Sterility Testing

SARASWATI INSTITUTE OF
PHARMACEUTICAL SCIENCES

Name of Teacher: Ms. Dipal M Patel

Designation: Assistant Professor

Department: Pharmaceutics

Name of Institute: Saraswati Institute of Pharmaceutical Sciences, Dhanap, Gandhinagar

Sterility Testing

Introduction

Sterilisation:

- Is the process of making something free from bacteria or other living microorganisms.

Sterility Testing:

- Are done to detect if viable forms of micro-organisms are present or not on or in the pharmaceutical preparations.

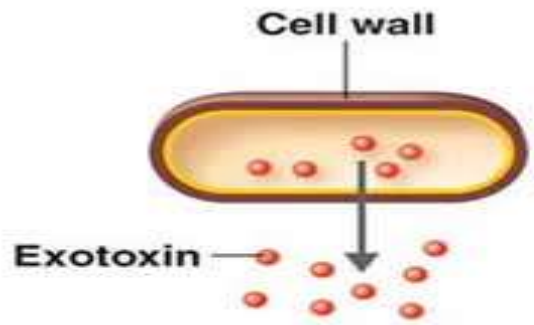
OR

- A test that critically assesses whether a sterilized pharmaceutical products is free from contaminating microorganism.

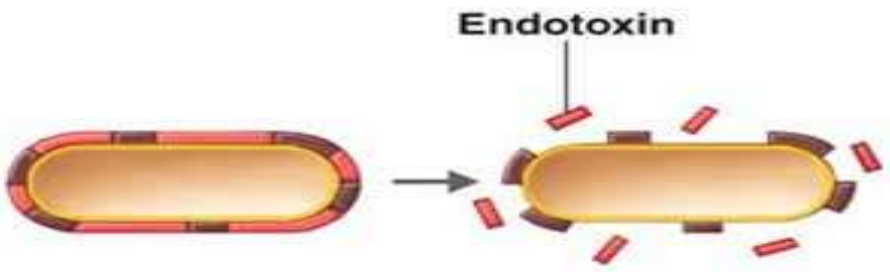
Pyrogen:

- A fever producing substances that cause febrile reactions when sufficient amount enter the circulatory system.

Differences Between Exotoxins and Endotoxins



(a) Exotoxins are proteins produced inside pathogenic bacteria, most commonly gram-positive bacteria, as part of their growth and metabolism. The exotoxins are then secreted or released into the surrounding medium following lysis.



(b) Endotoxins are the lipid portions of lipopolysaccharides (LPSs) that are part of the outer membrane of the cell wall of gram-negative bacteria (lipid A; see Figure 4.13c). The endotoxins are liberated when the bacteria die and the cell wall breaks apart.

- Bacterial endotoxin (pyrogen)
- Ex: **Streptococcal exotoxins, Staphylococcal enterotoxins, Bacterial endotoxin lipopolysaccharides (LPS), fungal products.**
- **BACTERIAL ENDOTOXIN TEST IS REPLACED BY RABBIT PYROGEN TEST.**
- Rabbit pyrogen test is based on rectal temperature of rabbits, measure temperature before and after IV injection of a test solution in the ear veins.(Response-fever)
- ADV: INEXPENSIVE, EASY TO HANDLE, LABILE THERMOREGULATORY MECHANISM

Why sterility test for pharmaceutical products?

- To reveal the presence of viable forms of bacteria, fungai and yeasts in a pharmaceutical products or devices.
- To monitored the quality of the pharmaceutical products before marketing
- To avoid the contamination
- To assure the safety of the products

Which products undergo sterility tests?

- Injections
- Implants
- Syringes
- Bandages
- Dressings
- Surgical Instruments
- Needles
- Injectables
- Bulk Solids
- Ophthalmic Products..etc

Precautions while performing sterility tests

- Ventilated aseptic rooms/regions supplied with bacteriologically cleaned air to avoid accidental contamination by microorganisms.
- Highly trained staff
- The working conditions in which the tests are performed are monitored regularly by appropriate sampling of the working area and by carrying out appropriate controls.

