Pharmaceutical Quality Management

IPQC FOR PARENTERALS

By:

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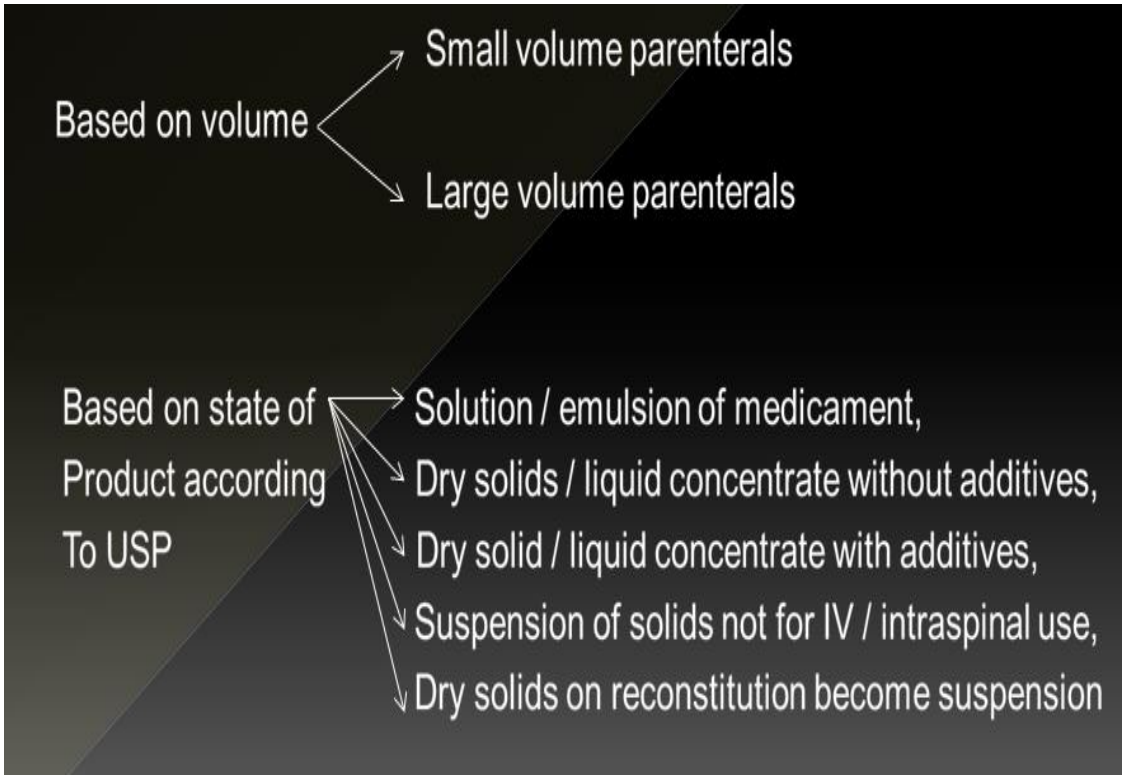
PARENTERALS

A parenteral dosage form can be defined as a sterile drug product which is presented in the form of solution, suspension, emulsion, or reconstituted lyophilized powder, suitable for administration by injection through skin or mucous membrane.

Para enteron = beside the intestine.



▸ IPQC FOR PARENTERAL PRODUCTS

1) Clarity test (for particulate matter)

1.1. By visual method

1.2. Light obscuration technique

1.3. Membrane Microscopy

2) Leakers Test (package integrity)

2.1. Visual methods

2.2. Bubble test

2.3. Dye test

2.4. Vacuum ionization method

3) Fill volume Testing

4) pH

5) Pyrogen testing

5.1. Rabbit fever response test

5.2. Limulus amoebocyte lysate test

6) Assay for drug content

7) Sterility test

7.1. Membrane filtration method

7.2. Direct inoculation method

8) Test for packing containers

8.1. For glass containers

8.1.1. Water attack test

8.1.2. Powder glass test

8.2. For plastic containers

8.2.1. Leakage test

8.2.2. Collapsibility test,

8.2.3. Transparency test,

8.2.4. Water vapor

8.2.5. Permeability test

9) Inspecting for label check

10) Uniformity of contents

11) Uniformity of weight.

