isopropanol, guanidium thiocyanate/t-octylphenoxypolyethoxyethanol, formaldehyde, povidone-iodine, 0.05% chlorhexidine, 0.1% benzalkonium chloride, acidic electrolysed water, Clyraguard copper iodine complex and hydrogen peroxide vapour have been shown to disinfect SARS-CoV-2.

Conclusions: Heating, UV light irradiation and chemicals can be used to inactivate SARS-CoV-2 but there is insufficient evidence to support one measure over others in clinical practice.

Keywords: cleaning, COVID-19, decontamination, SARS-CoV-2

Received: 5 February 2021; revised manuscript accepted: 5 February 2021.

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What is known about the topic?

- The global COVID-19 pandemic is a consequence of the rapid spread of the SARS-CoV-2 virus and cleaning is essential in breaking the cycle of infection.
- There is limited specific guidance regarding the proper disinfection methods that should be used.

What does the study add?

- Our review included 27 studies which inoculated SARS-CoV-2 virus onto different types of material including masks, nasopharyngeal swabs, serum, laboratory plates and simulated saliva, tears or nasal fluid, and then interventions were applied in an attempt to eliminate the virus.

- Several methods are effective in inactivating the SARS-CoV-2 virus, with these methods broadly classed as those related to heating, UV light irradiation and chemical agents.
- All of the studies took place in laboratory settings rather than real-life clinical settings, so there is insufficient evidence to recommend one type of cleaning procedure over another.

Introduction

As of November 2020, there have been more than 1.3 million deaths attributed to SARS-CoV-2.¹ The global COVID-19 pandemic is believed to have originated in Wuhan, China in December 2019 and is a consequence of the rapid spread of the SARS-CoV-2 virus.² Current evidence suggests that this virus is primarily transmitted between people through respiratory droplets and contact routes.³ Cleaning and disinfection is essential in breaking the cycle of infection so that the surfaces and objects which may be in contact with infected patients are decontaminated. While methods have been advised to protect oneself from COVID-19 including the washing of hands frequently, avoiding close contact, covering the nose and mouth with a mask, cleaning and disinfection,⁴ infected patients may still be spreading the illness in the community and in healthcare settings despite adherence to these methods. In hospital settings, where there is inevitable contact with patients infected with COVID-19, it is of critical importance that equipment and facilities are cleaned to avoid infecting healthcare staff and other patients. There is limited specific guidance regarding the proper disinfection methods that should be used.

Many studies have evaluated the reduction of SARS-CoV-2 using thermal, irradiation and chemical methods. Early studies of viral decontamination focused on N95 respirators because of concerns over inadequate supplies of personal protective equipment in outbreak areas. There were also concerns that this new virus may have different response to different methods of decontamination depending on the environment, including factors such as humidity, temperature and irradiation. The most commonly used measure to reduce viral transmission is application of chemical methods in the form of hand sanitiser or hand wash.

While the efficacy of different methods to clean and disinfect viruses has been previously studied, the extent to which these agents work for SARS-CoV-2 is less known. We therefore conducted a systematic

review of cleaning and decontamination methods that have been reported in the literature for SARS-CoV-2.

Methods

Eligibility criteria

We selected studies that evaluated the cleaning, disinfection or decontamination of SARS-CoV-2. There were no restrictions on what type of measure was used but the study had to evaluate the effectiveness of the measure on inactivating or eradicating SARS-CoV-2. There was no restriction based on study design, cohort type or language of the report, but original data had to be presented.

Search strategy

We searched MEDLINE and EMBASE using OVID with no date or language restriction in November 2020. The exact search terms were: (cleaning OR disinfection OR decontamination) AND (COVID-19 OR SARS-CoV-2). We reviewed the bibliography of relevant studies and reviews for additional studies that met the inclusion criteria.

Study selection and data extraction

Three reviewers screened all titles and abstracts retrieved from the search for studies that met the inclusion criteria. The studies that potentially met the inclusion criteria were reviewed and the final decision to include or exclude studies was made by consensus. Data were collected on the substrate of SARS-CoV-2 testing, the method of decontamination or disinfection and study results.

Data analysis

Data were extracted into pre-designed and piloted tables. Study findings were narratively synthesised.

