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Number : 44601208/319
Subject : Report April 25, 2014

Mutual Analysis of Sterilization of Medical Equipment with Ethylene Oxide and Hydrogen Peroxide

WHAT IS STERILIZATION?

Sterilization is killing all of the live forms at a medium/environment or on a material by means of physical or chemical methods. While killing microorganisms during sterilization, it is important to keep materials undamaged and not to effect environment negatively at medical applications.

STERILIZATION TECHNIQUES

Sterilization processes are primarily executed by means of physical and chemical methods. There are heat (dry or humid), filtration (for gases and liquids) and radiation (by ionized or non-ionized beams) applications for physical methods. The materials such as ethylene oxide, ozone, hydrogen peroxide and beta-propiolactone are utilized for chemical methods.

Achievement is directly proportional with heat at the sterilization by means of heat. Furthermore, the structure of the microorganism has a role in addition to the period, osmotic pressure and pH. For example, spore-forming bacteria are relatively more resistant to sterilization. Here, depending on heat, process works in the coagulation direction of intracellular proteins. Only high temperature (for example: 160-180 °C) is required for dry sterilization; additionally pressure is required (such as 121 °C, 1 atm., 20-30 min.) for the sterilization with humid heat. These conditions are provided by autoclave equipment.

Filtration is the decomposition process of particles and microorganisms available in liquids and gases by using filter. Although there are various filter materials, membrane filters, made up of materials with different peculiarities are preferred for sterilization in recent years. Micro-filtration enables to hold and decompose particles in between 0,1-1 μm ranges. In general, suspended particles and large colloids may not pass through the membrane while the decomposed solids and macro molecules are passing through the membrane. Also these filters are utilized to separate the flocked materials and suspended solid materials from water.

Although the sterilization techniques with heat are easy and economic, and as well as used for many materials such as glass and some plastics, they are not suitable for the products which are not resistant to heat and may be broken down/corrupted such as biologic materials, fiber optics, electronic materials and plenty of plastics. Filtration is preferred because of its easy application and practicability, enabling sterilization without changing the structure of liquids and gases.

Various effects are observed on microorganisms during the sterilization with radiation depending on the wave length, density and application period. Ionized radiation sources, such as gamma and X radiations, have high energy due to their small wave lengths. Especially, gamma radiation is preferred for packing materials and medical materials because of its high penetration characteristic.

Particularly, non-ionized radiation types, including ultraviolet (U.V.) rays are at the large wave length and has no penetration characteristic. For that reason, they are applied for only surface disinfections. Another disadvantage of U.V. rays is to damage some plastics such as polystyrene foam in case of exposed for long period of time.

Chemical sterilization becomes active by destroying and denaturing the structure of the cytoplasmic membranes and proteins of the microorganisms or inactivating their enzymes. Plenty of chemicals, used for surface sterilization don't kill bacterial spores. While selecting chemical sterilants, it is important to be careful for coherence of the material to be sterilized with chemical. Besides, these materials shouldn't have detrimental effects at the working area and don't risk the life of the employee. Because, it's known that these materials generally have irritant, toxic and carcinogenic effects.

Beta-Propiolactone (BPL) has bactericidal effect on the surfaces, subjected to liquid or gaseous phase. Its toxic effect was determined after being in use commonly for a long time, and it was reported that it should be used and applied carefully (1).

Ethylene Oxide (EtO) and Hydrogen Peroxide (H_2O_2) are among the chemicals, mostly used in this field. Liquid sterilization chemicals such as Hydrogen Peroxide include oxidizing agents. Hydrogen peroxide, potassium permanganate and ozone spoil enzyme activities with their oxidizing effects. These gaseous sterilants are used at low temperatures. The active agent of EtO has alkalizing feature, the active agent of H_2O_2 and ozone has oxidizing feature.

