Sterility criteria

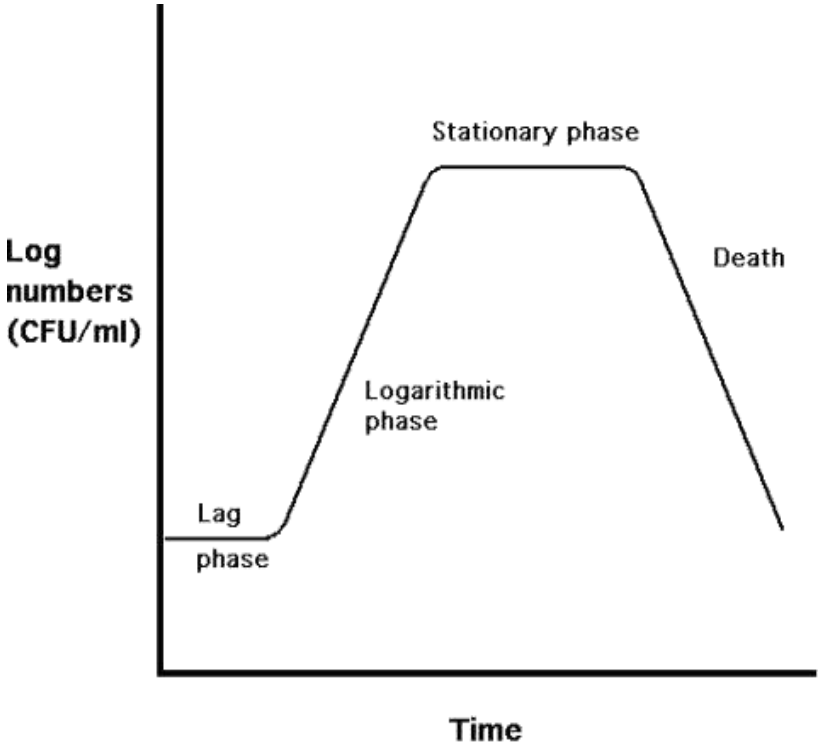
- **Bioburden** is normally defined as the number of bacteria living on a surface that has not been sterilized.
- The term is most often used in the context of **bioburden testing,** also known as microbial limit testing, which is performed on pharmaceutical products and medical products for quality control purposes.

Sensitivity of microorganisms

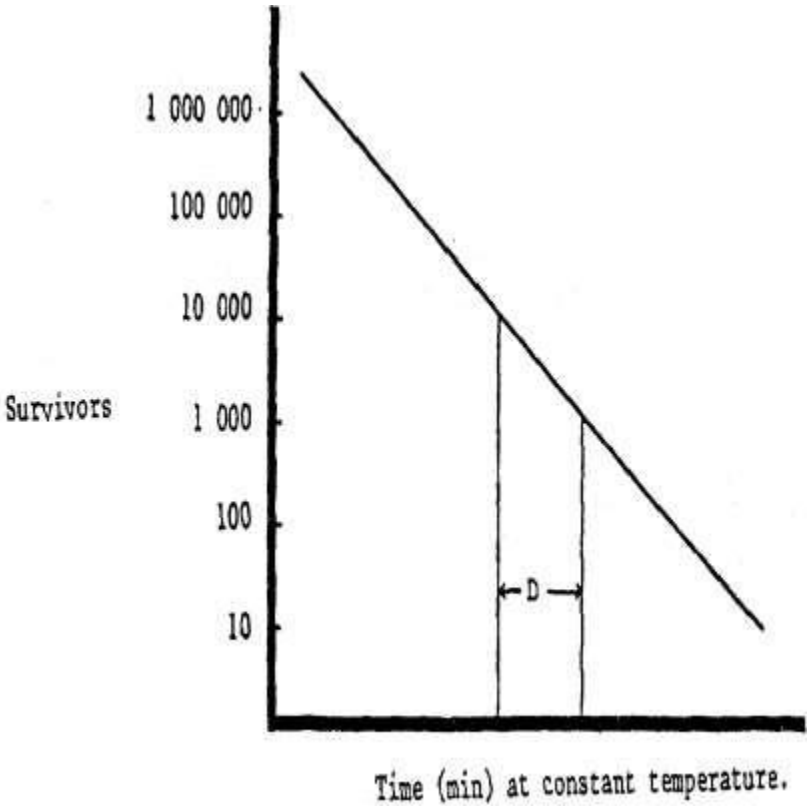
- Microorganisms show resistance to heat, radiation and chemicals.
- The vegetative forms of bacteria and fungi are the most sensitive.
- The thermophilic bacteria, smaller viruses and mould spores are killed at temperature between 70 to 90°C, while bacterial spores may be destroyed at 90 to 120°C temperatures.

