

UNIT-V

Assessment of a new antibiotic



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Assessment of microbial contamination and Spoilage

- Assessment of microbial content of a pharmaceutical product is very essential. **Sterile product should be perfectly free from micro-organism** and that can be assessed by a **test of sterility**.
- However, **non-sterile products** may contain some **micro-organisms**. These micro-organisms could be **pathogenic and non-pathogenic**. All these micro-organisms **can cause spoilage** and may cause **potential health hazards**.

- Number of total micro-organisms present in a products must be **low and should be within permissible limit. Types and nature of the microbes** should also be **assessed to determine the presence of specific microbes.**
- Total number of microbes and types of microbes is assessed by **microbial limit test.** Following tests are performed to assess the microbial contamination and subsequent spoilage.

1. Test of sterility

- Indian pharmacopoeia, British pharmacopoeia and United States pharmacopoeia recommends **test of sterility** for some pharmaceutical products.

Procedure are similar with little variations. Indian pharmacopoeia recommended tests are discussed briefly. Two methods are there i.e. **direct inoculation** and **membrane filtration**.

- **i) Direct inoculation-** In this method, little amount of sample is directly added to the culture media which was specified in pharmacopeia. This inoculated media is then incubated for specified period of time. Presence of growth indicates the presence of micro organism which is sourced from sample. Hence it may be concluded that sample is not sterile. Sample may be termed sterile in absence of any growth.

- **ii) Membrane filtration-** In this method sample is filtered through a membrane filter and washed with diluting fluid. Microbes, if present, will be there at the top of the filter paper. Now this filter paper is inoculated into specified culture media. If growth is observed then it indicates that product is not sterile.
- A positive and negative control test must also be conducted in both the above mentioned method.

2. Microbial limit test

- The European pharmacopoeia recommends both qualitative and quantitative assessment of micro-organism. United States Pharmacopoeia recommends microbiological Limit test. It has two parts, **(i) Total Aerobic microbial count** and **(ii) Test for specified microorganism.**

(i) Total Aerobic microbial count

- In this method specified amount of test sample (10gm) is taken and mixed with specified amount (90ml) of peptone water.
- This is sample dilution. There are specific procedure to make dilutions for water insoluble products and fatty products. Total microbial count is examined by following procedure-

- First, examination of sample by membrane filtration method- 10 ml of prepared dilution is mixed with 90 ml of peptone water and filtered through membrane filter. It is then washed 3 times with sterile peptone water.
- One filter paper is placed in petridish containing Soyabean Caesin Digest Agar media and incubated for 5 days at 30-35oC. Bacterial count is determined by counting colonies.
- Another filter paper is placed in Sabouraud Dextrose Agar Media and incubated for 5 days at 20-25oC. Fungal count is then determined.

- Second, Examination of sample plate count method- In this method prepared dilution is directly transferred to 4 petridishes.
- Two for bacteria and two for fungus. In first two petridishes 15 ml of Soyabean Caesin Digest Agar media is added. Colonies are counter after incubation for 5 days at 30-35°C. In remaining two petridish 15 ml of Sabouraud Dextrose Agar Media was transferred and incubated at 20-25°C. Colonies are counted.

(ii) Test for specified micro-organism

- Specific identification tests for *Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* is performed.
- ***E. coli*** is cultivated in **Macconkes** agar media. Colonies are identified by characteristics **metallic shine**.
- **Salmonella** are cultivated *on* **bismuth sulphite agar** media and identified by **black or green colonies**.
- ***Pseudomonas aeruginosa*** is identified by cultivating in **cetrimide agar** media. Colonies show **greenish** colour.
- ***Staphylococcus aureus*** is cultivated in **Vogel Johnson agar** media and identified by **black colonies surround by yellow zones**.

3. Periodic test

- Periodic test of the product for total microbial count must be done in order to determine continuing efficacy of the product.
- Many alternative tests may be done for detection and determination of micro-organism *e.g*
 - **Luciferase test**
 - **Epifluorescence test**
 - **Electrical impedance test**