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Major Article

Hand sanitizers: A review of ingredients, mechanisms of action, modes of delivery, and efficacy against coronaviruses

Andrew P. Golin BSc [#,a], Dexter Choi BHSc [#,a], Aziz Ghahary PhD [b,*]

[a] Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada
[b] BC Professional Firefighters' Burn & Wound Healing Research Laboratory, Department of Surgery, Division of Plastic Surgery, iCORD, Vancouver, British Columbia, Canada

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Background: The emergence of the novel virus, SARS-CoV-2, has posed unprecedented challenges to public health around the world. Currently, strategies to deal with COVID-19 are purely supportive and preventative, aimed at reducing transmission. An effective and simple method for reducing transmission of infections in public or healthcare settings is hand hygiene. Unfortunately, little is known regarding the efficacy of hand sanitizers against SARS-CoV-2.
Methods: In this review, an extensive literature search was performed to succinctly summarize the primary active ingredients and mechanisms of action of hand sanitizers, compare the effectiveness and compliance of gel and foam sanitizers, and predict whether alcohol and non-alcohol hand sanitizers would be effective against SARS-CoV-2.
Results: Most alcohol-based hand sanitizers are effective at inactivating enveloped viruses, including coronaviruses. With what is currently known in the literature, one may not confidently suggest one mode of hand sanitizing delivery over the other. When hand washing with soap and water is unavailable, a sufficient volume of sanitizer is necessary to ensure complete hand coverage, and compliance is critical for appropriate hand hygiene.
Conclusions: By extrapolating effectiveness of hand sanitizers on viruses of similar structure to SARS-CoV-2, this virus should be effectively inactivated with current hand hygiene products, though future research should attempt to determine this directly.

INTRODUCTION

The emergence of novel pathogens, bacterial or viral, has always posed serious challenges to public health around the globe. One of these dangerous pathogens is "severe acute respiratory syndrome coronavirus 2" or SARS-CoV-2, more commonly known for causing coronavirus disease 2019 or COVID-19, which has been declared a global pandemic by the World Health Organization in early 2020. Since its discovery in December 2019 in Wuhan, there have been over three million confirmed cases worldwide by April 2020.[1] With cases increasing exponentially around the world, it has caused significant burden on all aspects of society despite aggressive isolation methods to prevent the spread of the virus. Currently, therapeutic strategies to deal with COVID-19 are only supportive, making prevention aimed at reducing transmission the best method at this time.

One of the many ways implemented to prevent the spread of this virus, as with previous contagious pathogens, is frequent and effective handwashing. In both healthcare and community settings, alcohol-based hand sanitizers have become a popular alternative to the traditional handwashing with soap and water. Alcohol-based hand sanitizers have been utilized as an effective alternative to handwashing to prevent the spread of bacterial and viral infections, making it one of the essential protocols in decreasing healthcare burden.[2,3] A range of hand sanitizers are available with various combinations of ingredients and modes of delivery. Given the popularity of hand sanitizers during this pandemic, it is important to understand which types of hand sanitizers work best against this novel virus. In this

* Address correspondence to Aziz Ghahary, PhD, Faculty of Medicine, University of British Columbia, 4550 ICORD, 818 10th Avenue West, Vancouver, BC, Canada, V5Z 1M9

E-mail address: aghahary@mail.ubc.ca (A. Ghahary).

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[#] These authors contributed equally to this work.

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review, we will discuss the role of various types of alcohol-based hand sanitizers in effective elimination of bacterial and viral pathogens with the focus on the effectiveness against enveloped viruses, such as SARS-CoV-2.

VIRAL VERSUS BACTERIAL STRUCTURE

Viruses are relatively simple structural infectious agents with a minimum of 2 structural components (Fig. 1). First, they contain genetic material, such as DNA or RNA. The genetic materials inside viruses are either single stranded (ssDNA or ssRNA) or double stranded (dsDNA or dsRNA). The strands are also either positively or negatively sensed. Positive sense DNA suggests it is directly translatable into protein if it were RNA. Negative sense RNA, on the other hand, is the complementary strand for messenger RNA. In order to protect and encapsulate the genetic material, all viruses also contain a protein coat, called a capsid. Viruses can then further be divided by the presence or absence of a lipid envelope, which determines whether viruses are "enveloped" or "non-enveloped." Despite being composed of various structural and functional elements that are common to many life forms, such as genetic material and lipid envelopes, viruses must have a host in order to replicate, and hence are not typically described as living entities.