Death rates or survivor curve

- It is determined by assessing the reduction in the number of viable microorganisms resulting from contact with a given destructive force.



Hypothetical bacterial growth curve.



Sterility indicators

- Changing appearances in colour or pattern, the sterilization indicators visually show if cleaning conditions are passing or procedures have been completed.
- Eliminating any confusion or possibility that instruments will not be sterile, indicators are used routinely in clinical and research environments where contamination elimination is crucial.
- With the temperature resistance required to endure the purification, the sterilization indicators are available in different forms such as tapes, ampoules, and sticks.
- Monitoring of sterilization process can be achieved by the use of **physical, chemical or biological indicators.**

1. Physical indicators

i) Moist heat Indicator:

- ✓ A **Master Process Record (MPR)** is prepared as part of the validation procedure for a particular autoclave
- ✓ The MPR should be checked at annual intervals and whenever significant changes occur in the BPR(Batch Production **Records)**when compared with the MPR.
- ✓ Microprocessor-controlled sterilization cycles are now a part of modern autoclaves.

ii) Dry heat:

- ✓ In dry sterilization processes, a temperature record chart is made of each sterilization cycle and is compared against a master temperature record.

iii) Radio sterilization:

- ✓ A plastic dosimeter gives an accurate measure of the radiation that is absorbed and is considered to be the best technique currently available for the radio sterilization process.

iv) Gaseous methods:

- ✓ For gaseous sterilization procedures, elevated temperatures are monitored for each sterilization cycle by temperature probes and routine leak tests are performed to ensure gas-tight seals. Gas concentration is measured independently of pressure rise, often by reference to the weight of gas used. Pressure and humidity measurements are recorded.

v) Filtration:

- ✓ Bubble point pressure test is a technique employed for determining the pore size of filters and may also be used to check the integrity of certain types of filter devices immediately after use. The principle of the test is that the filter is soaked in an appropriate fluid and pressure is applied to the filter. The pressure difference when the first bubble of air breaks away from the filter is equivalent to the maximum pore size. When the air pressure is further increased slowly, there is general eruption of bubbles over the entire surface. The pressure difference is equivalent to the mean pore size.

