

# An Enzymatic Based Formulation for Cleaning and Disinfection of Medical Devices

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## Abstract

The high prevalence of healthcare-associated infections (HCAIs) is a major challenge globally. In Africa, the prevalence of HCAIs is between 2.5 - 14.8 %, which is twice as high as that in Europe. In this study, we aimed at developing an effective enzymatic detergent/disinfectant for medical devices. The components of the formulation consisted of tetric acid (surfactant), benzalkonium chloride (disinfectant), three enzymes (protease, amylase and lipase), and sodium citrate/bicarbonate (buffers). Through a series of experiments, the concentration of reagents, tetric acid and benzalkonium chloride were optimized. The optimized formulation composed of 1 % benzalkonium chloride, 3.5 % sodium citrate and 6 % sodium bicarbonate, 1M citric acid Monohydrous, and enzymes lipase, amylase and lipae (protease, amylase and lipase) making 15 % of the formulation. The biofilm of *S. aureus* was prepared by incubating sterile aluminum chips inoculated with the bacteria at 37 °C. The antimicrobial activity of the formulation in terms of log<sub>10</sub>-reduction of *C. albicans* was 5.87±0.52 and for *S. aureus* 7.29±0.44 respectively for non-biofilm surface. The negative control (double distilled water) showed no antimicrobial activity. The ability of the optimized formulation to

effectively clean and disinfect the biofilm was also determined. For biofilm treatment with the formulation for more than 50 minutes,  $\log_{10}$ -reductions of 6.57 (at 1 hr.) and 9.33 (at 24 hrs.) were noted. This formulation was stable after 4 weeks at pH (P-value = 0.00046) and antimicrobial activity (P-value = 0.0030). These properties make this enzymatic detergent/disinfectant suitable for use in cleaning and disinfecting medical devices. The product demonstrated superior efficacy to the positive control product Aniosyme. Aniosyme had a > 99.9999 % reduction on the both *C. albicans* and *S. aureus* but no clearance of artificial soil on the STF strip for up to 60 minutes. From these findings, use of enzymes in detergent/disinfectants could be better alternative to surfactants and may hence lead to more environmentally and user friendly cleaning agents.

**Keywords:** Benzalkonium chloride, Biofilm, Enzymes, Disinfectants, Healthcare associated Infections, *Staphylococcus aureus*.

## Introduction

According to the World Health Organization (WHO) Health care-associated infections (HCAIs) are the most frequent adverse event in health-care delivery worldwide with 7 % of hospitalized patients at any given time. In developed countries about 30 % of patients in intensive care units (ICUs) are affected by at least one HCAI with prevalence being at least 2 - 3 fold higher in developing countries [1-2]. Biofilms (blood and other body matrices) in medical instruments continue to present a major problem to the prevalence of HCAIs [1-3]. Disinfectants destroy or inhibit growth of pathogenic microorganisms when applied to inanimate objects [4]. A broad spectrum of antimicrobial activity is one of the indicators of the effectiveness of a disinfectant [5]. An ideal disinfectant should also be compatible with the surfaces to be disinfected, easy to prepare and use, stable, affordable, readily available, as well as lacking any unpleasant odor [6]. They should be efficient in the removal of biofilms from medical devices such as flexible endoscopes, which cannot be disinfected using harsh chemicals or high temperatures [7]. As such, enzyme-based detergent/disinfectants that would be more appropriate in health facilities. Such detergent/disinfectants should be safe to use and effective even at minimum concentrations [8-9]. Currently, detergent/disinfectants that are available in developing countries are mostly imported from developed countries and also have issues with stability, ease of use and their safety [7]. Hence it is important to further develop enzyme based disinfectants that are effective, stable and that are safe [10]. *C. albicans* has been reported to be a major pathogen in the fungal infections associated with HCAIs [11, 12]. *S. aureus* has ability to survive in constant contact with human beings and is one of most important pathogens that contributes to the high prevalence of HCAIs. With *S. aureus* biofilms being more common [7-14], effective disinfection of medical instruments is important in reducing chances of microbial growth and

biofilm formation. This study therefore aimed at developing an enzymatic detergent/disinfectant that is effective against of *S. aureus* (in biofilm and not) and also against *C. albicans*.

## Materials and Method

### Test Formulation of an enzymatic/disinfectant cleanser

The new formulation consisted of 2-Solution System;

- a) Solution A (Sol A) contained a 0.6% protease enzyme, EverisDuo (Novozyme, Bagsværd, Denmark), 0.6% of amylase, Stainzyme (Novozyme, Bagsværd, Denmark) and 0.6% lipase, Lipex (Novozyme, Bagsværd, Denmark). The enzymes were mixed at 6 ml each.
  
- b) Solution B (Sol B) contained a buffer 3.5 % sodium citrate (Merck, Darmstadt, Germany)/ 6 % sodium bicarbonate (Merck, Darmstadt, Germany), a nonionic surfactant 0.3 % tetronic acid (Merck, Darmstadt, Germany), and a quaternary ammonium compound disinfectant 1 % benzalkonium chloride (MP Biomedicals, Irvine, CA).

