STERILIZATION — AN UPDATE

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ABSTRACT

Sterilization refers to any process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses, spore forms etc.) from a surface, equipment, article of food or medication, or biological culture medium. Sterilization is the first and most important step in any dental procedure. There are different methods to sterilize the instruments but autoclaving is the recommended method, because almost all non-disposable dental items including UV light tip, plastic items, hand pieces, scalers, towels and even cotton rolls can easily be sterilized. This method is the easiest and cost effective. There are different models with printers; these printouts are very helpful for record keeping and in medico-legal cases.

Different types of autoclaves e.g. type N, S and B are available in different designs. Dentists working in remote areas in non-industrialized countries may not be familiar with different types of autoclaves. This article describes various methods of sterilizations with advantages and drawbacks.

Key words: *Sterilization, Autoclave, Dry heat sterilization, Radiation sterilization, Chemical sterilization, Ethylene Oxide*

INTRODUCTION

In today's world, the patients and the health care professionals have become more concerned about the transmission of pathogenic organisms than ever before. Controlling microbial contamination through sterilization has long been considered the most essential component of an infection control program. The result of proper instrument sterilization is the protection of the patient, and the health care professional from various infection diseases.

Since infections increase the severity of illness, complicate recovery, and prolong inpatient stays so they raise the cost of care. The ever-growing expense of health care has focused provider's attention, on reducing the costs. Often this means re-sterilizing and re-using expensive plastic items that might otherwise be discarded after use.

Sterilization refers to any process that effectively kills or eliminates transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) from a surface, equipment, article of food or medication, or biological culture medium. Sterilization however does not; remove prisons (protein particle believed to be the cause of encephalopathy). Sterilization can be achieved through application of heat, chemicals, irradiation high pressure or filtration.[1]

Classification There are two types of sterilization: physical and chemical.

A) Physical sterilization includes: Heat sterilization (Dry heat, Autoclave, Heated chemical vapour) Radiation sterilization

B) Chemical sterilization includes: Ethylene oxide, Chlorine bleach, Gluteraldehyde, Formaldehyde, Hydrogen peroxide

There are several types of sterilization equipment e.g. Autoclaves, dry heat sterilizers, heated chemical vapour sterilizers, and gas sterilizers.

1-Dry Heat sterilization

Dry heat can be used to sterilize items, but as the heat takes much longer to be transferred to the organism, both the time and the temperature must usually be increased, unless forced ventilation of the hot air is used. The standard setting for a hot air oven is at least two hours at 160 °C (320 °F). A rapid method heats air

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to 190 °C (374 °F) for 6 minutes for unwrapped objects and 12 minutes for wrapped objects.

This method is not generally regarded as being suitable for plastics. It is considered effective and safe for metal instruments because the process does not dull instrument edges or rust/corrode the instruments. The approximate cost is Rs 55,000/-. Center for Disease Control and Prevention (CDC) guidelines call for weekly monitoring the working of the dry heat sterilizer by doing a weekly spore test.[1]

2-Autoclaving (Steam sterilization)

A widely used method for heat sterilization is through autoclave. Autoclaves commonly use steam heated to 121°C or 134°C. To achieve sterility, a holding time of at least 15 minutes at 121°C or 3 minutes at 134 °C is required. Additional sterilizing time is usually required for liquids and instruments packed in layers of cloth, as they may take longer to reach the required temperature. After sterilization, autoclaved liquids must be cooled slowly to avoid boiling over when the pressure is released. Proper autoclave treatment will inactivate all fungi, bacteria, viruses and bacterial spores, which can be quite resistant. It will not necessarily eliminate all prions.

Some people substitute a pressure cooker for an autoclave, a pressure cooker is not properly gauged, and its moisture may damage the integrity of the autoclaving bags. There are several different "types or classes" of autoclaves called gravity displacement, positive pressure displacement, and negative pressure (vacuum) displacement. Brief description of each is given below.

