Sterilisation

What is sterilisation?

The **total** and **complete destruction of microorganisms** (**including spores**). Thus, materials undergoing sterilisation are **free from any form of life** (bacteria, fungi, viruses and spores).

What is sterilisation used for?

In clinical practice, sterilisation is important, whenever **invasive procedures** (i.e. instruments or material entering the patient's body) are performed:

- operating room: surgical instruments (scalpels, needles or implants), personal protective equipments - PPE (gloves, masks, gowns),
- endoscopic tubes,
- or when biological specimens are collected (swabs, needles, etc.)

Besides its application in the clinical setting, sterilisation in the microbiology laboratory is invariably necessary during **inoculation of culture media**. Bacteriological loops as well as the culture media have to be sterile in order to **prevent contamination** with other microorganisms and subsequently ensure an appropriate diagnosis. Furthermore, during collection of specimens, the transporting container has to be sterile for the same reason.

How is sterilisation achieved?

Physical methods:

- **Heat**
  - **Dry heat**: high temperature in absence of moisture or steam. By Bunsen burner or hot air oven
  - **Moist heat**: moderate temperature with moisture or steam. By autoclave, boiling or pasteurization
- **Filtration**: sterilisation with a **filter** or **membrane**, through which microorganisms cannot pass
- **Ultraviolet** and **gamma radiation**

Chemical methods:

- Ethanol oxide
- Formaldehyde

In the microbiology laboratory, sterilisation is mostly achieved by either **moist** (autoclave, boiling) or **dry heat** (hot air oven, flame).

How does sterilisation using a flame for inanimate objects work?

A flame from a Bunsen burner or an alcohol lamp should heat bacteriological loops made out of platinum until they are red.

How does sterilisation using a hot air oven work?

The instruments/materials are heated at 180 °C for one hour, so that denaturation of proteins and radical damage can ensure the death of spores and all microorganisms. A temperature of 180 °C is required because dry heat has a lower penetration power as compared to moist heat.

Preparation of the equipment to be sterilised: disinfection, washing, drying; with a sterile method so that recontamination cannot occur. The instruments are placed into metal boxes.

The control of the sterilisation process is done with the help of chemical indicators.

With this method, sterilisation of laboratory glassware, porcelain articles, metal instrumentation, some powders: talc, oils (paraffin) is ensured.

Figure 1. Hot air oven

How does sterilisation using an autoclave work?

The instruments/materials are subjected to hot steam (121°C) under vacuum conditions for 30 minutes. Less temperature than for the hot air oven is enough, because the generated steam produces a high pressure, which raises the boiling temperature. The packaging of the autoclaved instruments/material is carried out through temperature-resistant bags that are hermetically sealed.

The control of sterilisation is done with the help of chemical indicators with thermochemical agents or biological tests containing *Bacillus stearothermophylus* spores (Stearotest 120), which evaluates

the effectiveness of sterilization by verifying the viability of spores after inoculating them into broth tubes (tubes with liquid culture medium). Sterilisation is effective if the tubes remain sterile and no bacterial growth is observed (the broth remains clear).

Because less heat is applied, not only can metal instruments be sterilised, but also rubber articles or cotton fabrics.

Flash autoclaves, a variant of autoclaves, sterilise at a temperature of 134°C for 4 minutes and are mainly used in operating theatres.



Figure 2. Autoclave oven

Disinfectants and antiseptics

The term disinfectant includes both disinfectants in the strict sense and antiseptics. Both terms refer to products that share the **ability to inhibit or kill unwanted microorganisms**. **Disinfectants** in the strict sense are intended for **inert media** (instruments, surfaces); **antiseptics** are intended for **living tissues** (skin, mucous membrane). These products act shortly and do not protect against new contamination or natural proliferation (mitosis, replication). They must therefore be **reapplied regularly**. Antiseptics and disinfectants are capable of **inhibiting the growth** of microorganisms (bacterio**static**, fungi**static**, viru**static** action) or of **eliminating** them (bacteri**cidal**, fungi**cidal**, viru**cida**l, spori**cidal** action). Some products have both actions depending on the concentration used. Generally, the higher the concentration, the more lethal the effect (exception: ethanol 70% more active than 96%).

