Running title: Efficacy of hand sanitizers in food preparation setting

Efficacy of instant hand sanitizers against foodborne pathogens compared to hand washing with soap and water in food preparation settings: a systematic review

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Key words: Handwashing, hand sanitizers, foodborne pathogens, food settings, soiled hands

ABSTRACT

Hands can be a vector for transmitting pathogenic microorganisms to foodstuffs and drinks, and to the mouths of susceptible hosts. Hand washing is the primary barrier to prevent transmission of enteric pathogens via cross-contamination from infected persons. Conventional hand washing involves the use of water, soap and friction to remove dirt and microorganisms. Over recent years there has been an increasing availability of hand sanitizing products for use when water and soap are unavailable. The aim of this systematic review was to collate scientific information on the efficacy of hand sanitizers compared to hand washing with soap and water for the removal of foodborne pathogens from the hands of food handlers. An extensive literature search was carried out using three electronic databases - Web of Science, Scopus and PubMed. Twenty-eight scientific publications were ultimately included in the systematic review. Analysis of the literature showed various limitations in the scientific information due to the absence of a standardized protocol to evaluate efficacy of hand products, and variation in experimental conditions applied in different studies. However, despite the existence of conflicting results, scientific evidence seems to support the historical scepticism about the use of water-less hand sanitizers in food preparation settings. Water and soap appear to achieve greater removal of soil and microorganisms than water-less products from hands. Alcohol-based products achieve rapid and effective inactivation of various bacteria, but their efficacy is generally lower against non-enveloped viruses. The presence of food debris significantly affects inactivation rates of hand sanitizers.

Foodborne disease via consumption of contaminated food and beverages is considered one of the most common causes of human disease all around the world (*45*). Norovirus, non-typhoidal *Salmonella* spp. *Listeria* monocytogenes, *Clostridium perfringens, Campylobacter* spp. and *Toxoplasma gondii* are the foodborne pathogens most commonly reported in the USA, causing 9.4 million episodes of foodborne illness, 55,961 hospitalizations and 1,351 deaths (*53*). In the UK, the Food Standards Agency estimates there are more than 500,000 food poisoning cases each year, caused by *Campylobacter* spp. which is responsible for about 280,000 cases each year, followed by *Clostridium perfringens* with about 80,000 cases, Norovirus with about 74,000 cases and *Salmonella* which is responsible for the highest number of hospitalizations, about 2,500 each year (*27*). More than 320,000 cases of foodborne zoonotic disease are annually reported in the European Union. The most common microorganisms causing foodborne diseases in this region are *Campylobacter* spp., *Salmonella* spp. and viruses such as hepatitis A virus and norovirus (*17*). Among 31 different microorganisms causing foodborne diseases, five foodborne pathogens, known as the "Top 5", have been identified by food safety experts as highly infective agents that can easily be transmitted by infected food handlers and cause severe illness. The top five foodborne pathogens include: Norovirus, *Salmonella* Typhi (typhoid-like fever), *Escherichia coli* O157:H7 or other Enterohaemorrhagic and Shiga toxin-producing *E. coli*, *Shigella* spp., and Hepatitis A virus (*25*). Greig et al. (2007) reviewed a total of 816 reports of foodborne outbreaks from United States, Canada, Europe, Australia and identified 14 agents responsible for most of outbreaks where food workers were implicated. The 14 main agents were Norovirus (or probable Norovirus), *Salmonella enterica*, Hepatitis A virus, *Staphylococcus aureus*, *Shigella* spp., *Streptococcus* Lancefield A and G and parasites like *Cyclospora*, *Giardia* and *Cryptosporidium* (*30*).

The origins of pathogenic microorganisms in food include the food itself or its source, such as the growing, harvesting or processing environment, as well as cross-contamination and infected food handlers. In industrialized countries infected food handlers have been identified as an important cause of foodborne illness (*4, 31, 33*). Estimates suggest that up to one third of outbreaks in Ireland (*4*) and 12% of outbreaks in the United Kingdom (19) are caused by infected employees. Another study of foodborne illness outbreaks in restaurants in the United States identified food handling by infected workers as the main factor contributing to around two-thirds (65%) of foodborne illness outbreaks (*33*). Food service facilities including restaurants and catered events are the settings where most food worker associated-outbreaks occur (*56*), and contact with bare hands and failure to properly wash hands were the most frequently reported factors contributing to outbreaks (*57*). In light of this, good personal hygiene and safe food handling practices are essential for preventing foodborne illness.

Hand washing for hand hygiene is the most important practice to prevent the spread of pathogens (*6*). Hand washing with water and soap is generally considered to be the gold standard method to remove dirt and transient microorganisms from hands. Plain soaps have minimal or no antimicrobial activity against bacteria and viruses, but by surfactant action, friction and final rinsing under water can effectively remove dirt, soil and microbial load from the outer layer of hand skin (*39, 60*). Over the past two decades, increasing interest has been placed on the use of hand cleansing products possessing antimicrobial activity, like antimicrobial soaps, or instant hand sanitizers including both alcohol-based and alcohol-free preparations.

Antimicrobial soaps are preparations containing both a detergent and antiseptics or disinfectants with antibacterial activity, such as Triclosan, Chlorhexidine gluconate (CHG) or Para-chloro-meta-xylenol (PCMX). Antimicrobial soaps are considered to be effective against Gram positive microorganisms, to have moderate activity against viruses and tubercle bacilli, but to be less effective against Gram negative microorganisms (*34, 39*).

Alcohol-based hand sanitizers, or alcohol-based hand rubs (ABHRs), are instant hand hygiene products; the antimicrobial activity of which is due to the ability of alcohol to denature protein. These products usually contain a quantity of alcohol, varying from 60% to 95%, and a thickening agent or humectants such as polyacrylic acid, glycerin, or propylene glycol to decrease the drying effect of alcohol. ABHRs have documented microbiological activity against bacteria (*21, 51*), fungi and some enveloped viruses including HIV, herpes, adenovirus, influenza and parainfluenza viruses (*20*). Lower efficacy against non-enveloped "naked" viruses is generally reported in the literature, and the level of inactivation seems to vary a lot depending on the viruses tested, type of alcohol, concentration, and time of exposure (*12, 20, 21, 29, 32, 49, 50, 52*).

Finally, another group of instant hand products known as alcohol-free hand sanitizers, such as povidone-iodine-, triclosan- or quaternary ammonium-based compounds, has also attracted growing interest over recent years. Despite being historically recognised as less effective than ABHRs, more recent formulations prepared with benzalkonium chloride (BZK) have demonstrated many advantages over ABHRs including residual antimicrobial activity after use, less drying effect on hand skin, and lack of decrease in efficacy after repeated use (*13*).

Use of water-less hand sanitizers as an alternative to conventional hand washing has long been debated. Despite some potential advantages over conventional water and soap (quicker and easier usage), instant hand products are generally considered to more effectively meet needs in hospital and health-care, rather than food preparation, settings. ABHRs containing 60% to 95% alcohol are recommended as an alternative to hand washing in hospital and health-care settings when hands are not visibly soiled (*5*). In contrast, their use in food establishments has historically been refused because of their inability to remove fat and food debris from soiled hands (*23*). To date, little research has been conducted to examine the efficacy of hand disinfectants against transient microorganisms normally occurring on food workers' hands during food preparation. This systematic review was carried out to examine the performance of different hand hygiene products against foodborne pathogens in food preparation settings.

MATERIALS AND METHODS

An extensive literature review was conducted in November 2014 using the electronic databases Web of Science, Scopus and PubMed. The search was limited to articles published in English from 1990 to 2014. Search terms used were: "efficacy of hand washing", "efficacy of hand sanitizers", "evaluation of hand sanitisers", and "effect of hand hygiene products".

