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]

MICROBIAL CONTAMINATION TEST (MCT)

Centre for Quality Control

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OUTLINE

- **Introduction**
- **Certificate of Analysis**
- **Media Validation**
- **Test Method**
  - Total Viable Aerobic Count
  - Test for Specified Microorganisms
- **Method Validation**

Introduction - **Microbial Contamination Test (MCT)**

- **Microbial Contamination Test is conducted on non-sterile products to check:**
  - The level of microbial (bacterial and fungal) contamination
  - Presence/ absence of certain pathogenic microorganism

  in order to assure product safety.

- **Types of samples include:**

Capsule

Tablet

Aqueous preparation

Transdermal Patch

Cream

Pessary

Inhaler

Suppository

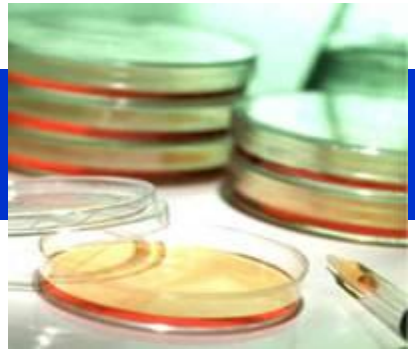
Certificate of Analysis

- **Specification and results**
  - refer **British Pharmacopoeia 2012**, Table 5.1.4-1 Acceptance criteria for microbiological quality of non-sterile dosage forms

Table 5.1.4.-1. – *Acceptance criteria for microbiological quality of non-sterile dosage forms*

Route of administration	**TAMC (CFU/g or CFU/mL)**	**TYMC (CFU/g or CFU/mL)**	**Specified micro-organisms**
Non-aqueous preparations for oral use	10^3	10^2	Absence of *Escherichia coli* (1 g or 1 mL)
Aqueous preparations for oral use	10^2	10^1	Absence of *Escherichia coli* (1 g or 1 mL)
Rectal use	10^3	10^2	-
Oromucosal use Gingival use Cutaneous use Nasal use Auricular use	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL)
Vaginal use	10^2	10^1	Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Candida albicans* (1 g or 1 mL)
Transdermal patches (limits for one patch including adhesive layer and backing)	10^2	10^1	Absence of *Staphylococcus aureus* (1 patch) Absence of *Pseudomonas aeruginosa* (1 patch)
Inhalation use (special requirements apply to liquid preparations for nebulisation)	10^2	10^1	Absence of *Staphylococcus aureus* (1 g or 1 mL) Absence of *Pseudomonas aeruginosa* (1 g or 1 mL) Absence of bile-tolerant gram-negative bacteria (1 g or 1 mL)
♦Special Ph. Eur. provision for oral dosage forms containing raw materials of natural (animal, vegetal or mineral) origin for which antimicrobial pretreatment is not feasible and for which the competent authority accepts TAMC of the raw material exceeding 10^3 CFU/g or CFU/mL.	10^4	10^2	Not more than 10^2 CFU of bile-tolerant gram-negative bacteria (1 g or 1 mL) Absence of *Salmonella* (10 g or 10 mL) Absence of *Escherichia coli* (1 g or 1 mL) Absence of *Staphylococcus aureus* (1 g or 1 mL)♦

Media Validation



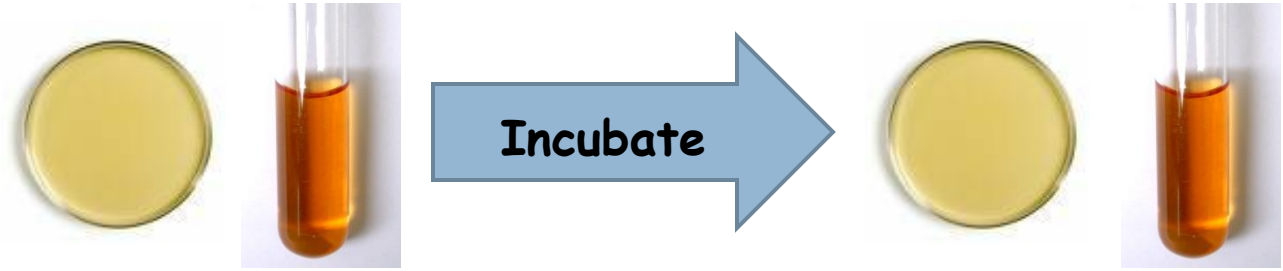
- **Prior to test, make sure that:**
  - ✓ Media is sterile
  - ✓ Media supports growth of microorganisms
  - ✓ Selective media is selective

