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EVALUATION OF THE EFFICIENCY OF AUTOCLAVING HEALTHCARE WASTE USING BIOLOGICAL AND CHEMICAL INDICATORS

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ABSTRACT

Autoclaving is among the techniques used to treat health services waste belonging to the most applied group of biohazards and sharps. However, a shortcoming of the autoclaving treatment involves the operational parameters and appropriate frequency for monitoring the process because there is limited scientific evidence for using the optimal parameters. The objective of this study was to evaluate the efficiency of the autoclaving process, using biological and chemical indicators, at different temperatures and exposure times, in three enterprises that provide services of autoclaving health care waste. To this end, partnerships were signed with three private enterprises and an operational procedure was developed to conduct real-scale tests under three different scenarios, using temperatures of 150, 132, and 125°C at 15-min exposure times and reduced exposure times (4 or 10 min). The results showed that among the three projects, a statistically significant difference between exposure times of 15 min and 4 min of the healthcare waste under Scenario 2, at a temperature of 132°C, only occurred in one case. However, modification of the exposure time and/or operational temperature requires further studies as well as confirmation from an environmental agency.

1. INTRODUCTION

Significant progress and improvement in health services for risk elimination has resulted in an increase in the rate of healthcare waste generation, particularly in developing countries that have deficient normative instruments for the proper management of this waste (Windfeld, 2015).

Several authors have argued that there is no evidence that healthcare waste is, in fact, more contaminated than urban solid waste or that it can cause environmental diseases and contamination (Zanon, 2002; Cussiol, 2005; Costa e Silva et al., 2011). However, most authors establish an exception to sharps and biological waste, which require treatment before final disposal because they are capable of containing potentially pathogenic microorganisms and serve as a vehicle for disease dissemination, putting at risk the professionals who manage it (Nascimento et al., 2009).

According to Ciplak and Kaskun (2015), the technologies used in the treatment of healthcare waste are categorized into two groups: high-temperature technologies, which covers incineration, and low-temperature technologies comprising alternative technologies such as autoclaving and microwave. However, the authors noted that in developing countries there is inadequate means of targeting healthcare waste as it is an easy and low-cost method.

According to Windfeld (2015), in developed countries such as the United States and the European Union, incineration is the main form of healthcare waste treatment. However, these countries have been searching for centralized forms of incineration treatment to have better equipped facilities to control atmospheric emissions. However, because of the enactment of stricter atmospheric emission limits, there is a trend towards the closure of incineration plants in favor of alternative treatments.

Autoclaves, among the main alternative technologies to incineration, are equipment that use heat and temperature to inactivate microorganisms and were originally developed for sterilization of equipment and surgical and laboratory materials (Zhao et al., 2009).

The autoclave treatment of healthcare waste consists of the use of humidity, pressure, and heat, in a controlled manner, for inactivation of the microbial load in the waste mass. To conduct the treatment, the residues are disposed in the equipment and exposed to water vapor for a predetermined time and at a predetermined temperature (Pichtel, 2005). Steam waste treatment equipment is typically operated at minimum standards, between 121°C to 134°C,

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for 30 min, as a basis for the already consolidated application of autoclaving for sterilizing medical products (WHO, 2014).

However, there is a lack of normative instruments that regulate alternative treatments, such as autoclaving. Moreover, as highlighted by Taghipour et al. (2016) and Oliveira (2017), there is a lack of data in the literature that specify ideal parameters for autoclaving treatment, such as exposure time, temperature, and ideal pressures for microbial inactivation of healthcare waste as well as the establishment of appropriate frequencies for monitoring of the process by means of efficiency tests. Such a situation may pose risks to public health, particularly to workers in these treatment enterprises, as well as environmental impacts.

The efficiency of an autoclaving process depends on controlling the physical measurements of time, temperature, and pressure, as well as biological indicators (BIs) and chemical indicators (CIs) used (Sandle, 2016).

This efficiency evaluated using BIs is measured by a representative rate of the number of inactivated or killed microorganisms following treatment (Teng et al., 2015). The quantitative assessment of the reductions in the microbial populations considers the

resistance of different species present, and is conducted according to predefined scales (Rodrigues, 2008).

The State and Territorial Association on Alternative Treatment Technologies (STAATT, 1998) defined and quantified microbial inactivation as "Log10 Inactivation," characterizing it as the difference, before and after treatment, between the logarithm of the number of viable microorganisms. The common standard of microbial inactivation for healthcare waste treatment is Level III, based on the criteria established by STAATT.

