

METHODS OF STERILIZATION

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INTRODUCTION :

Dental health care workers including dentists, are frequently exposed to life threatening microorganisms. Current methods of sterilization and asepsis if followed

ardently can significantly decrease the risk of infections for the patient, dentist and the other staff.

Historical Background:

Concern about the transmission of disease has been exposed for thousands of years.

- Written guidelines for disease control are found in the bible.

- The Israelites were required to follow principles of heat sterilizations, hand washing and isolation in the 16th century.

- More effective chemical disinfectants were introduced in the mid 19th century.
 - William Henry first utilized heat sterilization using heated water in a pressurized vessel in 1832.

 - Iodine was recommended as wound dressing by Davies in 1839.

 - Laverne used chlorine water in 1843.

 - Chlorinated lime was used by Semmelweis to reduce the incidence of childbirth infections in 1847.

 - Joseph Lister in 1900s, introduced the concept of aseptic surgery.

The field of asepsis and sterilization has undergone considerable growth and development in the last hundred years with the discovery of numerous chemical and physical agents and barrier techniques to prevent the transmission of infective organisms.

To fully understand the principles of disinfection and sterilization, we need to have the accurate definitions of these terms.

Sterilization:

Refers to the removal of all forms of life, including bacterial spores. By definition, there are no degrees of sterilization, it is an all or none process. Chemical or physical methods may be used to accomplish this form of microbial removal.

Disinfection:

Refers to the removal of pathogenic organisms but does not necessarily include removal of bacterial or other spores. Physical or chemical methods may be employed but most disinfectants are chemical agents applied to inanimate objects. (A disinfectant that is applied to living tissue is referred to as an antiseptic)

Factors that influence the degree of killing:

Before discussion of particular methods, a review of the factors that influence the degree of killing of organisms is important. The following factors play a significant role in the selection and implementation of appropriate method.

- Types of organisms.
- Number of organisms present.
- Concentration of disinfecting agent.
- Amount of organic soil present.
- Nature of surface to be disinfected.

Types of organisms:

Organisms vary greatly in their ability to withstand chemical and physical treatment. This variety is due to the biochemical composition of the cells and the protective mechanisms afforded by its constituents.

E.g. – Spores have coats rich in proteins, lipids and carbohydrates as well as dipicolinic acid and calcium, all, which offer protection to spores.

By contrast, viruses containing lipid rich envelopes are more susceptible to the effects of detergents and wetting agents.

Number of organisms:

Another factor to consider is the total number of organisms present, referred to as the microbial load. If the number of organism is plotted against time, they are exposed to the killing agent, logarithmically; the result is a straight line. As the microbial load, is composed of organisms of varying susceptibilities to killing agents, not all the organisms die at the same time. In general, higher numbers of organisms require longer exposure times

Concentrations of disinfecting agents:

Proper concentrations ensure the inactivation of target organisms and promote safe and cost effective practices. Hence, instructions on the preparation, dilution and use must be followed very carefully.

Organic soil present:

Organic soil such as blood, mucus and pus, affects the killing activity by actually inactivating the sterilization agent. It also prevents full contact between object and agent. Hence for optimal killing activity, instruments and surfaces should be cleansed of excess organic material before sterilization.

Nature of surface to be sterilized:

Certain instruments are manufactured of biomaterials that exclude the use of certain disinfection or sterilization methods because of possible damage to instruments.

STERILISATION METHODS:

A basic guideline for effective clinical infection control is “don’t *disinfect when you can sterilize.*” All instruments that contact saliva or blood should be sterilized; using ADA accepted methods of sterilization before storage and subsequent use.

Before referring to the actual methods of sterilization, the following preliminary steps are essential.

□ **Cleaning :**

Before any attempt at sterilization is made, it is essential that the item to be treated is in a condition that makes it susceptible to these procedures. As even the strongest sterilant will fail to be effective if blood or dental materials prevent it reaching the surface we want it to work on.

This can be achieved by either using a brush and detergent for scrubbing or by use of an ultrasonic cleaner. The instruments can be soaked in disinfectant solutions such as iodophors or phenolic compounds to prevent drying out of debris and then scrubbed using detergent under running water. Heavy gloves should be used for this purpose.

