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No: 44601208/319 25 / 04 / 2014
Subject: Report

INTERACTIVE ANALYSIS OF MEDICAL DEVICE STERILIZATION WITH ETHYLENE OXIDE AND HYDROGEN PEROXIDE

What is Sterilization?

Sterilization is a term that refers killing all forms of life on a material by either chemical or physical techniques. In medical applications, it is important not to damage the material or leave harmful effects to the environment while killing the microorganisms.

Sterilization Techniques

Sterilization is carried out by major physical and chemical methods. In physical methods, there are existing applications such as heating (dry or humid), filtration (liquids or gases), and radiation (ionized or non-ionized beam). Whereas in chemical methods, the materials such as ethylene oxide (EtO), ozone, hydrogen peroxide (H2O2), beta propiolactone (BPL) are being used.

Sterilization by heating is proportional to the temperature. In addition, duration, osmatic pressure, pH as well as the structure of the microorganism are also important factors during sterilization. For instance, a bacteria that forms bacterial spore is relatively more resistant to sterilization. In heating proses, protein structures coagulate as a function of temperature. It only requires high temperature in dry heating (e.g. 160-180°C).Whereas pressure is also required in humid sterilization (121°C, 1 atm, 20-30 min). These conditions can be ensured by autoc1ave.

Filtration is a technique that separates microorganisms and partic1es inside the liquids or gases by using a filter. Although there are many kinds of filters available in the market, membrane filters that are produced materials with different specifications are recently being preferred. Microfiltration ensures holding and separating of the partic1es in the range of 0.1-1 μm. Dissolved solids and macro molecules can usually pass through the filter, however large partic1es and large colloids cannot pass. Flocked materials and suspended solid materials can be separated from liquids and gasses by using these filters.

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Although sterilization via heat is an easy and economic method that can be applicable for glass and some plastic materials, it cannot be used for biologic materials, fiber optics, electronic materials and most of the heat indurable plastics. Filtration is being preferred because it is applicable, practical, and it ensures sterilization without changing the structures of liquids and gases.

Sterilization via radiation has variable effects on microorganisms depending on the wavelength, density and duration of the radiation. Ionized beams such as gamma and X beam have high energy level due to the small wavelength. Gamma beam is especially preferred for sterilization of packaging materials and medical devices because of their high penetration ability. Non-ionized radiation types that includes UV beam do not have penetration ability and have long wavelength. Therefore, they can be only used for surface sterilization. In addition, damaging the surface of some plastics such as polystyrol when applied longer time is another disadvantages of using UV beam.

Chemical sterilization either destroys the protein structures on cell membranes of the microorganisms or inactivating the enzymatic structure. Most of the chemicals that are being used for surface sterilization cannot kin bacteria spores. Therefore, coordination of the material and chemicals should be considered before determining the chemical that will be used for sterilization. Yet these chemicals can usually be toxic and carcinogenic.

Beta propiolactone (BPL) application either liquid or gas has bactericidal effect. After being widely used for long time, its toxic effect was detected and it was announced that BPL should be used IDare carefully (1).

Ethylene oxide (EtO) and hydrogen peroxide (H2O2) are being widely used for chemical sterilization. Ozone, H2O2, potassium permanganate damages the enzyme activity with their oxidant effects. These gases are being used in law temperature. The active material of EtO has alkylating feature whereas ozone and H2O2 are oxidants.

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Ethylene Oxide

EtO is in the group of alkyl materials. In this group formalin and BPL are being placed besides EtO. Formalin in high concentration has mortal effect on all microorganisms. It is being used for cadaver and keeping the tissues *in vitro.* EtO is generally being used for sterilization of polyethylene materials.

EtO is applied purely or by combining with other gases. During the application 30-85 % humidity is required. The operation is done by applying 25-1200 mg/L gas at 25-65°C temperature for 1-24 hours. Eta is mostly used for surface sterilization of non heat resistant pots, powder products such as spices and polymeric materials for medical usage.

However appropriate ventilation requires to be set in order to remove toxic wastes that are being produced after EtO sterilization (2). EtO is included in carcinogenic gases group by International Cancer Research Foundation (IARC) since it shows mutagenic effect when applied inveterately (3,4).

EtO sterilization has a larger market share for disposable medical device sterilization. It disrupts the ability of proliferation of microorganisms by inactivating the hydroxyl and sulflhydryl groups of proteins and nucleic acids.

Eta is a colorless and toxic gas which is relatively heavier than aif and has light smell. it was founded in 1859 and first being used for the purpose of filling the yapar inside the room. Bacteriocidal affect of Eta was founded during the World War II. it has been used for sterilization since 1960.

EtO reacts with the cell wall of microorganism and results irreversible alkalinization. It is applicable to most of the medical devices especially heat and humid resisting plastic materials. Pure form of Eta is highly toxic and explosive chemical. Eta is liquid under 10.8 °C and gas above this temperature. Firstly, it was being use d with chlorofluorocarbon (CFC) in order to reduce the inflammability, however, after determination of CFC hazardous effect, usage of CFC was terminated in 1995 (Hazardous Substances Data Bank, HSDB). Toxicology database files for dichlorodifluoromethane, trichlorofluoromethane, and trichlorotrifluoroethane (5).

EtO is highly toxic for bacteria, fungus and most of the heat resistant bacterial endospores. The

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success of the Eta sterilization depends on (a) gas concentration, (b)temperature, (c) relative humidity and (d)application time. Duration of the sterilizations can be variable depending on these four parameters.