    (promote certain organisms but inhibit non-target organisms)

- **In order to so,**
  - Test for Media Sterility
  - Test for Growth Promotion & Inhibitory Properties

Media Validation- Test for Media Sterility

- **To prevent False Positive result**
  - maybe due to contaminated media
- **To ensure the media is sterile**
- **Negative Control**
  - Use the chosen sterile diluents in place of the sample under test
  - Alternatively, incubate portions of the media for a few days at the specified temperature.
- **Acceptance criteria:** No growth observed



No growth of microorganisms observed

Media Validation-
Test for Growth Promotion and Inhibitory Properties

- **There are 2 categories of media used in MCT:**

1. General nutritive media

- used in Total Viable Aerobic Count
- suitable for cultivation of a wide variety of microorganisms
- e.g. Tryptone Soya Agar
- Test for Growth Promotion Properties

2. Selective media

- used in Test for Specified Microorganisms
- contains ingredients which promotes growth of certain organisms but inhibit other non-target microorganisms
- e.g. Mannitol Salt Agar, Cetrimide Agar, MacConkey Broth
- Test for Growth Promotion, Indicative and Inhibitory Properties

Media Validation- General Nutritive Media

❑ Test for Growth Promotion Properties

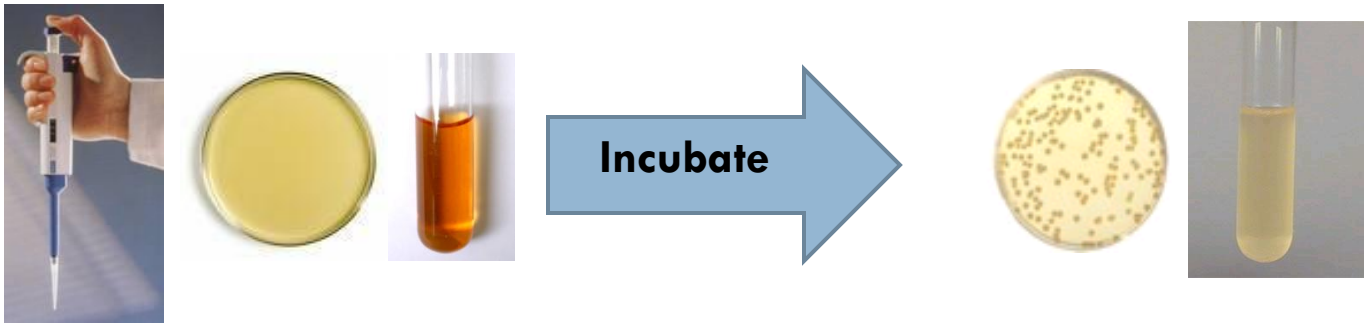
✓ To verify that media used are able to support growth of a wide variety of microorganisms

Test Method

• Inoculate portions/ plates of media with a small number (< 100 cfu) of microorganisms* indicated in Table 1.

• Use a separate plate of medium for each microorganism.

• Incubate at the specified temperature.