For combined mixture solution (A and B) various concentrations of disinfectant and surfactant, were tested. This was through a series of experiments to find the optimum formulation. Sol A and Sol B were mixed at a ratio of 1.5:8.5 of Sol A and Sol B respectively.

### Positive control formulations

The performance of the developed formulation was compared with that of two products in the Kenyan Market.

- a) An enzyme-free detergent Safikem, (KEMRI, Kenya) that contained sodium lauryl ether sulfate 5 % w/w (an anionic surfactant), linear alkyl benzene sulphonic acid 2.5 % (an anionic surfactant) and cetrimide 0.5 % w/w (disinfectant) stabilized with NaCl and NaOH.
- b) An enzymatic detergent/disinfectant Aniozyme (Laboratoires Anios, Lezennes, France), which contained quaternary ammonium propionate, chlorhexidine digluconate, non-ionic surfactants, enzyme complex (protease, lipase and amylase), perfume coloring and excipients.

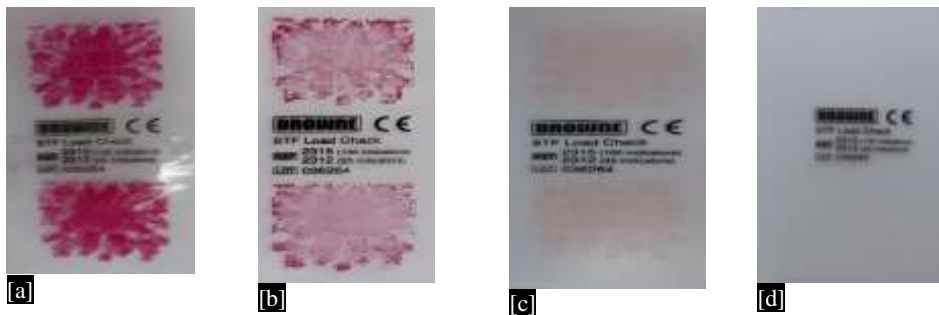
### Microorganisms

The microorganisms that were used in the study were *S. aureus* ATCC 25923, and *C. albicans* ATCC 1023. They were stored at minus 80 °C freezer. *S. aureus* ATCC 25923 strains were propagated in Trypticase Soy Agar (TSA) and grown at 37 °C on blood agar plate for 24 hrs. The *C. albicans* ATCC 1023 strains were propagated on Sabouraud's Dextrose agar plate and grown for 3 days at room temperature (25 - 26 °C).

### Determination of the cleaning efficacy of the formulations

The detergency performance of various formulations was tested using Brown STF (Soil Test Formula) Load Check Indicator strips (STERIS Animal Health, Derby, UK). These strips contain two sources of protein, lipids and polysaccharides and they mimic the cleaning efficacy soil tests for surgical instruments as described in ISO/TS 15883-5. Briefly, the strips were dipped into a dilution of 1litre double-distilled water containing 6 ml of the formulation and washed at intervals ranging from 10 to 60 minutes. The strips were removed from the solution, washed, and photographed. The time it took for the formulation to clear the STF strip was assessed by observation of the strips. This was taken as an indicator of the cleaning efficacy.

The interpretation of the results was based on visual observation of soil (red color) removal as illustrated on *Figure 1*. The cleaning efficacy end-point was considered achieved when the STF strips appeared clear as shown in *Figure 1[d]*.



**Figure 1.** The STF Strips appeared as [a] with complete ineffective cleaning efficacy, [b] with slight cleaning efficacy, [c] moderate efficacy, and [d] complete cleaning efficacy that was taken as the end-point.

### Optimization of the key parameters in the final formulation of the enzymatic based detergent/disinfectant

To determine the optimum dilution of enzymatic detergent/disinfectant formulation for use, 1 ml to 10 ml of it were diluted into 1litre double distilled water and tested for cleaning efficacy using STF Load Check Indicator Strips.

### Determination of the disinfection efficacy

The antimicrobial efficacy of the optimized formulation against *S. aureus* and *C. albicans* was tested using a carrier test method as described by G. Reybrouck [14]. Two hundred microliter (200  $\mu$ l) of phosphate buffer saline (PBS) solution in separate tubes containing 600 CFU/ml of *S. aureus* (ATCC 25923) and 9400 CFU/mL of *C. albicans* (ATCC 10231) was spread on the STF Load Check Indicator strips and left to dry for one and half hours. The strips were washed using the optimized formulation (6 ml of formulation in 1liter of double distilled water and washed for 10 minutes). The cleaned strips were swabbed and the swab suspended in PBS, vortexed and tested for sterility on blood agar and Sabouraud's Dextrose agar. A 100  $\mu$ l of the contents of test tube containing the swab was plated and incubated overnight at 37 °C for *S. aureus* and room temperature for 3 days for

