

INDUSTRIAL PHARMACY-I**UNIT IV-PARENTERALS****CLASS:32****TOPIC vials and infusion fluids. Quality control tests of parenteral products****VIALS**

- Vial is a glass or plastic container closed with a rubber stopper and sealed with an aluminum crimp.
- Vials are available for single or multiple dosing. Injection vials can be obtained in either neutral or soda glass and occasionally in treated soda glass.



Filling machine



Sealing machine

INFUSION FLUID

- PVC(polyvinyl chloride) collapsible bags are used to pack most infusion fluid. It is a part of large volume parenteral product(100ml-1000ml per day).

ADVANTAGES:

- Durable and light weight.
- No air interface. The bag collapses as it empties.

DISADVANTAGES:

- They adsorb some drugs.

- The permit high moisture penetration



- Semi rigid polythene bags are also used for parenteral solution(3L),dialysis solution(5mL) and 100mL electrolyte solution.
- The filling of the infusion fluid is done by filling machine only.
- When closures are to be inserted by machines the surface of closure is usually halogenated or coated with silicone to reduce friction
- Aluminium caps are used to hold rubber closures in space
- **Single caps**-permanent center hole or a center that is torn away at the time of use to expose the rubber closure
- **Double aluminium caps**-usually have inner cap with a caps-permanent center hole which in use exposed when the entire outer cap is torn off
- **Triple aluminium caps** are used for large bottles with rubber closures having permanent holes for attachments to administration sets
- The inner cap with a caps-permanent center hole remains in place during use to secure the rubber closure
- The thin disc is used in conjunction with a thin rubber disc to seal the holes through the closure
- The outer cap holds the disc in place is torn away at the time of use



Quality control tests of parenteral products

In process quality control test:

- Conductivity measurement
- Volume filled
- Temperature for heat sterilised product
- Environmental control test
- Osmolarity
- pH measurement

Conductivity measurement :

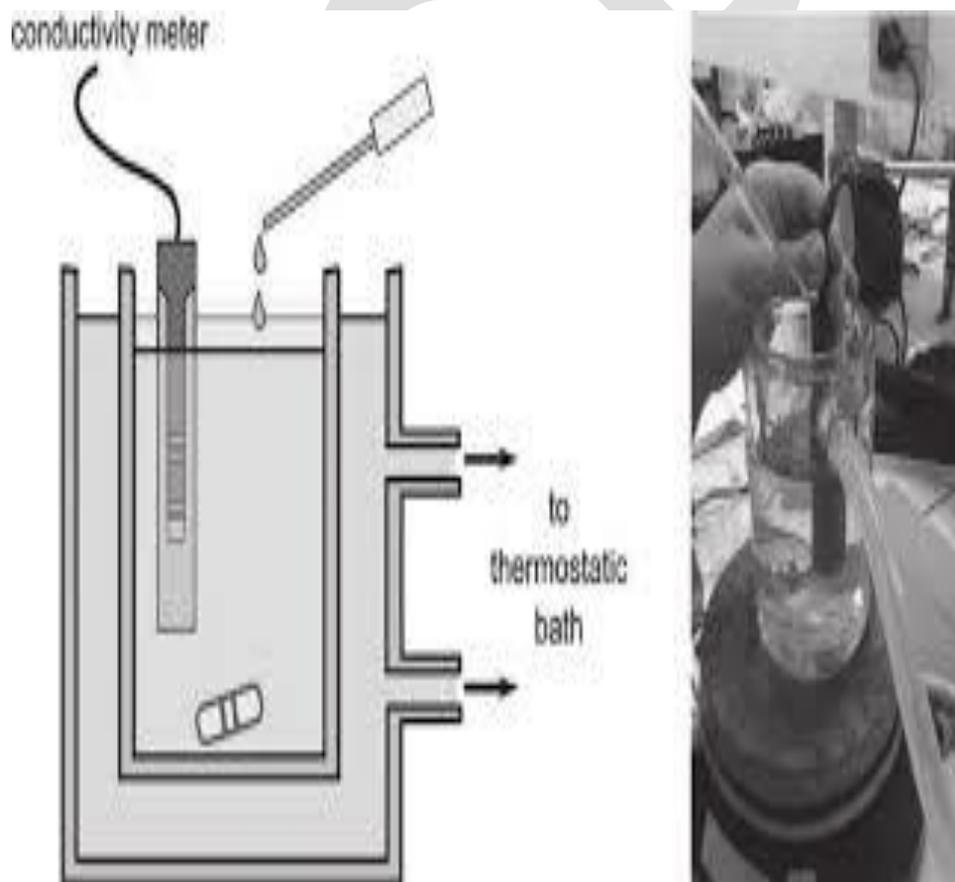
- Conductivity is measured by conductometer.
- It measures the conductivity of vehicle used in sterile preparation.
- Conductivity of pure water is 0.55 microsiemens /cm.