Ultrasonic cleaning is generally thought to be much safer, efficient and more effective than hand scrubbing. Operate the ultrasonic cleaner with closed lid for 10 minutes, following which the instruments are rinsed under running water and carefully dried before sterilizing to prevent rusting.

The cleansing capacity of the ultrasonic cleaner decreases with time, hence should be checked for the efficiency from time to time. It can be done by running the cleaner with detergent solution until it's warmed up followed by immersion of a regular strength aluminum strip for 20 seconds. It's then inspected for minute bubbles and holes when held up against light, which confirms the working of the cleaner.

Drying of instruments before sterilization is important for proper sterilization function and for rust inhibition.

□ **Packaging :**

Packaging instruments prior to sterilization is preferred because handling and storage after sterilization is simplified and chances of contamination are minimized.

There are numerous systems for instrument packaging including trays and cassettes that fit specific sterilizers and simplify packaging and reuse. Also various sterilizing bags and wraps from a variety of paper and plastic materials can be used.

The autoclaved instruments can be wrapped in a cloth and sealed with a paper sticker stating 'STERILISED'. A torn seal indicates tampering of sterilized instruments or that the instruments are not sterilized.

□ **Storage :**

Storing sterilized instruments in packaging in cassettes or trays has several advantages over loose storage. The safest, most efficient handling of storage is to use separate tray of instruments for each anticipated procedure. These should be stored after sterilization in closed cabinets and opened just prior to the procedure.

PHYSICAL METHODS OF STERILISATION:

The use of heat has been long recognized as the most efficient, reliable method of sterilization. Over the years, numerous investigations using bacterial test systems have shown that cell death is associated with heat inactivation of critical enzymes and other proteins within the microorganism.

All the organizations – CDC, ADA and OSAP (Office Sterilization Asepsis Procedures and Research Foundation) repeated stress use of heat sterilization for all instruments and items that go into a patient's mouth and can withstand repeated exposure to high temperatures.

* **STEAM STERILISATION :**

Efficient sterilization may be accomplished by the use of moist heat at higher temperatures in the form of saturated steam under pressure. The most commonly used is an autoclave.

The word autoclave means self-locking and is used to denote an apparatus that sterilizer by use of steam under pressure. They operate on the same principle as the pressure cookers.

Saturated steam is much more efficient for destroying microorganisms than either boiling or dry heat. Thus the presence of air in the packages hinders the penetration of steam and delays sterilization.

Parameters:

A temperature of 121 C (250 F) is applied for 15 – 20 mins, at 15 lbs.' of pressure.

Direct exposure to saturated steam at 121 C for 10 mins, normally destroys all forms of life, including highly resistant *clostridium botulinum* spores.

In case of heavily wrapped instruments, sterilization time of 30 minutes usually suffices.

Recommended packing:

Basic requirement is that the material must allow steam to penetrate.

Acceptable materials – paper, plastic, cloth or paper peel pouches.

Unacceptable materials – closed metal and glass containers.

Advantages:

- Most efficient and reliable method.
- Simple to operate and relatively inexpensive.
- Ability to process a wide range of materials without destruction.

Disadvantages:

- Corrosion of unprotected carbon steel instruments.
- Dulling of unprotected cutting edges.
- Possibility that packages may remain wet at the end of the cycle.
- Possible deposits from use of hard water.
- Possible destruction of heat sensitive materials.

Corrosion of metallic instruments can be prevented by use of coating emulsion chemicals that vaporize in the autoclave and protect metal from oxidation by hydrolysis.

Commonly used is 1% sodium nitrite.

Flash Priority Sterilization:

It is a device that is used in dental setup to quickly sterilize objects using steam under pressure.

Wrapped items – 8 minutes at 121 C, 15 lbs.

Unwrapped items – 3 minutes at 121 C, 15 lbs.

It is used for smaller instruments, such as individual hand pieces or forceps.

*** DRY HEAT STERILISATION :**

When used properly, dry heat is an effective and accepted method of instrument sterilization. This method requires more time to sterilize owing to the fact that dry air is not an efficient a heat conductor as moist air.

The ovens with stringent insulation and temperature criteria should be used, as it should meet the requirements of FDA and be capable of providing an even and rapid heating.

