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Effectiveness of Enzyme Detergents in Cleaning Medical Devices in Critical Use Conditions

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Abstract

Infections associated with health care are a major public health problem due to the occurrence morbidity and mortality and the burden that is impose to patients, to healthcare workers and to the health systems. According to the previous statement it has been identified a serious problem associated with the biomedical element's disinfection processes, therefore it was necessary to evaluate the efficacy of twelve enzymatic detergents used for cleaning medical devices in Colombia. A preliminary exploratory study was made to determine the effectiveness of the enzymes present in the twelve enzyme detergents (obtained commercially) by three methods: (i) Formulation and observation, (ii) Bioluminescence, which was used to assess the microbial load that cause a reaction with ATP and (iii) Enzymatic activity where the enzyme is in touch with detergents to produce a detected compound by spectrophotometry. Enzyme activity is evident in most commercial products of disinfection, being majority the activity in the product 7 (P7) (12.2761 mM/mL/h) and lower for the product 4 (1.8150 mM/mL/h). P7 represents more effective performance removing organic matter. In this regard, P1, P2, P6 and P9 products had a lower removal performance relative to others, according to the bioluminescence in response to the ATP detected (RLU).

Keywords: Biocharge; Detergents; Enzyme; Luminometry; Spectrophotometry

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Introduction

Healthcare-associated infections (HAIs) are a major health problem worldwide, not only for patients but also for their family, community and state. This pathology affects all health institutions being one of the leading causes of morbidity and mortality, with high costs of health care represented in prolonged hospital stay (1 million days in supplementary hospitalization each year), with expensive antibiotics, surgical reoperations, without social costs given for loss of wages, production, etc. [1-6].

According to this, it has been identified a serious problem associated with disinfection processes of biomedical elements. In different institutions that provides health services, it has been evidenced a problematic of incidence and alerts prevalence, incidents and adverse events due to an increase in the number of cases of postsurgical users reentering infected to different functional unit's hospitalization [7,8].

The main objective for manufacturers of enzymatic detergents is to provide effective cleaning with the detersive action and proteolytic effect with the removal of organic matter, in medical devices from surgical medical procedures. Currently, washing processes are recommendations based on cleaning methods which are already stipulated at a national and international level in Colombia Resolution 2183 of 2004 (Good Sterilization Practices-GSP); Decree 4725 of 2005 (Requests for medical records provided by the INVIMA for medical devices), Resolution 2003 of 2014, and international protocols and treaties, such as AAMI, PAHO, CDC Atlanta and APIC [9-14].

The variety of products used for washing medical devices, including a wide variety of components, such as enzymatic detergents, which focuses on testing the effectiveness of these products on the market and to guarantee the consumer a complete removal of organic matter and pathogens [15].

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Therefore, it is imperative to generate immediate correction of these situations, such as detergent formulation, to demonstrate effectiveness and efficiency in disinfection and elimination of pathogenic and infectious foci [16-20].

Material and Methods

Study area

Enzymatic detergents were evaluated in different parts of the country and its effectiveness to ensure that the results are relevant and the research objectives would be achieved, the cities where the sampling took place were Barranquilla, Cali and Bogota and clinics that had all standards for cleaning surgical material that had the same geometric characteristics **(Tables 1-3)**.

Determination of ATP in the pre-sampling

Starting with a pre-sampling validated with a luminometer, to determine the number of samples required and amount of replica to be taken as statistically significant. Enzymatic detergents were subjected to critical conditions of use, before submitting it to normal use, for this purpose, a series of tools with different geometries was used (Laryngo, Stripper, Tweezers), which were impregnated with organic material (blood) and bacteria (*Klebsiella pneumoniae*), to quantify the ATP present after washing of surgical materials with enzymatic detergents [21,22].

Disinfectants

The purpose of this research is to conduct a preliminary

Table 1 Characteristics and formulation of enzyme products.

