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Validation of the Ethylene Oxide Sterilization of Polypropylene Disposable Syringes

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Abstract

The current study describes the efficiency of the sterilization method by mixture of 30% ethylene oxide and 70% CO_2 gas in a single cylinder its capacity 25 kg for single-use polypropylene syringes. The sterilization process was performed within six hours at 53°C to 54°C, pressure of 1320 ml bar and relative humidity of 60%. The chemical and biological indicators were removed from the cartons after completing the sterilization process. The results of the chemical test gave a change in the color of the chemical indicators tape which adhesive on the surface of each carton from yellow to orange. The chemical indicators strips which are packaged with the syringes changed from red to green that means indication of the sterilization process successfully. The result of the biological indicators (a bacterium called Bacillus atrophaeus placed in special tubes in prepared conditions containing special food). The biological indicators placed for 48 hours on a special instrument containing an incubator and an ultraviolet light bulb to detect the presence of bacteria. The instrument gave a negative result (green light) to indicate the absence of bacteria or bacterial growth and this indicated the success of the sterilization process and the efficiency of sterilization method by ethylene oxide gas effective for medical products, especially for medical syringes.

Keywords: Sterilization, Hypodermic needles, Validation.

المستخلص

تصف الدراسة الحالية التحقق من كفاءة طريقة التعقيم لمحاقن البولي بروبلين البلاستيكية أحادية الاستخدام بواسطة خليط من غازي أكسيد الإيثيلين بنسبة 30% و غاز ثاني أكسيد الكربون بنسبة 70% في أسطوانة واحدة سعة 25 كيلو جرام. تمت عملية التعقيم في غضون ستة ساعات في درجة حرارة 53 الي 54 درجة مئوية وضغط 1320 ملي بار ورطوبة نسبية 6%. تم إخراج الأدلة الكيميائية والبيولوجية من الكراتين بعد أن تم إخراجها من غرفة التعقيم حيث أعطت نتائج الإختبار

الكيميائي تغير في لون الأدلة الكيميائية على شكل الشرائط الملصقة على سطح كل كرتونة من اللون الاصفر إلى اللون البرتقالي .الأدلة الكيميائية المغلفة مع الحقن تغير لون الدليل من الاحمر إلى اللون الأخضر دلالة على نجاح عملية التعقيم أعطت نتيجة الأدلة البيولوجية والتي هي عبارة عن بكتريا تسمي (باسليس أتروفيوس) موضوعة في أسطوانات خاصة في ظروف مهيئة تحتوي على غذاء خاص. حيث تم وضع الأدلة البيولوجية لمدة 48 ساعة على جهاز خاص يحتوي على حاضنة وعلى لمبة أشعة فوق بنفسجية للكشف عن وجود بكتريا حيث أعطى الجهاز نتيجة سلبية للدلالة على عدم وجود نمو بكتيري او جرثومي(ضؤ اخضر) ودل ذلك على نجاح عملية التعقيم والتحقق من كفاءة طريقة التعقيم بواسطة غاز اكسيد الايثلين الفعال للمنتجات الطبية وخاصة للمحاقن الطبية .

Introduction

Validation is verifying and documenting with a high degree of assurance that specific equipment will perform consistently according to predetermined specifications. Also validation means to check that something is officially true and acceptable , especially in order to approve it , the results of tes have been validated by independent experts . To validate asystem requirements is to make sure its contents translate correctly and to validate the design of a system is to demonstrate that it satisfies its system requirements,(Hucker and Axel ,2001, 150-153).

The guidelines on general principles of process validation,(Lang and Bolton ,1991, 357-361).

A comperhensive method validation strategy for bioanalytical applications in the pharmaceutical industry mentions four types of validation :

Prospective validation (or premarket validation) ,Retrospective validation ,Concurrent validation and Revalidation .

Sterilization is the elimination of all living organism, bacteria and fungi spores from the contents of the products (plastic disposable syringe).

Syringes have to be sterile because of their use in the administration of sterile and pyrogenic drugs by parenteral route. Recently, sterilization is defined as the presence probability of viable microorganism in one million products. A sterile medical device is one that is free of viable microorganisms. The purpose of sterilization is to inactivate the microbiological contaminants and thereby transform the non-sterile medical devices into sterile ones.

For many medical device the sterilization with ethylene oxide may be the only one method that effectively sterilizes and doesn't damage the device during the sterilization process. Medical devices made from certain polymer (plastic or resin) or that have multiple layers of packaging. (Peacock, 1999, 24-26).

