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Short Communication

Sterilization in Microbiology

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It has long been a challenge to control infectious diseases by destruction, decrease in their number or inhibition of microorganisms. It can be carried out with different methods depending on the place to be applied and the degree of microbial eradication that is intended to be achieved. This is why it is convenient to define some concepts:

Sterilization

A physical or chemical process that destroys all life forms of microbial life, including spores.

Disinfection

Aims at the destruction of microorganisms by chemical agents (disinfectants), in order to reduce the number of vegetative forms to minimum levels.

Disinfectant

It is the chemical substance that inhibits or destroys microorganisms when applied to inert material without significantly altering it.

Asepsis

Term applied to procedures used to prevent microorganisms from progressing in a given environment (operating room, laboratory, etc.)

Antiseptics

They are disinfecting agents that are used on body surfaces in order to reduce the amount of normal flora and microbial contaminants of a pathogenic nature. They have a lower degree of toxicity than disinfectants and generally a lower degree of activity. Certain preparations can be used as antiseptics or disinfectants interchangeably, but at different concentrations in each case.

Antimicrobials

Are chemical substances produced by microorganisms or chemically synthesized that at low concentrations are able to inhibit and even destroy microorganisms without producing toxic effects on the host.

The most important and most commonly used sterilization methods are:

Physicists

Flamed

It is a simple and effective procedure, it consists of the exposure of an object to the effect of the flame until incandescence. They are sterilized in this way, the ansas of sowing culture.

Incineration

It is the best system to sterilize all those products in which their destruction does not matter, such as, for example, a biological material.

Stove

Uses dry heat at high temperature, 20 minutes for 180ºC, 60 minutes at 160ºC, being sufficient sterilization for 60 minutes at 100-140ºC, it is used to sterilize glass material properly wrapped in paper, metal. etc.

Chamberl and Autoclave

Sterilization with wet heat (water vapor) is much faster and more efficient than dry heat because water molecules irreversibly denature proteins by breaking the H-junctions between peptide groups at relatively low temperatures. It consists of a chamber in which air can be replaced by pressurized water vapor. It operates at 121ºC and 1 atm. pressure for 20 minutes. In this way it is possible to destroy all vegetative forms and spores. It is used to sterilize any material resistant to that temperature and is widely used for the sterilization of culture media.

Tindalization

(Intermittent sterilization) consists of subjecting the product to intermittent heating between 56 and 100ºC for 30 minutes, which ensures that the vegetative forms are destroyed. In the intervals it is kept at room temperature or at 37ºC, the spores germinate and the resulting bacteria become more sensitive to subsequent heating.

Radiation

UV light

It is absorbed at a wavelength of 240 to 280 nm by nucleic acids causing genetic damage by altering the bases. It is used in the preparation of vaccines, biosafety booths, workplaces such as laboratory countertops, etc.

Ionizing radiation

They act by injuring nucleic acids. It is mainly used in industrial processes to sterilize surgical devices, gloves, syringes, etc.

Chemists

Chemical agents such as ethylene oxide, formaldehyde or glutaraldehyde react very easily with different functional groups of nucleic acids and proteins by alkylating these essential radicals.

Ethylene oxide

It is a flammable and potentially explosive gas, very penetrating that inactivates microorganisms by replacing labile hydrogen atoms with other groups such as hydroxyls, carboxyls, etc. The material is exposed to sterilization to 5-10% ethylene oxide in carbon dioxide at 50-60º under controlled humidity conditions for 4 to 6 hours. It is necessary to then subject it to a period of aeration due to its mutagenic nature. It is an effective agent in the sterilization of thermolabile material such as prostheses, catheters, etc.

Formaldehyde or formaldehyde

It is a gas easily soluble in water that is used at 40% (formalin). Used in gaseous form and in a closed chamber, it is used in hospital sterilization and in the pharmaceutical industry. It is also widely used as an environmental disinfectant of highly contaminated rooms that once treated must be aerated.