The 1994 STAATT rated acceptable levels for ensuring the performance of health care waste treatment technologies were revised in 1998. Level III microbial inactivation refers to "inactivation of vegetative bacteria, fungi, hydrophilic / lipophilic viruses, parasites and mycobacteria in a log 6 reduction or greater, and inactivation of Geobacillus stearothermophilus spores and Bacillus atrophaeus spores with reduction equal to or greater than 4Log10" (STAATT, 1998).

Chemical indicators assess whether the temperature, pressure, and time parameters have been adequately achieved; they completely change in color if the vacuum system is functioning properly, helping to identify faults (WHO, 2016).

Some studies have evaluated the application of biological and chemical indicators to monitor autoclaves. These include Garibaldi et al. (2017), who evaluated the standard configuration of two autoclaves for the healthcare waste treatment of Ebola-infected patients, considering different exposure times, temperatures, and pressures. The authors found that 16 out of 19 autoclave cycles with the factory default setting were positive for the biological indicators inserted in the center of the load. The optimized parameters for dry waste autoclaving were: a time of 30 minutes, temperature of 134°C, and pressure of 20 psi.

Experiments with both real wastes and simulated loads were also performed using both different combinations of exposure time and pressure pulses as well as biological indicators to evaluate the process efficiency. The authors concluded that deeper pressure pulses are more effective than shallow pulses for treatment (Emmanuel; Kiama; Heekin, 2008).

Considering that the absence of a systematic standardization and evaluation of healthcare waste treatment technology via autoclaving may result in permanent damage to public health, professionals, and the environment, this work aimed to evaluate the efficiency of the autoclaving process, using BIs and CIs, at different temperatures and exposure times, in three enterprises that provide services of autoclaving of health services waste.

2. MATERIALS AND METHODS

Considering the lack of standardization of test frequency using BIs and CIs between enterprises, as well as the non-standardization of the locations of insertion of these indicators in the mass of waste, it was proposed that three enterprises, which provide services of autoclaving of healthcare waste, be tested to evaluate the efficiency of the process, using BIs and CIs at different temperatures and exposure times.

The three enterprises used pre-vacuum horizontal type autoclaves with the following capacities: company I - 400 kg / hour; company II - 600 kg / hour; company III - 600 kg / hour.

As highlighted by Garibaldi et al. (2017), "cycles that evaluate treatment efficiency should contain the biological indicators disposed within the waste load, considering that indicators outside the mass of waste may not reflect actual treatment conditions," a fact that demonstrates the importance of assessment of the mass of waste.

Thus, for the insertion of indicators in the mass of waste, it was proposed for the enterprises the support be manufactured (Figure 1), such that the indicators were inserted in the middle and at the bottom of the container, considering that some ventures conducted monitoring using indicators on the external side of the container; therefore, it is necessary to evaluate the treatment conditions inside the waste mass, where it would be more difficult to contact the vapor with the waste.

The indicators were arranged in the mass of the waste, both at the bottom and in the middle of the containers, approximately 10 cm and 50 cm from the base of the containers, such that the conditions in which the healthcare wastes were exposed during the treatment were evaluated.

Tests using chemical, biological, and class IV indicators, which are multiparametric indicators that are capable of reacting to two or more critical process units, were conducted for 10 consecutive days under three scenarios in the three enterprises. It should be emphasized that the enterprises were oriented to acquire the same indicators, of brands that are commonly used, considering that each BI lot can present a variation in its population, resistance, and time of inactivation of the microorganisms, as caused by genotypic and phenotypic variations in spore cultures (Sandle, 2016).

In two ventures an exposure time of 10 min was applied as the shortest exposure time and in another venture an exposure time of 4 min was applied. An exposure time of 15

FIGURE 1: Supports for insertion of indicators.

min was common for all the enterprises, considering that it is the standard time used during the treatment processes, according to Figure 2.

The exposure time of 4 min was adopted based on the arguments reported by McKeen (2018). The authors presented the most used parameters for two types of autoclaves (gravitational and pre-vacuum) for sterilization of medical-hospital products, in which pre-vacuum autoclaves at temperatures from 132 to 135°C require exposure times of 3 to 4 min. In this manner, it is interesting to evaluate if such parameters would also be suitable for waste decontamination. The exposure time of 10 min was adopted because it was an intermediate time between the times previously proposed.