***Note: Microorganisms used should not be more than 5 passages removed from the original seed-lot.**

Media Validation- General Nutritive Media

Table 1- Media, Microorganisms and Test Condition for Growth Promotion Test

Test	Media Used	Microorganisms	Test Condition
Total Aerobic Microbial Count (TAMC)	Tryptone Soya Agar (TSA)	• Staphylococcus aureus • Pseudomonas aeruginosa • Bacillus subtilis • Candida albicans • Aspergillus brasiliensis	≤ 100 cfu 30 - 35°C, ≤ 3 days for bacteria and ≤ 5 days for fungi
	Tryptone Soya Broth (TSB)	• Staphylococcus aureus • Pseudomonas aeruginosa • Bacillus subtilis	≤ 100 cfu 30 - 35°C, ≤ 3 days
Total Yeasts and Moulds Count (TYMC)	Sabouraud Dextrose Agar (SDA)	• Candida albicans • Aspergillus brasiliensis	≤ 100 cfu 20 - 25°C, ≤ 5 days

Media Validation- General Nutritive Media

Acceptance Criteria

Solid media:

- Growth obtained must not differ by a factor of 2 (50-200%) from the calculated value for a standardized inoculum. (Quantitative)
- Growth of the microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs.

Liquid media:

- Clearly visible growth of microorganisms comparable to that previously obtained with a previously tested and approved batch of medium occurs

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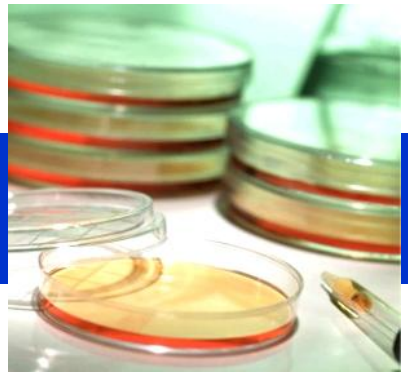
Media Validation - Selective Media



- **For media used in Test for Specified Microorganisms**
- **Tests for Growth Promotion, Indicative and Inhibitory Properties need to be conducted**

1. Test for Growth Promoting Properties

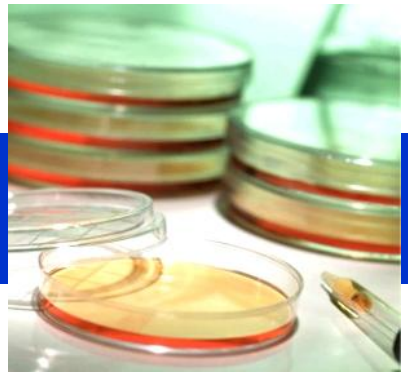
Liquid & Solid Media

1. Inoculate a portion of the medium with a small number (≤100 cfu) of the appropriate microorganism (Table 2). For Solid media, use surface spread method.

2. Incubate at the specified temperature for **not more than the shortest time** specified in the test.

Acceptance criteria: Clearly visible growth

Media Validation - Selective Media



2. Test for Inhibitory Properties

1. Inoculate the medium with **at least 100 cfu** of the appropriate microorganism (Table 2).
2. Incubate at the specified temperature for **not less than the longest time** specified in the test.

Acceptance criteria: No Growth of the test microorganisms occurs

3. Test for Indicative Properties

1. Inoculate each plate of medium using surface spread method with a small number **(≤100 cfu)** of the appropriate microorganism (Table 2).
2. Incubate at the specified temperature for a period of time **within the range** specified in the test.

Acceptance criteria: Colonies are comparable in appearance and indicative reactions to those previously obtained with a previously tested and approved batch of medium.

Media Validation - Selective Media

Table 2- Growth Promoting, Inhibitory and Indicative Properties of Media

Test for	Media	Property	Test Strain
Bile-Tolerant Gram Negative Bacteria	Enterobacteria Enrichment Broth (EEB)	Growth Promoting	E. coli P. aeruginosa
		Inhibitory	S. aureus
	Violet Red Bile Glucose Agar (VRBGA)	Growth Promoting & Indicative	E. coli P. aeruginosa
Escherichia coli	MacConkey Broth (MCB)	Growth Promoting	E. coli
		Inhibitory	S. aureus
	MacConkey Agar (MCA)	Growth Promoting & Indicative	E. coli
Salmonella	Rappaport Vassiliadis Salmonella Enrichment Broth (RVS)	Growth Promoting	Salmonella typhimurium or Salmonella abony
		Inhibitory	S. aureus
	Xylose, Lysine Deoxycholate Agar (XLD)	Growth Promoting & Indicative	Salmonella typhimurium or Salmonella abony
Pseudomonas aeruginosa	Cetrimide Agar (CETA)	Growth Promoting	P. aeruginosa
		Inhibitory	E. coli
Staphylococcus aureus	Mannitol Salt Agar (MSA)	Growth Promoting & Indicative	S. aureus
		Inhibitory	E. coli
Candida albicans	Sabouraud Dextrose Broth (SDB)	Growth Promoting	C. albicans
	Sabouraud Dextrose Agar (SDA)	Growth Promoting & Indicative	C. albicans