Glutaraldehyde

It is used by immersing the clean material in a 2% solution, it is mainly used in the sterilization of optical instruments and those used in respiratory therapy. The activity of compounds derived from heavy metals (such as silver, mercury, etc.) is due to the formation of salts that dissociate with difficulty from the sulfhydryl groups of proteins.

Silver nitrate and argentic derivatives

They are good bactericides. Silver nitrate has been used in the treatment of burns in 0.5% solutions and in the prophylaxis of oftalmia neonatorum by Neisseria gonorrhoeae.

Mercurial derivatives

The most used as a skin disinfectant is mercurochrome, it is non-toxic and remains active in the presence of organic matter.

Hydrogen peroxide (hydrogen peroxide)

It is an oxidizing agent with a fleeting effect due to being broken down by the catalases of the tissues.

Potassium Permanganate

An oxidizing agent that is inactivated in the presence of organic matter. It is little used. In dermatology it is used for its antifungal property.

Chlorinated derivatives

They are inactivated in the presence of organic matter. Chlorine and derivatives are oxidizing agents widely used in the purification of water in the form of chlorine gas in large establishments, and in the form of hypochlorite is used to discard biological material (blood, serum, etc.) Chloramine is a less potent antiseptic than hypochlorite, slower acting but better tolerated in topical application.

Iodinated derivatives

These are oxidizing agents that are used in the form of an aqueous solution, combining them with detergents or organic substances. Iodophores are compounds that are progressively released. Iodine is found in polyvinylpyrrolidone (povidone iodine). There are also alcoholic solutions.

Alcohols

They act by denaturing proteins. Their action is fast, but they evaporate easily. Ethyl alcohol is used in antisepsis at a concentration of 70%, at this concentration the surface tension of the bacterial cell is further reduced facilitating the denaturation process.

Phenols

They act by precipitating proteins. Hexachlorophene and phenol are not used because of their toxicity. Other phenolic derivatives are cresols, which together with soaps originate stable compounds.

Chlorhexidine

It is a phenolic derivative that acts by altering the permeability of the bacterial cell membrane. It has rapid inactivation and is well tolerated by the skin. It is widely used in hospitals in the washing of the skin surface in the form of a solution (aqueous or alcoholic) or associated with non-ionic detergents.

Anionic detergents

They act by disorganizing cytoplasmic membranes. They have little bacteriostatic power. They can be improved by combining them with disinfectants or other surfactants such as lauryl sulfate.

Cationic detergents

They have antiseptic action, are inactivated in contact with soap, cotton and organic matter. They are little used.

Glycols

Propylene glycol and Ethylene glycol, are applied by means of devices called glycosates or in the form of aerosols for environmental disinfection.

Products described as sterile must satisfy the so-called Sterility Test. These products are sterilized in their final container, except in cases where the product, due to its nature, cannot be subjected to the corresponding treatment in its container. Products that cannot be sterilized in their final container are prepared by methods and under certain conditions to avoid microbial contamination after undergoing a proper sterilization process all their components, if possible, as well as containers and closures.

- ✓ Working conditions must be properly controlled trying to prevent the introduction and growth of microorganisms,
- ✓ The level of microbial contamination of raw materials, equipment and all material used should be as low as possible before sterilization.
- ✓ Microbiological control should be carried out on raw materials likely to present a high level of contamination due to their nature or their mode of preparation.
- ✓ Each specific sterilization process must be validated.
- ✓ The procedures and precautions used must be such that a theoretical level of contamination is reached in the final product, corresponding to no more than 1 live microorganism per 1 x 106 units subjected to sterilization.

The effectiveness of sterilization procedures is significantly influenced by the initial degree of microbial contamination, and the following precautions must be observed:

For all sterilization methods, the critical conditions of the operation must be controlled in such a way as to ensure that all units in the batch have been subjected to at least the minimum sterilization conditions. The duration of the treatment is measured from the moment in which the prescribed conditions for sterilization are achieved in the set of products to be sterilized.

