



NIGERIAN INSTITUTE OF SCIENCE LABORATORY TECHNOLOGY,  
*FEDERAL MINISTRY OF SCIENCE AND TECHNOLOGY*

National Training on Infection Prevention and Control (IPC)

MODULE 3: APPLICATION OF INFECTION PREVENTION AND CONTROL  
TOPIC: DECONTAMINATION AND STERILIZATION

BY

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# Decontamination and sterilization



## Introduction:

- The right use of the laboratory and equipment, keeping them clean and sterile are fundamental to safety and quality result.
- The two processes are very crucial and play key roles in infection prevention and control management system.
- Effective decontamination and sterilization are necessary to protect laboratory personnel (as well as others) from infection and the release of infectious organisms to the outside environment.



# Decontamination

- **Decontamination** is the process of cleansing an object or substance to remove contaminants such as micro-organisms or hazardous materials, including **chemicals, radioactive substances, and infectious diseases.**
- It is a term used to describe a process or treatment that renders a **laboratory device, instrument, or environmental surface safe to handle.**
- All infectious materials and all contaminated equipment or apparatus should be decontaminated before being washed, stored, or discarded.
- **Hand washing** is a typical example of ways to decontaminate the hand from contaminant.

# Sterilization



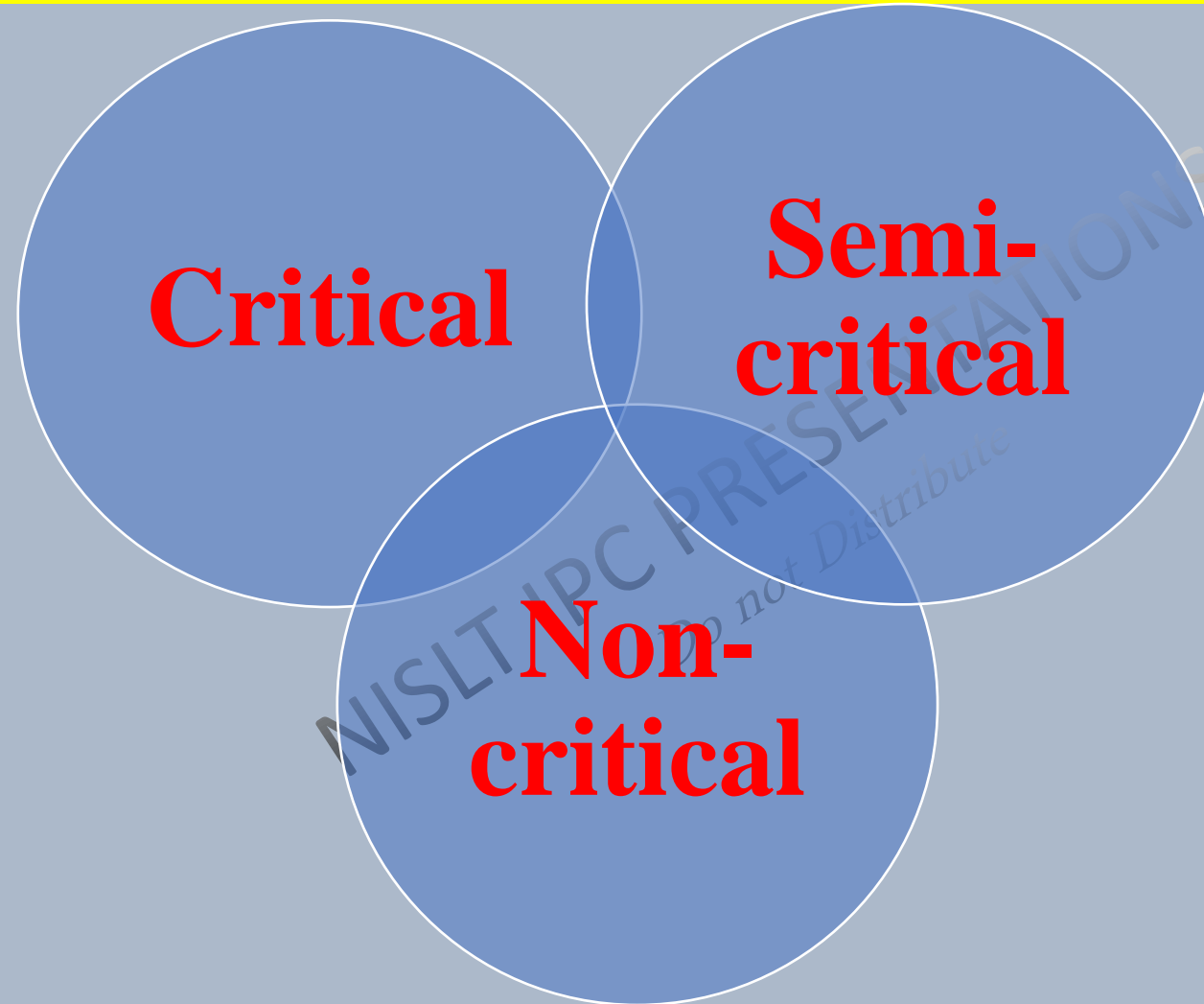
- **Sterilization** is the complete destruction or removal of microorganisms, including bacterial spores.
- It involves the use of **physical** or **chemical** procedure to destroy **all microbial life**, including highly resistant bacterial endospores.
- All materials, media, tubes, plates, loops, needles, pipettes, and other items used for culturing microorganisms should be sterilized before used to achieve desired result.

