Microbiology Team
Med441

Sterilization & disinfection

MED441
KING SAUD UNIVERSITY

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Editing File

Color Index:
- Main text
- **Boys slides only**
- **Girls slides only**
- **Doctor's notes**
- Extra information
- ***Important***

Objectives

- Define the terms sterilization, disinfectant, and antiseptic.
- Know the different methods of sterilization (physical and chemical methods)
- Know and realize that heat is the most important method of sterilization and its application in medical practice.
- Know dry heat as applied as applied in hot air oven and moist heat as applied in Autoclaves.
- Know the principles of Autoclave function and monitoring methods of sterilization
- Know the importance of non heat sterilization methods and their use for sterilization of heat sensitive objects.
- Know the differences between antiseptics and disinfectants..
- Know the factors affecting disinfectants and antiseptics.
- Know the scope of function and applications of different disinfectants and antiseptics in clinical practice.

Definitions

- **Sterilization**: complete killing of all forms of microorganisms, **<u>including bacterial spores</u>**
  Ex: clostridia and bacillus
- **Disinfection**: killing or removing of harmful vegetative microorganisms. **Without killing spores**
- **Disinfectant**: chemical substance used on inanimate objects. Toxic to human.
- **Antiseptic**: disinfectant that can be safely used on living tissues.

Methods of sterilization

Methods of sterilization

Physical methods

01 Heat (moist heat and dry heat)

02 U.V. (Ultra violet) light

03 Ionizing radiation

Chemical methods

(Used for heat sensitive equipment)

01 Ethylene oxide

02 Glutaraldehyde

Sterilization physical methods

Heat: most important method, should be used whenever possible.

Dry heat

Use temperature at **160°C for one hour**

Moist heat

Eg. Autoclaves, we use temperature at **121°C for 15 minutes** or **134°C for 10 minutes**

U.V. Light

Dr. Note: we can use U.V. to sterilize water

Has limited sterilizing power because of poor penetration into most materials. Generally used in irradiation of air in certain areas such as **operating rooms**, **virology**, and **tuberculosis labs**

Ionizing radiation

Dr. Note: Ionizing radiation is generally for plastics because they can't withstand heat

E.g. **Gamma radiation**: has greater energy than U.V. light, therefore more effective. Used mainly in industrial facilities e.g. sterilization of **disposable plastic syringes, gloves, specimens, containers, and Petri dishes.**

Dry heat

Dry heat kills microorganisms (how?) by destroying their **oxidative processes**

Simplest method is exposing item to be sterilized to the naked flame e.g. bunsen burner- for sterilizing bacteriological loops, knives, blades

Hot air oven exposes items to 160°C for 1 hour

Has **electric element in chamber** as source of heat plus a **fan** to circulate air for even distribution of heat in chamber. Oven without fan is dangerous.

Used for items that are lacking water such as:

01 Metals

02 Glassware

03 Ointment, oil, waxes, powder

Dr. Note: يشبه المايكرويف لكن له ارفف ومروحة

Moist heat

Heat kills microorganisms (how?) **by denaturing proteins**

Uses hot water

Autoclave: a tough double walled chamber in which air is replaced by pure saturated steam under pressure

Autoclaving: the standard sterilization method in hospitals

The equipment is called **Autoclave**. The process is called Autoclaving.

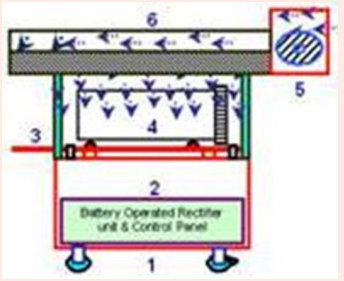
It works under the same principle as the pressure cooker where **water boils** at increased atmospheric pressure, because of **increased pressure,** the **boiling point of water is > 100**

Dr. Note:
-No air
-There is moist
-Temperature is >100

Dr. Note: مثل قدر الضغط

How do Autoclaves work?

