EFFECTIVENESS EVALUATION OF AUTOCLAVES AT NATIONAL INSTITUTE FOR CONTROL OF VACCINES AND BIOLOGICALS

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Abstract: National Institute for Control of Vaccines and Biologicals (NICVB) has utilized wet-heat sterilization methods for laboratory instruments, solution, and culture media. According to World Health Organization (WHO) recommendations, autoclaves used in sterilization processes require annual revalidation. Therefore, we conducted the study with the titled "Effectiveness Evaluation of Autoclaves at the National Institute for Control of Vaccines and Biologicals" to assess the operational performance and sterilization efficacy of autoclaves. The study was performed on two autoclaves: AC 10-TN and AC 11-TN. The results indicated that both autoclaves met operational performance requirements based on physical indicators, including: pressure ≥ 0.1 MPa, temperature ≥ 121°C, holding time ≥ 15 minutes and uniform heat distribution. Sterilization efficacy was also evaluated through biological indicators such as no appearance of microorganisms in both BI cultures and media that ensured sterility, while all positive controls met the required criteria and the risk of false negatives was eliminated. Consequently, both autoclaves were confirmed to be in high quality, reliable, and suitable for use in vaccine and biological product quality control testing.

Keywords: *Wet-steam sterilization, biological indicators, autoclave*

1. Introduction

The primary goal of sterilization process in medical and pharmaceutical fields is to completely eliminate microorganism from equipment, laboratory instruments, medical tools, biological waste and cultural environments, to ensure absolute sterility and to prevent contamination. Each steriled process should be evaluated to ensure the effecacy of sterilization and the integrity of the product being sterilized, including the container and outer packaging before test performance. Sterilization by saturated stream under high pressure is carried out in an autoclave. This method is priority to choose, especially for solution, culture media, laboratory tools, medical devices. Throughout the sterilization process, it is essential to monitor the temperature, pressure as well as the duration of sterilization. These parameters should demonstrate sterilization effectiveness as proven by validation results [1-7].

According to Wolrd health Orrganization (WHO) recommendation, autoclave plays an important role in wet sterilization [2,5]. In NICVB, this procedure has been used to sterile solution, media of sterility tests for vaccines and biological products, and other laboratory tools. Following WHO guideline, autoclave machines should be evaluated annually to validate sterility ability [5]. Based on these requirements, the project "Effectiveness Evaluation of

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Autoclaves at the National Institute for Control of Vaccines and Biologicals" was carried out with two main purposes: (1) evaluate the monitoring ability of autoclaves and (2) evaluate the sterilization ability of autoclaves.

2. Research method

Descriptive laboratory method.

2.1. Research object

Research object: Autoclaves at National Institute for Control of Vacciens and Biologicals.

Figure 1. Two autoclaves used in the study

2.2. Research duration and location

- Location: Experimental media Department, National Institute for Control of Vaccines and Biologicals.

- Duration: 12/2024

2.3. Materials and chemicals

2.3.1. Media, chemicals and microorganism strains

- BI (Bio Indicator): ProSpore Ampoule *Geobacillus stearothermophilus*1,3 x 10^6 CFU/vial (MesaLabs), expired date: 01/2026.

- *Bacillus subtilis* strain ATTC 6633, lot ATCC 6633-F1

- *Kocuria rhizophila strain* ATCC 9341, lot ATCC 9341-F2

- Fluid Thioglycollate Medium (FTM) prepared at Experimental Media Department.

- Tryptic Soy Broth (TSB) prepared at Experimental Media Department.

- Tryptic Soy Agar (TSA) prepared at Experimental Media Department.

- Steriled water (POLYVAC), expired date: 07 days from manufaction.

2.3.2. Materials and equipment

- Data Logger temperature measurement (MadgeTech), calibration date: 12/2025 .

- Glass tube Ø 18, Duran.

- Glass bottle 500ml, Duran.

- Glass bottle 1000ml, Duran.

- Wet-heat indicator tape: Contain chemical changed color based on temperature during sterilization.

- Autoclave machine code AC 10-TN (Sanyo), calibration date: 12/2025.

- Autoclave machine code AC 11-TN (Sanyo), calibration date: 12/2025.

2.4. Research design

In this study, each prupose was validated through different indicators.

For the first purpose: Observe the physical indicators during monitoring process of autoclave to demonstrate that the autoclave has worked correctly as the wet sterilization requirements, including pressure, temperature, maintain duration and heat distribution.

