Sterility Testing

Disclaimer

All the content material provided here is only for teaching purpose.

NTRODUCTION

- 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.

NTRODUCTION

Which products undergo sterility tests?

- The test is applied to substances or preparations which, according to the Pharmacopoeia, are required to be sterile. For example
  - Injections
  - Implants
  - Syringes
  - Bandages
  - Dressings
  - Surgical Instruments
  - Needles
  - Injectables
  - Bulk Solids

What precautions should be taken while performing sterility tests?

- The tests for sterility are carried out in aseptic regions to avoid accidental contamination by microorganisms.
- 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.

PRINCIPLE

- If microorganisms are placed in a media that provides nutrients and water and kept at a favourable temperature the organism will grow and their growth can be indicated by turbidity in originally clear medium.
- The sterility tests provide optimum conditions for the growth and multiplication of organisms, spores, etc that might be a contaminant.
- It is not possible to claim that a batch of products is sterile unless the entire content of each batch has been tested.
- But these conditions are not possible because the article or the preparation under test is either made unstable (like a syringe) or is destroyed (like an injectable solution).
- Thus only a part of the batch can be sampled for testing.

STEPS INVOLVED IN STERILITY TESTING

1. Selection of the samplesize.
2. Selection of the quantity of the product.
3. Method of testing.
4. Observation and Results.

1.SELECTION OF SAMPLE SIZE

Quantity per Container	Minimum quantity to be used foreach medium unless otherwise justified and authorised
Parenteral preparations: • Notmore than 100 containers • More than 100 but not more than 500 containers • More than 500 containers	• 10 per cent or 4 containers whichever is greater • 10 containers • 2 per cent or 20 containers (10 containers for large- volume parenterals) whichever isless
Ophthalmic and othernon-injectable: • Notmore than 200 containers • More than 200 containers • If the product is presented in the form of single- dose containers, apply the scheme shown above for preparations for parenteral use	• 5 per cent or 2 containers whichever is greater • 10 containers
Bulk solid products: • Up to 4containers • More than 4 containers but not more than 50 containers • More than 50 containers	• Each container • 20 per cent or 4 containers whichever is greater • 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 eachmedium unless otherwise justified andauthorised
Liquids: • Less than 1ml • 1-40ml • Greater than 40ml and not greater than 100ml • Greater than 100ml Antibiotics	• Whole contents of eachcontainer • Half contents of each container but not less than 1ml • 20ml • 10 per cent of the contents of the container but not less than 20ml • 1ml
Insoluble preparations, creamsand 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 300mg • 300mg-5g • Greater than 5g	• The whole contents of eachcontainer • Half the contents of each container but not less than 50mg • 150mg • 500mg

3.TESTMETHODS

- Method A: Membrane Filtration method
- Method B: Direct Inoculationmethod

MEMBRANE FILTRATION METHOD

- Membrane has a nominal pore size not greater than 0.45 micron and diameter of approximately 50mm.
- 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.
- 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.

CULTUREMEDIUM

- Properties:

  Must initiate and maintain vigorous growth of small numbers of aerobic or anaerobic bacteria including spores.

  Thus, must provide sufficient moisture, adequate pH, nutrients, suitable Redox potential.

- Classification:

1. For detection of AEROBES:

   Peptone Broth

   Glucose Peptone Broth

2. For detection of ANAEROBES: Cooked

   Meat Medium

   Semi Fluid Meat Medium Liver

CULTUREMEDIUM

3. For both AEROBES and ANAEROBES:

   Fluid Thioglycolate Media Thioglycolate

   Broth Media

   Corn Steep Liquor-Sodium Thioglycolate Media

   Semi-FLuid Hydrosulphite Media

4. For detect of AEROBIC and LOWER FUNGI:

   Soybean Caesin Digest Media Sabourould's

   Media

THIOGLYCOLATE MEDIUM

L-Cystine Agar	0.5 g
Sodium chloride	0.75 g
Glucose monohydrate/anhydrous Yeast	2.5 g
extract (water-soluble) Pancreatic digest	5.5/5.0 g
of casein Sodium thioglycollate or	5.0 g
Thioglycollic acid	15.0 g
Resazurin sodium solution (1 g/l of resazurin sodium), freshly p	0.5 g
Water R	0.3 ml
	1.0 ml
Sterilise in autoclave at 121 C for 20 mins pH	Upto 1000 ml

after sterilization 6.9 to 7.3.

ALTERNATIVE THIOGLYCOLLATE MEDIUM

- Contains no agar and no indicator
- Used with:

Turbid suspensions and viscid products (creams). For devices having tubes with small Lumina.

SOYBEAN CASEIN DIGEST MEDIUM

Pancreatic digest of casein Papaic	17.0 g
digest of soya-bean meal Sodium	3.0 g
chloride	5.0 g
Dipotassium hydrogen phosphate	2.5 g
Glucose monohydrate/anhydrous	2.5/2.3 g
Water R	Upto 1000 ml

pH after sterilization 7.1 to 7.5.

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.

4 INTERPRETATION OF RESULTS

After incubation and during the incubation period

If growth is not observed

Passes the sterility test (preparation sterile)

If growth is not observed (sample passed)

If they are not readily distinguishable from those growing in containers reserved in the first test

Preparation fails the test

If growth is not observed

Preparation passes the test

If growth is observed

Preparation fails the test

If growth is observed

If they are readily distinguishable from those growing in containers reserved in the first test

Second re-test is performed using twice the no. of samples

If growth is observed

Preparation fails the test

If growth is observed

Containers are reserved and re-test is performed as in the original test

If growth is observed

REFERENCES

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