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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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1 Running title: Efficacy of hand sanitizers in food preparation setting

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

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22 soiled hands

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26 **ABSTRACT**

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

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

76 *Staphylococcus aureus*, *Shigella* spp., *Streptococcus* Lancefield A and G and
77 parasites like *Cyclospora*, *Giardia* and *Cryptosporidium* (30).

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

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

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

107 Alcohol-based hand sanitizers, or alcohol-based hand rubs (ABHRs), are instant
108 hand hygiene products; the antimicrobial activity of which is due to the ability of
109 alcohol to denature protein. These products usually contain a quantity of alcohol,
110 varying from 60% to 95%, and a thickening agent or humectants such as polyacrylic
111 acid, glycerin, or propylene glycol to decrease the drying effect of alcohol. ABHRs
112 have documented microbiological activity against bacteria (21, 51), fungi and some
113 enveloped viruses including HIV, herpes, adenovirus, influenza and parainfluenza
114 viruses (20). Lower efficacy against non-enveloped “naked” viruses is generally
115 reported in the literature, and the level of inactivation seems to vary a lot depending
116 on the viruses tested, type of alcohol, concentration, and time of exposure (12, 20,
117 21, 29, 32, 49, 50, 52).

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

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

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MATERIALS AND METHODS

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

145 Three preliminary criteria were adopted to select journal papers. Only articles that
146 described levels of inactivation of foodborne pathogens (the actual pathogens not
147 surrogate microorganisms), used a research approach with quantitative outcomes,
148 and described studies undertaken in industrialized countries, were included in this
149 study. In contrast, all book chapters, studies carried out on microorganisms not

150 involved in foodborne illness, studies involving inactivation of foodborne
151 microorganisms from raw food or food contact surfaces were excluded before
152 analysis, based on the title and abstract.

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

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RESULTS

162 From amongst 2108 records originally matching the search terms, 38 unique
163 journal abstracts were preliminarily screened for eligibility after duplicates were
164 removed. Subsequent analysis of full text journal articles permitted selection of the
165 28 journal articles that are included in this review (Table 1). Among the selected
166 studies testing hand washing products against foodborne pathogens, ten papers
167 provided information on Norovirus, three on Hepatitis A virus, two on *Listeria*
168 *monocytogenes*, fourteen on *Escherichia coli*, eight on *Staphylococcus aureus* and
169 one on *Salmonella* spp. No scientific information was found for other pathogenic
170 bacteria like *Campylobacter* spp. and *Bacillus cereus*.

171 Besides the use of conventional water and soap or water only, products more
172 generally tested against pathogenic bacteria and viruses included antibacterial liquid
173 soaps, alcohol-based hand sanitizers and non-alcohol based sanitizers including
174 triclosan-, chlorexidine gluconate- (CHG), povidone-iodine- and quaternary

175 ammonium-based products like benzalkonium chloride (BZK) or benzethonium
176 chloride (BZT), 5-pyrrolidone-2-carboxylic acid (PCA) and copper sulphate
177 pentahydrate (CS). Hand washing practices considered also included use of soap
178 and nailbrush (40), *Wash-sanitise*, consisting of using hand sanitizers after hand
179 washing with water and soap (15, 30, 47), and a new hand hygiene regime known as
180 *SaniTwice* (a registered trademark of James Mann, Handwashing for Life,
181 Libertyville, IL) consisting of a two stage hand cleansing including application of an
182 excess of alcohol-based sanitizer, hand rubbing, cleaning hands with a paper towel,
183 and a final application of alcohol-based sanitizer (14).