Ethylene Oxide (EtO)

It is in the alkalizing agents group. Also, formalin and beta-propiolactone are in this group in addition to EtO. Formalin with high concentration has lethal effects on all microorganisms. It is used to keep cadavers and tissues. Ethylene oxide is utilized commonly for the sterilization of polyethylene materials.

Ethylene oxide (EtO) is applied purely or together with other gases. Humid at the rate of 30-85 % is required during the process. Process is realized in between 25-65 °C, 1-24 hour period and 25-1200 mg/L gas usage. Mostly it may be used for vessels/containers not resistant to heat, surface sterilization of the materials keeping powder/granulated products like spices, polymeric materials to be used for medical purposes. But, acceptable aeration conditions should be provided at the end of process to remove/clear toxic residues (2). The International Agency for Research on Cancer (IARC) has included this gas into the carcinogenic gases group because of showing mutagenic effect in case of chronic exposure to this gas (3,4).

This sterilization method has the largest market share for the industrial sterilization of single use medical materials. Effect mechanism of sterilization is that the microorganisms lose their reproduction capability as the result of inactivation due to the alkalization of their proteins and nucleic acids, especially hydroxyl and sulfhydryl groups. Ethylene oxide is a colorless, toxic, flammable, and explosive gas with a very light odor, and also it is a little bit heavier from air. It was discovered in 1989 and at first, it was used for the purpose of providing air into the room. It was found out during the 2. World War that it has bacteriocidal property. As it was at all of the science branches, the developments, experienced in surgery has created new technique and new tool requirements. Sterilization of the instruments/tools/devices/equipment, sensitive to heat and humidity with ethylene oxide has been brought to the agenda. Ethylene oxide gas has been used for sterilization since 1960. Ethylene oxide sterilization is to realize sterilization process at low temperatures (37-55 °C) in standard periods, under pressure, humidity and EtO gas.

Ethylene oxide works as a sterilant that enters reaction with the cell wall of the microorganisms and causes irreversible alkalization. It is compliant with a lot of medical materials, especially it is a preferred method for the plastic materials, sensitive to heat and humidity. EtO is used especially for the purpose of sterilization of medical materials. It is in liquid form under 10,8 °C and in gas form above this temperatures, and a very toxic, irritant and explosive chemical when it is pure. it was diluted with chlorofluorocarbon (CFC) (12 % EtO+88% CFC) to decrease its flammability. This compound, used very commonly in the beginning hasn't been used now due to its harmful effects to the health. (Hazardous Substances Data Bank (HSDB). Toxicology database files for dichlorodifluoromethane, trichlorofluoromethane, and trichlorotrifluoroethane (5).

Usage of CFC is forbidden beginning from 1995 with the acceptance of "Clean Air Agreement", prepared by Environmental Protection Agency (EPA) of the United States of America in 1990. In 1996, production of the sterilizers, operated with aforesaid 12 % EtO+88% CFC is stopped by Occupational Safety and Health Administration of USA (USA-OSHA) in the scope of studies to protect stratospheric ozone (6). EtO+HCFC and EtO+CO_2 compounds are still used in our country. Although the damages of the Hydrochlorofluorocarbons (HCFC), alternative of the Chlorofluorocarbons (CFC), to the ozone is low, it has a very high greenhouse effect. For this reason, import of this gas will be ended on 01.01.2015 and other alternative gases such as hydrofluorocarbon (HFC), CO_2 or 152+dimethyle ether which have no destructive effects on ozone will be used (7). Depending on this cause, pure EtO is being used in the produced new generation sterilizers.

The high reactivity of EtO, resulting from its exergonic combustion, which unites it with its highly effective diffusing feature is the most important factor that inactivates microorganisms. This gas has a directly alkalizing feature and doesn't need any metabolic activation. Its accepted that its microbiological inactivation feature -powerful alkalizing feature- unites with the cellular constituents of the microorganisms, including functional proteins such as nucleic acids and enzymes (8). As the result of adding alkyl groups into the proteins, DNAs and RNAs of the microorganisms, they are bound to sulfhydryl, hydroxyl, amino and carboxyl groups and prevents normal activation of the cell metabolism and its reproduction, and therefore this causes the microorganisms to die. Such similar chemical structures are not available in many medical equipment, so they are not subjected to this type of structural transformation (9,10).