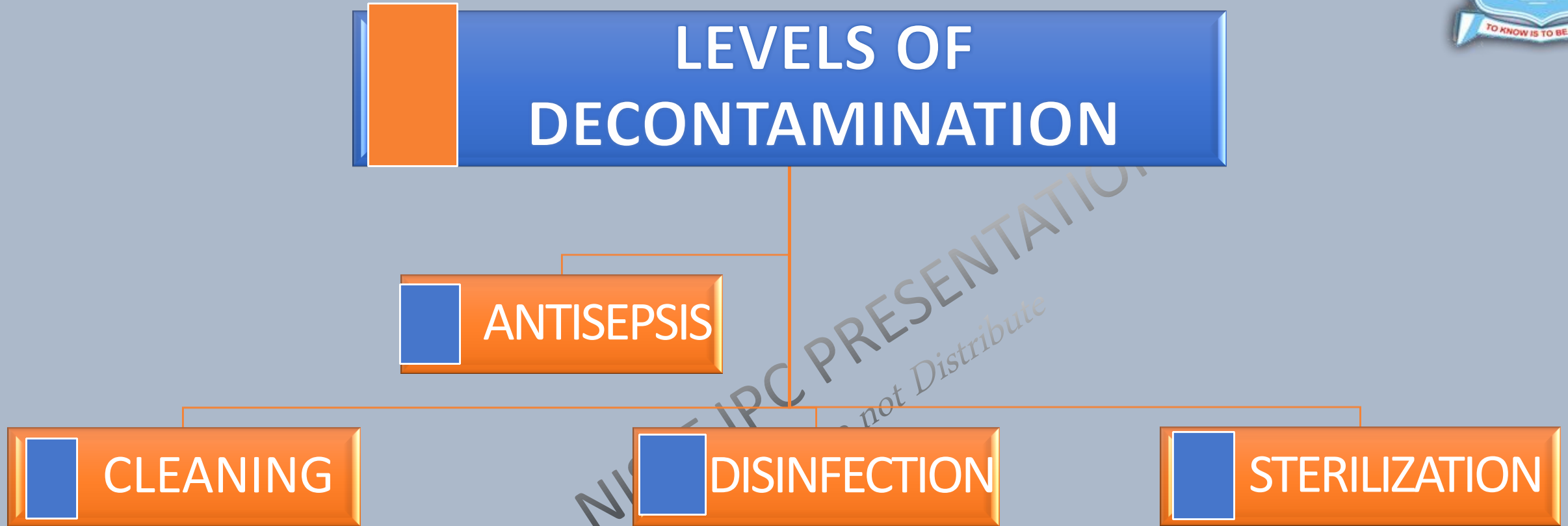
# Levels of risk for infection transmission



- The **method of decontamination** is determined by the level of risk for infection transmission.
- For example, devices that will be in contact with the bloodstream or sterile tissue/sterile body cavities must be **sterilized**.
- the instrument that will be in contact with intact mucous membranes e.g resuscitation masks require **high level of disinfection**
- Items that will only be in contact with intact skin, can undergo **low-level disinfection**, which should remove most pathogens.

# Spaulding Classification of Instrument







# Antisepsis

- **Antisepsis** is the application of a liquid antimicrobial chemical to skin or living tissue to inhibit or destroy microorganisms.
- It includes swabbing a site for collection of blood ( or injection) from (on) a person or animal and hand washing with germicidal solutions.
- Manufacturer recommendations for appropriate use of germicides should always be followed.



# CLEANING



- **Cleaning** - The physical removal of body materials, dust or foreign material.
- It is the removal of contamination from an item to the extent necessary for further processing or for intended use.
- It is often a required step before sterilization or disinfection of inanimate objects.
- Cleaning will reduce the number of microorganisms as well as the soil, therefore allowing better contact with the surface being disinfected or sterilized and reducing the risk of soil being fixed to the surface.
- Removal of soil will also reduce the risk of inactivation of a chemical disinfectant and the multiplication of microorganisms.

# Cleaning Cont'd



- **Application of cleaning** – surfaces, laboratory instrument and equipment
- Cleaning of these items can be manual or automated.
- Staff responsible for manual cleaning should be provided with personal protective equipment including heavy-duty gloves, plastic aprons, and eye protection.
- The use of an automated washer-disinfector is ideal, as this minimizes handling, saves time and achieves better disinfection than manual methods.

# Washer-disinfector



# SOME CLEANING PPE



# Cleaning Cont'd



- Any cleaning method that generates movement of dust, e.g. sweeping or dry dusting, should not be used.
- **Damp dusting** of surface and **mopping** of floors are the preferred method as these techniques do not generate dust movement.
- Most cleaners concentrate on clearing floor and surfaces, but risk area that should equally be taking care of include **the door handle, light switch, equipment button** which are frequently touched by people.