184 The relative efficacy of products was generally tested *in vitro*, *ex vivo* and/or *in*
185 *vivo*. Most of the *in vitro* studies involved experiments carried out using a suspension
186 assay consisting of a standardized quantity of the target microorganism treated with
187 increasing concentrations of the test product, with the aim of estimating the
188 inactivation rate for each product used (1, 10, 16, 20, 21, 28, 29, 54, 46, 55). One *in*
189 *vitro* study evaluated inactivation rates of tested products on latex gloves immersed
190 in a solution of phosphate buffered saline (PBS) or crab cooking water artificially
191 contaminated with 5 log₁₀ CFU *L. monocytogenes*/ml (44). *Ex vivo* tests included
192 experiments carried out on pig skin from a freshly killed pig (the pig skin method)
193 previously treated with sanitizing products, then artificially contaminated with
194 challenge microorganisms to test residual activity of tested products after use (9, 28,
195 35, 54). *In vivo* studies involved experiments carried out with selected human
196 volunteers to estimate the efficacy of each tested product to remove or inactivate
197 target microorganisms from artificially contaminated whole hands, finger pads or
198 gloves. The vast majority of *in vivo* studies retrieved in the literature were carried out
199 on hands or finger pads artificially contaminated with pure cultures of bacteria or

200 viruses without the presence of food components or organic material (9, 16, 22, 29,
201 37, 38, 41, 42, 43, 47, 55). Seven studies evaluated the efficacy of hand washing
202 products in a food preparation setting on naturally and artificially soiled hands or
203 gloves (7, 8, 14, 15, 40, 44, 48). Three studies evaluated inactivation rates of
204 products on hands contaminated with viral suspensions prepared with other organic
205 loads like fetal bovine serum or feces (15, 36, 40). Other factors pertaining to food
206 preparation settings like hygiene of nails (40) and wearing rings when handling food
207 have also been minimally considered (61). A summary of the experimental
208 conditions applied and main findings from *in vitro*, *ex vivo* and *in vivo* evaluations in
209 all studies included in this review are summarized in Table 2. Information relating to
210 specific pathogens will now be summarised.

211 **Norovirus.** Because human norovirus (HuNoV) cannot be routinely cultured *in*
212 *vitro*, determining the effectiveness of sanitizers and disinfectants against HuNoV is
213 difficult. Methodologies used to estimate level of virus reduction include the use of
214 reverse transcription-quantitative real time PCR to quantify the number of RNA
215 copies of HuNoV extracted and purified from tested samples (41, 42, 46) and the use
216 of cultivable surrogates like Feline Calicivirus (FCV) and Murine Norovirus (MNV).
217 Norovirus surrogates were generally tested alone as an alternative to HuNoV (9, 15,
218 29, 36, 38, 40, 55), or in parallel with HuNoV (46).

219 Liu et al. (41) compared the efficacy of an antibacterial soap, alcohol-based
220 sanitizer containing 62% ethyl alcohol, and water rinsing for the removal of HuNoV
221 from artificially contaminated finger pads. Ethanol-based hand sanitizer was the least
222 effective hand product tested ($0.34 \pm 0.22 \log_{10}$ reduction). The greatest \log_{10}
223 reduction was observed for water rinse only ($1.38 \pm 0.49 \log_{10}$) and antibacterial soap
224 ($1.1 \pm 0.49 \log_{10}$). A separate study by Liu et al. (42) tested various commercially

225 available hand hygiene products containing 62% to 95% alcohol on finger pads
226 against multiple HuNoV strains. The study showed a wide range of efficacy (0.10 to
227 3.74 log₁₀ reduction), varying according to different products and strains tested. The
228 highest level of RNA reduction was achieved by a 70% ethanol gel containing
229 additional ingredients that seem to potentiate the virucidal activity of alcohol alone. A
230 limitation of the study reported by the authors was the presence of PCR inhibitors in
231 the test products that may have affected PCR amplification and led to an
232 overestimate of virus reduction.

233 Eight papers evaluated the efficacy of hand sanitizers against FCV and MNV.
234 Experimental methods used to estimate viral inactivation included a virus-specific
235 cytopathic effect (CPE) test consisting of culturing post treatment samples on a serial
236 dilution of permissive host cells (9, 15, 29, 36, 38, 40, 55), and a plaque assay test in
237 parallel with TaqMan real-time reverse transcription PCR (46). Park et al. (46)
238 evaluated *in vitro* virucidal efficacy of seven hand sanitizers containing ethanol,
239 triclosan and chlorhexidine against both Norovirus surrogates (i.e. FCV and MNV)
240 and human norovirus (HuNoV). None of the products demonstrated significant RNA
241 reduction when tested against HuNoV, whereas results achieved for Norovirus
242 surrogates showed different levels of viral reduction measured by plaques assay and
243 RT-qPCR. A general lack of correlation between the two detection methods and
244 different degrees of viral inactivation of FCV or MNV were generally observed. Only
245 a 72% alcohol pH 2.9 ABHR reduced the infectivity of both FCV and MNV (3.4 and
246 2.6 log₁₀, respectively) by the plaque assay test, whereas no correlation was found
247 between reduced infectivity and RNA reduction measured by real-time reverse
248 transcription PCR.. Conflicting results were also reported in two studies evaluating
249 both *in vitro* and *in vivo* efficacy of hand products against FCV and MNV. Gehrke et