Three preliminary criteria were adopted to select journal papers. Only articles that described levels of inactivation of foodborne pathogens (the actual pathogens not surrogate microorganisms), used a research approach with quantitative outcomes, and described studies undertaken in industrialized countries, were included in this study. In contrast, all book chapters, studies carried out on microorganisms not involved in foodborne illness, studies involving inactivation of foodborne microorganisms from raw food or food contact surfaces were excluded before analysis, based on the title and abstract.

Once preliminary results matching search terms were obtained, data extraction was carried out in three steps. Firstly, duplicate articles were identified and removed. Secondly, remaining titles and abstracts were screened for eligibility against inclusion criteria. Thirdly, full text articles were retrieved and assessed in terms of their study design and scientific approach. All articles identified were then critically reviewed by the authors and included as appropriate to provide an overview of the topic.

RESULTS

From amongst 2108 records originally matching the search terms, 38 unique journal abstracts were preliminarily screened for eligibility after duplicates were removed. Subsequent analysis of full text journal articles permitted selection of the 28 journal articles that are included in this review (Table 1). Among the selected studies testing hand washing products against foodborne pathogens, ten papers provided information on Norovirus, three on Hepatitis A virus, two on *Listeria monocytogenes*, fourteen on *Escherichia coli*, eight on *Staphylococcus aureus* and one on *Salmonella* spp. No scientific information was found for other pathogenic bacteria like *Campylobacter* spp. and *Bacillus cereus*.

Besides the use of conventional water and soap or water only, products more generally tested against pathogenic bacteria and viruses included antibacterial liquid soaps, alcohol-based hand sanitizers and non-alcohol based sanitizers including triclosan-, chlorexidine gluconate- (CHG), povidone-iodine- and quaternary ammonium-based products like benzalkonium chloride (BZK) or benzethonium chloride (BZT), 5-pyrrolidone-2-carboxylic acid (PCA) and copper sulphate pentahydrate (CS). Hand washing practices considered also included use of soap and nailbrush (*40*), *Wash-sanitise*, consisting of using hand sanitizers after hand washing with water and soap (*15, 30, 47*), and a new hand hygiene regime known as *SaniTwice* (a registered trademark of James Mann, Handwashing for Life, Libertyville, IL) consisting of a two stage hand cleansing including application of an excess of alcohol-based sanitizer, hand rubbing, cleaning hands with a paper towel, and a final application of alcohol-based sanitizer (*14*).

The relative efficacy of products was generally tested *in vitro*, *ex vivo* and/or *in vivo.* Most of the *in vitro* studies involved experiments carried out using a suspension assay consisting of a standardized quantity of the target microorganism treated with increasing concentrations of the test product, with the aim of estimating the inactivation rate for each product used (*1, 10, 16, 20, 21, 28, 29, 54, 46, 55*). One *in vitro* study evaluated inactivation rates of tested products on latex gloves immersed in a solution of phosphate buffered saline (PBS) or crab cooking water artificially contaminated with 5 $\log_{10}$ CFU *L. monocytogenes*/ml (*44*). *Ex vivo* tests included experiments carried out on pig skin from a freshly killed pig (the pig skin method) previously treated with sanitizing products, then artificially contaminated with challenge microorganisms to test residual activity of tested products after use (*9, 28, 35, 54*). *In vivo* studies involved experiments carried out with selected human volunteers to estimate the efficacy of each tested product to remove or inactivate target microorganisms from artificially contaminated whole hands, finger pads or gloves. The vast majority of *in vivo* studies retrieved in the literature were carried out on hands or finger pads artificially contaminated with pure cultures of bacteria or viruses without the presence of food components or organic material (*9, 16, 22, 29, 37, 38, 41, 42, 43, 47, 55*). Seven studies evaluated the efficacy of hand washing products in a food preparation setting on naturally and artificially soiled hands or gloves (*7, 8, 14, 15, 40, 44, 48*). Three studies evaluated inactivation rates of products on hands contaminated with viral suspensions prepared with other organic loads like fetal bovine serum or feces (*15, 36, 40*). Other factors pertaining to food preparation settings like hygiene of nails (*40*) and wearing rings when handling food have also been minimally considered (*61*). A summary of the experimental conditions applied and main findings from *in vitro*, *ex vivo* and *in vivo* evaluations in all studies included in this review are summarized in Table 2. Information relating to specific pathogens will now be summarised.

Norovirus. Because human norovirus (HuNoV) cannot be routinely cultured *in vitro*, determining the effectiveness of sanitizers and disinfectants against HuNoV is difficult. Methodologies used to estimate level of virus reduction include the use of reverse transcription-quantitative real time PCR to quantify the number of RNA copies of HuNoV extracted and purified from tested samples (*41, 42, 46*) and the use of cultivable surrogates like Feline Calicivirus (FCV) and Murine Norovirus (MNV). Norovirus surrogates were generally tested alone as an alternative to HuNoV (*9, 15, 29, 36, 38, 40, 55*), or in parallel with HuNoV (*46*).

Liu et al. (*41*) compared the efficacy of an antibacterial soap, alcohol-based sanitizer containing 62% ethyl alcohol, and water rinsing for the removal of HuNoV from artificially contaminated finger pads. Ethanol-based hand sanitizer was the least effective hand product tested (0.34±0.22 $\log_{10}$ reduction). The greatest $\log_{10}$ reduction was observed for water rinse only (1.38±0.49 $\log_{10}$) and antibacterial soap (1.1±0.49 $\log_{10}$). A separate study by Liu et al. (*42*) tested various commercially available hand hygiene products containing 62% to 95% alcohol on finger pads against multiple HuNoV strains. The study showed a wide range of efficacy (0.10 to 3.74 log_{10} reduction), varying according to different products and strains tested. The highest level of RNA reduction was achieved by a 70% ethanol gel containing additional ingredients that seem to potentiate the virucidal activity of alcohol alone. A limitation of the study reported by the authors was the presence of PCR inhibitors in the test products that may have affected PCR amplification and led to an overestimate of virus reduction.

