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Review

# 손소독제(겔형, 액제형, 와이프형)의 효능 평가법 개선: 평가 전략 연구 사례 및 손 균총 정보 활용 등 최근 동향

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# Improvement of the Efficacy Test Methods for Hand Sanitizers (Gel, Liquid, and Wipes): Emerging Trends from *in vivo*/*ex vivo* Test Strategies for Application in the Hand Microbiome

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**ABSTRACT -** Skin sanitizers are effective in killing or removing pathogenic microbial contaminants from the skin of food handlers, and the progressive growth of consumer interest in personal hygiene tends to drive product diversification. This review covers the advances in the application of efficacy tests for hand sanitizers to suggest future perspectives to establish an assessment system that is optimized to each product type (gel, liquid, and wipes). Previous research on the *in vivo* simulative test of actual consumer use has adopted diverse experimental conditions regardless of the product type. This highlights the importance of establishing optimal test protocols specialized for the compositional characteristics of sanitizers through the comparative analysis of test methods. Although the operational conditions of the mechanical actions associated with wiping can affect the efficacy of the removal and/or the inactivation of target microorganisms from the skin's surface, currently there is a lack of standardized use patterns for the exposure of hand sanitizing wipes to skin. Thus, major determinants affecting the results from each step of the overall assessment procedures [pre-treatment – exposure of sanitizers – microbial recovery] should be identified to modify current protocols and develop novel test methods. The *ex vivo* test, designed to overcome the limited reproducibility of *in vivo* human trials, is also expected to replicate the environment for the contact of sanitizers targeting skin microorganisms. Recent progress in the area of skin microbiome research revealed distinct microbial characteristics and distribution patterns after the application of sanitizers on hands to establish the test methods with the perspectives on the antimicrobial effects at the community level. The future perspectives presented in this study on the improvement of efficacy test methods for hand sanitizers can also contribute to public health and food safety through the commercialization of effective sanitizer products.

**Key words:** Sanitizer, Efficacy test, Standardization, Personal hygiene, Hand microbiome

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The general perception of personal hygiene among food handlers and consumers has increased to prevent infectious diseases after the COVID-19 pandemic 2020[1]. Although a representative method to avoid contact-dependent infection is washing hands, the limited conditions that soap and water are required to wash make people use sanitizer[2]. Most product types of hand sanitizers (gel, liquid, and wipes) are convenient to carry and have the advantage of quickly keeping part of the body safe from the risk of microbial contaminants. However, public doubts about the effectiveness and potential health hazards of hand sanitizers have also emerged with the expanded use of the products[3,4]. Moreover, the increase in consumers' demands for effective and non-irritating hand sanitizers results in the diversification of products, and thus the efficacy test methods applicable to the various products are also needed[5].

This review article suggests the characteristic feature of the standardized method and/or in-house protocols of efficacy tests using hand sanitizers to establish strategies for improving the current test methods. Overall results from the previous relevant studies regarding the assessment of the efficacies of hand sanitizer products according to the typical product types of hand sanitizers (gel, liquid, and wipes) were compared with the perspectives of the principles for experimental methodologies (*in vitro*, *in vivo*, and *ex vivo* tests), target microorganisms, and treatment conditions. Various test methods have been developed and applied to hand sanitizers. Standardized efficacy test methods provided by the institutions governing the effectiveness of hand sanitizers are summarized in Table 1. Representative previous relevant studies using efficacy test methods including the modified and in-house protocols are also described in Table 2. As the methods of standard efficacy tests for hand sanitizer can be different according to the organizations (e.g., countries, institutions), we figure out the strategies for the improvement of current test methods by the comprehensive comparative analysis of strengths and limitations from those independent methods. Advances in the research associated with the efficacy of hand sanitizers to suggest key considerations for the modification of test methods were also identified and included in the design of future perspectives on the establishment of a reliable efficacy assessment system.