Product	P1	P2	P3	P4	P5	P6
Presentation	Liquid. Vial with 500 mL	Liquid. Vial with 120 mL	Liquid. Gallon with 4 L	Liquid. Vial with 1 L	Liquid. Vial with 1 L	Liquid. Vial with 1 L
Type of Enzyme	Lipase, protease and amylase	Protease, Lipase, Amylase, Cellulase	Amylase, Protease, Lipase and Carbohydrase	Protease	Protease, Lipase and Amylase	Amylase and Protease
Dissolution	5 mL/L	8 mL/L	15 mL/L	8 mL/L	5 mL/L	30 mL/L
pH	6.5 to 7.5	NR	7.0	7.0	7.0	6.0
Storage Temperature (°C)	<20	NR	NR	15 to 30	<35	<30
Wash Temperature (°C)	<30	NR	45	40	<60	<20
Contact Time (min)	10	5	2	1	5	3
Invima Register	NR*	2011DM-0008074	2007DM-0000565	NR*	2006DM-0000310	2007V-0004379
Product	**P 7**	**P 8**	**P 9**	**P 10**	**P 11**	**P 12**
Presentation	Solid. Pack of 12 water-soluble bags of 20 g	Liquid. Vial with 5 L	Liquid. Vial with 1 L	Liquid. Vial with 1 L	Liquid. Gallon with 5 L	Solid. Box with 100 bags of 25 g
Type of Enzyme	Protease	Protease	Protease, Lipase and Amylase	Protease, Lipase and Amylase	Amilase, Cellulase, Lipase, Mananase and Protease	Lipase, Protease and Amylase
Dissolution	5 g/L	1 to 4 ml/L	20 mL/L	7.5 mL/L	10 mL/L	5 g/L
pH	10.5	7.8	8.3 and 8.9	7.0	7.0	7,0
Storage Temprature (°C)	NR*	<32	50 to 60	<30	5 to 35	50
Wash Temperature (°C)	20	<55	<30	<50	55	50

*NR: No Register.

Table 2 Report of enzymatic activity (EA) for phosphatase and β-D-glucosidase for commercial enzymatic products.

Sample	EA Phosphatase (mM/mL/h)	EA β-D-Glucosidase (mM/mL/h)
Product 1	3,5795	0,0000
Product 2	3,0753	0,0000
Product 3	2,3191	0,0000
Product 4	1,8150	0,0000
Product 5	3,7055	2,5712
Product 6	0,0000	0,0000
Product 7	12,2761	0,0000
Product 8	0,0000	0,0000
Product 9	0,0000	3,5795
Product 10	0,0000	0,0000
Product 11	3,5669	6,1885
Product 12	0,0000	0,0000

Table 3 Forms and clinical characteristics of the devices used.

Geometry	Characteristic	Representation*
Geometry 1	Lock box	
Geometry 2	Depth measurement	
Geometry 3	Drill guides	
Geometry 4	Several boxes or containers	

*Photos captured by authors.

exploratory study to determine the effectiveness of the enzymes present in 12 enzyme detergents commonly used, obtained commercially, to which were determined to study contaminant removal: (i) Observation by three methods, in order to verify that no remaining traces of organic matter when handling the instrument after washing, (ii) Luminescence, which will be held in order to determine the microbial load as it enters contact with the device being cleaned, causing a reaction between ATP swabs and there on the surface and (iii) Enzymatic activity where enzymes produce a compound that can be detected by spectrophotometry and verify the action of enzymes. For this purpose, it was evaluated detergents with one, two, three and four enzymes. These products are treated according to the specifications in the Manufacturer's datasheet, to avoid mistakes by alterations in product and subsequently, in the analysis. All procedures were performed according to the notation and methodology proposed in the Standard Methods for the Examination of Water and Wastewater [23,24].

Determination of the enzymatic product functionality responsible for disinfection

It was evaluated 12 enzymatic detergents conventionally used for cleaning and disinfection of medical devices, to verify enzyme effectiveness. Each of the products present different formulations detailed in **Table 1** [25-27].

