BIO & PHARMA ANALYTICAL TECHNIQUES

Chapter 11
Microbiological Test

by
Dr. Siti Umairah Mokhtar
Faculty of Engineering Technology
umairah@ump.edu.my

Chapter Description

- Aims
  - Discuss theory, principles and application of analytical techniques used in material characterization, pre-formulation development, manufacturing process and storage stability.

- Expected Outcomes
  - Explain **general facts of microbiological test** including application in other field
  - Differentiate the **methodology of microbiological test** available in pharmaceutical laboratory
  - Categorize the **application of microbiological test** in pharmaceutical laboratory

- References

  - Gunzler H. & Williams A. (2002). Handbook of Analytical Techniques. Wiley-VCH, Weinheim, Germany.
  - Mullertz, A., Perrie, Y. and Rades, T. (2016) Analytical Techniques in the Pharmaceutical Sciences (Advances in Delivery Science and Technology). Springer, United States.

MICROBIOLOGICAL LIMIT TEST

- This test are designed to perform qualitative & quantitative **estimation of the no. of viable aerobic micro-organisms** present or **detecting the presence of designated microbial species** in pharmaceutical product.

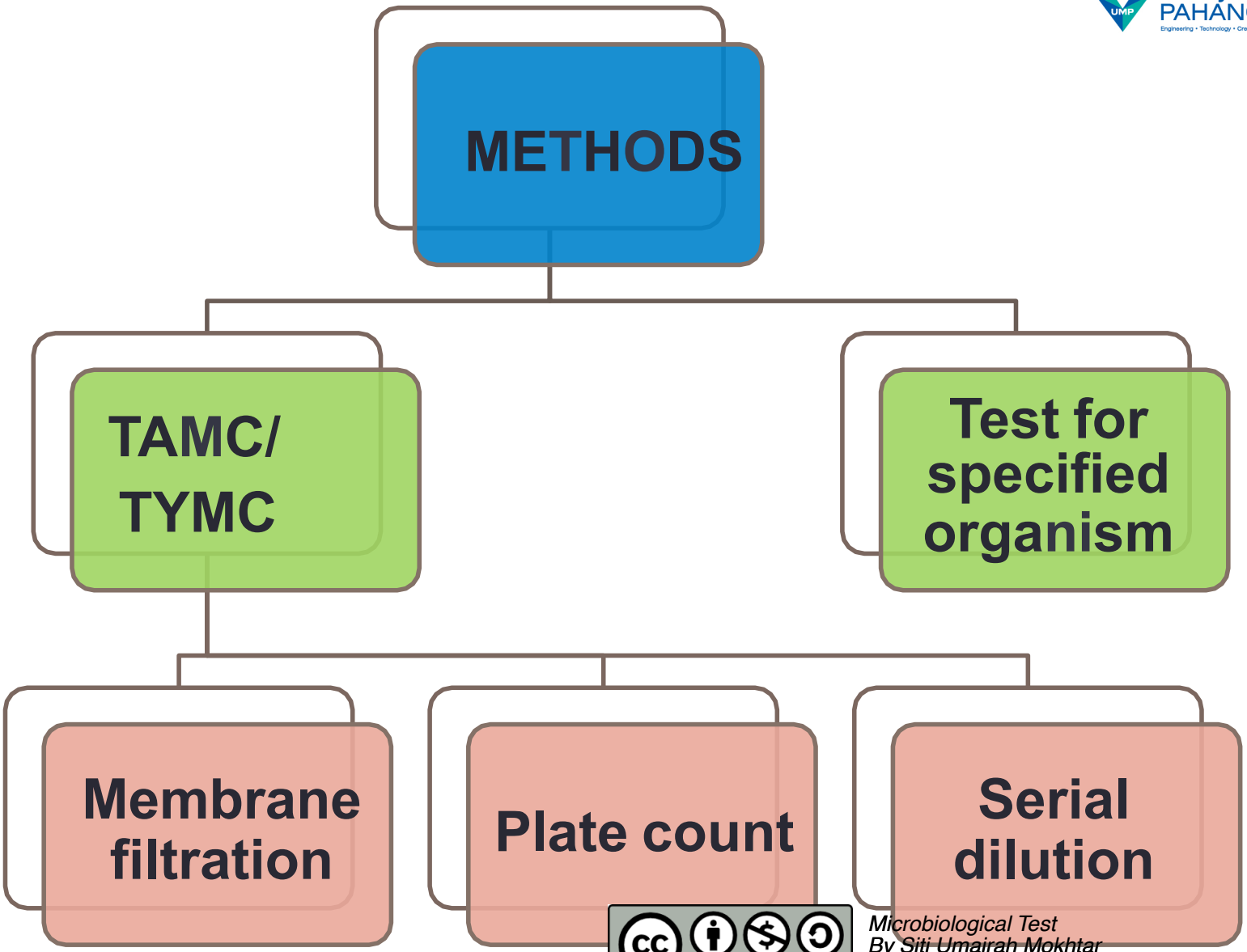
- The term 'growth' is used to designate the presence & presumed proliferation of viable micro-organism.