## Efficacy test methods applicable to hand sanitizers – *in vitro* tests

Representative *in vitro* test methods can be divided into disc diffusion, well diffusion, and suspension testing. Disc diffusion evaluates the efficacy of antimicrobials by the extent of the inhibitory area on the surface of a solid medium incubated with the antimicrobials-impregnated disc[6]. Antimicrobial components form a concentration gradient around the disc when a disc containing antibiotics is placed on a medium[7,8]. The sterile disc to be fully absorbed in each antimicrobial is one of the critical factors for the experimental results, and the volume of the product exposed to the disc is generally recommended to be set for obtaining a disc fully impregnated with samples[8]. The well diffusion is the method of loading antimicrobial substances in a well directly on the solid medium inoculated with target microorganisms[9]. Manaye et al.[10] made eight holes on the solid medium plate by using a sterilized 6 mm cork bore and filled holes with alcohol-based hand sanitizer except for one hole filled with sterilized water as control. Tests for the determination of minimum inhibitory concentration (MIC) and/or minimum bactericidal concentration (MBC) are also generally applied to the active substances of hand sanitizers[11-14]. In the case of quantitative suspension tests of sanitizing efficacies, direct killing effects by the exposure of target microorganisms to the antimicrobials under the soiling conditions can be assessed[14]. Representative standardized methods of the suspension tests for the active substances and/or biocidal products of hygienic handrub are provided by the guidance of the Biocidal Products Regulation from the European chemical agency according to the target microorganisms as follows: suspension tests for demonstrating bacteria (EN 13727)[15,16], yeast (EN 13624)[17], or virus (EN 14476)[18,19].

## Efficacy test methods applicable to hand sanitizers – *in vivo* tests

The target for the treatment of hand sanitizers for *in vivo* efficacy tests is the human body, and thus the major goal of the determination of experimental methods is to simulate the actual use by consumers. Representative test principles are the finger dipping method, fingerpad method, and glove juice method for the hand. Although the changes in the quantitative microbiological population level of hand microbiota after the treatment of sanitizers is regarded as the indicator of the efficacy, most studies mainly validate the sanitizing effects by the inactivation and/or removal of specific microbial strains artificially inoculated on human skin due to the individual diversity of hand microbiome-derived by internal (e.g., gender, age) and external factors (e.g., use of cosmetic products to be applied to hands, housemates, pets)[20,21].

The finger dipping method is standardized as a European standard (EN 1500) established for hygienic handrub, and the desirable treatment conditions (e.g., standard handrub procedure with the contact time of 30-60 sec after the addition of the products onto volunteers' palms) is set according to the relevant regulation[22]. The standard handrub

