National Pharmaceutical Control Bureau
MINISTRY OF HEALTH MALAYSIA

World Health Organization
WHO Collaborating Centre for Regulatory Control of Pharmaceuticals

PIC/S
Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme

QUALITY SYSTEM
SIRIM
Certified to ISO 9001:2000
Cert. No: AR 2293

STANDARDS MALAYSIA
ACCREDITED LABORATORY
MS ISO/IEC 17025:2005
[illegible]

STERILITY TEST (ST)

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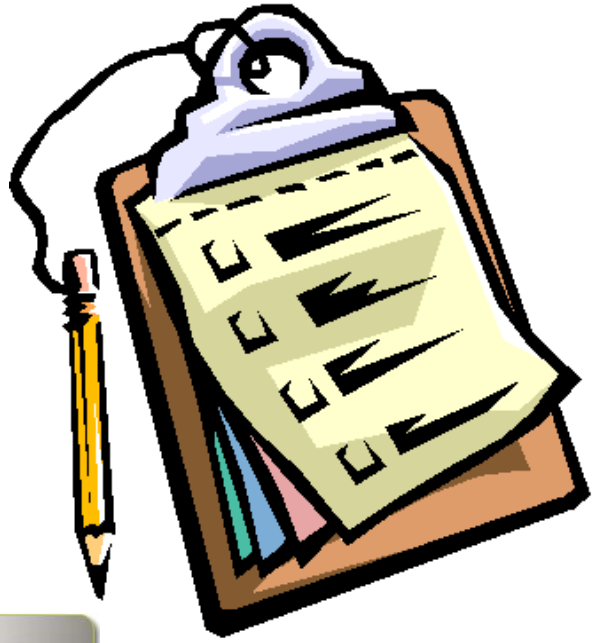


Sterility Test - Introduction

Definition : The sterility of a product is defined by the absence of viable and actively multiplying microorganisms when tested in specified culture media.

The test is applied to substance, preparations or articles which, according to the Pharmacopoeia, are required to be sterile.

Sterility Test - Introduction

Turbidity in the broth media usually indicates contamination.

- Test is performed on the end –product and is one of the quality control tests specified for release of a batch of sterile product.

Certificate of Analysis

Specification and Result

- As per British Pharmacopoeia or USP
- BP – **Appendix XVI A. Sterility**

Sterility Test – Media Validation

Media types

- Fluid Thioglycollate medium (FTM)
- Soybean Casein Digest Medium (SCD or TSB)

Sterility Test – Media Validation *(cont.)*

Prior to test, make sure that:

- Media is sterile
- Media supports growth of microorganisms

2 components in Media validation :

- Media sterility Test
- Growth Promotion Test

Sterility Test – Media Validation *(cont.)*

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

Sterility Test – Media Validation *(cont.)*

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.

Sterility Test – Media Validation (cont.)

Table 2.6.1.-1 – *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

Sterility Test – Test Methods

Methods are defined in Pharmacopoeia:

- Membrane Filtration Method
  - (open or a closed system)
- Direct Inoculation Method

**When the preparation to be tested has an antimicrobial effects, these effects must be reduced or neutralised by adding an appropriate substance to the specified test media, to diluents or solvents, or to the preparation prior to testing.*

Sterility Test – Test Methods *(cont.)*

- Membrane Filtration Method (Open Funnel Method)

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

Sterility Test – Test Methods *(cont.)*

- Membrane Filtration Method (Closed System Method)

Sample been filtered and rinsed → **Adding medium (FTM/SCD) into apparatus** → **Incubate**

Sterility Test – Test Methods *(cont.)*

Direct Inoculation of the culture medium

- Transfer the preparation directly into the culture medium
- Volume of the product is not more than 10% of the volume of the medium.

Table 2.6.1.-2 – *Minimum quantity to be used for each medium*

Quantity per container	Minimum quantity to be used for each medium unless otherwise justified and authorised
Liquids	
– less than 1 ml	The whole contents of each container
– 1-40 ml	Half the contents of each container but not less than 1 ml
– greater than 40 ml and not greater than 100 ml	20 ml
– greater than 100 ml	10 per cent of the contents of the container but not less than 20 ml
Antibiotic liquids	1 ml
Other preparations soluble in water or in isopropyl myristate	The whole contents of each container to provide not less than 200 mg
Insoluble preparations, creams and ointments to be suspended or emulsified	The whole contents of each container to provide not less than 200 mg
Solids	
– less than 50 mg	The whole contents of each container
– 50 mg or more but less than 300 mg	Half the contents of each container but not less than 50 mg
– 300 mg to 5 g	150 mg
– greater than 5 g	500 mg
Catgut and other surgical sutures for veterinary use	3 sections of a strand (each 30 cm long)

Sterility Test – Test Methods *(cont.)*

Incubation

- Period : At least 14 days incubation
- Temperature : 30-35°C for FTM
  20-25°C for SCD/TSB

Sterility Test – Test Methods *(cont.)*

Incubation and Examination

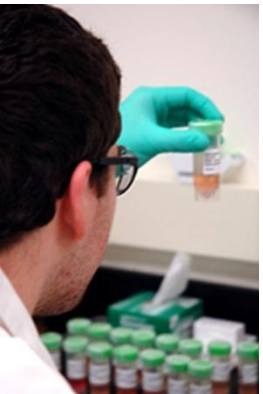
- All test & sterility control containers – incubated for at least 14 days (unless microbial contamination detected earlier)
- Examine for evidence growth
- Preparation not readily seen (turbid/cloudy due to its nature) – after 14 days of incubation ⇨ transfer a suitable portion (2-5% of contents) to fresh, same medium ⇨ incubate for 7 days

Sterility Test – Interpretation of results

- No evidence of microbial growth is found.

