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Research Article

Evaluation of Bactericidal Activity of Alcohol Based Hand Sanitizer (Microcleer ENP™) According to EN 1500 and EN 12791

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Abstract

Hands of an individual is the primary areas of contact with various surfaces thereby higher chances of spreading the microorganisms. In majority of the conditions, regular cleaning process of hands with soap and water eliminates the chances of being infected with contagious diseases. Whereas, when soap and water are not readily available, it has been suggested to use an alcohol-based hand sanitizer that contains at least 60% alcohol, popularly know as hand-sanitizer and received top-most attention during the pandemic of COVID-19. However, hand-sanitizers are being evaluated for their efficacy as per the international guidelines, of which the European standard test methods such as EN 1500, EN 12791 etc. are considered as hall mark tests. In this context, Microcleer ENP™ is an alcohol based hand sanitizer, developed by Sarvotham Care Ltd., comprised of Chlorohexidine Gluconate and Ethyl Alcohol. Microcleer ENP™ was evaluated for it's efficacy as per the EN 1500 and EN 12791 test methods. Results of the EN 1500 tests suggests significant log reduction (2.00 - 2.92) of viable non-pathogenic *Escherichia coli* upon a contact period of 15 seconds. Microcleer ENP™ has shown 99.88% reduction against non-pathogenic *E. coli*. Similarly, the test results of EN 12791, have shown significant log reduction of viable bacteria at '0' hour (2.13 - 2.40) and at '3' hour (3.78 - 4.70) time points. Microcleer ENP™ has shown more than 3.5 log reduction, suggesting it's efficacy as antimicrobial agent. The results of EN 1500 and EN 12791 concludes the Microcleer ENP™ has proven its efficacy as 'hand sanitizer'.

Keywords: Alcohol Based Hand Sanitizer (ABHS); EN 1500; EN 12791

Introduction

Microorganisms are present on skin, gut and other parts of human body, as transient or in the colonised form. Of all these, hands are the prime areas of contact of microorganisms constantly, includes pathogenic and non-pathogenic in nature. Regular handwashing is one of the best ways to remove microorganisms, which intern reduces the risk of prone to infections, further it prevents the spread of microorganisms to others [1]. In addition, the guidance for handwashing process has been brought into public awareness programme, as a result the rate of specific communicative infections such as diarrheal and bacterial respiratory infections were reduced drastically. Under certain conditions, such as the 'health care practitioners', the recent pandemic of COVID-19, etc. instead of water and soap, use of alcohol based hand-sanitizers/ hand rub are recommended. According to World Health Organization (WHO), the alcohol based hand sanitizer/hand rubs must contain alcohol (at least 60%), with optional of other active ingredients, excipients and humectants in the form of either liquid, gel or foams [2]. The hand rub/sanitizers are meant for application

on hands to inactivate the microorganisms and/or to suppress their growth for time being, as the hands are the prime area of contact with microorganisms with higher chances of transfer/contamination. It has been highly sensitised by WHO, CDC along with various independent organizations about the essentiality of 'hand-hygiene' during the pandemic COVID-19, of course, the practice of hand hygiene is continued post-COVID also. However, to meet the demand of supplying the quality hand rub/sanitizers was the concern, due to unauthorised suppliers especially in developing and undeveloped countries [3].

There are various international guidelines to assess the efficacy of hand-sanitizers, especially the European Standards viz. EN 1500, EN 12791, EN 1499 etc. are being followed more than two decades. However, majority of the scientific paternity are enlightened about these guidelines during the pandemic COVID-19. Each test procedure has meant for certain products intended to use based on their particular application in determining the suitability and efficacy of the test compound accordingly [4]. In addition, alcohol-based hand sanitizers efficacy was evaluated against various infectious microorganisms such as gram-positive bacteria, gram-negative bacteria, enveloped viruses, non-enveloped viruses, mycobacteria, and even fungi [5]. Hence, quality assessed hand sanitizers play a critical role in preventing the spread of contagious diseases and cross-infection under the hospital set up through health care workers [6].

In view of the above given background, M/s Sarvotham Care Ltd., Hyderabad, India has developed an alcohol-based hand sanitizer named as Microcleer ENP™. The efficacy of Microcleer ENP™ assessed following the EN 1500 and EN 12791 guidelines.

Methodology

Test compound and positive control

Microcleer ENP™ is comprised of Ethyl alcohol - 70%, Chlorhexidine Gluconate - 2.5%, and volume made with distilled water. Isopropyl alcohol was diluted to 60% with distilled water and used as the 'positive control' as per EN guidelines.