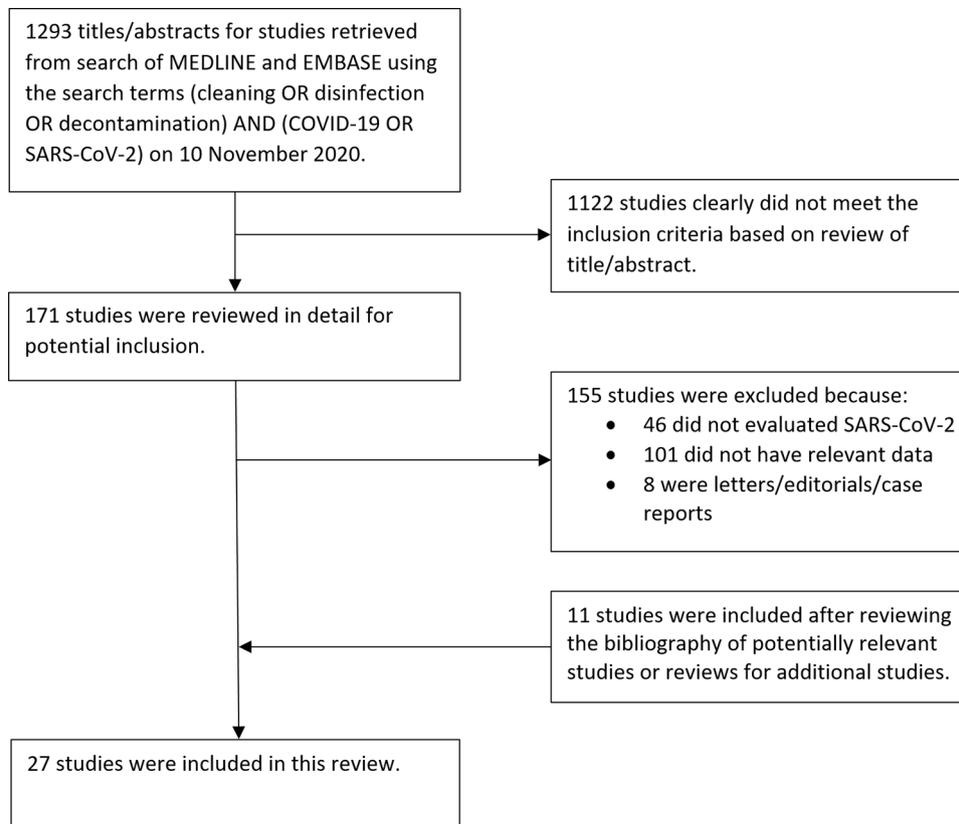


Figure 1. Flow diagram of study selection

Considerable heterogeneity in the study methodology meant that we did not perform statistical pooling or meta-analysis. However, the data were grouped according to type of measure to disinfect SARS-CoV-2 which included heat and humidity, light irradiation and chemical agents.

Results

A total of 27 studies were included in this review after screening and reviewing potentially relevant studies (as shown in Figure 1).^{5–31}

The description of the substrates for testing SARS-CoV-2 and methods for decontamination or disinfection are shown in Tables 1–3. Most studies used Vero E6 cells to culture SARS-CoV-2 and some studies inoculated the virus onto masks, nasopharyngeal swabs, serum, laboratory plates and simulated saliva, tears or nasal fluid. The methods used to eliminate virus included heat, humidity, light irradiation and chemical treatments.

Heat and humidity

The results of studies that evaluated the effect of heat and humidity on SARS-CoV-2 are shown in Table 1. Chin *et al.*⁹ studied the stability of the virus at different temperatures and found that it could be detected beyond 14 days at 4°C but not detectable after 2 days at 37°C. With respect to different surfaces, the virus could be detected for 3 h on paper or tissue paper and up to 7 days on stainless steel, plastic and masks. Heat inactivation of the virus was reported to take place at 2 min at 98°C, 10–15 min at 60–65°C, 15–30 min at 56°C, and 48 h at 42°C.^{5,6,30} Heating N95 masks at 70°C was reported by two studies to be effective in decontaminating the virus.^{10,11} The conditions of heating appear to be important, as Gamble *et al.*¹² found that the virus was not detected at 30 min in a closed 2 ml vial that was heated to 70°C, but if tested in a covered or uncovered plate in an oven the time needed to achieve decontamination increased (90 and 60 min, respectively). Also Campos *et al.*⁷ evaluated the influence of humidity and temperature,

Table 1. Results from included studies for heat and humidity.