STEPS INVOLVED IN STERILITY TESTING

1. Selection of the sample size.
2. Selection of the quantity of the product.
3. Media requirements
4. Test microbes
5. Method of testing.
6. Observation and Results.

1. SELECTION OF SAMPLE SIZE(IP)

Quantity per Container	Minimum quantity to be used for each medium unless otherwise justified and authorised
Parenteral preparations:	
• Not more than 100 containers	• 10 per cent or 4 containers whichever is greater
• More than 100 but not more than 500 containers	• 10 containers
• More than 500 containers	• 2 per cent or 20 containers
Ophthalmic and other non-injectables:	
• Not more than 200 containers	• 5 per cent or 2 containers
• More than 200 containers	• 10 containers
Bulk solid products:	
• Up to 4 containers	• Each container
• More than 4 containers but not more than 50 containers	• 20 per cent or 4 containers whichever is greater
• More than 50 containers	• 2 per cent or 10 containers whichever is greater

2. SELECTION OF QUANTITY OF THE PRODUCT

Quantity per Container	Minimum quantity to be used for each medium unless otherwise justified and authorised
Liquids: • Less than 1ml • 1-4ml • 5ml or more but less than 20ml •20ml or more but less than 50ml •50ml or more but less than 100ml •Antibiotics	 • Whole contents of each container • Half contents of each container • 2ml • 5ml • 10ml •1ml
Insoluble preparations, creams and ointments to be suspended or emulsified	Use the contents of each container to provide not less than 200mg
Solids: • Less than 50mg • 50mg or more but less than 200mg • 200mg/Greater	 • The whole contents of container • Half the contents of each container but not less than 50mg • 100mg

3. Media requirements

1. **Fluid Thioglycollate Medium(FTM)**

- It is used with clear fluid products.
- FTM is primarily intended for the culture of anaerobic bacteria; however it will also detect aerobic bacteria.

2. **Alternative Thioglycollate Medium(ATM)/ Thioglycolate Broth**

- Used with turbid or viscid products
- For the culture of anaerobic condition

3. **Soyabean Casein Digest Medium(SCDM)/ Tryptone Soya Broth(TSB)/ Casein Soybean Digest Broth(CSDB)**

- Used for turbid or viscid products
- For both fungi and aerobic bacteria

- **Medium 1 & 2 are adjusted to pH 7.1±0.2**
- **Medium 3 is adjusted to 7.3±0.2**
- **Autoclave at 121°C for 20mins**

Table 11.2: Composition of media used for sterility test

Components	FTM	ATM	SCDM
L-cystine	0.5 gm	0.5 gm	–
Sodium chloride	2.5 gm	2.5 gm	5.0 gm
Dextrose monohydrate/anhydrous	5.5 gm/5.0 gm	5.5 gm/5.0 gm	2.5 gm/2.3 gm
Yeast extract (water soluble)	5.0 gm	5.0 gm	–
Pancreatic digest of casein	15.0 gm	15.0 gm	17.0 gm
Sodium thioglycollate / thioglycollic acid	0.5 gm/0.3 ml	0.5 gm/0.3 ml	–
Papaic digest of soybean meal	–	–	3.0 gm
Dipotassium hydrogen phosphate	–	–	2.5 gm
Resazurin sodium soluble (0.1%)	1.0 ml	–	–
Distilled water to make	1000 ml	1000 ml	1000 ml
pH of the medium (After sterilization)	7.1 ± 0.2	7.1 ± 0.2	7.3 ± 0.2

Tests for culture media

- If freshly prepared media are not used within 2 days, store them in the dark, preferably 2 – 25°C.
- Visible test