**Table 1.** Standard test methods for the analysis on the efficacy of hand sanitizers

| Category | Test codes | Target product type | Target microorganisms | Treatment methods |
|---|---|---|---|---|
| *in vivo* (finger dipping) | EN 1500 | Gel | *Escherichia coli* | Subjects perform standard handrub procedures for 30-60 sec [palm to palm right palm over left dorsum and left palm over right dorsum (5 times) palm to palm with fingers interlaced (5 times) backs of fingers to opposing palms with fingers interlocked (5 times) rotational rubbing of right thumb clasped in left palm and vice versa (5 times) rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa (5 times)]. |
| *in vivo* (fingerpad) | ASTM E2276-10 | Gel | *Acinetobacter baumannii*, *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis* | Subjects put their hand on the vial containing the 1 mL test preparation and perform a 10-30 sec massage while turning it over about 10 times. |
| *in vivo* (fingerpad) | ASTM E1838-17 | Gel | Hepatitis A Virus, Human Adenovirus (Type 2 or 5), Human Rotavirus | The gel is distributed in a 35 mm*10 mm sterile petri dish, then the experimental target touches the gel and rubs 30 sec with the index finger. |
| | | Wipe | Feline calicivirus, Human Rhinovirus Type 37, Rhinovirus 14, Murine Norovirus Type 1 | A gloved technician removes the wipe from the wrapper so that they do not contaminate, with the wipe folded, carefully puts it on the sterilized side. The experimental targets rub the fingerpad inoculated with virus using wipe for 15 sec under moderate pressure, rub it up and down 4 times and rub it left to right 4 times, and repeat these rub process for 15 sec. The technician then turns the front of the wipe upside down, and the experimental targets wipe for another 15 sec. |
| *in vivo* (fingerpad) | ASTM E2613-14 | Gel | *Aspergillus niger*, *Candida albicans* | Subjects perform a 10-30 sec massage of hand putted on the vial containing the 1 mL test preparation while turning it over about 10 times. |
| *in vivo* (glove juice) | ASTM E2011−21 | Gel | Feline calicivirus Murine, Human Adenovirus Type 5, Human Rhinovirus Type 37 or 14, Human Rotavirus, Norovirus | Experimental targets rub gel on their hands softly for 90 sec. |
| *in vivo* (glove juice) | ASTM E2755-22 | Wipe | *Serratia marcescens*, *Staphylococcus aureus* | The tester wipes on the backside and frontside of both hands (approximately 5 sec). The subject should rub each finger and thumb, wrap each finger, and wet the entire surface completely (approximately 15 sec). Subjects turn the wipe over and rub the palm to the wrist, and then rub the back of the hand to the wrist (approximately 10 sec). |
| | | Gel | | Subjects rub the gel (1.5 mL) dispensed on subject's hands for 30 sec. |
| *in vivo* (swab or glove juice) | ASTM E1115-11 | Gel | Natural hand microorganisms | Subjects rub the gel (1.5 mL) dispensed on hands. |
| *ex vivo* (cup scrub) | ASTM E2897-22 | Liquid | - | Test formulation is applied in appropriate amount of volume on the pigskin and is rubbed for 30 sec to equally distribute to skin. |

**Table 2.** Research on the efficacy test methods for hand sanitizers

| Category | Target product type | Target microorganisms | References |
|---|---|---|---|
| *in vitro* (disc diffusion) | Gel | *Staphylococcus aureus* | Rahmasari et al.[8] |
| *in vitro* (disc diffusion) | Liquid | *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* | Surwase et al.[7] |
| *in vitro* (well diffusion) | Liquid | *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella boydii*, *Staphylococcus aureus* | Manaye et al.[10] |
| *in vitro* (time-kill test) | Gel and liquid | *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* | Kobayashi et al.[39] |
| *in vivo* (glove juice, swab) | Gel and liquid | Natural hand microorganisms | Zapka et al.[45] |
| *in vivo* (swab) | Liquid | SARS-CoV-2 | Kratzel et al.[73] |
| *in vivo* (glove juice) | Gel and liquid | Natural hand microorganisms | Christie and Sidhu[40] |
| *in vivo* (hand-rinse method) | Wipe | Natural hand microorganisms | Mihalache et al.[42] |
| *in vivo* (glove juice) | Wipe | Clostridium difficile | Oughton et al.[74] |
| *in vivo* (glove juice) | Wipe | Natural hand microorganisms | Butz et al.[29] |
| *in vivo* (glove juice) | Gel and wipe | *Geobacillus stearothermophilus*, *Serratia marcescens* | D'Antonio et al.[21] |
| *in vivo* (glove juice) | Gel and wipe | Influenza A (H1N1) virus | Larson et al.[20] |
| *ex vivo* (pig skin model), *in vivo* (human trials) | Gel | *Serratia marcescens*, *Staphylococcus aureus* | Kaiser et al.[43] |
| *in vitro* (disc diffusion) | Gel | *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis* | Jain et al.[75] |
| *in vitro* (well diffusion) | Liquid | *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* | Vuai et al.[76] |
| *in vitro* (disc diffusion) | Gel | *Clostridium difficile*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* | Jain et al.[75] |
| *in vitro* (well diffusion) | Liquid | *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* | Vuai et al.[76] |
| *in vitro* (well diffusion) | Gel and liquid | *Escherichia coli*, *Staphylococcus aureus* | Chojnacki et al.[77] |
| *in vitro* (well diffusion) | Liquid | *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* | Selam et al.[78] |
| *in vitro* (disc diffusion) | Gel and liquid | *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella boydii*, *Staphylococcus aureus* | Manaye et al.[10] |
| *in vitro* (disc diffusion) | Gel | *Acinetobacter baumannii*, *Candida albicans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis* | Booq et al.[79] |
| *in vivo* (swab) | Gel and liquid | Natural hand microorganisms | Babeluk et al.[80] |