a- Gravity displacement autoclave or **class "N"** has a heating element fully or partially submerged in a pool of water in the bottom of the autoclave chamber, along with a hole that transfers water from a reservoir to the autoclave chamber. As the water in the pool is heated, it begins to evaporate, forming steam. Steam is lighter than air, so when the chamber fills with steam the majority of air in the chamber is pushed to the bottom of the chamber, and escapes via the hole. This hole is connected to a temperature sensitive diaphragm that closes once it is sufficiently heated. Once the diaphragm closes, pressure builds up inside the autoclave chamber.[2] The benefit of this type of autoclave is its simplicity; the drawback with gravity displacement autoclaves is they are only designed to function properly with solid unwrapped instruments. The price of this type autoclave starts from Rs 65,000/ and onwards.

b- A **positive pressure displacement** autoclave improves on the design of a gravity displacement autoclave by creating the steam in a separate internal unit, called a "steam generator". Once the amount of steam needed to displace air in the chamber is produced a valve opens and a pressurized burst of steam enters the autoclave chamber, resulting in a higher percentage of air from the chamber being removed as compared to a gravity displacement autoclave, this decreases autoclave cycle times. The **disadvantages** are its high initial cost and generally have a smaller chamber.

c- Negative pressure, or **vacuum displacement** autoclaves, also known as **class "S"**, have a separate internal "steam generator", and a "vacuum pump". After the autoclave chamber is closed, the vacuum pump removes all air from the chamber and then steam is injected into the chamber. Negative pressure displacement autoclaves are able to attain some of the highest sterility assurance level (SAL). Negative displacement autoclaves generally have a forced filtered air drying system that allows the autoclave packages to be thoroughly dries before contacting any ambient air.[1-5] **Drawbacks** are, high initial cost and sometimes the small size of these systems.

Another type of steam autoclave is called **class "B"**. These systems are more or less enlarged negative pressure displacement autoclaves. The steam generator for class "B" autoclaves is usually a separate standalone unit. Class B autoclave has three times more vacuum than class S to create vacuum in hollow instruments.

To ensure the autoclaving process most auto claves have meters and chart that record or display pertinent information such as temperature and pressure as a function of time. Indicator tape is often placed on packages of products prior to autoclaving. A chemical in the tape will change colour when the appropriate conditions have been met. Some types of packaging have built-in indicators on them.

Biological indicators ("bio indicators") can also be used to confirm autoclave performance. Simple bio-indicator devices are commercially available based on microbial spores. Most contain spores of the heat resistant microbe Bacillus Stearothermophilus, among the toughest organisms for an autoclave to destroy. [6,7] Typically, these devices have a self-contained liquid growth medium and a growth indicator. After autoclaving, an internal glass ampule is shattered, releasing the spores into the growth medium. The vial is then incubated (typically at 56 °C (132 °F)) for 48 hours. If the autoclave destroyed the spores, the medium will remain its original colour. If autoclaving was unsuccessful, the B. Sterothermophilus will metabolize during incubation, causing a colour change during the incubation.

For effective sterilization, steam needs to penetrate the autoclave load uniformly, so an autoclave must not be overcrowded, and the lids of bottles and containers must be left ajar. During the initial heating of the chamber, residual air must be removed. Indicators should be placed in the most difficult places for the steam to reach to ensure that steam actually penetrates there.

For autoclaving, as for all disinfection of sterilization methods, cleaning is critical. Extraneous biological matter or tissue debris may shield organisms from killing them, by physical or chemical method. Cleaning can also remove a large number of organisms. Proper cleaning can be achieved by physical scrubbing. This should be done with detergent and water to get the best results. Instruments with organic matter cleaning require cool water because warm or hot water may cause organic debris to coagulate. Treatment with ultrasound or pulsed air can also be used to remove debris.[7-10]

Autoclaving uses saturated steam, which allows lower temperatures and shorter times than in the dry heat method. Steam penetrates well into a product. The temperatures and times used for autoclaving vary depending on the particular cycle chosen (lower temperatures must be held for longer times). Conditions that prevent the steam from reaching the surfaces e.g. poor cleaning, improper packaging or over packing of the autoclave can seriously reduces the effectiveness of autoclaving as a sterilization method. Some materials will lose structural integrity at the temperatures used for autoclaving and even products where the softening temperature is higher than the autoclaving temperature produces stresses and subsequent distortion. Where autoclaving is to be used, the effect of multiple sterilization cycles needs to be considered to prevent cumulative effects of the treatment on the plastic. If the devices are to be packaged before autoclaving then the packaging material and packaging method needs to be carefully chosen. The suitability of a package for autoclaving will depend on the material, the size of the package, the wall thickness of the package and the contents. Steam sterilizers (autoclaves) are the most popular method of sterilization. CDC guidelines call for weekly monitoring the working of the steam sterilizer by doing a weekly spore test.