Helpful video



01 **Air** in the chamber is evacuated and filled with saturated steam

02 The chamber is closed tightly

03 The steam keeps on filling into it and the **pressure gradually increases**

04 The items to be sterilized completely surrounded by **saturated steam** (moist heat)

05 The surface of the material to be sterilized **condenses** to **release its latent heat of condensation.**

06 Which adds to already raised temperature of steam, Eventually, **all microorganisms and spores are killed**

The usual temperature achieved is 121°C for 15 minutes or 134°C for 10 minutes

Advantages of Autoclaves

01 Temperature > 100°C therefore **spores killed**

02 Condensation of steam **generates extra heat**

03 The condensation also allows the steam to **penetrate** into **porous** materials

Note: Autoclave items must be used for invasive procedures in operating room, dental equipment, and the laboratories

Helpful video

Monitoring of Autoclaves

Chemical method

Use **heat sensitive chemical** that **changes color** at the right temperature and exposure time

- Autoclave tape (commonly used)

- Browne's tube

Physical method

Use **thermocouple** to measure accurately the temperature **and pressure.**

Biological method: spore test

A **kit containing a spore** bearing organism is added during the sterilization process, then **cultured later** on to ensure that spores have been killed

Dr. Note: نعقم المواد بالماء والصابون ونلفها بالغطاء الازرق

Dr. Note: autoclave tape يشبه اللزاق وفيه خطوط بيضاء فاتحة

Dr. Note: نغلف المواد وندخلها جهاز التعقيم وبعد نهاية دورة التعقيم اذا طلعت الخطوط سوداء معناه انه الحرارة دخلت كل أجزاء المواد وبالتالي تعقمت بشكل صحيح
واذا طلعت الخطوط بيضاء (ما تغيرت) يعني ال autoclaving method is not sufficient

Dr. Note: فيها مادة كاشفة لونها احمر توضع في جهاز التعقيم وبعد التعقيم لو صار لونها اخضر يعني التعقيم صحيح

Autoclave tape

Browne's tube

Spore test (biological indicator)

Dr. Note:
كيس يحتوي على spores محضرة بطريقة تجارية
توضع في جهاز التعقيم وبعد ما يخلص التعقيم ناخذ ال spores ونحطها في culture media لعدة أيام
اذا نمت البكتيريا معناه جهاز التعقيم ما اشتغل كويس

Chemical Sterilization methods

strong chemicals
may be used to achieve ***sterilization*** (i.e kill spores).

Useful *for heat sensitive equipments.*
Eg: Lensed Endoscopes and Plastics.

درجات الحرارة **ممكن**
ماتجي بالاختبار

01

Ethylene Oxide Chambers

Ethylene oxide **alkylates** DNA molecules and thereby inactivates microorganisms. Possibly explosive. It's a gas.
Temperature: **55-60° C**
Exposure period: **4-6 hours**

02

Activated Alkaline Glutaraldehyde 2%

Immerse item in solution for **20 mins**.
If ***Mycobacterium Tuberculosis or Spores are present then immerse for 2-3 hours***

03

Other uses

Hypochlorite used for drinking water supply, house cleaning and swimming pools.
Note: Their use depends on their concentration.

Methods of Disinfection

Used to kill most bacteria and viruses, but not spores

Methods of Disinfection

Physical methods

01 Pasteurization

02 Filtration

03 Boiling

04 Steam sterilizers

Chemical methods

01 Alcohol

02 Hydrogen peroxide

03 Glutaraldehyde and Formaldehyde

04 Halogens

05 Phenolics

06 Surface active compounds

Disinfection physical methods

Helpful video

Pasteurization

Use heat at temperatures sufficient to ***inactivate harmful organisms in milk*** (Including most viruses, most vegetative bacteria and fungi) ***temp of sterilization is not achieved***.

