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Performance evaluation of the sterilization process with Bowie & Dick test and biological indicator in the quality control of a blood bank in Peru

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Abstract

Managing contaminated waste in blood banks is a crucial process that must end with the safe disposal of blood products. In this sense, practical methods and indicators must be used to guarantee the operability of autoclave equipment and the sterility of treated waste to mitigate the risk of biological exposure and hospital biosafety. We designed a cross-sectional study to evaluate the performance the autoclaving process with Bowie & Dick test and biological indicator at the Hemotherapy and Blood Bank Unit of the Cayetano Heredia Hospital in Peru. Fifty autoclaving processes were carried out independently for the Bowie & Dick tests and biological indicators based on *Geobacillus stearothermophilus* spores. Autoclaving was programmed at 134°C for 3.5 minutes for the Bowie & Dick test, while, for the biological indicator, it was programmed at 121°C for 5 minutes. Both in the presence of contaminated waste. The autoclaving process evaluated by the Bowie & Dick test had a compliance rate of 80% (CI95: 66.3%–90.0%), while, by the biological indicator, 90% (CI95: 78.2%–96.7%). We did not find significant differences between the results of both tests ($P = .689$; Fisher exact test). The compliance rate in the autoclaving process within the blood bank of a Peruvian Hospital is acceptable; however, there are actions for continuous improvement, especially at the vacuum level in the autoclaving process.

Keywords: autoclave, biological indicator, Bowie & Dick test, sterilization

1. Introduction

Hospital biomedical waste involving human-derived biological matrices must be adequately disposed to limit contamination risks and environmental impact.[1] Approximately 10% to 25% of hospital waste is estimated to be hazardous; however, under the principle of universality, all biological waste is considered potentially infectious.[2] The World Health Organization biosafety manual establishes that the clinical laboratory generates the most significant amount of biological waste at the hospital level and, in particular, the blood bank.[3] This situation is due to the management of units of blood or heme components for transfusion that, under certain conditions, are not used; due to expiration, seropositive screening, and incomplete donation, among others.[4] In this sense, the management and disposal of this contaminated waste must be ensured through sterilization processes before its final disposal, which results in a weakness in most clinical laboratories and blood banks.[5]

The sterilization of contaminated waste is commonly carried out in autoclaves, equipment that generates steam at high temperatures and pressure that manages to penetrate the biological material, denaturing proteins and inactivating microorganisms.[6] Autoclaving is the most robust and cost-effective method for sterilizing biomedical waste and devices. However, it may be <100% effective in eliminating all microorganisms under various circumstances.[7] Frequently, this process is not objectively monitored in clinical laboratories of low- and medium-income countries.[8] Consequently, the effectiveness of the process cannot be demonstrated or guaranteed.[9] There are various commercial, physical, chemical, and biological methods for the control and monitoring of each sterilization cycle, with

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the use of indicator paper or the Bowie & Dick test being the most widely used, even when it shows low specificity and high rates of false negatives.[10] This test challenges the sterilizer ability to remove air (non-condensable gases) from a dense package and displace it with steam.[11] The non-condensable gases NCG reduce the efficiency of heat transfer affecting thermal coagulation of microorganisms.[12] Reports show that 3.5 mL of NCG for every 100 mL of condensable gases in a sterilizer could reduce the efficiency by 21%.[13] The Bowie & Dick test type 2 uses cellulose papers impregnated with a chemical indicator in the most difficult-to-penetrate sections within the system, which is made up of papers laid one on top of the other. Evidence of adequate steam penetration is indicated by homogeneous color change, while if there is a partial change, it is considered incomplete steam penetration.[11]

In the last 2 decades, biological indicators based on thermo-tolerant microorganisms such as Bacillus stearothermophilus have been implemented, which under the spore stage resists high temperatures that allow monitoring of sterility processes, validating it when degraded at 121°C, thus that the evidence of active bacterial metabolism by the color change in the indicators reflects an ineffective autoclaving process.[14] These biological indicators benefit the evaluation and quality control of sterilization processes in clinical laboratories seeking accreditation, due to their more excellent resistance to the contaminating bioburden, according to International organization for standardization 14937:2009 and International organization for standardization 22441 standards: 2022.[15] However, the evaluation of the process must be comprehensive and based on an analysis of causes, identifying variables that can generate contamination in the products that are sterilized, such as the time, pressure, and temperature of the autoclave programming, handling and quantity of loads, preventive maintenance and operators.[16]

There needs to be more information on the performance of autoclaving and analysis of efficacy, a determining aspect in the quality of sterilization of contaminated waste.[17] In this sense, our research aimed to evaluate the steam sterilization process through Bowie & Dick tests and biological indicators based on Bacillus stearothermophilus. Our results contribute to improving the handling and management processes of contaminated waste generated in blood banks.