*C. albicans* respectively. Then colony counts of viable cells on plates after disinfection were determined. The experiment was repeated 10 times. The colony counts, CFU/ml, and Log reductions were determined after cleaning. Log reductions were determined using the formula as described by Hamilton in [15]:  $\text{Log reduction} = \log_{10} A - \log_{10} B$ , where A is the number of viable microorganisms before disinfection and B is number of viable microorganisms after disinfection

### **Determination of the effectiveness of the formulation on cleaning and disinfection of the *S. aureus* Biofilm**

The *S. aureus* biofilm was prepared using a modified method by [7] . Briefly, ten aluminium chips (19 cm<sup>2</sup>) were cleaned, disinfected with 70 % ethanol, and sterilized in an autoclave for 15 minutes at 121 °C. A pure culture of *S. aureus* containing  $2.16 \times 10^9$  CFU/mL was made. Five chips were immersed into 60 mL Tryptic Soy Broth (TSB) and then into a petri dish of 10 mL of the bacterial suspension. The petri dish was incubated at 37 °C for 3 days. The chips were then washed with PBS. This was repeated five times. The strips were then washed using the optimized formulation (6 ml of formulation in 1liter of double distilled water) for varying durations (10 minutes, 20 minutes, 30 minutes, 50 minutes, 60 minutes and 24 hours). The cleaned strips were swabbed and the swab suspended in PBS and tested for sterility on blood agar. The colony count, CFU/mL, and log<sub>10</sub> reductions were calculated to indicate the disinfection efficacy.

### **Stability Test of the Formulated Enzymatic Detergent/Disinfectant**

The stability study was done over time to determine storage period (shelf-life) of the formulation. The efficacy of disinfection and pH of the stored reagent were determined from day 0 (initial test) then weekly for 3 weeks. For disinfection efficacy, *S. aureus* was used as a marker with the concentration of  $6.0 \times 10^6$  (week 0 and 1) and  $2.16 \times 10^9$  CFUs/ml inoculum being coated on the STF strip. Using the optimized formulation (6 ml of formulation in 1liter of double distilled water and washed for 10 minutes) the strip was subjected to cleaning and disinfection. The pH was determined by using a pH meter. Each experiment was repeated three times each week.

### **Statistical Analysis**

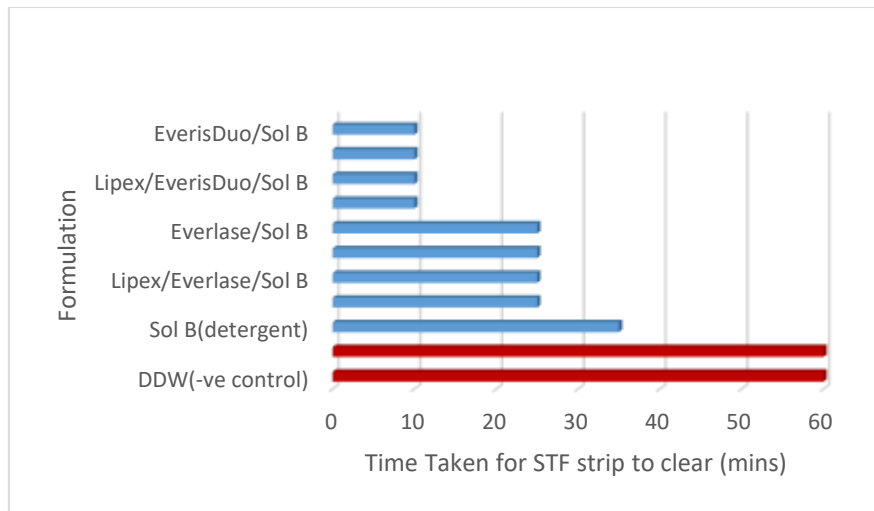
Data was expressed as the mean  $\pm$  standard error of the mean for log reduction. To determine Standard errors for antimicrobial efficacy, JupyterLab was used. Graphs were made using excel and graph pad.

## **Results and Discussion**

### **Determination of the cleaning efficacy of the formulation**

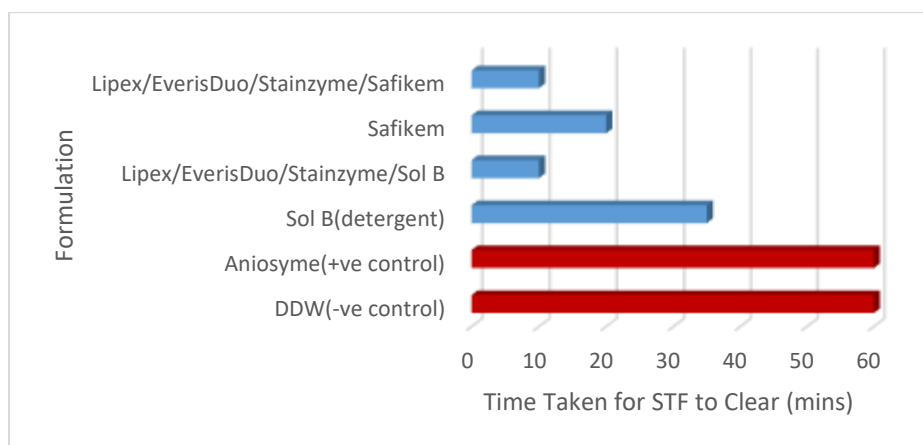
The negative control (double distilled water) did not clear the STF strips within the 60 minutes' experimental period. Formulations containing only one of the enzyme without a detergent did not also clear the STF strips in 60 minutes' experimental period. The detergent alone (Sol B) demonstrated a cleaning period of 35 minutes.

