International Journal of Life science & Pharma Research

STRATEGIES FOR THE VALIDATION OF STEAM STERILIZATION PROCESS IN AUTOCLAVES FROM THE PHARMACEUTICAL INDUSTRY

LEONARDO TEIXEIRA RODRIGUES[1,2], ADERVAL SEVERINO LUNA[1] , CRISTIANE ASSUMPÇÃO HENRIQUES[1] AND ANTONIO CARLOS AUGUSTO DA COSTA*

[1] PPG-EQ/IQ - Universidade do Estado do Rio de Janeiro Rua São Francisco Xavier 524, Pavilhão Haroldo Lisboa da Cunha, Maracanã, Rio de Janeiro, RJ CEP: 20550-013,
[2] Biomanguinhos – Fundação Oswaldo Cruz.

ABSTRACT

The process of sterilization in the asepsis pharmaceutical ensures the materials used in the production of medicines. The method of saturated steam sterilization is always the first choice in the pharmaceutical industry because it is the most effective, faster, with better cost / benefit and with less environmental impact. In order to ensure necessary sterility, the qualification of autoclaves and validation of requirements when their loads are required by national and international regulatory bodies. The regulations of these bodies are not always objective and clear, indicating what should be done without specifying how it should be done. The focus of this work was to create a validation methodology, change control and revalidation in horizontal autoclaves, highlighting the critical points of the process, exploring the theoretical concepts of bioengineering. For this, we used statistical techniques for analyzing data collected in a study of thermal distribution, heat penetration and microbiological challenges to determine a fast, safe and effective that meets the requirements of regulatory bodies without affecting the production capacity of industrial plants and quality of the sterilization process.

KEYWORDS : Autoclave; Sterilization; Validation; Microbes; Pharmaceutical Industry.

INTRODUCTION

In the nineteenth century surgeons began to disinfect hands, tools and environment in order to prevent infection during surgery, thus resulting in the surgery asseptic covering sterilization by physical and chemical instruments and the surgical environment. Therefore, one can say that sterilization is the physical or chemical attempts to destroy or eliminate all forms of life, especially microorganisms. Even with the advancement of science and technology, there are still cases of infections and hospital products. This is due to the lack of studies and tests, in some companies and hospitals, showing that the sterilization processes are really effective. With this, we highlight the importance of sterilizing equipment qualification and validation of sterilization processes.

1.1 Steam and Sterilization

The heat sterilization is widely used in the pharmaceutical industry. Table 1 shows the equivalence of mortality among the different temperatures with the respective exposure times, ie mathematically all have the same potential sterilization. It can be concluded that the sterilization in the presence of steam is more efficient since it requires less time and temperature as the dry heat.

Table 1. ***Dry and steam sterilization***

Steam sterilization				**Dry condition**		
Temperature (°C)	121	126	134	160	170	180
Time (min)	15	10	3	120	60	30

This is due to the fact that as water is heated, more energy is absorbed to the point the temperature rises until the boiling state (latent heat). This energy is used to increase the temperature, but for transition from liquid to gas. The spent energy is too high this transition, causing the reverse process - condensation - the energy obtained is also very high, confirming that the sterilization by heat in the presence steam has a high yield. The yield can be further increased if the internal pressure of the chamber is maintained above atmospheric pressure, since the boiling point is to be greater than 100 °C. This is the case of industrial work with autoclaves relative pressures around 1.1 kgf/cm ² so that the boiling point of water to occur near 121 °C. The saturation temperature increases with pressure, but there is a limit, called the critical point, above which there is no defined transition between the two states. Other important points refer to the dilations that take place when the water passes into the gaseous state and back to the contraction when liquid (Figure 1).

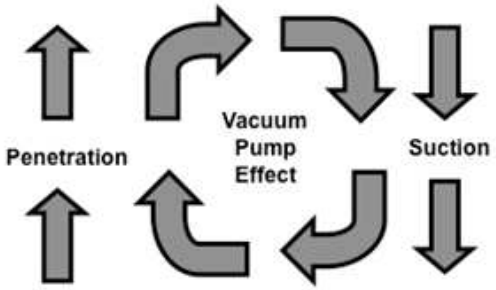

Figure 1. ***Cycle of steam penetration***

The steam to condense and wet the surface of the instant product undergoes a contraction of volume (in the order of 1500 times). This causes the penetration of steam into the object to be processed to produce an effect of "vacuum pump" that captures more steam which continues heating the product and condensing. To condense the vapor creates a partial vacuum which tends to be occupied by more steam which, in turn, provides more energy. Thus, one cycle is fulfilled. This phenomenon is also called successive "heat pump" (Luqueda, 2004).