Bacteria are single-celled living organisms that, unlike viruses, do typically survive without a host and thus are viewed as living agents. The genetic material is freely floating DNA, and similarly to viruses, bacteria lack nuclei (Fig. 2). Like viruses, bacteria are diverse in their structure. They typically have an inner cell membrane and an outer cell wall, though exceptions do exist. Peptidoglycan, a component of the outer cell wall, is a polymer consisting of sugars and amino acids. Bacteria contain varying thicknesses of peptidoglycan which partly explains whether bacteria stain purple or pink during the Gram-stain procedure, and thus determines the classification of "gram-positive" or "gram-negative" bacteria (Fig. 3). There are, however, bacteria that lack peptidoglycan and therefore do not stain. These are known as "atypical bacteria."

HAND SANITIZER INGREDIENTS

There are 2 large categories of hand sanitizers: (1) non-alcohol-based hand sanitizers (NABHS) and (2) alcohol-based hand sanitizers (ABHS). The most common primary active ingredient of NABHS, benzalkonium chloride, a quaternary ammonium, is a commonly used disinfectant.[4] Disinfectants with benzalkonium chloride are generally less irritating than those with alcohol, though more recent evidence suggests it may cause contact dermatitis more often than previously thought.[5] Although ABHS are less user-friendly on skin than NABHS, ABHS predominate in health care settings given their low cost and efficacy of reducing infectious transmission.[6] NABHS, however, are less worrisome regarding their flammability and abuse potential.[6]

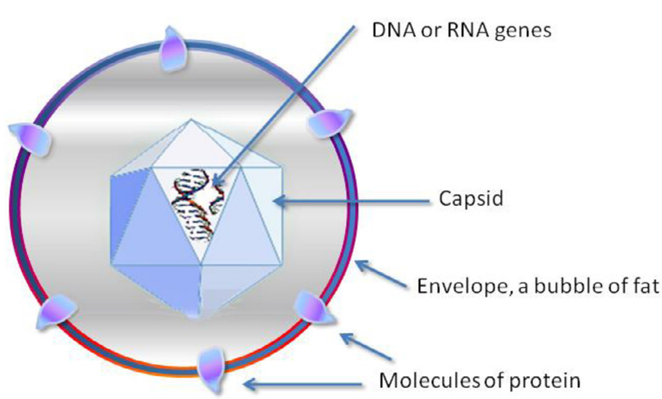

Fig 1. Generic structure of a virus with a lipid envelope. Image by Graham Beards, distributed under a CC BY-SA 3.0 license.

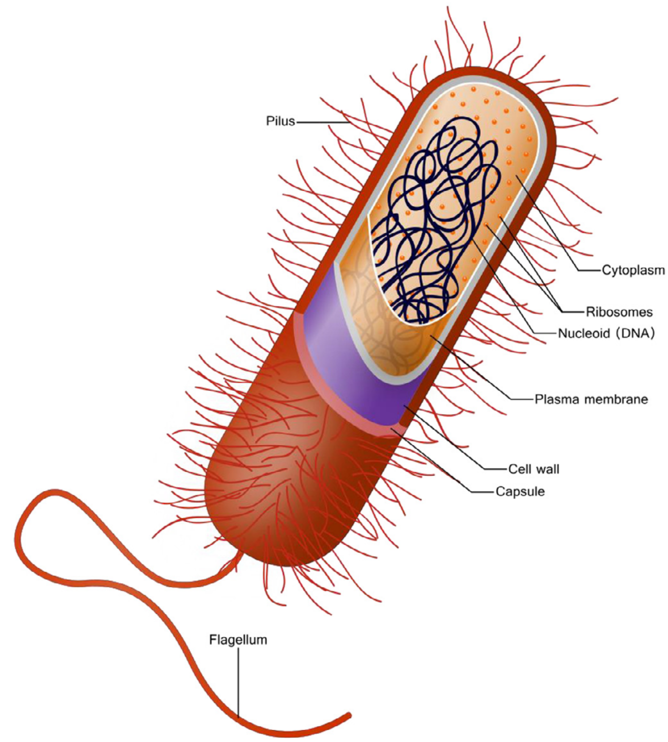

Fig 2. Generic structure of a gram-negative bacterium. Image by Ali Zifan, distributed under a CC-BY-SA 4.0 license.