250 al. (29) tested three types of alcohol - ethanol, 1- propanol and 2-propanol. *In vitro*
251 experiments showed higher effectiveness achieved by 50% and 70% 1-propanol
252 (10^4 -fold reduction) over ethanol and 2-propanol. In contrast, 70% ethanol achieved
253 higher viral inactivation ($3.78 \log_{10}$ reduction) *in vivo* than either 1-propanol or 2-
254 propanol (3.58 and $2.15 \log_{10}$ reduction, respectively). Steinmann et al. (55)
255 compared virucidal activity of three ABHRs and three antimicrobial soaps. Results
256 from suspension tests demonstrated $\geq 5 \log_{10}$ reduction of both FCV and MNV
257 achieved by two of three ABHRs tested which was greater efficacy than soaps
258 tested (typically $\leq 3 \log_{10}$ reduction). Conversely, the modified finger pad test carried
259 out against MNV only, showed superior antimicrobial activity of a povidone-iodine
260 soap ($4.62 \log_{10}$ reduction) compared to the other ABHRs and soaps tested. Two
261 studies evaluated *in vivo* efficacy of hand hygiene products against FCV only. Lages
262 et al. (38) tested four ABHRs, three non-alcoholic sanitizers and two triclosan-
263 containing antimicrobial liquid soaps after 30 s and 2 min exposure times. Limited
264 efficacy of all the products tested was generally observed; only one antimicrobial
265 soap containing 10% povidone-iodine ($\leq 2.67 \log_{10}$ reduction) and one ABHR
266 containing 95% ethanol ($\leq 1.30 \log_{10}$ reduction) achieved appreciable viral reduction
267 compared to water rinse tested in parallel. Czerwinski and Cozean (9) compared a
268 novel hand sanitizer containing benzethonium chloride (BZK), a 62% ABHR, an
269 antibacterial liquid soap, and water rinse. Apart from a promising level of inactivation
270 shown by the novel hand sanitizer ($3.49 \log_{10}$ reduction), generally viral reductions
271 were $< 1 \log_{10}$ in all the other cases. Two studies evaluated efficacy of products on
272 hands artificially contaminated with a fecal suspension of FCV. Kampf et al. (36)
273 tested efficacy of three ABHRs; greatest reduction in FCV was achieved by a 95%
274 alcohol containing hand sanitizer ($2.17 \pm 1.06 \log_{10}$). Lower concentrations of alcohol

275 did not demonstrate more than 1 log₁₀ viral reduction. Lin et al. (40) compared six
276 hand washing practices on contaminated natural and artificial nails. Use of soap and
277 nail brush (2.54±0.57 log₁₀ reduction) achieved the highest log₁₀ viral reduction,
278 followed by hand washing with antibacterial soap (2.26±0.42 log₁₀ reduction), and
279 then combined use of soap and hand sanitizer (2.13± 0.93 log₁₀ reduction). In
280 contrast, the use of hand sanitizer alone demonstrated limited efficacy (0.86±55 log₁₀
281 reduction). Presence of long nails on treated hands was found to significantly impact
282 efficacy of all the hand products tested. Finally, one study carried out by Edmonds et
283 al. (15) compared four hand hygiene regimes on hands contaminated with a viral
284 suspension of MNV prepared with 0.5% fetal bovine serum to mimic soiling with
285 organic matter. Hand hygiene practices included an antimicrobial soap, a 70%
286 alcohol gel, hand washing followed by hand sanitizing, and SaniTwice. Sanitizing
287 with 70% alcohol gel was slightly more effective (2.6±0.41 log₁₀ reduction) than hand
288 washing with antimicrobial soap (1.79±0.29 log₁₀ reduction). A higher level of viral
289 reduction was achieved by SaniTwice (4.04±0.33 log₁₀) and by the combination of
290 conventional hand washing and sanitizing (3.19±0.31 log₁₀).