Different families of antiseptics and disinfectants are distinguished according to their mechanism of action on microorganisms: **membrane damage**, **coagulation of intracellular constituents**, and **denaturation of enzymes**. Apart from the concentration, various factors can influence the activity of the products:

- contact time (increased activity with increased contact time)
- temperature (increased activity with increased temperature)
- pH (decrease or increase in activity depending on the family of microbes)
- liposolubility (penetration of the stratum corneum i.e. the outer layer of the skin)
- presence of fluids or other biological materials (blood, pus)
- presence of soap (increased activity of quaternary ammoniums, chlorhexidine and chlorinated products.

Families of disinfectants and antiseptics

Table 1. Families of disinfectants and antiseptics

Families	Examples	Target and mechanism of action
Alcohols	Ethanol, Isopropanol	Denaturation of cytoplasmic and membrane proteins Inhibition of nucleic acid and protein synthesis
Aldehydes	Formaldehyde	Cell wall damage Inhibition of nucleic acid and protein synthesis

Quaternary ammonium salts	Benzalkonium chloride	Binding to fatty acids and phosphate groups of the cell membrane leakage of cellular constituents and lysis of cell
Biguanides	Chlorhexidine	Binding to fatty acids and phosphate groups of the cell membrane leakage of cellular constituents Coagulation of cytosol
Halogens (chlorine and iodine)	Sodium hypochlorite, Povidone-iodine	Destruction of membrane proteins and chromosomes (halogenation)
Oxidants	Hydrogen peroxide	Production of free radicals that interact with lipids, proteins and DNA

Spectrum of activity of disinfectants and antiseptics

Table 2. Spectrum of activity of disinfectants and antiseptics

Families	Spectrum of activity							
	Gram positive	Gram negative	Mycobacteria	Yeasts	Moulds	Viruses	Enveloped viruses	Spores
Alcohols	+	+	+	+/-	+/-	+/-	+	-
Aldehydes	+	+	+	+	+	+	+	+
Quaternary ammonium salts	+	+/-		+	+	+/-	+	
Biguanides	+	+	+/-	+	+/-	+/-	+	-
Halogens (chlorine and iodine)	+	+	+	+	+	+	+	+
Oxidants: disinfection	+	+	+	+	+	+	+	+
Oxidants: antiseptics	+	+	-	+	+	+/-	+	-

+ active products

+/- inconstantly active products

- inactive products

Notes: Aldehydes - only for disinfection; iodine halogens - only for antisepsis

a 

b 

c 

Figure 3. Vials with various disinfectants: a. HMI Scrub NP - Hygienic antiseptic, surgical by rubbing, b. Anios Oxy'Floor - high level surface detergent disinfectant, c. Desderman Pure Gel - alcohol solution for hygienic and surgical disinfection of the hands

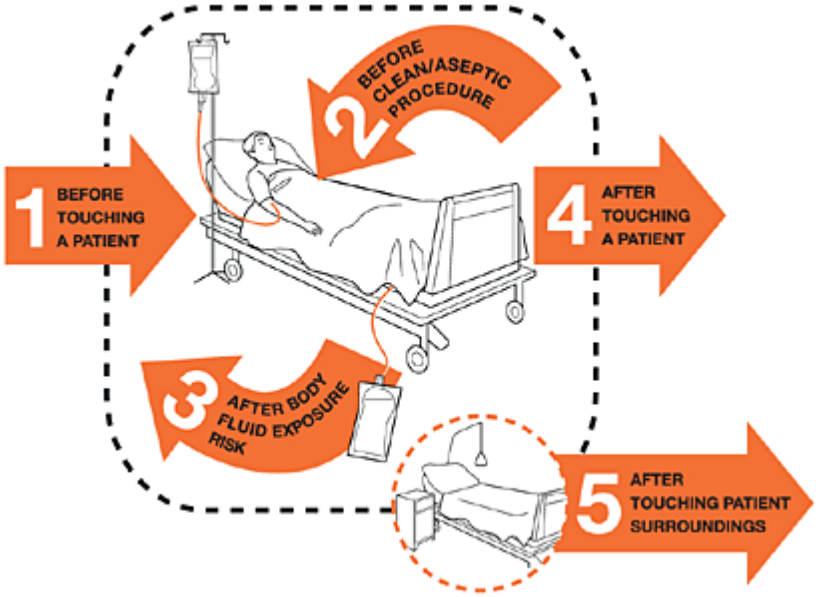


Figure 4: The 5 moments of hand hygiene (http://www.who.int/gpsc/5may/background/5moments/en/)

Culture Media

A culture medium is a preparation in which microorganisms can **multiply**. It must therefore satisfy the **nutritional requirements** of the microorganism studied, which involves:

- covering **mineral requirements** and **growth factors**,
- providing a source of **carbon** and **energy**,
- presenting a **pH** close to the **optimal pH**,
- having an **optimal ionic strength** (the medium can be isotonic but it is not obligatory).