For the second purpose: Evaluate the sterilization ability by adding a thermally-resistant spore (BI) and start the sterilization process.

The detailed research design was shown in Figure 2.

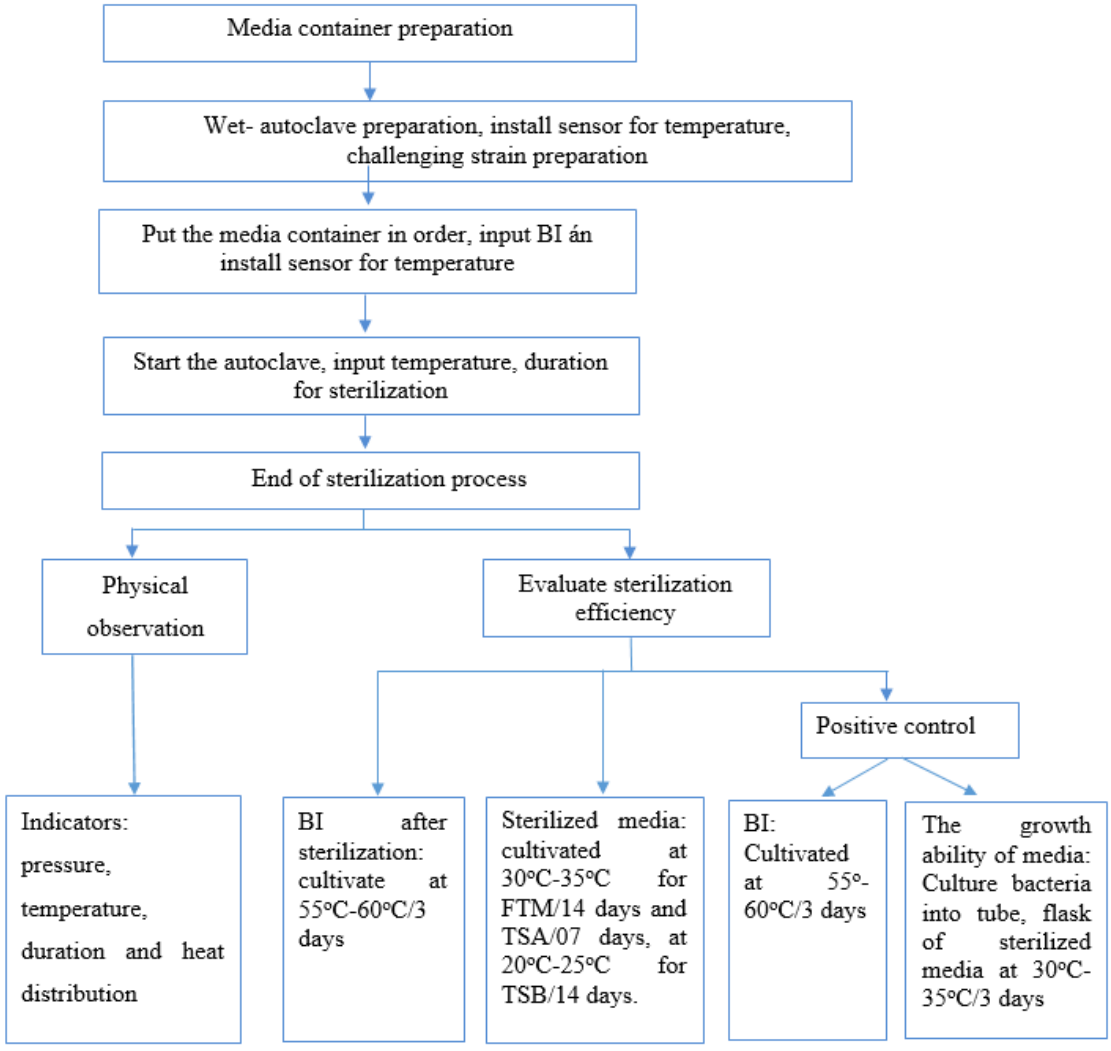


Figure 2. Summary of research design

The study was carried out on 2 autoclave machines with 3 duplicates.

Protocol

- Media container preparation for wet sterilization:

+ AC 10-TN: 260 tubes Ø 18 x 20ml (FTM, TSB)/ tube.

+ AC 11-TN: 150 tubes Ø 18 x 20ml (FTM, TSB)/ tube, 04 glass bottles 500ml (400ml TSA/bottle), 01 glass bottle 1 liter (900ml TSA/bottle).

- BI preparation and set up Sensor: temperature, date, time start.