Eight papers evaluated the efficacy of hand sanitizers against FCV and MNV. Experimental methods used to estimate viral inactivation included a virus-specific cytopathic effect (CPE) test consisting of culturing post treatment samples on a serial dilution of permissive host cells (*9, 15, 29, 36, 38, 40, 55*), and a plaque assay test in parallel with TaqMan real-time reverse transcription PCR (*46*). Park et al. (*46*) evaluated *in vitro* virucidal efficacy of seven hand sanitizers containing ethanol, triclosan and chlorhexidine against both Norovirus surrogates (i.e. FCV and MNV) and human norovirus (HuNoV). None of the products demonstrated significant RNA reduction when tested against HuNoV, whereas results achieved for Norovirus surrogates showed different levels of viral reduction measured by plaques assay and RT-qPCR. A general lack of correlation between the two detection methods and different degrees of viral inactivation of FCV or MNV were generally observed. Only a 72% alcohol pH 2.9 ABHR reduced the infectivity of both FCV and MNV (3.4 and 2.6 log_{10}, respectively) by the plaque assay test, whereasno correlation was found between reduced infectivity and RNA reduction measured by real-time reverse transcription PCR.. Conflicting results were also reported in two studies evaluating both *in vitro* and *in vivo* efficacy of hand products against FCV and MNV. Gehrke et al. (*29*) tested three types of alcohol - ethanol, 1- propanol and 2-propanol. *In vitro* experiments showed higher effectiveness achieved by 50% and 70% 1-propanol (10^4-fold reduction) over ethanol and 2-propanol. In contrast, 70% ethanol achieved higher viral inactivation (3.78 $\log_{10}$ reduction) *in vivo* than either 1-propanol or 2-propanol (3.58 and 2.15 $\log_{10}$ reduction, respectively). Steinmann et al. (*55*) compared virucidal activity of three ABHRs and three antimicrobial soaps. Results from suspension tests demonstrated ≥5 $\log_{10}$ reduction of both FCV and MNV achieved by two of three ABHRs tested which was greater efficacy than soaps tested (typically ≤ 3 $\log_{10}$ reduction). Conversely, the modified finger pad test carried out against MNV only, showed superior antimicrobial activity of a povidone-iodine soap (4.62 $\log_{10}$ reduction) compared to the other ABHRs and soaps tested. Two studies evaluated *in vivo* efficacy of hand hygiene products against FCV only. Lages et al. (*38*) tested four ABHRs, three non-alcoholic sanitizers and two triclosan-containing antimicrobial liquid soaps after 30 s and 2 min exposure times. Limited efficacy of all the products tested was generally observed; only one antimicrobial soap containing 10% povidone-iodine (≤2.67 $\log_{10}$ reduction) and one ABHR containing 95% ethanol (≤1.30 $\log_{10}$ reduction) achieved appreciable viral reduction compared to water rinse tested in parallel. Czerwinski and Cozean (*9*) compared a novel hand sanitizer containing benzethonium chloride (BZK), a 62% ABHR, an antibacterial liquid soap, and water rinse. Apart from a promising level of inactivation shown by the novel hand sanitizer (3.49 $\log_{10}$ reduction), generally viral reductions were <1 $\log_{10}$ in all the other cases. Two studies evaluated efficacy of products on hands artificially contaminated with a fecal suspension of FCV. Kampf et al. (*36*) tested efficacy of three ABHRs; greatest reduction in FCV was achieved by a 95% alcohol containing hand sanitizer (2.17±1.06 $\log_{10}$). Lower concentrations of alcohol did not demonstrate more than 1 $\log_{10}$ viral reduction. Lin et al. (*40*) compared six hand washing practices on contaminated natural and artificial nails. Use of soap and nail brush (2.54±0.57 $\log_{10}$ reduction) achieved the highest $\log_{10}$ viral reduction, followed by hand washing with antibacterial soap (2.26±0.42 $\log_{10}$ reduction), and then combined use of soap and hand sanitizer (2.13± 0.93 $\log_{10}$ reduction). In contrast, the use of hand sanitizer alone demonstrated limited efficacy (0.86±55 $\log_{10}$ reduction). Presence of long nails on treated hands was found to significantly impact efficacy of all the hand products tested. Finally, one study carried out by Edmonds et al. (*15*) compared four hand hygiene regimes on hands contaminated with a viral suspension of MNV prepared with 0.5% fetal bovine serum to mimic soiling with organic matter. Hand hygiene practices included an antimicrobial soap, a 70% alcohol gel, hand washing followed by hand sanitizing, and SaniTwice. Sanitizing with 70% alcohol gel was slightly more effective (2.6±0.41 $\log_{10}$ reduction) than hand washing with antimicrobial soap (1.79±0.29 $\log_{10}$ reduction). A higher level of viral reduction was achieved by SaniTwice (4.04±0.33 $\log_{10}$) and by the combination of conventional hand washing and sanitizing (3.19±0.31 $\log_{10}$).

Hepatitis A virus (HAV). Little information is available in the scientific literature about relative effectiveness of hand washing products against HAV. Only three studies describing efficacy of hand washing products against HAV were retrieved (*20, 21, 43*). Fendler et al. (*21*) and Fendler & Groziak (*20*) demonstrated limited *in vitro* efficacy of a commercially available alcohol-based hand sanitizer containing 62% alcohol and emollients against HAV. The levels of inactivation achieved by 30 s timed exposure were 1.75 and 1.25 $\log_{10}$ reduction, respectively, corresponding to 94.37% (*21*) and 94.4% (*20*) reduction of original inoculum.

A study by Mbithi et al. (*43*) evaluated elimination rates of 10 different products on whole hands or finger pads artificially contaminated with a mixture of viruses and feces. Formulations tested included a non-medicated soap, five ethanol-based hand sanitizers, and four antibacterial liquid soaps, compared to tap water without soap used as a control. None of the tested products reached a level of inactivation of 99.9%, which is generally desired. Inactivation rates observed from both whole-hand and finger pad methods ranged from 79% to 94%. One antibacterial soap and non-medicated soap attained a higher level of virus reduction (≤94.56±5.75% and ≤91.39±2.65%, respectively) than alcohol based hand sanitizers (≤90.67±2.08%) and tap water (≤81.57±4.5%). Residual infectivity, estimated as a mean number of Plaque Forming Units through a plaque assay test, ranged from 0 to 0.64 PFU for ABHRs, 0.63 to 1.74 PFU for antimicrobial soaps, 1.57 PFU for plain soap and 3.88 PFU for tap water. No information was found in the literature about the efficacy of hand washing and hand sanitizers against HAV on hands soiled with food components.

Listeria monocytogenes. Only two papers describing *in vitro* and *in vivo* efficacy of sanitizing products against *L. monocytogenes* were found in the literature (*21, 44*). Fendler et al. (*21*) reported > 5 $\log_{10}$ reduction of *L. monocytogenes* achieved *in vitro* by a commercially available hand sanitizer containing 62% alcohol on a 30 s timed exposure kill test. McCarthy (*44*) compared the *in vivo* efficacy of one hand sanitizer and five disinfectants, including two chloride-based, one iodine-based, one peroxide-based, one quaternary ammonium-based sanitizer, on contaminated latex gloves. The impact of the organic compounds on inactivation rates of the tested products was estimated through immersion of gloves in both sterile phosphate buffered saline (PBS) and crab cooking water artificially contaminated with 5 $\log_{10}$ CFU/ml of *L.*

monocytogenes. Of the different products tested, only the peroxide-based product achieved 5 $\log_{10}$ reduction of attached *L. monocytogenes* on both soiled and non-soiled contaminated gloves. The two chloride-based and the quaternary ammonium-based products achieved 5 $\log_{10}$ reduction on gloves contaminated with PBS suspensions of *L. monocytogenes* (i.e. no food residue present) but demonstrated lower efficacy (≤ 1-2 $\log_{10}$ reduction) in the presence of crab cooking water. Iodine-based sanitizer and alcohol-based instant hand sanitizer demonstrated lower efficacy in both cases. No data about the efficacy of conventional hand washing in removing *L. monocytogenes* from gloves or hands was found in the literature.

Staphylococcus aureus* and *Escherichia coli. Six studies assessed *in vitro* and/or *ex vivo* efficacy of hand sanitizers against *St. aureus* and *E. coli*. Hand formulations included conventional ABHRs and new generation hand products containing a combination of active antimicrobials and other compounds like thickening agents, emollients and natural compounds. Fendler et al. (*21*) reported that > 5 $\log_{10}$ reduction was achieved by a 62% alcohol based sanitizer against both methicillin-resistant and vancomycin-tolerant and methicillin-resistant *St. aureus*, non-pathogenic *E. coli* and *E. coli* O157:H7. High *in-vitro* inactivation rates were also reported by Biagi et al. (*1*), Czerwinski et al. (*10*), Gaonkar et al. (*28*), Kaiser et al. (*35*), and Shintre et al. (*54*). Biagi et al. (*1*) tested the *in vitro* efficacy of a new combination of two natural compounds, pyrrolidone-2–carboxylic acid (PCA) and copper sulphate pentahydrate (CS). The combination of PCA and CS demonstrated higher efficacy than 70% ethanol and 60% isopropanol used alone. Czerwinski et al. (*10*) tested the efficacy of a novel alcohol-based antiseptic and a novel water-based antiseptic lotion prepared with a synergistic combination of ingredients centred on Benzethonium chloride (BZT). The novel water-based product demonstrated equivalent antimicrobial (99.9%) activity against *E. coli* and *St. aureus* strains compared to the other alcohol-based product. Gaonkar et al. (*28*) tested an ABHR prepared with an emollient (Octoxy) and other additional ingredients against *E. coli* and methicillin-resistant *St. aureus*. *In vitro* evaluations showed > 7 $\log_{10}$ reduction of both *E. coli* and *St. aureus* and *ex vivo* tests showed higher antimicrobial activity and superior residual activity after use of the novel Octoxy compared to the two other ABHRs applied in parallel as a control. Kaiser et al. (*35*) compared *ex vivo* a combination of a surgical scrub containing 4% Chlorexidine gluconate (CHG) and ABHRs prepared with and without thickening agents against *St. aureus*. Hand sanitizers thickened with anionic polymers were found to negatively impact persistent activity of CHG. In contrast, no negative effect was observed for ABHRs alone or thickened with non-ionic compounds. Shintre et al. (*54*) tested the synergistic effect of alcohol and quaternary ammonia in combination with moisturizers or essential oils *in vitro* and *ex vivo*. Synergistic combination of farnesol and BZT demonstrated better prolonged activity (i.e. 20-35 min post application) against *St. aureus and E. coli* than other hand sanitizers and chemicals compounds used alone.