291 **Hepatitis A virus (HAV).** Little information is available in the scientific literature
292 about relative effectiveness of hand washing products against HAV. Only three
293 studies describing efficacy of hand washing products against HAV were retrieved
294 (20, 21, 43). Fendler et al. (21) and Fendler & Groziak (20) demonstrated limited *in*
295 *vitro* efficacy of a commercially available alcohol-based hand sanitizer containing
296 62% alcohol and emollients against HAV. The levels of inactivation achieved by 30 s
297 timed exposure were 1.75 and 1.25 log₁₀ reduction, respectively, corresponding to
298 94.37% (21) and 94.4% (20) reduction of original inoculum.

299 A study by Mbithi et al. (43) evaluated elimination rates of 10 different products on
300 whole hands or finger pads artificially contaminated with a mixture of viruses and
301 feces. Formulations tested included a non-medicated soap, five ethanol-based hand
302 sanitizers, and four antibacterial liquid soaps, compared to tap water without soap
303 used as a control. None of the tested products reached a level of inactivation of
304 99.9%, which is generally desired. Inactivation rates observed from both whole-hand
305 and finger pad methods ranged from 79% to 94%. One antibacterial soap and non-
306 medicated soap attained a higher level of virus reduction ($\leq 94.56 \pm 5.75\%$ and
307 $\leq 91.39 \pm 2.65\%$, respectively) than alcohol based hand sanitizers ($\leq 90.67 \pm 2.08\%$) and
308 tap water ($\leq 81.57 \pm 4.5\%$). Residual infectivity, estimated as a mean number of
309 Plaque Forming Units through a plaque assay test, ranged from 0 to 0.64 PFU for
310 ABHRs, 0.63 to 1.74 PFU for antimicrobial soaps, 1.57 PFU for plain soap and 3.88
311 PFU for tap water. No information was found in the literature about the efficacy of
312 hand washing and hand sanitizers against HAV on hands soiled with food
313 components.

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

324 *monocytogenes*. Of the different products tested, only the peroxide-based product
325 achieved 5 log₁₀ reduction of attached *L. monocytogenes* on both soiled and non-
326 soiled contaminated gloves. The two chloride-based and the quaternary ammonium-
327 based products achieved 5 log₁₀ reduction on gloves contaminated with PBS
328 suspensions of *L. monocytogenes* (i.e. no food residue present) but demonstrated
329 lower efficacy (\leq 1-2 log₁₀ reduction) in the presence of crab cooking water. Iodine-
330 based sanitizer and alcohol-based instant hand sanitizer demonstrated lower
331 efficacy in both cases. No data about the efficacy of conventional hand washing in
332 removing *L. monocytogenes* from gloves or hands was found in the literature.

333 ***Staphylococcus aureus* and *Escherichia coli*.** Six studies assessed *in vitro*
334 and/or *ex vivo* efficacy of hand sanitizers against *St. aureus* and *E. coli*. Hand
335 formulations included conventional ABHRs and new generation hand products
336 containing a combination of active antimicrobials and other compounds like
337 thickening agents, emollients and natural compounds. Fendler et al. (21) reported
338 that > 5 log₁₀ reduction was achieved by a 62% alcohol based sanitizer against both
339 methicillin-resistant and vancomycin-tolerant and methicillin-resistant *St. aureus*,
340 non-pathogenic *E. coli* and *E. coli* O157:H7. High *in-vitro* inactivation rates were also
341 reported by Biagi et al. (1), Czerwinski et al. (10), Gaonkar et al. (28), Kaiser et al.
342 (35), and Shintre et al. (54). Biagi et al. (1) tested the *in vitro* efficacy of a new
343 combination of two natural compounds, pyrrolidone-2-carboxylic acid (PCA) and
344 copper sulphate pentahydrate (CS). The combination of PCA and CS demonstrated
345 higher efficacy than 70% ethanol and 60% isopropanol used alone. Czerwinski et al.
346 (10) tested the efficacy of a novel alcohol-based antiseptic and a novel water-based
347 antiseptic lotion prepared with a synergistic combination of ingredients centred on
348 Benzethonium chloride (BZT). The novel water-based product demonstrated