Temperatures within a range between 145 and 150°C were adopted under Scenario 1 because they are temperatures commonly used by developments during the treatment process. The temperature of 132°C was adopted under Scenario 2 as the maximum temperature recommended by the manufacturers for use of the BIs, while the temperature of 125°C was used under Scenario 3 because it was within the temperature range (from 121 to 127°C) commonly recommended and used in sterilization processes for medical and hospital materials.

For the tests, the indicators commonly used by the companies were used. Two different brands of BIs (3M and Cristófoli) and CIs (class IV of the 3M and Cristófoli brands) were used, and the same lots were used in case of acquisition needs later. Each scenario was evaluated over 10 days to verify several possible situations of loads received, in relation to the variation in composition, by the enterprises.

The enterprise 1 uses BI 1262 of 3M, containing 105 spores of G. stearothermophilus, with a reading in 48 hours. The BI changes color to yellow if there are surviving spores in the ampoule, indicating positive results. The CI used by the enterprise is of class IV and also from the same manufacturer.

In enterprise 2 the BI and CI were fixed to the outside of the container and two stainless-steel supports, one of 70

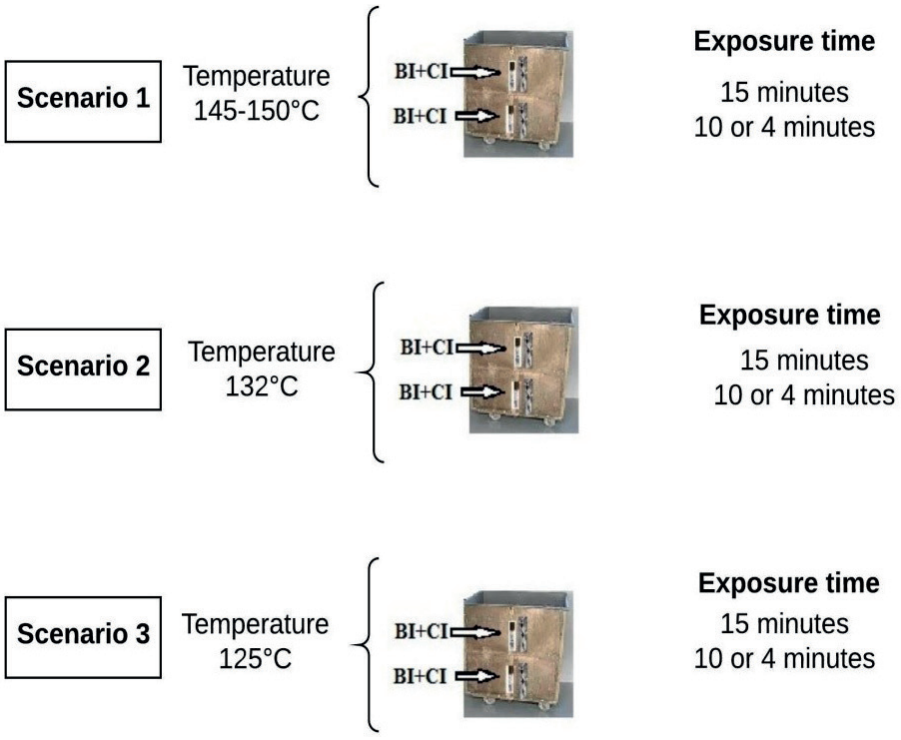

FIGURE 2: Tests plan.

cm and one of 1 m, were constructed for the insertion of the indicators in the mass of waste. The supports have a device for insertion of the BI, in the internal part, and another for the insertion of the CI, in the external part.

The enterprise 3 uses the 3M BI (Attest 1492 Super Fast Reading; American Type Culture Collection 7953 biological index) with a 1hour reading. The CI used by the enterprise is of class IV and also from the same manufacturer.

Following the treatment process, the indicators were removed from the containers. The CIs presented immediate results and the BIs were incubated, according to the manufacturers' instructions, for further reading of the results.

After all tests, the results were statistically analyzed using the Association or Independence Test (Chi-square test), which is used to evaluate the correlation between categorical variables. For this, two qualitative variables and the data were organized in a contingency table (Callegari-Jacques, 2003). The test statistic chi-square (χ^2), describes in a single number how the frequencies observed in each table cell differ from the frequencies that would be expected if there were no relationship between the treatments and results that define the lines and the table columns.

