Sterility Test - Introduction

Sterility – Process of removing all viable forms of microorganisms.

Sterility test – A test that critically assesses whether a sterilized pharmaceutical product is free from contaminating microorganisms.

(or)

Acc to IP – The sterility tests are intended for testing the absence of viable forms of microorganisms in or on the pharmacopeial preparations.

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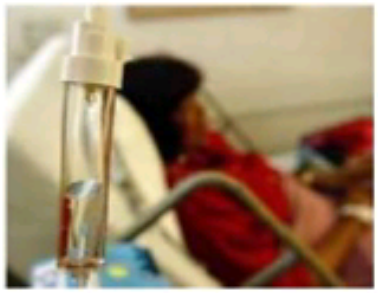
Products which are necessary to be sterilized :



- Injections
- Implants
- Syringes
- Ophthalmic preparations
- Ointments & creams
- Bandages
- Surgical dressings & devices
- needles

PRINCIPLE

- These tests are based upon the principle that if micro-organisms (present in the sample) are placed in a medium which provides nutritive material and water, and kept at a favorable temperature, the organisms will grow and their presence can be indicated by a turbidity in the originally clear medium.
- The interpretation of results is based on the assumption that the contents of every container in the batch, had they been tested, would also have given the same results.
- Since every container cannot be tested, a sufficient number of containers should be examined to give a suitable degree of confidence in the results of the tests.

CULTURE MEDIA (IP/USP/BP)

A. FLUID THIOGLYCOLLATE MEDIUM

Ingredients	Qty	Function
L-Cystine	0.5g	antioxidant
NaCl	2.5g	Isotnicity
Dextrose	5.5g	Reducing agent, carbon source
Granular agar	0.75g	Viscosity enhancer
Yeast extract	5.0g	Growth promoter
Pancreatic digest of casein	15.0g	Nitrogen source
Sodium thioglycollate	0.5g	Reducing agent
Thioglycollic acid	0.3ml	–
Resazurin(0.1%)	1.0ml	Oxidation-reduction indicator
Distilled water	1000ml	

→ Primarily intended for the culture of aerobic & anaerobic bacteria

B. ALTERNATIVE THIOGLYCOLLATE MEDIUM

→ It Contains no agar

C. SOYABEAN CASEIN DIGEST MEDIUM

Ingredient	Quantity	Function
Pancreatic digest of Casein	17g	"C" , "N" & essential amino acids
Pancreatic digest of Soya bean meal	3g	"C", "N" & essential amino acids
Sodium chloride	5g	Isotonicity
Dibasic Potassium Phosphate	2.5g	Reducing agent "C" source
Distilled water	1000ml	

→ Suitable for culture of both fungi & aerobic bacteria

MEDIA SUITABILITY TEST

Prior to test, make sure that :

- Media is sterile
- Media supports growth of microorganisms

2 components in Media suitability test :

- Media sterility test
- Growth Promotion test

MEDIA SUITABILITY TEST - ctnd

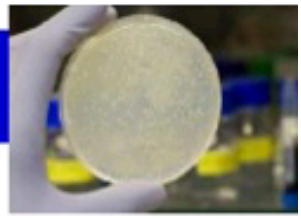
Media sterility

- Negative Control - may be used to identify a "false positive" test result
- Incubate for 14 days prior to use, may be conducted concurrently with test
  - 30 - 35°C for Fluid Thioglycollate medium (FTM)
  - 20 - 25°C for Soybean Casein Digest Medium (SCD/TSB)

Acceptance criteria:

- Should be sterile, no growth observed

MEDIA SUITABILITY TEST - ctnd



Growth Promotion Test

- To test the ability of media to support the growth of micro-organisms
- The media should be inoculated with <100 cfu of challenge organisms. The challenge inoculum should be verified by concurrent viable plate counts
- Growth promotion challenge organisms should show clearly visible growth in the test media within 3 days for bacteria and 5 days for fungi.

