

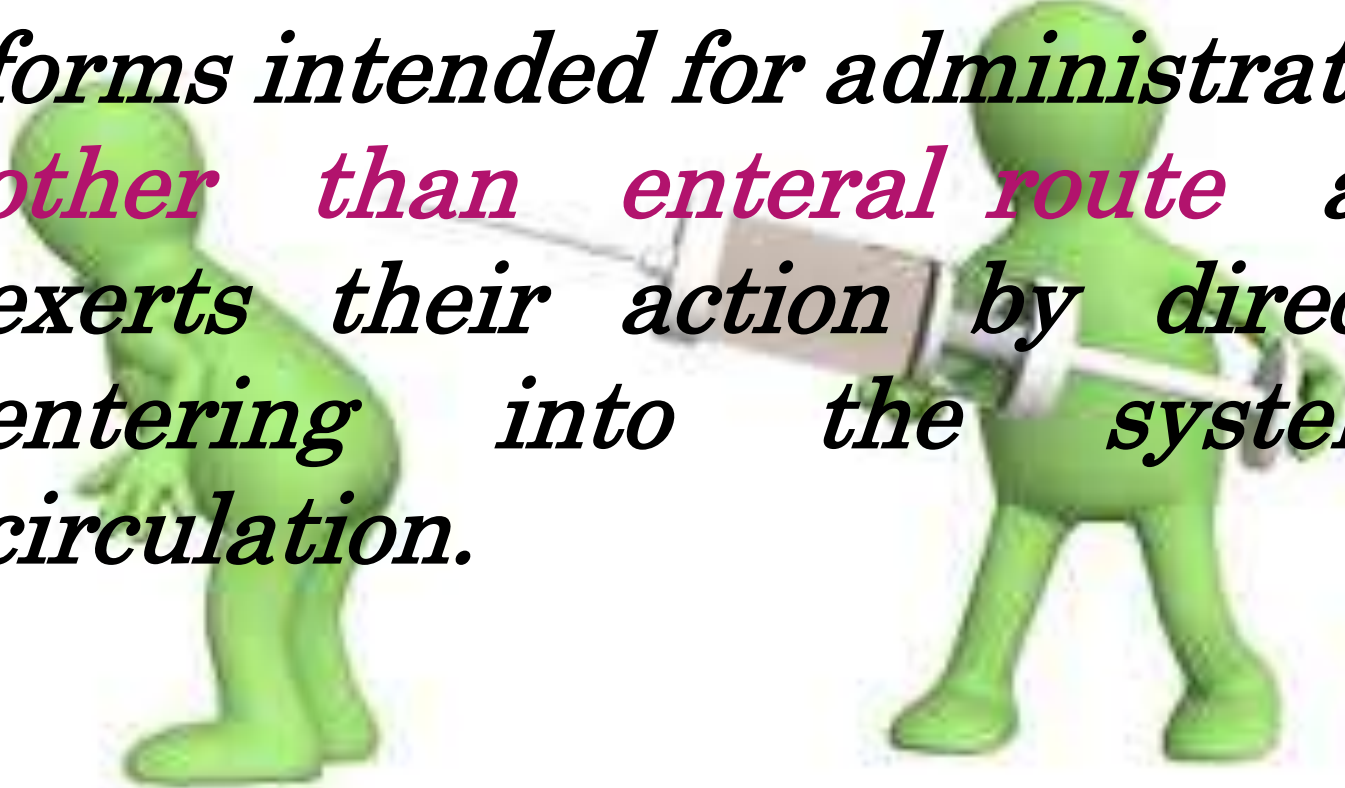
The background of the slide features a collection of pharmaceutical glassware. In the foreground, a syringe with a clear barrel and a grey plunger is positioned horizontally, its needle pointing towards the left. To its left, a small glass vial lies on its side, with its dark grey cap removed and the needle inserted into the opening. Behind these, several other vials stand upright, some containing clear liquids. The entire scene is set on a highly reflective surface, creating clear mirror images of the objects below them. The lighting is bright and even, highlighting the transparency of the glass and the metallic sheen of the syringe.