- Turn on the autoclaves

- Input media containers in order, stick the wet-heat indnsoricator tape on each container.

- Add BI and install Sensor for temperature:

+ Add BI: 3 BI was sticked with a sensor part of Sensor for temperature by tape

+ Install Sensor for tempersture: Put 03 sensor devices to:

* AC 10-TN (Bottom up): 1 sensor at

the first container Ø 18 + 1 sensor at the second container Ø 18 + 1 sensor in the middle of the third container Ø 18.

* AC 11-TN: 1 sensor in the glass bottle 1 litre + 1 sensor in the glass bottle 500ml + 1 sensor in the middle of container Ø 18.

- Install sterilized temperature: 121^{o}C

- Install duration: 15 minutes.

- Run the autoclaves

- Observe results after performance.

- Evaluation of sterilization:

2.4.1. Evaluation by indicators: pressure, temperature, duration and heat distribution

- Accepted criteria [1,8-11]:

+ Pressure indicator ≥ 0,1MPa

+ Temperature: All position of Sensors should be ≥ 121^{o}C.

+ Duration: Maintain within ≥ 15 minutes.

+ Heat distribution: In the range of 121^{o}C - 125^{o}C.

+ Wet sterilization indicator tape: Indicator line turns black.

2.4.2. Evaluation of productivity

- Productivity evaluation is used as proven of sterilization effeciency of an quipment during validation process to eliminate complete contanimation to ensure the sterility of a product.

2.4.2.1. BI tubes after sterilization

- All BI tubes after sterilization were cultivated at 55^{o}C-60^{o}C in 3 days.

- Accepted specification: No appearance of microorganism in all BI tubes after 3 days cultivation. Biological indicator BI tube should be clear, no precipitation, no contamination or changing color [1,9-12].

2.4.2.2.Tubes, culture media after sterilization

- Media tubes, containers after sterilization should be cultivated to observe any appearance of microorganism:

+ Media cultivation: Incubate 05 FTM tubes at 30^{o}C-35^{o}C + 05 TSB tubes at 20^{o}C-25^{o}C in 14 days.

+ Incubate 01 TSA bottle at 30^{o}C-35^{o}C for 07 days.

- Accepted criteria:

+ There is no appearance of microorganism in all FTM and TSB media, tubes after 14 days [13].

+ There is no appearance of microorganism in all TSA media plates after 7 days [14].

2.4.2.3. Possitive control

a) Positive control BI tubes

- Incubate 01 BI tube at 55^{o}C-60^{o}C forr 3 days.

- Accepted criteria: There is appearance of microorganism in BI tube after 3 days. The tube is turbid, precipitation at the bottom, contaimination sign, the color changes from purple to yellow [1,9-12].

b)The growth of media after sterilization

- Put 100µl challenging strain *Bacillus subtilis at dilution* $10^{-4,5}$*, Kocuria rhizophila* at dilution 10^{-6} into media: 04 FTM tubes, 04 TSB tubes and 02 TSA bottle then incubate at 30^{o}C-35^{o}Cfor 3 days.

- Accepted criteria: There is appearance of microorganism in tubes and media bottle after 3 days of incubation [13].

3. Result

3.1. The result of observation of physical indicators (pressure, temprature, duration and heat distribution) of Sensors at different spots in 2 autoclaves.

3.1.1 Autoclave machine code AC 10-TN

Table 1. Result indicator of autoclave machine code AC 10-TN

No.	Indicator	Result			Specification	Conclusion
		Performance 1	Performance 2	Performance 3		
1	Pressure	0,1 MPa	0,1 MPa	0,1 MPa	Pressure should be ≥0,1MPa	Pass
2	Temperature	Min: 121,01°C Max: 121,82°C	Min: 121,04°C Max: 123,42°C	Min: 121,08°C Max: 121,63°C	All Sensor in different spots should be ≥ 121°C	Pass
3	Maintained duration at 121°C	15 mins	15 mins	15 mins	Maintained duration at 121°C of all sensors in different spots should be 15 mins	Pass
4	Heat distribution of 03 Sensors	Min: 121,01°C Max: 121,82°C	Min: 121,04°C Max: 123,42°C	Min: 121,08°C Max: 121,63°C	Heat distribution of all sensors in different spots should be in a range of 121°C - 125°C	Pass
5	Wet sterilized indicator tape	Indicator line turned black			Temperature and duration of sterilization should be ≥ 121°C/15 mins	Pass