After the Eta sterilization, ventilation is required in order to remove the gas from the materia1. Ventilation time was set for 14 days initially but with invention of new ventilation systems this time is tried to be reduced shorter time (2-5 days).

The Limitation of Sterilization

If the ethylene oxide sterilization is being done in the hospital and properly controlled, it is being accepted reliable in terms of sterility and toxicity. Even though the toxic wastes on the plastic materials are not being checked regularly, the general methods need to be applied in order to minimize the risk factors. Short application of high pressure gas and keeping the material at law temperature (20-30°C) at least 4 days after sterilization procedure are important factors for the success of CO2/EtO mixture sterilization. All the materials that are going to be used for gas steriIization need to be clean and the plastics should preferentially be disposable.
All the machines that are being used in EtO sterilization requires to be full automatic and they need to be checked by expert microbiologist from packaging operation to sterility tests (11).

Hydrogen peroxide (H2O2)

Hydrogen peroxide is pale blue compound, having higher viscosity than water that becomes colorless when diluted in water. Even though H202 is present in the nature, it can be produced using chemical processes. it has an unstable nature, which can be decomposed to water and oxygen endothermically. Due to its decomposition property, it is an oxidizing agent and may trigger ignition when comes in contact with organic materials. Since hydrogen peroxide is ignitable, the oxygen, released in case of decomposition, may cause fire and high pressure.

The product is actually not combustible, however oxygen that is released following the decomposition, may enhance fire. Especially when it is heated up, it may cause burning or explosion when contacts with organic vapor or liquid. EU-OSHA and NIOSH have determined the maximum

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concentration in the air that can be exposed is as 1 ppm. According to data from IARC, it is certain that, hydrogen peroxide has mutagenic and carcinogenic effect on animals and cause serious irritation, inflamation and systemic toxicity when inhaled. Based on the data obtained from mammalian experiments, it is known that hydrogen peroxide may cause cancer in human as well.

The compound is a very weak acid and mainly produced for bleaching purpose in paper industry. In addition, it is also used as disinfectant, oxidative agent and production of antiseptic solutions and rocket fuel.

In human body it is decomposed by catalase, preseni in liver, which converts hydrogen peroxide to water and oxygen. H2O2 is a corrosive, combustible, a strong oxidizing agent. High concentrations of it cause irritation on skin and eyes. Even though, it has been used as a disinfection agent previously, due to its harmful effects on human health, currently it is listed as dangerous chemical. it is dangerous for eyes, skin, throat, and lungs, attention must be paid while working with it. It may cause permanent damage in eyes including blindness. When it is inhaled, it causes serious irritation on trachea.

Even though H2O2 has positive effect on wound healing if used in small doses (less than 3%) when it is used as irrigation solution for the surgical treatment of deeper wounds and administrated by intravenous injection, it causes clotting in vessels, apnoea, ulcerative colitis, shock, cardiac arrest and death. Also there are studies reporting that it may cause permanent lung damage and arterial embolism when continuous admission of 0,120-0,144 ppm (mg/L) of hydrogen peroxide. In another case study, it was reported that symptoms of embolism have been observed 30 minutes after surgical closer of a bone wound that was irrigated with 6% H2O2 and followed with 0.9% saline irrigation.

In the light of aforementioned informations, H2O2 usage for treatment, disinfection and sanitation purposes has to be limited, material safety data sheet has to be read before using it.

The Specific Conditions for Sterile Medical Devices:

The institution has to establish documentated procedures to validate sterilization processes. Sterilization processes has to be validated before first usage. Each sterilization validation document should be kept.

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Ethylene oxide sterilization:

Ethylene oxide is a very toxic, irritant and explosive compound having a boiling point of 10.8°C when it is pure. Therefore pure form of it is not available commercially. lts application is held in autoclave or such devices under particular temperature, humidity, pressure and time. Volume of the device determines the required amount of the EtO. 500 mg/L ethylene oxide provides enough sterilization at 58 °C under 40% relative humidity, when applied for 4 hours. EtO can diffuse through plastic bag. The material that is sterilized, should be packed in a plastic bag and then put in to EtO autoclave.

Following condition has to be considered while operating EtO autoclave:

- Materials have to be placed properly into autoclave, which should allow proper diffusion of the gas.
- Autoclave lid has to be tightly closed and then should be vacuum pumred **.** Enough aif should be taken into autoclave through a valve should be provided to obtain desired humidity
- Enough EtO gas, that is needed for sterilization should be given into alitedave
- Autoelave should be heated up for desired sterilization
- When the sterilization process completed, gas in the alitedave has to be emptied.

Heat sensitive materials such as polyethylene, plastic, rubber can be sterilized using EtOH sterilization.

Validation of Sterilization:

Chemical and biological indicators are used to validate sterilization process. Bacterial spores are placed in the middle *of* device. All bacterial spores have to be inactivated to conc1ude a successful sterilization process. This validation test has to be carried out in a microbiology lab. In order to decide the success *of* sterilization process, no growth *of* bacteria from spores should be observed in 7 days.

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Conclusion:

Gas sterilization is adviced when any other sterilization method is impossible to apply. Before sterilization process, it has to be assured that the material shouldn't be affected by sterilization; material should be packed using a bag allowing the diffusion *of* gas and humidity; the material should be conditioned; proper heat distribution should be present in the device; enough gas concentration should be provided and a proper biological indicator should be used.

While working with EtO and H2O2, all safety precautions has to be taken into account. Following gas sterilization, the product has to be ventilated. For that process, ventilation conditions, residual gas amount limits has to be determined; the time period inc1uding validation time has to be defined; safety and toxicity has to be evaluated.

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