The high level of bacterial inactivation generally observed *in vitro* does not necessarily reflect the actual capacity of products to remove transient microorganisms from the outer layers of skin of hands. Incomplete effectiveness against target microorganisms from cleaned hands is generally reported in all studies carried out on hands artificially contaminated with *E. coli*. Edmonds et al. (*16*) compared the efficacy of two novel 70% alcohol gel and foam, seven commercially available ABHRs, and two World Health Organization recommended formulations containing 60-90% alcohol against one methicillin-resistant *St. aureus* strain. Results showed superior efficacy of the novel gel and foam preparations after single and multiple uses compared to the other products. However none of the products exceeded 3 $\log_{10}$ reduction of the target microorganism. Fishler et al. (*22*) evaluated the effectiveness of two hand washing regimes in reducing transient bacteria after single wash and subsequent potential transfer of bacteria to a ready-to-eat food. The antimicrobial soap achieved a higher level of bacterial removal (>3 $\log_{10}$ reduction) than plain soap (≤2 $\log_{10}$ reduction), but failed to avoid the transfer of seeded bacteria to a ready-to eat food item. Kampf et al. (*37*) reported limited efficacy of two ABHRs on hands artificially contaminated with *E.coli*. Bacterial inactivation achieved by two 62% alcohol containing products was only slightly better (≤3.5±0.45 and 3.58±0.71 $\log_{10}$ reduction) than rubbing under running water applied in parallel (2.39±0.57). Paulson et al. (*47*) examined the abilities of four hand washing regimes including plain soap, an antimicrobial soap, an alcohol hand sanitizer, and combined use of an antimicrobial soap and a ABHR (used after hand washing). All the products used alone performed equally and none exceeded 2 $\log_{10}$ reduction. Higher efficacy (3.28 $\log_{10}$ reduction) was observed by combined use of hand washing and hand sanitizing.

***Salmonella* spp.** Little information about the efficacy of sanitizing products against *Salmonella* spp. is available in the literature. Only one *in vitro* study (*21*) reporting > 5 $\log_{10}$ reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium being achieved by a 62% ABHR was retrieved.

Efficacy of hand products on hands soiled with food components. Experimental conditions described in the literature to mimic food preparation settings include contamination of food workers hands with natural soil encountered in the food service industry (*7*), or hands artificially inoculated with pure cultures of bacteria mixed with crab cooking water (*44*), chicken or beef broth (*14, 15, 40*), ground beef

(*7, 14, 15, 40*), dirt or cooking oil (*48*). Efficacy of hand products was estimated based on the enumeration of microorganisms released from treated hands, or based on the enumeration of bacteria remaining on hands. Methods for enumerating released bacteria included the glove juice (*14, 15, 61*) or the hand rinse (*8, 48*) techniques. Both techniques consist of enumerating bacteria released from washed hands previously placed into a glove or a bag filled with sterile water or buffer. Conversely, enumeration of bacteria remaining on the hands after hand washing or hand sanitizing is usually estimated through image analysis or by pressing washed hand palms onto the surface of an agar plate (*7*).

Four studies compared efficacy of hand hygiene products on soiled hands. Courtenay et al. (*8*) compared eliminating abilities of three hand washing regimes including rinsing with warm water, rinsing with cold water and hand washing with water and soap on hand and gloves contaminated with *E. coli* and ground beef. Water and soap achieved a higher level of removal than other hand hygiene regimes, but the level of bacterial removal was higher from hands (99.98%) than from gloves (99.13%). The efficacy of four hand sanitizers containing 62% ethanol was also compared on clean hands contaminated with 10^6 $\log_{10}$ cfu of *E. coli*/ml of broth. The level of bacterial reduction achieved by the four hand sanitizers ranged from 96.44 - 90.40% and was consistently lower than that observed for water and soap. Charbonneau et al. (*7*) tested eliminating abilities of a plain soap, a 70% alcohol hand sanitizer, and combined use of hand washing and ABHS on hands naturally contaminated with raw chicken and ground beef. The study showed higher efficacy achieved by plain soap over the other hand hygiene regimes. Limited efficacy of ABHRs on clean hands or hands soiled with dirt and oil was also reported in a study by Pickering et al. (*48*). Bacterial reduction achieved did not exceed 2 $\log_{10}$

of seeded *E.coli* (10^7 CFU/ml) in all cases. Efficacy of hand hygiene practices in moderately and heavily soiled conditions has been evaluated in two studies (*14, 15*). Edmonds et al. (*15*) demonstrated superior efficacy of combined use of water and soap and hand sanitizing than water and soap or antimicrobial soap used alone. Reported levels of bacterial inactivation achieved from both moderately and heavily soiled hands were >5.0 and >4.6 $\log_{10}$ reduction, respectively. Edmonds et al. (*14*) tested eliminating capacity of SaniTwice carried out with three 62% to 70% alcohol products compared to a plain soap, an antibacterial soap and a 70% alcohol gel used alone. SaniTwice with 70% alcohol foam showed higher efficacy than water and soap and other alcohol-based regimes. The level of bacterial reduction observed on moderately and heavily soiled hands was 4.61 and 3.92 $\log_{10}$, respectively. Heavy soil condition was found to impact efficacy of all the practices tested (<1-2 $\log_{10}$ reduction).

Other considerations in relation to effective hand cleansing. Only two studies evaluated the efficacy of hand washing techniques in the removal of bacteria or viruses from natural and artificial nails (*40*) or from hands with rings present (*61*). Wongworawat et al. (*61*) compared the efficacy of three hand sanitizers, including a povidone-iodine, a water-aid alcohol and an alcohol-chlorhexidine hand sanitizer, on hands with and without rings. The alcohol-chlorhexidine hand sanitizer showed slightly higher efficacy than other products. No significant difference in the number of bacteria retrieved from cleansed hands with and without rings was generally observed. Results reported suggest that the presence of rings should not significantly impact effectiveness of hand sanitizers.

Lin et al. (*40*) assessed effectiveness of different cleansing products and hand practices from natural and artificial nails on hands inoculated with *E. coli* or FCV.

Use of a nailbrush and soap achieved the highest removal of target microorganisms. However the presence of long nails significantly impacted efficacy of all regimes tested, suggesting that maintaining short fingernails is essential to reduce the risk of transmitting hazardous microorganisms when handling food.

DISCUSSION

Effective hand washing is extremely important to help prevent harmful microorganisms from spreading from people's hands to food. Contact with bare hands and failure to properly wash hands have been reported as the main risk factor contributing to foodborne disease caused by food handlers (*57*). European Union food safety legislation requires every person working in a food handling area to maintain a high standard of personal cleanliness, and food business operators to provide an adequate number of washbasins suitably located and designed for cleaning hands (*18*). The Food Code 2009, published by the Food and Drug Administration to standardize food safety and food hygiene procedures, states that the total time recommended for proper hand washing is at least 20 seconds, of which 10-15 seconds should be used for rubbing followed by rinsing under running warm water and drying hands (*24*).