3. RESULTS AND DISCUSSION

3.1 Enterprise 1

The support, in wood, used by the enterprise to insert the indicators in the masses of waste presented some irregularities during the tests. The enterprise reported that the support did not withstand some tests and broke, requiring fabrication of new supports. It was observed that the material considerably deteriorated as it was subjected to the tests. However, the indicators were not lost amid the masses of waste, allowing their recovery and insertion in the incubator to analyze the results.

Table 1 shows the results of the tests applied during the project. It was possible to observe that some BIs were dry follows the incubation period, which made it impossible to read the results. We were informed that this situation has been frequently occurring in the enterprise. According to the manufacturer of the indicators, this may have occurred because of inadequate storage conditions.

From the analysis of Table 1, it is possible to observe that all the BIs evaluated under Scenario 1 at 150°C over 15 min of exposure, inserted at the bottom of the containers (10 cm from the base), showed inactivation of Geobacillus stearothermophilus spores. However, three BIs showed dryness, which impaired the reading of the results. The CIs, inserted under the same conditions to which the BIs were submitted, presented 90% satisfactory results.

Under this same Scenario 1, under the condition in which the BI was inserted within 50 cm of the container base, the results were similar to those of the BIs inserted at the bottom of the container. Five BIs showed dryness, rendering the reading unfeasible. As for the CIs, inserted under the same conditions, they presented 90% satisfactory results.

As for the 10-min exposure time under Scenario 1, none of the BIs showed spore growth for those inserted 10 cm from the base. However, under these conditions, five BIs were dry. The CIs, inserted under the same conditions to which the BIs were submitted, presented 100% satisfactory results.

For those BIs inserted in the middle of the containers (50 cm from the base), under the same aforementioned conditions, the problem of dryness in five BIs occurred; of the valid BIs, one showed spore growth. The CIs, inserted under the same conditions to which the BIs were submitted, presented 90% satisfactory results. Notably, the CI that presented an unsatisfactory result was not the one inserted in the same medium in which the BI showed spore growth.

Regarding Scenario 2, in which a temperature of 132°C was evaluated, considering an exposure time of 15 min,

TABLE 1: Results tests enterprise 1.

Tests	**Enterprise1**											
Scenario 1-150°C (Exposure time)	BI 10 cm			CI 10 cm			BI 50 cm			CI 50 cm		
	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]
15 minutes	7	7	0	10	9	1	5	5	0	10	9	1
10 minutes	5	5	0	10	10	0	5	4	1	10	9	1
Scenario 2-132°C (Exposure time)	BI 10 cm			CI 10 cm			BI 50 cm			CI 50 cm		
	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]
15 minutes	6	4	2	10	9	1	7	7	0	10	10	0
10 minutes	6	6	0	10	10	0	9	9	0	10	10	0
Scenario 3-125°C (Exposure time)	BI 10 cm			CI 10 cm			BI 50 cm			CI 50 cm		
	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]
15 minutes	10	3	7	10	6	4	7	7	0	10	10	0
10 minutes	10	3	7	10	8	2	9	9	0	10	9	1

** N: Number of indicators (biological and chemical) evaluated*
[1] NGS: No growth of spores of Geobacillus stearothermophilus
[2] GS: Growth of spores of Geobacillus stearothermophilus
[3] S: Satisfactory result for chemical indicator
[4] UNS: Unsatisfactory result for chemical indicator

66.7% of the BIs showed satisfactory results and 33.3% showed unsatisfactory results. Under this scenario and at the time of exposure, four BIs showed dryness. The CIs, inserted under the same conditions to which the BIs were submitted, presented 90% satisfactory results. Notably, the CI that showed an unsatisfactory result was inserted in the same support as that BI that showed spore growth.

Under the condition in which the BI was inserted at 50 cm from the base of the container, none of the BIs showed growth; however, three BIs showed dryness, rendering the reading unfeasible. Regarding the CIs, all showed satisfactory results.

As for the results of the 10-min exposure time under Scenario 2, none of the BIs presented spore growth for those BIs inserted 10 cm from the base. However, under these conditions, four BIs were also dry. The CIs, inserted under the same conditions to which the BIs were submitted, showed 100% satisfactory results.

In the BIs inserted 50 cm from the base of the container, for the same aforementioned condition, none of the BIs showed growth and only one showed dryness. Regarding the CIs, all showed satisfactory results.