procedure requires to follow 6 steps [palm to palm – right palm over left dorsum and left palm over right dorsum (5 times) – palm to palm with fingers interlaced (5 times) – backs of fingers to opposing palms with fingers interlocked (5 times) – rotational rubbing of right thumb clasped in left palm and vice versa (5 times) – rotational rubbing, backward and forwards with clasped fingers of the right hand in left palm and vice versa (5 times)][23].

The fingerpad method guides to treat hand sanitizers by the contact of the same finger between the left and right hands to mimic rubbing of hand sanitizers by consumers[24,25]. Fingerpad tests have been established through ASTM-approved methods according to the objectives for the application of products (e.g., gel, liquid, and wipes) as follows: bactericidal effects (ASTM E2276-10)[26], virucidal effects (ASTM E1838-17)[27], and fungicidal effects (ASTM E2613-14)[28]. Although the recapitulation of consumers' usage patterns is limited because the microbial inoculation and the treatment of products are conducted on the part of the hand (i.e., fingers), the experimental conditions can be easily controlled compared with typical simulative analysis methods for hand sanitizers. To simulate the application of sanitizers through rubbing hands, the standard fingerpad test method to expose products to finger skin was revised from the use of a plastic vial containing the hand sanitizers contacted with the finger by the specific number of full inversions (ASTM E1838-10)[27] to rubbing fingers from both hands (ASTM E1838-17)[24,29]. Thus, the standardized protocols for the exposure of gel and liquid products are similar among the test methods. Whereas the operating conditions for the treatment of wipes including the direction, time, and number of repetitions of rubbing are also standardized in the test protocols but the mechanical force applied by the tester to the skin of the human subject during the wiping is indicated as moderate pressure, highlighting the importance of the assessment of the impact of this factor to the test results for the improvement of the methodological guidelines to be clarified[20].

The glove juice method for hand is designed to evaluate the hand sanitizing effects on the microorganisms distributed on the whole part of the hand after the use of sanitizer by the elution of residual microorganisms on the hands through the massage in gloves filled with dilution buffers. Standard test methods describe recommended protocols for determining the microorganism-eliminating effectiveness of handrub (ASTM E2755-22)[30]. Since glove juice method has been mainly adopted for the exploration of the efficacy of hand sanitizers by allowing human subjects to use the products with conventional methods for the recapitulation of consumers' actual usage pattern, the differences in the experimental design and treatment methods have been reported as follows: treatment amount and time [manufacturer's instructions (e.g., wiping the entire hand using one sheet of wipe according to the recommended use specified by manufacturer)[21], quantified values (e.g., 1.5 mL for 10 min)[20], minimum criteria (e.g., 1 mL for at least 20-30 sec until the complete drying)[13], specific unit (e.g., applying one pump from the commercial product of the sanitizer gel followed by the massage and sufficient drying process)[21]], number of subjects participating in the test according to the minimum criteria for standard methods (e.g., no fewer than six subjects)[25], comparative analysis on the different product types (e.g., sequential treatment of multiple products to same human subject)[30]. In terms of the experimental factors which are not regulated as specific quantitative values, comparative analysis on the impact of the different conditions within the range for the applicable value of those factors to the achievement of efficacy is expected to improve the test methods by the clarification of the determinant factors to be regulated for the accurate results[20]. Especially the efficacy of hand sanitizer wipes can be affected by the different forces and the repetition of wiping which may result in a lack of uniformity for the test protocols and the different results among individuals.