EtO is highly toxic for most of the virus, bacteria, fungus and bacterial endospores, resistant to heat. Its efficiency is possible with its special packing

type, permitting penetration of the gas and strict control of 4 parameters during the process. These parameters may be summarized as; (a) gas concentration, (b) heat, (c) relative humidity and (d) application period. Sterilization period may show alterations depending on to these 4 parameters.

A significant period of aeration is required after process to remove EtO gas residues on material. This quarantine period was determined 14 days at the beginning, nowadays, it is tried to be reduced to the shorter periods (2-5 days) with the developed aeration rooms.

Usage Limits at Sterilization.

If EtO sterilization is executed at a hospital and controlled with the correct methods, it is accepted safe in terms of sterility and toxicity. Although the toxic residues on plastic materials may not be inspected regularly, applying general method is essential to minimize risk factors.

After completion of the process, it is important to keep materials, exposed to high pressure for a short period of time, waiting at least 4 days at low temperature (20-30°C) for the achievement of sterilization with CO_2/EtO mixture. All materials to be sterilized with gas should be clean and preferably the plastic materials should be single-use. The faultlessness of all devices, used in EtO sterilizers should be guaranteed and be full automatic, and all process, from packing to tests, should be checked by a competent microbiologist (11).

Hydrogen Peroxide (H_2O_2)

Hydrogen Peroxide (H_2O_2) is a pale blue color compound and becomes colorless when it is diluted, and its viscosity value is higher than water. Although it is available in air in natural gas form, hydrogen peroxide is a colorless liquid produced by means of chemical methods in room temperature. It has an unstable feature and decomposes to oxygen and water easily by releasing heat. It is oxidant due to this future and may cause fire when touched to a organic material (12). Contacting with flammable materials may cause fire. Oxygen, released as the result of decomposition may cause the organic materials to burn and excessive pressure.

Basically, the product is not flammable but the oxygen released as the result of decomposition may intensify fire. Especially when it is heated, as the result of contact with the organic liquid and humidity, it may cause sudden fire and explosion. Release of oxygen from H_2O_2 may pull organic steams and hydrogen steams to the explosion range. One of the leading organizations of the World, "The European Agency for Safety and Health at Work (EU-OSHA")", and "The

National Institute for Occupational Safety and Health (NIOSH) in USA" has indicated that the maximum limit to be exposed in air may be 1 ppm (13,14). According to the data of the International Agency for Research on Cancer (IARC), it is certain that H_2O_2 has mutagenic and carcinogenic effects on experimental animals and when inhaled by human being, it causes serious irritations and inflammation in nose and throat, and systemic toxication. Furthermore, It is also stated depending on the findings on experimental mammals that this gas may be carcinogen on human being as well (15).

The compound which is a very weak acid is produced especially for paper industry to give white color to papers. Besides, this compound is used for the production of disinfectants, oxidants, antiseptics and rocket fuels.

This molecule is decomposed body by catalase enzyme, produced in liver in human body. This enzyme decomposes hydrogen peroxide molecule and transforms into water and oxygen molecules.

Hydrogen peroxide which may cause burns is a corrosive, flammable and very strong oxidizing material. Intensive H_2O_2 burns skin and creates blisters on skin, causes permanent damages inside the eyes.

Although it was used as disinfectant in previous years, it was taken into the "hazardous chemicals" list, because its harmful effects was confirmed with various researches (16).

It is irritating for eyes, skin, nose, throat and lungs, and should be careful. It may cause permanent damages in eyes including blindness. When swallowed, it causes irreparable damages and tissue destructions. Although inhaling vapor by way of respiratory system is relatively less harmful, tissue destructions on the surface of trachea are observed (17).