Flash method:
Temp. **74**°C for **3-5 sec**

Conventional method:
Temp. **62**°C for **30 min**

Boiling

Commonly used in domestic circumstances (in homes) doesn't kill spores

steam sterilizers

For baby bottles

Filtration

Use of ***membrane filter*** made of cellulose acetate or other materials.

Generally removes most bacteria but viruses and some small bacteria
(E.g: **Chlamydia** and **Mycoplasma**) require ***smaller pore sizes***

Thus filtration ***does not technically sterilize*** the items but is adequate for circumstances under which is used.

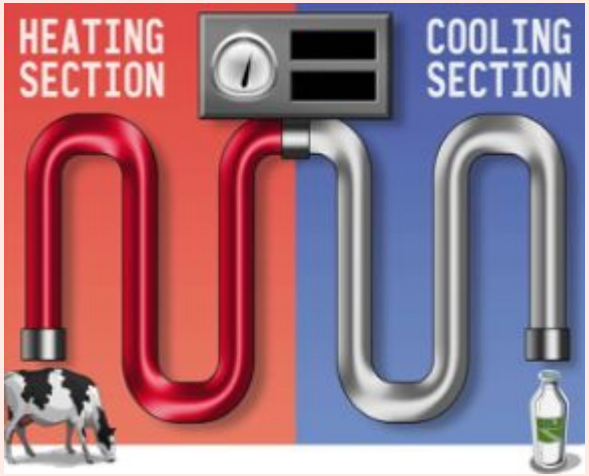
Main use: ***heat labile substances*** (degrade with heat)
Other use: ***Air conditioning*** of certain operating theatres

Pasteurization of milk

Dr. Note:
Typhoid fever is because of salmonella typhi.

To prevent transmission of the following important ***zoonotic*** diseases to humans

1. **Typhoid fever**
2. **Brucellosis**
3. **Bovine tuberculosis**
4. **Q fever**



Females side only

Chemical Disinfection methods

- Classes of Disinfectants /Antiseptics:

01 **Alcohol**:
Isopropyl alcohol (90-95%)

02 **Hydrogen peroxide**

03 **Glutaraldehyde and Formaldehyde.**
Glutaraldehyde can be used to achieve sterilization

Dr. Note:
يمكن أيضاً أن تستخدم Glutaraldehyde للمواد الي ماتتحمل sterilization بالحرارة العالية to kill spores and achieve sterilization

04 **Halogens**:
Iodine, Chlorine, Hypochlorite.

05 **Phenolics**:
Phenol, Chlorhexidine, Hexachlorophene.

06 **Surface active compounds**:
Quaternary ammonium compounds.

Dr. Note: weak, low-level disinfectants

Factors influencing activity of disinfectants

01 Activity ***directly proportional to temperature***.

02 Activity ***directly proportional to concentration*** up to an ***optimum concentration*** (After this level there is no advantage in increasing the concentration).

03 ***Disinfectants may be inactivated***, (**They inactivate by covering the surface of an instrument**):
- Dirt (needs to be cleaned before disinfecting)
- Organic matter: Proteins, pus, blood, mucus and feces
- Non-Organic matter: Cork, Hard-water and some plastics

04 ***Time,*** Disinfectants need time to work.

05 ***Range of action***, Disinfectants not equally effective against the whole spectrum of microbes.
E.g: **Chlorhexidine** is less effective against ***gram negative (-ve)*** bacteria than ***Gram positive (+ve)*** cocci.
Hypochlorite and **glutaraldehyde** are more active against hepatitis virus than most other disinfectants.