Study ID	Substrate of SARS-CoV-2 tested	Results
Auerswald <i>et al.</i> ⁵	Vero E6 cells were used to culture SARS-CoV-2.	Results all methods inactivated the viruses: <ul style="list-style-type: none"> • Heat at 56°C for 30 min • Heat at 56°C for 60 min • Heat at 98°C for 2 min
Batéjat <i>et al.</i> ⁶	Vero E6 cells were used to culture SARS-CoV-2 on cell culture supernatants, nasopharyngeal swab and serum.	Heated cell culture supernatant: <ul style="list-style-type: none"> • 56°C not detected at 30 min • 65°C not detected at 15 min Heated nasopharyngeal swab: <ul style="list-style-type: none"> • 65°C not detected at 10 min • 95°C not detected 15 min Heated serum: <ul style="list-style-type: none"> • 56°C not detected at 15 min
Campos <i>et al.</i> ⁷	Droplet of SARS-CoV-2 (1 × 10 ⁶ PFU/sample) placed on meltblown fabric from N95-grade filtering facepiece respirators.	Reduction from 25°C in log 10: <ul style="list-style-type: none"> • Ambient humidity (60%)–BSA: 60°C 30 min 2.16 ± 0.23, 75°C 30 min 3.69 ± 0.32, 85°C 20 min >4.77, 95°C 5 min >4.77 • Ambient humidity (60%) + BSA: 60°C 30 min 1.07 ± 0.06, 75°C 30 min 2.89 ± 0.31, 85°C 20 min 4.3 ± 0.55, 95°C 5 min 4.8 ± 0.44 • 100% humidity–BSA: 60°C 30 min 2.82 ± 0.09, 75°C 30 min >4.97, 85°C 20 min >4.97, 95°C 5 min >4.97 • 100% humidity + BSA: 60°C 30 min 2.27 ± 0.09, 75°C 30 min 4.92 ± 0.12, 85°C 20 min >5.02, 95°C 5 min >5.02
Chin <i>et al.</i> ⁹	SAR-CoV-2 culture (7.8 log unit of TCID50/ml) 15 µl droplet pipetted on surfaces with 200 µl virus transport medium and 135 µl of disinfectants.	At different temperatures: <ul style="list-style-type: none"> 4°C virus detected after 14 days 22°C virus not detected at 14 days 37°C virus not detected at 2 days 56°C virus not detected at 30 mins 70°C virus not detected at 5 min Surfaces and when virus undetected: <ul style="list-style-type: none"> • Paper 3 h • Tissue paper 3 h • Wood 2 days • Cloth 2 days • Glass 4 days • Banknote 4 days • Stainless steel 7 days • Plastic 7 days • Mask inner 7 days • Mask outer layer detected at 7 days
Daeschler <i>et al.</i> ¹⁰	4 N95 respirators models pieces and inoculated with 5 µl of SARS-CoV-2 (7.8 log TCID50/ml).	<ul style="list-style-type: none"> • Heating 70°C for 60 min virus was not detected
Fischer <i>et al.</i> ¹¹	Vero E6 cells inoculated on samples of N95 masks and stainless steel surfaces.	Median time to one millionth (mins) for N95 mask and stainless steel: <ul style="list-style-type: none"> • Control 1.56 × 10³ and 4.66 × 10³ min • Heat 70°C 95.6 and 176 min
Gamble <i>et al.</i> ¹²	SARS-CoV-2 suspension in culture medium which was heat treated in 24-well plate and closed vials.	Heat treatments at 70°C: <ul style="list-style-type: none"> • Uncovered 24-well plate in oven: virus reduced by 1 log₁₀ TCID50/ml at 60 min • Covered 24-well plate in oven: virus not detected at 90 min • Closed 2 ml vial in oven: virus not detected at 30 min • Closed 2 ml vial in water: virus not detected at 30 min
Wang <i>et al.</i> ³⁰	SARS-CoV-2 with infectious titre 1.4 × 10 ⁷ was heated at different temperatures.	Viral titres (TCID50/ml) <ul style="list-style-type: none"> • 37°C present at 24 h (1.8 × 10⁶), 320 at 48 h • 42°C present at 60 min (1.9 × 10⁶), undetectable at 48 h • 56°C undetectable at 30 min • 56°C in 50% human serum undetectable at 30 min • 60°C undetectable at 15 min • Unheated 1.4 × 10⁷ at 120 min

PFU, plaque forming-unit; BSA, bovine serum albumin; TCID, tissue culture infective dose; .

Table 2. Results from included studies for ultraviolet light irradiation.