Test Method

- **MCT consists of 2 tests:**

1. Total Viable Aerobic Count (TVAC)

- Enumeration of bacteria and fungi present in the product
- Total Aerobic Microbial Count (TAMC)
- Total Yeast and Mould Count (TYMC)

2. Test for Specified Microorganism

- Qualitative: Presence or absence of specified microorganisms
- Semi Quantitative: Test for Bile-Tolerant Gram Negative Bacteria

***The type of specified microorganisms tested depends of the route of administration and the type of preparation**

Test Method - **Total Viable Aerobic Count (TVAC)**

- **The choice of method is based on factors such as the nature of product and the required limit of microorganisms.**

Membrane Filtration	Plate Count	Most Probable Number (MPN)
Suitable for soluble and filterable samples	Surface Spread & Pour Plate	Low precision and accuracy
Filter pore size ≤ 0.45 µm	Perform test at least in duplicate for each medium	Only for Total Aerobic Microbial Count (TAMC)
Bacteria retaining efficiency of filter not affected by sample	Take arithmetic mean count for each medium	May be suitable for samples with very low bioburden

Test Method- **TVAC Membrane Filtration**

- **Use sterilized filtration apparatus.**
- **Membrane pore size ≤ 0.45µm.**

- **Filter sample preparation containing 1g of product.**
- **Rinse the filter with an appropriate volume of diluent.**

- **Transfer the membrane filter to the surface of TSA and SDA for enumeration of TAMC and TYMC respectively.**
- **Incubate TSA at 30 - 35°C for ≥ 3 days and SDA at 20 - 25°C for ≥ 5 days.**

Test Method - TVAC Plate Count Method

or

Make a 1 in 10 dilution, using at least 10g or ml of product

Serial dilution (1:10)

30- 35˚C
3-5 days

TSA (duplicate)

TAMC: Count all colonies including fungi on TSA

SDA (duplicate)

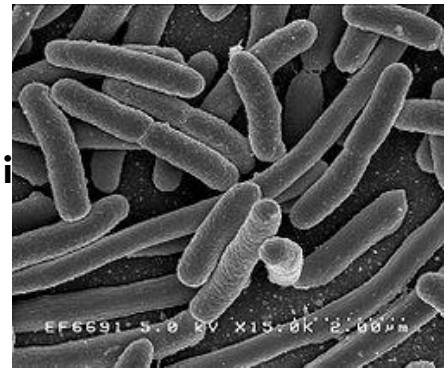
TYMC: Count all colonies including bacteria on SDA

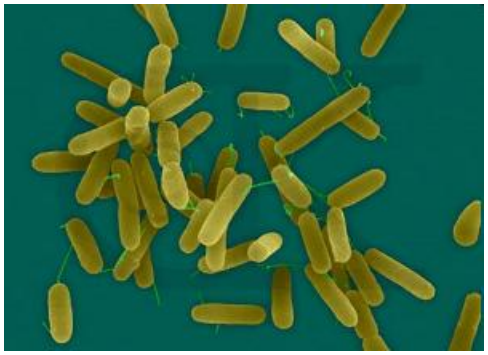
20- 25˚C
5 -7 days

Test Method - Test for Specified Microorganisms

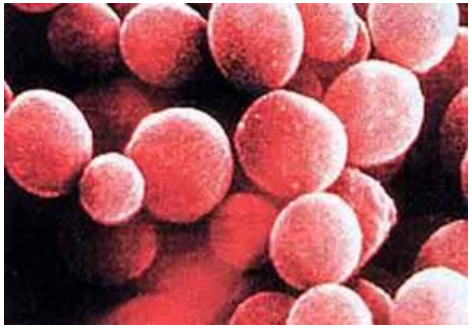
Specified microorganisms tested for in MCT are…