Parameters:

170 C (340 F) for 1 hour or 160 C (320 F) for 2 hours.

Packaging:

Instruments should be packaged loose or located at a finger's space apart for minimum warm up time. Lightly wrapping with paper or aluminum foil increases time required from 45 – 60 minutes.

Advantages:

- Low cost equipment and is the least expensive form of heat sterilization.
- Best for sterilizing metal instruments, which are resistant to high temperatures, which would otherwise rust or dull in the presence of water vapor.

Disadvantages:

- Requires long cycle for sterilization.
- Requires careful loading, packaging and temperature monitoring.
- Temperature above 175 C may melt solder in instruments and impression trays.
- Has poor penetration.

Rapid Heat Transfer Sterilizers:

It is a more new design of the dry heat sterilizer. It has the advantages of shorter sterilizer cycles as there is controlled internal air flow within the chamber.

Parameters: temperature – 190 C (375 F)

Cycle time – 12 minutes for wrapped items.

- 6 minutes for unwrapped items.

Glass bead sterilizers:

For small metal items, such as endodontic instruments and drills, a variation of the dry heat principle can be applied. The glass bead sterilizer consists of an insulated container filled with small silica beads and fitted with a heating element. The beads retain the heat and distribute it evenly, as well as providing support for the fine instruments to be sterilized.

The instruments are sterilized at temperature specified by the manufacturers.

Temperature – 218 C – 240 C

Time cycles – Root canal instruments – 5 sec.

Absorbent points and cotton pellets – 10 sec.

A variation of this is the hot salt sterilizer, where the commonly and easily available sodium chloride replaces the glass beads.

*** UNSATURATED CHEMICAL VAPOUR :**

This system depends on heat, water and chemical synergism for its efficiency and has a major advantage of greatly reducing corrosion of metal items processed.

The principle of operation is similar to that of steam sterilizer but it uses a mixture of chemical that is a solution of alcohol, formaldehyde, ketone, acetone and water is used to produce the sterilizing vapors.

Parameters:

Temperature – 132 C (270 F) at 40 – 20 psi for 20 minutes. The unit must be preheated before use.

Packaging:

Instruments should be bare or packed with paper or muslin. Material should allow vapor condensation on the instruments.

Advantages:

- Has a short cycle time.
- Ideal for instruments such as files, burs, wires bands etc as it doesn't damage their structural integrity.

Disadvantages:

- Residual chemical vapors containing formaldehyde and methyl alcohol can be released when chamber door is open which leaves unpleasant odors.

MONITORING OF STERILISATION:

Proper functioning of the sterilization cycles should be verified by periodic use of monitoring devices, the monitoring is basically done through the use of

- Heat sensitive chemical indicators.
- Biological indicators.

Chemical monitoring:

Heat sensitive indicators consist of paper strips, labels and steam pattern cards impregnated with chemicals designed to change color when exposed to heat or chemical vapors. The chemical formulation of the indicator ink on a strip used to monitor autoclaves for e.g., makes it sensitive to the correct combination of the three factors necessary for sterilization i.e.

- Time

- temperature
- saturated steam

The use of specific chemical indicators serves as a routine check for each load of items processed through the sterilizer. When indicators are used on the outside and inside of every package, gross sterilizer and process malfunctions can be usually detected very quickly.

Biological indicators:

These preparations contain bacterial spores, which are more resistant to heat than viruses or vegetative bacteria. They are available in the form of either glass vials containing spore suspensions or bacterial spore impregnated paper strips. The test medium contained includes a pH indicator. This chemical changes color when spores germinate and produce acids, thereby providing a visual demonstration of the sterilization cycle success or failure. The spore vehicle designed is specific to each sterilization procedure and it can't be interchanged, if a proper feed back is desired.

The organisms *Bacillus stearothermophilus* spores are the appropriate biological for monitoring autoclaves and chemicallaves, while *Bacillus subtilis* preparations provide a rigorous challenge of the dry heat ovens and ethylene oxide units.

Proof of destruction of these heat resistant forms after exposure to the sterilization cycle is used to infer that all microorganisms exposed have been destroyed, and represents the most sensitive check of the sterilizer efficacy.

As a baseline, periodic biological monitoring checks the most important piece of infection control equipment in practice, the results of such analysis provide quality control feedback for preparatory practices.