Study ID	Substrate of SARS-CoV-2 tested	Results
Fischer <i>et al.</i> ¹¹	Vero E6 cells inoculated on samples of N95 masks and stainless steel surfaces.	Median time to one millionth (mins) for N95 mask and stainless steel: <ul style="list-style-type: none"> Control 1.56×10^3 and 4.66×10^3 min UV light 128 and 9.79 min
Helingloh <i>et al.</i> ¹³	Vero E6 cells used to culture SARS-CoV-2 (5×10^6 TCID50/ml) and 600 μ l virus stock placed in 24-well plates.	<ul style="list-style-type: none"> UVC and UVA treatment: inactivation in 9 min. 50% reduction in 1.4 min with treatment
Inagaki <i>et al.</i> ¹⁶	Vero E6 cells infected with SARS-CoV-2 diluted with phosphate buffered saline and adjusted to 2.0×10^4 plaque-forming units/ml and 150 μ l were placed in a Petri dish and irradiated.	Infectious titre with different irradiation time with deep ultraviolet light (280 nm) (reduction ratio %, log PFU ratio): <ul style="list-style-type: none"> 1 s 4.7×10^3 (87.4%, 0.9) 10 s 2.7×10^1 (99.9%, 3.1) 20 s 6.7×10^0 (99.9%, >3.3) 30 s <20 (99.9%, >3.3)
Kitagawa <i>et al.</i> ¹⁸	Vero E6 cells were infected with SARS-CoV-2 and viral suspension made with viral titres of 5×10^6 TCID50/ml. 100 μ l of viral suspension transferred on plates which were irradiated.	Mean viable SARS-CoV-2 by time (log reduction) with 222 nm UV disinfection: <ul style="list-style-type: none"> Control 2.05×10^4 10 s $2.34 \pm 0.86 \times 10^3$ [0.94] 30 s $6.32 \pm 0.0 \times 10^1$ [2.51] 60 s $6.32 \pm 0.0 \times 10^1$ [2.51] 300 s $6.32 \pm 0.0 \times 10^1$ [2.51] RNA did not change after UV irradiation compared with controls
Ozog <i>et al.</i> ²⁴	Vero-E6 cells were infected with SARS-CoV-2 virus. 10 μ l drop of SARS-CoV-2 viral stock (8×10^7 TCID50/ml) inoculated on four different locations of five different N95 filtering facepiece respirators.	Low-pressure mercury lamp ultraviolet germicidal irradiation device (254 nm) with irradiation for 60–70 s each side and log ₁₀ TICD50/4 mm punch (300 μ ml) for location 1, 2, 3, 4 (range for respirator A, B and C) and control by mask type: <ul style="list-style-type: none"> 3M 1860 <1.3, <1.3, <1.3, <1.3–2.28, 2.98–5.48 3M 8210 <1.3–2.38, \leq1.3, <1.3, <1.3, 2.38–5.48 Moldex 1511 <1.3, <1.3, <1.3, <1.3, 2.58–4.68 3M 8511 <1.3, <1.3, \leq1.3, 2.28–2.78, 3.38–4.48 3M 9211 <1.3, <1.3, \leq1.3, 2.28–2.78, 2.28–3.88
Rathnasinghe <i>et al.</i> ²⁵	Vero-E6 cells infected with SARS-CoV-2 and virus was adsorbed to N95 mask coupons and mask squares.	Infectious SARS-CoV-2 was not detected by plaque assays on N95 respirator snippets when irradiated with UVC for 120 s per side or longer suggesting 3.5 log reduction in 240 s of irradiation
Ratnesar-Shumate <i>et al.</i> ²⁶	Vero cells infected with SARS-CoV-2 with concentration 1.5×10^6 TCID50/ml. Viral solution spike 1:10 on simulated saliva or complete growth medium.	With UVB irradiation of saliva, inactivation rates ranged from 0 in darkness to 0.15 log ₁₀ TCID50 loss/min Ninety percent of infectious virus would be lost every 6.8, 8.0, and 12.8 min for integrated UVB irradiances of 1.6, 0.7, and 0.3 W/m ² With UVB irradiation of growth medium, inactivation rates in ranged from 0 in darkness to 0.07 log ₁₀ TCID50 loss/min. Ninety percent of infectious virus would be lost every 14.3 and 17.6 min for integrated UVB irradiances of 1.6 and 0.7 W/m ² , respectively Inactivation rate of virus was greater when suspended in simulated saliva than growth medium
Simmons <i>et al.</i> ²⁷	Vero E6 cells infected with SARS-CoV-2 with viral titre of 1.3×10^7 plaque-forming units. 0.020 ml of virus deposited as droplet on chamber slide and N95 respirator, 3M 1860.	Pulsed-xenon UV light on (log ₁₀ PFU/ml) <ul style="list-style-type: none"> Slide at 0 min 6.2 Slide at 1 min 2.67 (reduction 99.97%, log₁₀ 3.53) Slide at 2 min <1.66 (reduction >99.997%, log₁₀ >4.54) Slide at 5 min <2.08 (reduction >99.992%, log₁₀ >4.12) N95 respirator at 0 min 6.35 N95 respirator at 5 min <1.56 (reduction >99.998, >4.79)
Smith <i>et al.</i> ²⁸	Vero E6 cells infected with SARS-CoV-2 inoculated on N95 masks (1860, 1870+, 8511).	Infectivity (Δ RNA in Vero E6 cells from input log ₁₀) with 254 nm UV light on mask type: <ul style="list-style-type: none"> 1860 log reduction of \sim3 1870+ log reduction of \sim2 8511 log reduction of \sim3

PFU, plaque forming-unit; UV, ultraviolet; UVC, ultraviolet C; UVB, ultraviolet B; UVA, ultraviolet A; RNA, ribonucleic acid.

Table 3. Results from included studies for chemical or other agents.