The formulations containing a protease in Sol B (alone or with other two enzymes) cleaned the STF strips within a period of 10 minutes. The protease Everlase alone in Sol A in the detergent (Sol B) demonstrated cleaning in 25 minutes. The addition of Stainzyme and Lipex to formulations containing Sol B/EverisDuo and Sol B/Everlase demonstrated no apparent change to the cleaning time. Aniosyme did not show any clearance of the soil when used to clean for the maximum set time of the experiment (60 minutes) as shown in *Figure 2*. The manufacturer's inserts for Aniosyme recommends the soaking of instruments to be cleaned to be carried out for a period of 24 hrs.



**Figure 2.** Cleaning duration of various formulations in comparison to the commercial enzymatic detergent and disinfectant (Aniosyme). Red bars represent the formulations (controls) that did not clear the STF strip within the experimental set time of 10 – 60 minutes.

When Sol B was replaced with the Safikem detergent the STF strips cleared within a period of 20 minutes which was a better performance (of 15 minutes) than that of Sol B alone that cleared the STF strips after 35 minutes (*Figure 3*). However, the performance of Sol B and Safikem was the same upon the addition of EverisDuo containing enzymes formulation. This implied that the formulations containing tetric acid could potentially be replaced by those containing the ingredients in Safikem.



**Figure 3.** The cleaning efficacy of various enzymes (in detergent) and Safikem. Formulation containing Sol B (Tetronic acid 0.3 %), sodium bicarbonate, tri-Sodium Citrate, (Benzalkonium chloride 0.024 %) and citric acid Monohydrous (0.1M) at pH 8.0 and the enzymes EverisDuo, Stainzyme, Lipex separately (Sol A). Two STF strips were cleaned using the KEMRI Detergent (Safikem) as a positive control and double distilled water (negative control). Red bars represent the formulations (controls) that did not clear the STF strip within the experimental set time of 10 – 60 minutes.

**Determination of the optimum formulation for disinfection**

The optimized formulation Sol B was added into disinfectants, benzalkonium chloride (BAC) and chlorhexidine (CHX) separately. Both disinfectants were tried at the concentrations of 1 % to 5 %. The results are shown on *Table 1*. Chlorhexidine containing formulations showed better cleaning efficacy even at higher concentrations of up to 5% within a period of 10 - 60 minutes while the cleaning efficacy of benzalkonium chloride containing formulations of above 1% did not.

**Table 1.** Cleaning efficacy of BAC and CHX at different concentrations between 10 - 60 minutes cleaning.

Disinfectant	Concentrations of disinfectant added to the detergent					
	1 %	1.5 %	2 %	3 %	4 %	5 %
BAC	√	X	X	X	X	X
CHX	√	√	√	√	√	√

Key: √ = Effective cleaning within 60 minutes, x = No cleaning after 60 minutes

Disinfection efficacy studies (*Table 2*) showed that all the concentrations of disinfectant prepared except for 0.024% chlorhexidine were efficacious against the test bacteria *S. aureus* (ATCC 25923) after cleaning for 10 minutes. From these results, 1% BAC showed complete efficacy (no bacterial growth observed on plate).

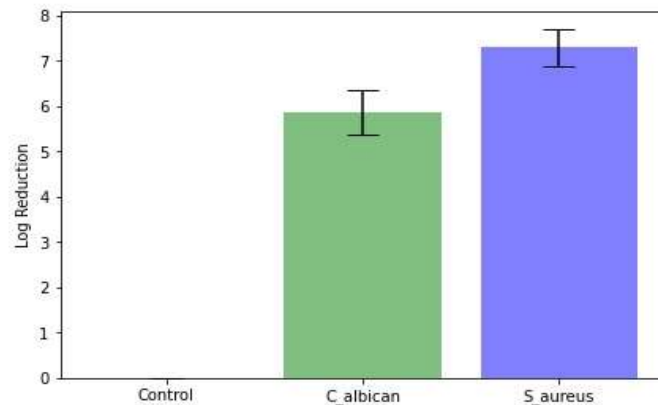
For the 1 % - 2 % CHX there was noticeable reduction of bacteria on plates. Therefore 1 % BAC was considered to be the most appropriate disinfectant and concentration to use for the product prototype.

**Table 2.** Disinfection efficacy of BAC and CHX against *S aureas*

Disinfectant	Concentrations of disinfectant added to the detergent			
	0.024 %	1 %	1.5 %	2 %
BAC	√	√	ND	ND
CHX	X	√	√	√

Key: √ = Effective disinfection, x = Ineffective disinfection, ND = Not done

The disinfection efficacy of the formulation prototype against *C. albicans* demonstrated a (> 99.999 % reduction) with 5.87-log reduction. For *S. aureus* the formulation antibacterial efficacy was (> 99.9999 % reduction) with a 7.29-log reduction (*Figure 4*). Where the initial inoculated CFU/ml before disinfection were  $6 \times 10^8$  (*S. aureus*) and  $9.4 \times 10^7$  (*C. albicans*).