## Efficacy test methods applicable to hand sanitizers – *ex vivo* tests

Pig skin has been mainly used as an *ex vivo* test for the efficacy tests of hand sanitizers. The standard method (ASTM E2897-12)[31] using fur-removed pig skin cut (sizes as 13.85 $cm^2$) describes the hand sanitizers by the dispense of sample products in the cylinder fixed to the skin surface followed by rubbing for 30 sec, and the results are analyzed using the cup-scrub technique (ASTM E1874)[32,33]. Although other *ex vivo* models mimicking the human skin have been reported to be applied as alternatives to animal or human skin tests (e.g., toxicity tests for cosmetics) including the synthetic skin[34-36] and artificial skin tissue models[37,38], the application study to evaluate the efficacy of hand sanitizers is scarcely reported.

## Major research findings supportive of the development and the application of efficacy test methods for hand sanitizers

Findings from the results of the efficacy test of hand sanitizers conducted in previous studies imply key considerations to conduct the accurate assessment and test protocols need to be improved. Microorganisms revealed as highly resistant against hand sanitizers by the efficacy tests can be suggested as the target microbial species required to

be controlled by the sanitizing procedures. Escudero-Abarca et al.[24] evaluated the capability of alcohol-based commercial hand sanitizer products to inactivate human norovirus (hNoV) but all products tested couldn't eliminate hNoV, highlighting the role of efficacy tests using resistant microorganisms as the confirmatory strategy of the limited spectrum of the sanitizers. Research on the influence of the storage and handling conditions of the product to be assessed prior to the initiation of the test can provide clues for the establishment of methods to assess the stability of sanitizers. The method for managing the sample product is needed to be regarded as a control variable because the change in the composition and antimicrobial capacity of ingredients affect the efficacy of hand sanitizer products after opening even though the susceptibility to the environmental conditions inducing undesirable changes can be variable according to the physicochemical characteristics of active substances and/or formulated products[39].

There have been reported cases about the necessity of the improvement of the test method through the modification of the experimental conditions[40,41]. To reflect the practical hand sanitizer exposure time of healthcare workers using hand sanitizer, Christie and Sidhu[40] modified the standardized glove juice method (ASTM E2755-22)[30] by the reduction of contact time of sanitizers from 30 sec [i.e., time set in the standard method and also recommended by centers for disease control and prevention (CDC)] to 8 sec, and the shorter contact time did not significantly affect the effect. Although most of the commercial hand sanitizer products showed immediate and transient effects as evaluated by *in vivo* short-term efficacy evaluation, sanitizers feasible to work with residual effects should also be evaluated with the perspectives of the long-term sustained effects and their durability. Bondurant et al.[41] reported the long-term efficacy of hand sanitizer on human skin after the application of hand sanitizer containing benzalkonium chloride (1, 2, and 4 h).

The expected outcome of using the hand sanitizing wipe can be represented as not only the microbiological safety but also cleaning of the skin surface by removing soils, and the elimination of residual microorganisms on the hand after wiping can also be considered as the indicator for the efficacy of products. The bioluminescence measurement method established by Mihalache et al.[42] evaluated the cleaning effect of hand sanitizing wipes by the analysis of the organic dirt removed after the wiping treatment which can be applied as proper hygiene practices not only in the kitchens but also in other daily lives situations.

Previous studies on the development and application of the *ex vivo* test methods have suggested the strategy to alleviate the burden of the implementation of the *in vivo* tests by using repetitive *ex vivo* experiments as the preliminary steps to determine the treatment conditions verified by human trials[43,44]. Kaiser et al.[43] evaluated the incompatibility of chlorhexidine gluconate in hand sanitizers by the comparison between using excised pig skin as a surrogate skin substrate model (*ex vivo*) and a human subject (*in vivo*). Cheeseman et al.[44] reported that the efficacy of alcohol handrubs could be predictable by using *ex vivo* tests available for the analysis of the potential residual activity and friction effects.