# Method of cleaning laboratory devices and instruments that can be safely immersed in water



- Wear domestic gloves, aprons and visors to protect your mucous membranes
- Fill a sink or tub with warm water
- Add detergent according to the manufacturer's instructions
- If applicable, disassemble the instrument fully
- Hold the item below the surface of the water
- Using a soft nylon brush, clean all surfaces, grooves and hinges of the instrument
- Inspect the instrument thoroughly to ensure all visible organic material is removed
- Prepare the item for disinfection or sterilization as needed

# Decontamination of Blood Spills



- **The following principles should be applied:**
- All blood spillages should be immediately cleaned up using domestic gloves
- Glass and solids should be removed using a brush and pan, and discarded in a sharps container or if too large, wrapped in newspaper before safely disposing
- The remaining fluids should be blotted using as many paper towels as needed; these should be discarded in the laboratory waste
- Water and detergent should be used to remove all visible blood
- The area should be wiped over with a chlorine-based solution (at a concentration of **10 000 parts per million**) and allowed to dry.

# Biosafety Level chart

Biosafety level	BSL 1	BSL 2	BSL 3	BSL 4
Sample organism	Nonpathogenic strain of E. coli	HIV, influenza, lyme disease	yellow fever virus, SARS coronavirus, and tuberculosis bacteria	Ebola virus, the Lassa virus, and any agent with unknown risks of pathogenicity and transmission
Pathogen type	Agent that present minimal potential hazard to personnel & Environment Unlikely to cause disease	Agent associated with human diseases, pose moderate hazard to personnel & Environment	Agent (indigenous) that present a potential for aerosol transmission, causing lethal disease.	Dangerous agent pose a high risk of aerosol-transmitted lab infections, life threatening
Autoclave requirements	None	None	Pass through autoclave with bioseal required in the lab room	Pass through autoclave with bioseal required in the lab room



# General Spill Clean-Up Guidelines

- **Wear gloves, protective eyewear and a lab coat**
- **Use forceps or pan and brush to pick up broken glass and discard in sharps container**
- **Cover spilled material with paper towels**
- **Add diluted disinfectant in sufficient quantity to ensure effective microbial inactivation, and leave it for 15 minutes**
- **Dispose of towels, wipe spill with disinfectant and discard clean up material in waste container.**
- **Wash hand with soap and water after removal of gloves**

# *Decontamination of Spill from BSL-1 Materials*

- Wearing gloves and a lab coat, pick up broken glass with forceps and place in sharps container.
- Absorb the spill with paper towels or other absorbent material.
- Add diluted disinfectant in sufficient quantity to ensure decontamination, let sit for 15 minutes.
- Discard these materials into waste container.
- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism. Discard of clean-up materials in waste container.
- Autoclave all gloves and other materials worn to clean up the spill, Wash hands with soap and water

## *Decontamination of Spill from BSL-2 Materials*

- Keep other workers out of the area to prevent infection
- Remove contaminated clothing and put in a biohazard bag
- Wash hands and any exposed skin and inform the PI of the spill.
- Wear gloves, face protection and lab coat to clean up spill.
- If broken glass is present, use forceps to pick up and place in sharps container.
- Absorb the spill with paper towels and add diluted disinfectant insufficient quantity to ensure decontamination, allow for 15 minutes.

## *Decontamination of Spill from BSL-2 Materials Cont'd*

- Discard all materials into waste container.
- Wipe the spill area with the appropriate dilution of a disinfectant effective against the organism. Discard of clean-up materials in waste container.
- Autoclave all gloves and other materials worn to clean up the spill.
- Wash hands with soap and water.
- If an injury has occurred, complete an Occupational Injury/Illness Report and seek medical evaluation

## *Decontamination of Spill from BSL-3 Materials*

- Stop work immediately.
- Avoid inhaling airborne material while quickly leaving the room. Notify others to leave. Close door, and post with warning sign
- Remove contaminated clothing, turn exposed area inward, and place in a biohazard bag. Wash hands with soap and water.
- Do not reenter the lab until given permission.
- Following instruction from the Biological Safety Officer, allow 30 minutes for aerosols to disperse before re-entering the laboratory to begin clean-up.

## *Decontamination of Spill from BSL-3 Materials Cont'd*

- put on personal protective equipment (HEPA filtered respirator, gown, gloves, and shoe covers) and assemble clean-up materials (disinfectant, autoclavable container or bag, forceps, sharps container, and paper towels).
- Contain the spill with absorbent paper towels or disposable pads. Carefully add appropriate disinfectant to the spill; avoid creating aerosols when pouring the disinfectant.
- Leave the room and allow 30 minutes for the disinfectant to inactivate the material.
- Autoclave (or soak in 10% bleach solution) lab coat, gloves, and other protective equipment that was worn for clean-up.
- Wash hands thoroughly with soap and water.

# Disinfection

- **Disinfection** -The destruction or removal of microorganisms at a level that is not harmful to health and is safe to handle.
- This involves uses a liquid chemical to eliminate virtually all pathogenic microorganisms, **with the exception of bacterial spores**, on work surfaces and equipment.
- Soaking of instrument in disinfectant does not guarantee effective disinfection or sterilization unless it has been thoroughly cleaned because most disinfectants cannot penetrate organic matter.

# The Effectiveness of the Liquid Chemical Is Influenced by



- The kinds and numbers of organisms.
- The amount of organic matter,
- The object to be disinfected,
- Chemical exposure time,
- Temperature,
- And concentration.