3.1.2. Autoclave machine code AC 11-TN

Table 2. Result indicator of autoclave machine code AC 11-TN

No.	Indicator	Result			Specification	Conclusion
		Performance 1	Performance 2	Performance 3		
1	Pressure	0,1 MPa	0,1 MPa	0,1 MPa	Pressure should be ≥0,1MPa	Pass
2	Temperature	Min: 121,01°C Max: 121,52°C	Min: 121,14°C Max: 21,42°C	Min: 121,04°C Max: 121,16°C	All Sensor in different spots should be ≥ 121°C	Pass

3	Maintained duration at 121°C	15 mins	15 mins	15 mins	Maintained duration at 121°C of all sensors in different spots should be 15 mins	Pass
4	Heat distribution of 03 Sensors	Min: 121,01°C Max: 121,52°C	Min: 121,14°C Max: 121,42°C	Min: 121,04°C Max: 121,16°C	Heat distribution of all sensors in different spots should be in a range of 121°C - 125°C	Pass
5	Wet sterilized indicator tape	Indicator line turned black			Temperature and duration of sterilization should be ≥ 121°C/15 mins	Pass

Conclusion: Presure, temperature, duration and heat distribution reached to the requirements.

+ Pressure indicator ≥ 0,1MPa

+ Temperature: All position of Sensors should be ≥ 121°C.

+ Duration: Maintain within ≥ 15 minutes.

+ Heat distribution: In the range of 121°C - 125°C.

+ Wet sterilization indicator tape: Indicator line turns black.

Initial indicator line **Indicator line after sterilization**

Figure 3. The figure of indicator line before and after sterilization

3.2. Sterilization result

3.2.1. Cultivation of BI after sterilization

Result of BI cultivation that used in 2 autoclaves/ 3 lots:

- No appearance of microorganism in all BI tubes after 3 days of cultivation and after sterilization process.

- Comment: All biological indicator BI tubes were clear, no precipitation, no contamination, no changing color.

- Conclusion: Spores were eliminated completely in all BI tubes.

Initial BI tubes **BI tubes after sterilization**

Figure 4. BI tubes before and after

3.2.2. The result of sterility of meadia and tubes after sterilization

Table 3. The result of sterility media/ 2 autoclaves

No.	Media	Result (3 lots of media)			Conclusion
		Lot 1	Lot 2	Lot 3	
1	FTM	No microorganism appearance in all FTM, TSB media tubes in 14 days after sterilization by AC 10-TN and AC 11-TN (Media was clear, no changing color or appear precipitation). Pass			Pass
2	TSB				
3	TSA	No microorganism appearance in all TSA media bottles in 07 days after sterilization by AC 11-TN.			Pass

Comment: Media after sterilization reached the requirement.

Conclusion: FTM, TSB, TSA media met the criteria for sterility

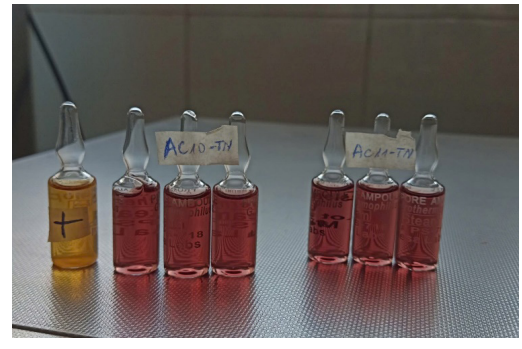
3.2.3. Positive control

3.2.3.1. Positive control BI tubes

- There was a growth of microorganism in all BI tubes in 3 days cultivation and the color changed from purple to yellow.

- Comment: The biological indicator BI tubes were turbid, contained precipitation, contamination appearance and the color changed from purple to yellow.

- Conclusion: There was a growth of microorganism, the color changed from purple to yellow.



Possitive control BI tubes after sterilizaiton

Figure 5. Positive control BI tube and BI tubes before and after sterilization

3.2.3.2. The result of the growth of media after sterilization

Table 4. The result of the growth of meida/ 2 autoclaves

No.	Media	Microorganism strain	Result (3 lots of media)	Conclusion
1	FTM	*Bacilus subtilis*	3/3 (The media were turbid, colorless indicator)	Appearance of bacteria in all tubes after 3 days of challenging microorganism cultivation
2		*Kocuria rhizphila*		
3	TSB	*Bacilus subtilis*	3/3 (The media were turbid, precipitation at the bottom)	
4		*Kocuria rhizphila*		
5	TSA	*Bacilus subtilis*	3/3 (Appearance of bacteria in culture media bottle)	Appearance of bacteria in all tubes after 3 days of challenging microorganism cultivation
6		*Kocuria rhizphila*		

Conclusion: Microorganism appearance was clearly observed in media tubes, bottles contained bacteria after 3 days cultivation.