# QUALITY CONTROL TESTS FOR PARENTERALS

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M PHARM 1<sup>ST</sup> YEAR

# PARENTERALS

*Parenterals are the **sterile dosage forms** intended for administration **other than enteral route** and exerts their action by directly entering into the systemic circulation.*



# **ADVANTAGES**

- **Quick onset of action.**
- **Suitable for the drugs which are not administered by oral route.**
- **Useful for uncooperative , nauseous, or unconscious patients.**
- **Useful for emergency situation.**
- **Duration of action can be prolonged by modifying formulation.**
- **Means of correcting serious disturbances of fluid and electrolyte balance.**

## **DISADVANTAGES**

- **Only trained personnel is required.**
- **Pain on injection.**
- **Difficult to reverse physiologic effect of drugs.**
- **Sensitivity or allergic reaction at site of injection.**
- **Require strict control of sterility and non pyrogenicity than other formulation.**
- **More expensive and costly to produce.**

# **CATEGORIES**

- ❖ **Injections**
- ❖ **Infusions**
- ❖ **Concentrates for injections and infusions**
- ❖ **Powder for injections and infusions**

# **QUALITY CONTROL TESTS**

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**Uniformity of content**

**Test for volume of liquid**

**Test for pyrogen**

**Test for sterility**

**Clarity of solution**

**Uniformity of weight**

**Test for bacterial endotoxin**

**Leakage test**

# **UNIFORMITY OF CONTENT**

- **30 sterile units are selected from each batch.**
- **The weight of 10 individual sterile units is noted and the content is removed from them and empty individual sterile unit is weighed accurately again.**
- **Then net weight is calculated by subtracting empty sterile unit weight from gross weight.**
- **The dose uniformity is met if the amount of active ingredient is within the range of 85-115.0% of label claim.**

- **Relative standard deviation is equal to or less than 6.0%.**
- **If one unit is outside the range of 85-115.0%, and none of the sterile unit is outside the range of 75-125.0% or if the relative standard deviation of the resultant is greater than 6.0% ,or if both condition prevail, an additional 20 sterile unit should be tested.**
- **The sterile units meet the requirements if not more than one unit is out side the range of 85-115%, no unit is outside the range of 75-125.0% and the calculated relative standard deviation is 7.8%.**



# **TEST FOR VOLUME OF LIQUID**

- **Test applies to liquid supplied in single dose , only part of the content is used**
- **Empty the contents of one container& determine the volume of contents**
- **Emulsions & suspensions shake the container before the determination**
- **The volume is not less than the amount stated on the label.**

<b>Parenteral preparations</b>	<b>Minimum number of items tested</b>
<b>Not more than 100 containers</b>	<b>10% or 4 container</b>
<b>More than 100 but not more than 500 containers</b>	<b>10 containers</b>
<b>More than 500 containers</b>	<b>2% or 20 containers whichever is less</b>
<b>For large volume parenterals</b>	<b>2% or 20 containers whichever is less</b>

# **UNIFORMITY OF WEIGHT**

- **Remove the labels & wash the container & dry**
- **Weigh the container along with content**
- **Empty the container completely**
- **Rinse with water & ethanol, dry at 100°C to a constant weight**
- **Cool & weigh**
- **Net weight should be calculated**

# **TEST FOR PYROGEN**

- **The test involves measurement of the rise in body temperature of rabbits following the IV injection of a sterile solution into ear vein of rabbit.**
- **Dose not exceeding 10 ml per kg injected intravenously within a period of not more than 10 min**
- **Test animals: Use healthy, adult rabbits of either sex, preferably of the same variety.**
- **Recording of temperature: Clinical thermometer**

# PRELIMINARY TEST(SHAM TEST)

- **If animals are used for the first time in a pyrogen test or have not been used during the 2 previous weeks condition them 1 to 3 days before testing the substance by injecting IV 10ml per kg pyrogen free saline solution warmed to about 38.5°**
- **Record the temperature of the animals beginning at least 90 min before injection and continuing for 3 hours after injection.**
- **Any animal showing a temperature variation of 0.6° or more must not be used in main test**

# MAIN TEST

Carry out the test using a group of 3 rabbits.