OBJECTIVES OF MLT

1. Microbial limit tests are designed **to estimate the number of viable aerobic organisms** present in pharmaceutical products and raw materials.

2. The microbial limit testing of raw material as well as finished pharmaceutical products can help **to determine whether the product complies with requirement of regulatory.**

3. The most care must be taken while performing microbial test so that **contamination from outside can be avoided**.



TAMC/TYMC

Unit: cfu/ml or gm

- colony-forming unit (CFU or cfu) is a measure of viable bacterial or fungal numbers.

- Unlike direct microscopic counts where all cells, dead and living, are counted, CFU measures viable cells.

- For convenience the results are given as CFU/ml (colony-forming units per milliliter) for liquids, and CFU/g (colony-forming units per gram) for solids.

PRE-TREATMENT OF SAMPLE:-

Solid/tablet:

- Reduce the substances to a moderately fine powder, suspend it into the vehicle solution specified (water/ hydroalcoholic)

Ointments/Creams

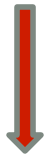
- Prepare a suspension with the aid of a minimal quantity of a suitable sterile emulsifying agent (polysorbates),

Fluid specimen in aerosol form

- Chill the container in an alcohol-dry ice mixture for 1 hr, cut open the container, allow it reach to RT to permit the propellant to escape, transfer/collect the test material

A. Membrane filtration method

10 ml or dilution containing 1 gm sample



membrane filter(50 mm in diameter, pore size NGT 0.45 µm)

Residue

- Wash it with *buffered sodium chloride-peptone solution pH 7.0* [For fatty substances add to the liquid *polysorbate 20* or *polysorbate 80*.]
- Transfer the filter on media for ***enumeration***

for the enumeration of microorganism

Media bacteria

casein soyabean digest agar

Incubation time

5 days,

at 30°c to 35°c

Media fungi

Sabouraud dextrose agar with antibiotics.

Incubation time

5 days,

at 20°c to 25°c

- Count the **number of colonies** that are formed.
- Calculate the **number of micro-organisms per gm or per ml** of the preparation being examined.

B. Plate count method

a) Pour-plate method

b) Surface-spread method

- a)Pour plate method:

Take petri dishes 9 to 10 cm in diameter

↓

1 ml of the pretreated preparation + 15ml (15-20 ml as per U.S.P) of liquified media agar

↓

If necessary, dilute the pretreated preparation.

b) Surface-spread method:-

- Spread the pretreated preparation on the surface of the solidified media in a Petri dish of the same diameter.
- Prepare at least two such Petri dishes using the same dilution and incubate.
- If necessary dilute the pretreated preparation

For bacteria	• Count the 300 colonies per plate as the maximum consistent with good evaluation.
For fungi	• Calculate the results using plates with not more than 100 colonies.

for the enumeration of microorganism

Media

bacteria

casein soyabean digest agar NMT 45°c

Media

fungi

Sabouraud dextrose agar with antibiotics.