Experimental Procedure

To determine the enzymatic activity a calibration curve was made from the stock solution of 4-Nitrophenol ($O_2NC_6H_4OH$). Dilutions of the stock solution were made from a mixture of 2 parts of the NaCl solution and 1-part Na_2CO_3 solution.

Regarding the measurement of EA, this method consisted of a sample (enzyme detergent) which was taken to the Laboratory of Environmental Studies in Water and Soil of the University of Caldas. In these samples, the substrate solution at a defined concentration (4-Nitrophenyl-β-D-Glucopyranose (conc.>99%, M=301.26, Quantity=1 g., Laboratory: Carl Roth GmbH) and 4-nitrophenylphosphate (conc.>98%, M=371.12, Quantity=2.5 gr. Laboratory: Carl Roth GmbH, colorless) was added. 4-Nitrophenol separate (hydrolysis product) was detected at 405 nm in spectrophotometer Nanocolor UV/Vis. To do this, samples were prepared according to the respective data sheet, subsequently they were taken 5 mL of each of the products tested (enzyme detergent) and it was dissolved in 50 mL solution of sodium chloride (NaCl). Of this suspension was taken 2 mL and mixed with 2 mL of the substrate solution. It was incubated for 3 hours at 30°C with stirring in a water bath (Model D-20K) for 3 hours, after the incubation time, the reaction was stopped by adding 2 mL of solution of sodium carbonate (Na_2CO_3). Then the samples were centrifuged (Hettich 1205-1; Rotofix 32) at 4500 rpm for 10

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min at room temperature, then, spectrophotometric reading at 405 nm against a blank was performed [28]. Measuring EA was *in vivo*, i.e., it operates under the conditions of original pH of the enzyme detergent, using deionized water, in order to avoid interferences [29,30].

Estimate of the enzymatic extracellular activity

According to Marxsen et al. [28], the enzymatic activity, as measured by the spectrophotometric method, can be calculated using the following equation:

$$EA_X = \frac{(Abs_x \times D \times F)}{t}$$

Where,

EAx: Enzymatic activity of the enzyme × (mol/h)

Absx: Absorbance of the final product from incubation measured λ=405 nm

D: Dilution factor

F: Photometric factor given by the inverse of the slope calibration curve of 4–nitrophenol (mol/L)

t: Time in hours

Determination and validation of ATP

The adenosine triphosphate (ATP) to be exposed in a bioluminescence reaction in a system comprising luciferin/luciferase produces light emission, as this is directly proportional to the amount of light emitted (emitted photons depending on the number of molecules ATP transformed). This process was determined by luminometry, expressing the results as Relative Light Units (RLU), in ranges 4.6 × 10^{-18} moles of ATP. The main advantage of using bioluminescence compared to other conventional methods of reading biological dirt is to get an answer instantly avoiding mistakes of analysis that can even take [21,22,31,32].

Bioluminescence equipment characteristics

For this analysis it was used Luminometer Higyena System Sure Plus which has the best features for measuring ATP. The main parameters that was used are high selectivity, linearity, repeatability in measuring, sensitivity and precision, ease use of the equipment and short period of measure (15 seconds for the analysis and obtaining results). A total of 39 samples (in triplicate) with a total of 117 results at all locations where study was conducted is taken [21,22].

Statistical analysis

Data processing applied the Statgraphics Plus for Windows version 5.1 (DEMO) and R version 3.2.3. Normality tests of Shapiro-Wilk were performed and analysis of variance (ANOVA) and pos hoc Tukey tests (p<0.05), to differentiate between treatments.