**Preparation of the sample:** Dissolve the substance in or dilute with pyrogen free saline solution . Warm the liquid to approximately  $38.5^{\circ}$  before injection.



## **PROCEDURE**

- **Inject the solution under examination slowly into the marginal veins of the ear of each rabbit over a period not exceeding 4 min.**
- **Record the temperature of each animal at half-hourly intervals for 3 hours after injection.**
- **The difference between the initial temperature and the maximum temperature which is the highest temperature recorded for a rabbit is taken to be its response.**

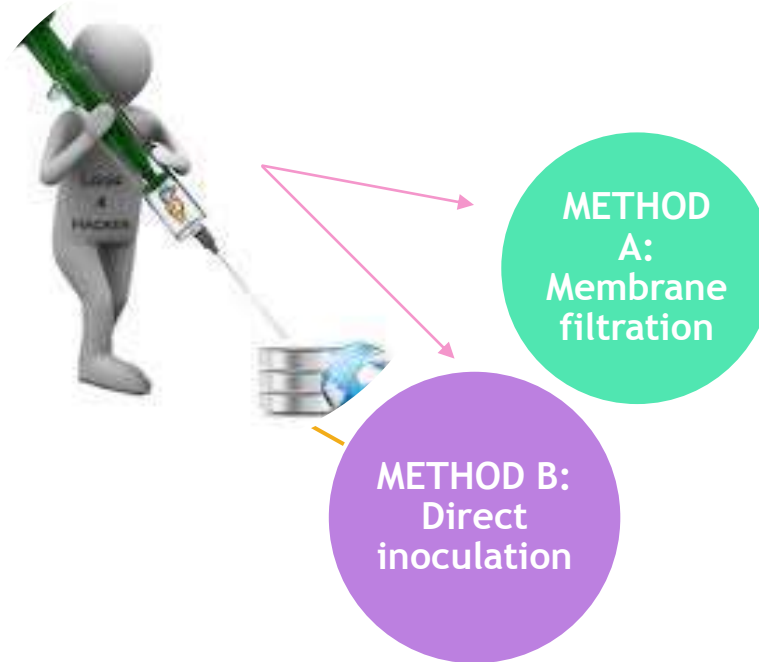
# INTERPRETATION OF RESULT

No. of Rabbits	Individual Temperature rise (°c)	Temperature rise in groups (°c)	Test
3 Rabbits	0.6	1.4	Passes
If above not passes 3+5 =8 rabbits	0.6	3.7	Passes



# TEST FOR STERILITY

**Sterility is defines as freedom from the presence of viable microorganism**



# Media to be used in the sterility test

Fluid  
Thioglycol  
ate  
Medium

Soyabean-  
casein digest  
Medium

## MEMBRANE FILTRATION METHOD:-

- A membrane has a nominal pore size not greater than  $0.45\mu$  and diameter of approximately 50mm.
- This method basically involves filtration of Sample through membrane filters.
- The filtration is assisted under strict aseptic condition, after filtration complete the membrane is cut into 2 halves and one halve is placed in suitable volume of ( 100 ml usually)FTM, SCDM medium.
- Incubate the media for not less than 14 days.