Ethylene oxide (EO) is an organic compound with the formula C_2H_4O.It is a colorless flammable gas at room temperature, with a faintly sweet odor; it is the simplest epoxide: a three-membered ring consisting of one oxygen atom and two carbon atoms. Because of its special molecular structure, ethylene oxide easily participates in addition reactions; e.g., opening its ring and thus easily polymerizing an isomeric of acetaldehyde and alcohol.

Ethylene oxide is industrially produced by direct oxidation of ethylene in the presence of silver catalyst. It is extremely flammable and explosive and is used as a main component of thermo baric weapons ;(Ox borrow, 1983, 546-549).

Therefore, it is commonly handled and shipped as a refrigerated liquid. As a poisonous gas that leaves no residue on items it contacts, pure ethylene oxide is a disinfectant that is, widely, used in hospitals and the medical equipment industry to replace steam in the sterilization of heat-sensitive tools and equipment, such as disposable plastic syringes.

Ethylene oxide is important or critical to the production of detergents, thickeners, solvents, plastics, and various organic chemicals such as ethylene glycol, ethanol amines, simple and complex glycols, poly glycol ethers and other compounds.

Ethylene oxide sterilization process was intended to sterilize medical devices, to eliminate pathogenic microorganisms and bacterial activity. Furthermore, compliance with the requirements ensures that validations conducted following this International Standard will provide products that meet the defined requirements for sterile products with a high degree of confidence. Sterilization is an example of such a process. For this reason, sterilization processes are validated for use.

There are several sterilization methods such as dry air, oven, and steam in autoclave at 121°C, UV radiation, ethylene oxide and ionized radiation.

Sterilization of products is possible in the terminal packages. Bacteria control is evaluated by this method and has the lowest risk. Because of these reasons, terminal sterilization method is recommended. There are two methods used for the sterilization of syringes. One of them is EO sterilization which has been used since the 1950s.

Pure EO gas is flammable and explosive, also according to Environmental Protection Agency (EPA) it is a toxic and carcinogenic gas, so EO gas is diluted with some agents like hydro chlorofluoro carbon (HCFC) and carbon dioxide. There are some disadvantages of this method; for instance it is complex because of depending on some critical parameters such as temperature (3°C 0-65°C), relative humidity (30-99%).

Validation method of ethylene oxide sterilization cycle is to verify that the system (Automatic E.O – Sterilizer) and the Gas mixture are able to perform a complete sterilization cycle successfully) which prepared it by treating 2-chloroethanol with hydroxide.

Temperature is a major factor in sterilization, so it increases the rate of product exposure for gas, it is between 53°C to 54 °C C especially Ethylene oxide effect.

Relative humidity is very important factor in process of penetrating of Ethylene oxide inside the package and bacteria cell at the same time to damage DNA by cooling the bacteria cell, (Furuhashi and Miyamae, 1982, 23-35).

1320 mbar of gas pressure should be reach to accelerate the ethylene oxide sterilization process inside the chamber.

Materials and Methods

Materials

Polypropylene plastic syringes, locally produced by Avamed for medical manufacturing. Sterilization chamber, ethylene oxide 30% + 70% carbon dioxide mixed, chemical strips, biological strip as indicators and Auto reader instrument.

Chemical Indicator Strips

Chemical indicator, any substance that gives a visible sign , usually by a color change, of the presence or absence of a threshold concentration of a chemical species.

Comply Chemical Indicator Strips EO – 1251, made in USA by 3M Health Care, St. Paul. MN 55 144 – 1000. This indicator is paper strip 1.5 cm wide by 20 cm long, printed with chemical indicator ink that turns from red to green when exposed to ethylene oxide sterilization process. It is designed to indicate whether ethylene oxide (EO) has penetrated to the point of placement of the strip, usually the center of the pack.

Chemical Indicator Strip is an action as acidic agent when EO gas action as a powerful alkylation agent.

Biological Indicators

Rapid Readout Biological Indicator EO – 1294, made in USA by 3M Health Care, St. Paul MN 55144 – 1000. The Rapid Readout Biological Indicator is specifically designed for the rapid reliable monitoring of ethylene oxide sterilization process; it detects the activity of a naturally occurring Bacillus *atrophaeus* enzyme, beta – glycosidase which is one of the enzymes involved in spore outgrowth and normal vegetative cell, (Heider etal,2002,pp.158-167).

Method

Before loading the chamber, hot water is circulated to increase the chamber temperature to the desired degree 50 °C – 55 °C. Chamber is loaded by 60 corrugated cartons of 5 ml syringes. The chamber is vacuumed once to remove the air from inside the chamber and then load. The chamber is humidified and the load passing steam to increase the humidity up to 50 % – 65 %. Where the sterilization chamber was filled with (60 cartons) single-use plastic syringes and chemical indicators was placed in the form of strips glued to the outside of all cartons. Another chemical indicator sought and wrapped in plastic with syringes to make sure the processing gas passage on the surface of the cartons and inside the product (syringes). Biological indicators were also placed inside the product after they were wrapped in plastic.