Steam sterilization

In the autoclave, temperature and steam pressure should be measured independently with an accuracy greater than ± 2°C and ± 10 KPa (0.1 atm) respectively; preferably a continuous record of these parameters should be obtained. The temperature should be measured in the coldest part of the autoclave which is usually located near the steam outlet conduction. The temperature should also preferably be measured in two or more containers, located in different places of the autoclave, so that the measured temperatures represent the extreme values of all the containers in the batch.

When it is difficult for an autoclave to quickly achieve the displacement of air by steam (for example, when treating porous materials, textiles, utensils of various types), it is necessary to evacuate the air from the autoclave before the intake of steam. The effectiveness of the procedure can be confirmed by the use of appropriate biological indicators.

Dry heat sterilization

The furnace should normally be equipped with a forced air circulation system and filled in such a way as to achieve an even tem-

perature distribution throughout the load. Temperature should be measured and preferably recorded in at least two locations where sterilization conditions are least likely to be met. The effectiveness of the procedure can be confirmed using appropriate biological indicators.

Radiation sterilization

During the sterilization procedure, the radiation dose should be monitored regularly. This control involves dosimetric procedures, independent of the radiation rate, which allow a quantitative measurement of the dose received by the product itself. It must be demonstrated that the radiation dose applied is effective and appropriate for the nature of the product to be sterilized and its packaging material. The effectiveness of the procedure can be confirmed by the use of appropriate biological indicators. The dosimetry system is compared with the help of physical, chemical or microbiological methods with the same system arranged in a reference radiation facility. This check is done every time a change in radiation procedures is made and at least once a year.

Gas sterilization

Significant physical and chemical parameters (time, temperature, relative humidity, pressure, gas concentration) should be measured and recorded as often as possible. Products that cannot be sterilized in their final container need special precautions. They must be prepared under conditions designed to avoid any microbial contamination. Production premises and the ventilation system must be designed to minimise microbial contamination and must be regularly monitored appropriately. Equipment, containers and stoppers and, if possible, components should be subjected to a proper sterilization process.

Filtration through filters that retain bacteria

Solutions can be filtered through membranes of nominal porosity less than or equal to 0.22 µm, or through another type of filter that retains bacteria. Precautions should be taken to ensure that the properties of the filter are maintained during use. In the case of filtration of a liquid in which microbial growth can develop, the same filters should not be used if the duration of the procedure is longer than one working day.

Preparation in aseptic conditions

Products that undergo the filtration process described above and some others, are prepared under aseptic conditions. They can undergo a final heat treatment compatible with their thermostability, if this treatment proves justified. The conditions of preparation may, in certain cases, be controlled with the help of an appropriate culture medium, previously sterilized distributed under the same conditions as the product to be examined; the medium is incubated and then examined for possible contamination.

Biological indicators are preparations of microorganisms selected for their high resistance to one or more sterilization methods. They can be used to confirm the effectiveness of a sterilization process. The biological indicator must be clearly distinguishable from the product to be sterilized, in order to avoid any mixing or contamination of the product.

The growth of the control microorganisms that are subjected to the sterilization process shows that it is insufficient. A biological indicator may consist of artificially inoculated units of the product to be examined or fibrous substances, sand, glass, metal sheets that support the control microorganisms.

Control microorganisms should be deposited at sites considered to be the most difficult to sterilize. The choice of control microorganisms is based on the following criteria:

- ✓ The resistance of the control strain to the particular method of sterilization must be large, compared to the resistance of all pathogenic microorganisms and that of the microbial contaminants of the product.
- ✓ The control strain must not be pathogenic.
- ✓ The control strain should be easily grown.

A biological indicator is characterized by the strain of control microorganisms it incorporates, the number of colony-forming units per indicator unit, the D(1) value and the expiration date. Only the indicated microorganisms should be present. All information concerning the culture medium and incubation conditions must be specified.