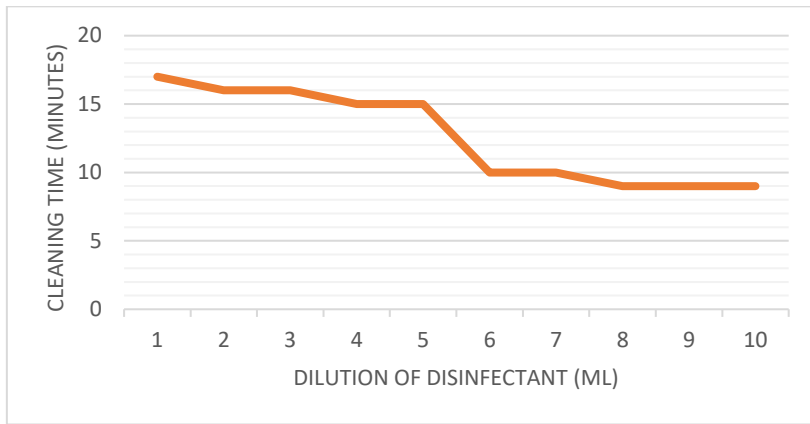


**Figure 4.** Disinfection performance of the newly formulated detergent/disinfectant on *C. albicans*, *S. aureus* and against the control (double distilled water). Data expressed as mean  $\log_{10}$ -reduction.

#### Optimization of the key parameters in final formulation of the enzyme cleaner

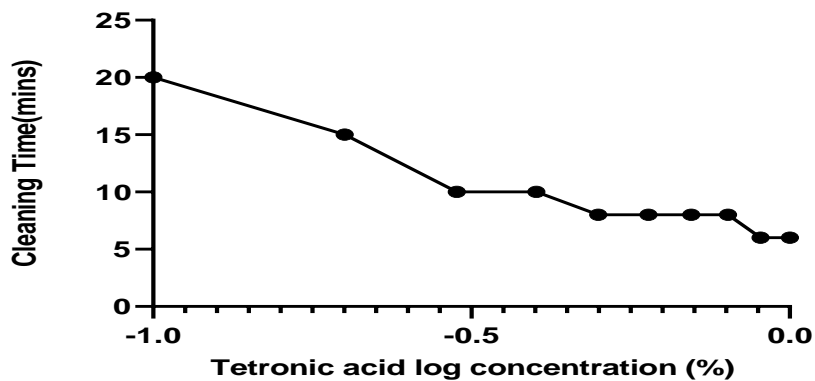
To optimize the volume for use in 1liter double distilled water, cleaning was carried using the volumes 1ml detergent/disinfectant formulated in 1liter double distilled water. This was done until 10 ml dilution. The dilutions (6:1 and 7:1) indicated a cleaning time of 10 minutes while the dilution lower than 6ml:1liter water indicated a longer than 10 minutes cleaning time. Dilution higher than 7ml into 1liter water showed a shorter than 10 minutes cleaning time *Figure 5*.





**Figure 5.** Performance of cleaning efficacy of various volume combinations of detergent/disinfectant (**Sol B**) in 1 litre double distilled water to determine the most minimal effective volume to use in final detergent/disinfectant formulation.

To optimize the concentration of tetrionic acid the cleaning was carried using the concentrations 0.1 %, 0.2 %, 0.3 %, 0.4 %, 0.5 %, 0.6 %, 0.7 %, 0.8 %, 0.9 %, and 1.0 %. The cleaning efficacy increased with increasing concentration of tetrionic acid (*Figure 6*). The concentrations 0.3 % to 0.5 % Tetrionic were effective with 10 minutes cleaning time of the soil on STF strip. The higher concentrations from 0.6 % to 1 % indicated a faster cleaning time of 5 minutes compared to the lower concentrations (0.1 % - 0.2 %) which indicated a longer cleaning time of 20 minutes.



**Figure 6.** Cleaning performance of varying concentrations of tetrionic acid in formulation prototype to determine the most minimal effective concentration to use in the final formulation.

**The cleaning and disinfection efficacies of formulation prototype on Biofilm**

The results of the biofilm formation after 15 days were determined by counting CFU/m<sup>2</sup> of the aluminum chip. The cell adhesion was 3.79×10<sup>9</sup> CFU/m<sup>2</sup>. This indicated biofilm formation on the surfaces of the chips. The incubation tempera-

ture for *S. aureus* was 37 °C which provided favorable conditions for it to grow. As seen in *Table 4*, at 20 minutes, the biofilm removal efficacy of the detergent/disinfectant was practically existent as the colonies noted were over 3.55 CFU/ml and 0 after 24hrs of disinfection. The maximum biofilm removal of *S. aureus* was achieved after 1440 minutes (24 hrs) of soaking the chips in the formulated detergent/disinfectant. This resulted in only one viable bacterial cell after exposure of chip to the detergent/disinfectant.