Study ID	Substrate of SARS-CoV-2 tested	Results
Auerswald <i>et al.</i> ⁵	Vero E6 cells were used to culture SARS-CoV-2.	Results all methods inactivated the viruses: <ul style="list-style-type: none"> • 560 µl of viral lysis buffer for 10 min at room temperature • 200 µl of inactivating sample buffer containing 50% guanidium thiocyanate and 6% t-oxyphenoxypolyethoxyethnaol for 15 min at room temperature
Chan <i>et al.</i> ⁸	Vero E6 cells infected with SARS-CoV-2.	Log 10 reduction in virus at 1 and 5 min: <ul style="list-style-type: none"> • Ethanol (75%) $\geq 1.83 \pm 0.29$ and $\geq 2.00 \pm 0.00$ • Bleach (10%) $\geq 3.25 \pm 0.00$ and $\geq 3.25 \pm 0.00$ • Virkon (2%) $\geq 3.00 \pm 0.00$ and $\geq 3.00 \pm 0.00$ • Formalin (10%) $\geq 1.25 \pm 0.00$ and $\geq 1.25 \pm 0.00$ • Lysis buffer (EasyMAG) $\geq 2.00 \pm 0.43$ and $\geq 2.25 \pm 0.00$ • AVL (viral lysis buffer, Qiagen) $\geq 3.00 \pm 0.43$ versus $\geq 3.25 \pm 0.00$ • Liquid hand soap (Funchem) $\geq 2.00 \pm 1.56$ versus $\geq 2.25 \pm 0.00$ • Hand wash (Mannings) $\geq 0.83 \pm 0.29$ versus $\geq 0.92 \pm 0.38$ • Hand rub (WHO formulation I) $\geq 2.17 \pm 0.14$ and $\geq 2.25 \pm 0.00$ • Advanced had sanitiser (Purell) $\geq 2.50 \pm 0.0$ versus $\geq 2.50 \pm 0.00$ • Disinfecting solution (Dermo docyn) $\geq 2.30 \pm 0.50$ and $\geq 3.75 \pm 0.43$ • Hand wash (Walch) $\geq 0.83 \pm 0.29$ and $\geq 0.92 \pm 0.14$
Chin <i>et al.</i> ⁹	SARS-CoV-2 culture (7.8 log unit of TCID50/ml) 15 µl droplet pipetted on surfaces with 200 µl virus transport medium and 135 µl of disinfectants.	Household bleach (1:49), household bleach (1:99), pure ethanol (70%), povidone-iodine (7.5%), chloroxylenol (0.05%), chlohexidine (0.05%) and benzalkonium chloride (0.1%) all virus not detected at 5 mins Hand soap (1:49) virus not detected at 15 min
Fischer <i>et al.</i> ¹¹	Vero E6 cells inoculated on samples of N95 masks and stainless steel surfaces.	Median time to one millionth (mins) for N95 mask and stainless steel: <ul style="list-style-type: none"> • Control 1.56×10^3 and 4.66×10^3 min • Ethanol (70%) 6.97 and 17.7 min • Vaporised hydrogen peroxide 10.1 and 8.55 min
Ibanez-Cervantes <i>et al.</i> ¹⁴	SARS-CoV-2 undiluted and diluted 1:10, 1:100, 1:1000, and 1:10000 inoculated on N95 masks.	Hydrogen peroxide plasma after mask placed in sterilised bag results in virus not detected after treatment at all concentrations
Ijaz <i>et al.</i> ¹⁵	SARS-CoV-2 suspension and dried on glass surface with 5% FBS organic load.	Log 10 reduction in infectious SARS-CoV-2 titre: <ul style="list-style-type: none"> • Antiseptic liquid (0.094 w/v para-chloro-meta-xyleneol) for 5 min at 21°C ≥ 4.7 • Hand sanitiser gel (49% w/w ethanol) for 1 min at 21°C ≥ 4.2 • Liquid hand wash (0.025% w/w salicylic acid) for 1 min at 37°C ≥ 3.1 • Bar soap (0.018 w/w para-chloro-meta-xyleneol) for 1 min at 38°C ≥ 3.0 • Surface cleanser (0.077 w/w QAC) for 5 min at 21°C ≥ 4.1 • Disinfectant wipes (0.19% w/w QAC) for 2 min at 21°C ≥ 3.5 • Disinfectant spray (50% w/w ethanol QAC) for 2 min at 21°C ≥ 4.5
Jahromi <i>et al.</i> ¹⁷	SARS-CoV-2 suspension prepared by infecting monolayers of A549 cells with viral titres 10^5 – 10^{10} TCID50/ml. 10 µl inoculum was pipetted into test field and sanitising fluid was sprayed on.	Reduction factor: <ul style="list-style-type: none"> • Water <1 • 70% ethanol/water ~5 • 70% isopropanol/water ~5 • 35% ethanol/35% isopropanol/water ~6 • 35% ethanol/35% isopropanol/3% glycerin/water ~6 • 3% SDBS/70% ethanol/water ~6 • 3% SDBS/70% ethanol/3% glycerine/water ~6 • 3% SLS/70% ethanol/water ~6 • 3% hand soap/70% isopropanol/water ~6 • 3% dish soap/70% ethanol/water ~7 • 3% dish soap/35% ethanol/35% isopropanol/3% glycerin/water ~7 • 3% dish soap/water <1 • 3% hand soap/water <1
Kratzel <i>et al.</i> ¹⁹	Vero E6 cells were infected with SARS-CoV-2 to prepare a viral suspension. Viral suspension mixed with 1:1 organic load and 1:8 disinfectant solution for 30 s.	Log 10 reduction factor: <ul style="list-style-type: none"> • Original WHO formulation I (80% ethanol/1.45% glycerol/0.125% hydrogen peroxide) ≥ 3.8 • Modified WHO formulation I (80% ethanol, 0.725% glycerol/0.125% hydrogen peroxide) ≥ 5.9 • Original WHO formulation II (75% 2-propanolol 1.45% glycerol, 0.125% hydrogen peroxide) ≥ 3.8 • Modified WHO formulation II (75% 2-propanolol/0.725% glycerol/0.125% hydrogen peroxide) ≥ 5.9 • $\geq 30\%$ ethanol ≥ 5.9 • $\geq 30\%$ 2-propanolol ≥ 5.9

(Continued)

Table 3. (Continued)