MATERIALS AND METHODS

The horizontal autoclave is basically a combination of two pressure vessels sealed, contained in the other. The outer vessel is called outer chamber, but also is known as a shirt or jacket. The vessel built is the place to accommodate the load for sterilization and is called the inner chamber. The normal process of steam sterilization is basically composed of three phases. The first stage is known as pre-conditioning, and constitutes the air is sucked into the inner chamber through repetitive cycles of alternating vacuum pulses of injection steam. At least three vacuum pulses are sufficient, since the third pulsed vacuum already ensures removal of 99.994% of the air inside the chamber (Luqueda, 2004). It is essential to eliminate the air within the chamber, because the air is considered the best thermal insulation. The next step includes the exposition time, where the maximum temperature is kept at 121°C. At this stage, a temperature sensor installed in the drain valve controls the steam inlet in about 1.1 kgf/cm², so that the sterilization temperature

remains close to 121° C. If it was maintained at 134°C, the pressure should be adjusted to 2.1 kgf/cm². The drain is the best place to control temperature, as air and condensate, heavier than steam, are removed by gravity through the drain located at the bottom of the camera, turning at the coldest point of the camera. If the drain is at 121°C, it is expected that the other points within the chamber are greater than or equal to 121°C. The last phase of the sterilization cycle is the post-conditioning, where the vacuum system is switched on while the steam valve is closed. The presence of vacuum with a heat radiation from the walls of the chamber takes all moisture from the chamber (drying). After drying time, the pressure is equalized to atmospheric pressure, even outside air through the filter without aeration. The quality and proper maintenance of this filter are vital to the success of the sterilization, since the injection end of the contaminated air cycle affects the sterilization process (Figure 2).

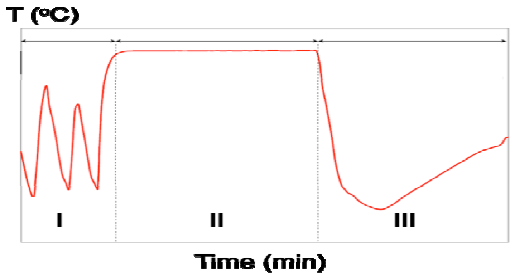

Figure 2 ***Stages of steam sterilization. I) Pré-conditioning; II) Time of exposure; III) Post-conditioning.***

2.1 Quantification of Steam Sterilization

With the knowledge of the kinetics of microbial destruction, it is possible to use mathematical models to predict the behavior microbial steam sterilization. According Luqueda (2004) the kinetics of microbial death does not necessarily follow a linear model and that there is a mathematical model entirely linear. In practice it is found that the microbial death behaves as a first-order reaction. The decimal reduction time (D) is defined as the exposure time required to cause a 90% reduction in the population of a microorganism. All the values for D are specific to a temperature, usually cast to 121.1°C. This is calculated by Equation 1 (Russell, 2004):

$$D = \frac{\Delta t}{\log_{10} N_0 - \log_{10} N} \qquad (1)$$

Where:

Δt = Duration of the thermal treatment

N_0 = Initial population of microorganisms

N = Final population of microorganisms

The value z is another way to determine the microbial resistance to sterilization by moist heat. Is defined as the number of degrees of temperature required for switching an output logarithmic value. The z value is used to determine the mortality assessment, it is not suitable above 135°C and is not suitable their extrapolation (Baumer, 2006). For verification purposes, been standardized in the pharmaceutical z value of 10. The unit that quantifies the sterilization is called Lethality (F_{zero} or F_0), expressed in minutes. According to the definition Parenteral Drug Association (PDA, 2007), F_0 is the exposure time (min) equal to 121°C with z value of 10°C. For example, when a sterilization cycle accumulated value F_0 to 15

min, then the product was exposed theoretically 15 minutes at 121°C. In this case, 121°C maintained for 15 min, 115°C maintained for 30 min and maintained at 134°C for 3 min, have the same value F_0 equal to 15 min. The calculation of the lethality of the sterilization process by saturated steam is given by Equation 2.

$$F_0 = \int_{t_1}^{t_2} 10^{\frac{T-121.1°C}{Z}} dt \qquad (2)$$

Where:

F_0 = Accumulated lethality at 121.1°C with a Z value equal to 10°C and D equal to 1 min.
t_1 = Time when the lower temperature is higher than 100°C.
t_2 = Time just after t_1, when the lower temperature is lower than 100°C.
Z = Absolute number of degrees of temperature required for a change in one logarithm in the value of D. In sterilization processes, this value is equal to 10°C.
T = Instant temperature (°C).

