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Sterilization Method for Borosilicate Glass Vials and Chlorobutyl, Bromobutyl Rubbers for Medical Purposes

I.S. Karapetyan[1]* and H.R. Petrosyan[2]

[1]Radioisotope Production Center, 38/7 Halabyan str., 0036 Yerevan, Armenia
[2]Yerevan State University, 1 A. Manoogian str., 0025 Yerevan, Armenia

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Abstract

For surgical and medical tools to prevent the spreading of infectious microorganisms to patients, disinfection and sterilization must be achieved by the use of disinfectants and sterilization procedures. Health care regulations must determine whether cleaning, disinfection, or sterilization is essential based largely on the item's intended use because sterilizing all patient care equipment is unnecessary. In this work, successful sterilization of vials and rubbers used for medical purposes has been implemented. It can be claimed that the sequence of moist and dry heat is more practical for sterilizing primary packaging materials used in diagnostic or treatment facilities, especially for borosilicate vials and chlorobutyl, bromobutyl rubbers. It has been shown that the suggested sterilization method provides sterile and endotoxin-free vials and rubbers that can be used for medical purposes. Endotoxins and sterility tests have been implemented for microbiological analysis of borosilicate vials and chlorobutyl, and bromobutyl rubbers. Endotoxin levels in all vials and rubbers tested were within acceptable limits, and no microbial growth was observed in test specimens based on sterility tests. The suggested sterilization method was effective in producing sterile and endotoxin-free vials and rubbers suitable for medical use.

1. Introduction

Sterilization is a procedure that eliminates all microorganisms that could cause a health risk by contaminating packaging materials. It efficiently kills all germs, including fungus, bacteria, viruses, and spore forms, and saves prions, from a surface, equipment, food, medication, or biological culture media. The selection of an appropriate sterilization method is dependent on the type of material utilized. Each material must undergo a thorough evaluation of its sterilization susceptibility, as well as an examination of all packaging characteristics [1–2].

Numerous invasive procedures are carried out in various healthcare facilities [3–4]. The risk of introducing pathogens into the patient's body is increased when a medical device or surgical instrument comes into contact with the sterile tissue or mucous membrane of the patient during the various processes [5–8]. Therefore, to stop the spread of these pathogens, healthcare professionals should have better knowledge of these techniques [9].

Several methods of sterilization are known, which are widely used in the world both for medical purposes and for experiments in scientific laboratory conditions. For example, sterilization with moist heat (autoclaving) [10], dry heat [11], irradiation with γ or X-rays [12], e-beam [13], certain chemicals in solution, certain gases or vapors (ethylene oxide, vaporized hydrogen peroxide, chlorine dioxide, ozone, etc.) [14]. The goal and physico-chemical characteristics of the sterilized sample determine which of these sterilization techniques will be employed [15–16].

*Corresponding author.
E-mail address: isabellakarati@gmail.com

Gamma radiation provides a number of benefits over conventional sterilizing techniques, including higher penetration, greater sterility assurance, and efficacy regardless of temperature and pressure [12]. Despite this, several studies show the harmful consequences of gamma radiation on sterilized items [17].

High-energy electrons are used in the electron beam (E-beam) irradiation process for a number of purposes, including sterilizing single-use medical equipment, preventing product contamination, and modifying materials like heat shrink tubing, wire and cables, and molded components [13, 18]. It must be noted that due to the less penetrating power than Gamma rays, thick and densely packed materials cannot be sterilized. Attenuation diminishes sterilization power [19–20].

Vaporized hydrogen peroxide (VHP) is a commonly used sterilization method for medical devices and pharmaceuticals [21–22]. While it has many advantages, such as being effective against a wide range of microorganisms and being a non-toxic and environmentally friendly option, there are also some disadvantages that need to be considered when selecting a sterilization method, including equipment cost, regulatory compliance, material compatibility residual toxicity, as well as drying time [23–24].

Ethylene oxide (ETO) sterilization is a common chemical technique. In healthcare facilities, ETO is used to sterilize heat- or moisture-sensitive critical objects (and occasionally semicritical items) that cannot be disinfected with steam sterilization [25]. ETO's main drawbacks include its lengthy cycle time, high cost, and potential risks to patients and employees [26].