349 equivalent antimicrobial (99.9%) activity against *E. coli* and *St. aureus* strains
350 compared to the other alcohol-based product. Gaonkar et al. (28) tested an ABHR
351 prepared with an emollient (Octoxy) and other additional ingredients against *E. coli*
352 and methicillin-resistant *St. aureus*. *In vitro* evaluations showed $> 7 \log_{10}$ reduction of
353 both *E. coli* and *St. aureus* and *ex vivo* tests showed higher antimicrobial activity and
354 superior residual activity after use of the novel Octoxy compared to the two other
355 ABHRs applied in parallel as a control. Kaiser et al. (35) compared *ex vivo* a
356 combination of a surgical scrub containing 4% Chlorhexidine gluconate (CHG) and
357 ABHRs prepared with and without thickening agents against *St. aureus*. Hand
358 sanitizers thickened with anionic polymers were found to negatively impact persistent
359 activity of CHG. In contrast, no negative effect was observed for ABHRs alone or
360 thickened with non-ionic compounds. Shintre et al. (54) tested the synergistic effect
361 of alcohol and quaternary ammonia in combination with moisturizers or essential oils
362 *in vitro* and *ex vivo*. Synergistic combination of farnesol and BZT demonstrated
363 better prolonged activity (i.e. 20-35 min post application) against *St. aureus* and *E.*
364 *coli* than other hand sanitizers and chemicals compounds used alone.

365 The high level of bacterial inactivation generally observed *in vitro* does not
366 necessarily reflect the actual capacity of products to remove transient
367 microorganisms from the outer layers of skin of hands. Incomplete effectiveness
368 against target microorganisms from cleaned hands is generally reported in all studies
369 carried out on hands artificially contaminated with *E. coli*. Edmonds et al. (16)
370 compared the efficacy of two novel 70% alcohol gel and foam, seven commercially
371 available ABHRs, and two World Health Organization recommended formulations
372 containing 60-90% alcohol against one methicillin-resistant *St. aureus* strain. Results
373 showed superior efficacy of the novel gel and foam preparations after single and

374 multiple uses compared to the other products. However none of the products
375 exceeded 3 log₁₀ reduction of the target microorganism. Fishler et al. (22) evaluated
376 the effectiveness of two hand washing regimes in reducing transient bacteria after
377 single wash and subsequent potential transfer of bacteria to a ready-to-eat food. The
378 antimicrobial soap achieved a higher level of bacterial removal (>3 log₁₀ reduction)
379 than plain soap (≤2 log₁₀ reduction), but failed to avoid the transfer of seeded
380 bacteria to a ready-to eat food item. Kampf et al. (37) reported limited efficacy of two
381 ABHRs on hands artificially contaminated with *E.coli*. Bacterial inactivation achieved
382 by two 62% alcohol containing products was only slightly better (≤3.5±0.45 and
383 3.58±0.71 log₁₀ reduction) than rubbing under running water applied in parallel
384 (2.39±0.57). Paulson et al. (47) examined the abilities of four hand washing regimes
385 including plain soap, an antimicrobial soap, an alcohol hand sanitizer, and combined
386 use of an antimicrobial soap and a ABHR (used after hand washing). All the products
387 used alone performed equally and none exceeded 2 log₁₀ reduction. Higher efficacy
388 (3.28 log₁₀ reduction) was observed by combined use of hand washing and hand
389 sanitizing.

390 ***Salmonella* spp.** Little information about the efficacy of sanitizing products
391 against *Salmonella* spp. is available in the literature. Only one *in vitro* study (21)
392 reporting > 5 log₁₀ reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium
393 being achieved by a 62% ABHR was retrieved.