**Table 3.** Log reduction of *S. aureus* biofilm after 10,20,30,50,60 and 1440 minutes of being exposed to detergent/disinfectant formulated.

Detergent/Disinfection Time(minutes)	Colony Counts	Final Log (CFU/mL)	Log reduction
10	>...	-	0
20	352	3.55	5.78
30	228	3.36	5.97
50	188	3.27	6.06
60	58	2.76	6.57
1440	1	0	9.33

Key: >...= Too numerous to count

It is worth to note that while the cleaning and disinfection efficacy could be satisfactorily achieved within 10 minutes (*Figure 2*) with a log reduction of 7.29 for *S. aureus* and 5.87 for *C. albicans* respectively, the same could only almost be achieved after 50 minutes (log reduction of 6.06). After 24 hours of disinfection of the biofilm, it resulted in almost 100 % efficacy.

### Stability Test of the Formulated Enzymatic Detergent/Disinfectant

The stability study was done over time to determine the storage (shelf-life) of the formulation. The cleaning efficacy was carried out for 3 weeks, starting from day 0 (initial) while the disinfection efficacy *Table 5* and pH testing were done from 0 (initial test) then weekly for 4 weeks. The cleaning efficacy *Figure 7* indicated that the formulation was effective (clearing the STF strip at 10 -15 minutes) for 3 weeks. The disinfection ability of the formulation was proven to have improved at week 4 with a mean  $7.536 \pm 0.816$  log-reduction *Table 5* from 6.68 log-reduction at week 0.

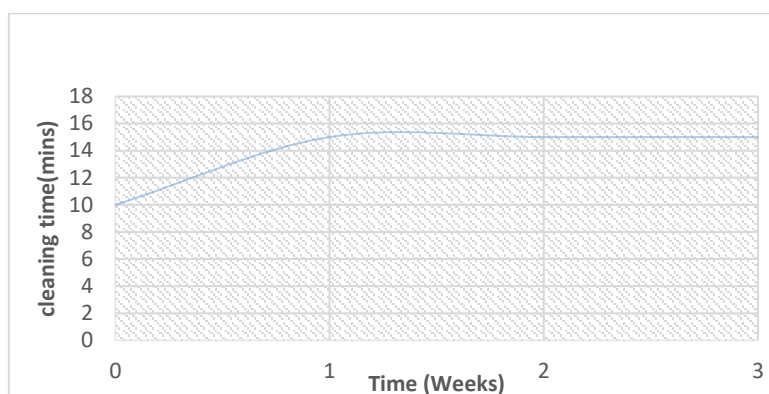
**Table 5.** pH and antimicrobial activity (expressed as log<sub>10</sub>-reduction) of formulated detergent/disinfectant over a period of 4 weeks. Data expressed as Mean±Std (standard deviation) and mean values considered statistically different when  $P \leq 0.05$ .

	N	Mean±Std	P-value
<b>PH</b>	5	8.428±0.403	0.00046
<b>Antimicrobial Activity</b>	5	7.536±0.816	0.0030

Initial PH for week 0-8.04

Initial Antimicrobial activity-log reduction of 6.68

Each experiment was repeated three times



**Figure 7.** Cleaning performance of the formulation over a period of 4 weeks beginning with the initial week (0). The trend shows that the cleaning time at week 0 was 10 minutes and it began to go up to 15 minutes (week 2-3). At week 4 the cleaning of the formulation was ineffective.

## Discussion

This study, through a series of optimization experiments, successfully developed an enzymatic detergent/disinfectant containing Tetronic acid (0.3 %), EverisDuo/Stainzyme/ Lipex and the disinfectant BAC (1 %) with a better performance attributes to the currently leading brand of enzymatic cleanser, Aniosyme. The initial screening of formulation prototypes was established mainly by relying on the literature review.

The study demonstrated that 1 % BAC had the ability to clean the STF load check indicator strip soil while the higher concentrations failed to hence they could not be used for development of product prototypes. The recommended concentration of BAC is up to 3 % as a disinfectant [16] hence the established concentration in this study was within this concentration. The antimicrobial efficacy of benzalkonium chloride in this study against *C. albicans* is supported by [17] who reported that treatment of medical devices with benzalkonium chloride reduced *C. albicans* adhesion to plastic surfaces.

When the formulations containing at least one enzyme without a detergent were used for cleaning the STF strips applying the standard protocol established in this study, there was no clearance of the STF strips after sixty minutes. This may be because enzymes alone are not cleaners but are used as catalysts to aid in digesting targeted soil presented in medical devices. Hence they are incorporated in detergents to improve their cleaning efficacy as proven by [18]. Similarly, the cleaning efficacy of detergent alone was less than that of the detergent plus enzyme, clearly demonstrating the benefits of combining the detergent and enzyme to produce a superior cleaner. The cleaning duration of Lipex/Stainzyme/EverisDuo in Sol B was comparable to that of Lipex/Stainzyme/EverisDuo in Safikem (10 minutes). The clearance duration of Safikem alone was 20 minutes. These results infer that the detergent Sol B could be substituted with Safikem.