Hospital disinfection methods

Article	Disinfectant
Floors, walls	Phenolics fluids 1-2%
Surfaces tables	Hypochlorite, Alcohol
Skin: Surgeons' hands, Patient skin	Chlorhexidine, Iodine alcohol, 70% Alcohol,Iodine
Endoscopes	Glutaraldehyde 2% (Cidex), Subatmospheric steam.
Thermometers	70% Alcohol

Important to know:

Any instrument or item used for sterile body site should be ***sterile***.
(Anything that's in the body is sterile)

Any instrument or item used for non-sterile body site can be **disinfected**.
(for example, mucus membrane and the skin)

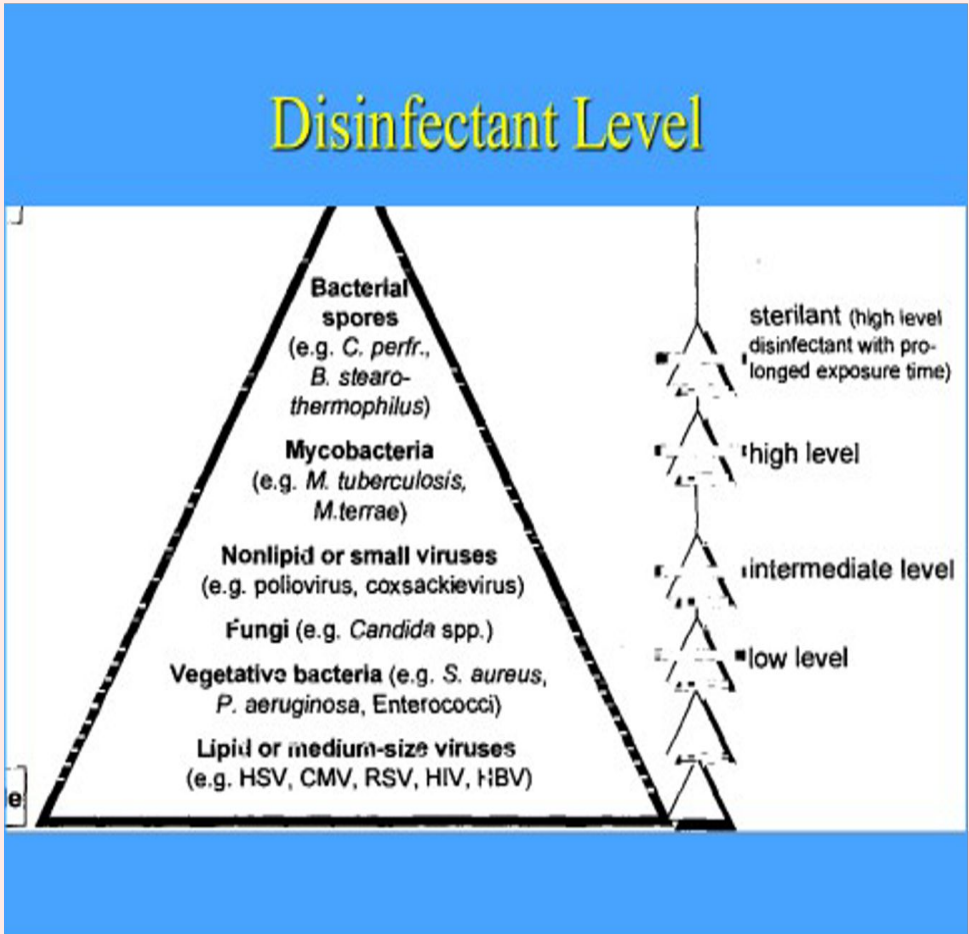
Dr. Note: alcohol more than 70% evaporate fast, less than 70% less effective

Summary

1-Sterilization Monitor by physical, chemical or biological)	A-Physical	Heat	Dry Metals,Glassware,Ointment / Oils/ Waxes/Powder	160˚C /60 mints
			Moist heat	121° C /15 mints or 134° C /10 mints
			Pasteurization For milk	74°C for 3-5 seconds or 62°C /30 mints
		UV Light	TB lab	254 nm waves
		Ionizing Radiation	sterilization of disposable	X- rays, gamma rays & cosmic rays
	B-Chemical (heat sensitive)	Ethylene Oxide (gas)	endoscopes	55-60°C /4-6 hours.
		Gluteraldehyde (Aldehydes)	Endoscopes anesthetic tubes	2% 20 mints –TB 2 hrs
		Filtration	For sera and antibiotics	0.22-0.24 µm pores
2-Disinfectant		Phenolics fluids	Surfaces Surgical and neonatal units	1-2%
		Gluteraldehyde	surfaces	2%
		Alcohole	Surfaces/thermometer	
3-Antiseptic		Chlorhexidine	skin	0.5%
		Iodine (halogens)	skin	10%
		Alcohole	skin	70%