2. Methodology

2.1. Design and place of study

We designed a cross-sectional study to assess the performance of autoclaving sterilization process comparing the results of 2 methods (biological indicators and Bowie & Dick test) used at the Blood Bank of the Cayetano Heredia National Hospital during the months of November and December of 2019. This Hospital is a level III Center (the highest category in the Peruvian health system). It is the most important in the northern area of Lima, the capital of Peru. The sterilization processes for each method were pre-established by the Hemotherapy and Blood Bank Unit in its internal procedures. The autoclave is exclusive for biological materials derived from the Hospital blood bank (tubes with blood samples, polypropylene vials, and blood collection bags).

The number of tests to evaluate was estimated by comparison of proportions in independent groups, with a confidence and power level of 95% and 80%. We consider a difference in proportions of 15%, a variance of 10%, and a rejection rate of 10%, obtaining a sample of 50 measurements.

2.2. Procedures and techniques

Sterilization process. We used an 80 L capacity autoclave (JP Selecta, TM), a temperature programming range of up to 150°C, and a pressure of 2.5 psi. The contaminated material sterilization process was carried out at 121°C at 15 pounds of pressure for 20 minutes, as established in the autoclaving procedure of the Hemotherapy and Blood Bank Unit of the Cayetano Heredia Hospital.

Bowie & Dick test. We use a commercial Bowie & Dick test (Famos Medizintechnik, Germany) in pre-vacuum sterilizers. During the execution, an initial test, the autoclave was carried out unloaded (empty) and was programmed at 134°C for 3.5 minutes, according to the manufacturer instructions. After the accepted result, the challenge tests were placed on all cycles, being positioned next to the load. The results were reported as compliant or non-compliant evidence. Compliance is established by observing the complete color change of the lines or stripes on the paper (See Fig. 1. Supplemental digital content, http://links.lww.com/MD/K258, which shows the autoclaving process using the Bowie & Dick test). The judgment of the results was stablished by authors in agreement with the paper patterns.

Biological indicator. We used a commercial biological indicator prepared with Geobacillus stearothermophilus spores (American type culture collection 7953, EZTest Mesalabs, Germany) in a minimum population of 1×10^5 spores in a culture medium, modified trypticase soy broth, with a pH-sensitive indicator (purple of bromocresol).[18] The indicator was used in each loaded autoclaving process (with contaminated residues) with the parameters previously described. After the process was completed, the ampoules were incubated at 60°C for 24 hours, and they were read, reporting as compliant or non-compliant (See Fig. 2. Supplemental digital content, http://links.lww.com/MD/K259, which shows the autoclaving process using the biological indicator).

The assays for both methods were performed in different days but in pairs, one after the other (randomly) under similar environmental conditions and with the same operator and charge. The qualification of the installation and operation of the equipment was carried out by the supplier company.

This research did not involve the participation of human beings or the manipulation of biological matrices. Therefore, informed consent was not required. However, administrative permission was obtained from the Cayetano Heredia National Hospital, and the project was approved by the Review Committee of Alas Peruanas University through Resolution No. 192-2019-EPTM-FCS-UAP.

2.3. Statistical analysis

The 2 tests used in the autoclaving process were evaluated by the compliance rates and their 95% confidence interval. The compliance rate obtained by each process was compared with Fisher exact test, considering a probability value of $< .05$ as a significant difference. The agreement between the results of both tests was evaluated using the rate of agreement and expected agreement with its standard error. The analysis used Stata software (StataCorp College Station, TX) version 17.0.

3. Results

We carried out 50 autoclaving tests evaluated by the Bowie & Dick test and biological indicator independently (See Table 1. Supplemental digital content, http://links.lww.com/MD/K257, which shows individual results of autoclaving process and accordance between both procedures). We observed that 80% of the autoclaving process with B& D resulted passed and 90% through the biological indicator. The combined evaluation of both methods generates a compliance of 72% (CI95%: 57.5%–83.8%) which is a considerable agreement rate between both methods (Table 1). We found no significant differences between the compliance rates evaluated by both methods (Fisher exact

Table 1

Compliance frequencies of the Bowie & Dick test and biological indicator in autoclaving.

Compliance	N	%	SE	CI95
Bowie & Dick test	50	80.0%	5.7%	66.3%–90.0%
Biologic indicator	50	90.0%	4.2%	78.2%–96.7%

CI95 = confidence interval 95%, SE = standard error.