Results

According to **Table 2**, the activity of the enzymes presented in most commercial products of cleaning and disinfection was evidence, being majority the activity in the product 7 (12.2761 mM/mL/h) and lower for the product 4 (1.8150 mM/mL/h). Only products 5 (2.5712 mM/mL/h) and 9 (3.5795 mM/mL/h) have both glucosidase and phosphatase activity. For which it is inferred both enzymes are active to start the cleaning process. The product 11 has greater enzymatic activity for glucosidase (6.1885 mM/mL/h), where, the lower catalytic activity for this enzyme presented in product 5 (2.5712 mM/mL/h).

Most samples from Bogota had lower concentration of ATP (Relative Light Units within a 0-2500 scale) with dispersed behavior. On the contrary, most samples of Barranquilla showed a higher concentration of Relative Light Units (7500 to 10,000 scale), reflecting greater dispersion of data relating to products in the city of Bogota. The performance of product P7 in both cities (Bogota and Barranquilla), whose data were similar near zero represents more effectiveness in removing organic matter. P1, P2, P6 and P9 products had lower removal performance compared to the other **(Figure 1)**.

According to the analysis of variance performed in the program Statgraphics Plus v. 5.1 (DEMO), significant differences (p<0.05) for catalysis by phosphatase among the 12 products tested are evident **(Table 4)**. Tukey pos hoc analysis was performed, showing a significant difference in the product 7. It was evident

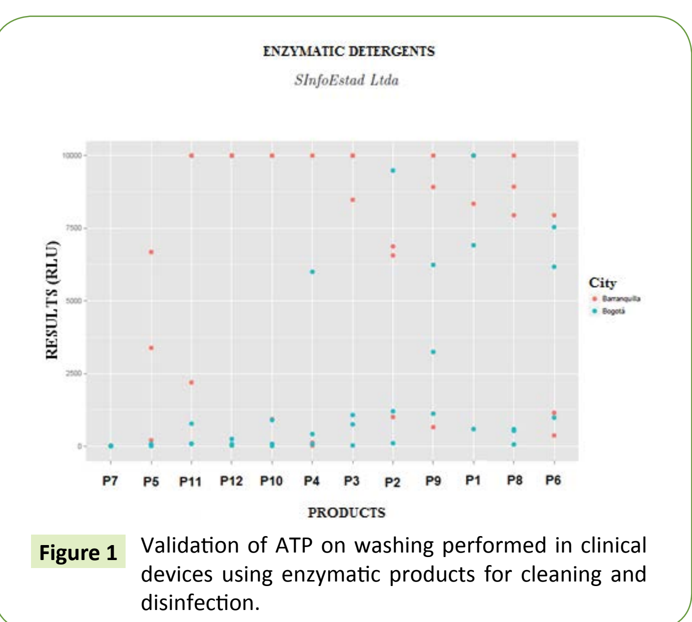

Figure 1 Validation of ATP on washing performed in clinical devices using enzymatic products for cleaning and disinfection.

Table 4 Comparison between the enzymatic activity of the phosphatase present in the 12 evaluated products.

Resource	Summ of squares	GL	Media square	F-Coefficient	P-Value
Main effects A: Product	72,7103	11	6,61003		
Residues	3,3252	12	0,2771	23,85	0,0000
Total(Corrected)	76,0355	23	-		

the homogeneity of the P6, P8, P10 and P12 products classifying them into one group tending to normal **(Table 5)**.

Regarding the enzyme activity evaluated on glucosidase, significant differences ($p<0.05$) among the 12 products tested are presented in **Table 6**. According to post hoc Tukey analysis, significant differences imprinted by product P7 in addition to the disparity between P5 and P11 products relative to others assessed it is evident **(Table 7)**.

Table 5 Tukey post hoc analysis for the enzymatic activity of the phosphatase.

Method: 95,0 Product	percentage Re-count	Tukey HSD LS Media	LS Sigma	Homogeneous Groups
6	2	0,0	0,372223	X
7	2	0,0	0,372223	X
8	2	0,0	0,372223	X
12	2	0,0	0,372223	X
10	2	0,0	0,372223	X
3	2	1,84015	0,372223	XX
4	2	1,9221	0.372223	XX
2	2	2,52705	0.372223	X
5	2	3,0753	0,372223	X
9	2	3,09425	0,372223	X
1	2	3,2644	0,372223	X
11	2	5,5646	0,372223	X

Table 6 Comparison between the enzymatic activity of β-D-Glucosidase present in the 12 evaluated products.