12) Passiability of parenteral preparation from needle

13) Conductivity test

1. PARTICULATE MATTER

"*Particulate matter in injections and parenteral infusions consists of mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions." (USP <788>) (EP 2.9.20)*

- Monitor the Visible and Subvisible Size Domains
- *What is Being Seen?*

  Substances detected by unaided human vision within a small time window

- *What is being counted?*

  Not just single, hard particles any immiscible to semi-solid to solid material, soft to hard, transparent to opaque may be counted as a particle

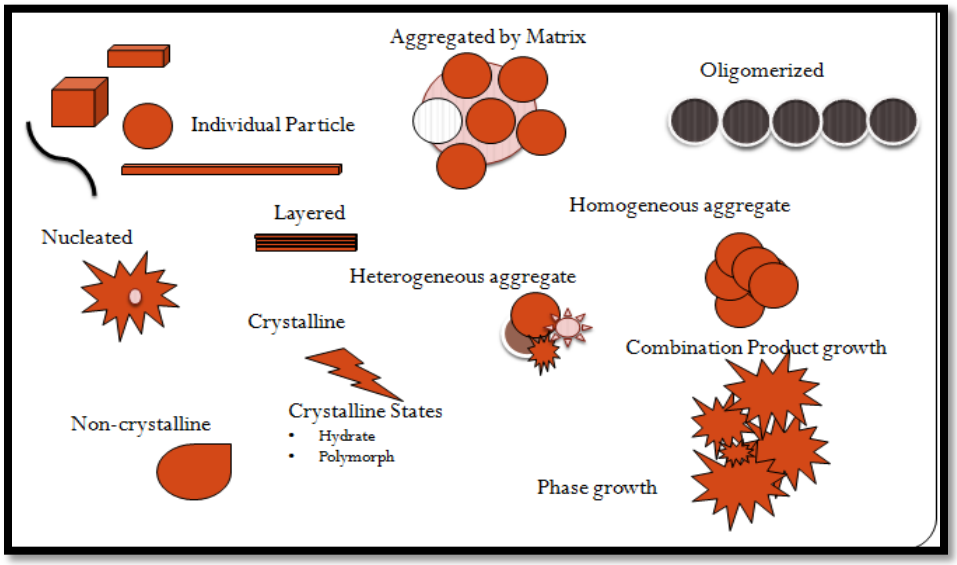
  singular solids, aggregates, drug solids, salts, polymorphs, gels, lubricants, plasticizers

Particle Definition

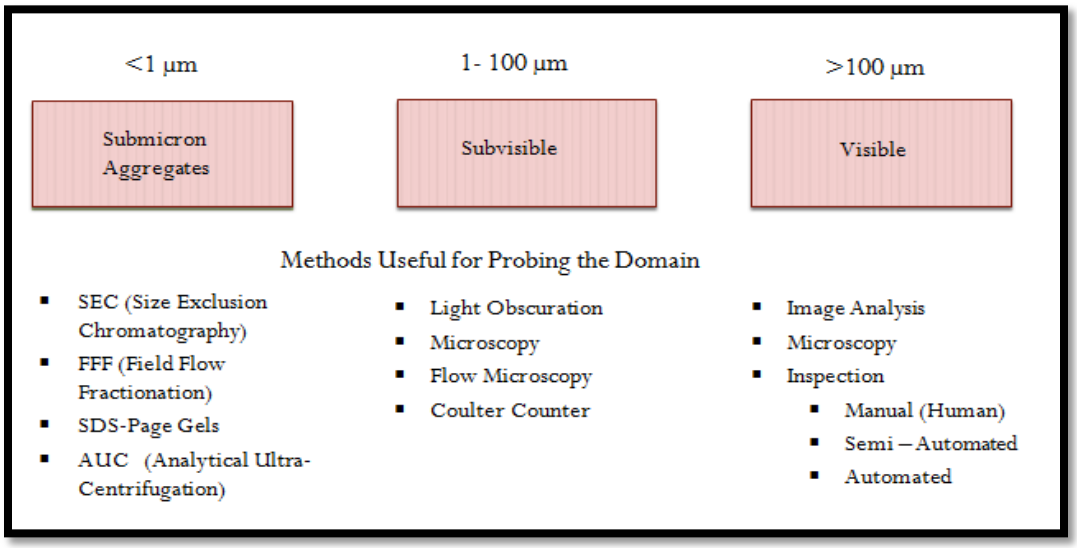
- For All Particles:
  - Mobile (not attached?)
  - Undissolved (Recrystallization? Immiscibles?)
  - Not air bubbles (not oil?)
- Particles may be many things
  - Single
  - Aggregated
    - ✓ Matrix?
    - ✓ Heterogeneous?
  - Non-crystalline vs. crystalline