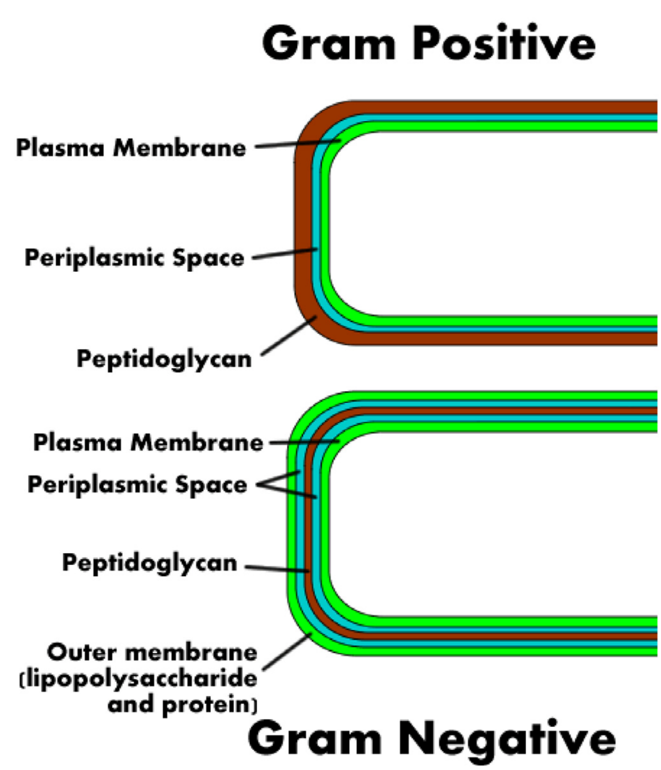

Fig 3. Gram-positive versus gram-negative bacteria. Image by Julian Onions, Wikimedia Commons, Public Domain.

Hand sanitizer preparations containing alcohol on the other hand can include ethanol, isopropyl alcohol, *n*-propanol, or a combination of these,[7] water, as well as excipients and humectants. Solutions containing alcohols between 60% and 95% in volume are most prevalent and effective. Humectants are included to prevent skin dehydration and excipients help stabilize the product as well as prolong the time needed for the evaporation of alcohol, thereby increasing its biocidal activity.[8]

ALCOHOL MECHANISM OF ACTION AGAINST BACTERIA

The compound, *n-propanol,* is the most commonly used alcohol compound in biocides.[7] It is not known with much confidence the exact mechanism of alcohol's antimicrobial activity, however, it may be related membrane damage, and inhibition or uncoupling of mRNA and protein synthesis through effects on ribosomes and RNA polymerase,[9] or associated with protein denaturation.[7] For activity against bacteria, its optimal bactericidal efficacy is achieved at concentrations between 60% and 90%.[10] In fact, absolute alcohol, or alcohol that is no more than one percent water, is less bactericidal than alcohol between the aforementioned range.[10] Water is thus critical in the protein denaturation process. No matter which process, if not multiple, are affected by alcohol, essential metabolic pathways, membrane damage and loss of cellular integrity ultimately occur.[7] It is important to note, however, that alcohols exhibit bactericidal activity against vegetative bacteria—those undergoing metabolism and binary fission—but not against spores.[11]

ALCOHOL MECHANISM OF ACTION AGAINST VIRUSES

The viral targets of alcohol-based hand sanitizers are predominantly the viral envelope, if present, which is derived from host lipid envelopes, the protein capsid, which contains and protects the genetic material, and the genetic material itself.[7] Given that all these components are necessary for the viral life cycle (eg, attachment, penetration, biosynthesis, maturation, lysis), and thus critical for its ability to transmit to another host, it can be presumed that altering the structure or function of any of the aforementioned components will typically render the virus ineffective.

