

Microbiological Standard and Microbiology Test for Pharmaceutical Product

Marlia Singgih Wibowo

INTRODUCTION



- Raw material and Pharmaceutical products have their quality requirement to guarantee their safety, quality and benefit
- One of the quality requirement : quality in microbiology
- Possibility of microbial contamination in Pharmaceutical products can be originated from : the raw material, production process, storage , distribution.

PHARMACEUTICAL INDUSTRY and their environment

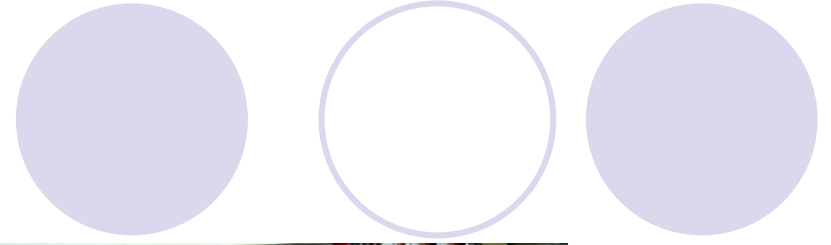
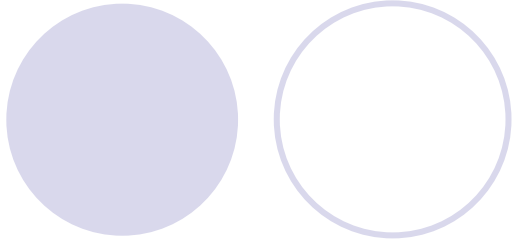


Contamination from production process : from utilities, instruments, or during production process



Material and Pharmaceutical Product

- Raw Material (Bahan baku)
- Purified Water (Air murni)
- Sterile Pharmaceuticals (Produk Farmasi Steril)
- Non-Sterile Pharmaceuticals (Produk Farmasi Non-Steril)
- Medical Devices (Alat kesehatan)



Pharmaceutical Raw Material

- Raw Material for Pharmaceuticals can be synthetic raw material or material originated from nature (plants, animal, mineral, etc)
- Natural materials are tend to be easily contaminated by microorganisms , unlike synthetic material



Classification of natural raw material (Grigo, 1976)

1. Raw material from synthetic process or extract from nature that has been purified (average 10^1 cfu/g or mL)
2. Raw material from synthetic process or extract from nature (average 10^2 cfu/g or mL)
3. Plant Extracts (average 10^3 cfu/g or mL)
4. Animal Product or Plants with slightly process (average 10^4 cfu/g or mL)
5. Animal Product or Plants without any process (average 10^5 cfu/g or mL)

Some examples of raw material which have microbial content limit in British Ph and European Ph (amount of microorg. per g or per mL, except for *Salmonella* per 10g or 10 mL)

Raw Material	TVC	<i>E.coli</i>	<i>Salmonella</i>	<i>Enterobac</i>
Acacia	10 ⁴	+		
Al hidroksida	10 ³	+		+
Bentonit	10 ²			
Serbuk Digitalis		+	+	
Gelatin	10 ³	+	+	
Laktosa	10 ³	+		
Pankreatin	10 ⁴	+	+	
Tepung beras	10 ²			
Tepung gandum	fungi			

TVC=total viable count, + = must be free from the microbes

Contaminant Microorganisms which are commonly found in natural raw material

- *Bacillus*
- *Enterobacteriaceae*
- *Staphylococcus*
- *Aspergillus*
- *Penicillium*
- *Mucor*
- *Rhizopus*



E.coli



Salmonella

WATER



- ❖ Potable water (Air minum) : no *Coliform bacilli* per 100 ml
- ❖ Purified Water (Water for non-steril product):
 - ✓ Allow <10 sampai < 100 cfu per 100 ml
 - ✓ No *Pseudomonas*
- ❖ Water for injection :
 - ✓ < 0,25 endotoksin unit (EU) per ml.
 - ✓ Batas mikroba < 10 cfu per 100 ml
 - ✓ Tidak ada *Pseudomonas*

Sterile Pharmaceutical Product

- Including **parenteral** product, ophthalmic preparation, contact lens solution, and other products for open wound or for irrigation process
- **Sterility test** should be applied
- Sterility requirement is stated as ***Sterility Assurance Level*** : means the probability is as same as or less than 10^{-6} , which means in one million sterile products, only 1 product maximum allowed to be not sterile.
- The sterility analysis is based on any microbial growth in media **Fluid Thioglycollate** (FTM) and **Soyabean Casein Digest** (SCD) at 30-35°C (bacteria) and 20-25°C (fungi) for 7 and 14 hari.

Non-Sterile Pharmaceutical Product

- There is no single regulation for non-sterile product, it is depend on each country regulation
- No microbial contaminant which can be sources for infection due to medication process (*medication-borne infection*)
- TVC (*Total Viable Count*) is allowed in limited amount and no enteric pathogen in the raw material.