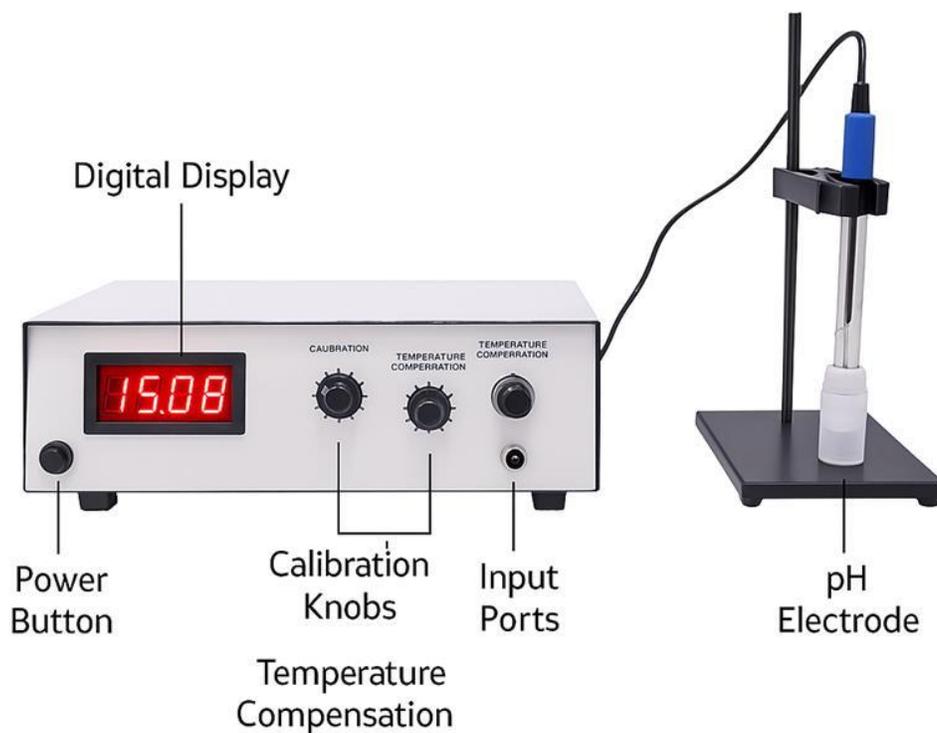
pH measurement

2 different types of methods used in measurement of pH.

1. Dip a piece of pH paper into the sample.

2. pH meter

Conductivity meter

pH meter**Temperature for heat sterilization-**

- It is important to maintain the constant temperature during heat sterilization of product.
- The temperature changes may cause some undesirable changes like change in potency, change in isotonicity.
- The temperature can be determined by normal thermometer
- Volume filled –

An injection container is filled with a volume in slight excess of the labeled size

Labeled Size (ml)	Mobile Liquid	Viscous Liquid
0.5	0.1ml	0.12ml
1	0.1ml	0.15ml
2	0.15ml	0.25ml
5	0.3ml	0.5ml
10	0.5ml	0.7ml
20	0.6ml	0.9ml
50	2%	3% ml

FINISHED PRODUCT QUALITY CONTROL TEST

- There are mainly five quality control test for parenterals are performed.
- Content Uniformity Test
- Leaker test
- Clarity test
- Pyrogen test
- Sterility test
- Particulate test

CONTENT UNIFORMITY TEST

- 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 NMT 7.8%.