While less is known regarding the specific mechanism of action of alcohols agents against viruses compared to bacteria, it is understood that ethanols have a broader and stronger virucidal activity than propanols. In fact, high concentration of ethanol has shown to be highly effective against enveloped viruses[12] and thus is effective against the majority of clinically relevant viruses.[13] It is also interesting to note that adding acids to ethanol solutions can increase its efficacy against viruses that are more resistant to ethanol alone.[13,14] Despite the potential synergy of ethanol and acidity, it remains known that most hand sanitizers continue to be ineffective against nonenveloped viruses.[15]

BENZALKONIUM CHLORIDE MECHANISM OF ACTION

Similar to alcohol-based hand sanitizers, benzalkonium chloride (BC), the primary ingredient of NABHS, is generally not effective against nonenveloped viruses,[16,17] though a study demonstrating its efficacy against the nonenveloped human coxsackie virus suggest exceptions exist.[18] Despite this exception, it appears that the lipid envelope of either bacteria or viruses are critical structures for BC's effectiveness.

The cationic "headgroup" of BC is progressively adsorbed to the negatively charged phosphate heads of phospholipids in the lipid bilayer, and as a result, increase in concentration.[19] The consistent increase of BC concentration results in reduced fluidity of the membrane and thus the creation of hydrophilic gaps in the membrane.[19] In addition, the alkyl chain "tail" component of BC further perturbs and disrupts the membrane bilayer by permeating the barrier and disrupting its physical and biochemical properties.[19] Protein function is subsequently disturbed and the combination of the aforementioned effects results in the solubilization of the bilayer constituents into BC/phospholipid micelles.[19] BC also interrupts intercellular targets and compromises the conformational behavior of DNA.[20]

EFFICACY OF HAND SANITIZERS

Bacteria and fungi

Traditionally, bacteria on hands can be categorized as resident and transient floras. Common resident floras include *Staphylococcus aureus, Staphylococcus epidermidis*, and *Enterococcus faecalis*, which colonize deep layers of the skin and are resistant to mechanical removal.[21] On the other hand, transient floras such as *S. aureus, Escherichia coli*, and *Pseudomonas aeruginosa*, colonize the superficial layers of skin.[21] There are also numerous bacterial strains that can be transmitted to the host from other sources that can potentially develop into a variety of bacterial infections. ABHS are very effective for quickly destroying many pathogens by the action of the aqueous alcohol solution without the need for water or drying with towels. According to the Centers for Disease Control and Prevention (CDC), ABHS have excellent in vitro antimicrobial activity, including multidrug-resistant pathogens, such as methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus*.[22] Specific in vitro studies show that hand sanitizers containing 60%-80% ethanol produced 4 to 6 log reduction in 15-30 seconds against a range of bacterial and fungal species.[23] Numerous studies have also documented in vivo antimicrobial activity from contaminated hands.[24,25] While different alcohol-based hand sanitizers all demonstrated antimicrobial effects against various gram-positive and gram-negative bacteria using the Kirby-Bauer method, which uses antibiotic-impregnated disks to test the susceptibility of strains, propanol-based sanitizers were more effective compared to ethanol with the greatest zone of inhibition.[4,21]

With increasing use of hand sanitizers as an infectious control measure, it is also important to note any potential tolerance mechanisms from bacteria. An in vitro alcohol tolerance assay using a lower concentration of isopropanol showed that newer isolates of *E. faecium* were more alcohol-tolerant than their predecessors.[26] Other similar studies on other pathogens have also demonstrated increasing tolerance when exposed to lower concentrations of alcohol.[27] Tolerance is not only limited to alcohols, but also exists for BC.[28,29] The presence of any selective pressure in environments encourage microbes to adapt and evolve resistance to such pressures, and in the case of BC, researchers have observed resistant strains that were able to survive certain concentrations of BC (0.1%-0.4%) since the 1960s.[30,31] Given this, tolerance to quaternary ammonium compounds is not a novel observation. As time goes on and the use of both alcohol and BC continue in hand sanitizers and disinfectants, it is inevitable that tolerance will only increase. While future studies are conducted to determine novel mechanisms of tolerance, it is essential to emphasize adherence to hand hygiene protocols that require adequate exposure, volume, and concentrations of hand sanitizers to minimize selective pressures and thus tolerance.

