

Bacterial Death

Death of any living organism can be defined as an **irreversible inactivation** of it, so it does not **grow** nor **reproduce** anymore.

To confirm the death of bacteria:

We grow it on a **suitable media** (agar plate for example) and see if it **forms colonies** or **not** so then we can confirm whether the bacteria is **alive or dead** originally.

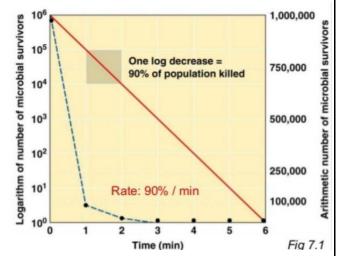
<u>Note:</u> The setup (media) must be of **suitable conditions** to the bacteria we are studying, because if it wasn't suitable the bacteria would be **dead** and we won't know if it was originally dead or not.

Exponential decrease through bacterial death

As seen in the following graph, An exponential decrease in microbial survivors takes place and varies in degree according to **the killing agent**.

The **rate** of bacteria death is **90%** per every **minute**.

<u>Note:</u> The curve is **not linear** because at some point the bacteria will **adapt** to the conditions applied by the killing agent.



Formula of cell death

$$S = S_o * e^{-kt}$$

- S → Number of cells survived after using a killing agent
- S^o → Number of cells originally

Antimicrobial Agents Definitions

1	Sterilization	A process to make an object free from any living organism on a surface.
2	Disinfection	Removal of some microorganism to get a surface that is appropriate to use level . It is less effective than sterilization. <u>Note:</u> Some microorganisms may persists since they are highly resistant microbes like spores and prions.
3	Cleaning	Removal of organic and inorganic materials (biofilm) from objects mechanically with water or fire for example.

Bacteriostatic: It is the **reversible inactivating** of an organism process. Once we remove the agent the bacteria will **resume** its growth and replication.

Bactericidal: It is the **irreversible inactivating** of an organism process. *Even if we remove the agent the bacteria will still be dead.*

Sterilization Processes

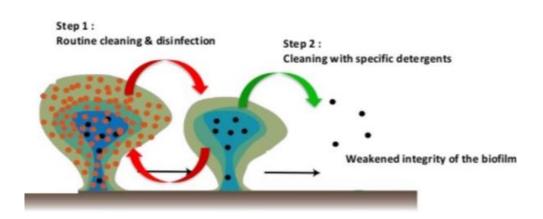
1	Autoclaves	They are strong containers that use pressurized and saturated steams at almost 121 C° for around 15-20 min. It's the most efficient way to kill microorganism using heat and pressure.				
2	Filters	With pores size of 0.2 μm filters mostly, microorganisms will be retained .				
3	Chemicals	Hydrogen peroxide is used in high concentrations (10%-30%).Glutaraldehyde is used with long contact times (3-12hr). <u>Note:</u> Both when used at lower concentration/time may be considered disinfectants since they wont be as effective to be considered sterilizing agents.				
4	Radiations	UV radiation damages DNA by crosslinking adjacent pyrimidines. Ionizing radiation like Gamma radiation for example causes strand breaks <u>Note:</u> Gamma radiation has better penetration into materials than UV radiations.				
5	Gas plasma /Vapor phase	This process shows high efficiency in killing spores. Example: Hydrogen peroxide and Peracetic acid in vapor form The graph shows that Plasma kills bacteria better than UV				

<u>Note:</u> Sterilization processes are used **depending on the objects' nature**, for example some objects' are **heat sensitive** therefore we cannot use **autoclaves**, so filters or gas plasma are considered **more suitable**.

Factors that affect efficacy of both disinfection and sterilization:

1) Prior cleaning

The presence of **biofilm** makes it harder for sterilizing agents and disinfectants to **penetrate** the bacteria and even sometimes **inactivates** the agents, so we have to **clean** the object before sterilizing/disinfecting to get rid of biofilms, that's why cleaning is **important**.



2) Organic and inorganic load present

This **decreases** the **efficacy of action** for both disinfection and sterilization.

3) Type and level of microbial contaminations.

Example: Prions, whom are highly resistant.

- 4) Concentration of and exposure time to the germicide (killing agent) as discussed before.
- 5) Physical nature of objects If they have crevices, hinges or lumens, its hard to reach them properly.
- 6) Presence of biofilm
- 7) Temperature and pH of the disinfection process

Antimicrobial Agents Definitions part 2

1	Is the presence of pathogenic microbes in living tissues associated fluids. Septic Note: A septic shock is a serious medical condition that occurs when living tissues are damaged in response to infection by microbes.				
2	Antiseptic	 Destroys or inhibits the growth of microorganisms in or on living tissues or fluids. They are similar to disinfectants, but they work on living tissues unlike disinfectants. <u>Example:</u> Alcohol, Iodine and Chlorhexidine. 			
3	Aseptic	Is an adjective describing a technique free of microorganisms <u>Example:</u> Surgery			
4	Preservation	Is the prevention of multiplication of microorganisms in formulated products using preservatives .			

Mechanism of action of antimicrobial agents

1) Denaturation of proteins

Heat destroys the **tertiary structure** which gives a protein its **function**.

2) Oxidation damage interferes with most biological processes of the cell (membrane/DNA synthesis)

Example: Hydrogen peroxide interferes with **some enzymes** causing **biological** interferences.

3) DNA damage

Example: UV and Ionizing radiation.

4) Cell membrane and cell wall disruption <u>Example</u>: Alcohol dissolves membrane lipids.

This table is only for further explanation of how each Antimicrobial agent affect different microorganism and others

Agents	Bacteria	Mycobacteria	Bacterial Spores	Fungi	Viruses
Disinfectants					
Alcohol	+	+	-	+	+/-
Hydrogen peroxide	+	+	+/	+	+
Phenolics	+	+	-	+	+/
Chlorine	+	+	+/	+	+
lodophors	+	+/	-	+	+
Glutaraldehyde	+	+	+	+	+
Quaternary ammonium compounds	+/	_	-	+/-	+/-
Antiseptic Agents					
Alcohol	+	+	-	+	+
lodophors	+	+	-	+	+
Chlorhexidine	+	+	-	+	+
Parachlorometaxylenol	+/-	+/	-	+	+/
Triclosan	+	+/-	-	+/-	+

+ \rightarrow The microbe is **killed**

→ The microbe is **not killed**

+/- \rightarrow Means that the effect **depends** on the **concentrations/time** used.