LEAKER TEST

- Leaker test for ampoules is intended to detect **incompletely sealed ampoules** so that they can be discarded in order to maintain sterile condition of the medicines.
- Tip seals are more likely to be incompletely closed than pull seals.

Open capillaries or cracks at the point of seal result in LEAKERS

- The leaker test is performed by immersing the ampoules in a dye solution, such as 1% Methylene blue, and applying at least 27 inches of Hg vacuum for a minimum of 30 mins.
- Detection of leaker is prominent when ampoules are immersed in a bath of dye during autoclaving as this has advantage of accomplishing both leaker detection and sterilization in one operation.
- Another means of testing for leakers is a high frequency spark test system which detect presence of pinholes in ampoules.
- Bottles and vials are not subjected to such a vacuum test because of the flexibility of the rubber closure.

Clarity test

- It is performed to ensure that the parenterals are free from visible foreign particles.

- Each parenteral preparation in its final container is subjected individually to a visual inspection to exclude the possibility of foreign particles.
- The unlabelled containers are held by the neck against strongly illuminated black (for dark particles)& white screen(for light colour particles).
- The contents of the container are slowly inverted & rotated ,then examined. . It may be dangerous when the particle size is larger than R.B.C. & may block the blood vessel. This type of products are immediately rejected from the batch.
- The limit test for particulate matter is prescribed in I.P. 1996 (A- 125)
- Applicable for: 100 ml or more volume containers of single dose IV given by IV infusion.
- Not applicable for: Multi-dose injections Single dose SVP Injectable solutions constituted from sterile solids



PYROGEN TEST

- Pyro” (Greek = Fire) + “gen” (Greek = beginning). v Fever producing, metabolic by-products of microbial growth and death. v Bacterial pyrogens are called “Endotoxins”.
- Gram negative bacteria produce more potent endotoxins than gram + bacteria and fungi.
- Endotoxins are heat stable lipopolysaccharides (LPS) present in bacterial cell walls, not present in cell-free bacterial filtrates
- Stable to at least 175oC; steam sterilization ineffective
- Water soluble; monomer unit of LPS can be 10,000 Daltons (1.8 nm) so endotoxins can easily pass through 0.22µm filters
- Sources: Water (main),contaminated solutes, raw materials, equipment, process environment, people, and protein expression systems if using gram negative bacteria.
- Water is free from pyrogens if it has been distilled so that condensed molecules have gone through vapour state protected from the inadvertent contamination
- Open containers of solutes capable of supporting growth of microoraganisms invites such contamination
- Containers renerded free from pyrogens by adequate cleaning and heating usually at 210oc for 3 to 4 hrs
- Autoclaving temperatures do not destroy pyrogens during a normal cycle
- Selective solvent extraction methods are useful in the production of antibiotics –heavy pyrogen contamination result of fermentation process-
- ultrafiltration –separation and elimination of pyrogens
- 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 mins.
- Test animals: Use healthy, adult rabbits of either sex, preferably of the same variety.
- Recording of temperature: Clinical thermometer, thermistor.

- Rabbits are used to perform this test because their body temp increases when pyrogen are introduced into their bodies by parenteral route
- 3 healthy adult rabbits of either sex, each weighing NLT 1.5 kg are selected
- Do not use any rabbit having a temp higher than 39.8 o C
- Showing temp variation >0.2 o C between two successive reading in the determination of initial temp
- Same test is performed within 7 days of actual test

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 mins 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° before injection

PYROGEN TESTING



PROCEDURE

- Inject the solution under examination slowly into the marginal veins of the ear of each rabbit over a period not exceeding 4 mins.
- 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

BACTERIAL ENDOTOXIN TEST(IP&BP)

- BET measures the concentration of bacterial endotoxin that may be present in the sample

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

using a lysate derived from the amoebocytes of the horseshoe crab, *Limulus polyphemus*.