Study ID	Substrate of SARS-CoV-2 tested	Results
Kumar <i>et al.</i> ²⁰	Inoculum of 10 µl of viral suspension containing 6.5 log TCID ₅₀ SARS-CoV-2 was spotted on N95 respirator masks (3M 1860, 3M Aura 1870, 3M Vflex 1804S, AO Safety 1054S).	Standard autoclaving, vaporous hydrogen peroxide, peracetic acid dry fogging system, ethylene oxide gassing and low-temperature hydrogen peroxide gas plasma all resulted in ≥6 log reduction of infectious virus on all treated masks. Autoclaving resulted in functional failure of the 3M 1860/8210 models after 3+ cycles, low-temperature hydrogen peroxide gas plasma all mask failed at 5+ cycles
Liang <i>et al.</i> ²¹	Vero 76 cells infected with SARS-CoV-2. Povidine iodine gels were tested at full strength (90% sample versus 10% virus solution), 1/1.8, 1/3.2 and 1/10 dilution in simulated tears or nasal fluid incubated at 37°C for 30s, 2 min and 10 min.	Log reduction value at 30s, 2 min and 10 min: <ul style="list-style-type: none"> • Control 0 • 90% drug (1.0% Povidine iodine gel (PVP-I)) 3.5, 2.9, 3.3 • 50% drug (1.0% PVP-I) 3.2, 2.9, 3.3 • 28% drug (1.0% PVP-I) 2.2, 2.6, 3.3 • 9% drug (1.0% PVP-I) 1.2, 0.8, 1 • 90% drug (0.6% PVP-I) 3.1, 2.9, 3.3 • 50% drug (0.6% PVP-I) 3.1, 2.9, 3.3 • 28% drug (0.6% PVP-I) 2.9, 2.8, 3.3 • 9% drug (0.6% PVP-I) 2.3, 1.9, 1.6 • Ethanol 3.5, 1.9, 3.3
Mantlo <i>et al.</i> ²²	Vero cells infected with SARS-CoV-2 with concentration of 1 × 10 ⁶ TCID ₅₀ /ml. 10 µl of virus stock missed with 90 µl of Clyra, diluted Clyra or control.	Mean TCID ₅₀ /ml: <ul style="list-style-type: none"> • Clyraguard 1:1 at 30 min mean: <7.5 × 10¹ • Clyraguard 1:10 at 60 min mean: 5.83 × 10⁴ • Clyraguard 1:100 at 60 min mean: 5.00 × 10⁴ • Saline at 60 min mean: 2.25 × 10⁴ • Boiling saline at 10 min mean: <7.5 × 10¹
Mukherjee <i>et al.</i> ²³	Vero-E6 cells infected with SARS-CoV-2 with initial viral load from 5.86–8.19 log TCID ₅₀ .	Log reduction <ul style="list-style-type: none"> • 8% soap bar total fatty matter 67 at 40°C for 20s ≥3.14 • 8% soap bar total fatty matter 68 at 40°C for 20s ≥3.06 • 8% soap bar total fatty matter 72 at 40°C for 20s ≥4.06 • Liquid cleanser 10% surfactant at 20°C for 20s ≥3.10 • Liquid cleanser 12% surfactant at 20°C for 10s ≥3.01 • Liquid cleanser 19% surfactant at 20°C for 10s ≥3.42 • Alcohol-based sanitiser 60.5% alcohol at 20°C for 10s ≥3.25 • Alcohol-based sanitiser 65% alcohol at 20°C for 10s ≥4.01 • Alcohol-based sanitiser 95% alcohol at 20°C for 15s ≥4.01
Smith <i>et al.</i> ²⁸	Vero E6 cells infected with SARS-CoV-2 inoculated on N95 masks (1860, 1870+, 8511).	Infectivity (ΔRNA in Vero E6 cells from input log 10) <ul style="list-style-type: none"> • Ethanol 1860 undetectable • Ethanol 1870+ undetectable • Ethanol 8511 undetectable • Vaporised hydrogen peroxide 1860 log reduction of -2 • Vaporised hydrogen peroxide 1870+ log reduction of -2 • Vaporised hydrogen peroxide 8511 log reduction of 3 • Ethanol altered mask integrity
Takeda <i>et al.</i> ²⁹	Vero E6 cells infected with SARS-CoV-2 then cultured in growth medium with viral titre 5.75–7.25 log ₁₀ TCID ₅₀ /ml mixed with solutions for 1 min at 25°C.	Acidic electrolysed water with free available chlorine 74 ppm for 1 min viral titres 9:1 ≥99.99% inactivation and decrease of ≥4.25 log ₁₀ TCID ₅₀ /ml inactivation lost with 5:1 (decrease ~2 log ₁₀ TCID ₅₀ /ml)
Welch <i>et al.</i> ³¹	3D printed materials in disks had virus (100 µl) added and subjected to chemical treatment.	Virus infectivity reduction (log 10) by treatment: <ul style="list-style-type: none"> • 10% bleach >5.5 • 70% isopropanol 1.4 • Ammonium quarternary >5.5 • H₂O₂ wipe 5.5

TCID, tissue culture infective dose; RNA, ribonucleic acid; PVP-I, Povidine iodine gel.

and found that humidity has a profound effect on heat inactivation of dried SARS-CoV-2. In particular, meltblown fabric, a key component of N95-grade filtering facepiece respirators, could be heated to 75–85°C to inactivate the virus in 20–30 min under 100% relative humidity without lowering filtration efficiency.

Light irradiation

The results from studies evaluating viral inactivation through ultraviolet light (UV) are shown in Table 2. UV light was used at different wavelengths including 222 nm, 254 nm, 260–285 nm and 365 nm. On N95 masks, Fischer *et al.*¹¹ found that UV light was much slower at reducing virus

levels compared with virus on stainless steel (128 min *versus* 9.79 min). For viral suspension, a reduction of 2–3 log in virus could be achieved with exposure to 222 nm for 30 s and 280 nm light at 10 s.^{16,18,25} On N95 masks, SARS-CoV-2 viral load could be reduced by 2–3 log units by irradiation with 254 nm ultraviolet light.²⁸ Simmons *et al.*²⁷ found that virus deposited on chamber slide and N95 respirators could be reduced by pulsed-xenon UV light with more than a 4 log reduction at 5 min of exposure. Helingloh *et al.*¹³ reported that virus in well plates could be inactivated with UVC (254 nm) and UVA (365 nm) light (inactivation in 9 min and 50% reduction in 1.4 min). Ratnesar-Shumate *et al.*²⁶ considered simulated sunlight on SARS-CoV-2 and found that 90% of infectious virus was inactivated every 6.8 min in simulated saliva and every 14.3 min in culture media when exposed to simulated sunlight.