Testing as per EN 1500

Heathy participants were selected, then the participants were asked to wash their hands with soft soap to remove natural transient flora. Hands were thoroughly dried with paper sterile paper towels, and then randomly assigned either to positive control (Propan-2-ol 60% v/v) or the test solution i.e. Microcleer ENP™ to evaluate the activity. Pure culture of non-pathogenic strain of *Escherichia coli* (ATCC-8739) inoculum was prepared at a strength of $2x10^5$, $3x10^5$ and $5x10^5$ cfu/mL. Care was taken while immersing the hands of participants in to a volume of the specified inoculum, that the hand must immerse up to the mid-carpals by spreading the fingers apart, for a period of 5 seconds. This procedure was carried out in the biosafety cabinet. Then, hands of participants were allowed to air dry for 3 minutes without touching any surface in the biosafety cabinet. The pre-values of the viable bacteria on the inoculum-immersed-air-dried hands were obtained, by rubbing the fingertips into a petri dish containing sterile tryptic soy broth (TSB).

Immediately after pre-value sampling the process of immersion into inoculum and air-drying of hands was repeated, followed by assigning to either positive control or test solution i.e. Microcleer ENP™, as per the standard practice of hand-sanitiser application. After 15 sec. of contact time with test compound and/or positive control, once again the fingertips are then sampled for post-values in the same manner as the pre-values. Meanwhile, the TSB was added with chemical neutralizer, to avoid its extended activity in the TSB. The pre- and post-samples were then diluted appropriately and plated onto tryptic soy agar (TSA) medium, followed by incubation at 36 ± 1ºC for a period of 24 - 48 hours. The pre- and post-values recovered from the fingertips were evaluated against one another, as per the standard mathematical calculation called the reduction factor, is the quantitative measure of the antimicrobial efficacy.

Testing as per EN 12791

Another test of bactericidal effectiveness was evaluated as follows, by collecting the viable bacterial count at three time points i.e. Initial, '0' hour-Post Appliance and 3 hour-Post appliance, from each participant. As the regular process, the participants washed their hands by applying soft soap, rinsing with tap water, followed by drying with disposable paper towels. Immediately after drying, participants asked to dip the fingertips of both hands into two different petri plates containing 10 mL of TSB, in order to detect the number of microorganisms present on the hands before treatment i.e. Initial Time point.

Then, participants were assigned to either reference-control (Propanol 60% v/v) group or test compound i.e. Microcleer ENP™. Either the propanol or Microcleer ENP™ was applied on hands of participants as standard practise of applying the hand-disinfectant and allowed to get dry. Then, participant's left hand fingertips were dipped into petri plate containing 10 mL TSB with neutralizer. This sampling was considered as '0-hour post-application' of either reference or test compound. Sterile surgical glove was used to cover the right hand of participants for a period of 3 hours, then the sampling was obtained as mentioned above procedure for the determination of 3 hours' post-application of either reference or test compound. The TSB was diluted appropriately and 1 mL of TSB was plated to TSA petri plates. Then the plates were incubated at 36 ± 1ºC for a period of 24 - 48 hours. The number of colonies for each Petri plate and the number of cfu/mL of sampling liquid was determined. The calculated cfu/mL value was transformed into common logarithm.

Results

EN 1500

The inhibitory activity of Microcleer ENP™ against different levels of inoculum size of non-pathogenic *Escherichia coli* (ATCC 8739) is given in Fig. 1. Upon contact with Microcleer ENP™ for a period of 15 seconds, a log reduction of 2.155 with Percent reduction of 99.3 was noted against the inoculum size of 2×10^5 cfu/mL. Similarly, a log reduction of 2.0 and 2.9 and percent reduction of 99.0 and 99.9 was noted against the inoculum size of 3×10^5 and 3×10^5 cfu/mL, respectively (Figure 1).

EN 12791

Inhibitory effect in relation with time of exposure to Microcleer ENP™ against flora present on hands after washing with soft soap solution was given in Fig. 2. The microbial inhibitory data of 1st Volunteer before application, after application at 0 h and 3 h of Microcleer ENP™ were $4x10^6$, $3x10^4$ and $2x10^2$ cfu/mL, respectively. The log reduction with reference to pre-application for post-application of Microcleer ENP™ at 0 h and 3 h were 2.1 and 4.3. Similarly, data of 2nd volunteer at before application, 0 h and 3 h of post application of Microcleer ENP™ were $6x10^6$, $3x10^4$ and $1x10^3$ cfu/mL, respectively with log reduction of 2.3 and 3.7 (Figure 2). In the 3rd volunteer, pre-application, 0 h and 3 h post-application of Microcleer ENP™ were $5x10^6$, $2x10^4$ and $1x10^2$ cfu/mL, respectively with log reduction of 2.4 and 4.7. In all the volunteers positive control propanol has reduced the microorganisms to as low as $1x10^1$ cfu/mL, with log reduction of 5.6.