The presence of food components like fat, oil or other dirt is considered the main factor affecting removal and inactivation rates of hand hygiene products against microorganisms occurring on the hands of food workers (*26*). The levels of microbial contamination reported for hands of food workers can vary between 2 and >5 $\log_{10}$ cfu/hand across various food settings, and the bacterial flora generally encountered on the hands of food handlers is a mixture of Enterobacteriaceae and other mesophilic bacteria in the presence of fat and other soil (*11*). Various pathogens with very low infective doses (1 to 100 units) including viruses, parasites and enteric bacteria can be present on contaminated hands in high numbers (*58*). Pathogens carried by contaminated hands can be easily transferred to food and hand contact surfaces and can survive for long periods (*58, 59*).The ideal hand hygiene regime to be used in a food setting should ensure maximum removal of food components and food flora from cleaned hands in order to minimize the level of transferable microorganisms. Most of the hand disinfectants, including medicated soaps and instant hand sanitizers, have a broader antimicrobial activity than plain soaps but are generally considered not to properly meet the needs of food workers because they are unable to remove food soil from cleansed hands (*23*).

This systematic review evaluated the scientific information available in the literature about the efficacy of conventional and improved hand hygiene products in relation to their use in food preparation settings. Analysis of the literature showed the existence of conflicting reports about the efficacy of soaps and hand sanitizers against foodborne pathogens. No standardized method to estimate removal and inactivation rates of target pathogens seems to be available and the varying experimental conditions (including quantity of product used, duration of treatment, type of food soil used) between different studies makes comparison of results difficult. Hand washing with water and soap is generally reported to achieve effective removal of bacteria and soil from hands (*7*, *8*, *14*, *15*) and gloves (*8*) and to be superior to other products in the removal of bacteria and viruses from fingernails when used with a nailbrush (*40*). However, a residual level of microorganisms even after proper washing is generally reported (*7, 8, 14, 15*, *40*), suggesting that hand washing alone cannot ensure elimination of risk in relation to bacterial transmission from hands to food. Conventional hand washing is more effective on contaminated hands than on gloves (*8*) suggesting that frequent changes of gloves rather than washing gloves when they become visibly soiled would more effectively minimize risk of bacterial contamination between different food preparation steps.

Information on the efficacy of antimicrobial soaps over conventional plain soaps is also controversial and the existence of conflicting results has been previously reported in two other reviews *(31, 60).* Apart from one study reporting lower efficacy compared to plain soap (*47*), the evidence seems to indicate that antimicrobial or medicated soaps can achieve a slightly higher level of microbial inactivation on artificially contaminated hands without food residue present (*22, 43*), whereas their efficacy on soiled hands is similar to conventional soaps (*15, 40*).

Instant hand sanitizers have shown high and rapid *in vitro* efficacy against various target bacteria (*10, 16, 21, 54*), whereas their efficacy against naked viruses seems to be lower (*20, 21, 38, 46*) and vary according to different viruses tested, type of alcohol and concentration used (*29, 46, 55*). These findings are in general agreement with four other reviews (*2, 3, 31, 60*). Apart from some improved formulations (*9, 32*), instant hand sanitizers used *in vivo* do not usually exceed 2-3 $\log_{10}$ microbial reduction (*16, 37, 41, 42, 47, 48*) and their efficacy seems to be affected by the presence of food debris, as observed on both moderate (*44*) and heavily soiled hands (*7*, *14*, *40*), as only one study included in this systematic review reported similar rates of bacterial inactivation on both clean and soiled hands (*48*). Instant hand sanitizers used alone seem not to be a reliable substitute for conventional hand washing in food establishments (*7*). In contrast, their application after hand washing, previously carried out with either antimicrobial or plain soap (i.e. wash-sanitize regimes), seems to be more effective than hand sanitizer or soaps used alone (*15*, *47*); levels of bacterial inactivation have been demonstrated to significantly increase up to 4 or 5 $\log_{10}$ reduction on both moderately and heavily soiled hands (*15*).

Preliminary results reported for SaniTwice are also encouraging (*14*). The method tested on hands moderately and heavily soiled with a mixture of food components and *E. coli* showed good levels of bacterial reduction (~4 $\log_{10}$ reduction). A similar level of inactivation is also reported against MNV on artificially contaminated hands. These findings suggest that this hand hygiene regime could be used as an alternative to wash-sanitize when water and soap are not available. However, no evidence about the efficacy of this hand hygiene regime against HuNoV or HAV on soiled hands seems to be available in the literature. For this reason, further studies would be needed to prove the effectiveness of SaniTwice in different food settings and against different foodborne pathogens.

Finally, a new generation of alcohol-free lotions is attracting more and more interest (*1, 28, 35).* Evidence from *in vitro* and *ex vivo* studies showed similar efficacy against target bacteria compared to alcohol-based products, with prolonged activity after application, and potentially less skin irritation. However, very little is known about their efficacy against viruses, and no evidence about their inactivation rates on soiled hands seems to be available in the literature currently.

ACKNOWLEDGEMENTS

This material is based upon works supported by ***safe*food**, The Food Safety Promotion. Board, under Grant No. 06-2014

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Table 1. Number of scientific publications matching search terms retrieved from three different electronic databases.

Search term	Web of Science	Scopus	PubMed	Total
“Efficacy of hand washing”	351	690	456	1497
“Efficacy of hand sanitizers”	63	62	23	148
“Evaluation of hand sanitisers”	28	30	2	60
“Effect of hand hygiene products”	166	160	77	403
Number of unique articles retrieved	21	10	7	38
Number of articles excluded*	4	4	3	10
Number of articles reviewed	17	6	5	28

*Three of the excluded papers were review articles, other seven did not meet inclusion criteria.

Table 2. Summary of results regarding efficacy of hand sanitisers presented in the scientific papers included in this systematic review.

References	Microorganism	Test method	Hand hygiene products and disinfectants	Reduction observed
Czerwinski & Cozean (*9*)	FCV	Finger pad	Novel alcohol-based antiseptic containing BZT	3.49 Log_{10}
			Hand sanitizer (62% ethanol)	0.14 Log_{10}
			Hand washing with antibacterial soap	0.67 Log_{10}
			Water rinse	1.09 Log_{10}
		Pig skin method	Novel alcohol-based antiseptic containing BZT	
	E. coli	2 min post application		1.65 Log_{10}
		1h post application		1.34 Log_{10}
		4h post application		1.15 Log_{10}
	St. aureus	2 min post application	Novel alcohol-based antiseptic containing BZT	1.87 Log_{10}
		1h post application		2.14 Log_{10}
		4h post application		1.62 Log_{10}
Edmonds et al. (*15*)		Moderate food soil load	Non-antimicrobial hand wash	3.10±0.61 Log_{10}
			PCMX hand wash	3.56±0.64 Log_{10}
			WS (non-antimicrobial hand wash + 62% EtOH foam)	3.81±0.89 Log_{10}
			WS (PCMX hand wash+62% EtOH foam)	4.16±0.91 Log_{10}
			WS (non-antimicrobial hand wash +70% EtOH AF gel)	5.13±0.71 Log_{10}
			WS (PCMX hand wash +70% EtOH AF gel)	5.22±0.60 Log_{10}
		Heavy food soil load	WS (non-antimicrobial hand wash + 62% EtOH foam)	4.11±0.48 Log_{10}
			WS (Triclosan hand wash+62% EtOH foam)	3.97±0.45 Log_{10}
			WS (PCMX and wash +70% EtOH AF gel)	4.60±0.52 Log_{10}
			WS (Triclosan hand wash +70% EtOH AF gel)	4.51±0.43 Log_{10}
	MNV	Organic soil load (5% fetal bovine serum)	Non-antimicrobial hand wash	1.79±0.29 Log_{10}
			ABHRs (70% EtOH AF gel)	2.60±0.41 Log_{10}
			WS (non-antimicrobial hand wash + 70% EtOH AF gel)	3.19±0.31 Log_{10}
			STW (70% EtOH AF gel)	4.04±0.33 Log_{10}

