Experiment 9

Sterility testing of a pharmaceutical product

Firstly, watch this video:

https://www.youtube.com/watch?v=RAH0FLfiig0&list=PLf-BeYf_KaMt02E_ApBp_XpMX-5xFo-l0&index=3

➢ Objectives:

1. To understand the importance of sterility in pharmaceutics.
2. To learn and perform sterility testing methods.

➢ Introduction:

Pharmaceutical industries based on their manufacturing pattern can be broadly classified into the following categories:

1. **Sterile products**
   - Injectables, sterile ophthalmic drops, monoclonal antibodies (MAbs) and vaccines.
   - The sterile products are produced in the **cleanroom** environment free from any microbial contamination.
   - The testing methods which ensure the product sterility state that a positive result (microbial growth) after 14 days at appropriate temperature (7 days incubation at 20 °C - 25 °C followed by 7 days incubation at 30 °C - 35 °C) is considered a fail, whereas negative results shows that the product is sterile.
   - The sterile products can be produced either by following aseptic manufacturing techniques or terminal sterilization.

2. **Non- sterile products**
   - Tablets, capsules, ophthalmic drugs
   - Unlike sterile pharma manufacturing, non-sterile pharma products do not require the cleanroom environment to be completely free from any microbial presence.
   - Non- sterile products tend to have moderate to low contamination control
   - Acceptance criteria
     - Bioburden _ number of recoverable viable organisms
     - Absence of objectionable organism

A microbial control strategy and risk management are used identify and prevent microbial contamination that could compromise the quality of the product. .

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محاضرة: exp 9

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لجان الدُفعات

Soul Academy

Providing adequate and reliable **sterility test data** is an important quality assurance issue.

In this experiment we will take about sterility of the media, Sterility testing of sterile and Non-sterile pharmaceutical products, and endotoxin Test (LAL Test).

Sterility of the media

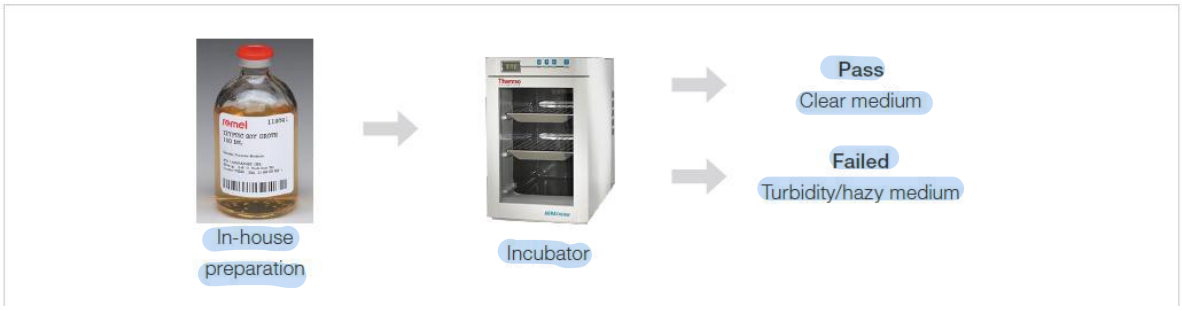
There are mainly two types of media that are used in sterility:

a. Fluid Thioglycollate Medium (FTM)
   For detection of both aerobic and anaerobic bacteria.

b. Tryptone Soya Broth or Soyabean Casein Digest Medium (TSB or SCDM).
   Primarily intended for the culture of both fungi and aerobic bacteria.

Dehydrated culture media sterility for 14 days

Ready-to-use media or PPM sterility testing for 14 days



Sterility testing of sterile pharmaceutical products

The analysis must be carried out by using:

1. Membrane Filtration
2. Direct inoculation

In each of the mentioned methods, the product sterility is investigated using appropriate culture media for recommended temperature and duration.

Membran Filtration

الخطوات حق

Step 1: Pass the liquid sample through filter membrane (with pore size not more than 0.45 µm)

Step 2: To neutralize and remove any antimicrobial residue from the filter membrane (with pore size not more than 0.45 µm) by washing it with 100 mL of suitable rinsing fluid

Step 3: Repeat step-2

Step 4: Upon completion of step 3, remove the filter with help of sterile forceps and cut the filter into two pieces with sterile seizer and place each piece in appropriate medium

Step 5a: Incubate half of the filter with Tryptone Soya Broth (CM0129B) at 22.5 °C ± 2.5 °C for 14 days

Step 5b: Incubate the other half of the filter in Fluid Thioglycollate Medium (CM0173B), at 32.5 °C ± 2.5 °C for 14 days

PASSED	FAILED
No growth	Growth, repeat

1. **Membrane filtration:**

2. **Direct inoculation:**

Step 1: Sample were spiked in the suitable media directly with the help of syringe

Step 2a: Tryptone Soya Broth (CM0129B) spiked with product incubated at 22.5 °C ± 2.5 °C for 14 days

Step 2b: Fluid Thioglycollate Medium (CM0173B) spiked with product incubated at 32.5 °C ± 2.5 °C for 14 days

PASSED	FAILED
No growth	Growth, repeat

Sterility testing of Non- sterile pharmaceutical products

Unlike sterile product manufacturing, the manufacturing of non-sterile pharmaceutical products does not require the cleanroom environment to be completely free from any microbial presence.