# Disinfectant



## Advantages

They are cheap,

They act rapidly

and can be used on heat-sensitive items,

## Disadvantages

They are less effective than heat

They can cause allergic reactions

High concentration can corrode metal surfaces ( e.g Chlorine)

Frequent use lead to development of resistant strains of micro-organisms

# Chemicals use for disinfection



- **Alcohol** and **chlorine-based** disinfectants are the most widely available.
- Chlorine may not be suitable for all types of disinfection, as it can be **corrosive** (causing damage to **metal surfaces**).
- Other types of disinfectants available include **quaternary ammonium compounds** (QACs) and **phenolics**.
- For semi-critical items like endoscopes, high-level disinfection is needed using aldehydes, peracetic acid
- Any re-usable device or instrument should have a specified process for decontamination.



# Sterilization

- **Sterilization** - The complete destruction or removal of microorganisms, including bacterial spores.
- It involves the use of physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores.
- The most widely used method for the final step in decontamination of **heat-stable items** is **steam sterilization.**
- Steam is a reliable, non-toxic and cost-effective method of sterilization.

# Autoclave

- The machines used for steam sterilization are known as **autoclaves**, and can be **downward displacement (gravity)** or **high-vacuum autoclaves**.
- Items to be autoclaved must be wrapped in materials (cloth or paper) that allow penetration of steam.
- The most commonly used standard temperature-time cycles for porous specimens and bottled fluids are **134°C for 3 minutes**, **126°C for 10 minutes** and **121°C for 15 minutes**.
- In this method, the microorganisms are killed by **coagulating their proteins**, and this method is much more effective than dry heat sterilization where microbes are killed through oxidation.

# Laboratory waste colour-coded containers

- **The recommended colours are;-**
- **Yellow** - for incineration
- **Light blue or transparent with blue inscription** - for autoclaving
- **Black** - normal household waste: local authority refuse collection
- **White or clear plastic**- soiled linen