Table 2 continued				
Gehrke et al. (*29*)	FCV	Suspension 30 s exposure	EtOH 50%	2.19 Log_{10}
			EtOH 70%	3.55 Log_{10}
			EtOH 80%	2.19 Log_{10}
			1-Propanol 50%	≥ 4.13 Log_{10}
			1-Propanol 70%	≥ 4.06 Log_{10}
			1-Propanol 80%	1.90 Log_{10}
			1-Propanol 50%	2.31 Log_{10}
			1-Propanol 70%	2.35 Log_{10}
			1-Propanol 80%	1.35 Log_{10}
		Finger tips	EtOH 70%	3.78±0.83 Log_{10}
			EtOH 90%	2.84±0.64 Log_{10}
			1-Propanol 70%	3.58±0.92 Log_{10}
			1-Propanol 90%	1.38±0.33 Log_{10}
			2-Propanol 70%	2.15±0.50 Log_{10}
			2-Propanol 90%	0.76±0.19 Log_{10}
			Water	1.23±0.44 Log_{10}
Kampf et al. (*36*)	FCV	Fingerpad Organic soil 5% fetal bovine serum	Reference alcohols (70% Ethanol)	1.45±0.41 Log_{10}
			Sterillium Virugard (95% Ethanol)	2.17±1.06 Log_{10}
			Sterillium Rub (80% Ethanol)	1.25±0.28 Log_{10}
			Desderman N (75.1% Ethanol)	1.07±0.61 Log_{10}
Lages et al. (*38*)	FCV	Finger tips 30 s and 2 min contact periods	ABHRs (99.5% Ethanol)	1.00 (30s) - 1.30 (2 min) Log_{10}
			Hand sanitizer (62% Ethanol)	0.50 (30s) - 0.55 (2 min) Log_{10}
			Antiseptic (91% Isopropanol)	0.00 (30s) - 0.43 (2 min) Log_{10}
			Antiseptic (70% Isopropanol)	0.67 (30s) - 0.55 (2 min) Log_{10}
			Antiseptic (3% Hydrogen peroxide)	0.09 (30s) - 0.47 (2 min) Log_{10}
			Antiseptic (0.13% Benzalkonium chloride + 2% lidocaine hydrochloride	0.00 (30s) - 0.22 (2 min) Log_{10}
			Antiseptic (10% Povidone-iodine)	2.67 (30s) - 2.39 (2 min) Log_{10}
			Antimicrobial soap (0.60% Triclosan)	0.25 (30s) - 0.50 (2 min) Log_{10}
			Antimicrobial soap (0.115% Triclosan)	0.42 (30s) - 0.17 (2 min) Log_{10}
			Water	0.33 (30s) - 0.42 (2 min) Log_{10}
Lin et al. (*40*)	FCV	Fingertips (Artificial feces)	Tap water	1.22±0.86 [(1)] Log_{10} 1.97±0.68[(2)] Log_{10}
			Soap	1.89±0.31 [(1)] Log_{10} 1.82±0.46[(2)] Log_{10}

Table 2 continued

			Antibacterial soap (Triclosan)	1.65±0.19 (1) Log_{10} 2.26±0.42(2) Log_{10}
			Hand sanitizers (62% Ethanol)	0.43±0.47 (1) Log_{10} 0.86±0.55(2) Log_{10}
			Soap plus sanitizer	1.85±0.69 Log_{10} (1) 2.13±0.93 Log_{10} (2)
			Soap plus nail brush	0.41±0.49 Log_{10} (1) 2.54±0.57 Log_{10} (2)
	E. coli	Fingertips Heavy food soil load	Tap water	1.29±0.53 Log_{10} (1) 1.18±0.14 Log_{10} (2)
			Soap	1.09±0.51 Log_{10} (1) 1.18±0.24 Log_{10} (2)
			Antibacterial soap (Triclosan)	1.26±0.47 Log_{10} (1) 1.45±0.59 Log_{10} (2)
			Hand sanitizer (62% Ethanol)	1.16±0.63 Log_{10} (1) 1.31±0.68 Log_{10} (2)
			Soap plus sanitizer	1.59±0.45 Log_{10} (1) 1.85±0.84 Log_{10} (2)
			Soap plus nail brush	2.54±0.54 Log_{10} (1) 3.07±1.18 Log_{10} (2)
Liu et al. 2010 (*41*)	HuNoV	Fingerpad	Hand sanitizer (62% Ethanol)	0.27±0.12 Log_{10} (3) 0.34±0.22 Log_{10} (4)
			Antibacterial soap (0.5% Triclosan)	0.67±0.47 Log_{10} (3) 1.10±0.49 Log_{10} (4)
			Water rinse	0.58±0.37 Log_{10} (3) 1.38±0.49 Log_{10} (4)
Liu et al (*42*)	HuNoV	Fingerpad	Hand sanitizer (VF481 - 70% Ethanol)	3.74±0.85 Log_{10}
			Hand sanitizer (VF447 - 70% Ethanol)	2.04±0.78 Log_{10}
			Hand sanitizer (Endure 300 - 70% Ethanol)	1.49±0.62 Log_{10}
			Hand sanitizer (Sterillium Virugard - 95% Ethanol)	0.10±0.17 Log_{10}
			Hand sanitizer (Germstar Noro - 63% Ethanol)	0.11±0.22 Log_{10}
			Hand sanitizer (Anios Gel 85 NPC - 85% Ethanol)	1.27±0.22 Log_{10}
Park et al. (*46*)	HuNoV	Suspension (1 min exposure)	Hand sanitizer (79% Ethanol - pH 7.1)	0.1±0.2 Log_{10} (5)
			Hand sanitizer (72% Ethanol - pH 4.1)	0.0±0.2 Log_{10} (5)
			Hand sanitizer (72% Ethanol - pH 2.9)	0.1±0.1 Log_{10} (5)
			Hand sanitizer (67% Ethanol - pH 7.4)	0.2±0.2 Log_{10} (5)

Table 2 continued				
			Hand sanitizer (0.1% Triclosan - pH 3.0)	0.0±0.3 Log_{10} (5)
			Hand sanitizer (0.2% Triclosan - pH 3.0)	0.0±0.1 Log_{10} (5)
			Hand sanitizer (4% Chlorexidine - pH 5.4)	0.0±0.1 Log_{10} (5)
	MNV		Hand sanitizer (79% Ethanol - pH 7.1)	3.01±0.05 Log_{10} (5) >3.6 Log_{10} (6)
			Hand sanitizer (72% Ethanol - pH 4.1)	0.0±0.5 Log_{10} (5) >3.6 Log_{10} (6)
			Hand sanitizer (72% Ethanol - pH 2.9)	0.1±0.5 Log_{10} (5) >2.6 Log_{10} (6)
			Hand sanitizer (67% Ethanol - pH 7.4)	1.9±0.4 Log_{10} (5) 2.0±0.2 Log_{10} (6)
			Hand sanitizer (0.1% Triclosan - pH 3.0)	0.4±0.3 Log_{10} (5) 1.1±0.1 Log_{10} (6)
			Hand sanitizer (0.2% Triclosan - pH3.0)	0.0±0.2 Log_{10} (5) 0.2±0.1 Log_{10} (6)
			Hand sanitizer (4% Chlorexidine - pH 5.4)	0.0±0.1 Log_{10} (5) 0.0±0.3 Log_{10} (6)
	FCV		Hand sanitizer (79% Ethanol - pH 7.1)	0.8±0.7 Log_{10} (5) 0.0±0.2 Log_{10} (6)
			Hand sanitizer (72% Ethanol - pH 4.1)	0.7±0.9 Log_{10} (5) 0.0±0.2 Log_{10} (6)
			Hand sanitizer (72% Ethanol - pH 2.9)	0.9±0.8 Log_{10} (5) >3.4 Log_{10} (6)
			Hand sanitizer (67% Ethanol - pH 7.4)	0.8±0.4 Log_{10} (5) 0.4±0.2 Log_{10} (6)
			Hand sanitizer (0.1% Triclosan - pH 3.0)	0.0±0.7 Log_{10} (5) >3.4 Log_{10} (6)
			Hand sanitizer (0.2% Triclosan - pH 3.0)	0.2±0.2 Log_{10} (5) >3.4 Log_{10} (6)
			Hand sanitizer (4% Chlorexidine - pH 5.4)	0.1±0.3 Log_{10} (5) 0.0±0.2 Log_{10} (6)
Steinmann et al. (*55*)	FCV	Suspension (30s exposure)	Hand sanitizer (45% Ethanol)	> 5 Log_{10}
			Hand sanitizer (55% Ethanol)	> 5 Log_{10}
			Hand sanitizer (90% Ethanol)	< 1 Log_{10}