There are acceptance limits set for these industries, which align with the regulatory acceptance guidelines to manufacture non-sterile products. Further, they test for objectionable microorganism presence in the product.

The basic tests done in non-sterile pharmaceutical industry for microbial identification is a **Microbial enumeration test** (microbial counting test) for the quantitative counting of viable microorganisms and the determination of the absence of specified microorganisms in finished pharmaceutical products and raw materials.

لازم عدد الـ MO يكون متوافق مع
كم لازم يكون في MO بهذا الـ Product

The methods for enumeration of microorganisms from pharmaceuticals includes membrane filtration and conventional plate count (including pour-plate method, surface-spread method).

The specific enrichment procedures depending on the target specified microorganism that must be absent, as required by a product monograph. Products which are insoluble or immiscible in water must be appropriately treated to obtain a suspension suitable for the test procedures.

Endotoxin test (LAL test)

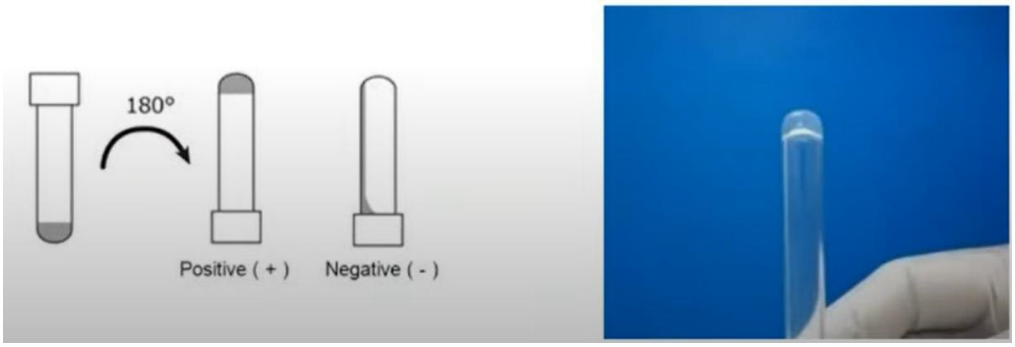
Bacterial endotoxin is a lipopolysaccharide found in the cell membrane of Gram-negative bacteria which may cause adverse events in patients such as, but not limited to fevers, headaches, inflammation, nausea, chills, vomiting, hypotension, lung toxicity, Toxic Anterior Segment Syndrome, abortion or death when injected into the blood stream.

Therefore, all sterile drugs, medical devices and combination products must meet bacterial endotoxin specifications. Historically, in vitro Limulus Amoebocyte Lysate (LAL) assays based on the **gel clot method** have been used to detect and quantify bacterial endotoxin in pharmaceutical products.

The LAL reagent is derived from the amoebocyte lysate of the horseshoe crab (Limulus Polyphemus), and it can react specifically with microbial lipopolysaccharides to give a gel even at very high dilutions.

The **Gel-Clot Method** is a qualitative and Quantitative assay that detects Gram-negative bacterial endotoxin based upon a reaction between lysate and endotoxin which results in a firm clot formation.

For samples with endotoxin, the endotoxin amount present in a test sample is calculated by diluting the sample to determine the assay endpoint where a clot does not form . If no clot forms in the verified dilution from the inhibition and enhancement testing, the sample does not contain detectable endotoxin.



Practical Part

Part 1: Microbial Count of non-sterile product

I. **For Water Soluble Non Sterile Solid Product**

1. Apply aseptic technique
2. Weight 3 tablets and record their weight “approximately 1 g”
3. Grind the weighted tablets using mortar and pestle
4. Transfer the powder and dissolve it in 9 ml sterile distilled water
5. Make 1:10 serial dilution two times
6. Transfer 1 ml from each tube into the surface of agar plate
7. Use L- shape rod to spread the sample over the agar plate
8. Incubate the plates at 37 °C for 48 h
9. Count the cells and identify them under the microscope

شرح مبسط لاختبار السم الداخلي (اختبار LAL)

ما هو السم الداخلي البكتيري؟

- مادة سامة توجد في جدار خلايا البكتيريا سالبة الجرام
- قد تسبب مشاكل خطيرة للمرضى مثل:
- حمى، صداع، غثيان
- التهابات، انخفاض ضغط الدم
- مضاعفات خطيرة قد تصل للوفاة

لماذا نستخدم هذا الاختبار؟

- لفحص الأدوية والأجهزة الطبية المعقمة
- للتأكد من خلوها من السموم البكتيرية قبل استخدامها

كيف يعمل الاختبار؟

- يستخدم مادة مستخلصة من دم حيوان "سرطان حدوة الحصان"
- عند وجود سموم بكتيرية، يتفاعل معها مكوناً جل (كتلة متخثرة)

طريقة العمل:

1. يتم تخفيف العينة تدريجياً
2. إذا ظهر تخثر (جل) في أي تركيز، فهذا يدل على وجود سموم
3. إذا لم يظهر تخثر في التركيز المطلوب، تكون العينة آمنة

النتيجة:

- كلما احتجنا لتخفيف أكثر لاكتشاف السموم، يعني أن العينة أنظف

لا تنسوا نصيبنا منكم من دعائكم