# Autoclave validation

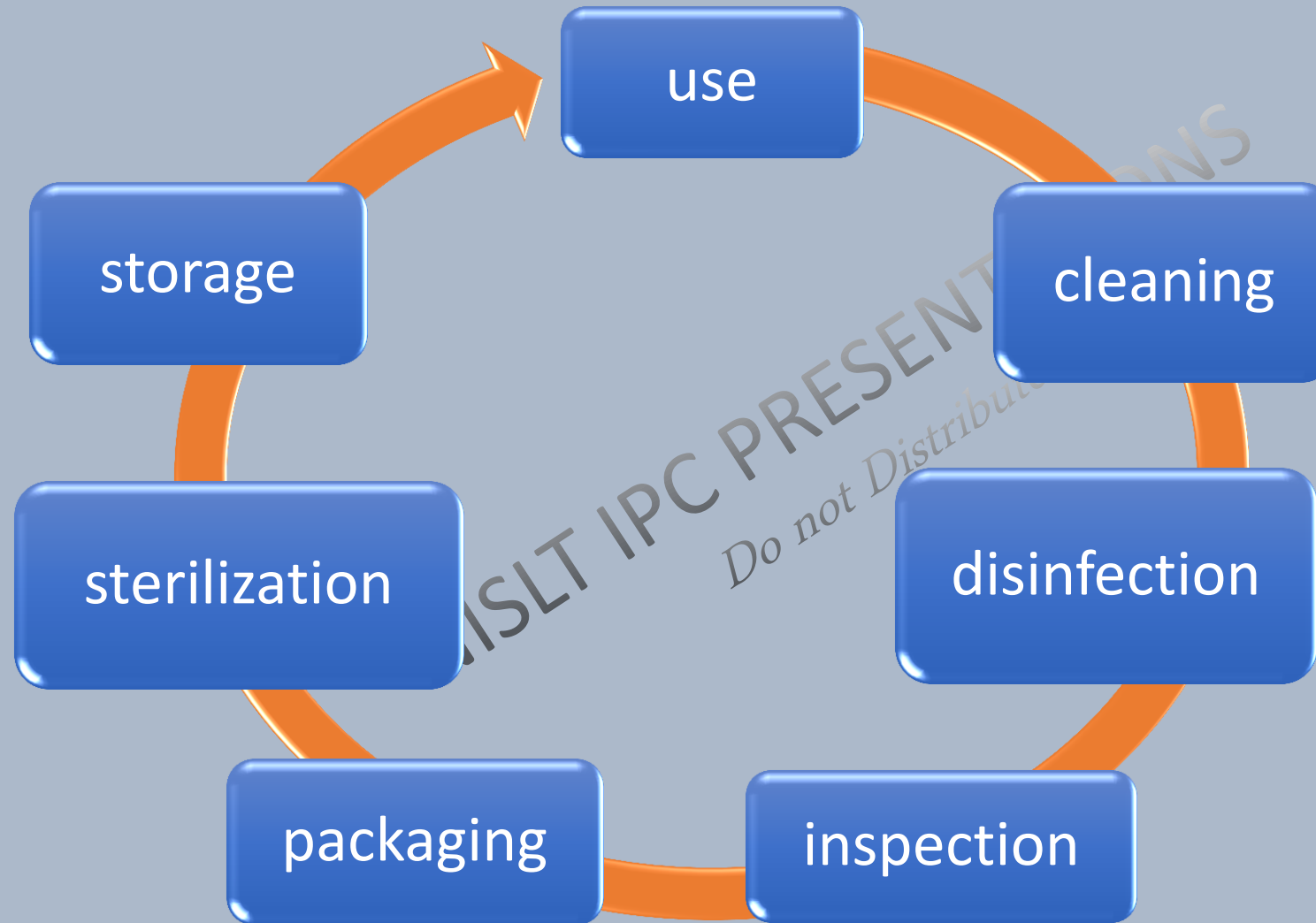


- **Autoclave Validation:** A quality assurance procedure used to ensure that the autoclave reaches adequate temperature for an adequate amount of holding time to sterilize biological agents and wastes.
- Each autoclave should be validated every 40 operating hours.
- Autoclave that fails validation test should be labelled “Out of Service” until it **pass** validation test.

# validation methods for Autoclave

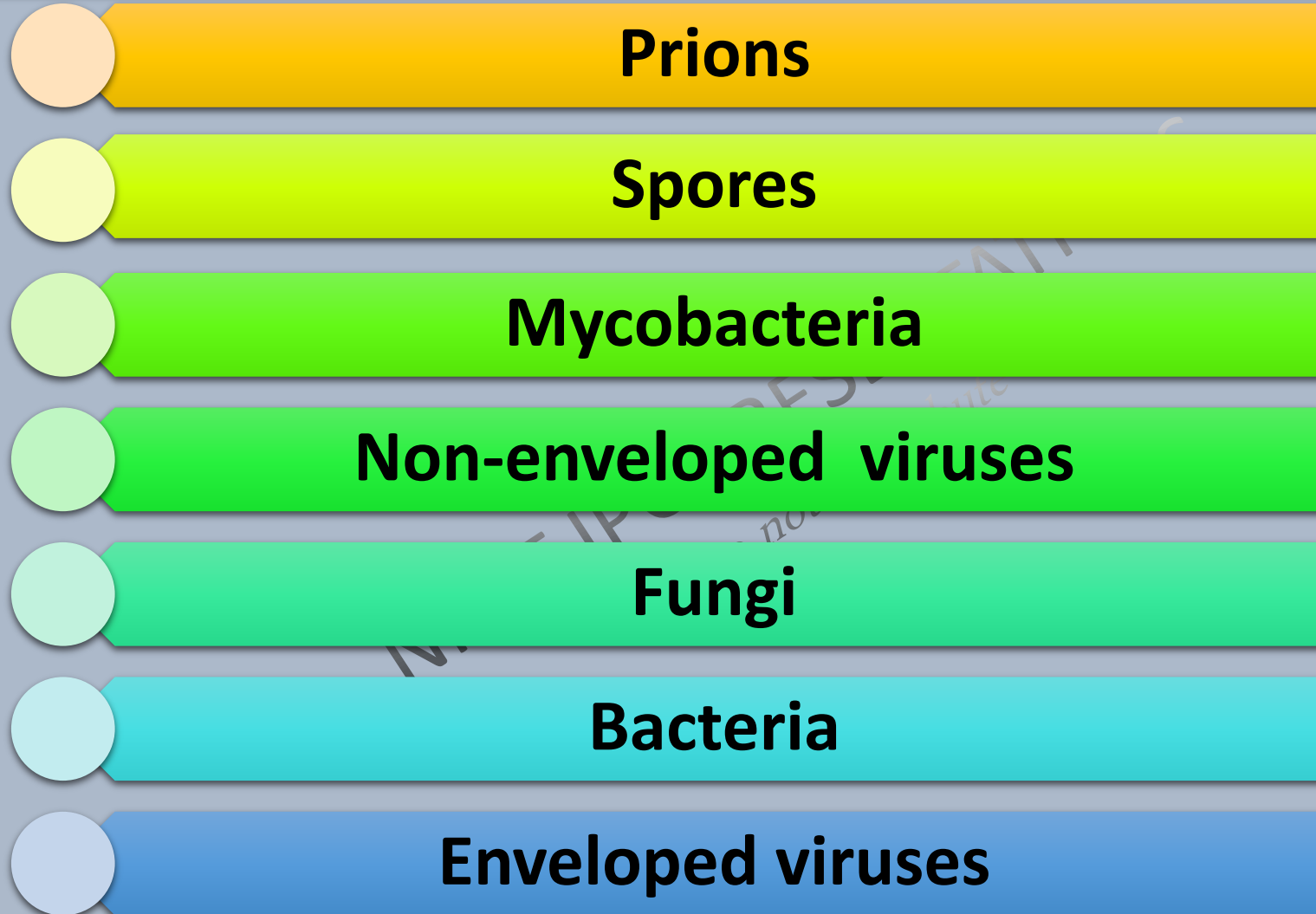
- There are several validation methods used to ensure that the sterilization process is effective.
- **Biological indicators** measure the effectiveness of the autoclave in killing bacterial spores (E.g. *Bacillus stearothermophilus*).
- **Chemical indicators** are used to verify that the items have been exposed to heat (e.g. autoclave tape) and that steam has penetrated the packs (e.g. the 'Bowie Dick' test).