## **DIRECT INOCULATION METHOD:-**

©The DT method is the more traditional sterility test method. Basically, the DT method involves three steps:

- 1. Aseptically opening each sample container from a recently sterilized batch of product.**
- 2. Using a sterile syringe and needle to withdraw the required volume of sample for both media from the container**
- 3. Injecting one-half of the required volume sample into a test tube containing the required volume of FTM and the other half volume of sample into a second test tube containing the required volume of SCD.**

## MINIMUM QUANTITY TO BE USED FOR EACH MEDIUM

Quantity per container	Minimum quantity to be used for each medium
Liquids	
1. less than 1 ml	The whole contents of each container
2. 1-40 ml	Half the contents of each container but not less than 1 ml
3. Greater than 40 ml and not greater than 100 ml	20 ml
4. Greater than 100 ml	10 per cent of the contents of the container but not less than 20 ml
Antibiotic liquids	1 ml

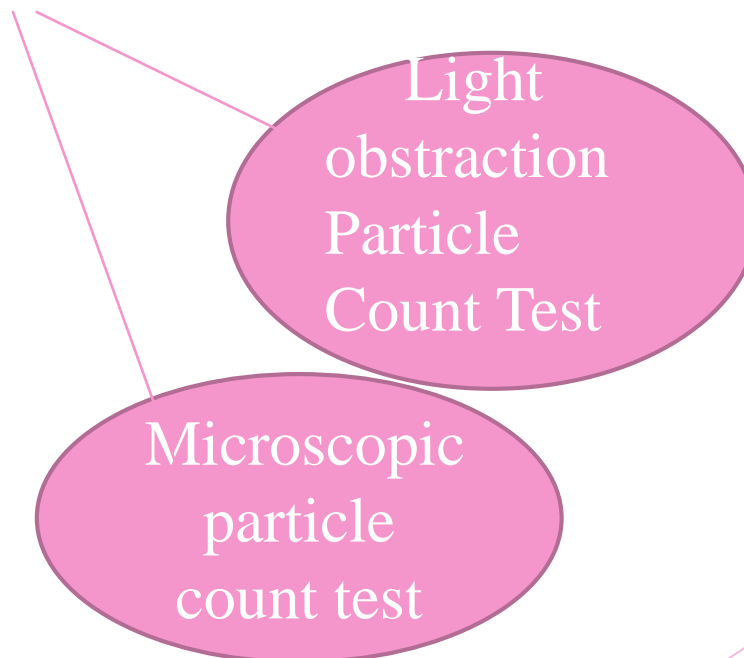
## **INTERPRETATION OF RESULTS**

- **If the material being tested renders the medium turbid so that the presence or absence of microbial growth cannot be easily determined by visual inspection, 14 days after the beginning of incubation, transfer portion (< 1 ml) of the medium to fresh vessels of the same medium and then incubate original and transfer vessel for not less than 4 days.**
- **If No evidence of microbial growth is found- complies with test for sterility.**
- **If evidence of microbial growth is found- does not complies with test for sterility.**

# PARTICULATE MATTER TEST

**Particulate matter refers to the extraneous, mobile, undissolved particles, other than gas bubbles, unintentionally present in the solutions.**