The results of this study agree with those of Magazine who tested the cleaning efficacy of non-enzymatic, enzymatic and alkaline detergents using STF load check indicator strips as well as other industry recognized indicator soils. The findings demonstrated the benefits of two types of proteases to commercial detergents where the cumulative rank index for using the two enzymatic detergents was 97 % while neither the detergent base nor the alkaline detergent reached 90 %. In this study, the benefit of addition a protease (EverisDuo) resulted in fast cleaning at 10 minutes and was found to be more efficacious than that of adding other enzymes Everlase (a protease), Stainzyme (an amylase) and Lipex (a lipase). Although there is not a lot of literature explaining why different proteases might outperform each other, it is suspected that the performance difference between the two protease (EverisDuo and Everlase) might be because of changes in their molecular structure.

It is important to optimize the amount of surfactants and volume usage of the detergent/disinfectant used for cleaning because if not optimized it can lead economic losses due to high production costs. From the results increasing concentration of the Tetronic acid surfactant corresponds with the faster cleaning of the artificial soil on STF strip. This can be attributed to the fact that high surfactant concentration reduce surface tension therefore making it easier to wet the surface and remove soil [19].

The difficulty of cleaning and disinfecting the biofilms was demonstrated in this study. While the cleaning and disinfection efficacy could be satisfactorily achieved within 10 minutes with a log reduction of 7.29 for *S. aureus* and 5.87 for *C. albicans* respectively, the same could only almost be achieved after 60 minutes (log reduction of 6.06). The soaking of the strips for 24 hours resulted in almost 100 % efficacy with a log reduction of 9.33. This finding is in line with the study by [20] who found that bacterial spores and biofilms exhibit less susceptibility to disinfectants hence in this study, more exposure time of the biofilm to disinfectant was required.

In regards to the product stability, the evidence so far indicates that the product is stable after four weeks in terms of pH and antimicrobial activity. The alkaline pH of the product over time and disinfection ability against the *S. aureus* when used as a marker for this study is in line with [21] who found out that alkaline disinfectants have favorable bactericidal activity.

The cleaning of the STF soil by the formulated detergent/disinfectant declined at week 4 which is suspected to be because of insufficient exposure time of the STF strip to detergent/disinfectant. The stability of the product prototype will be carried for the next two years in order to determine the final shelf-life of the product.

## Conclusion

This study has developed an enzyme based disinfectant with better cleaning and disinfection efficacy than supposedly products in the Kenyan market. The product is effective against *S. aureus* (in biofilm and not) and *C. albicans*. The developed product is also effective in cleaning and disinfection of the microorganisms in the biofilm albeit with a longer cleaning period of at least 60 minutes. The study has

also demonstrated the effectiveness of the benzalkonium chloride 1% as a disinfectant when used with a tetronic detergent.

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## References

- [1] Phillips, K. S., Patwardhan, D., & Jayan, G., Biofilms, medical devices, and antibiofilm technology: Key messages from a recent public workshop. *American Journal of Infection Control*, **43** (1) (2015), 2–3. <https://doi.org/10.1016/j.ajic.2014.09.019>
- [2] Chen, M., Yu, Q., & Sun, H., Novel Strategies for the Prevention and Treatment of Biofilm Related Infections. *OPEN ACCESS Int. J. Mol. Sci*, **14**, (2013), 14. <https://doi.org/10.3390/ijms140918488>
- [3] Khatoun, Z., McTiernan, C. D., Suuronen, E. J., Mah, T. F., & Alarcon, E. I., Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. *Heliyon*, **4** (12) (2018), e01067. <https://doi.org/10.1016/j.heliyon.2018.e0106>
- [4] Weber, D. J., Rutala, W. A., & Sickbert-Bennett, E. E., Outbreaks associated with contaminated antiseptics and disinfectants. *Antimicrobial Agents and Chemotherapy*, **51** (12) (2007), 4217–4224. <https://doi.org/10.1128/AAC.00138-07>
- [5] McDonnell, G., & Russell, A. D., Antiseptics and disinfectants: Activity, action, and resistance, in: *Clinical Microbiology Reviews*, **12** (1) (1999), 147–179. <https://doi.org/10.1128/cmr.12.1.147>
- [6] W. A. Rutala and D. J. Weber, Selection of the Ideal Disinfectant, *Infect. Control Hosp. Epidemiol.*, **35** (7) (2014), 855–865. <https://doi.org/10.1086/676877>
- [7] Marques, S. C., Rezende, J. D. G. O. S., Alves, L. A. D. F., Silva, B. C., Alves, E., De Abreu, L. R., & Piccoli, R. H., Formation of biofilms by *Staphylococcus aureus* on stainless steel and glass surfaces and its resistance to some selected chemical sanitizers. *Brazilian Journal of Microbiology*, **38** (3) (2007), 538–543. <https://doi.org/10.1590/S1517-83822007000300029>
- [8] Borkar, I. V., Dinu, C. Z., Zhu, G., Kane, R. S., & Dordick, J. S., Bionanoconjugate-based composites for decontamination of nerve agents. *Biotechnology Progress*, **26** (6) (2010), 1622–1628. <https://doi.org/10.1002/btpr.498>
- [9] Grover, N., Douaisi, M. P., Borkar, I. V., Lee, L., Dinu, C. Z., Kane, R. S., & Dordick, J. S., Perhydrolase-nanotube paint composites with sporicidal and antiviral activity, *Applied Microbiology and Biotechnology*, **97** (19) (2013), 8813–8821. <https://doi.org/10.1007/s00253-012-4573-3>