Table 2 continued				
			Antimicrobial liquid soap (1% Triclosan)	< 1 Log_{10}
			Antimicrobial liquid soap (4% Chlorexidine)	< 1 Log_{10}
			Antimicrobial liquid soap (0.75-0.81% available iodine)	3 Log_{10}
	MNV	Suspension (30s exposure)	Hand sanitizer (45% Ethanol)	5 Log_{10}
			Hand sanitizer (55% Ethanol)	> 5 Log_{10}
			Hand sanitizer (90% Ethanol)	> 4 Log_{10}
			Antimicrobial liquid soap (1% Triclosan)	< 1 Log_{10}
			Antimicrobial liquid soap (4% Chlorexidine)	< 1 Log_{10}
			Antimicrobial liquid soap (0.75-0.81% available iodine)	> 2 Log_{10}
	MNV	Modified fingerpad	Hand sanitizer (45% Ethanol)	4.25 Log_{10}
			Hand sanitizer (55% Ethanol)	3.94 Log_{10}
			Hand sanitizer (90% Ethanol)	3.91 Log_{10}
			Antimicrobial liquid soap (1% Triclosan)	3.42 Log_{10}
			Antimicrobial liquid soap (4% Chlorexidine)	0.96 Log_{10}
			Antimicrobial liquid soap (0.75-0.81% available iodine)	4.62 Log_{10}
			Water	3 Log_{10}
Mbithi et al. 1993 *(43)*	HAV	Fingerpad	Alcare (62% emolliented ethanol foam)	89.27 ± 4.38%
			Aquaress (nonantimicrobial soap)	77.96 ± 7.17%
			Bacti-Stat soap (0.1% Chlorhexidine gluconate, 0.50% Didecyl dimethyl ammonium chloride, 5% Isopropanol)	92.04 ± 4.02%
			Bioprep hand soap	83.35 ± 2.76%
			Dettol (4.8% 4-Chloro-3,5-xylenol, 9.4% Isopropanol)	88.63 ± 5.38%
			70% Ethanol	87.40 ± 4.59%
			Savlon (1.5% Chlorhexidine gluconate, 15% Cetrimide)	90.91 ± 5.08%
			Scrub Stat IV (4% Chlorhexidine gluconate, 4% Isopropanol)	89.57 ± 6.70%
			Septisol (0.75% Hexachlorophene)	88.60 ± 5.36%
			Tap water	79.74 ± 4.80%
			Triclosan hand soap (Triclosan 0.5%)	91.29 ± 4.47%

Table 2 continued

		Whole hand	Alcare (62% emollientеd ethanol foam)	86.17 ± 4.28%
			Aquaress (nonantimicrobial soap)	91.39 ± 2.65%
			Bacti-Stat soap (0.1% Chlorhexidine gluconate, 0.50% Didecyl dimethyl ammonium chloride, 5% Isopropanol)	94.56 ± 5.75%
			Bioprep hand soap	81.44 ± 1.59%
			Dettol (4.8% 4-Chloro-3,5-xylenol, 9.4% Isopropanol)	90.67 ± 2.08%
			70% Ethanol	86.92 ± 1.63%
			Savlon (1.5% Chlorhexidine gluconate, 15% Cetrimide)	86.53 ± 3.44%
			Scrub Stat IV (4% Chlorhexidine gluconate, 4% Isopropanol)	81.15 ± 1.15%
			Septisol (0.75% Hexachlorophene)	89.20 ± 0.81%
			Tap water	81.57 ± 4.55%
			Triclosan hand soap (Triclosan 0.5%)	88.98 ± 1.73%
Fendler & Groziak (*20*)	HAV	Suspension (30s exposure)	Purell Instant Hand Sanitizer (62% Ethanol+emollients)	1.25 Log_{10}
McCarthy (*44*)	*L. monocytogenes*	Glove immersion (30s)		
		PBS (PBS) and	50 PPM Chloride (50ppm Sodium hypochlorite)	5 $^{(PBS)}$ - 3 $^{(CCW)}$ Log_{10}
		Crab cooking water (CCW)	100 PPM Chloride (50ppm Sodium hypochlorite)	5 $^{(PBS)}$ - 3 $^{(CCW)}$ Log_{10}
			Zep-I-dine^{TM} (25 iodine)	3 $^{(PBS)}$ - 3 $^{(CCW)}$ Log_{10}
			Zepamine A^{TM} (195 ppm active quaternaries)	5 $^{(PBS)}$ - > 4 $^{(CCW)}$ Log_{10}
			Zep^{TM} hand sanitizer (62% Ethanol)	4 $^{(PBS)}$ - 3 $^{(CCW)}$ Log_{10}
			Ultra Kleen (Peroxide-based powder 56g/3.8 L of water)	5 $^{(PBS)}$ - 5 $^{(CCW)}$ Log_{10}
Edmonds et al (*14*)	*E. coli*	Hand test		
		Moderate food soil load	Non-antimicrobial hand wash	2.86 Log_{10}
			STW (62% Ethanol gel)	2.84 Log_{10}
			STW (62% Ethanol foam)	3.84 Log_{10}
			70% AF foam	4.44 Log_{10}
			STW (70% AF foam)	4.61 Log_{10}
		Heavy food soil load	Non-antimicrobial hand wash	2.65 Log_{10}
			STW (62% Ethanol gel)	2.69 Log_{10}
			STW (62% Ethanol foam)	2.87 Log_{10}
			70% AF foam	2.99 Log_{10}
			STW (70% AF foam)	3.92 Log_{10}

Table 2 continued

Kampf et al. (*37*)	*E. coli*	Hand test	Purell Instant Hand Sanitizer (62% Ethanol)	3.05 ± 0.45 Log_{10}
			Alcare plus (62% Ethanol)	3.58 ± 0.71 Log_{10}
			Water	2.39 ± 0.57 Log_{10}
Czerwinski et al. (*10*)	*E. coli*	Suspension (15 s)	Hand sanitizer (Zylast Antiseptic, 76% Ethanol)	>6.14 Log_{10} (99.9%)
			Water-based antiseptic lotion (Zylast-Lotion, 0.2% BZT)	>6.14 Log_{10} (99.9%)
	St. aureus	Suspension (15s)	Hand sanitizer (Zylast Antiseptic, 76% Ethanol)	>6.14 Log_{10} (99.9%)
			Water-based antiseptic lotion (Zylast- 0.2% BZT)	4.09 Log_{10} (99.9%)
Courtenay et al. (*8*)	*E. coli*	Hand test	Cool water	94.96% [7]
		Heavy soil load		40.1% [8]
		Ground beef	Warm water	99.78% [7]
				79.7% [8]
			Hand washing with plain soap	99.98% [7]
				91.3% [8]
		Hand test	Hand sanitizer B (62% Ethanol+skin conditioner)	94.44% [7]
		(not soiled hands)	Hand sanitizer c (62% Ethanol+skin conditioner)	96.33% [7]
			Hand sanitizer P (62% Ethanol+skin conditioner)	96.07% [7]
			Hand sanitizer S (62% Ethanol+skin conditioner)	90.40% [7]
Fishler et al. (*22*)	*E.coli*	Hand test	Hand washing with plain soap	<2 Log_{10}
			Antimicrobial soap (0.46% Triclosan)	>3 Log_{10}
Gaonkar et al. (*28*)	*E. coli*	Suspension (15 s exposure)	Octoxy hand rub	7 Log_{10}
		Pig skin method (15 min post application)	Hand sanitizer (60% EtOH +Phenoxyethanol+BZK)	Residual 4.96 Log_{10}
			Hand sanitizer Avagards (61% EtOH +CHG)	Residual 5.04 Log_{10}
			Octoxy hand rub	Residual 0 Log_{10}
	St. aureus	Suspension (15 s exposure)	Octoxy hand rub	7 Log_{10}
		Pig skin model	Hand sanitizer (60%EtOH +phenoxyethanol+BZK)	Residual 5.11 Log_{10}
		15 min post application	Hand sanitizer Avagards (61% EtOH+CHG)	Residual 5.68 Log_{10}
			Octoxy hand rub	Residual 0 Log_{10}