It should also be noted that the types of sterilization described are not suitable for the sterilization of primary packaging materials used in diagnostic or treatment centers, as they require special equipment and, in some cases, poisonous gases. Based on the foregoing, it can be argued that for the sterilization of primary packaging materials used in diagnostic or treatment centres, in particular borosilicate vials and chlorobutyl, bromobutyl rubbers, it is more convenient to use moist and dry heat sterilization methods.

Moist sterilization or autoclaving was the original technique used to sterilize medical supplies. The primary fatal processes include irreversible coagulation, denaturation, and loss of vital enzymes, as well as the breakdown of protein and lipid complexes and bacterial endotoxins. Moisture concentration has a significant impact on the temperatures at which proteins coagulate and at which microorganisms become fatal. In addition to the above, this type of sterilization is very convenient for use in diagnostic or treatment facilities and is relatively inexpensive [27–28].

Dry heat sterilization is a process that uses high temperatures to eliminate microorganisms and spores from medical equipment, surgical instruments, and other materials that cannot withstand moisture. The main advantage of dry heat sterilization is that it is a relatively simple and straightforward process that can be used on a wide variety of materials, including powders, oils, and metals. It also does not leave behind any residue, making it ideal for materials that are sensitive to moisture or chemicals [29].

Thus, based on the advantages and disadvantages of all possible methods, and the currently existing problems, in this work it was proposed to apply both moist and dry heat methods, one after the other. This approach will make it possible to achieve better sterility using inexpensive and effective methods. The simultaneous use of these two methods also is more convenient to use in hospitals and other medical facilities.

2. Experimental part

15 ml borosilicate type A class vials (FILL-EASE, China), chlorobutyl and bromobutyl rubbers (FILL-EASE, China), and primary washing deionized water (18.2 mΩ, Smart2Pure, Thermo Scientific, USA) were used for sterilization. Silvery aluminum covers (Huayi Isotopes Co., China) were used for sealing.

LAB 500 C washer disinfector (Steelco, Italy), Elma S30H Ultrasonic (Elma Schmidbauer GmbH, Germany), YX-18LD portable pressure steam sterilizer (Redsun, China), SNB-100 Thermal Oven (Memmert, USA), dry heat kraft paper (Sterit, Vinar, Russia) were used during the sterilization process.

Sterility tests of investigated vials and rubbers were done by direct inoculation method using 9 ml Trypcase Soy Broth (TSB-T, Biomérieux, France), 9 ml Clear-Thio (THIOC-T, Biomérieux, France) mediums, 2 ml sterile syringes (Promed, Armenia) as well as sterile gloves (AccuTech, Belgium).

Endotoxin-free water (LAL water, Pirotest, Russia), Endosafe Charles River Portable Test System (Charles River Laboratories International, USA), Endsafe LAL cartridges (Charles River Laboratories International, USA), pyrogen-free 2–200 µL pipette tips (Eppendorf, Germany), Eppendorf safe-lock tubes (Eppendorf, Germany) were used for endotoxin analysis.

An isolator with A and B grades from "Comecer" (Bologna, Italy) was used during the packaging process. Grade isopropanol wipes (Prosat, France) were used for the surface cleaning process. For the particle control and microbiological monitoring of laminar flow cells accordingly MET ONE 3415 particle counter (HACH USA), 55 and 90 mm irradiated Count-Tact Agar plates (Biomérieux, France) were used. Memmert UFE 600 (Memmert, USA) equipment was used to incubate 55, 90 mm plates as well as Trypcase Soy Broth, and Clear-Thio mediums for testing aerobic and anaerobic bacteria.

Borosilicate vials washing procedure: 10 none sterile borosilicate vials were placed in the LAB 500 C washer disinfector. Vials were cleaned using cold and warm rinses with alkaline and acid detergents, followed by 40 min of dry heating in «Intensive» mode. After this procedure, vials were double packaged into kraft paper for further sterilization.

Rubbers washing procedure: 10 pcs of none sterile rubbers (chlorobutyl, bromobutyl) were washed at running water for about 10 min. Afterwards, each rubber was rinsed with deionized water, moved into ultrasonic cleaner (37 kHz), and filled with purified deionized water. Ultrasonic cleaning was carried out for 15 min in «Sweep» mode at 75-80 °C. Then chlorobutyl (5 pcs) and bromobutyl (5 pcs) rubbers were double packaged into kraft paper for further sterilization.