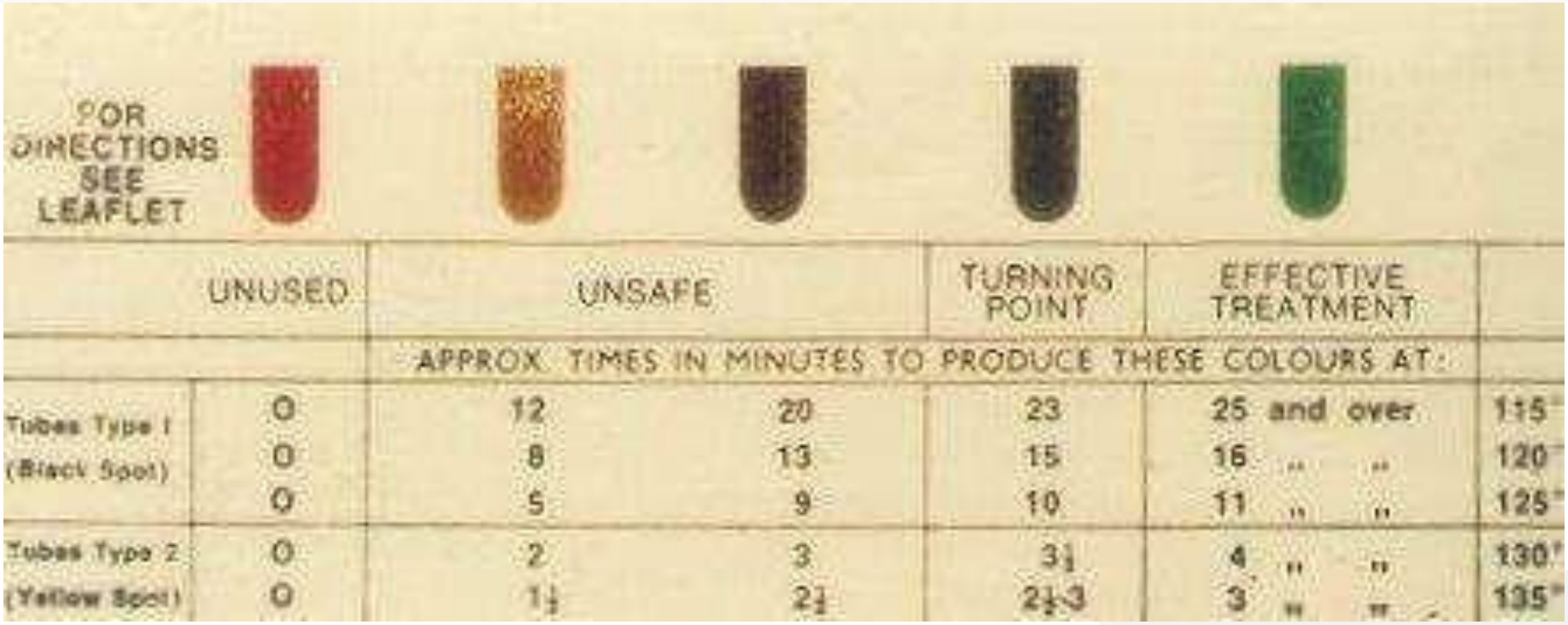
2. Chemical indicators

- Chemical monitoring of a sterilization process is based on the ability of heat, steam, gases and ionizing radiation to alter the chemical or physical characteristics of a variety of chemical substances.

i) Browne's tubes:

- Most commonly used chemical indicator for heat process
- Contains small sealed coloured tubes having a reaction mixture and an indicator
- Exposure to high temperature results in the change of colour of the indicators (Red to green)

TYPES OF Browne's tubes:



		UNUSED	UNSAFE		TURNING POINT	EFFECTIVE TREATMENT	
		APPROX. TIMES IN MINUTES TO PRODUCE THESE COLOURS AT:					
Tubes Type 1 (Black Spot)	O	12	20		23	25 and over	115°
	O	8	13		15	16 " "	120°
	O	5	9		10	11 " "	125°
Tubes Type 2 (Yellow Spot)	O	2	3		3½	4 " "	130°
	O	1½	2½		2½-3	3 " "	135°

II) WITTNESS TUBES

- Consist of single crystalline substances of known melting point contained in glass tubes
- Ex: Sulphur(115°C), Succinic anhydride(120°C), Benzoic acid(121°C), etc.
- A dye may be included to show more clearly that the crystals have melted.
- Indicates that a certain temperature has been reached.

WITTNESS TUBES

3. Biological indicator

- Consists of a suitable organism deposited on a carrier and are distributed throughout the sterilizer load
- At the end of the sterilization process, the units are recovered and cultured to determine the presence or absence of survivors.
- Confirm the ability of the sterilization process to kill microbial spores
- Can check large number of spores
- Includes all the parameters of the sterilization process
- Most critical test of sterilization process

Table 7.6: Biological indicators for monitoring sterilization processes

Sterilization process	Species	D-value
Autoclave at 121°C	*Bacillus stearothermophilus*	1.5 min
	Clostridium sporogenes	0.8 min
Dry heat at 160°C	*Bacillus subtilis* var. niger	5 – 10 min
Ethylene oxide at 600 mg/lit. (Temperature - 54°C, 60% - relative humidity)	*Bacillus subtilis* var. niger	2.5 min
Ionizing radiation	*Bacillus pumilus*	3 kGy (0.3 M rad)
Membrane filter (0.45 µm pore size)	*Serratia marcescens*	–
Membrane filter (0.22 µm pore size)	*Pseudomonas diminuta*	–