Chemical agents

The impact of a variety of chemical agents on the inactivation of SARS-CoV-2 is shown in Table 3. Studies have shown that SARS-CoV-2 could be inactivated with viral lysis buffer, guanidium thiocyanate/t-octylphenoxypolyethoxyethanol, formaldehyde, 70% ethanol, vaporised hydrogen peroxide, hydrogen peroxide plasma, standard autoclaving, peracetic acid dry fogging system, ethylene oxide gassing, 10% bleach, 2% Virkon, lysis buffer, AVL, WHO formulation I hand rub, advanced hand sanitizer and disinfecting solution, 7.5% povidone-iodine, 0.05% chlorhexidine, 0.1% benzalkonium chloride treatment, povidone-iodine gel, Clyraguard copper iodine complex, 9:1 ratio of acidic electrolysed water of pH 2.5 with free available chlorine 74 ppm, original WHO formulation, modified WHO formulation, original WHO formulation II, modified WHO formulation II, 30% ethanol and 30% 2-propanolol.^{5,8,9,11,14,19–22,28,29,31} However, autoclaving and low-temperature hydrogen peroxide gas plasma results in failure of masks after three and five or more cycles, respectively.²⁰ In the study, by Chan *et al.*⁸ of multiple agents, the least effective agents (defined as less than a 10-fold reduction in the viral load at 5 min of exposure) were Mannings hand wash and Walch hand wash. Interestingly, 1:49 hand soap required 15 min before virus was not detected.⁹ Ijaz *et al.*¹⁵ studied SARS-CoV-2 in suspension and dried on glass and found that antiseptic liquid (0.094 w/v para-chloro-meta-xyleneol) for 5 min at 21°C, hand

sanitiser gel (49% w/w ethanol) for 1 min at 21°C, liquid hand wash (0.025% w/w salicylic acid) for 1 min at 37°C, bar soap (0.018 w/v para-chloro-meta-xyleneol) for 1 min at 38°C, surface cleanser (0.077 w/w QAC) for 5 min at 21°C, disinfectant wipes (0.19% w/w QAC) for 2 min at 21°C and disinfectant spray (50% w/w ethanol QAC) for 2 min at 21°C all resulted in more than 3.1 log reduction in the viral load. Jahromi *et al.*¹⁷ evaluated different solutions of ethanol, isopropanol, SDBS, glycerine and soap and found that the most effective agents in reducing SARS-CoV-2 were combinations of dish soap/ethanol/isopropanol/water with or without glycerine, which resulted in a ~7-fold in reduction factor in SARS-CoV-2 suspension. The least effective were 3% dish soap or hand soap and water (1-fold in reduction factor). Mukherjee *et al.*²³ studied the impact of various concentrations of soap, liquid cleanser and alcohol-based sanitiser on the reduction of SARS-CoV-2 and found that use of soap with ≥ 67 total fatty matter at 40°C for 20 s could reduce virus by more than 3 log units while similar reduction could be achieved with liquid cleanser of $\geq 10\%$ surfactant at 20°C for 20 s and alcohol-based sanitiser with $\geq 60.5\%$ alcohol at 20°C for ≥ 10 s.

Other notable findings

Chin *et al.*⁹ also reported the time when the virus could not be detected on different surfaces. After 2–3 h the virus was not detected on paper, tissue paper, wood and cloth. However, it could last for 7 days on stainless steel, plastic and both the inner and outer layer of masks, and could be detected up to 4 days on glass or bank notes.

Discussion

Our review has several key findings. First, several methods are effective in inactivating the SARS-CoV-2 virus, with these methods broadly classed as those related to heating, UV light irradiation and chemical agents. Second, all the studies took place in laboratory setting rather than real-life clinical settings, so there is insufficient evidence to recommend one type of cleaning procedure over another. Third, there is clear evidence that the ability of the virus to remain active depends on the environment, as it can last much longer on plastic or steel compared with paper or wood. Finally, the ideal cleaning or decontamination method really depends on what we are trying to

make virus free, and to date there are no studies detailing how to clean rooms or hospital items other than masks. These findings suggest that many of the traditional agents that have been used to destroy viruses are effective for SARS-CoV-2, but whether certain approaches should be supported in clinical settings is not clear.

We have shown that a variety of different methods have been tested for their antiviral activities for SARS-CoV-2. There are broadly several treatment classes including heat, light irradiation and chemical agents. Heat appears to be effective in reducing virus, but in terms of clinical practice it can only be used for objects that can be placed in an autoclave oven or other heating element as heating patient rooms and their contents to high temperatures is impractical. However, heat can potentially damage heat-sensitive materials, especially when heated to high temperatures for prolonged periods. Light irradiation appears to be a more novel approach which seems to be effective in eliminating virus. Light could be delivered from a lamp onto objects or placed in clinical environment to decontaminate light-exposed areas. However, there appears to be significant heterogeneity in the wavelength, power and duration of light exposure in the studies evaluated. There are also risks of damaging items with prolonged exposure to high-energy and high-intensity light and a carcinogenic risk to humans, so it cannot be used on items patients are wearing or as a measure to clean body parts. Reassuringly, many typical household items such as soap, cleansers and alcohol gel have been shown to reduce virus. However, laboratory test conditions are very different from real life, as people are not guaranteed to wash their hands for 20s and there may be areas which are not adequately washed.³²