Viruses

Although viruses are more difficult to directly study in vivo compared to bacteria, numerous studies have attempted to validate the effectiveness of hand sanitizers on viruses. The World Health Organization recommends alcohol-based hand sanitizer formulations against bovine viral diarrhea virus, hepatitis C virus, Zika virus, murine norovirus, and coronaviruses as shown with effective inactivation in quantitative suspension tests.[32,33] Other formulations from Sterillium that contain isopropanol as the main ingredient also completely inactivated enveloped enteric and respiratory viruses, such as H1N1 influenza A virus,[34] but failed to inactivate nonenveloped viruses, except rotavirus. A number of in vivo studies have also been conducted where the virus is applied to fingertips and the efficacy of the hand sanitizers in reducing the numbers of viral particles recoverable from hands is determined.[35] Many of these finger pad tests show moderate efficacy against most nonenveloped viral strains, which are known to be more resistant to disinfectants than enveloped viruses.[36,37] It is crucial to keep note of the type of viral strains as high concentrations of ethanol has shown to be highly effective against enveloped viruses[12] and thus is effective against the majority of clinically relevant viruses.[13] That being said, although nonenveloped viruses such as Hepatitis A and enteroviruses require 70%-80% alcohol to be reliably inactivated, Sattar et al suggest that 60% ethanol was sufficient to reduce the titers of rotavirus, adenovirus, and rhinovirus by >3 log10 within a 10-second contact period.[36] Even with nonenveloped viruses, satisfactory activity can be achieved with higher alcohol concentrations and extended contact times.[3,38]

As evidence on the novel SARS-CoV-2 continues to rapidly emerge, data from previous coronaviruses can be extrapolated in the context of the efficacy of hand disinfection given their structural similarity. A systematic review examining the 2002-04 SARS outbreak indicated that 9 out of 10 small case-control studies pointed towards the idea that hand washing decreases the likelihood of nosocomial and community transmission, although only three showed statistical significance, partly explained due to the small sample sizes of the studies.[39] A portion of the studies varied in the specific method of hand washing; some studies used hand sanitizers, while others did not specify whether it was achieved through soap and water or sanitizers. Although direct in vivo confirmation of virus inactivation after hand sanitizer use is infeasible to achieve in a standardized method, in vitro studies have confirmed that alcohol-based hand sanitizers can be effective in decreasing the viral load. Specifically, in vitro studies using sputum cultures of SARS-CoV infected patients with four different alcohol-based hand sanitizer formulations were all able to inactivate the virus below the limit of detection.[40]

Transmissions of SARS-CoV-2 have been described with incubation times of up to 10 days, facilitating its spread via droplets, contaminated hands, or surfaces.[41] As such, it is important to note the efficacy of inactivating viruses on all modes of transmission. Alcohol-based disinfectants have also been shown to effectively inactivate SARS-CoV and MERS-CoV (Middle East respiratory syndrome-related coronavirus) on inanimate surfaces, such as metal, glass, and plastic.[42]

One of the key limitations for analyzing the true efficacy of hand disinfection arises from the method of data collection via retrospective self-reporting, which can lack standardization and objectivity in frequency and method of handwashing.[39] There is also a myriad of confounding variables, especially in hospital settings, such as frequency and extent of contact with infected persons and the use of personal protective equipment. As hand hygiene is one aspect of a multicomponent intervention to reduce infection rates, it is difficult to truly assess the effectiveness of hand sanitizers independently.

HAND SANITIZERS VERSUS SOAP

Numerous hand sanitizers, consisting of different ingredients and methods of application, have been compared. However, the CDC recommends washing hands with soap and water whenever possible over hand sanitizers.[22] The superiority of handwashing stems from various factors, such as elimination of a wider spectrum of pathogens and chemicals, and removal of bioburden on soiled hands. A 2016 systematic review supports the historical skepticism about the use of hand sanitizers in food preparation settings and suggests that hand washing with soap and water is more effective than alternative hand disinfection methods for removal of soil and microorganisms from hands.[43]

Nonetheless, we wanted to specifically compare the efficacy of hand soaps and sanitizers on their efficacy on inactivating enveloped viruses. An in vitro quantitative suspension test comparing 3 different ethanol-based hand sanitizers and 3 different antimicrobial soaps all demonstrated a 4 log10 (>99.99%) reduction in the test enveloped viruses.[44] Although there is limited evidence in direct comparison between soap and sanitizers, numerous studies have confirmed the effectiveness of sanitizers on various enveloped viruses.[13,32] When pertaining to nonenveloped viruses, finger pad testing showed that hand washing with soap and water is sufficient to remove >5 log10 virus particles of human norovirus GI and MNV1, while alcohol-based hand disinfectants were not able to effectively inactivate these same viruses.[34] Alcohol-based products achieve rapid and effective inactivation of various bacteria, but their efficacy is generally lower against nonenveloped viruses. This might even explain the speculation that the use of alcohol-based hand sanitizers might be a risk factor for norovirus outbreaks that were previously described in long-term care facilities.[45,46]