Figure 1: Bactericidal Activity of Microcleer ENP™ as per EN 1500.

The bactericidal efficacy of Microcleer ENP™, was determined by artificial contamination of hands of participants with non-pathogenic *E. coli* (ATCC 8739) with different levels of initial inoculum. Hands immersed in to inoculum were allowed to dry and then taken initial value of viable bacterium followed by application of Microcleer ENP™, to be exposed for 15 seconds. Then the viable bacterium levels were assessed. The bars of each participant determined the levels of bacterium at initial and end of exposure period of 15 seconds for Microcleer ENP™. Data sets suggests the Log reduction and % reduction of viable bacterium from each participant.

Discussion

Knowledge about 'Hand Hygiene' gained utmost importance, especially during COVID-19, as hands are the prime area of contact with infectious agent i.e. SARS-CoV-2. A significant reduction in 'Nosocomial Infections' was noticed with better hand hygiene condition of healthcare workers (HCW), this could be due to reduction of transmission of microorganisms to patients, thereby ultimate reduction of morbidity, mortality and costs associated with Healthcare Associated infections (HCAI) [5]. The World Health

Figure 2: Bactericidal Activity of Microcleer ENP™ as per EN 12791.

The bactericidal efficacy of Microcleer ENP™, against the natural skin flora (no artificial contamination) of hands of participants by applying Microcleer ENP™. The data of viable bacterium was obtained at initial, '0' and '3' h of application of test compound. The bars represent the viable bacterium levels at each time point of individual participants. Data sets suggests the Log reduction of viable bacterium, at different time points from each participant.

Organization has recommended alcohol-based hand sanitizers (ABHS) in view of its cost-effectiveness, and microbicidal activity with added advantage of 'ease of application', in absence of water [2]. During the COVID-19, the advice by Healthcare agencies for use of ABHS to prevent the spread of infection, the demand and sale of ABHS has raised and resulted shortage of such category of products in most markets [7]. This in turn lead to huge production of ABHS by various un-authorised manufacturers, with higher chances of substandard products. In view of the protection of public health, law enforcers reaffirmed, adherence to guidelines to meet the legislation of biocidal products.

In this context, Microcleer ENP™ was developed as per the WHO guidelines of ABHS with Ethyl alcohol - 70%, Chlorhexidine Gluconate - 2.5%, and volume made with distilled water. The product was tested as per the EN 1500 and EN 12791 guidelines to ensure the efficacy of the product. Outcome of EN 1500 testing of Microcleer ENP™ suggest, the formulation is effective in reducing the viability of ATCC strain of *E. coli*. These results are corroborated with an ethanol based gel (Sterillium® Comfort Gel) tested for 15 seconds for its effectiveness against the ATCC strains and clinical isolates [8]. In addition, it was confirmed that ABHS comprised of lesser than 70% alcohols have shown compromised efficacy [9]. Efficacy determination of Microcleer ENP™ in 15 seconds is further assures that even the shorter application time, must possess the effective hand sanitization function, which is key factor to be the best hand sanitiser.

Another test EN 12791, intended to determine the efficacy of ABHS against the viable bacteria present on hands in relation with exposure period of test formulation up to 3 hours. Results of Microcleer ENP™ - EN 12791 test suggests that significant reduction at 0 hour and 3 hour time points in comparison to initial viable bacterium levels (Fig. 2). This data suggests that Microcleer ENP™ has fulfils the requirement of 'surgical hand preparation category' as it reduced resident skin flora by more than 3.5 log reduction, the bench mark reduction as per EN 12791 guideline [2]. Marchetti., *et al*. (2003) have evaluated about five different ABHS for their efficacy as per EN 12791 guidelines, wherein the results suggest that one of the product with a combination of Propanol (45% 2-propanol, 30% 1-propanol, 0.2% mecetronium etilsulphate) have superior activity over the n-propanol (60%) as standard positive control [10]. The results of current study were in line with observations of Marchetti., *et al*. (2003).

Conclusions

The results of the efficacy of Microcleer ENP™ in terms of EN 1500, have significantly reduced non-pathogenic viable *E. coli* strain (ATCC 8739) as artificial contaminant. In addition, EN 12791 testing of Microcleer ENP™ have shown it's efficacy in elimination of native flora of skin (no artificial contaminant) intended for surgical hand preparation.

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Conflicts of Interest

The authors declare no conflict of interest.

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