New insights into the survival and inactivation of microorganisms on hands by the metagenomic approaches (i.e., hand microbiome) are expected to support the improvement of efficacy test methods of hand sanitizers[45,46]. Zapka et al.[45] proposed recommendations for the best practices in hand microbiome studies based on results from sampling of hands (50 human subjects) using a swab and glove juice methods before and after the treatment of alcohol-based hand sanitizer. Ramadhani et al.[46] reported the potential effects of hand hygiene practices using soap products and alcohol-based handrubs on the microbiota of human skin's homeostasis. Since there may be a considerable number of microbial species in the hand, hand microbiome data can be used to suggest novel microorganisms to be adopted as the target of efficacy tests (e.g., pathogens, dominant microbial species during the dysbiosis)[47,48].

## Future perspectives

Standardization of the overall procedures of efficacy assessment specialized for the types and composition of hand sanitizers is needed to ensure the repeatability and reliability of the tests, especially for the *in vivo* human trials. Differences in *in vivo* laboratory-based test protocols have been regarded as the major challenge for the evaluation of the reliability of data and the development of a well-designed protocol closely simulating the actual use by food handlers should be followed[49]. Comprehensive analysis of the previous relevant research regarding the efficacy test for hand sanitizer implied the diversification of the experimental factors to assess the spectrum of antimicrobial effects including the target microorganisms (i.e., various species and strains of bacteria, fungi, and virus has been used), pre-treatment, the method for the exposure of sanitizers (e.g., the amount of product used for the contact with skin, simulated usage patterns), and sampling after treatment (e.g., the components of elution medium). Especially previous research that provided the strictly-controlled protocols for the exposure of hand sanitizing wipes is scarcely reported although the efficacy of the removal and/or inactivation of target microorganisms from the skin surface can be affected by various operational conditions of the experiments (e.g.,

treatment time, the order of contact with skin, pressure applied to the skin during the wiping procedures)[50-52]. Major determinant factors for the wiping effects with the perspectives to the mechanical actions have been reported by the standardized tests of wet wipes to be applied on inanimate surfaces (e.g., 4-field tests), and thus the adoption of the key factors should be followed for the improvement of current methods for hand sanitizing wipes[53-57]. Whereas the variation in the components of hand sanitizers (e.g., active compounds, emollients, viscosity controllers, neutralizing agents, dyes, moisturizers) and recent trends regarding the formulation of sanitizers (e.g., alcohol-free products) also require the modification of test methods optimized for the target samples[52,58]. Comparative analysis of the methodologies and results among different test methods which can be applied to the same products is expected to provide clues for the strengths and limitations of each method[14]. Then adjustment of regulations according to the development and improvement of test methods should be followed[59,60] because the recommended usage conditions determined by the efficacy tests are directly linked to the level of potential health risk derived by the exposure of hand sanitizers to skin surface[61]. The development of advanced test methods can also be widely applied for the estimation of community-level hand hygiene levels by observational studies and/or simulation-based studies with educational interventions for food handlers to establish infection-control policies[62].

The *ex vivo* tests using animal skin (e.g., pig), synthetic artificial skin, or skin cell culture is promising alternative to the *in vivo* tests to overcome the difficulties in human trials[36,63,64]. The establishment of systematic test procedures using *ex vivo* models will also allow the repetitive tests under precisely controlled environments and time-resolved experimental measurement with the short-term sampling interval to figure out the determinant factors on efficacies and efficiency for the use of hand sanitizers. To broaden the range of applicable hand sanitizer products, however, the accumulation of data demonstrating the reliability of *ex vivo* test results compared with the *in vivo* tests is further needed due to the limited research reports related to this issue of equivalence. Although the availability of animal or artificial skin surface models allowing the efficacy assessment of hand sanitizers without the consideration of the interference from hand microflora can be regarded as one of the strengths of the *ex vivo* test, however, the recapitulation of the skin model inoculated with human skin commensal bacteria is expected to improve the assessment system for simulative research.

