STERILITY TEST

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Introduction, Principle, Test For Sterility, Method For Sterility Test, Interpretation of result of sterility test.

INTRODUCTION :-

The test for sterility are intended for detecting the presence of viable forms of microorganism in pharmaceutical preparation. A number of product are necessary to be sterilized such as ophthalmic preparation, injection, infusion, implants, syringes, bandages, dressing, needles, surgical instruments, etc. and must be checked for the contamination of microorganism (bacteria and fungi). The test must be carried out in an aseptic area to avoid accidental contamination of the product during the test.

PRINCIPLE:-

1.This test are base upon the principle that if microorganism (present in the sample) are place in a medium which provide nutritive material and water and kept at a favorable temperature, the organism will grow and their presence can be indicated by a turbidity in the originally clear medium.

2.The interpretation of result is based on the assumption that the contents of every container in the batch, had they have been tested, would also given the same result.

3. since every container cannot be tested, a suffiint number of container should be examined to give a suitable of confidence in the result of testes.

TEST FOR STERILITY:-

The test for sterility are as follows :-

1. Culture Media
2. Sutability of Media

Table 1 – Number of items recommended to be tested in the batch (as per I.P)

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

1) Culture Media :-

The following media are used for test of sterility. The composition of media used for sterility test is given in the table.

A. Fluid thioglycollate medium (FTM)

B. Alternative thioglycollate medium (ATM)

C. Soyabean casein digest medium (SCOM)

Table 2 – Composition of media used for sterility test

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	

2) Sutability of Media :-

The media used for sterility test should comply the following test.

A. Sterility of Media

B. Growth Promotion Test

Table 3 – Test Microorganism for growth promotion test of media

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

METHODS FOR STERILITY TEST :-

Sterility test can be carried out by using following methods :

1. Method A : Membraine Filtration.

2. Method B: Direct Inoculation.

Method A : Membraine Filtration :

This method is to be preferred where the substances being examine is (a) an oil, (b) an ointment that can be put into solution, (c) a non-bactirio static solid not readily soluble in the culture medium, and (d) a soluble powder or liquid that possesses inherent bacteriostatic and fungistatic Properties.

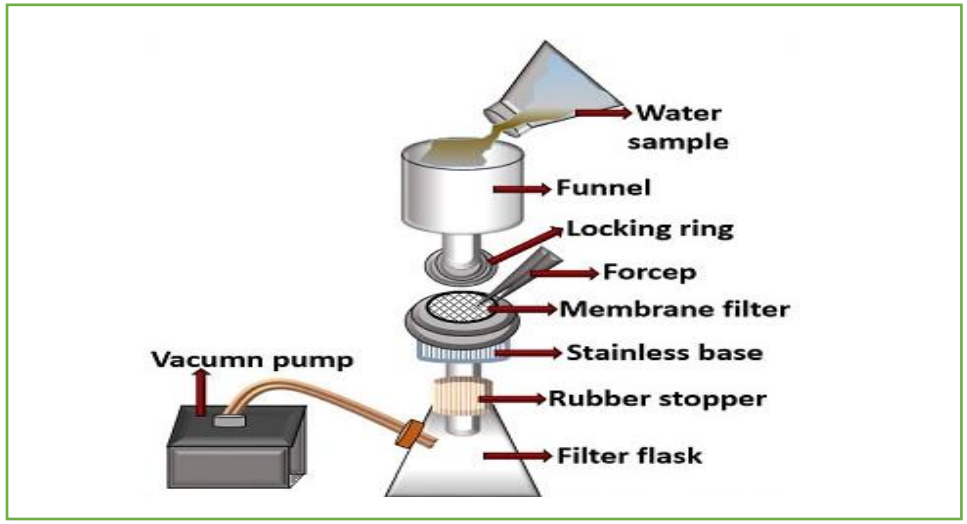


Fig.no.1 - Membraine Filtration

Table 4 – quantites of injectable preparation used for sterility testing

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

Method B: Direct Innoculation :-

Quantities of sample to be used :

The quantities of substance or preparation being examined which is to be used for innoculation in the culture media varies according to the quantity in each container

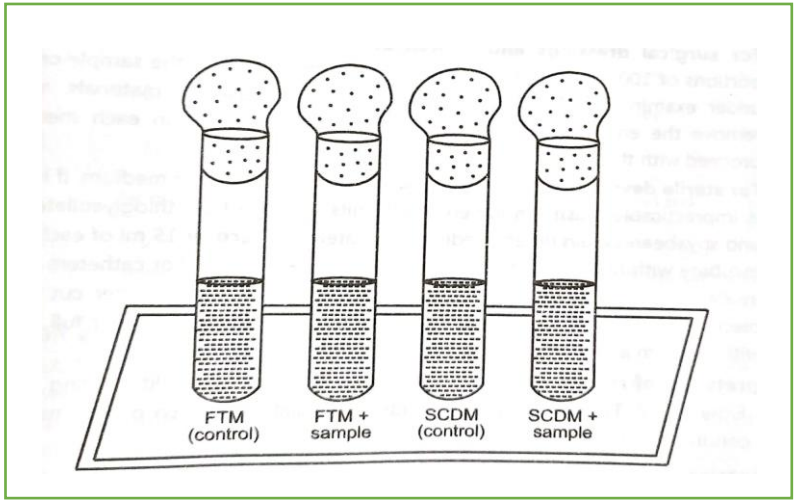


Fig no. 2 – Sterility Testing by Direct Innoculation

Method of Test :

1. For aqueous solution and suspensions.

2. For oils and oily solution.

3. For ointment and creams.

INTERPRETATION OF RESULTS :-

Observatioon and interpretation of sterility testing results are shown in figure.

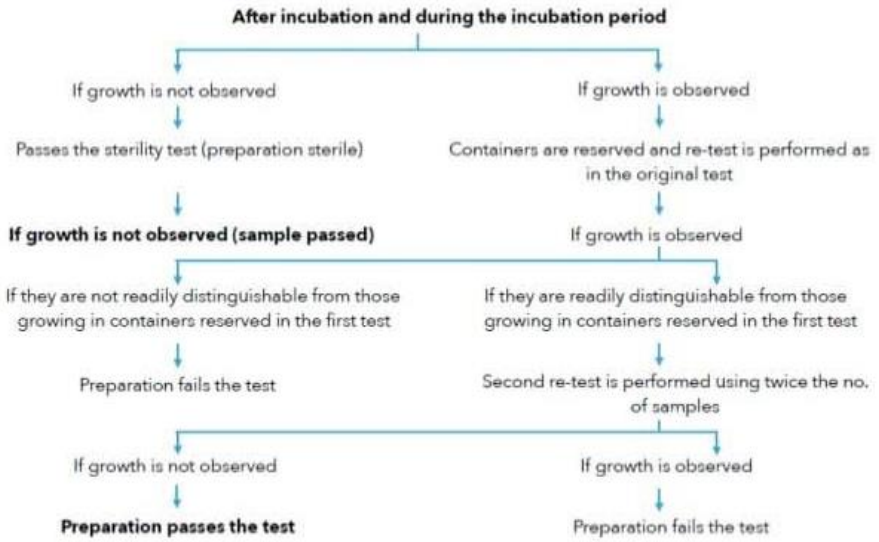
The test may be considered invalid only when one or more of the following condition are fulfilled :

1. Microbial growth is found in the negative controls.

2.Data on microbial monitoring of the sterility testing facility show a fault.

3. A review of the testing procedure used for the test in question revels of fault.

Table 5 – Interpritation of results of test for sterility



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