According to Equation 2, it can be seen that the calculation of mortality has a significant value only above 100 ° C. For this reason, the data acquisition system automatically performs the calculation of lethality only when the temperatures are above 100°C.

$$F_0 = 10^{\frac{100°C-121.1°C}{10°C}}$$

$$F_0 = 0.008\,\text{min}$$

In this case we can say that sterilization at 100°C is 100 times less effective than 121°C, for 1 minute to 100°C is approximately 0.01 minutes at 121°C (Figure 3).

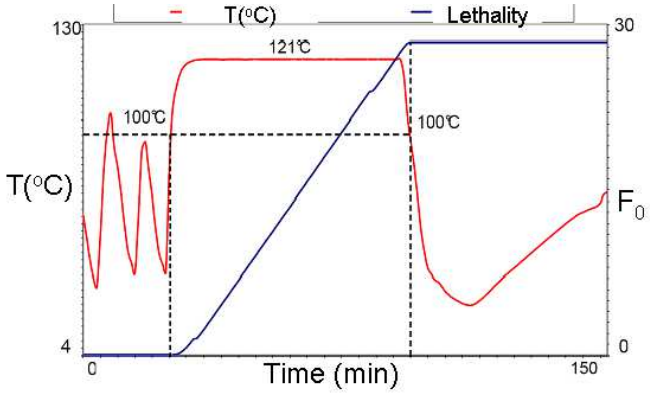

Figure 3 ***Ramp of accumulated lethality.***

The concept of absolute sterilization is being replaced by the probability of the load to be sterile. Due to theoretical and practical limitations, no similar statement regarding the absence of microorganisms can be proved (Luqueda, 2004). In the sterilization process, the killing of microorganisms is described by an exponential function. Therefore, the presence of viable microorganisms on any item should be expressed in probabilities. Although this

probability can be reduced to a very small number can never be reduced to zero. This is why the term sterility assurance level is used to estimate in the pharmaceutical or evaluate the efficacy of the sterilization process. He is often portrayed by its English abbreviation SAL (Sterile Assurance Level) and expresses the probability of survival of viable microorganisms.

The standardized value of the SAL, the majority of the International Pharmacopoeia is 6.10. To achieve a SAL of 10^{-6} cycle, must be able to reduce 12 log units of an initial population of one million, thus expressing a first probability of survival in 1,000,000 (FDA, 1994) (Figure 4). The probability calculation is extracted from Equation 3.

$$F_0 = D_{121°C}(\log_{10} N_0 - \log_{10} B) \qquad (3)$$

Where:
F_0 = Minimum required lethality
$D_{121°C}$ = Thermal resistance of the bioindicator
N_0 = Initial population of the bioindicator
B = Probability of survival of the bioindicator

To estimate the probability of survival should rearrange the formula Equation 4.

$$\log_{10} B = \log_{10} N_0 - \frac{F_0}{D_{121°C}} \qquad (4)$$

As an example, a microbial challenge was determined using a biological indicator value D of 121°C = 1.3 min with initial population of 1.5 x 10^6. To obtain a sterilization cycle which is able to provide a SAL of 10^{-6} to scale a cycle that is capable of accumulating the following minimum required lethality (Equation 5).

$$\begin{aligned} F_0 &= D_{121°C}(\log_{10} N_0 - \log_{10} B) \\ F_0 &= 1.3(\log_{10} 1.5x10^6 - \log_{10} 10^{-6}) \qquad (5) \\ F_0 &= 15.8\,\text{min} \end{aligned}$$

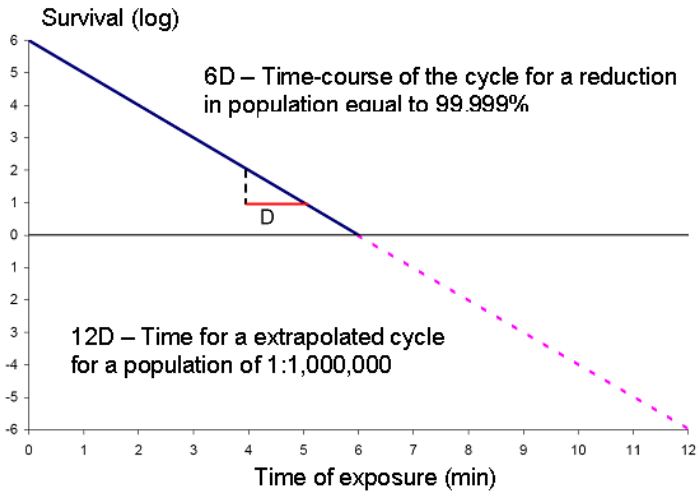

Figure 4 : ***Sterile Assurance Level at 10^{-6}.***

Exemplifying an opposite manner, one can estimate the probability of a given sterilization cycle which has accumulated a mortality rate of 25.3 min and was challenged with the same biological indicator (Equation 6).