ACCORDING TO IP

Table 2

Medium	Test micro-organism	Incubation		
		Temp (°)	Duration	Type of microorganism
Fluid Thioglycollate	1. *Clostridium sporogenes* (ATCC[1] 19404)	30 to 35	3 days	Anaerobic
	2. *Staphylococcus aureus* (ATCC 6538)	30 to 35	3 days	Aerobic
	3. *Pseudomonas aeruginosa* (ATCC 9027)[2]	30 to 35	3 days	Aerobic
Alternative Thioglycollate	1. *Bacteroides vulgatus* (ATCC 8482)[3]	30 to 35	3 days	Anaerobic
	2. *Clostridium sporogenes* (ATCC 19404)	30 to 35	3 days	Anaerobic
	3. *Bacillius subtilis* (ATCC 6633; NCIMB[4] 8054)	30 to 35	3 days	Aerobic
Soyabean-Casein Digest	1. *Aspergillus brasiliensis* (ATCC 16404)	20 to 25	5 days	Aerobic
	2. *Candida albicans* (ATCC 10231; ATCC 2091; NCYC[5] 854)	20 to 25	5 days	Aerobic
	3. *Bacillius subtilis* (ATCC 6633; NCIMB 8054)	30 to 35	3 days	Aerobic

BP/USP *Strains of the test micro-organisms suitable for use in the Growth Promotion Test and the Validation Test*

Aerobic bacteria	
Staphylococcus aureus	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518
Bacillus subtilis	ATCC 6633, CIP 52.62, NCIMB 8054
Pseudomonas aeruginosa	ATCC 9027, NCIMB 8626, CIP 82.118
Anaerobic bacterium	
Clostridium sporogenes	ATCC 19404, CIP 79.3, NCTC 532 *or* ATCC 11437
Fungi	
Candida albicans	ATCC 10231, IP 48.72, NCPF 3179
Aspergillus niger	ATCC 16404, IP 1431.83, IMI 149007

VALIDATION TEST (IP/BP/USP)

- Carry out a test as described below under Test for Sterility of the Product to be Examined using exactly the same methods, except for the following modifications.

Membrane Filtration

- After transferring the content of the container or containers to be tested to the membrane, add an inoculum of a small number of viable microorganisms (not more than 100 cfu) to the final portion of sterile diluent used to rinse the filter.

Direct Inoculation

- After transferring the contents of the container or containers to be tested to the culture medium, add an inoculum of a small number of viable microorganisms (not more than 100 CFU) to the medium

VALIDATION TEST - ctnd

This validation is performed

(a)when the test for sterility has to be carried out on a new product; and

(b whenever there is a change in the experimental conditions of the test. The validation may be performed simultaneously with the Test for Sterility of the Product to be Examined

A. MEMBRANE FILTRATION

It is preferred where the substance under examination is :

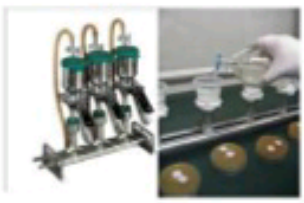
- An oil
- An ointment that can be put into the solution
- A non bacteriostatic solid not readily soluble in the culture medium.
- A soluble powder or a liquid that possess inherent bacteriostatic or fungistatic properties.
- For liquid products where the volume in a container is 100m1 or more.

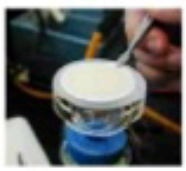
Sterility Test – Test Methods (cont.)

PROCEDURE :

- A membrane has a nominal pore size not greater than 0.45µ and diameter of approximately 50mm.
- This method basically involves filtration of Sample through membrane filters.

Sample been filtered and rinsed → Membrane filter is cut into half → Membrane into medium → Incubate





Sterility Test – Test Methods (cont.)

- The filtration is assisted under strict aseptic condition. After filtration gets completed, remove the membrane from the holder aseptically.
- Cut into 2 halves. Place each half in a suitable volume (usually 100ml) of FTM and SCDM respectively and incubate.

Incubation

- **Period** : at least 14 days incubation
- **Temperature** : 30-35° C for FTM
  20-25 ° C for SCD

B. DIRECT INNOCULATION

It is the more traditional sterility test method. Basically, it involves 3 steps:

1. Aseptically opening each sample container from a recently sterilized batch of product.
2. Using a sterile syringe and needle to withdraw the required volume of sample for both media from the container
3. Injecting one-half of the required volume sample into a test tube containing the required volume of FTM and the other half volume of sample into a second test tube containing the required volume of SCD and incubating both.

INTERPRETATION OF RESULTS

After the incubation and during the incubation period

If growth is not observed

Passes the test of sterility
(preparation is sterile)

If growth is not observed
(passes the test)

If growth is observed

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

If growth is not observed

Preparation passes the test

If growth is observed

microorganisms are isolated & identified

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

Preparation fails the test

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 not observed

Preparation passes the test

If growth is observed

Preparation fails the test