Moist heat and dry heat: After washing procedures, kraft papers were moved into a steam sterilizer (0.105–0.14 MPa). Autoclaving was carried out for about 40 min, then papers were moved into the thermal oven preheated to 180 °C for 2 h for endotoxin elimination. It should be noted, that paper with chlorobutyl rubbers was kept in the oven about 2–3 min then place on the top of the oven surface. This was done to avoid the physical deformation of chlorobutyl rubbers. After cooling all papers, vials, and rubbers were cleaned with sterile isopropanol wipes and moved into the "Comecer" B grade laminar flow cell which was cleaned with sterile wipes.

Sterilized vials preparation: First kraft packages of sterilized products were removed in the B grade cell. Second kraft packages were moved into pre-cleaned A grade area (cell). The closing process of vials with rubbers was implemented in A grade cell by using isopropanol pre-cleaned gloves. Then vials were sealed with silvery aluminum covers. For the sterility and endotoxin tests, endotoxin-free water (LAL water) has been filed into 10 vials via 10 ml syringes.

It should be noted that for environmental and microbiological monitoring of A, and B grades (cells, gloves) MET ONE 3415 particle counter and 55, 90 mm irradiated Count-Tact Agar plates have been used. Samples were stored in Memmert UFE 600 stove at 32±2 °C for a 5-day incubation period.

Endotoxin test has been implemented by Endosafe Charles River analyzer using Endsafe LAL cartridges with the sensitivity of 5-0.05 EU/ml. Meanwhile, sterility tests were performed using 9 ml Trypcase Soy Broth and 9 ml Clear-Thio mediums. Sterility samples were stored in Memmert UFE 600 stove, at 32±2 °C for a 14-day incubation period.

3. Results and discussion

To make sure that the bottle capping process is carried out under sterile conditions, microbiological control of A and B grade cells was carried out by measuring particles and sampling microbiological media with a size of 55 mm. Samples were taken from the right and left walls of grades A and B, while one sample in each grade was left open to assess the sterility of the boxes. Since the primary packaging and sealing processes for vials and rubbers were carried out with built-in gloves, the microbiological purity of the gloves was also checked with 90 mm contact plates. As expected, the A and B grades were in perfectly clean microbiological condition. In all cases, there were no microbial growths on the 55 mm contact plates (Fig. 1). Glove samples also showed no microbiological growth on 90 mm settle plates (Fig. 1).

Particle counter measurements determined particles in both, A and B grade cells which were in permitted limits for noted grades according to "USP Guidances on Environmental Control including related USP, FDA, EMEA & PDA Activities facilities [30]. The results are shown in Table 1.

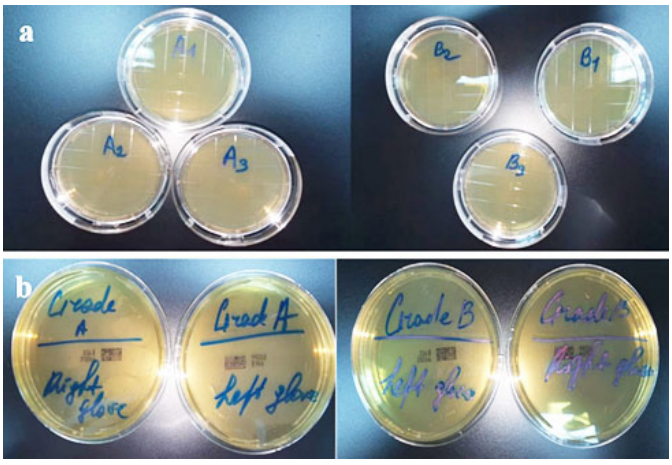

Fig. 1. Microbiological samples taken from A and B grade cells (a) and gloves (b).