Recent progresses in the analysis of hand microbiomes broadens our knowledge regarding the responses of skin microflora on hands against the exposure of hand sanitizers[65,66]. Most previous research on the analysis of the hand microbiome has reported the variable distribution of different microbial species and the burden of microbial contaminants according to the skin sites (e.g., fingers, palm, opisthenar)[67,68], and thus the effects of hand sanitizers are also likely to be variant. The results from the analysis on the similarity of the microbial community composition between the left and right hands of the same individual also reported intraindividual differentiation, and thus the difference in microbial abundance between dominant and nondominant hands should be considered to improve the *in vivo* test methods for hand sanitizer using each side of hands as control and treatment experimental group from same human subject[69-71]. Moreover, understanding the contamination of microorganisms on hands and their hand-to-hand transmission is the prerequisite for the establishment of strategies modulating microorganisms on the left and right hand to be equalized before the treatment of hand sanitizers[72]. Hand microbiome data can also be used for the identification of novel target microorganisms to be protected or inactivated after the treatment of hand sanitizers to explore the species- or strain-dependent microbial susceptibility and resistance against the active substances of sanitizers[12].

## 국문요약

피부를 대상으로 한 살균을 목적으로 하는 외용소독제의 경우 식품 취급자에 오염된 미생물의 사멸 또는 제거를 목적으로 활용될 수 있으며, 최근 개인위생에 대한 관심 증가에 따라 제품 소비 증가와 제품 다양화가 두드러지게 나타나고 있다. 살균 효능은 소독제의 핵심 품질 평가 요소로서 수행 절차 및 조건에 따라 상이한 결과가 나타날 수 있기 때문에 시험법의 효율성과 정확성을 높이기 위한 연구가 필요하다. 이에 본 총설논문에서는 주요 제형별(겔형, 액제형, 와이프형) 시험법 개발 현황을 파악하고 시험법별 특장점 분석 결과와 최근 관련 연구를 통하여 제시된 시사점을 기반으로 향후 효능 평가 체계의 발전 방향을 제시하고자 하였다. 인체 대상 시험법의 경우 시험 유형에 따라 소독제를 시험 대상 피부 표면에 처리하는 조건이 다양화되어 있어 시험법 간 동등성에 대한 평가를 통해 소독제 제품의 성분이나 특성에 따라 최적의 시험 유형을 파악하고 그에 대응되는 적절한 평가 체계 및 관련 규제의 표준화의 필요성을 시사하였다. 특히 와이프형 소독제의 경우 처리 방식이 미생물 제거 및 살균에 직접적으로 영향을 미침에도 불구하고 피부에 노출하는 손 대상 처리를 위한 사용 패턴의 표준화 사례가 부족하였다. 한편 [전처리 - 소독제 노출 - 미생물 회수] 등 각 시험 절차별로 결과에 영향을 미치는 주요 결정 요인을 발굴하는 연구의 지속 수행을 통해 기존 시험법을 개선하

고 신규 시험법을 개발하고자 하는 노력이 요구된다. 최근 활발하게 개발되고 있는 *ex vivo* 시험법은 인체 시험의 제한적인 연구 재현성과 같은 한계를 극복하면서도 인간 피부 환경을 구현하기 위한 기술의 적용을 통해 연구 결과의 신뢰도를 확보할 수 있을 것으로 판단된다. 한편 손 피부를 대상으로 한 균총 연구 등 소독제 처리 전후 미생물의 특성과 분포 분석 관련 연구가 최근 다수 보고되고 있어 이를 활용한 미생물 군집 단위의 소독제 효능 평가 시험법의 확립이 기대된다. 본 연구를 통해 제시된 소독제 효능 시험법의 현황 기반 발전 전략은 보다 효과적인 개인위생 관리 확립을 통해 손을 통해 교차 오염되는 미생물에 의한 감염성 질병 발생을 최소화하여 공중보건 및 식품 안전성 향상에 기여할 수 있다.

## Conflict of interests

The authors declare no potential conflict of interest.

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