Escherichia coli



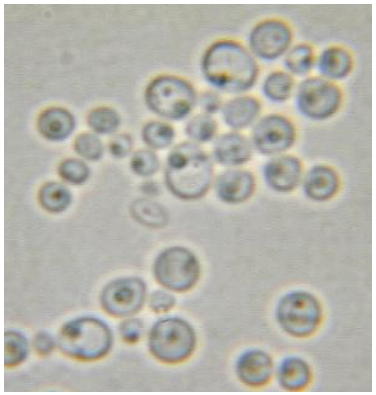


Pseudomonas aeruginosa



Staphylococcus aureus

Salmonella



Candida albicans

Test Method – Test for Staphylococcus aureus & Pseudomonas aeruginosa

Preparations tested include those of orocumosal, gingival, cutaneous, nasal, auricular and vaginal use and transdermal patches.

10 g sample

90ml of Buffered NaCl Peptone Solution

10 ml

90ml of Tryptone Soya Broth (TSB),

30 -35°C, 18 – 24hrs

Subculture on

30 -35°C, 18 – 72hrs

Mannitol Salt Agar (MSA)

Cetrimide Agar (CETA)

Test Method - **Test for** Escherichia coli

Preparations tested include aqueous and non- aqueous preparations, oral dosage forms containing natural origin and solely herbal medicinal products.

10g/10 ml sample

↓

90ml of Buffered NaCl Peptone Solution

↓ **10 ml**

90ml of Tryptone Soya Broth (TSB),

30 -35°C, 18 – 24hrs

↓ **1 ml**

100ml of MacConkey Broth (MCB),

42 - 44°C, 18 – 72hrs

↓ **Subculture on**

30 -35°C, 18 – 72hrs

MacConkey Agar (MCA)

Test Method - **Test for** Candida albicans

Preparation tested is those of vaginal use.

10 g sample

90ml of Buffered NaCl Peptone Solution

10 ml

100ml of Sabouraud Dextrose Broth (SDB), 30 -35°C, 3 – 5 days

Subculture on

30 -35°C, 24- 48hrs

Sabouraud Dextrose Agar (SDA)

Test Method - Test for Bile Tolerant Gram-Negative Bacteria

Preparations tested include products for inhalation, oral dosage forms containing natural origin and solely herbal medicinal products.