Drawbacks of Steam sterilization includes corrosion of unprotected instruments and unprotected cutting edges to dull. Excess water in the steam can provide a portal for microorganism to penetrate wet instrument packages. The price of this type autoclave starts from Rs 1,50,000 to 5000,000 and onwards

3-Heated Chemical Vapour Sterilizers

These types of sterilizers also offer relatively short cycle times. Metal instruments can be processed with minimal rust or corrosion, and cutting edges remain sharp; however, instruments must be dried completely before processing. Although heating provides the most reliable way to rid objects of all transmissible agents, but heat is not always appropriate, because it will damage heat-sensitive materials such as biological materials, fiber optics, electronics, and many plastics. CDC guidelines call for weekly monitoring the working of the chemical sterilizer by doing a weekly spore test. **Drawbacks** of this sterilizer are; it requires a special solution, heat sensitive plastics may be destroyed and the unit must be placed in a well-ventilated area to diffuse the chemical odour.

B-Chemical sterilization

Chemicals are also used for sterilization.

1- Gaseous Chemicals -Ethylene Oxide (EO)

Ethylene oxide is the most common sterilization method, used for over 70% of total sterilizations, and for 50% of all disposable medical devices.

EO gas is commonly used to sterilize objects sensitive to temperatures greater than 60 C° such as plastics, optics and electrics. Ethylene oxide treatment is generally carried out between 30°C and 60°C with relative humidity above 30% and a gas concentration between 200 and 800 mg/L for at least three hours (requires a longer time to sterilize than any heat treatment). Ethylene oxide penetrates well, moving through paper, cloth and some plastic films. EO can kill all known viruses, bacteria and fungi, including bacterial spores and is satisfactory for most medical materials, even with repeated use. The effectiveness of EO sterilization depends on many variables such as time, gas concentration, temperature and relative humidity (necessary to moisten bacteria to insure effective destruction). This has made monitoring EO sterilization difficult and time consuming.

The two most important ethylene oxide sterilization methods are: the gas chamber method and the micro-dose method.

(i) The gas chamber method

EO sterilization requires evacuation of the sterilization chamber, the introduction of moisture with EO gas (either in the pure state or as a 10 to 15% mixture with an inert gas), and keeping the internal pressure of the chamber lower than atmosphere to prevent leakage of the EO to the atmosphere. After the specified exposure time, the EO is purged and the chamber is flooded with filtered sterile air to remove any residual EO. The majority of plastics are unaffected by EO sterilization treatment, but some can absorb EO and these must be treated to eliminate any EO before use.

(ii) The micro-dose method

This method was developed in the late 1950s, using a specially designed bag to eliminate the need to flood a larger chamber with EO. This method is also known as gas diffusion sterilization, or bag sterilization. This method minimizes the use of gas. **Drawbacks** of this method is, EO is regarded by the EPA(Envirmental Protection Agency) and OSHA (Occupational Safety and Health administration) a toxic and possibly carcinogenic gas. When mixed with air, EO is not only flammable but can also be explosive. This method releases residual EO in environment. Flammability and storage issues require special handling. It also requires operator exposure risk and training costs.

Spore testing for EO

Bacillus Subtilis, a very resistant organism, is used as a rapid biological indicator for EO sterilizers. If sterilization fails, incubation at 37 °C causes fluorescent change within four hours, which is read by an auto-reader and after 96 hours, a visible colour change occurs. Fluorescence is emitted if a particular (EO resistant) enzyme is present, which means that spores are still active. The colour change indicates a pH shift due to bacterial metabolism.

2- Use of Bleach

Chlorine bleach is another accepted liquid sterilizing agent. Household bleach consists of 5.25% Sodium hypochlorite. It is usually diluted to 1/10 immediately before use; however to kill Mycobacterium tuberculosis and prions, it should be diluted only 1/5, and 1/2.5 (1 part bleach and 2.5 parts water) respectively. Bleach will kill many organisms immediately, but for full disinfection, it should be allowed to react for 20 minutes. Bleach will kill many, but not all spores. It is highly corrosive and may corrode even stainless steel surgical instruments. It damage the plastics (produces cracks) Bleach decomposes over time when exposed to air, so fresh solutions should be made daily.

