



# Inactivation of SARS-CoV-2 by 2 commercially available Benzalkonium chloride-based hand sanitizers in comparison with an 80% ethanol-based hand sanitizer

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## SUMMARY

**Background:** The CDC and WHO recommend alcohol-based hand sanitizers to inactivate severe acute respiratory syndrome coronavirus-2 [SARS-CoV-2].

**Aim:** Benzalkonium chloride [BAK] is another hand sanitizer active ingredient that could be used in response to the global pandemic. Deployment of BAK-based hand sanitizers could reduce shortages of alcohol products and increase hand hygiene options where there are social, physical, and toxicological constraints on alcohol use.

**Methods:** Two commercially available BAK-based hand sanitizers, a concentrate diluted on-site with water and a ready-to-use product, were tested for activity against SARS-CoV-2 in the European Norm Virucidal Activity Suspension Test [EN14476]. A WHO and CDC-recommended 80% alcohol-based hand sanitizer formulation was tested in parallel.

**Findings:** Both BAK formulations demonstrated a  $\geq 4.0 \log_{10}$  reduction of SARS-CoV-2 in 30 seconds, meeting the EN14476 performance standard for virucidal activity against SARS-CoV-2 and matching the in vitro effectiveness of the ethanol-based sanitizer.

**Conclusion:** These findings indicate that a commercial BAK hand hygiene formulation may be another effective means of inactivating the SARS-CoV-2 virus and could be considered as option for pandemic response.

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## Introduction

At the time of this writing the novel coronavirus SARS-CoV-2 had resulted in 133,389,722 COVID-19 infections and 2,892,713 deaths worldwide [1]. Availability of vaccines is expected to reduce future impact, but global viral prevention measures remain urgent. The Center for Disease Control and Prevention [CDC] and the World Health Organization [WHO] recommend

the use of alcohol-based hand sanitizers for personal hygiene and infection control during the COVID-19 pandemic [2,3]. More specifically, the CDC [2] recommends use of alcohol-based hand rubs [AHBR] at a concentration of 60%–95% alcohol, or greater than 70% isopropanol and notes a preference for ABHR over soap and water “where hands are not visibly soiled” ... “in most clinical situations due to evidence of better compliance compared to soap and water. Hand rubs are generally less irritating to hands and are effective in the absence of a sink.” Furthermore, in 2020 the FDA issued guidance to make hand sanitizers (hand rubs) more readily available to Americans by instructing industry on how to temporarily produce alcohol

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for use in hand sanitizers and how to produce hand sanitizer containing 80% alcohol recommended by the WHO for the pandemic response [4].

Unprecedented global demand for alcohol-based hand sanitizers during the pandemic caused dramatic shortages, hoarding and price gouging. In addition, products containing unsafe contaminants (methanol or 1-propanol) appeared in the marketplace [5,6]. Hoarding and shortages impacted availability of these products for both consumer and professional healthcare use [7] and the list of commercial alcohol-based hand sanitizers posing a risk of serious adverse events from misuse/ingestion substantially increased [5,6].

In addition, alcohol-based hand sanitizers are considered undesirable in certain situations where these products may violate religious beliefs, where the potential for abuse is high and where facilities are not able to accommodate increased flammability risk [8]. Ingestion of alcohol-based hand sanitizers can cause alcohol poisoning [9]. From 2011 – 2015, U.S. poison control centers received nearly 85,000 calls about alcohol hand sanitizer exposures among children [9,10]. In addition, older children and adults might purposefully swallow hand sanitizers to become intoxicated [9,11–13]. In March 2020 (during the COVID-19 pandemic), calls to Poison Control related to hand sanitizer increased by 79% compared to March of 2019, with the majority of these calls for unintentional exposures in children 5 years of age and younger [14]. The CDC [2] acknowledges the issue of ABHR shortages and recognises the eligibility of benzalkonium chloride (BAK) and other alternative active ingredients for use in the formulation of hand sanitizers for healthcare personnel but the CDC does not recommend BAK or any other alternatives to alcohol. A review published early in the pandemic by Kampf *et al.* [15] raised a concern about the reliability of BAK against certain viruses. More recently, reports by Chin *et al.* [16], Ogilvie *et al.* [17], and Ijaz *et al.* [18] add to the weight of evidence supporting the effectiveness of BAK in inactivating SARS-CoV-2. A review of the effectiveness of BAK against viruses by Schrank *et al.* [19] urges further research and re-evaluation of BAK effectiveness against SARS-CoV-2.