On the contrary, scientific studies have shown that after hand washing, as many as 80% of individuals retain some pathogenic bacteria on their hands.[47] Moreover, hand washing removes the body's own fatty acids from the skin, which may result in cracked skin that ultimately provides a potential entry portal for pathogens.[48] To overcome the limitations of plain hand washing, hand sanitizers were introduced claiming to be effective against those pathogenic microorganisms as well as to improve skin condition due to the addition of emollients.[49]

FOAM VERSUS GEL

Three common modes of delivering the active ingredient in hand sanitizers, whether alcohol or other disinfectants, are foams, gels, and sprays. There is limited research on comparing the efficacy of various sanitizer delivery systems on virucidal efficacy. One study with 30 human volunteers showed >3 log10 reductions of H1N1 viral counts on finger pads with foam, gel, and wipes with no differences in efficacy among delivery types.[50] Similar findings were seen by Grayson et al, comparing ethanol based gel and liquid forms on H1N1.[51] Both of these studies suggest that there could be some varying differences in efficacy due to the method of mechanical friction that could contribute to physical removal of pathogens, but there needs to be further research conducted to compare the efficacy between the various hand sanitizer delivery systems.

Aside from virucidal efficacy, the formulations of hand sanitizers were identified as one factor influencing compliance to hand hygiene, though conflicting results exist. A single pump from foam dispensers provides approximately 1.1 mL of hand sanitizer.[52] Manufacturers suggest different recommended volumes for application, but a recent study compared gel and foam hand sanitizers and found no statistically significant difference of complete hand coverage upon usage of equal volumes.[52] It did, however, conclude that volumes <2 mL resulted in high rates of poor coverage (67%-87%), whereas volumes ≥2 mL generated a lower rate of incomplete coverage (13%-53%). The World Health Organization does not have a specific volume of sanitizer they suggest but recommend to "cover all surfaces of the hand" (https://www.who.int/gpsc/5may/tools/who_guidelines-handhygiene_summary.pdf). Contrary to the previous results,[52] a study comparing foam and gel with equal concentrations of ethanol determined that foam spreads more than gel and that the average log10 reduction of foam was superior against *E. coli* bacteria than gel.[53] To translate these experimental findings to clinical practice, however, the compliance of utilizing adequate amounts of foam sanitizer must be met. A study reviewing the potential compliance of various hand sanitizer varieties found that foams containing 62% ethanol have a long drying time, and given this, health care workers in clinical practice likely use inadequate volumes of the product in order to quicken the drying time, and consequently do not use a sufficient amounts of foam hand sanitizer for complete hand coverage.[54] Indeed, a focus group of nurses strongly rejected high volumes (3 mL) of hand sanitizers in gel, foam, or liquid form as "there is not enough time to apply such large doses whilst working on a busy ward."[55] Moreover, the gel and foam formats were considered more desirable than the liquid due to some key desirable properties, including fast absorption, soft/moisturized hand feel, clean nonsticky feel, and low smell.[55] Another study compared the tolerability of gel and liquid forms with varying ingredients (ethanol or propanol) among dental students and found that all types were tolerated.[56] Multiple studies suggest that there are other factors, such as sanitizer ingredients, addition of skin moisturizer, and accessibility in the workplace, that affect compliance and ultimately make it difficult to directly compare the different types of hand sanitizers. Further studies need to be conducted to determine the long-term compliance of the various sanitizer forms, which are more useful in settings where consistent hand hygiene is crucial.

CONCLUSIONS

With the current research in the literature, it is difficult to confidently suggest one mode of hand sanitizing delivery over the other. What we can state, however, is that soap and water is superior to sanitizer, and when hand washing is unavailable or inconvenient, a sufficient volume of sanitizer is important to ensure complete hand coverage, and compliance is critical for appropriate hand hygiene. And finally, with extrapolating the virucidal data on viruses of similar structure to SARS-CoV-2, this virus can be effectively inactivated with current hand hygiene products, though future research should attempt to determine this directly.

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