Microbiology Quality Requirement for Pharmaceutical Product (according to FIP,1976)

Cat	Product	Regulation
1a 1b	Injection Ophthalmic product, or for microbe-free organs, for open wound, ulcer	Sterile – based on Pharmacopeia Free from viable microbes per g or mL
2	Topical product for skin lesion, nasal product, or throat (high risk)	Viable Microbes max. 10² /g or mL, and no <i>Enterobacteriaceae</i> , <i>P.aeruginosa</i> , <i>S.aureus</i>
3	Other products	Viable Microbes max. 10³ – 10⁴ anaerob bacteria, 10² of yeast and moulds /g or mL, Limit for specific microbes : no <i>E.coli</i> , and no <i>Salmonella</i> , no <i>P.aeruginosa</i> , no <i>S.aureus</i> , Other <i>Enterobacteriaceae</i> max. 10 ² /g atau mL

Microbial contamination limit in raw material and products from plants (according to UNIDO, 1990)

Material/ Product	Bacteria	Yeast and Mould	Coliform Bacteria	<i>Salmonella</i>	<i>Staphylo coccus</i>
Plant originated products	$< 10^4$ /g	$< 10^2$ /g	-	-	-
Plant originated raw material	$< 10^7$ /g	$< 10^4$ /g	-	-	-

Note : (-) must be free from the microorganism

UNIDO = (United Nation Industrial Development Organization)

Microbiology Tests in Farmakope Indonesia edisi IV, 1995

- **Mikrobiological test**

- <51> Uji Batas Mikroba (Microbial Limit Test)

- <61> Uji Efektivitas Pengawet (Preservative efficacy test)

- <71> Uji Sterilitas (Sterility test)

- **Biological test**

- <91> Vitamin B12 Activity test

- <121> Calcium Pantothenat Determination

- <131> Antibiotic Potency Test

<51> Uji Batas Mikroba (Microbial Limit test)

- Purpose : to determine amount of **viabel** aerobic microbes in any Pharmaceutical preparations, from raw material to end-products
- To claim that the pharmaceutical preparation is free from certain species of microorganisms
- The work should be done in **aseptic condition**
- If no other statement, term “**incubation**” is placing the container in thermostatic controlled place at temperature of 30 – 35°C for 24 – 48 hours
- Term “**tumbuh (growing)**” is any indication of development / replication of viable microorganisms on media

<61> Preservative Efficacy Test

- **Definition of Antimicrobial Preservative** : substances added into pharmaceutical product to protect the product from microbial contamination.
- Preservatives are used mostly for multi-dose products (**wadah dosis ganda**)
- Preservatives cannot be used in purpose of decreasing viable microbes as a replacement of un-compliance production process
- **Amount of preservatives used** should be as low as possible
- Test for preservative efficacy is dedicated for multi-dose products with liquid solution (aqueous)
- Test and regulation is only applied for “original sealed product” (**“wadah asli yang belum dibuka”**) , which is distributed by the producer

<71> Sterility Test

- The test is used to determine whether raw material or pharmaceutical products which have to be sterile are fulfill the requirement as stated in each monography of the material or product.
- To use sterility test as a part of quality control in industry, should be refer to Pharmacopeia **<1371> Sterilisasi dan Jaminan Sterilitas Bahan Kompendia** (Sterility and Compendia Sterility Assurance)
- Considering the positive result can be caused by false procedures or environmental contamination, there are two steps of test should be done, and it is stated in section: **Penafsiran Hasil Uji Sterilitas (Estimation/Interpretation of Sterility Test Result)**



- Alternative Procedures can be used as long as the result obtained is reliable (equal) → See “**Prosedur**” on “**Uji dan Penetapan dalam Ketentuan Umum**”
- But if any difference appear, and the contamination is happened or resulted from Pharmacopeia procedure, → the result should be decided as not fulfill the requirement (should be rejected)

<91> Determination of Vitamin B12 Activity

- The test is carried out using *Lactobacillus leichmanii*, with turbidimetric method
- Standard Solution used : **Sianokobalamin BPFI** (Baku Pembanding Farmakope Indonesia) with range of 0.01 – 0.04 ng per mL. Blank used is purified water.
- Instrument : Spectrophotometer UV-VIS.
- Method : Spectrophotometric method at wavelength 530 nm.
- The Concentration is determined and calculated using standard curve

<121> Determination of Calcium Pantothenat

- The test is carried out using *Lactobacillus plantarum* with turbidimetric method
- Standard Solution used : Calcium pantothenat BPHI (Baku Pembanding Farmakope Indonesia) with range of 0.01 – 0.04 ng per mL. Blank used is purified water.
- Instrument : Spectrophotometer UV-VIS.
- Method : Spectrophotometric method at wavelength 660 nm.
- The Concentration is determined and calculated using standard curve

<131> Antibiotic Potency Test

- Potency of an antibiotic can be measured according to the susceptibility of certain microorganisms against the antibiotic
- Comparing the unknown potency toward the standard of the known potency of antibiotic
- Concentration VS Potency
- Two general methods : **Plating method** dan **Turbidimetric method**
- Plating method : Antibiotic solution is absorbed on paper disc or poured into stainless steel cylinder, diffusion effect of antibiotic is observed on agar medium.
- Turbidimetric method : effect of antibiotic solution against growth of microbes showed in intensity of turbidity