3- Glutaraldehyde and Formaldehyde

Glutaraldehyde and formaldehyde solutions are accepted liquid sterilizing agents, provided that the immersion time is sufficiently long. To kill all spores in a clear liquid can take up to 12 hours with glutaraldehyde and even longer with formaldehyde. The presence of solid particles may lengthen the required period or render the treatment ineffective. Glutaraldehyde and formaldehyde are volatile, and toxic by both skin contact and inhalation. Glutaraldehyde has a short shelf life (< 2 weeks) and is expensive. Formaldehyde is less expensive but is much more volatile and has a much longer shelf life if some methanol is added to inhibit polymerization to formaldehyde. GIutaraldehyde cannot be used for all medical materials. Typical cycle times and concentrations only provide disinfecting, which can fail to kill resistant microorganisms. The solutions are highly corrosive and toxic; since they come in liquid form, they cannot be used with barrier

packaging. The moment an instrument is removed from the liquid, its sterility is compromised. Immersion in a liquid steriliant is not recommended because sterilization by liquid chemicals cannot be monitored biologically. In addition, instruments disinfected by liquids must be handled aseptically, rinsed in sterile water, and dried with a sterile towel. Furthermore, instruments immersed in liquid sterilants are not wrapped and, therefore, must be used immediately or stored in a sterile container. [1]

4- Phthaladehyde: Ortho-phthalaldehyde (OPA) is a chemical sterilizing agent that received food and Drug Administration (FDA) clearance in late 1999. Typically used in a 0.55% solution, OPA shows better mycobactericidal activity than glutaraldehyde. It also is effective against glutaraldehyde-resistant spores. OPA has superior stability, is less volatile, and does not irritate skin or eyes. It acts more quickly than glutaraldehyde but is more expensive, and will stain proteins (including skin) gray in colour.

Glass bead sterilizer has long been employed for instruments re-used on the same patient at a single appointment; however, they are not suitable for terminal sterilization of instruments prior to re-use on other patients. It is not approved by the U.S Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) to be used as inter-patients sterilizer since 1997. There are no current evidence based guidelines for using this sterilizer.[11]

Irradiation is commonly used for sterilization and can be generated by either gamma ray from a Cobalt (Co_{60}) source or an electron beam (E-beam). In both cases, the cost of capital equipment is great. Dosage for either process is measured in Megarad (Mrad) and as a rule, a radiation dose of around 2.5 Mrad will sterilize clean articles in air. The required dosage will be approximately twice as high in anaerobic conditions. It is important to recognize that this is the minimum dosage and equipment will be set to deliver this as a minimum dosage – the actual delivered dosage is often much higher.

Both gamma and E-beam sterilization use radiation and the effect on plastic materials is same for both however complete sterilization and radiation damage of some magnitude will inevitably occur. The effect of radiation is cumulative and for items that must be repeatedly sterilized the total dosage can rise rapidly. For these items records need to be kept to insure that safe limits are not exceeded. Irradiation is very effective for fully packaged and sealed single-use items. [12]

Plastic devices subjected to irradiation sterilization will inevitably be affected by the radiation and the environment used during sterilization, and will experience changes in the polymer structure such as chain scission and cross-linking. These processes will lead to changes in the tensile strength, elongation at break and impact strength.

The changes in mechanical properties may not be immediately apparent and there can be some time delay in their development. One visible side effect of irradiation sterilization is that many plastics will discolour (although this may fade with time).Irradiated devices are completely safe to handle and can be used immediately after sterilization.

Gamma Rays are produced from a Co60 source and have a high penetrating power (up to 50 cm). This allows a high packing density in the sterilization chamber. This can also mean that products at the outer edges of the packing can be subjected to much higher radiation doses than those at the center of the pack. Materials to be gamma sterilized need a margin of error in their resistance to radiation to insure that there is no excessive degradation if items are at the outer edges.

E-beam sterilization uses an E-beam generator (between 1 MeV2 and 12 MeV) to produce a beam of high energy electrons that destroys organisms. The E-beam electrons have a much lower penetrating power, but higher dose rates than gamma rays and will only penetrate around 5 cm. This means that the packing density must be low to insure that the electrons reach the center of the pack. The higher dose rates and shorter times used for E-beam sterilization can slightly improve the dosage to produce substantial damage due to the reduced exposure to oxygen during the process.