# Decontamination cycle





# Decreasing Order of Resistance of Microorganisms Disinfectants/Sterilant

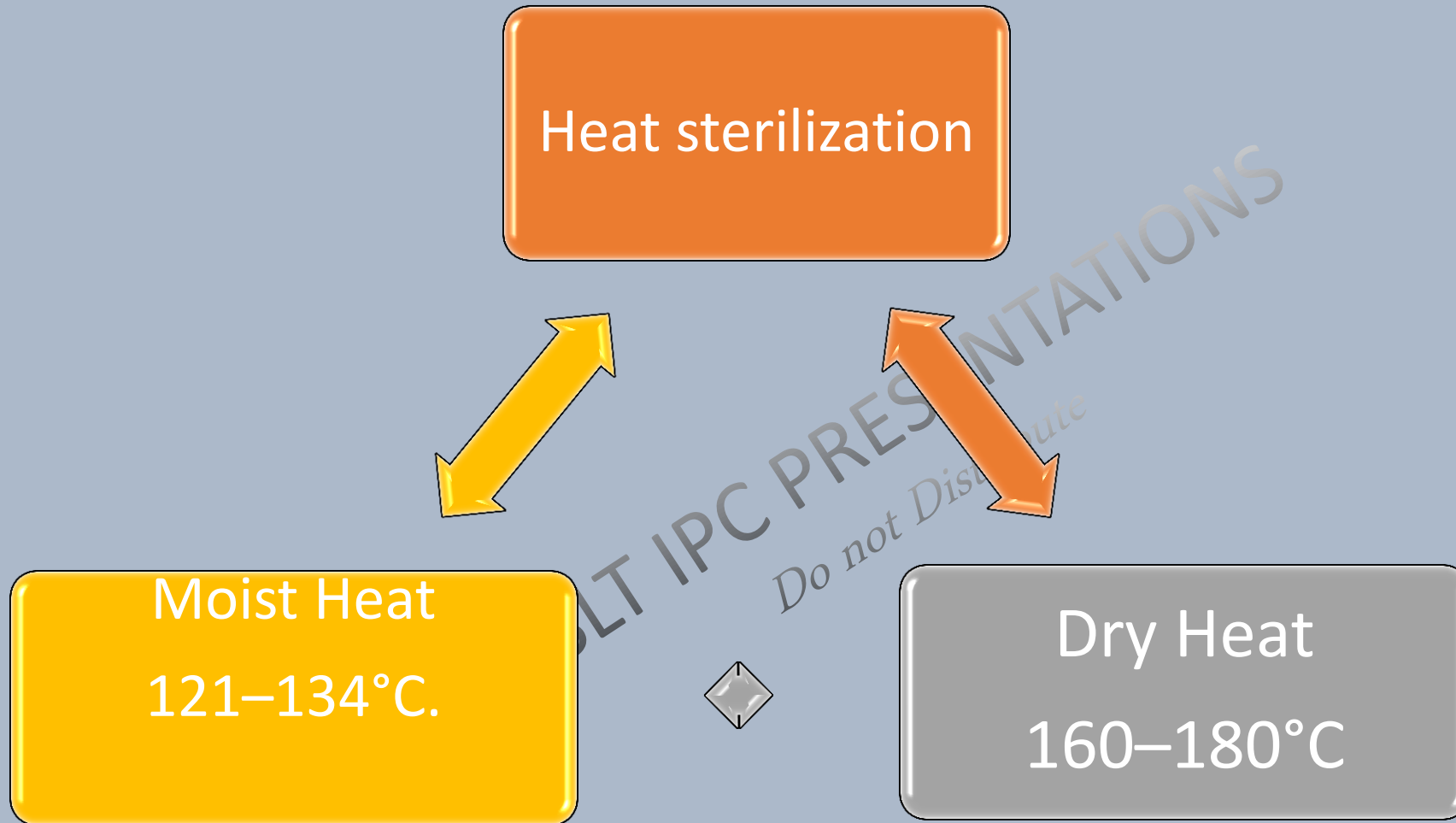


# Methods of Sterilization

## Physical and chemical method

### Heat Sterilization

- Heat sterilization is the most effective and widely used method of sterilization, where the bactericidal activity results through **the destruction of enzymes and other essential cell constituents.**
- This method of sterilization is applicable to thermostable products.
- It can be applied to both **moisture-sensitive** and **moisture-resistant** products,



# Moist Heat Sterilization



- **Moist heat sterilization** is one of the most effective methods of sterilization where the steam under pressure acts as a bactericidal agent.
- Moist heat sterilization usually involves the use of steam at temperatures in the range 121–134°C.
- High pressure also facilitates the rapid penetration of heat into deeper parts of material and moisture present in the steam causes the coagulation of proteins, **irreversible loss of function** and activity of microbes.

## Moist Heat sterilization

### Below 100°C

Pasteurization of milk at 63°C for 30 min or

73°C for 20 sec, serum and body fluids at 56°C for 1 hour, vaccines of non-spore forming bacteria at 60°C for 1 hour

### At 100°C

**Boiling** to remove pathogenic vegetative microbes and some spores in distilled water for 30-40 min

Tyndallization e.g. media with sugar and gelatin at 100°C for 30 minutes on three successive days

### Above 100°C

This principle is employed in an autoclave where the water boils at 121°C at the pressure of 15 psi or 775 mm of Hg.