If turbidity or other evidence of growth is seen:

- Streak on solid media
- Examine the suspected growth microscopically by Gram stain
- Identify the isolates, as far as the genus and preferably species level

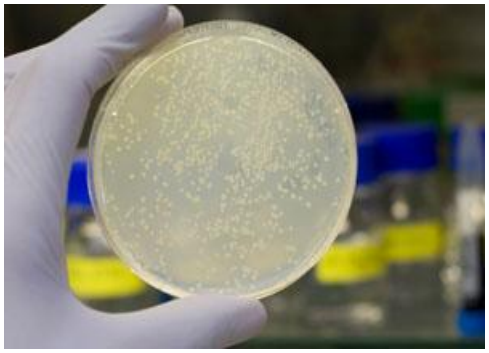


Sterility Test – Method Validation

Validation (bacteriostasis & fungistasis)Test

- The test should be validated by inoculation with <100 cfu of challenge organism strains to the media/product container at the beginning of the test incubation period.

- The challenge inoculum should be verified by concurrent viable plate counts.



Sterility Test – Method Validation *(cont.)*

Validation (bacteriostasis & fungistasis)Test

- The challenge organisms, preferably, should be added directly to the product prior to membrane filtration or direct inoculation. If this is not practicable, the challenge organisms should be added to the last rinse solution (membrane filtration) or directly to media containing the product (direct inoculation).
- Validation done should mimic the test proper in every detail.
- Perform a growth promotion test as a positive control. Incubate all the containers containing medium for not more than 5 days.

Sterility Test – Method Validation (

Interpretation of results

- Challenge organisms should clearly show visible growth of bacteria within 3 days, and fungi within 5 days in the test media containing product.

- Visually comparable to that in the control vessel without product.

Sterility Test – Method Validation *(cont.)*

Validation (bacteriostasis & fungistasis)Test

- If performed concurrently with ST should confirmed validation tests as successful before the results of the ST are interpreted
- Validation to be performed on all new product and repeated whenever there is a change in the experimental conditions.

Checklist

Test	Document Required	Method	Results (Raw data)
CoA	1. Specification and Results	-	-
Validation Test	1. Sterility Test (Bacteriostasis and Fungistasis Test)	√	√
Routine Test	1. Sterility Test	√	√
	2. Growth Promotion Test	√	√
	3. Test for Media Sterility	√	√

Comments for ST:

Ujian Steriliti(ST):

1. Sila kemukakan tatacara pengujian(SOP) untuk yang berikut, berserta keputusan ujian (raw data) bagi satu kelompok keluaran siap:

   - Growth Promotion Test dan Media Sterility Test untuk semua media yang digunakan.

   - Ujian steriliti.

   - Validasi untuk ujian steriliti (Bacteriostasis & Fungistasis Test)

   Tatacara hendaklah spesifik kepada produk. Salinan terus dari farmakopoeia tidak diterima.

2. Tatacara ujian steriliti dan Validasi ujian steriliti perlu lengkapkan dengan butiran seperti di bawah bagi:

   i) Bilangan sample/ volum produk yang diguna untuk ujian.

   ii) Tatacara yang diguna (Membrane Filtration/ Direct Inoculation)

   iii) Composition rinsing buffer.

   iv) Volum rinsing buffer yang diguna untuk setiap membrane.

   v) Cara penyediaan sampel

3. Kesemua raw data yang dikemukakan perlu mengandungi nama dan nombor kelompok bagi Finished Product, tarikh mula dan selesai pengujian, keputusan pemerhatian setiap hari (contoh: pemerhatian selama 14 hari bagi Media Sterility Test dan ujian steriliti, berserta pemerhatian selama 3-5 hari bagi Growth Promotion Test dan Validasi ujian steriliti) & tandatangan/ nama penganalisis.

4. Sila kemukakan terjemahan bahasa Inggeris sekiranya data adalah dalam bahasa negara asing.

Comments for ST:

Sterility Test (ST):

1. Please provided test method (SOP) and 1 batch result in raw data for below:
   - Growth Promotion Test and Media Sterility Test for all the medium used.
   - Sterility Test.
   - Validation of sterility test (Bacteriostasis & Fungistasis Test)

   Test method must specific to this product and photocopy from Pharmacopoeia is not acceptable.
2. Test method for sterility test and validation must stated in details as below:
   i) Number of sample tested or volume sample
   ii) Method used (Membrane Filtration/ Direct Inoculation)
   iii) Composition of rinsing buffer.
   iv) Volume of rinsing buffer used in each membrane
   v) Sample preparation
3. All the results in raw data must include product's name, batch number, starting date and finished date, observation result in interval period (ex: observation for 14 days in sterility test and Media Sterility Test, observation for 35 days in Growth Promotion Test and validation of sterility test) & analyst's name and signature.
4. Please translate into English or BM if data in others language.


Any questions?


Thank you!