Scenario 3, in which a temperature of 125°C was evaluated, showed the most problems in terms of microbial inactivation. At an exposure time of 15 min, for the BIs inserted 10 cm from the base, 70% of the BIs evaluated showed spore growth. Under this condition, none of the BIs showed dryness which may have been related to the temperature at which the BIs were submitted, considering that the manufacturer establishes the maximum temperature as 132°C. Regarding the CIs, 40% showed unsatisfactory results, of which only two (i.e. one-half) were inserted together with BIs that also showed unsatisfactory results, demonstrating spore growth.

Under the condition in which the BI was inserted at 50 cm from the base of the container, none of the BIs that could be evaluated showed growth, but three BIs showed dryness, rendering the reading unfeasible. Regarding the CIs, all showed satisfactory results. Such a situation indicates that the bottom of the container (approximately 10 cm from the base) is a critical point for vapor penetration.

Regarding the results of the 10-minute exposure time under Scenario 3, the results of the BIs inserted at 10 cm from the container base were similar to those of the 15-min exposure time, in which 70% showed spore growth. However, only 20% of the CIs showed unsatisfactory results, both related to BIs that showed spore growth.

Under the condition in which the BI was inserted 50 cm from the base of the container, none of the BIs showed growth; however, a BI showed dryness, rendering the reading unfeasible. Regarding the CIs, 90% showed satisfactory results.

Notably, under all scenarios, the control BIs were also inserted in the incubator and all cases showed positive results with the presence of G. stearothermophilus in the ampoules.

From the statistical analysis of the BIs, it was possible to conclude that there was no significant difference in the inactivation of G. stearothermophilus spores between exposure times of 15 and 10 min., in relation to the different temperatures, in the three Scenarios evaluated. Thus, all the tests demonstrated that the inactivation of G. stearothermophilus is independent of the exposure times evaluated.

However, when there was a significant difference between the insertion positions of the indicators (10 and 50 cm from the container base), a statistically significant difference was observed between the position of the BIs of Scenario 3, at both exposure times. This result reveals that the BI position interfered with the inactivation of G. stearothermophilus spores, with the bottom of the container being a critical point for vapor penetration.

The enterprise ensured that, for those unsatisfactory BI results, the wastes were again submitted to the treatment process, until microbial inactivation efficiency was obtained, so that the healthcare waste were sent to final disposal.

3.2 Enterprise 2

During the validation phase of the supports, during the study, the results of the indicators were not satisfactory; thus, it was necessary to adapt and develop holes in all their extensions to enable steam insertion inside the supports.

Another necessary adaptation was the insertion of a BI in the support, considering that some BIs were showing water inside the ampoule, which made it impossible to read the results. In this sense, the BIs were repositioned on the supports with the lid facing upwards.

To perform the tests under Scenario 2, research of the new holes in the supports was conducted because of the sealing of the holes caused by the deformation of the plastic packages following the treatment process.

After adaptation, the indicators demonstrated good performance when they were in the treatment process, and did not deteriorate, which can cause the loss of indicators.

After the entire treatment cycle, the indicators were removed from the supports and evaluated and the BIs were inserted into the incubator to read the results. Quick use of the 3M mark, 1292 fast reading, containing 105 spores of G. stearothermophilus, with 3 h of reading, and CI class IV, from the same manufacturer, was completed. Table 2 shows the results of the proposed scenarios.

Notably, the enterprise was the first to conduct such tests in this manner; a trial period of 30 days was initially proposed under Scenario 1, to validate the supports and the test plan. However, because of operational issues, tests were performed for 24 and 25 days.

It is important to note that some BIs inserted in the support that evaluated the conditions at 10 cm from the base of the containers, under Scenario 1 for the exposure time of 15 min, showed water inside, which compromised reading of the results.

In this manner, the enterprise chose to perform most of the tests at the reduced time (4 min of exposure) under Scenario 1 with the indicators inserted in the middle of the waste mass (supported 50 cm from the base of the container) to avoid compromising the reading of the BIs.

From the analysis of Table 2, it can be observed that 76.2% of the BIs evaluated under Scenario 1, at 150°C over 15 min of exposure, inserted at the bottom of the containers (10 cm from the base), showed inactivation of Geoba-

TABLE 2: Results tests enterprise 2.