CHEMICAL STERILISATION:

* **ETHYLENE OXIDE STERILIZERS :**

Gas sterilization with ethylene oxide at ambient temperature is useful for sterilizing virtually any material including plastics, rubber, hand pieces, casts and appliances. It is toxic to all bacteria, fungi, viruses and spores at room temperature.

Commercial preparations previously incorporated carbon dioxide with ETO (90% CO and 10% ETO) to form a more stable active combination, however most current mixture current mixture contain 12% ETO and 88% chlorofluorocarbon (Freon) to further reduce product flammability.

Ethylene oxide functions as an alkylating agent by irreversibly, inactivating cellular nucleic acids and proteins. Multiple chemical sites on these molecules are most susceptible to this binding including exposed -NH, -COOH, -SH and -OH groups.

Parameters:

Heat at 120 F for 2-3 hrs for sterilization.

At room temperature, sterilization time is 12 hours.

Adequate humidity is required for sterilization. Ventilation to the out doors is required.

Advantages:

- High capacity for penetration
- Does not damage heat liable materials?
- Evaporates without leaving a toxic residue.
- Suitable for materials that cannot be exposed to moisture, such as radiographic film holders and prosthetic appliances.

Disadvantages:

- Before exposure to ETO cycle, items must be cleaned and dried. Residual organic matter such as blood, saliva or exudates can protect microorganisms from the gas for prolonged intervals and thus delay sterilization.
- It is also thought to be potentially mutagenic and carcinogenic.
- Can cause tissue irritation if not well aerated.

- Some units require special spark shield because ETO can be explosive in presence of flame or sparks.

* **GLUTRALDEHYDE STERILISATION :**

Glut aldehyde has two aldehyde units, one at each end of the carbon chain. Different commercial preparations are active at an acid, alkaline or neutral pH. The latter two types use an activator that brings the final 2 to 3.2% glutraldehyde to the desired pH.

At these concentrations, it is effective against vegetative bacteria, including M. tuberculosis, fungi and viruses and destroy microbial spores after a 10 hour immersion period.

Parameters:

6 – 10 hours required for sterilization at room temperature.

Advantages:

- Heat sensitive plastics, rubbers and fiber optics can be safely sterilized.
- No expensive equipment is required.
- The cost of use is relatively low.
- They have surprising resistance to inactivation by organic matter such as blood and exudates.

Disadvantages:

- Post exposure rinsing and handling are required.
- May damage metallic instruments i.e., carbon steel burs and metal bands often discolor and corrode when immersed for prolonged periods.
- Irritation of hands and discoloration of cuticles is common sequel when gloves are not worn during handling.
- Cannot be recommended for high-speed hand pieces, porphy angles or most dental instruments and devices.

WATERLINE BIOFILMS:

Biofilms are microorganisms that accumulate on surfaces inside moist environments such as dental unit water lines, allowing bacteria, fungi and viruses to multiply. This can significantly increase a patient's susceptibility to transmissible diseases.

Fungi, algae, protozoans and nematodes are found in fresh water aquatic biofilms, as well as *Pseudomonas aeruginosa*, *E coli*, *Legionella*. These organisms have almost no effect on healthy individuals but can be fatal for debilitated and immune-suppressed patients. They also pose a hazard in case maneuvers, which involve bone cutting etc. thus, reduction of waterline biofilms is essential in implementing an infection control program in the dental office.

Recommendations for reduction of biofilms: (ADA Guidelines)

- At start of each day, run and discharge water from dental unit waterlines for several minutes.
- Avoid using dental unit water for performing procedures involving bone cutting (i.e. sterile water or saline can be used)
- Weekly disinfections of waterlines using household bleach.

CONCLUSION:

The dental office is not only a setting which harbors a reservoir for spread of infections to the patient but also is a potent route through which various transmissible infections can be transferred to the working dental staff.

Hence, the use of simple sterilization techniques only has to be over emphasized in order to prevent the onset of various fatal diseases such Hepatitis-B, AIDS, being only a few to mention.

If nothing else, the human immunodeficiency virus has made the profession sit up and rethink its approach to clinical hygiene. To retain its credibility as a profession we have to demonstrate that we can safely provide dental treatment for the community as a whole.

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