

# Microbiological Analysis of Pharmaceutical Preparations

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- Microbiological Analysis of Pharmaceutical Preparations

- While there are no publications about the use of medicines for the treatment of microorganisms and the possibility of infection.
- Orally taken orally administered contaminant products after 1963 caused some infections.
- It was understood that the drugs could also be a source of infection.
- Oral medications include food type inf.-  
Salmonella,
- Eye ointments containing *P. aeruginosa*, eye drops are common eye inf.

- In the past - when the pharmacist prepared the medicine according to the patient's prescription and consumed it in a short time
- Today - the drug is being prepared in factories and used by a large patient population after a long time in the factory.

- Standard, set of rules for quality production = GMP (Good Manufacturing Practice)
- Reduce the risk of error in production to a minimum
- A concept that provides quality production suitable for its intended use
- First introduced in 1963 by the Food and Drug Administration (FDA) in the United States,
- It was accepted and published by the World Health Organization (WHO) in 1968,
- In 1984, practiced in our country as a compulsory drug producer.

- The rules governing the minimum requirements of the methods, installations and controls applied to the production, packaging and presentation of a product (medicine)
- The aim is; It is safe to use the drug, and it ensures that it carries the desired purity and quality

- GMP; A quality system that directly influences human health is a quality system that guides the conditions under which products such as medicines, cosmetics, food, medical devices should be produced.
- The quality of each serial product in the production depends on its suitability to all required standards.

So;

- Adequate training of staff, provision of suitable buildings and equipment
- Use of the right materials
- Implemented trial actions
- Availability of suitable storage and transport equipment
- Correct record keeping means - GMP

- Microbiological quality controls should be performed at each stage of production to minimize microbial contamination and microbial quality in pharmaceutical products and to minimize the risk of secondary infection.
- The microbial contamination in the pharmaceutical product causes the product and the patient's health to deteriorate, causing material and moral loss for the manufacturer.
- A statistically insignificant error in the medication may pose a serious hazard to the patient using the product.



# Causes of Microbiological Contamination of Pharmaceutical Preparations

- Raw material properties and characteristics:
- Many drug substances and adjuvants are suitable for the proliferation of microorganisms.
- The most important factors that play a role in the microbiological contamination of medicines are natural raw materials with a broad microflora of vegetable and animal origin.

- Pharmaceutical form:
- It is directly related to the microbiological contamination of a drug.
- For example; Liquid and semi-solid preparations are extremely dangerous. Antimicrobial substances such as ethanol and sugar are added to some preparations to inhibit the growth of bacterin.
- Sterile products and non-sterile products can not be produced in the same environment.

- For non-sterile preparations; The need for microbiological control depends on the route of administration of the drug.

	Aqueous solution	Anhydrous solution	Solid drug
Application path			
• Inhalation	*****	****	****
• Intranasally, intrabuccally	****	***	***
• Topical	****	**	**
• Oral	***	*	
• Anal	**	*	*

\* ; The form with the least control

\*\*\*\*\*; The form you need to check most

- For sterile preparations:
  1. For injectable preparations:
    - \* Sterility test
    - \* Pyrogenicity test
    - \* Toxicity test
  2. For ophthalmic preparations:
    - \* Sterility test

- For non-sterile preparations:

Microbiological limit test;

1. Aqueous solutions, water / for appropriate solvent-soluble substances

- Filter filtering

2. For distributed systems (Tablet, syrup, etc.)

- Can counting bacteria

3. For small amounts of preparations containing microorganisms

- Manufacturing stage- Fabricated Hygiene:

During the manufacture of medicines

1-unsuitable environmental conditions

2-used tools and equipment

3-staff

4-Raw

5-Water

6-packaging

7-storage and waiting time to raft; the causes of

- All factors that cause contamination during manufacture should be removed.
- The water used must comply with microbiological standards.
- Deionized water used for the preparation of non-injectable drugs and freshly drawn (4 hours prior) distilled water for injectable and eye preparations which must be sterile should be used after microbiological controls.
- Filtered air should be delivered to the area where the production is made.
- Trained personnel should be employed.

- Sterile production should be done in units built separately and purposefully from other production areas.
- Attention should be paid to particulate contamination during sterile production.
- This is why walls, ceilings and floors; Dust and other particulate matter.
- Provides continuous cleaning and disinfection
- The surfaces must be smooth and air, non-water permeable.



- Staphylococcus, Micrococcus and Diphtheroid bacilli, which are present in the normal hand flora, cause contamination of the drug and reach the organism through contaminated hands.
- Cross-contamination: Pathogenic bacteria or viruses are said to pass from a contaminant surface to another surface.
- - The employee is asymptomatic

- Spread of contamination; The material used at the hospital may be sourced.
- Therefore, the contamination spread can be reduced by methods such as not using the spoon, needles, injectors for the second time, and disposing of the applicators after the use of the topical products - disposing of the applicators.