The current study was undertaken to determine the effectiveness of two commercially available BAK containing hand sanitizing products, against SARS-CoV-2 virus, utilising the method used to show in vitro effectiveness of the WHO's 80% ethanol hand sanitizer for pandemic response [20–22].

## Methods

Leave on hand sanitizer solutions including Hand Sanitizer Product A (Ecolab Foaming Hand Sanitizer, Ecolab, St. Paul, USA), Hand Sanitizer Product B (Ecolab Concentrated Hand Sanitizer, Ecolab, St Paul, USA) and Hand Sanitizer Product C (ethanol hand sanitizer WHO formulation) [23] were used throughout the study. Hand Sanitizer Products A [0.1% BAK] and C [80% ethanol] are ready to use formulations. The CAS identity for the BAK in both Product A and Product B is 68424–85-1. Product C ethanol CAS identity is 64–17-5. Hand Sanitizer Product B is sold commercially as a 1:10 concentrate. This concentrate is diluted on site through an automated dilution system at a ratio of 1:10 using water available on site, generally not exceeding 10 grain per gallon (gpg) hardness, producing a ready to use solution of hand sanitizer containing 0.089% BAK. For the current study a 1:10 dilution in 10 grain per gallon AOAC

synthetic hard water was made. SARS-CoV-2 (Strain USA-WA1/2020) was obtained from BEI resources NR-52281.

All three products were tested for virucidal activity against SARS-CoV-2 using the quantitative suspension test protocol described under the European Norm EN14476:2013-A2:2019 (EN14476) [22]. Virus, suspended in minimal essential medium (MEM) with 2% newborn calf serum (NCS) and 0.3 g/L bovine serum albumin was exposed to Hand Sanitizer Product A, B or C for 30 seconds at 20–21°C. Following exposure, a 1 ml aliquot of the mixture was removed and neutralised with 1 ml of MEM + 10% NCS with 0.5% lecithin and 0.5% polysorbate 80 (Neutraliser), and serially diluted for enumeration of surviving virus. A sample [0.05 mL] of each dilution was transferred to microtiter plates containing Vero E6 cells. Plates were incubated at 36°C (+/- 2) with 5.0% (+/-3%) carbon dioxide. Characteristic cytopathic effect (CPE) as well as product specific cytotoxic effect (CTE) controls were assessed for each dilution following incubation between 6 and 8 days to enumerate viral survivors. Each BAK containing product (Products A or B) was paired with a sample of ethanol containing product (Product C) and each pair of samples was tested on three independent test dates, for a total of 12 independent samples. Experiments including products A and C are collectively termed Test 1. Experiments including products B and C are collectively termed Test 2 in results section below. The 50% tissue culture infective dose per ml (TCID<sub>50</sub>/ml) was calculated using the Spearman-Kärber method and Poisson distribution and converted to log<sub>10</sub> TCID<sub>50</sub>.

## Results

For Test 1 (Table I) inoculum titers of SARS-CoV-2 ranged from 6.98 to 7.23 log<sub>10</sub>TCID<sub>50</sub>. Virus recovery was ≤2.87 log<sub>10</sub>TCID<sub>50</sub> for both Hand Sanitizer Products A and C with a final average log<sub>10</sub> reduction in 30 seconds of ≥4.23 for both Products (Table I). In Test 2 (Table II) inoculum titers of SARS-CoV-2 ranged from 6.00 to 6.50 log<sub>10</sub>TCID<sub>50</sub>. Virus recovery was ≤1.50 log<sub>10</sub>TCID<sub>50</sub> for both Hand Sanitizer Products B and C with a final average log<sub>10</sub> reduction in 30 seconds of ≥4.67 for both products (Table II). These results indicate the same performance between both BAK based Products and the 80% ethanol hand sanitizer control.