Incubation time

4 days(48-72hr as per USP)

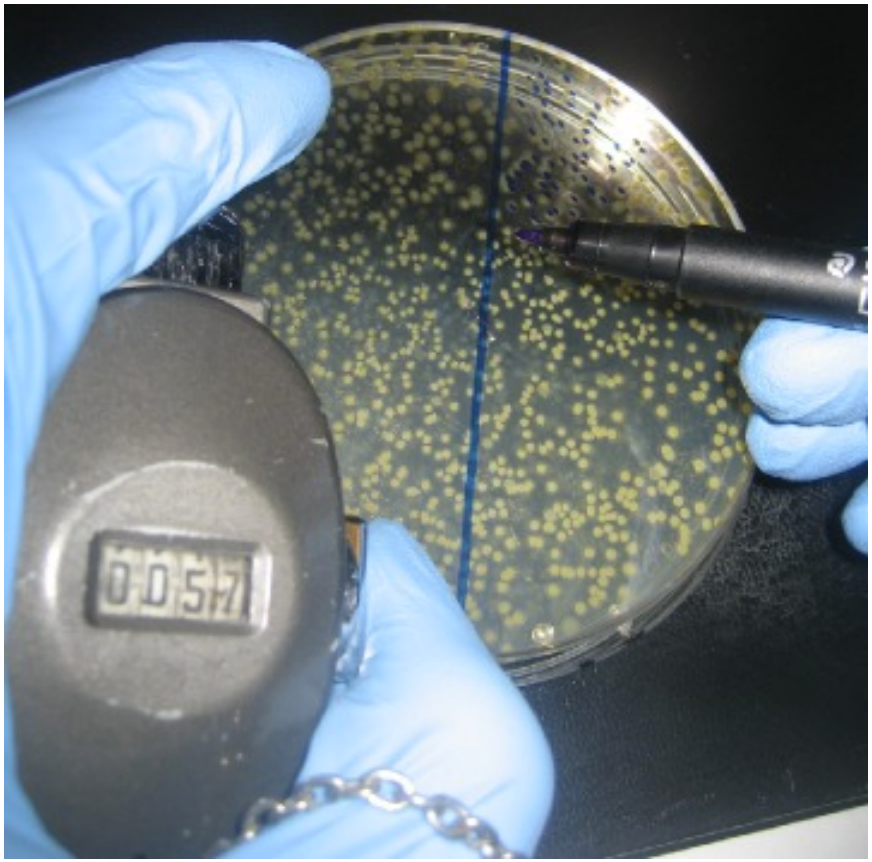
at 30°c to 35°c

Incubation time

5 days(48-78hr as per USP)

at 20°c to 25°c

Plate showing the colony



Source: https://en.wikipedia.org

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C. Serial Dilution Method (Multiple tube method)

1. Use **12 test tubes**: 9 containing 9 ml of soybean-casein digest medium each and 3 containing 10 ml of the same medium each for control.
2. Prepare dilutions using the 9 tubes.
3. First, add 1 ml of the test fluid to each of three test tubes and mix to make **10- times** dilutions.
4. Second, add 1 ml of each of the 10-times dilutions to each of another three test tubes and mix to make **100-times** dilutions.
5. Third, add 1 ml of each of the 100-times dilutions to each of the remaining three test tubes and mix to make **1,000-times dilutions.**

6. Incubate all 12 test tubes for at least 5 days at 30 – 35°C. No microbial growth should be observed for the control test tubes.