Tests	Enterprise 2											
Scenario 1-150°C (Exposure time)	BI 10 cm			CI 10 cm			BI 50 cm			CI 50 cm		
	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]
15 minutes	21	16	5	24	24	0	24	23	1	24	24	0
4 minutes	1	1	0	1	1	0	25	21	4	25	25	0
Scenario 2-132°C (Exposure time)	BI 10 cm			CI 10 cm			BI 50 cm			CI 50 cm		
	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]
15 minutes	20	12	8	20	20	0	20	12	8	20	20	0
4 minutes	20	2	18	20	20	0	20	2	18	20	20	0

** N: Number of indicators (biological and chemical) evaluated*
[1] NGS: No growth of spores of Geobacillus stearothermophilus
[2] GS: Growth of spores of Geobacillus stearothermophilus
[3] S: Satisfactory result for chemical indicator
[4] UNS: Unsatisfactory result for chemical indicator

cillus stearothermophilus spores and 23.8% of the tests evaluated did not show spore inactivation. However, the CIs, inserted under the same conditions to which the BIs were submitted, showed 100% satisfactory results. Under this same scenario, under the condition in which the BI was inserted 50 cm from the base of the container, 95.8% of the BIs showed satisfactory results of spore inactivation. In addition, the CIs evaluated under the same conditions also showed 100% satisfactory results. However, the results of the 4-min exposure time under Scenario 1 showed more spore growth (16% of the total BIs evaluated at a height of 50 cm from the container base), when compared to that of the 15-min exposure time. However, all CIs showed satisfactory results.

In relation to Scenario 2, in which the temperature of 132°C was evaluated at exposure times of 15 and 4 min, the enterprise chose to perform two tests per day, totaling 20 tests over 10 days. Under this scenario, it was possible to notice a greater divergence between the results. At an exposure time of 15 min, in both positions, 60% of the BIs did not show spore growth and 40% showed problems of microorganism inactivation, out of a total of 20 tests. However, the CIs showed all satisfactory results.

At an exposure time of 4 min, the situation was very unsatisfactory, considering that in both insertion positions the BIs showed 90% G. stearothermophilus spore growth, out of a total of 20 tests performed. Once again, the CIs showed 100% satisfactory results.

From the statistical analysis of the BIs, it was possible to conclude that for Scenario 1 there was no significant difference in inactivation of G. stearothermophilus spores between exposure times of 15 and 4 min under the two scenarios evaluated. However, under Scenario 2, there was a significant difference between exposure times, demonstrating that the inactivation of G. stearothermophilus spores is not independent of the exposure time employed. Thus, the exposure time of 4 min at a temperature of 132°C was not shown to be adequate for spore inactivation.

Notably, under all scenarios, the control BIs were also inserted in the incubator and all cases showed positive results, with the presence of G. stearothermophilus in the ampoules.

Considering that all the CIs showed satisfactory results, it was not possible to apply statistical tests for comparison. These findings demonstrate that using CIs only to assess the efficiency of the treatment process may not guarantee microbial inactivation in the mass of residues, although physical parameters are attained.

3.3 Enterprise 3

Enterprise 3 already used a support to perform BI tests. According to the managers of the project, BI loss was commonly recorded, both in the mass of the residue, because it became quite dense after treatment, and because of the dryness of the BI, which compromised the reading of the results. According to those responsible, after manufacturing of the supports, such problems were remedied.

However, the enterprise did not use CI to evaluate operational conditions. In this manner, it was proposed to acquire the indicator for conducting the tests. Considering that in one holder it was possible to insert only one indicator, two supports were required; one for insertion of the CI and another for insertion of the BI. Both were inserted in the mass of residues in the same position, being fixed to each other and in the container by a wire.

Following the treatment, the indicators were removed from the containers and evaluated, and the BIs were inserted into the incubator to read the results. The enterprise uses the BI of the mark 3M, super-fast reading Attest 1492, with readings of 1 h, containing 105 spores of G. stearothermophilus. The project opted to acquire the CI of the same manufacturer, in which the CI class IV was acquired. Table 3 shows the results obtained under the proposed scenarios.

Table 3 shows that, in general, the results of all the scenarios were satisfactory. It is possible to notice that all the BIs evaluated under Scenario 1, at 150°C over 15 min and 4 min of exposure, inserted at the bottom of the containers (10 cm from the base), showed inactivation of G. stearothermophilus spores. The CIs, inserted under the same conditions to which the BIs were submitted, showed 80% satisfactory results for both exposure times.

Under the condition in which the BI and CI were inserted within 50 cm of the container base, the results were also

TABLE 3: Results tests enterprise 3.