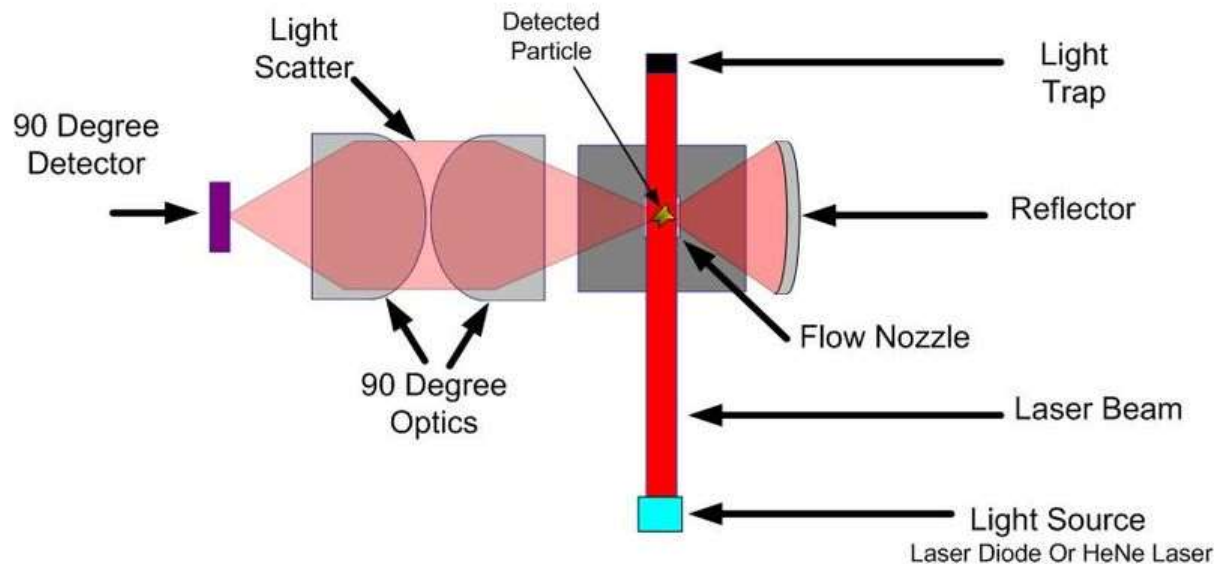
**2 methods are used:**



# LIGHT OBSTRUCTION PARTICLE COUNT TEST

Use a suitable apparatus based on the principle of light blockage which allows an automatic determination of the size of particles and the number of particles according to size.

Top Down View of Particle Counter





## Limits

Sample	Particle size in $\mu\text{m}$	Maximum no. of particles.
LVP $\geq$ 100 ml	10 25	Average in the units tested 25 per ml 3 per ml
SVP - 100 ml and less than 100 ml	10 25	6000 per container 600 per container

# MICROSCOPIC PARTICLE COUNT TEST

- **Wet the inside of the filter holder fitted with the membrane filter with several milliliter of particle-free water .**
- **Transfer the total volume of a solution pool or of a single unit to the filtration funnel, and apply vacuum.**
- **Place the filter in a Petri dish and allow the filter to air-dry.**
- **After the filter has been dried, place the Petri dish on the stage of the microscope, scan the entire membrane filter under the reflected light from the illuminating device, and count the number of particles**

## Limits :

Sample	Particle size in $\mu\text{m}$	Maximum no. of particles.
LVP $\geq$ 100 ml	10 25	Average in the units tested 12 per ml 2 per ml
SVP - 100 ml and less than 100 ml	10 25	3000 per container 300 per container