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

399 (7, 14, 15, 40), dirt or cooking oil (48). Efficacy of hand products was estimated
400 based on the enumeration of microorganisms released from treated hands, or based
401 on the enumeration of bacteria remaining on hands. Methods for enumerating
402 released bacteria included the glove juice (14, 15, 61) or the hand rinse (8, 48)
403 techniques. Both techniques consist of enumerating bacteria released from washed
404 hands previously placed into a glove or a bag filled with sterile water or buffer.
405 Conversely, enumeration of bacteria remaining on the hands after hand washing or
406 hand sanitizing is usually estimated through image analysis or by pressing washed
407 hand palms onto the surface of an agar plate (7).

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

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

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

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

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

453

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DISCUSSION

455 Effective hand washing is extremely important to help prevent harmful
456 microorganisms from spreading from people's hands to food. Contact with bare
457 hands and failure to properly wash hands have been reported as the main risk factor
458 contributing to foodborne disease caused by food handlers (57). European Union
459 food safety legislation requires every person working in a food handling area to
460 maintain a high standard of personal cleanliness, and food business operators to
461 provide an adequate number of washbasins suitably located and designed for
462 cleaning hands (18). The Food Code 2009, published by the Food and Drug
463 Administration to standardize food safety and food hygiene procedures, states that
464 the total time recommended for proper hand washing is at least 20 seconds, of which
465 10-15 seconds should be used for rubbing followed by rinsing under running warm
466 water and drying hands (24).

467 The presence of food components like fat, oil or other dirt is considered the main
468 factor affecting removal and inactivation rates of hand hygiene products against
469 microorganisms occurring on the hands of food workers (26). The levels of microbial
470 contamination reported for hands of food workers can vary between 2 and $>5 \log_{10}$
471 cfu/hand across various food settings, and the bacterial flora generally encountered
472 on the hands of food handlers is a mixture of Enterobacteriaceae and other
473 mesophilic bacteria in the presence of fat and other soil (11). Various pathogens with

474 very low infective doses (1 to 100 units) including viruses, parasites and enteric
475 bacteria can be present on contaminated hands in high numbers (58). Pathogens
476 carried by contaminated hands can be easily transferred to food and hand contact
477 surfaces and can survive for long periods (58, 59). The ideal hand hygiene regime to
478 be used in a food setting should ensure maximum removal of food components and
479 food flora from cleaned hands in order to minimize the level of transferable
480 microorganisms. Most of the hand disinfectants, including medicated soaps and
481 instant hand sanitizers, have a broader antimicrobial activity than plain soaps but are
482 generally considered not to properly meet the needs of food workers because they
483 are unable to remove food soil from cleansed hands (23).

484 This systematic review evaluated the scientific information available in the
485 literature about the efficacy of conventional and improved hand hygiene products in
486 relation to their use in food preparation settings. Analysis of the literature showed the
487 existence of conflicting reports about the efficacy of soaps and hand sanitizers
488 against foodborne pathogens. No standardized method to estimate removal and
489 inactivation rates of target pathogens seems to be available and the varying
490 experimental conditions (including quantity of product used, duration of treatment,
491 type of food soil used) between different studies makes comparison of results
492 difficult. Hand washing with water and soap is generally reported to achieve
493 effective removal of bacteria and soil from hands (7, 8, 14, 15) and gloves (8) and to
494 be superior to other products in the removal of bacteria and viruses from fingernails
495 when used with a nailbrush (40). However, a residual level of microorganisms even
496 after proper washing is generally reported (7, 8, 14, 15, 40), suggesting that hand
497 washing alone cannot ensure elimination of risk in relation to bacterial transmission
498 from hands to food. Conventional hand washing is more effective on contaminated

499 hands than on gloves (8) suggesting that frequent changes of gloves rather than
500 washing gloves when they become visibly soiled would more effectively minimize
501 risk of bacterial contamination between different food preparation steps.