4. TEST MICROBES

Medium	Test microbes	Genomic Strains	INCUBATION		
			Temperature (°C)	Duration (Days)	Conditions
Fluid thioglycollate	***Staphylococcus aureus***	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518,	30-35	3	Aerobic
	Clostridium sporogenes	ATCC 19404, CIP 79.3, NCTC 532 *or ATCC 11437,*	30-35	3	Anaerobic
	Pseudomonas aeruginosa	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275	30-35	3	Aerobic
Alternative thioglycollate	***Clostridium sporogenes***	ATCC 19404, CIP 79.3, NCTC 532 *or ATCC 11437*	30-35	3	Anaerobic
	Bacillus subtilis	ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134	30-35	3	Aerobic
Soyabean-Casein Digest	***Aspergillus brasiliensis***	ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455	20-25	3	Aerobic
	Candida albicans	ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594	20-25	3	Aerobic

Full form of microbial strains

- **ATCC: American type culture collection**
- **NCTC: National Collection of Type Cultures**
- **NCIB: National Collection of Industrial Bacteria**
- **NCIMB: National Collection of Industrial, Food and Marine Bacteria**
- **NCYC: National Collection of Yeast Cultures**
- **NCL: National Chemical Laboratory**
- **CIP: Collection of Institute Pasteur**
- **MTCC: Microbial Type Culture Collection and Gene Bank**

❖**Microbial genome sequencing strains**

IF TEST SAMPLES IS BACTERIOSTATIC/FUNGISTATIC,USE A SUITABLE STERILE NEUTRALIZATING AGENTS

Table 11.5: Inactivation of antimicrobial agents in sterility testing

Antimicrobial agent	Method of inactivation/inactivating agent
Penicillin	Penicillinase
Cephalosporins	Cephalosporinase
Streptomycin	Streptomycin adenyltransferase/ Streptomycin phosphotransferase
Aminoglycosides	Acetyl-coenzyme 'A'
Barbiturates	Dilute to 0.2% in culture medium with a pH 7.0
Sulfonamides	p-aminobenzoic acid
Chloramphenicol	Acetyl transferase
Phenolic disinfectants	Dilution
Halogens	Sodium thiosulphate
Quaternary ammonium compounds	Lecithin + Lubrol or Tween 80
Dyes	Membrane filtration
Heavy metals	Thioglycollic acid
Ethyl alcohol	Dilute to less than 1%
Other antibiotics	Membrane filtration

4. TEST METHODS

- **Method A: Membrane Filtration method**
- **Method B: Direct Inoculation method**

MEMBRANE FILTRATION METHOD

- Membrane has a nominal pore size not greater than **0.45 micron and diameter of approximately 50mm.**
- **Flow rate: 55-75ml/min at pressure 70mm Hg**
- **Cellulose nitrate filters are used**
- This method basically involves filtration of sample through membrane filters.
- The filtration is assisted under Vacuum after filtration completion the membrane is **cut into 2 halves and one halve is placed in two test tubes containing FTM, SCDM medium.**
- **For bacteria 20-25°C, for fungi 30-35°C**
- Incubate the media for not **less than 14 days.**

Used for:

- An oil or oily preparation.
- Ointments that can be put into solutions.
- Soluble powder.
- Liquid products where volume in a container is 100ml or more.
- Non bacteriostatic solid not readily soluble in culture media.