At the same time, even if hands are clean, faucet taps and door handles may become contaminated with virus. An interesting approach is to use hydrogen peroxide vapour as a method of decontamination, which appears to be effective to clean objects as well as rooms. These methods appear to be the best approach to clean rooms but may be more expensive compared with simpler methods. This also has the advantage in that, unlike liquid chemicals, it may be effective in cleaning airborne or droplet virus in rooms. While we have assessed the literature on decontamination more studies are needed from the clinical practice setting perspective, including what the ideal cleaning

procedures are for different healthcare items and settings.

It is important to consider that disinfection products may be effective in removing virus but they should not cause harm, and these agents are not without risks to people and the environment.³³ Since March 2020, there have been increased calls to poison centres in the United States for exposures to both cleaners and disinfectants such as bleach products, non-alcohol disinfectants and hand sanitisers.³⁴ While most cases involved ingestion and affected children, these increased exposures may be related to using more than recommended on labels, mixing multiple chemical products, not wearing protective equipment and use in poorly ventilated areas.³⁴ Bleach and hand sanitisers can cause irritation of the skin, eyes and respiratory tract,³³ and some sanitisers had to be recalled because of their methanol content which can cause vomiting, headaches, blindness, seizures, coma and death.³⁵ In a descriptive study of 1090 Iranian participants, 87% of participants used the incorrect proportion of water and alcohol to make disinfectants at home and 42% experienced at least one disorder of their hands, feet, eyes, respiratory or gastrointestinal systems after sequential uses of disinfectants.³⁶ Furthermore, there are concerns that antibacterial hand sanitisers may contribute to antibiotic resistance in bacteria and loss of a barrier to protect against coronavirus.³⁷ In the report by Samara *et al.*³³ it was suggested that the ideal disinfectant should have low toxicity profile with short- and long-term exposure, and that there is no safe chemical, so there is a need to enhance consumer awareness and develop disinfectants that are less harmful to humans and the environment.

One of the drivers for doing this review was to try to understand if there was any evidence regarding cleaning rooms where patients had been infected with COVID-19, and how they should be cleaned. This is important, as a room where a patient with COVID-19 had stayed could be a reservoir for infecting staff and future patients if it was not cleaned adequately. We found no studies that evaluated how rooms were cleaned and how they should be cleaned. Similarly, there were no evaluations of how other instruments and objects should be cleaned aside from N95 respirators. While spread of infection by direct contact is expected, preventing indirect transmission is

more challenging as there is no evidence to guide best practice in this area.

Failure to adequately clean areas where patients with COVID-19 are present or have been in contact with can have drastic consequences. This review suggests that many of the traditional methods such as heat, irradiation and chemical agents effectively employed to decontaminate viruses are also effective for SARS-CoV-2. However, it is not good enough simply to know that they are effective; it is important that these methods are sufficiently used to the best degree to eliminate viruses, and that the people responsible for cleaning are trained properly. Our ability to control the spread of the infection is only as strong as the weakest link. It may not be the doctors, nurses, and other healthcare professionals such as paramedics or allied health professionals, but the administrative team, maintenance workers, porters, cleaners, catering team or public visitors who may be this weak link. Similarly, the problem exists for surfaces and objects like stethoscopes, blood pressure cuffs and other monitoring equipment, paper notes, keyboards, ward telephones, curtains, hospital beds, chairs, the floor, and the list goes on. As cleaning is a major control component for outbreaks of infection, lack of vigilance and inadequate cleaning by a member of the staff or public will result in the virus continuing to spread. Worryingly, some of the most important healthcare workers, such as those in cleaner roles, have minimal specific training or guidance on how to minimise the spread of COVID-19. We hope that the research highlighted in this review and future work will encourage more support for these important key workers.

This review has a few limitations. There are a number of different methods of assessing viral fomite density, which means that it may be impossible to draw like-for-like comparisons between studies. While there are many studies in this review, many of the studies were only published on pre-print servers or as letters, and may lack rigorous peer review or comprehensive detail. Although it is understandable that there may be a pressing need for information, the quality is important, so more high-quality studies are needed. In addition, we originally aimed to identify studies of cleaning from actual clinical settings; however, we only found studies from *in vitro* settings. We hope that this review will prompt researchers to focus on this important area that has been neglected for future research.

Furthermore, this review may be limited by publication bias, as studies which show that agents are effective in eradicating the virus are more likely to be published compared with those which fail to eradicate the virus.

Conclusions

In conclusion, methods including heat, UV light irradiation and chemicals can be used to disinfect or inactivate SARS-CoV-2, but there is insufficient evidence to support one measure over others in clinical practice. In particular, there is a lack of studies from clinical settings about cleaning hospital facilities and sterilisation of equipment, so more studies are needed to understand how to reduce the spread of COVID-19.

Author Contributions

CSK conceptualized the study. MD and JT screened the search results and identified the included studies. CSK and MD extracted the data from included studies. CSK wrote the first draft of the manuscript. MN, EAM, NW, TK, GH and CDM critically revised the manuscript for intellectual contents.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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