4. Discussion

The results of this study were shown that two autoclave machines met the requirements.

Firstly, physical indictors including temperature monitoring during sterilization process, pressure, duration of sterilization, heat distribution of sensors at all different spots inside the autoclave. All these indicators reached to the criteria: sterilized at 121°C for 15 minutes with pressure 0,1MPa, and the temperature of diferent spots in the range of accepted specification. Therefore, two autoclaves passed the standards of stable ability, safety, and remain correctly temperature and duration of sterilization.

Secondly, biological indicators including cultivation of BI and media for validation process. The results determined the media after sterilization were all sterile: BI tubes after sterilization had no appearance of microorganism and no false negative results. Hence,two autoclave machines had goos efficiency in sterilization and contamination elimination.

The results of the study demonstrated processing ability and sterilized efficacy of two autoclaves. It is a proven that the validation of sterilization ability of two autoclaves in NICVB was high accurate and reliable.

5. Conclusion and suggestion

5.1 Conclusion

Based on the physical and biological indicators, two autoclaves code AC 10-TN and AC 11-TN are all reached to the requirements of maintained ability and sterilization. Therefore, these two equipments can be used for wet-heat sterilization of solution, culture media in NICVB.

5.2 Suggestion

NICVB would continuously utilises these two autoclaves for solutions, media sterilzation for vaccines and biological products quality control. It is also neecessary to revalidate autoclave machines annually according to the recommendation of WHO.

References

[1] Hội đồng Dược điển – Dược thư Việt Nam. Dược điển Việt Nam V, Phụ lục 16.1: Các phương pháp tiệt khuẩn. Nhà xuất bản Y học; 2017. tr. 414–417.

[2] The UnitedStates Pharmacopeial Convention. USP 41-NF36, <1222> Terminally sterilized pharmaceutical products parametric release. 2020.pp.8142-8144.

[3] The UnitedStates Pharmacopeial Convention, USP41-NF36, <1229> Sterilization of compendial articles. 2020.pp. 8194-8240.

[4] MadgeTech. Hitemp140 High temperature Data Logger: Hướng dẫn sử dụng bộ ghi nhiệt độ Data Logger. 2019.

[5] World health Organization, WHO good manufacturing practices for sterile pharmaceutical products, WHO TRS 961, Annex 6.2011.pp. 261-284.

[6] Phùng Thị Thanh Vân, Giáo trình Kỹ thuật xét nghiệm cơ bản. Trường Cao đẳng Y tế Hà Đông. 2020.tr. 42-46.

[7] Trương Văn Đạt, Đỗ Quang Dương, Huỳnh Văn Hóa. Nghiên cứu quy trình thẩm định hiệu lực phương pháp tiệt khuẩn bằng nhiệt. T*ạp chí Y Dược học*. 2012; 9: 62-65.

[8] Visitech. Băng keo chỉ thị hấp ướt 3M. [Internet]. (Truy cập: 19 tháng 08 năm 2025). Có sẵn tại: https://visitech.vn/sp/bang-keo-chi-thi-nhiet-hap-uot/

[9] Lê Viết Thành. SOP TB 07-01: "Quy trình chuẩn thẩm định nồi hấp". Viện Kiểm định Quốc gia Vắc xin và Sinh phẩm y tế. 2019.

[10] POPYVAC. PQ protocol: Thẩm định lò sấy ướt. 2011.

[11] Visitech. Hướng dẫn sử dụng ống chỉ thị sinh học trong hấp khử trùng.[Internet]. (Truy cập: 27 tháng 07 năm 2025). Có sẵn tại :https://visitech.vn/chi-thi-sinh-hoc-duoc-dung-nhu-the-nao/

[12] MesaLabs. Technical Report ProSpore. 2019.

[13] Phạm Thị Hằng. SOP MT02-43: "Quy trình kiểm tra chất lượng môi trường FTM và TSB". Viện Kiểm định Quốc gia Vắc xin và Sinh phẩm y tế. 2024.

[14] Nguyễn Thu Quỳnh. SOP MT 02-36: "Quy trình kiểm tra cảm quan và vô khuẩn môi trường nuôi cấy". Viện Kiểm định Quốc gia Vắc xin và Sinh phẩm y tế. 2024.