10 g/ 10ml sample

90ml of Tryptone Soya Broth (TSB),
20 -25°C, 2 - 5hrs

Enterobacteria Enrichment Broth- Mossel (EEB),
30 -35°C, 24 - 48hrs

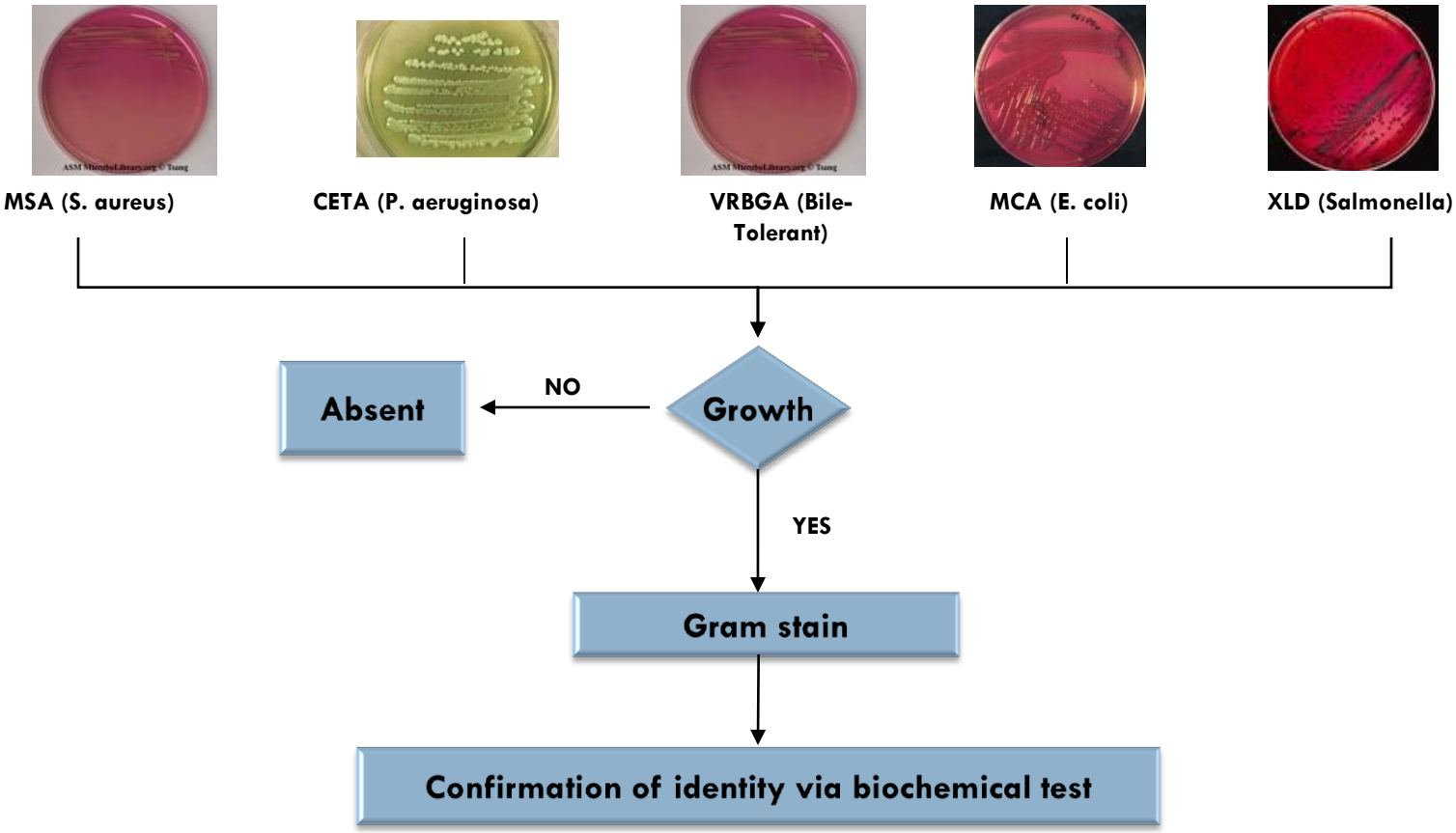
Subculture on

30 -35°C, 18 – 24hrs

Violet Red Bile Glucose Agar (VRBGA)

Test Method - **Test for Specified Microorganism**

- If growth is observed on selective agar, gram stain and identify the bacteria



Method Validation

- **Also known as 'Suitability of the Counting Method/ Test Method'**
- **To establish the ability of the chosen test method to detect microorganisms in the presence of product**
- **Product specific, i.e. need to conduct MCT validation on every product**
- **If the product contains antimicrobial ingredient/activity (e.g. antibiotic, preservative), this should be insofar possible removed or neutralised**
- **If surface active substance are used for sample preparation, their absence of toxicity for organisms and their compatibility with inactivators must be demonstrated**
- **Suitability must be confirmed if any changes which may affect the test outcome is introduced (e.g. change in formulation, change in API or preservative content)**

How?
Spike a small number of microorganisms into the product, run the test as per the chosen method, and check if the method is able to recover the microorganisms

Validation of Total Viable Aerobic Count by Plate Count Method

Objective: To demonstrate the ability of the test method to detect microorganisms present in the product

- Conducted in the presence & absence of product
- Spiked known number of microorganisms

(to obtain an inoculum of **NMT 100 CFU**. The volume of the suspension of the inoculum **should not exceed 1%** of the volume of diluted product.)