Summary

Level	uses	application	example
High-level disinfectants Kill all including spores	Used for items involved in invasive procedures but NOT withstand sterilization (critical)	Endoscopes, Surgical instruments	Moist heat Gluteraldehyde Hydrogen peroxide Chlorine dioxide Formaldehyde Peracetic acid
Intermediate-level disinfectants Kill all including mycobacterium non-enveloped viruses fungus and bacteria	Used for cleaning surface or instruments without bacterial spores and highly resilient organism (semicritical)	Laryngoscopes, Anesthesia breathing circuits...etc	Phenol compounds Alcohol Iodophor
Low-level disinfectants Enveloped viruses and bacteria	Used to treat noncritical instruments and devices, not penetrating into mucosa surfaces or sterile tissues (noncritical)	Hospital surfaces	Quaternary ammonium compounds



Summary

Summary :Disinfectants & Antiseptics

Disinfectants/ Antiseptics	GPC	Activity against		TB	Inactivated by		Corrosive Action
		GNB	Spores		Protein	Soap	
Phenolics Sudol	++	++	–	+	±	–	+
Izal	++	++	–	–	±	–	–
Soluble Phenolic* e.g. clearsol	++	++	–	+	±	–	± to +
Chlorine compound	++	++	++	+	++	–	++ or ± (buffered Solution)
Lodophor	++	++	+ (Slow)	+	+	–	–
Chlorhexidine (Hibitane)	++	+	–	–	–	–	–
70° alcohol	++	++	–	±	++	–	–
Formaldehyde	++	++	++ (slow)	++	+	–	++
Gluteraldehyde (Cidex)	++	++	++	++	±	–	+

Questions and answers

Q1) complete killing of all forms of microorganisms, including bacterial spores.			
A) Disinfection	B) Antiseptic	C) sterilization	D) pasteurization
Q2) Dry heat kills organisms by			
A) denaturing proteins	B) destroying oxidative processes	C) pure saturated steam	D) none
Q3) Dry heat sterilization uses temperature			
A) 121°C for 15 minutes	B) 160°C for one hour	C) 134°C for 10 minutes	D) a or c
Q4) which is incorrect about autoclaves?			
A) air in the chamber is filled with saturated steam	B) the chamber is closed tightly	C) pressure gradually decreases	D) all microorganisms and spores are killed
Q5) which is not a way of monitoring autoclaves?			
A) autoclave tape	B) flash method	C) browne's tube	D) spore test

1-C 2-B 3-B 4-C 5-B

SAQs

Q1) What are the factors influencing activity of disinfectants?	A1 1- Activity directly proportional to temperature. 2-Activity directly proportional to concentration up to an optimum concentration. 3- Disinfectants may be inactivated by dirt, organic matter and non-organic matter. 4- Time. 5- range of action.
Q2) Pasteurization of milk can prevent?	A2 1- Typhoid fever 2- Brucellosis 3- Bovine tuberculosis 4- Q fever
Q3) What is the meaning of disinfectant?	A3 chemical substance used on inanimate objects. And they're toxic to human.
Q4) What is the membrane used to filter sera?	A4 Cellulose acetate
Q5) Why sterilization is better that disinfection?	A5 Because sterilization kills spores.
Q6) What are the chemicals that can be used as disinfection/antiseptic?	A6 1- Alcohol 2- Hydrogen peroxide 3- Glutaraldehyde and Formaldehyde 4- Halogens 5- Phenolics 6- Surface active compounds.

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