E.g. Contaminated instruments and different culture media

# Dry Sterilization

- **Dry sterilization** is the process of removing microorganisms by applying moisture-free heat which is appropriate for moisture-sensitive substances.
- The dry heat sterilization process is based on **the principle of conduction**; that is the heat is absorbed by the outer surface of an item and then passed onward to the next layer.
- Dry moisture-less heat destroys microorganisms by **causing denaturation of proteins** and also lyses the proteins in many organisms, **causes oxidative free radical damage**, causes drying of cells, and can even burn them to ashes, as in incineration

# Dry Heat Sterilization Cont'd



- Substances like oil, powder, and related products cannot be sterilized by moist heat because moisture cannot penetrate into deeper parts of oily materials, and powders are destroyed by moisture.
- Similarly, laboratory equipment like Petri dishes and pipettes are challenging to sterilize by moist heat due to the penetration problem.
- Higher temperatures in the range 160–180°C are employed and also require exposure times of up to 2 hours depending upon the temperature employed.

# Dry Heat Sterilization Cont'd



- This principle is used in instruments like **hot air oven** and **incineration**, which generates very hot moisture-free air.
- The primary industrial application of dry heat sterilization is in the sterilization of glass bottles which are to be filled aseptically.
- In addition to the fact that this method achieves an adequate sterility assurance level, **this method also destroys bacterial endotoxins** (which are the products of Gram-negative bacteria also called pyrogens, which cause fever when injected into the body) which are difficult to eliminate through other sterilization techniques



# Depyrogenation



- The removal or inactivation of bacterial endotoxins from gram negative bacteria.
- Through high heat or extended heating time
- The temperature range 160–400°C

# Types of Dry Heat Sterilization



**Hot air oven** is a method of dry heat sterilization which allows the sterilization of objects that cannot be sterilized by moist heat.

It uses the principle of conduction in which the heat is first absorbed by the outer surface and is then passed into the inner layer.

The commonly-used temperatures and time are 170°C for 30 minutes, 160°C for 60 minutes, and 150°C for 150 minutes.

**Applications** in the sterilization of glassware, Petri plates, and even powder samples.

# Types of dry heat sterilization cont'd



**Red Heat:** by holding the instruments in a Bunsen flame till they become red hot e.g incubation loops, wires, and points of forceps

**FLAMING:** involves dipping of metallic objects into alcohol and expose it to flame for some time where the flame burns microbes and other dust presents in the instrument.

**INCINERATION** is the process of sterilization along with a significant reduction in the volume of the wastes. use for final disposal of waste /scrap

# Types of Dry Heat Sterilization Cont'd



**Infrared radiation** (IR) is a method of thermal sterilization in which the radiation is absorbed and then converted into heat energy, about 180°C for about 17 minutes.

IR is applicable for **mass sterilization** of packaged items like **pipette tips**, **syringes** and **catheters**.

# Physical Sterilization Cont'd

**Filtration:** The process of filtration is unique among sterilization techniques in that it removes, rather than destroys, microorganisms.

It can be used for clarification and sterilization of liquids and gases.

The principal application of sterilizing-grade filters is the treatment of heat-sensitive injections solutions, biological products, air, and other gases for supply to aseptic areas.

# Filtration



- **Other applications of filters include**
- sterilization of venting or displacement air in tissue and microbiological culture (carbon filters and hydrophobic membrane filters);
- decontamination of air in mechanical ventilators (glass fiber filters);
- Treatment of exhausting air from microbiological safety cabinets (HEPA filters)
- HEPA filters mask (97.5% efficient)

# Irradiation

**Irradiation** is the process of exposing surfaces and objects to different kinds of radiation for sterilization.

**Non-ionizing** waves (Ultraviolet radiation) have a very little penetration power, so microorganisms only on the surface are killed..

**Application:** for the surface sterilization of aseptic work areas, and the treatment of manufacturing-grade water.

# Ionizing Radiation



X-ray and gamma rays are the commonly used **ionizing radiation** for sterilization.

The ionization results in the formation of a large number of toxic  $O_2$  metabolites like hydroxyl radical, superoxide ion, and  $H_2O_2$  through ionization of water.

**Application:** Radiation sterilization is generally exposed to items in the **dried state** which include surgical instruments, unit-dose ointments, plastic syringes, and dry pharmaceutical products.





- **Sound (sonic) waves Vibration:** Sonic waves can be used as bactericidal agents which employ ultrasound (usually from 20–40 kHz) to vibrate a fluid.
- This method is routinely used by healthcare facilities to clean surgical and dental instruments before the terminal sterilization.
- **Pascalization or High-Pressure Processing (HPP)** is a method employed for preservation and sterilization of food
- **Solar disinfection** is a process used for the removal of microorganisms with the help of sunlight.
- This process is commonly used to purify or disinfect drinking water.