$$\text{Recovery} = \frac{\text{mean no. of colonies in presence of product}}{\text{mean no. of colonies in absence of product}} \times 100\%$$

Acceptance Criteria: Mean count of any test organisms not differing by a factor greater than 2 (50% – 200%)

Validation of Total Viable Aerobic Count by Plate Count Method

In presence of product

10 g/ 10ml sample

90ml buffered NaCl- peptone (1: 10)

+ 1 ml of microorganisms suspension

TSA / SDA (duplicate)

Incubate

Count the mean no. of colonies on plates

10ml diluents

In absence of product

90ml buffered NaCl- peptone (1: 10)

+ 1 ml of microorganisms suspension

TSA / SDA (duplicate)

Incubate

Count the mean no. of colonies on plates

Validation of Total Viable Aerobic Count by Plate Count Method

In presence of product

10 g/ 10ml sample

90ml buffered NaCl-peptone (1: 10)

1 ml **1 ml**

TSA / SDA (duplicate)

+ 10 µL microorganisms suspension

Incubate

Count the mean no. of colonies on plates

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Validation of Total Viable Aerobic Count by Plate Count Method

In presence of product

10 g/ 10ml sample

90ml buffered NaCl-peptone (1: 10)

10 ml

+ 100 µL microorganisms suspension

TSA / SDA (duplicate)

Incubate

Count the mean no. of colonies on plates

Validation of Total Viable Aerobic Count by Plate Count Method

British Pharmacopoeia 2012:

4-5 Suitability of the counting method in the presence of product

4-5-1 Preparation of the sample The method for sample preparation depends upon the physical characteristics of the product to be tested. If none of the procedures described below can be demonstrated to be satisfactory, an alternative procedure must be developed.

↓

4-5-2 Inoculation and dilution Add to the sample prepared as described above (4-5-1) and to a control (with no test material included) a sufficient volume of the microbial suspension to obtain an inoculum of not more than 100 CFU. The volume of the suspension of the inoculum should not exceed 1 per cent of the volume of diluted product.

To demonstrate acceptable microbial recovery from the product, the lowest possible dilution factor of the prepared sample must be used for the test. Where this is not possible due to antimicrobial activity or poor solubility, further appropriate protocols must be developed. If inhibition of growth by the sample cannot otherwise be avoided, the aliquot of the microbial suspension may be added after neutralisation, dilution or filtration.

↓

4-5-4-2 Plate-count methods Perform plate-count methods at least in duplicate for each medium and use the mean count of the result.

4-5-4-2-1 Pour-plate method

For Petri dishes 9 cm in diameter, add to the dish 1 mL of the sample prepared as described under 4-5-1 to 4-5-3 and 15-20 mL of casein soya bean digest agar or Sabouraud-dextrose agar, both media being at not more than 45 °C. If larger Petri dishes are used, the amount of agar medium is increased accordingly. For each of the micro-organisms listed in Table 2.6.12.-1, at least 2 Petri dishes are used. Incubate the plates as indicated in Table 2.6.12.-1. Take the arithmetic mean of the counts per medium and calculate the number of CFU in the original inoculum.

Validation of Total Viable Aerobic Count by Plate Count Method

Media, Microorganisms and Test Condition for Validation of Total Viable Aerobic Count

Test	Media Used	Microorganisms	Test Condition
Total Aerobic Microbial Count (TAMC)	Tryptone Soya Agar (TSA)	• Staphylococcus aureus • Pseudomonas aeruginosa • Bacillus subtilis • Candida albicans • Aspergillus niger	≤ 100 cfu 30 - 35°C, ≤ 3 days for bacteria and ≤ 5 days for fungi
Total Yeasts and Moulds Count (TYMC)	Sabouraud Dextrose Agar (SDA)	• Candida albicans • Aspergillus niger	≤ 100 cfu 20 - 25°C, ≤ 5 days