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

509 Instant hand sanitizers have shown high and rapid *in vitro* efficacy against various
510 target bacteria (10, 16, 21, 54), whereas their efficacy against naked viruses seems
511 to be lower (20, 21, 38, 46) and vary according to different viruses tested, type of
512 alcohol and concentration used (29, 46, 55). These findings are in general
513 agreement with four other reviews (2, 3, 31, 60). Apart from some improved
514 formulations (9, 32), instant hand sanitizers used *in vivo* do not usually exceed 2-3
515 log₁₀ microbial reduction (16, 37, 41, 42, 47, 48) and their efficacy seems to be
516 affected by the presence of food debris, as observed on both moderate (44) and
517 heavily soiled hands (7, 14, 40), as only one study included in this systematic review
518 reported similar rates of bacterial inactivation on both clean and soiled hands (48).
519 Instant hand sanitizers used alone seem not to be a reliable substitute for
520 conventional hand washing in food establishments (7). In contrast, their application
521 after hand washing, previously carried out with either antimicrobial or plain soap (i.e.
522 wash-sanitize regimes), seems to be more effective than hand sanitizer or soaps
523 used alone (15, 47); levels of bacterial inactivation have been demonstrated to

524 significantly increase up to 4 or 5 log₁₀ reduction on both moderately and heavily
525 soiled hands (15).

526 Preliminary results reported for SaniTwice are also encouraging (14). The method
527 tested on hands moderately and heavily soiled with a mixture of food components
528 and *E. coli* showed good levels of bacterial reduction (~4 log₁₀ reduction). A similar
529 level of inactivation is also reported against MNV on artificially contaminated hands.
530 These findings suggest that this hand hygiene regime could be used as an
531 alternative to wash-sanitize when water and soap are not available. However, no
532 evidence about the efficacy of this hand hygiene regime against HuNoV or HAV on
533 soiled hands seems to be available in the literature. For this reason, further studies
534 would be needed to prove the effectiveness of SaniTwice in different food settings
535 and against different foodborne pathogens.

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

542

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755 Table 1. Number of scientific publications matching search terms retrieved from
 756 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

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

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765 Table 2. Summary of results regarding efficacy of hand sanitisers presented in the scientific papers included in this systematic
 766 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	
	<i>E. coli</i>	Pig skin method 2 min post application 1h post application 4h post application	Novel alcohol-based antiseptic containing BZT		1.65 Log ₁₀
					1.34 Log ₁₀
					1.15 Log ₁₀
	<i>St. aureus</i>	2 min post application 1h post application 4h post application	Novel alcohol-based antiseptic containing BZT		1.87 Log ₁₀
					2.14 Log ₁₀
				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
		Finger tips	1-Propanol 80%	1.35 Log ₁₀
			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
Kampf et al. (36)	FCV	Fingerpad Organic soil 5% fetal bovine serum	2-Propanol 90%	0.76±0.19 Log ₁₀
			Water	1.23±0.44 Log ₁₀
			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
Lages et al. (38)	FCV	Finger tips 30 s and 2 min contact periods	Desderman N (75.1% Ethanol)	1.07±0.61 Log ₁₀
			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
Lin et al. (40)	FCV	Fingertips (Artificial feces)	Water	0.33 (30s) - 0.42 (2 min) Log ₁₀
			Tap water	1.22±0.86 ⁽¹⁾ Log ₁₀
			Soap	1.97±0.68 ⁽²⁾ Log ₁₀
				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⁾
	<i>E. coli</i>	Fingertips	Tap water	1.29±0.53 Log ₁₀ ⁽¹⁾
		Heavy food soil load		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% Chlorhexidine - 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 - pH 3.0)	0.0±0.2 Log ₁₀ ⁽⁵⁾ 0.2±0.1 Log ₁₀ ⁽⁶⁾
		Hand sanitizer (4% Chlorhexidine - 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)
		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% Chlorhexidine - 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)