- Drugs that are kept open may be contaminating with airborne microorganisms.
- In terms of homes and hospitals, the drugs used in hospitals are more likely to be infected with pathogenic microorganisms.
- -In the investigations conducted, it has been determined that the drugs are mostly in high-level contaminants during use.
- - Bacillus subtilis, yeast in the majority of daily used tablets and the land was found

# Validation

- Validation is the proof of the authenticity / reliability / reproducibility of any process, apparatus or method, system or activity.
- In the pharmaceutical industry, buildings, support units, equipment, computer systems, cleaning, disinfection, security alarm systems, products, methods etc. should be considered.

- When should the validation be done?
- Validation is mandatory according to GMP rules
- It should be routinely done at least once or twice a year under normal conditions
- Situations that require validation outside the routine:
  - In the first establishments of buildings and processes
  - When a new tool, machinery, equipment is started to be used

## Microbiological validation;

- Air and ventilation system
- Water and water system
- Machinery, equipment and surfaces
- Staff
- Cleaning and disinfection
- Sterilization Autoclave, tunnel, liquid filling, dust filling validations are done
- Each system should be microbiologically controlled by taking a large number of samples from different and especially critical points for the process

- Validation of ventilation system
  - In the production of sterile pharmaceutical products, the production area must be sterile. For this, a certain volume of air is taken from the areas determined according to the  $m^3 / \text{area}$  formula in the sterile field or with the aid of an air sampler. The air samples taken are analyzed for bacteria and fungi.
- Machines, equipment, counters, places such

- Cleaning Validation
  - The effectiveness of the disinfectant is evaluated by taking samples from various surfaces before and after cleaning and disinfection.
- Water System Validation



- Validation of sterilization procedures
  - Autoclave Control
  - No microorganisms, including spores, should survive.
  - The efficiency and homogeneous distribution of heat in the autoclave should be controlled by the aid of receivers and biological indicators.
  - The autoclave must kill the *B. stearothermophilus* spore suspension for proper

# Microbiological Degradation in Drugs

A pharmaceutical preparation;

- Contains pathogenic or potentially pathogenic microorganisms
- Possession of toxic metabolic residues of microorganism
- In the case of obvious and obvious physical and chemical changes, the preparation is regarded as completely degraded in terms of microbiology.
- Contamination is the activation of the active substances in the drug and may lead to some

# Microorganisms Encountered in Pharmaceutical Preparations

- Types of microorganisms contained in a drug that is contaminated; Air, water, human, animal and vegetal fluids.
- Aerobes are the dominant microorganisms. The majority, except *Bacillus anthracis*, are saprophytic bacteria.
- Spore forms are particularly resistant to heat and antimicrobial agents.

- Gram (-) basil is another group of bacteria that can be found in contaminating prep. *E. coli*, Klebsiella, Enterobacter, Hafnia, Serratia, Citrobacter, Salmonella, Proteus and Pseudomonas group microorganisms.
- Most of these microorganisms are opaque (opportunistic, potential pathogen).
- These bacteria, which are found in human and animal normal microflora, gain pathogenicity

- Gram (+) cocci (Micrococcus, Staphylococcus, D group Streptococcus)
- Yeast and Mold (Aspergillus, Penicillium, Saccharomyces) Are among the microorganisms encountered in medicines and most of them are heat resistant.

- Once the prevention of microbiological contamination of medicines is understood, it is desirable to limit the amount of microorganisms that may be present in medicines.
- To this end, the International Federation of Pharmacy (FIP) has published a report on the general problems of microbiological purity of

## Pharmaceutical Preparation Groups and Microbiological Purity Grades

Category	Pharmaceutical Shape	Amount of microorganisms allowed
1a	Injectable preparations	Sterile and apyrogen
1b	Ophthalmic preparations	Sterile
2	Prepared wound injured Ear, nose and throat prep Gynecological prep Inhalation prep Oral and other way applied	The amount of live microorganism in gr or ml $10^2$ cfu / ml-gr will not pass. cfu(coloni forming unit) M.O's of Enterobacteriaceae family with <i>P.aeruginosa</i> and <i>S.aureus</i> will not be found.
3	Other preparations	Live m.o amount: For aerob bacteria: $10^3$ - $10^4$ cfu / ml-gr For yeast and mold: 100 cfu / ml-gr  In any case; Pathogenic bacteria and fungi will NOT!