A culture medium is composed of a mixture of **nutritive substrates** (amino acids, peptides, nucleic bases, and sugar), a **buffer system** to avoid excessive variations in pH, **minerals** and **vitamins**.

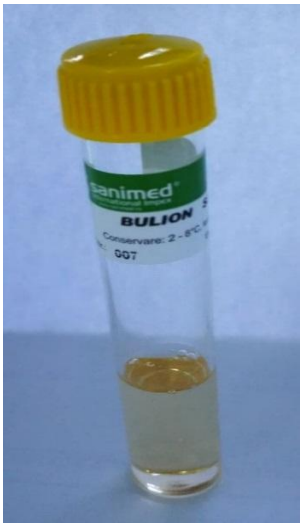
Various additions can be made to promote or allow the growth of bacteria requiring "growth factors" (vitamins, proteins, haemoglobins, etc.).

Culture media must provide **aerobic** or **anaerobic** conditions (depending on the microorganisms to be cultured).

The optimum pH of a culture medium lies within **7.2 - 7.4** (approximately neutral, close to the pH in the human body) – with some exceptions: *Brucella* grows at a pH of 6.8 and *Vibrio cholerae* that grows at a pH of 9.

Classification

- **physical state**: liquid, semi-solid (5% agar), solid (10% agar)
- **complexity:** simple, enriched
- **goal**: diagnostic, special, selective

a 

b 

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Figure 5. Liquid media. a. Simple liquid medium - Bulion, b. Storage liquid medium - Microbank ™, c. Liquid medium - BD BACTEC bottles

Examples of culture media:

- **liquid**: peptone water, broth
- **solid**: simple agar, Columbia agar with sheep blood, blood agar + nalidixic acid and colistin
- **selective**:

  - **Chapman's media** (for *Staphylococcus*):

  - **Sabouraud's media** (for yeasts and fungi)

  - **Lactose media** (Mac Conkey's medium, Hektoen Agar - for *Enterobacteriaceae*)