$$\log_{10} B = \log_{10} N_0 - \frac{F_0}{D_{121°C}}$$
$$\log_{10} B = \log_{10} 1.5x10^{6} - \frac{25.3}{1.3} \qquad (6)$$
$$B = 1x10^{-13.28}$$

COMPLIMENTARY RESULTS AND DISCUSSION

3.1 Classification of the Types of Matters to be Sterilized

Because they present peculiarities in the pre-conditioning, exposure time and post-conditioning loads of autoclaves were classified according to their with your final objective (purpose of the load) and its state of matter. If the material is used for product handling in a sterile environment (clean room or cabin laminar flow) or formulation of product ingredients, the sterilization process is called. If the material is classified as Waste Health Service - RSS, which are usually remnants of production that had contact with biological materials and must be discarded, are called and Decontamination Process (ANVISA, 2006). The term state of matter refers to solid and liquid states of the items of charge. They were divided into dry cargo (which is subdivided into solid and porous) and liquid cargoes (also subdivided into hermetically closed and open). Dried porous loads are intended to put the steam in contact with microorganisms through their pores and/or its packaging. There is a need to remove air, steam penetration, the steam and re-evaporation of moisture from the material, for example packed garments and glassware. Dry solids loads are intended to bring steam into direct contact with the surface of the load, for instance in trays. The processes used to load porous solid materials or have a common goal to put the steam in contact with the microorganism. For liquids, the goal is different - the water in the product reacts with the microorganism to kill him, so the steam is used to evenly heat the liquid. Figure 5 shows the possible combinations of categories to classify the matters.

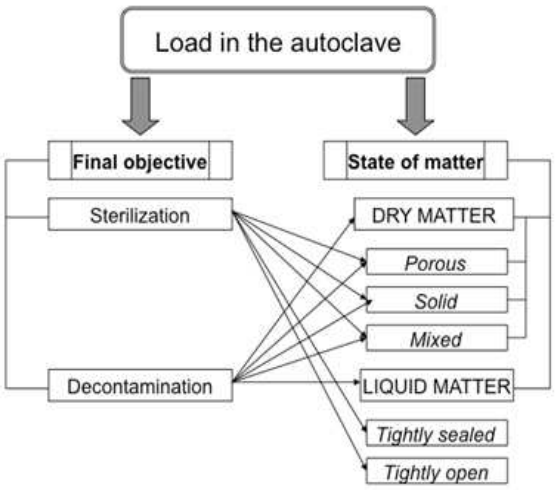

Figure 5 ***Classification of autoclavable loads***

3.2 Thermal Distribution

The thermal study with the empty autoclave is essential to evaluate the performance of the autoclave. The evaluation of the thermal uniformity of the load depends on the autoclave is used, since the positions of the cargo items can hinder the transit of the steam. The thermal study of the autoclave provides the thermal distribution in the inner chamber highlighting the hot and cold spots. This procedure was performed with three races to show the reproducibility of the system. Each type of cycle (dry, liquid and decontamination), which present different specificities, was tested separately. The points to be monitored are instruments of control and the ends of the chamber. The control instrument for sensing the load is dried and drain liquid loads is the load sensor. For each study, 12 were spread equidistantly type T thermocouples into the inner chamber, as shown in Figure 6. Maximum limits have been defined (124°C) at least (120°C) and the limit of variation between the sensors (± 2°C), only during the exposure time. A variation greater than ± 2°C has no impact on sterilization, since the cycle is sized according to the lowest value of accumulated lethality, but a malfunction of the autoclave. The presence of air in the chamber can cause large temperature variation.

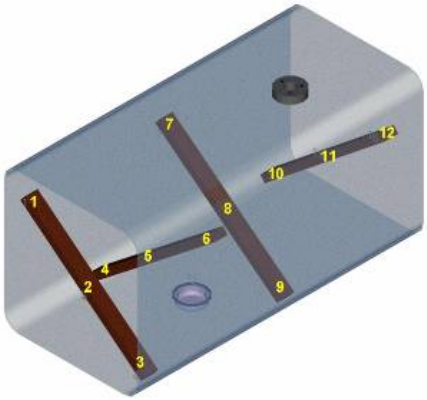

Figure 6 : ***Distribution of temperature sensors inside the autoclave***

The use of a large number of sensors can affect the nature of the leak-camera, as all the sensors are in the same hole in the autoclave, called validation gate. Today this problem is being solved with data loggers with wireless technology, but unfortunately it is too expensive. Since the steam distribution being evaluated in this study took care that the sensors do not touch to the inner wall surface of the chamber, as this would lead to false measurements.