## Discussion and conclusions

Intensive focus on hand hygiene measures during the course of the COVID-19 pandemic has led to increased interest in the effectiveness of BAK against SARS-CoV-2, with important new data developed by Chin *et al.* [16] and Ogilvie *et al.* [17] and calls for additional review by Schrank *et al.* [19]. A review by Kampf *et al.* [15] describes what they consider the inconsistency of BAK against enveloped viruses by including findings from Wood *et al.* [24] demonstrating <1 log inactivation of Human Coronavirus using 0.2% BAK for 10 min compared to a study by Saknimit *et al.* [25] that reports complete inactivation (>3.7 log) of Canine Coronavirus virus and Mouse Hepatitis virus using BAK at 0.05% in 10 minutes. The review by Schrank *et al.* [19] notes that data cited by Kampf *et al.* [15] on BAK is not current and that the Wood *et al.* study [24] was possibly an outlier. The authors go on to examine a larger set of studies, paying particular attention to the variation in methodologies of the researchers. Schrank *et al.* [19] urge both a reevaluation of

**Table I**

Inactivation of SARS-CoV-1 by hand sanitizers containing BAK or alcohol in EN14476 protocol testing [Test 1]

Test 1 <sup>a</sup>	Active Ingredient Concentration	Test Day <sup>b</sup>	Initial inoculum [Log <sub>10</sub> TCID <sub>50</sub> <sup>c</sup> ]	Virus recovery/ [30 second] [Log <sub>10</sub> TCID <sub>50</sub> ]	Virus Log Reduction <sup>d</sup> [Log <sub>10</sub> TCID <sub>50</sub> ]	Average Log <sub>10</sub> Reduction <sup>d</sup>
Hand Sanitizer Product A	0.1% Benzalkonium Chloride	1	6.98	≤2.87	≥4.11	≥4.23
		2	7.10	≤2.87	≥4.23	
		3	7.23	≤2.87	≥4.36	
Hand Sanitizer Product C	80% Ethyl Alcohol	1	6.98	≤2.87	≥4.11	≥4.23
		2	7.10	≤2.87	≥4.23	
		3	7.23	≤2.87	≥4.36	

<sup>a</sup> Test 1 executed under GLP by Microbac labs, VA.<sup>b</sup> The test was run in full, on three separate test days.<sup>c</sup> TCID<sub>50</sub> – Median Tissue Culture Infectious Dose.<sup>d</sup> ≥ Inactivation beyond the limit of detection.**Table II**

Inactivation of SARS-CoV-1 by hand sanitizers containing BAK or alcohol in EN14476 protocol testing [Test 2]

Test 2 <sup>a</sup>	Active Ingredient Concentration	Test Day <sup>c</sup>	Initial inoculum [Log <sub>10</sub> TCID <sub>50</sub> <sup>d</sup> ]	Virus recovery/ [30 second] [Log <sub>10</sub> TCID <sub>50</sub> ]	Virus Log Reduction <sup>e</sup> [Log <sub>10</sub> TCID <sub>50</sub> ]	Average Log <sub>10</sub> Reduction <sup>e</sup>
Hand Sanitizer Product B <sup>b</sup>	0.089% Benzalkonium Chloride	1	6.00	≤1.50	≥4.50	≥4.67
		2	6.50	≤1.50	≥5.00	
		3	6.00	≤1.50	≥4.50	
Hand Sanitizer Product C	80% Ethyl Alcohol	1	6.00	≤1.50	≥4.50	≥4.67
		2	6.50	≤1.50	≥5.00	
		3	6.00	≤1.50	≥4.50	

<sup>a</sup> Test 2 executed under GLP by Analytical Lab Group, MN.<sup>b</sup> Diluted 10:1 from concentrate 0.89% BAK to = 0.089% active BAK in test.<sup>c</sup> The test was run in full, on three separate test days.<sup>d</sup> TCID<sub>50</sub> – Median Tissue Culture Infectious Dose.<sup>e</sup> > Inactivation beyond the limit of detection.