- [10] Grover, N., Dinu, C. Z., Kane, R. S., & Dordick, J. S. (2013a). Enzyme-based formulations for decontamination: Current state and perspectives. *Applied Microbiology and Biotechnology*, **97** (8), 3293–3300. <https://doi.org/10.1007/s00253-013-4797-x>
- [11] Aboualigalehdari, E., Ghafourian, S., Sadeghifard, N., & Sekawi, Z., Is candida albicans a cause of nosocomial infection in Iran? *Reviews in Medical Microbiology*, **24** (4) (2013), 85–88. <https://doi.org/10.1097/MRM.0b013e328364243>
- [12] Perlroth, J., Choi, B., & Spellberg, B., Nosocomial fungal infections: Epidemiology, diagnosis, and treatment, in: *Medical Mycology*, **45** (4) (2007), 321–346. <https://doi.org/10.1080/13693780701218689>
- [13] Otto, M., Staphylococcal biofilms. In *Current Topics in Microbiology and Immunology*, **322** (2008), 207–228. *Curr Top Microbiol Immunol*. [https://doi.org/10.1007/978-3-540-75418-3\\_10](https://doi.org/10.1007/978-3-540-75418-3_10).
- [14] Reybrouck, G., The testing of disinfectants. *International Biodeterioration and Biodegradation*, **41** (3–4) (1998), 269–272. [https://doi.org/10.1016/S0964-8305\(98\)00024-9](https://doi.org/10.1016/S0964-8305(98)00024-9)
- [15] “Standardized Biofilm Methods Laboratory in the CBE The log reduction (LR) measure of disinfectant efficacy,” 2010. Accessed March 15, 2021.
- [16] Pereira, B. M. P., & Tagkopoulos, I., Benzalkonium chlorides: Uses, regulatory status, and microbial resistance, in: *Applied and Environmental Microbiology*, **85** (13) (2019). <https://doi.org/10.1128/AEM.00377-19>
- [17] Imbert, C., Lassy, E., Daniault, G., Jacquemin, J.-L., & Rodier, M.-H., Treatment of plastic and extracellular matrix components with chlorhexidine or benzalkonium chloride: effect on *Candida albicans* adherence capacity in vitro, *Journal of Antimicrobial Chemotherapy*, **51** (2003), 281–287. <https://doi.org/10.1093/jac/dkg088>
- [18] Zhang, J., Zhang, Y., Li, W., Li, X., & Lian, X., Optimizing detergent formulation with enzymes. *Journal of Surfactants and Detergents*, **17** (6) (2014), 1059–1067. <https://doi.org/10.1007/s11743-014-1626-x>
- [19] “23. Magazine, O. T. Surgical Reprocessing Washer Disinfector Trials Comparing Non-enzymatic, Enzymatic, and Alkaline Detergents Using Industry Recognized Test Soils. - Google Search.” [Online]. Available: <https://www.google.com/search?q=23.+Magazine%2C+O.+T.+Surgical+Reprocessing+Washer+Disinfector+Trials+Comparing+Non-enzymatic%2C+Enzymatic%2C+and+Alkaline+Detergents+Using+Industry+Recognized+Test+Soils.&oq=23.%09Magazine%2C+O.+T.+Surgical+Reprocessing+Washer+Disinfector+Trials+Comparing+Non-enzymatic%2C+Enzymatic%2C+and+Alkaline+Detergents+Using+Industry+Recognized+Test+Soils.&aqs=chrome..69i57.1645j0j9&sourceid=chrome&ie=UTF-8>. [Accessed: 07-Apr-2021].
- [20] Jensen, D. A., Rogers, M. A., & Schaffner, D. W., Surfactant concentration and type affects the removal of *Escherichia coli* from pig skin during a simulated hand wash, *Letters in Applied Microbiology*, **65** (4) (2017), 292–297. <https://doi.org/10.1111/lam.12777>

- [21] Kırmusaoğlu, S., Introductory Chapter: Overview of Disinfection, in: *Disinfection*, InTech, (2018). <https://doi.org/10.5772/intechopen.81051>
- [22] Yoo, J., Antimicrobial efficacies of alkaline disinfectant solution and commercial disinfectants against *Brucella ovis*, *Korean Journal of Veterinary Service*, **32** (4) (2009), 347–351.

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