Resource	Sum of squares	GL	Media square	F-Coefficient	P-Value
Main effects A: Product	355,704	11	32,3367	75,64	0,0000
Residues	5,13032	12	0,427527		
Total (Corrected)	360,834	23	-		

Table 7 Tukey post hoc analysis for the enzymatic activity of glucosidase.

Method: 95,0 Product	percentage Re-count	Tukey HSD LS Media	LS Sigma	Homogeneous Groups
3	2	0,0	0,462346	X
6	2	0,0	0,462346	X
2	2	0,0	0,462346	X
4	2	0,0	0,462346	X
9	2	0,0	0,462346	X
10	2	0,0	0,462346	X
8	2	0,0	0,462346	X
12	2	0,0	0.462346	X
1	2	0,0	0,462346	X
5	2	2,76655	0,462346	X
11	2	4,2601	0,462346	X
7	2	13,7066	0,462346	X

Discussion

Significant differences between enzyme activity for phosphatase and glucosidase in the 12 enzyme products, which was corroborated by the pos hoc Tukey analysis, are presented, determining that there are significant differences in catalysis by phosphatase in samples evaluated, where increased activity is evidenced by this enzyme in contrast with the results of the enzymatic activity of the glucosidase.

With the experimental data obtained, it is possible to establish clearly the most activity of phosphatase, attributed to the limiting factor that provides the presence/absence of phosphorus for microbial metabolism enzyme. The phosphatase activity was generally higher also highlighting the action of enzymes in monoenzyme products. Indicating that multienzyme systems do not allow the activation of protein compounds and its catalysis, it follows the impossibility of cleaning and disinfection, showed no enzyme activity, since its reaction mechanisms attributed to the compounds present in the products.

Multienzyme complexes in living organisms are efficient, because they are regulated by other factors such as genes that stop catalysis, changes in pH and substrate concentration [33,14]. These systems to be dynamic self-regulate, contrary to what happens when multiple enzymes are in an aqueous solution, because they compete for the substrate, in addition, in multienzyme systems, it is not possible to adjust the pH of the water efficiently, bearing in mind that water in Colombia has a variable pH (6.6-8.1), making the correct use of multienzyme detergents even more impossible.

The products evaluated, in their safety records, affirmed the presence of six types of enzymes, which have variable pH ranges, such as amylase (5.5-8.0), lipase (7.0-11), protease (8.5-10.5), cellulase (7.0), carbohydratase (9.0-10.0) and mannanases (6.0) [34-36], which compared with the pH reported for drinking water in Colombia, denaturation of enzymes can be inferred, since they do not adjust to the physicochemical conditions of the solvent, especially at the pH they require the enzymes to achieve the maximum catalytic activity, ratifying the fact that multiple enzymes at a defined pH do not work correctly [37].

Additionally, proteolytic enzymes (hydrolases), also called proteases, carry out the transfer of functional groups to water, in order to break the peptide bonds of the amino acids present in the proteins, thus having the ability to degrade any protein substrate that is found in the middle, the proteolytic effect is described by many authors as "promiscuous" [38,39], which indicates that under uncontrolled conditions, the protease enzymes use other enzymes as a substrate even from the same family that are present in the environment, which can respond to the values reported in multienzyme systems.

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Conclusion

According to the above, it becomes clear that the enzymatic activity in most commercial products of disinfection, being majority in the product 7 (P7) (Monoenzymatic bactericidal detergent in water-soluble doses of 20 g.), which is more effective performance removing organic matter. In this regard, P1, P2, P6 and P9 products had a lower removal performance relative to others, according to the bioluminescence in response to the ATP detected (RLU).

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