Table 1. MET ONE 3415 particle counter results for A and B grade cells

Particle size (μm)	Grade A	Grade B
0.5	1	2
1.0	0	0
2.0	0	0
3.0	0	0
5.0	0	0
10.0	0	0

10 vials were filled with endotoxin-free water for sterility and endotoxin tests. All vials were shaken for 1–2 min so that the LAL water touches the surfaces of rubbers and bottles as much as possible. First, a rapid endotoxin test was performed. According to the results presented in Table 2, the levels of endotoxin identified for the first five vials sealed with bromobutyl rubbers were 0.250, 0.05, 0.05, 0.05, and 0.05 EU/mL, respectively. At the same time for vials sealed with bromobutyl rubbers, the amount of endotoxins in all samples was less than 0.250 EU/ml (Table 3). As expected, all vials and rubbers (bromobutyl, chlorobutyl) passed the endotoxin test as the results were less than the permitted 17.5 EU/ml according to USP regulations related to endotoxin limits for common injectable [31].

In addition to the rapid endotoxin test, sterility sampling was also performed for all 10 vials sealed with chlorobutyl and bromobutyl rubbers. After 14 days of incubation, it was found that no bacterial growth was observed in either 9 ml of Trypcase Soy Broth or 9 ml of Clear-Thio mediums (Fig. 2).

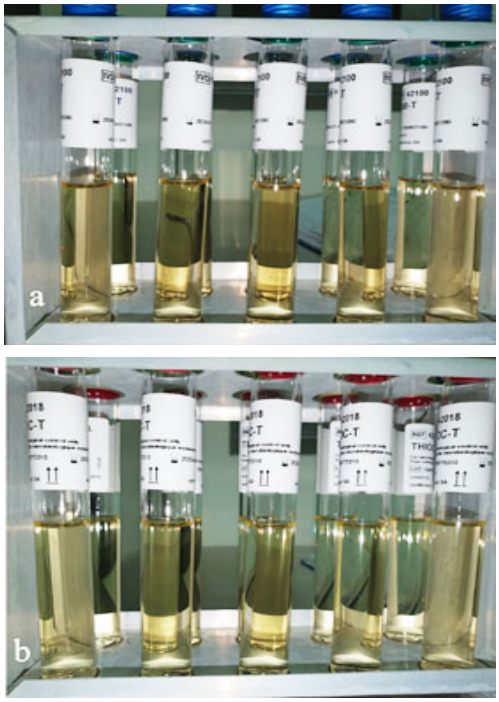

Fig. 2. Trypcase Soy Broth (a) and Clear-Thio mediums (b) after 14 days' incubation.

Table 2. Endosafe Charles River Portable Test System results for vials sealed with chlorobutyl rubbers

	Dilution factor	Reaction time of 4 channels (sec)	Sample reaction time CV (%)	Spike value (EU/ml)	Spike reaction time CV (%)	Spike recovery (%)	Test Suitability	Sample value (EU/ml)
Vial 1	1	756	0	0.504	6.5	67	Pass	<0.250
Vial 2	1	756	0	0.540	4.4	72	Pass	<0.050
Vial 3	1	756	0	0.748	5.9	100	Pass	<0.050
Vial 4	1	756	0	0.579	5.9	77	Pass	<0.050
Vial 5	1	808	0	0.527	12.3	61	Pass	<0.050

Table 3. Endosafe Charles River Portable Test System results for vials sealed with bromobutyl rubbers

	Dilution factor	Reaction time of 4 channels (sec)	Sample reaction time CV (%)	Spike value (EU/ml)	Spike reaction time CV (%)	Spike recovery (%)	Test Suitability	Sample value (EU/ml)
Vial 1	1	808	0	0.589	2.7	68	Pass	<0.250
Vial 2	1	808	0	0.509	0	58	Pass	<0.250
Vial 3	1	808	0	0.660	8.5	76	Pass	<0.250
Vial 4	1	808	0	0.991	0	114	Pass	<0.250
Vial 5	1	808	0	0.699	0	80	Pass	<0.250

4. Conclusions

Thus, it was studied a sterilization technique for vials and rubbers used in medical facilities. It was shown that sequential application of moist and dry heat is a practical and very effective option for sterilizing packaging materials like borosilicate vials and chlorobutyl, as well as bromobutyl rubbers in diagnostic or medical facilities. The suggested sterilization method was effective in producing sterile and endotoxin-free vials and rubbers suitable for medical use. All the vials and rubbers tested met the acceptable limit for endotoxin levels, and the sterility tests showed no microbial growth in the specimens.

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