Table 2 continued

Paulson et al (*47*)	*E.coli*	Hand test	Hand washing with plain soap	2.12 Log_{10}
			Antibacterial soap (PCMX)	1.9 Log_{10}
			Purell Hand sanitizer gel (62% Ethanol)	2.24 Log_{10}
			WS (antibacterial soap+hand sanitizer)	3.28 Log_{10}
Pickering et al. (*48*)	*E.coli*	Hand test:		
		Clean hand	ABHS	2.33 Log_{10}
		Dirt-covered hand	ABHS	2.32 Log_{10}
		Oil-coated hand	ABHS	2.13 Log_{10}
Shintre et al. (*54*)	*E.coli*	Suspension	ZBF hand rub (60% Ethanol+Farnesol+Benzethonium) chloride	>7 Log_{10}
	E.coli	Pig skin model (20 m post application)	ZBF hand rub (60% Ethanol+Farnesol+Benzethonium chloride)	Residual 3.26 Log_{10}
			Avagard™	Residual 4.70 Log_{10}
			Prevacare™	Residual 5.65 Log_{10}
			Triseptins	Residual 5.12 Log_{10}
			Alcohol gel base	Residual 5.60 Log_{10}
	St. aureus	Suspension	ZBF hand rub (Ethanol+Farnesol+Benzethonium chloride)	>7 Log_{10}
		Pig skin model (20 min post application)	ZBF hand rub (Ethanol+Farnesol+Benzethonium chloride)	Residual 1.89 Log_{10}
			Avagard™	Residual 4.94 Log_{10}
			Prevacare™	Residual 5.16 Log_{10}
			Triseptins	Residual 5.51 Log_{10}
			Alcohol gel base	Residual 5.37 Log_{10}
Edmonds et al. (*16*)	*St. aureus*	Suspension (15 s exposure)	Purell advanced hand sanitizer - 70% Ethanol gel	≥5.8 Log_{10}
			Purell advanced hand sanitizer - 70% Ethanol foam	≥4.2 Log_{10}
			Ethanol 70%	≥4.2 Log_{10}
		Hand test After $1^{(1A)}$ and 10 applications $^{(10A)}$	Purell advanced hand sanitizer - 70% Ethanol gel	$3.58^{(1A)}$ Log_{10} - $3.50^{(10A)}$ Log_{10}
			Purell advanced hand sanitizer - 70% Ethanol foam	$3.55^{(1A)}$ Log_{10} - $3.48^{(10A)}$ Log_{10}
			Sterillium comfort gel (90% ethanol gel)	$3.12^{(1A)}$ Log_{10} - $1.80^{(10A)}$ Log_{10}
			WHO recommended hand rub (80% Ethanol)	$3.07^{(1A)}$ Log_{10} - $2.39^{(10A)}$ Log_{10}
			WHO recommended hand rub (75% Ethanol)	$3.12^{(1A)}$ Log_{10} - $2.03^{(10A)}$ Log_{10}

Table 2 continued

			Purell advanced hand sanitizer - 70% Ethanol gel	$3.35^{(1A)}$ Log_{10} - $4.09^{(10A)}$ Log_{10}
			Purell advanced hand sanitizer - 70% Ethanol foam	$3.48^{(1A)}$ Log_{10} - $4.41^{(10A)}$ Log_{10}
			Endure 300 antimicrobial rinse - 62% Ethanol	$2.99^{(1A)}$ Log_{10} - $1.97^{(10A)}$ Log_{10}
			Avagard foam instant hand antiseptic (70% Ethanol)	$2.83^{(1A)}$ Log_{10} - $1.94^{(10A)}$ Log_{10}
			Avagard D (68% Ethanol)	$2.48^{(1A)}$ Log_{10} - $1.31^{(10A)}$ Log_{10}
			Alcare OR Foamed antiseptic hand rub (62% Ethanol)	$2.86^{(1A)}$ Log_{10} - $2.71^{(10A)}$ Log_{10}
			Rio gel antiseptico (70% Ethanol)	$2.88^{(1A)}$ Log_{10} - $2.47^{(10A)}$ Log_{10}
			Cutan Alcohol foam antiseptic hand rub (60%Ethanol)	$3.26^{(1A)}$ Log_{10} - $2.54^{(10A)}$ Log_{10}
Kaiser et al. (*35*)	*St. aureus*	Pig skin model	CHG wash only	4.22 Log_{10}
			CHG wash + 60% alcohol gel product and 0.25% Hydroxypropyl cellulose	4.12 Log_{10}
			CHG wash + 0.25% Carbomer in alcohol solution	1.07 Log_{10}
			CHG wash + 0.25% C10-30 Alkyl acrylate crosspolymer in alcohol solution	0.44 Log_{10}
			CHG wash + unthickened alcohol solution	4.11 Log_{10}
			CHG wash + Carbomer containing marketed Product A	0.54 Log_{10}
			CHG wash + Carbomer containing marketed Product B	0.56 Log_{10}
			CHG wash + Hydroxypropyl cellulose containing marketed Product C	4.26 Log_{10}
Fendler et al. (*21*)		Suspension	Purell Instant Hand Sanitizer (62% Ethanol+emollients)	
	Escherichia coli	(30 s exposure)		>5 Log_{10}
	Escherichia coli (O157;H7)			>5 Log_{10}
	L. monocytogenes			>5 Log_{10}
	St. aureus - methicillin-resistant strain.			>5 Log_{10}
	St. aureus - vancomycin-tolerant–methicillin-resistant			>5 Log_{10}
	Salmonella Enteritidis			>5 Log_{10}
	Salmonella Typhimurium			>5 Log_{10}
	Hepatitis A virus			1.75 Log_{10}
Charbonneau et al (*7*)	Natural food flora	Hand test Heavy soil load	Hand wash with non-medicated soap	W&S > WS> ABHSs
			Hand sanitizer (70% Ethanol)	ABHSs < W&S < WS
			WS (non-antimicrobial hand wash + 70% ETOH foam)	WS < W&S > ABHSs
Wongworawat et al. (*61*)	Natural flora	Hand test	Povidone-iodine scrub	Residual 2.5 $CFU^{(WR)}$ - 7.5 $CFU^{(R)}$
			Water-aided alcohol wash	Residual 0.5 $CFU^{(WR)}$ - 1.0 $CFU^{(R)}$
			Water-less alcohol-chlorexidine lotion	Residual 0.0 $CFU^{(WR)}$ - 0.0 $CFU^{(R)}$

*Hand product: BZT, Benzethonium chloride; W & S, water and soap; ABHRs, alcohol-based hand rubs; WS, Wash-sanitise; CHG, Chlorhexidine gluconate; STW, Sani-twice; W, PCMX, Para-chloro-meta-xylenol.

FCV, feline calicivirus; MNV, murine norovirus; HuNoV, human norovirus; HAV, Hepatitis A virus

(1) Artificial fingernails

(2) Natural fingernails

(3) Standard American Society for Testing and Materials (ASTM) finger pad method with rubbing (ASTM) finger pad method

(4) Modified American Society for Testing and Materials (ASTM) finger pad method (with rubbing)

(5) Viral reduction estimated through RT-qPCR

(6) Viral reduction estimated through Plaque assay

(7) Bare hands

(8) Gloves

(R) = Hands with ring(s)

(WR) = Hands without rings