Ultraviolet light irradiation (UV, from a germicidal lamp) is useful only for sterilization of surfaces and some transparent objects. UV irradiation is routinely used to sterilize the interiors of biological safety cabinets between uses, but is ineffective in shaded areas, including areas under dirt (which may become

polymerized after prolonged irradiation and is very difficult to remove). It also damages many plastics.[4]

CONCLUSIONS

- It is concluded that autoclaving is the method of choice for sterilization of dental instruments. It is effective, fast, safe and uses an uninterrupted cycle.
- The dry-heat oven is effective, but using an uninterrupted cycle is recommended.
- Cold sterilization chemical disinfectant is not suitable for sterilization in dental practice.
- Boiling-water devices are not effective for sterilization of dental instruments; they should be discarded and condemned.

Different types of autoclaves are available in market like class N is without vacuum system (for open instruments only), class S is with single vacuum (for open and packed instruments) and class B with triple vacuum (for open, packed instruments and hollow instruments). As presence of air may prevent penetration of steam in all parts, so more work has been done to develop strong vacuum system in recent years. The strong vacuuming ensures steam penetration into the smallest instrument cavity that is why researchers now recommended Class B autoclave for dental surgery in which there are triple vacuum to eliminate any air in chamber.

There are few important points, which must be kept in mind

1 Allow the washed instruments to dry completely before autoclaving to prevent rusting and compromised sterilization.

2 Oiling of hand pieces must be done after sterilization because oil may prevent steam to reach in internal parts.

3 Run hand pieces for 60 seconds before use to discard contaminated water in tubing system of dental unit.

4 Check the efficacy of autoclave on regular basis in any good laboratory by testing autoclaved instrument for sterility, fungus culture and bacterial culture. Instruments autoclaved in properly working sterlizer will be sterile and show no growth. This is very easy and cost effective method to check the performance of any autoclave specially in underdeveloped country where there is may be no special laboratory to check the efficacy of autoclaves and indicator strips and pouches may not be available in market.

REFERENCES

1 J AHarte. Looking inside the 2003 CDC dental infection control guidelines. J Calif Dent Assoc 2004;32(11):919-30.

2 Kohn WG, Harte JA, Malvitz DM, Collins AS, Clevel and JL. Eklund KJ. Guidelines for infection control in dental health care settings-2003. J Am Dent Assoc 2004; 135, (1): 33-47.

3 AlNegrish A, Al Momani, Al Sharafat. Compliance of Jordanian dentists with infection control strategies. Int Dent J Oct 2008;58(5):231-6.

4 J Falak I, R Taha, Q Rasha, A Hani and F J Daameh. Testing of several methods of sterilization in dental practice 1995; 1(1):80-86.

5 Raju G.K. and Cooney C L. Media and air sterilization: In Biotechnology, ed. Stephanopoulos G 1993; 3: 157-84.

6 Shalhoub SY and Al-Bagieh NH. Cross-infection in the dental profession. Dental instruments sterilization: Assessment Part I. Tropical Dent J 1991; 14:13-16.

7 C L Whitworth, M V Martin, M Gallagher and H V Worthington. A Comparison of decontamination methods used for dental burs. *BDJ*.2004;**197**, 635 – 40.

8 Roscoe DL, Gibson GC, Noble MA and Mathias RG. Hepatitis and HIV: Prevalence of infection and changing attitudes towards infection control procedures in British Columbia. J Canadian Dent Assoc 1991; 57: 863-70.

9 Bagieh NH and Shalhoub SY. Cross-infection: Contamination in the dental clinics. A pilot study, assessment Part 11. Tropical Dent J 1992; 15:11-13.

10 Deskins-Knebel D and Rosen S. Comparison of handpiece asepsis among Ohio dentists 1991-1992. Focus Ohio Dental J 1993; 67:8-12.

11 Zadik Y, Peretz A. The effectiveness of glass bead sterlizer in dental practice. J Isr Dent Assoc April 2008; 25(2): 36-9.

12 Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition U.S. Department of Health and Human Services, Centers for Disease Control and prevention and National Institute of Health, 2007.