BAK for use against SARS-CoV-2 by CDC and a continuation of research in this area.

The inconsistency in methodologies throughout the literature led the authors of this study to choose the EN14476 method entitled Quantitative suspension test for the evaluation of virucidal activity in the medical area, for this work. The EN14476 method is used in the European Union to demonstrate in vitro activity and support registration of hand sanitizers for virucidal activity and has recently been used to evaluate alcohol-based hand sanitizers for the COVID-19 pandemic in studies referenced by the CDC [2,20,21] and in other recent evaluations [25–27]. While neither an in vitro nor in vivo effectiveness standard is established for SARS-CoV-2, the EN14476 virucidal activity testing standard stipulates a product must demonstrate a ≥4.0 log<sub>10</sub> reduction against test virus and that hand sanitizers must achieve this reduction in a time-frame between 30 seconds and two minutes [22]. Both BAK-containing solutions (0.089% and 0.1%) achieved ≥4.0 Log<sub>10</sub> reduction in 30 seconds, meeting the EN14476 performance standard for virucidal activity against SARS-CoV-2 and matching the in vitro effectiveness of the 80% WHO ethanol-based sanitizer also identified by the FDA pandemic response. Our work builds on the earlier work by Chin *et al.* [16] and Ogilvie *et al.* [17] who demonstrated complete inactivation of SARS-CoV-2 using 0.1% BAK at 5 min and complete inactivation (>2.9 log) of 0.13% BAK (hand sanitizing wipe extract) within 15 sec, respectively. Ijaz *et al.* [18] also found

that a 0.19% alkyl dimethyl benzyl ammonium chloride solution was effective against SARS-CoV-2 on a glass surface after a two minute contact time.

We believe that this new data is a valuable piece of evidence further supporting in vitro effectiveness of BAK hand sanitizers against SARS-CoV-2 but there are limitations that could be explored in future work. For example, all of the data herein is built from the same EN standard, virus and internal ethanol control substance, but each pair of BAK and ethanol products were tested at separate independent laboratories which provided an additional variable between tests. Future work would include testing of all products at a single laboratory or all products at multiple laboratories to allow increased robustness of data. Each set of products was also tested in triplicate over three separate days (one sample and one control per day), in contrast they could have been tested multiple times on a single day. Future investigations of additional products or formats could include replication and side by side testing. But beyond this work, much additional opportunity to understand the utility of BAK as an alternative to ethanol remain.

We propose the potential exploration of additional viral pathogens and considerations of the impact on user dermal microbiome following use of ethanol or BAK based hand sanitizer products in the future. We also believe in vivo effectiveness remains an area of strong need but it contains many challenges, namely, safety and ethical constraints of working

with pathogenic human viruses and human subjects though future surrogate models might open the opportunity for further study.

We applaud the CDC's timely and necessary recommendations for the use of ethanol hand sanitizers as an intervention in the SARS-CoV-2 pandemic. We further applaud the FDA's provision of interim guidelines for the production of WHO's 80% ethanol based formulation to help meet the unprecedented demand for hand sanitizer during this pandemic. However, considering existing and recent research, including the current work, non-alcohol hand sanitizers, in particular BAK-containing products, should also be considered as suitable alternatives for this and potential future pandemic responses [16–19].

## Credit author statement

**Brandon L. Herdt:** Conceptualization, Methodology, Resources, Writing – original draft preparation, Writing - review and editing, Supervision, Project administration, Funding acquisition, **Elaine P. Black:** Conceptualization, Methodology, Writing - review and editing, **Sifang S. Zhou:** Investigation, Validation, Formal Analysis, **Cameron J. Wilde:** Investigation, Validation, Formal analysis.

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## Conflict of interest statement

Ecolab Inc. is a manufacturer and seller of the commercial sanitizing hand sanitizers based on benzalkonium chloride [BAK] included in this manuscript. Ecolab Inc. provided financial compensation to Microbac Laboratories, Inc. and Analytical Lab Group to conduct this research and to Ramboll for providing technical and editorial assistance.

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