Validation for Test for Specified Microorganisms

Example: Test for Specified Microorganisms in Topical Preparation

The specified microorganisms tested are:

i) Staphylococcus aureus

ii) Pseudomonas aeruginosa

Acceptance criteria:

S. aureus & Ps. Aeruginosa must be detected

10 g/ 10ml sample

90ml of Buffered NaCl Peptone Solution

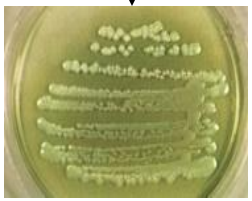
+ ≤100 cfu Ps. aeruginosa

10 ml

Enrichment:

90ml of Tryptone Soya Broth (TSB), 30 - 35°C, 18 – 24hrs

Subculture on



30 -35°C, 18 – 72hrs

Selective Agar: Cetrimide Agar (CETA)

Validation for Test for Specified Microorganisms

Example: Test for Specified Microorganisms in Pharmaceutical Oral Dosage Forms

The specified microorganisms tested are:

i) Escherichia coli

Acceptance Criteria:

E. coli must be detected

10 g/ 10 ml sample

↓

90ml of Buffered NaCl Peptone Solution

+ ≤ 100 cfu E. coli

10 ml ↓

Enrichment:

90ml of Tryptone Soya Broth (TSB), 30 -35°C, 18 – 24hrs

1 ml ↓

Selective Enrichment:

100ml of MacConkey Broth (MCB), 42 -44°C, 24 – 48hrs

Subculture on ↓

30 -35°C, 18 – 72hrs

Selective Agar: MacConkey Agar (MCA)

Checklist for MCT

Test	Document required	Method	Results (Raw data)
CoA	1. Specification and Results	-	-
Routine Test	1. Total Viable Aerobic Count (TAMC and TYMC)	√	√
	2. Test for Specified Microorganism	√	√
	3. Test for Growth Promoting, Indicative and Inhibitory Properties of Media	√	√
	4. Test for Media Sterility	√	√
Validation Test	1. Total Viable Aerobic Count (TAMC and TYMC)	√	√
	2. Test for Specified Microorganism	√	√

Comments for MCT (BM)

Ujian Kontaminasi Mikrobial (MCT):

1. Sila kemukakan tatacara pengujian (SOP) dan keputusan ujian (raw data) untuk yang berikut:

   - Test for Growth Promoting and Inhibitory Properties dan Media Sterility Test bagi semua media yang digunakan.

   - Total Viable Aerobic Count (TAMC & TYMC)

   - Test for Specified Microorganisms

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

2. Sila kemukakan tatacara validasi untuk ujian Total Viable Aerobic Count & Test for Specified Microorganisms, berserta acceptance criteria dan keputusan dalam bentuk raw data yang menunjukkan bahawa kandungan produk ini tidak merencatkan pertumbuhan mikroorganisma semasa MCT dijalankan.

   (Sila rujuk British Pharmacopoeia – Suitability of the Counting Method in the Presence of Product & Suitability of the Test Method)

   Kesemua raw data yang dikemukakan perlu mengandungi nama dan nombor kelompok bagi Finished Product, tarikh mula dan selesai pengujian, keputusan pemerhatian setiap hari & tandatangan/ nama penganalisis.

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

Comments for MCT (English)

Microbial Contamination Test (MCT):

1. Please provide method (SOP) and result in raw data for below:

   - Test for Growth Promoting and Inhibitory Properties dan Media Sterility Test for all the media used.

   - Total Viable Aerobic Count (TAMC & TYMC)

   - Test for Specified Microorganisms

   Method must be specific to the produck and photocopy from pharmacopoeia is not acceptable.

2. Please provide the validation method for Total Viable Aerobic Count & Test for Specified Microorganisms, together with acceptance criteria and the result in raw data.

   (Please refer to British Pharmacopoeia – Suitability of the Counting Method in the Presence of Product & Suitability of the Test Method)

   All the raw data provided must include product's name, batch number of finished product, starting date and finishing date, observation result in interval period, analyst's name and signature.

3. Please translate into English or BM if the raw data provided are in others language.

THANK YOU!