Steam sterilization

Bacillus stearothermophilus spores, e.g. ATCC 7953 OR CIP 52.81, are recommended as control microorganisms. The number of viable spores should be greater than 1 x 10 exp5 per indicator unit and the D value at 121° C should be approximately 11/2 min. Dry heat sterilization. The spores of Bacillus subtilis, for example, var. niger ATCC 9372 0 CIP 77.18, are recommended as control microorganisms. The number of viable spores should be greater than 1 x 10 exp5 per indicator unit and the D value should be at 160°C approximately 5 to 10 min.

Gas sterilization

The spores of Bacillus subtilis, for example, var. niger ATCC 9372 0 CIP 77.18, or the spores of Bacillus stearothermophilus ATCC 7953 or CIP 52.81, are recommended as control microorganisms. It is essential that the biological indicator is able to reveal insufficient humidification in the sterilizer and in the product to ensure that even dehydrated microorganisms are inactivated.

Radiation sterilization

Bacillus pumilus spores, e.g. ATCC 14884 or CIP 3.83, are recommended for a minimum dose of 25 KGy (2.5 Mrad). The number of spores should be 1 x 10 exp7 to 1 x 10 exp8 per indicator unit and the D value should be approximately 3 KGy (0.3 Mrad). Other sporeforming strains (Bacillus cereus mutants, e.g. SSI C1/1), Bacillus sphaericus, e.g. (SSI C1A) that exhibit higher resistance, can be used for higher radiation doses.

(1) The D-value is the value of a sterilisation parameter (duration or absorbed dose) necessary to reduce the initial value of the number of viable micro-organisms to 10%. The D-value only acquires significance under well-defined experimental conditions.

The Sterility Test applies to substances, preparations and objects that, according to the Pharmacopoeia, must be sterile, but a favorable result only means that no microorganism has been found in the sample examined under the conditions of the test. The extension of this result to an entire batch of product requires the certainty that all the units that compose it have been prepared in such a way that there is a high degree of probability that they would have satisfied the test. It is evident that this depends on the precautions taken in the course of manufacturing. For products subjected to a sterilization process in their final and sealed containers, the physical, biologically based and automatically registered test that testifies to the correct development of the sterilization treatment in the entire batch, is of a reliability superior to that of the sterility test. The latter however is the only analytical method available to any authority that has to control the sterility of a product. A sterility test should be carried out under the conditions studied to eliminate any risk of accidental contamination of the product in the course of the test, for example by using sterile laminar air flow hoods.

The precautions taken to avoid such contamination should not affect micro-organisms whose presence is to be revealed in the test. The effectiveness of the precautions observed must be regularly verified by an air and work surface control, carrying out checks on preparations known to be sterile. Culture media suitable for the growth of anaerobic and aerobic bacteria and for fungi, as well as their preparation methods are described below. Other means may be used provided that their ability to ensure the growth of a wide range of microorganisms has been demonstrated. They must satisfy the tests indicated, carried out on each batch of the chosen media, before use, or in parallel with the test of the product to be examined.

Sterility

They are incubated in each case for at least 7 days, at 30-35 ° C, portions of the means intended to show bacteria and at 20-25 ° C, the portions of the media intended mainly to show contamination by fungi. They should not have any microbial growth.

Nutritional properties

Tubes of the chosen media are sown respectively with approximately 100 viable microorganisms (aerobes, anaerobes and fungi) and incubated for a maximum of 7 days at the temperatures indicated above (Sterility). The medium is suitable if it allows a rapid and abundant growth of the corresponding microorganisms.

If the medium intended primarily for the search for fungi is also used for the bacterial sterility test, it must be tested with both types of microorganisms. If in the course of incubation, microbial growth is similar in the presence and absence of the product to be examined (early and abundant growth), the latter has no antimicrobial activity and the sterility test can be performed without modification. If the cultures containing the product to be examined have a weaker, retarded, or totally inhibited growth compared to crops that do not contain this product, the latter has an antimicrobial activity so it must be eliminated by filtration, dilution, or neutralization, before or during the course of the sterility test. The efficacy of elimination should be controlled by repeating the test.

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