Although positive results were obtained when it is used in less doses (less than 3 %) and short period to heal the wounds (18), It was reported that the events resulting with death such as atherosclerosis, asphyxia, cerebral vascular occlusion, duodenal ulcers, small intestinal gangrene, shock, heart arrest were experienced when it is used as irrigation solution during the treatment of deep wounds or applied by means of vein (19-25). In addition to that, there are studies reporting that 0,120-0,144 ppm (mg/L) hydrogen peroxide, mixing into the blood continuously causes arterial gas emboli and irreversible lung destructions (26, 27). Another report indicates that just after the irrigation with 6% hydrogen peroxide solution, in 30 minutes after the closure of a bone wound with surgical methods, which was irrigated with 0,9 saline, emboli symptoms was shown up (28).

In the lights of acts, usage of hydrogen peroxide should be limited as far as possible at laboratories, workplaces and health institutions for the purposes of treatment, sanitation and disinfecting, and "Material Information Form", prepared for chemicals and received together with the product should be read carefully and evaluated.

Special conditions for sterile medical equipment

Institution/organization should constitute documented procedures for the validation of the sterilization processes. Sterilization processes should be validated before the first usage. Validation records of each sterilization process should be kept.

Sterilization with Ethylene Oxide: Ethylene oxide is a liquid under 10,8 °C and gas over this degree, and very toxic, irritating and explosive when its pure. For that reason, its pure form is not available for commercial purposes. It mixtures with carbon dioxide gas are sold. It is applied in the autoclaves or similar equipment under specific humidity, pressure and period. Internal volume of equipment affects directly the amount of ethylene oxide, required to be used. A good sterilization for one liter equipment volume can be ensured with 500 mg ethylene oxide, 58 °C temperature, 40 % relative humidity and 4-hour period.

Ethylene oxide normally penetrate through nylon/plastic and doesn't cause any damages. First, the tools/materials to be sterilized are packed and hermetically sealed from outer environment with the nylon/plastic. Later, it is placed inside the ethylene oxide autoclave.

The rules to be applied may be listed as follows while operating ethylene oxide autoclave.

* The materials to be sterilized are place inside the autoclave intermittently to let the gas pass easily.
* The doors of the autoclave is closed tightly and the air inside is sucked with vacuum.
* Entry of air which will form the required amount of humidity into the autoclave is provided by the help of a valve.
* Calculated amount of ethylene oxide for sterilization is injected inside the autoclave.
* Formation of estimated heat for sterilization is provided and sterilization period is set.
* All gas in autoclave is discharged with vacuum at the end of determined period.

Temperature sensitive materials such as polyethylene, plastic, rubber tools and etc. are reused after sterilization with ethylene oxide.

Control of the sterilization

For this purpose, chemical (chemical indicator inside the bag/package) and biologic indicators (*Bacillus subtilis*) are used (6). Bacterial spores inside the special tubes are placed in the middle of equipment. If the sterilization is realized properly, all spores should die. The realization control of this event should be searched at microbiology laboratory. In case of non-reproduction of these bacteria, it is decided that the sterilization process is satisfactory. 7 days should be waited to decide for the non-reproduction of the spore-forming bacteria.

RESULT

Sterilization processes with gas should be applied for the cases if there isn't another available proper method. First, be sure that the process will not affect the product negatively. Packing should be made with the material allowing the penetration of gas and humidity. Product should be reached to the suitable heat and humidity (conditioning) before sterilization. The suitable heat distribution inside the equipment should be provided and gas concentration should be at proper level and necessarily a biological indicator, such as *Bacillus subtilis, Bacillus atropheus* should be used.

The required safety measures should be taken while working with EtO or H_2O_2 without ignoring the reality that these gases are risky for human life depending on the dose and exposure time. Products, sterilized with gas techniques should definitely be aerated after process. At gas processes, it is important to consider aeration conditions, level of residue gas amount (whether it is at the determined levels), completion of procedures including validation and major safety/toxicity matters.

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