Table 2

Comparison of compliance rates for the Bowie & Dick test and biological indicator in autoclaving.

	Biologic indicator		
Bowie & Dick test	Not conforming	Conforming	*P* value*
Not conforming	1	9	.689
Conforming	4	36	

*Fisher exact test.

test, $P = .689$) (Table 2). Several Bowie & Dick sheets showed non uniform color change after used, even if the discoloration was relatively week the result was considered fail as manufacturer indicate. There no were discrepancies in the judgment of authors for these results, which could support to absence of "false negative" results.

4. Discussion

The decontamination of biological samples is a fundamental process in any health facility. Even more so in hospital blood banks, which have many potentially infectious blood products.[3,19] The study evaluate the performance of autoclaving process through the use of 2 commercial methods and comparing the rate of agreement between the 2.

Previous evaluations have shown variability in the performances of autoclaves. Reports, mainly in dental areas, using biological indicators, show failure rates between 1.4% and 43%.[20–22] In some primary or secondary hospital settings, up to 71% of ineffective autoclaving process have been found with chemical indicators[23] and Sheth et al reported 100% pass results using indicators based on *Bacillus pumilus* spores,[24] a different strain from the one used in our study. Specific studies of the sterilization of products in blood banks are not available.

In our case, 80% autoclaving process were pass with the Bowie & Dick test and 90% with the Geobacillus stearothermophilus biological indicator. This difference could be explained for operational problems in pre-vacuum. Hassan et al report that 38.9% of Bowie & Dick test failed because inspected autoclaves had problems in air leaks, inadequate steam and vacuum pump.[25] Another study that compares operational autoclaving conditions with chemical (no Bowie & Dick) and biological indicators showed that chemical present more pass test that biological indicator, but it could change with the type of loading even when using the manufacturer settings.[26] One evidence of a loss of vacuum in our test is the uniformity of Bowie & Dick sheet after use. It represents an entrapped air (non-condensable gases) inside the chamber during a sterilization cycle.[27,28]

Evaluating the pre-vacuum and steam-filling stages may not be effective, but it only evaluates some of the autoclaving processes. On the other hand, it is essential to highlight that the biological indicator response depends on the amount of material to be autoclaved, the position and location of the indicator ampoule, and the incubation temperature at 60°C and the post-autoclaving time. Some reports show that the inefficiencies of the autoclaving process would be related to human error, such as incorrectly wrapping and loading an autoclave, and not to the machine malfunction.[29] In our case, non-compliant processes could be due to problems with air filters, heating jackets, door seal and among others related to vacuum.

If we analyze the global autoclaving process through both tests, 72% of the processes achieved efficacy in the decontamination of blood products, so the control that is carried out should ideally include both tests to characterize the entire autoclaving process comprehensively and identify potential errors that are solved later.

Indicators are not a common practice in public health establishments in Peru and other countries with similar characteristics. Jabbari et al reported that only 63.77% of hospitals used biological indicators to control autoclaving.[14] And in the other hand is the knowledge of operation that is deficient too.[29] The autoclaving process can present problems between each use, so a maintenance program for the equipment, appropriate uses, and control of each process through indicators is recommended.

Among the limitations of this study, obviously the use of the tests in independent sterilization processes does not allow a clear agreement to be evaluated. however, we tried to maintain the same conditions for both tests as much as possible. On the other hand, there was no technical support or calibration of the operation of the equipment prior to the study, this is a condition of the hospital efforts. In some cases, the visibility of the results for the Bowie & Dick test, indeterminate results, however, were attached to the manufacturer criteria to define.

Our results are relevant, given the context of quality assurance in biosafety processes, since the autoclaving process at the Blood Banks are not reported in Peruvian context did not have a monitoring system for this activity. Our research has shown the usefulness of the Bowie & Dick test and the biological indicator as fundamental tools to improve the process and guarantee the safety of the products that go to final disposal as hospital waste.

5. Conclusion

Whilst autoclaving remains the most widely used method of sterilization it is also susceptible to operational issues. Quality control of the process is a critical activity that guarantees correct sterilization. Methods such as Bowie & Dick and biological indicators are relevant and complementary since they simultaneously monitor the interaction of sterilization parameters (temperature, pressure, and time).

The importance of quality assurance with the use of monitoring methods has been discussed. Evidence like that is important for the regulatory authorities place considerable weight upon the regular and successful use of monitoring process even more in low and middle-income countries.

Author contributions

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