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-xyleneol, 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% emolliented 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-xyleneol, 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)	<i>L. monocytogenes</i>	Glove immersion (30s)		
		PBS (PBS) and Crab cooking water (CCW)	50 PPM Chloride (50ppm Sodium hypochlorite)	5 ^(PBS) - 3 ^(CCW) Log ₁₀
			100 PPM Chloride (50ppm Sodium hypochlorite)	5 ^(PBS) - 3 ^(CCW) Log ₁₀
			Zep-I-dine™ (25 iodine)	3 ^(PBS) - 3 ^(CCW) Log ₁₀
			Zepamine A™ (195 ppm active quaternaries)	5 ^(PBS) - > 4 ^(CCW) Log ₁₀
			Zep™ 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)	<i>E. coli</i>	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)	<i>E. coli</i>	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)	<i>E. coli</i>	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%)	
	<i>St. aureus</i>	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)	<i>E. coli</i>	Hand test	Cool water	94.96% ⁽⁷⁾	
			Heavy soil load	40.1% ⁽⁸⁾	
			Ground beef	99.78% ⁽⁷⁾	
				Warm water	79.7% ⁽⁸⁾
				Hand washing with plain soap	99.98% ⁽⁷⁾
					91.3% ⁽⁸⁾
				Hand test (not soiled hands)	Hand sanitizer B (62% Ethanol+skin conditioner)
			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)	<i>E.coli</i>	Hand test	Hand washing with plain soap	<2 Log ₁₀	
			Antimicrobial soap (0.46% Triclosan)	>3 Log ₁₀	
Gaonkar et al. (28)	<i>E. coli</i>	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	
		<i>St. aureus</i>	Suspension (15 s exposure)	Octoxy hand rub	7 Log ₁₀
				Pig skin model 15 min post application	Hand sanitizer (60%EtOH +phenoxyethanol+BZK)
			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)	<i>E.coli</i>	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)	<i>E.coli</i>	Hand test:	Clean hand	2.33 Log ₁₀	
			Dirt-covered hand	2.32 Log ₁₀	
			Oil-coated hand	2.13 Log ₁₀	
Shintre et al. (54)	<i>E.coli</i>	Suspension	ZBF hand rub (60% Ethanol+Farnesol+Benzethonium chloride)	>7 Log ₁₀	
			Pig skin model (20 m post application)	ZBF hand rub (60% Ethanol+Farnesol+Benzethonium chloride)	Residual 3.26 Log ₁₀
	<i>St. aureus</i>	Suspension	Pig skin model (20 min post application)	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
				ZBF hand rub (Ethanol+Farnesol+Benzethonium chloride)	>7 Log ₁₀
				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)	<i>St. aureus</i>	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 ^(10A) applications	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)	<i>St. aureus</i>	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)	<i>Escherichia coli</i> <i>Escherichia coli</i> (O157;H7) <i>L. monocytogenes</i> <i>St. aureus</i> - methicillin-resistant strain. <i>St. aureus</i> - vancomycin-tolerant-methicillin-resistant <i>Salmonella</i> Enteritidis <i>Salmonella</i> Typhimurium Hepatitis A virus	Suspension (30 s exposure)	Purell Instant Hand Sanitizer (62% Ethanol+emollients)	>5 Log ₁₀ >5 Log ₁₀ >5 Log ₁₀ >5 Log ₁₀ >5 Log ₁₀ >5 Log ₁₀ >5 Log ₁₀ 1.75 Log ₁₀
Charbonneau et al (7)	Natural food flora	Hand test Heavy soil load	Hand wash with non-medicated soap Hand sanitizer (70% Ethanol) WS (non-antimicrobial hand wash + 70% ETOH foam)	W&S > WS > ABHSs ABHSs < W&S < WS WS < W&S > ABHSs
Wongworawat et al. (61)	Natural flora	Hand test	Povidone-iodine scrub Water-aided alcohol wash Water-less alcohol-chlorexidine lotion	Residual 2.5 CFU ^(WR) - 7.5 CFU ^(R) Residual 0.5 CFU ^(WR) - 1.0 CFU ^(R) Residual 0.0 CFU ^(WR) - 0.0 CFU ^(R)

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

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

770 ⁽¹⁾ Artificial fingernails

771 ⁽²⁾ Natural fingernails

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

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

774 ⁽⁵⁾ Viral reduction estimated through RT-qPCR

775 ⁽⁶⁾ Viral reduction estimated through Plaque assay

776 ⁽⁷⁾ Bare hands

777 ⁽⁸⁾ Gloves

778 ^(R) = Hands with ring(s)

779 ^(WR) = Hands without rings

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