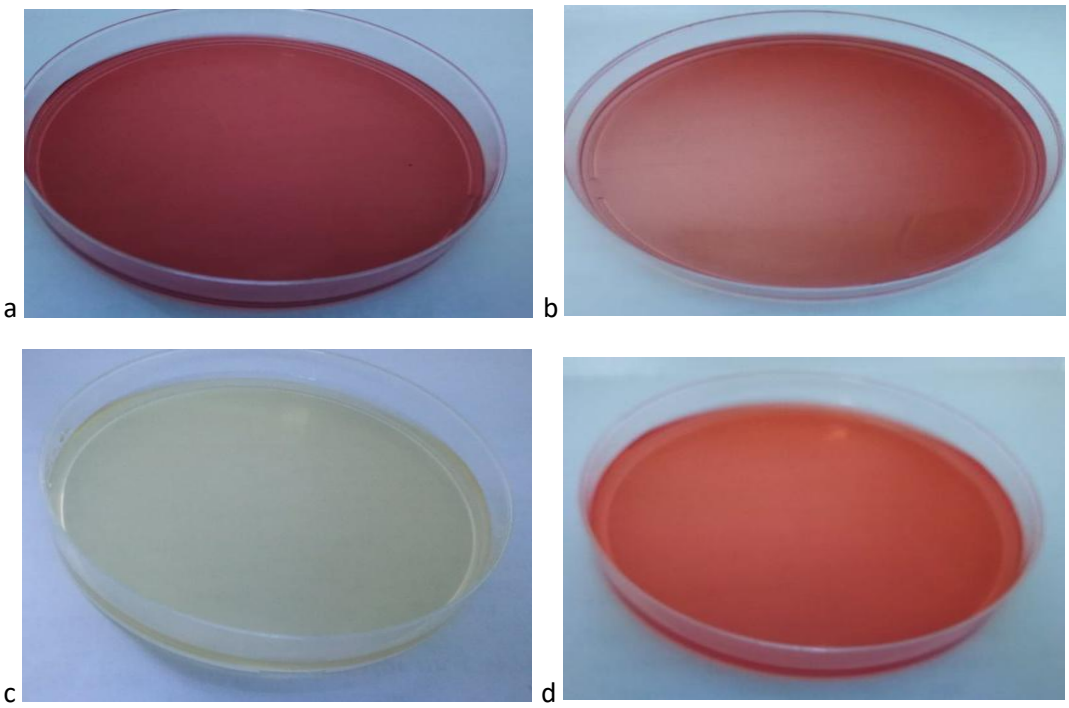

Figure 6. Differential media a. Selective-differential solid media - MacConkey, b. Selective differential media - Chapman (mannitol-salt-agar), c. Differential solid media - CHROMagar UTI, d. Compound solid media - Blood jelly (blood) -agar)

- **special**: Löwenstein-Jensen for *Mycobacterium tuberculosis* (Koch Bacillus)

The most commonly used culture media in clinical practice are:

Blood-containing culture media

- **Differential** culture medium
- Contains: horse/sheep blood (5-10%), meat extract, NaCl, tryptone, agar
- Used to detect **haemolytic activity**
- Haemolysis**: α-haemolysis** (= **partial** lysis, e.g.: *Streptococcus viridans*, *Streptococcus pneumoniae*), **β-haemolysis** (= **complete** lysis, e.g.: *Streptococcus haemolyticus*, *Streptococcus pyogenes*), **γ-haemolysis** (= **no** lysis)

Mac Conkey agar

- **Differential** and **selective** culture medium
- Used to differentiate between **Gram-negative bacilli**
- Contains: **lactose** (disaccharide), a **pH indicator** (neutral red dye) and **bile salts**. When certain bacteria ferment lactose, acid is produced causing the pH indicator within the medium to change its colour from pale pink to **pink**. The bile salts make the culture medium selective by **inhibiting** the growth of **gram-positive organisms**.
- Differentiation between **lactose positive** and **lactose negative** bacteria.

Chapman agar (Mannitol salt agar)

- **Differential** and **selective** culture medium
- Used to differentiate ***Staphylococcus aureus*** from other Gram-positive bacteria
- Contains: **mannitol** and a **high salt concentration**. The high salt concentration inhibits the growth of most bacteria i.e. other Gram-positive cocci such as *Streptococcus* but it does not inhibit the growth of genus *Staphylococcus.* Mannitol fermentation (revealed by the colour change of the medium from pink to yellow) indicates the presence of *S. aureus* which is the only member of the genus that ferments the sugar mannitol; so, mannitol fermentation differentiates ***S.aureus*** from other **staphylococci**

Hektoen agar

- **Differential** and **selective** culture medium used for **Enterobacteria**
- Contains: indicators of **lactose fermentation** and **hydrogen sulphide** (H_2S) production
- Selective: Inhibits growth of Gram-positive bacteria
- Differential:
    - Distinction between **lactose fermenting** (lactose-positive) and **lactose non-fermenting** (lactose-negative) **enterobacteria**; lactose fermentation is revealed by bacterial growth changing the colour of the medium from green to yellow/orange as in the case of *E.coli, Klebsiella*; in the absence of lactose fermentation, the colour of the medium does not change, hence enterobacteria which do not ferment lactose will produce colourless colonies which appear as green due to the unchanged colour of the medium, as in the case of *Salmonella* and *Shigella*;
    - Distinction between enterobacteria which produce **H_2S** and those which do not; H_2S production is revealed by colonies with a **black centre** e.g. *Salmonella, Proteus*; in the absence of H_2S production the colonies will appear as colourless, transparent, "borrowing" the green colour of the medium as described above e.g. *Shigella*

Table 3. Differential and selective culture medium used for Enterobacteria

	Lactose	**H_2S**
Salmonella	**(-); alkaline** reaction**:** **blue-green** colonies	**(+);** colonies with **black center**
Shigella	**(-); alkaline** reaction**:** **blue-green** colonies	**(-)**
***E.coli* and others**	**(+); acidic** reaction**:** **yellow-orange** colonies	**(-)**