# Chemical Sterilization

- **Chemical Sterilization** is the process of removal of microorganisms by the use of chemical bactericidal agents.
- Even if physical methods of sterilization are more appropriate for effective sterilization, it is not always appropriate to use for heat-sensitive materials like plastics, fiber optics, and biological specimens.
- The chemical method of sterilization can be categorized as **liquid** and **gaseous** sterilization

# Gaseous Sterilization



- **Gaseous sterilization** involves the process of exposing equipment or devices to different gases in a closed heated or pressurized chamber.
- Gaseous sterilization is a more effective technique as gases can pass through a tiny orifice and provide more effective results.
- However, there is an issue of release of some toxic gases during the process which needs to be removed regularly from the system.

# Some common gases used for sterilization:



- **Nitrogen dioxide (NO<sub>2</sub>)**

- **Formaldehyde**

- **Ozone**

- **Ethylene oxide**

# Ethylene oxide

- **Ethylene oxide** (EO) gas is a common gas used for chemical treatment applied to sterilize, pasteurize, or disinfect different types of equipment and surfaces because of its **wide range of compatibility** with different materials.
- EO treatment **often replaces other sterilization techniques** like heat, radiation, and even chemicals in cases where the objects are sensitive to these techniques.
- EO treatment is usually conducted at the temperature range of 30-60°C for several hours which aids in the activity of the gas.



## Ethylene oxide Cont'd

- The efficacy of the gas depends on the concentration of gas available for each article which is greatly assisted by the good penetrating nature of the gas, which diffuses readily into many packaging materials including rubber, plastics, fabric, and paper.
- Ethylene oxide kills all known microorganisms, such as bacteria (including spores), viruses, and fungi (including yeasts and molds), and is compatible with almost all materials even when repeatedly applied.

# Ethylene oxide – limitations

- This process, however, is not without drawbacks as the level of gas in the sterilizer goes on decreasing due to absorption, and the treated articles need to undergo a process of desorption to remove the toxic residual wastes.
- Organisms are more resistant to ethylene oxide treatment in a dried state, as are those protected from the gas by inclusion in crystalline or dried organic deposits.

# Formaldehyde

- **Formaldehyde** is another important highly reactive gas which is used for sterilization.
- This gas is obtained by heating formalin (37%w/v) to a temperature of 70-80°C.
- It possesses broad-spectrum biocidal activity and has found application in the sterilization of **reusable instruments, electrical equipment**, and the surface sterilization of powders.
- Formaldehyde works on the same principle of **modification of protein and nucleic acid**.
- As a result of the **low penetrating power**, its use is often **limited to paper and cotton fabrics**.



# Nitrogen dioxide (NO<sub>2</sub>)

- **Nitrogen dioxide** is a rapid and effective sterilant that can be used for the removal of common bacteria, fungi, and even spores.
- NO<sub>2</sub> has a low boiling point (20°C) which allows a high vapor pressure at standard temperature.
- The biocidal action of this gas involves **the degradation of DNA by the nitration of phosphate backbone**, which results in lethal effects on the exposed organism as it absorbs NO<sub>2</sub>.
- An **advantage** of this gas is that no condensation of the gas occurs on the surface of the devices because of the low level of gas used and the high vapor pressure.

# Ozone



- **Ozone** is a highly reactive industrial gas that is commonly used to sterilize air and water and as a disinfectant for surfaces.
- Ozone is a potent oxidizing property that is capable of **destroying a wide range of organisms including prions**, without the use of hazardous chemicals as ozone is usually generated from medical-grade oxygen.
- However, because ozone is an unstable and reactive gas, it has to be produced on-site, which limits the use of ozone in different settings.
- It is also **very hazardous** and thus only be used at a concentration of 5ppm, which is 160 times less than that of ethylene oxide

# Liquid Sterilization

- **Liquid sterilization** is the process of sterilization which involves the submerging of equipment in the liquid sterilant to kill all viable microorganisms and their spores.
- Although liquid sterilization is not as effective as gaseous sterilization, it is appropriate in conditions where a low level of contamination is present.

## Chemicals for Liquid Sterilization Cont'd:

**Hydrogen peroxide** ( $\text{H}_2\text{O}_2$ ) :  $\text{H}_2\text{O}_2$  has a short sterilization cycle time (28 minutes).

Vaporized hydrogen peroxide (VHP) is used to sterilize largely enclosed and sealed areas, such as entire rooms and aircraft interiors

**Glutaraldehyde** is an accepted liquid sterilizing agent which requires comparatively long immersion time. For the removal of all spores, it requires as long as 22 hours of immersion time. the use of glutaraldehyde is thus limited to certain surfaces with less contamination.

# Chemicals for liquid sterilization cont'd:



- **Hypochlorite** It is an oxidizing agent and thus acts by oxidizing organic compounds which results in the modification of proteins in microbes which might ultimately lead to death.
- **Appropriate concentrations** of hypochlorite can be used for the disinfection of workstations and even surfaces to clean blood spills and other liquids



# CONCLUSION

- Considering the importance of decontamination and sterilization in prevention of infectious agent from spreading, we should strictly adhered to safety precautions indoor and outdoor, most especially in this era of corona virus.
- We should endeavor to decontaminate reusable nose mask after single use.
- Wash hands frequently especially when in contact with surfaces or objects
- Observe appropriate distance as recommended by WHO.

**THANK YOU**

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