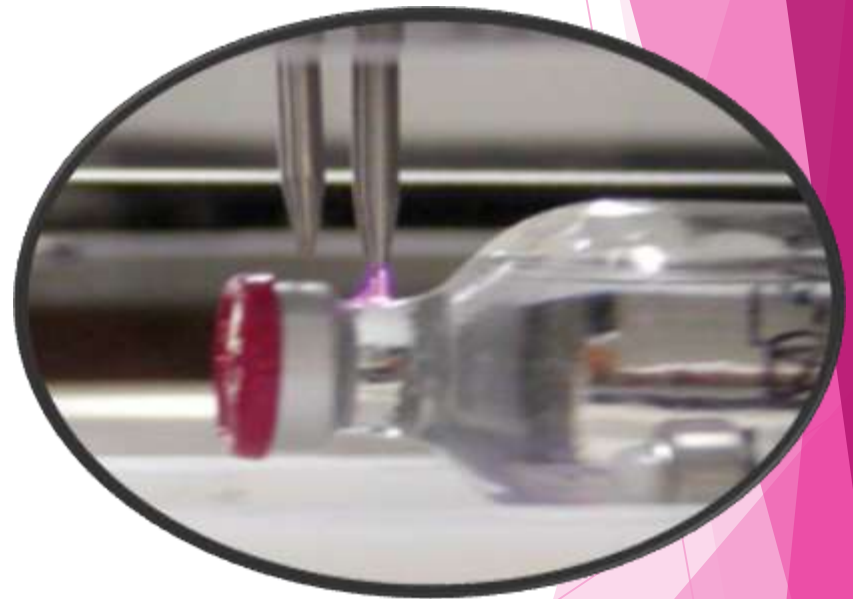
# **LEAKAGE TEST**

- Leakage test is employed to test the package integrity.
- Package integrity reflects its ability to keep the product in and to keep potential contamination out.
- Which is the flow of matter through the barrier itself.
- Leakage tests are 4 types
  - a) visual inspection
  - b) bubble test
  - c) dye tests
  - d) vacuum ionization test

# Leakage test apparatus



## High voltage leak detection



# **TEST FOR BACTERIAL** **ENDOTOXIN**

- **Measures the concentration of bacterial endotoxin**
- **Test is using lysate derived from hemolymph cells or amoebocytes of horse shoe crab**
- **Endotoxin limit calculated by**

**K/M**

**K** → maximum no. of endotoxin which receive the patient without suffering toxic reaction

**M** → maximum dose administered to a patient/kg/hr

# Mechanism of LAL Test

The test is based on the primitive blood-clotting mechanism of the horseshoe crab

enzymes located within the crab's amoebocyte  
blood cells

+

endotoxins



initiation of an enzymatic coagulation cascade



proteinaceous gel

# Horse shoe crab





## LAL reagent

- Bleeding adult crabs blood into an anticlotting solution
- Washing and centrifuging to collect the amoebocytes
- Lysing in 3% NaCl
- Lysate is washed and lyophilized for storage

# Procedure

## Test:

- Equal volume of LAL reagent and test solution (usually 0.1 ml of each) are mixed in a depyrogenated test-tube
- Incubation at 37°C, for 1 hour
- Remove the tube - invert in one smooth motion (180°)
- Observe the result

## Different Techniques

- Three different techniques:
  - The **gel-clot technique** - gel formation
  - The **turbidimetric technique** - the development of turbidity after cleavage of an endogenous substrate
  - The **chromogenic technique** - the development of color after cleavage of a synthetic peptide - chromogen complex

# Gel Clot Technique

- A solid gel is formed in the presence of endotoxins
- This technique requires positive and negative controls
  - Positive controls - a known concentration of endotoxin added to the lysate solution
  - Negative controls - water, free from endotoxins, added to the lysate solution

# Turbidimetric Technique

- The test is based on the measurement of opacity change due to the formation of insoluble coagulin
- Opacity is directly proportional to the endotoxin concentration
- This technique is used for water systems and simple pharmaceutical products

# Chromogenic Technique

- This is based on the measurement of color change which is caused by the release of the chromogenic chemical
- *p*-nitroanilide
- The quantity of the *p*-nitroanilide produced is directly proportional to the endotoxin concentration

## CONCLUSION

- **Quality control should be a fundamental segment of parenteral products manufacturing.**
- **All of the 5 basic tests which are performed are essential and have its own importance in parenteral production .**
- **All of these tests ensure that product meet its quality which has been judged to satisfactory also.**
- **Each test is unique and provides detailed assessment of quality control for parenteral products.**

# REFERENCE

- [www.pharmainfo.net/lal-test](http://www.pharmainfo.net/lal-test)
- [www.who.int/phint/en/d/Jb.7.3.4/](http://www.who.int/phint/en/d/Jb.7.3.4/)
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- **USP. Appendix 788, 56.**
- **IP. page no: 659 to 660**
- **Remington. The science and Practice of Pharmacy. 21<sup>st</sup> ed. Page no. 1367 to 1374**
- **[www.gpattutor.com](http://www.gpattutor.com)**

**THANK YOU**