7. If the determination of the result is difficult or if the result is not reliable, take a 0.1ml fluid from each of the 9 test tubes and place it to an agar medium or fluid medium, incubate all media for 24- 72 hours at 30°-35°c, and check them for the absence or presence of microbial growth.

8. Calculate the most probable number of microorganisms per ml or gram of the sample.

2. TESTS FOR SPECIFIED MICRO ORGANISMS

- Salmonella
- Staphylococcus aureus
- Candida albicans
- Pseudomonas aeruginosa
- Escherichia coli
- Clostridia

- **Preparation of test fluid:-**
  - Proceed as described under the test for total aerobic microbial count .
  - Method: Refer USP<62>

Environmental monitoring

- Environmental monitoring describes the processes and activities that need to take place to characterise and monitor the quality of the environment.

- Environmental Monitoring is a surveillance system for microbiological control of cleanrooms and other controlled environments. It is a process which provides monitoring, testing and feedback to the microbiological quality levels in aseptic environments.

- Routine environmental monitoring ensures a safe compounding environment.

SOURCES OF CONTAMINATION

1. Air
2. Personnel
3. Equipment
4. Cleaning agents
5. Containers
6. Water
7. Compressed gases amongst other things.

ENVIRONMENTAL MONITORING: SURFACE

Surface monitoring

- Product contact surfaces, floors, walls, and equipment should be tested on a regular basis

i. Touch plates (RODAC plates) - used for flat surfaces

ii. Surface Swabs - used for irregular surfaces

- Surface monitoring should be performed at conclusion of **aseptic processing** (to minimise risk of contaminating critical surfaces during production) swabs and contact plates can be used

ENVIRONMENTAL MONITORING: AIR

- Active air monitoring:

- Impaction, centrifugal and membrane (or gelatin) samplers
- A certain volume of air is samples (volume and location should be meaningful)
- Instruments should be calibrated.
    i. Slit-Agar Air Sampler (STA)
    ii. Sieve Impactor
    iii. Centrifugal Sampler
    iv. Sterilizable Microbiological Atrium
    v. Surface Air System Sampler
    vi. Gelatin Filter Sampler

Passive Air Monitoring

- Settle plates exposed for 2 hours and replaced for duration of activity
- Media should be capable of growing a range of bacteria and molds e.g. Soybean Casein Digest Agar (SCDA)
- Should consider use of medium specific for molds if shown to be a problem in the environment
- Only give qualitative or semi-quantitative results
- Data generated considered in combination with active air sampling results.

ENVIRONMENTAL MONITORING: WATER

- Microbiological quality of water is very important
- Should be extensive, **comprehensive water testing programme.**
- Feed water, pre-treatment, reverse osmosis (RO), deionized (DI), purified/highly purified and water for injection (WFI) should be tested
- For purified/highly purified water and WFI, limits defined in pharmacopoeia:
  - purified <100CFU/mL
  - Highly purified and WFI 10CFU/100mL (but is usually kept at high temperatures)

- Water should also be tested for presence of *coliforms* and/ or *pseudomonads* if appropriate (may cause biofilm)

- Water used for parenterals should be tested for pyrogens

- Water should be tested using R_2A agar (Reasoner's 2A) incubated for at least 5 days at 30-35°C

ENVIRONMENTAL MONITORING: PERSONNEL

- For each session - gloves should be monitored (but not immediately after sanitising!)
- Periodic sampling for other locations on gown.
- Clean room operators should be regularly validated to demonstrate that they do not contaminate gowns during gowning up (gowning qualification).
- The challenge in aseptic processing is always personnel: as a source of microbial and particle contamination.

APPLICATION

- Microbiological assay of antibiotic drugs
- Disinfection efficacy test of disinfectants and antiseptics
- Sterility test of sterilized pharmaceuticals
- Tests for microbial limits for non-sterile pharmaceutical and biological products
- Testing of water quality

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Any Question?

Please refer to:

Dr. Siti Umairah Mokhtar

umairah@ump.edu.my