3.3 Penetration of steam

The materials in thermal study were recorded via sensors inserted into the load of the items during the measuring cycle and the heat supplied to the cargo items. The objective of this procedure is not to assess the homogeneity among the items of thermal loads, but to evaluate the penetration of steam in packaging and loads through the lethality accumulated in each item. Is typically used as the reference minimum cumulative mortality and has been calculated at all times that the sensors were above 100 ° C. Twelve were randomly scattered sensors (one on each item of the load), where the positions were different in each study, covering all areas of the autoclave. In order to evaluate the behavior of the vapor in several items with different shapes, it was placed more than one sensor on the same item or identical items were used for most random positions of sensors and a sensor fixed in the cold spot. For a satisfactory safety margin, all the cycles were sized to provide a SAL of 10^{-6} for a population of microorganisms with $1x10^{6}$, a D value of 2 min; all cycles had a mortality of at least 24 minutes (Equation 7).

$$F_0 = D_{121°C}(\log_{10} N_0 - \log_{10} B)$$
$$F_0 = 2x(\log_{10} 1x10^6 - \log_{10} 10^{-6}) \quad (7)$$
$$F_0 = 24\,\text{min}$$

For microbial challenge was positioned one at each end of the bioindicator thermocouples.

3.4 Determination of the "Worst Case"

After completing the studies of the thermal distribution, penetration of steam and microbiological challenge, split the charges into three categories: Porous, Solid and Liquid. The solid cargo, because they are easy sterilization, were excluded from the process of selecting the worst case. The porous and liquid loads were evaluated for a relationship Lethality vs. Time, one for dry cargo and liquid cargo to other. It was then possible to determine the dry cargo and liquid cargo which the highest resistance to penetration of steam. That it took more time to accumulate F0 equal to 12 min was considered the "worst case".

3.5 Revalidation

Revalidation is the repetition of part or all of the validation tests intended to reconfirm the reliability of the process (ANVISA, 2009). Unfortunately, the regulators do not regulate which parts of the tests should be repeated, giving rise to recommendations that propose different every time the plant is inspected and must be done every time a repair in the autoclave can significantly affect the efficiency of the process. Revalidation should also be performed at least once every year. Based on the determination of the worst case, the annual revalidation process consists in calibrating instruments critical autoclave conduct a thermal distribution, a study of the penetration of steam and dry worst case study of penetration of liquid worst case.

CONCLUSIONS

This validation methodology provided sufficient data to evaluate the thermal performance of the autoclave with or without load. She brings more accurate diagnosis of any problems and can determine whether the cause is linked to the wear of the components of the autoclave or in the assembly and configuration of the loads. It also covers new concepts for selecting the "worst case" because contrary to what many believe, is not always the maximum load has a lower growth rate of lethality. Another important aspect was the revalidation. It created a methodology for revalidation fast, safe and effective, highlighting the critical points of the process, exploring the physical and microbiological concepts and concentrating efforts where they really needed. Therefore, it was possible to meet the requirements of regulatory bodies without affecting the production capacity of industrial plants and the quality of the sterilization process. This fact can be proven as this methodology has undergone several national and international audits, and none made any recommendations.

ACKNOWLEDGEMENTS

Authors are thankful to CNPq, FAPERJ and Bio-Manguinhos for support to conduct this work.

REFERENCES

1. ANVISA Consulta Pública nº 3, de 13 de janeiro de 2009. D.O.U de 17/02/09
2. ANVISA, Gerenciamento dos Resíduos de Serviços de Saúde. Brasília: Editora Anvisa, 2006.

3. BAUMER, Apostila C.B.E._Port_2006-07_Rev.A, 2006.
4. LUQUEDA, G. R. Conceitos Básicos de Desinfecção, Esterilização e Qualificação. São Paulo: Editora Baumer, 2004.
5. PDA Technical Report No. 1, Revised 2007, Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control.
6. RUSSELL, A.D.; HUGO, W.B.; AYLIFE, G.A.J. Principles and practice of disinfection, preservation and sterilization. Oxford: Editora Blackwell Scientific Publications, 2004.
7. US. FDA, Guidance for the Submission Documentation for Sterilization Process Validation in Applications for Human and Veterinary Drug Products, 1994.