- The addition of a solution containing endotoxins to a solution of a lysate produces turbidity, precipitation or gelation of the mixture.
- Only endotoxins from gram negative bacteria reacts in this way
- 5 to 10 times more sensitive than rabbit test
- Method A-Gel clot limit test method.
- Method B-Semi quantitative gel clot method.
- Method C-Kinetic Turbidimetric method.
- Method D-Kinetic Chromogenic Method



- Method E- End point chromogenic method

Gel clot limit test method

Preparation of test solution-

- Preparation of test solution by dissolving or diluting active substances
- Adjust the pH of test solution (about 6 to 8) pH adjust with use of acid, base, buffer.
- Prepare a sample solution at any dilution at or below Maximum Valid Dilution.
- Use water bacterial endotoxin as –ve control and
- 2 positive control. One of the +ve control consist of the control standard endotoxin at a conc. of 2λ. (labelled sensitivity)
- Other consist of test solution spiked with control standard endotoxin to give a conc. Of 2λ (ppc)

Interpretation Of Result

- The product under examination complies with the bacterial endotoxin test if the +ve control is +ve and –ve control as well as test solution are –ve.
- The test is not valid if the +ve control is –ve or if the –ve control is +ve
- **KINETIC TURBIDIMETRIC METHOD-** A photometric assay measuring the increase in turbidimetry caused by the reaction of the endotoxin with the lysate.

- **KINETIC CHROMOGENIC METHOD-** A photometric assay measuring a colour developed by the chromophore released from a chromogenic substrate by the reaction of the endotoxin with the lysate.

Advantages of LAL Test

- Greater Sensitivity
- Less Variability
- Much Less False Positives
- Much Less Expensive
- Alternative to Animal Model
- cheaper,
- more accurate than other
- is performed in the pharmaceutical laboratory
- specific for endotoxins of gram-negative origin
- particularly useful for: Radiopharmaceuticals and cytotoxic agents
- Products with marked pharmacological or toxicological activity in the rabbit (e.g. insulin)
- Blood products which sometime give misleading results in the rabbit
- Water for injection where LAL test is potentially more stringent and readily applied

STERILITY TEST

- Sterility is defines as freedom from the presence of viable microorganism.
- Sterility test is define as microbiological test applied to sterile product to show are the product manufactured and processed under specification guided by cGMP.
- Sterility test is destructive test thus,it is impossible to test every item for sterility.

Media to be used in the sterility test

- Fluid Thioglycollate Medium
- Soyabean-casein Digest Medium

Methods:

B.PHARMACY 5TH SEMESTER (2025-2026)

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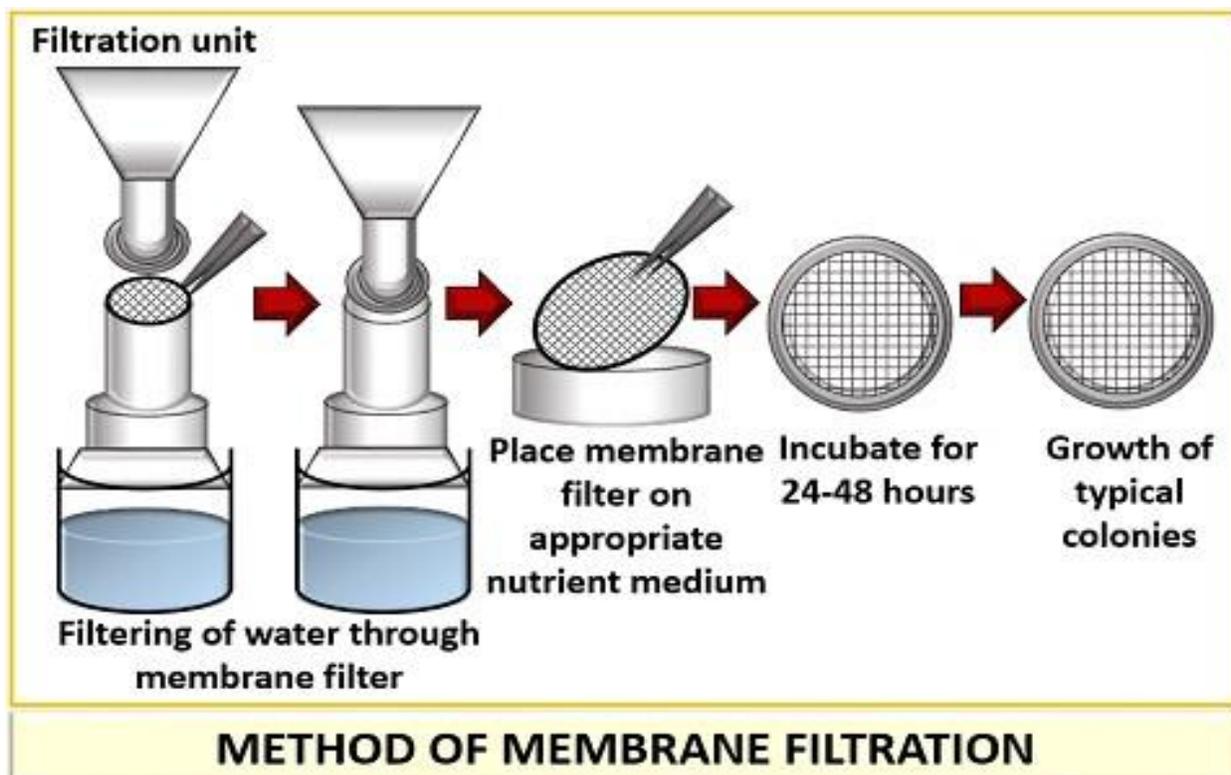
- Method A- Membrane filtration method
- Method B- Direct inoculation method