Tests	Enterprise 3											
Scenario 1-150°C (Exposure time)	BI 10 cm			CI 10 cm			BI 50 cm			CI 50 cm		
	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]
15 minutes	10	10	0	10	8	2	10	10	0	10	10	0
4 minutes	10	10	0	10	8	2	10	10	0	10	10	0
Scenario 2-132°C (Exposure time)	BI 10 cm			CI 10 cm			BI 50 cm			CI 50 cm		
	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]
15 minutes	10	10	0	10	10	0	10	10	0	10	10	0
4 minutes	10	10	0	10	10	0	10	9	1	10	10	0
Scenario 3-125°C (Exposure time)	BI 10 cm			CI 10 cm			BI 50 cm			CI 50 cm		
	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]	N*	NGS[1]	GS[2]	N*	S[3]	UNS[4]
15 minutes	10	10	0	10	9	1	10	10	0	10	10	0
4 minutes	10	10	0	10	8	2	10	10	0	10	10	0

* N: Number of indicators (biological and chemical) evaluated
[1] NGS: No growth of spores of Geobacillus stearothermophilus
[2] GS: Growth of spores of Geobacillus stearothermophilus
[3] S: Satisfactory result for chemical indicator
[4] UNS: Unsatisfactory result for chemical indicator

satisfactory for both exposure times, with no readability or unsatisfactory conditions occurring.

Regarding Scenario 2, in which the temperature of 132°C at an exposure time of 15 min was evaluated, all conditions also showed satisfactory results, showing only growth of G. stearothermophilus spores at an exposure time of 4 min, for the BI inserted in the middle of the container (50 cm from the base).

Scenario 3, in which a temperature of 125°C was evaluated, also showed satisfactory conditions similar to those of the other scenarios, presenting unsatisfactory results only in some CIs.

The developers stated that the manufacturer guarantees satisfactory treatment conditions at temperatures up to 121°C, which may justify the results of the three scenarios.

From the statistical analysis of the BIs, inactivation of G. stearothermophilus spores occurred between the exposure times of 15 and 4 under the three scenarios evaluated at the different temperatures evaluated.

In enterprises 1 and 3, it was observed that at the temperature and exposure time commonly used in the operation of the enterprises (145 or 150 °C, with healthcare waste exposure time of 15 minutes), both biological and chemical indicators presented satisfactory results.

From the results it was not possible to establish an ideal frequency for the use of the indicators in all scenarios, considering that in some enterprises there was a considerable variation between satisfactory and unsatisfactory indicators during the proposed test days.

However, in all the enterprises analyzed it was observed that at the temperature and exposure time commonly used in the operation of the projects (145 or 150 ° C, with exposure time of 15 minutes), the indicators, both biological and chemical, presented satisfactory results. This may indicate that the use of biological indicators at a frequency of use of twice a week would be appropriate for process monitoring.

4. CONCLUSIONS

The technique of autoclaving healthcare waste, although widely used for the treatment of healthcare waste from the biological and sharps group, presents some shortcomings in relation to the lack of specific legislation, to establish criteria and guidelines, mainly operational, to standardize the activity.

Although only one enterprise had a statistically significant difference between the exposure times for healthcare wastes of 15 min and 4 min, under Scenario 2 (at a temperature of 132°C), the results did not show that the ventures can, therefore, reduce the exposure time and / or the operating temperature, considering that the temperature of 125°C also showed the highest number of unsatisfactory results for enterprise 1.

The results also warned of the limitation of vapor penetration at the bottom of the containers, which must be observed and monitored by the enterprises, to evaluate, mainly, excess cargo in the containers.

In addition, another point to be evaluated by the enterprises is the use of operating temperatures above those suggested by the indicators' manufacturers, considering that in enterprise 1 the temperature of 150°C may have been a preponderant factor for the dryness of the indicators which impaired reading them.

Considering that some authors have reported on the lack of conclusive studies regarding the reliability of CIs, the results of the CIs obtained in this study indicate a limitation because they do not present similar responses to those of the BIs. Thus, it is clear that CIs should not act as a substitution for BIs but should be used for supportive monitoring to avoid false-negative or false-positive results.

It is necessary to conduct additional studies before any modification in the treatment process is carried out, mainly evaluating the insertion of indicators at different points and in higher quantities of containers per cycle, in addition

to the evaluation and validation of an environmental agency, considering the licensing of the development.

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