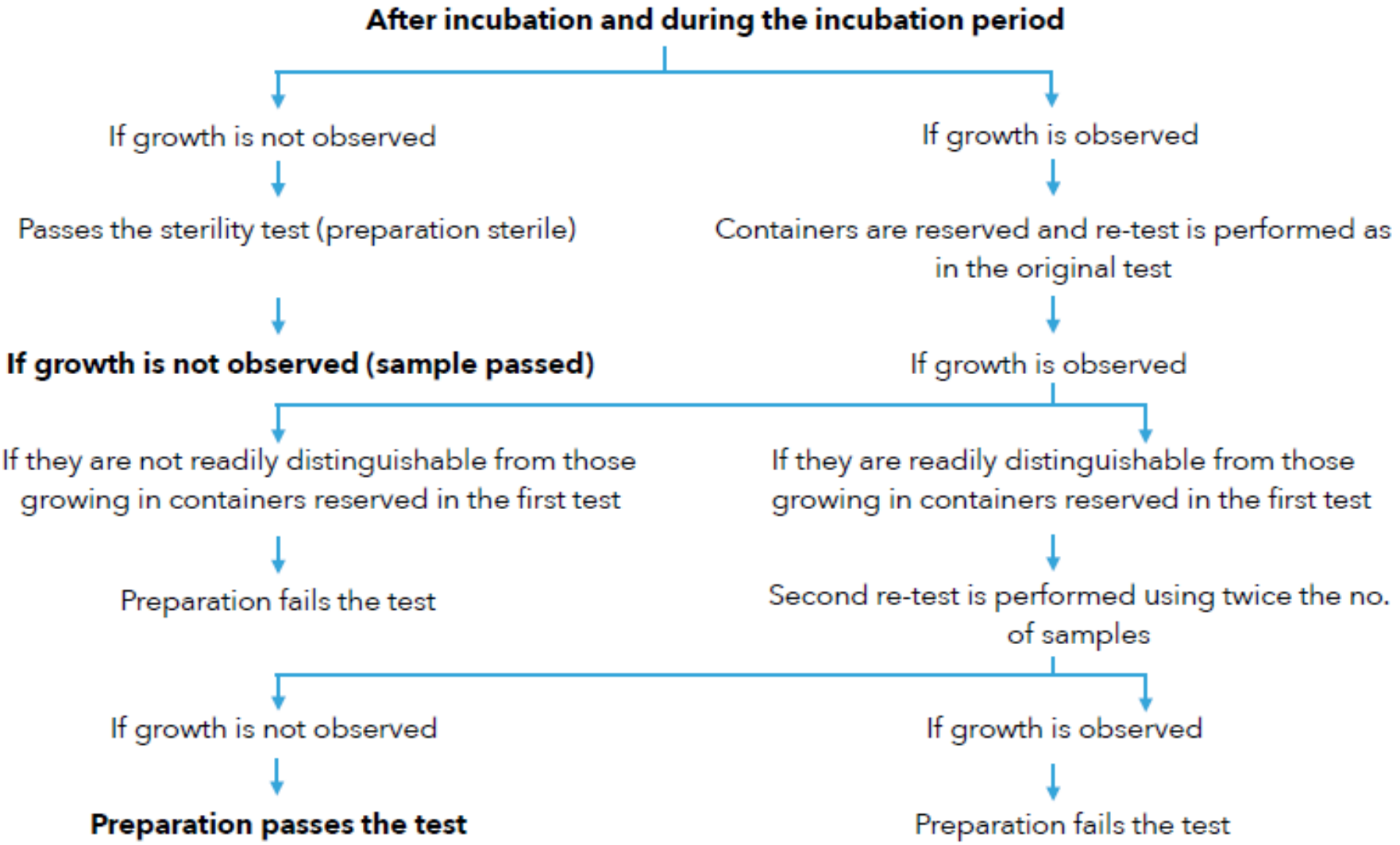
❖ Products ,volume & quantities, media preparation are given in the above tables

DIRECT INOCULATION METHOD

- It involves a **direct inoculation of required volume of a sample in two test tubes containing a culture medium that is FTM, SCDM.**
- Volume of the preparation under examination is not more than 10% of the volume of the medium.
- Incubate the inoculated media for not less than 14 days.

❖ Products, volume & quantities, media preparation are given in the above tables.

5. INTERPRETATION OF RESULTS



THANK YOU

You don't know the real stress until you have a phuchka in your mouth, a phuchka in your hand, a phuchka in your bowl and the phuchka wala is standing in front of you with another phuchka.