Table 23.7: Composition of culture medium for sterility testing

Components	Culture medium	
	Fluid thioglycollate	Soybean-casein digest
L-cystine	0.5 g	–
Sodium chloride	2.5 g	5.0 g
Dextrose	5.0/5.5 g	2.3/2.5 g
Pancreatic digest of casein	15.0 g	17.0 g
Papaic digest of soya bean	–	3.0 g
Dibasic potassium phosphate	–	2.5 g
Granular agar (moisture < 15%)	0.75 g	–
Yeast extract (water-soluble)	5.0 g	–
Sodium thioglycollate or thioglycolic acid	0.5 g or 0.3 ml	–
Resazurin (0.10% (w/v) fresh solution)	1.0 ml	1000 ml
Purified water	1000 ml	

MEMBRANE FILTRATION METHOD

- A membrane has a nominal pore size not greater than 0.45μ and diameter of approximately 50mm.
- This method basically involves filtration of Sample through membrane filters.
- The filtration is assisted under Vacuum, after filtration completion the membrane is cut into 2 halves and one half is placed in two test tubes containing FTM, SCDM medium.
- Incubate the media for not less than 14 days.
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DIRECT INOCULATION METHOD

- It involves a direct inoculation of required volume of a sample in two test tubes containing a culture medium that is FTM, SCDM.
- Volume of the preparation under examination is not more than 10% of the volume of the medium.
- Incubate the inoculated media for not less than 14 days.

Quantity per container	Minimum quantity to be used for each medium
liquids	
less than 1 ml	The whole contents of each container

1-40 ml	. Half the contents of each container but not less than 1 ml
Greater than 40 ml and not greater than 100 ml	20ml 20 ml
Greater than 100 ml	10 per cent of the contents of the container but not less than 20 ml
Antibiotic liquids	1 ml

Number of items in the batch	Minimum number of items to be tested for each medium
Parenteral preparations	
Not more than 100 containers	10 per cent or 4 containers whichever is the greater
More than 100 but not more than 500	10 containers
More than 500 containers	2 per cent or 20 containers (10 containers for large-volume parenterals)

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 comply with test for sterility

PARTICULATE TEST

- Particulate matter refers to the extraneous, mobile, undissolved particles, other than gas bubbles, unintentionally present in the solutions.
- 2 methods are used:
- Method A-Light Obscuration Particle Count Test
- Method B-Microscopic particle count test

LIGHT OBSCURATION 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.

BP, JP and USP limits for particulate matters determined by LOPC tes

Nominal Volume	$\geq 10 \mu\text{m}$	$\geq 25 \mu\text{m}$
More than 100 mL	25 particles/mL	3 particles/mL
100 mL or less than 100 mL	6000 particles/container	600 particles/container

MICROSCOPIC PARTICLE COUNT TEST

- Wet the inside of the filter holder fitted with the membrane filter with several millilitre 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

Sample	Particle size in μm	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