- The competition between the normal flora of the various body regions and the microorganisms that come here incidentally, etc., can be explained by the organism, bactericidal action of the saliva, gastric acidity and enzymes, pancreatic juice, bile, hair, skin, As well as defense against microorganisms.
- In some pathological conditions, this defensive mechanism is disturbed and the



# Examination Methods of Microbiological Rawness of Pharmaceutical Forms

- Methods used in microbiological analysis of drugs  
It is necessary to cover the mentioned items.

Methods:

- It should be examined especially depending on the characteristics of the drugs.
- It must be based on the nature of each of the constituent substances and the solubility property of the product.
- It should be able to detect the presence of antimicrobial in the sample being analyzed.
- The drug should be able to determine the current degree of contamination.

# Separation of Samples

- The samples should be separated by asepsis and antisepsis prior to the start of the analysis and should be able to repeat all the experiments, if necessary.
- It is recommended to take a sample of 10 gr or ml for each trial. For very expensive or powerable samples, it should be at least 1 gr or ml.

# Preparing Samples

a) Water soluble samples:

Samples are prepared with 10% homogenizate with phosphate buffer pH 7.

b) Samples not soluble in water but not oily:

Weigh 10 g of the sample to be investigated and prepare a homogenous mixture by adding approximately 85 ml of a phosphate buffer (containing a surfactant such as tween 20 or 80) of approximately pH 7.

### c) Oily samples:

Glass beads and 10 g of Tween 20 or 80 are placed in a sterile conditioner containing 10 grams of sterile pomade, cream, lotion, etc. to be examined.

-It is first heated with a pilot tube in a water bath up to 40 ° C.

-Mixed.

-80 ml of warm buffered solution to 40 [deg.] C. is added.

# Microorganism Census Methods

- 1) Counting on agar plates
- 2) Filtration and culturing on membranes
- 3) Counting with serial tiller and static evaluation

-A variety of microorganism counts to be made in the samples media are used.

- Total bacteria count: Casein-peptone Soy Agar (CSA) or Tryptone Glucose Extract Agar (TGEA) 30-300 colonies count
- For yeast and mold count: Antibiotic Sabouraud Dextrose Agar (SDA) 10-100 colonies count

- After these media are prepared in appropriate conditions and sterilized, sterilization controls are carried out at 37 ° C for 1 night. The time of use is in the water bath above 90 ° C and it is used by cooling to 45 - 50 ° C.
- The use of selective media samples [Salmonella-Shigella Agar (SS Agar)] is required for the identification of the m.o. species in the pharmaceutical preparations to

# Microorganism Census Methods

## 1) Counting on Agar Plates

A serial dilution is prepared with pH 7 phosphate buffer according to the probable contamination level of the 10% homogenate prepared. One milliliter of each diluent is transferred to 3 separate petri dishes. They are melted and cooled to 45-50 °C. The sample is poured into 15ml of medium and the sample and the mixture are shaken by rotation for homogenous mixing and frost is expected. After the addition, 6-7 ml of medium is added and frozen to form a layer on the surface.

- Petri dishes are incubated at 20-25 ° C for yeast and molds and at 35-37 ° C for bacteria. Colonies count 2-5 days later. The dilution factor of the aerobic bacteria amount of the sample in 1 gr or ml is also determined by



- For Maya and mold count, Sabouraud Dextrose Agar with a few of the antibiotics chloramphenicol, tetracycline, penicillin G is used.
- Plates for yeast and mold counts are incubated at 20-25 °C. Colonies count 2-5 days later.
- The number of yeast and mold in 1gr or ml is determined by multiplying the dilution ratio. For the yeast and mold, petriol containing 10 -

## 2) Filtration and culturing on membranes

This method is used for water soluble samples. A 10% homogenization of the sample is prepared. Dilutions are prepared here.

Each dilution is filtered through a membrane filter with a pore diameter of 0.45  $\mu\text{m}$ .

CSA or TGEA is poured into the membrane filters and carefully placed on the surface of the frozen petri dishes and held with sterile forceps.

Yeast and mold counts are also made in the same manner as petrol with antibiotic SDA nutrient. The petri dishes are kept at appropriate incubation temperatures for certain periods of time.

### 3) Counting with serial tiller and static evaluation

It is a method which is used to include less than 100 piece microorganisms in gram or ml or near-close counts in pharmaceutical preparations or raw materials which can be easily checked. (Most Probable Number Method (MPN))

In this method, the liquid form of the media is used.

10% homogenization and dilutions of the sample are prepared.

Peptone - Casein Soybean (CSB) and Saboraud Dextrose Supplement (SDB) medium are added to each decimal diluent. The seeds added to the CSB medium are incubated at 35 ° C and the SDB medium is incubated at 25 ° C.