The gas valve was opened to pass the gas mixture (ethylene oxide 30 % and carbon dioxide 70 %) to get the desired gas pressure of 1320 mbar. The gas valve was closed and the sterilization cycle is started to the left. The load was exposed to the gas for 6 hours. At the end of the sterilization cycle, the system is vacuumed and aerated four times.

At the end of the above steps, the chamber was unloaded. Biological indicator was evaluated by Auto Reader instrument used to read and detect the activity of biological indicators that contain (Bacillus *atrophaeus* enzyme) by reading a fluorescent product produced by the enzymatic breakdown of a substrate in the media, (Fritze and Rudiger, 2001, 35-37).

The colour change of the chemical indicator was observed.

Results and Discussion

Table 1. The results of Biological indicator vial

Vial No	Position of the Biological Indicator vial inside the Chamber	Readings	Results
1	Left Side Top	–ve	Pass the test
2	Left Side Bottom	–ve	Pass the test
3	Left Side Top	–ve	Pass the test
4	Middle To the front side	–ve	Pass the test
5	Middle Centre	–ve	Pass the test
6	Middle To the back side	–ve	Pass the test
7	Right Side Bottom	–ve	Pass the test
8	Right Side Top	–ve	Pass the test
9	Right Side Bottom	–ve	Pass the test
10	Front Side Up	–ve	Pass the test
11	Back Side Down	–ve	Pass the test
12	With Plain Box To the back side	–ve	Pass the test

Table 1 shows all the Rapid Readout Biological Indicator vials are giving negative readings that indicate the absence of the fluorescent product produced by beta glycosidase enzyme of Bacillus atrophaeus. There was no growth after incubation period and the biological indicator results of all tested positions gave negative reading, hence the test result passed according to the reference (British pharmacopoeia,2016).

Table 2. The results of Chemical Indicator Tape on the outer cartons

Tape No	Position of the Chemical Indicator Tape inside the Chamber	Color Change		Results
		Color Before Sterilization	Color After Sterilization	
1	Left Side Top	Yellow	Orange	Pass the test
2	Left Side Bottom	Yellow	Orange	Pass the test
3	Left Side Top	Yellow	Orange	Pass the test
4	Middle To the front side	Yellow	Orange	Pass the test
5	Middle Centre	Yellow	Orange	Pass the test
6	Middle To the back side	Yellow	Orange	Pass the test
7	Right Side Bottom	Yellow	Orange	Pass the test
8	Right Side Top	Yellow	Orange	Pass the test
9	Right Side Bottom	Yellow	Orange	Pass the test
10	Front Side Up	Yellow	Orange	Pass the test
11	Back Side Down	Yellow	Orange	Pass the test
12	With Plain Box To the back side	Yellow	Orange	Pass the test

Table 2 shows all the results of Chemical Indicator Tape on the outer cartons surfaces passed the test due to the change of color from yellow to orange after the sterilization cycle because of the reaction between EO gas and the chemical indicator tape.

Table 3. The results of Chemical Indicator strip

Strip	Position of the Chemical indicator Strips inside the Chamber	Color Change		Results
		Color Before Sterilization	Color After Sterilization	
1	Left Side Top	Red	Green	Pass the test
2	Left Side Bottom	Red	Green	Pass the test

3	Left Side Top	Red	Green	Pass the test
4	Middle To the front side	Red	Green	Pass the test
5	Middle Centre	Red	Green	Pass the test
6	Middle To the back side	Red	Green	Pass the test
7	Right Side Bottom	Red	Green	Pass the test
8	Right Side Top	Red	Green	Pass the test
9	Right Side Bottom	Red	Green	Pass the test
10	Front Side Up	Red	Green	Pass the test
11	Back Side Down	Red	Green	Pass the test
12	With Plain Box To the back side	Red	Green	Pass the test

Table 3 shows all the Chemical Indicator Strips color has been changed from red to green color after exposed to ethylene oxide sterilization process in all positions inside the chamber , that is mean sterilization cycle is valid.

Conclusion

This study demonstrated the possibility of sterilization process cycle using ethylene oxide and carbon dioxide (mixture), high performance and valid for medical device (hypodermic, plastic, disposable syringes).
The chemical indicators gave accepted results according to the color changes from red to the green in chemical strips and Biological indicator vial (kill of bacteria) because of effectiveness of EO gas.

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