- Complex
  - ✓ Layered
  - ✓ Nucleated

Particle Complexity



Particulate matter size range



Particle Categories

Extrinsic	Intrinsic	Inherent
Wild, Outside the System	Inside the System	**Is** the System: Solution Micelles Emulsion Colloid Protein Assembly
Extremes are "Filth"	Product-contact	n/a
Microbial Vector	May have Microbial Content	Formulation-Relevant
Uncontrolled	Unplanned	Controlled, Expected
Additive	Additive or Changing	Stable
Single to Many Particles	Various Physical States	Defined active ingredient
May be Considered Most Objectionable	Needs Planning & Control	Most Acceptable, Known

Compendial Guidance

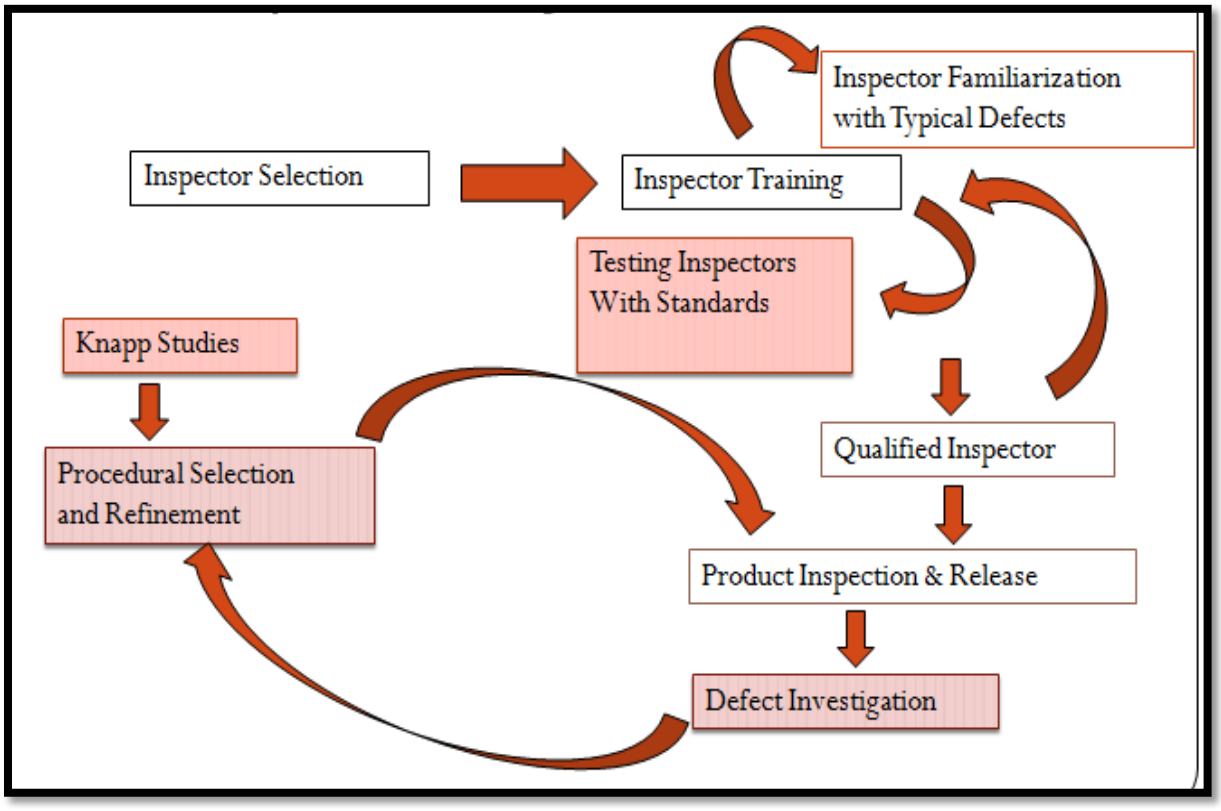
VISIBLE PARTICLE CONTENT

- USP <1> Injections
  - "…presence of observable foreign and particulate matter…"
  - "…every lot of all parenteral preparations is essentially free from visible particulates."
- EP <6.0> Parenteral Preparations, Injections
  - "…clear and practically free from particles."
- JP <6.06> Foreign Insoluble Matter Test for Injections
  - "…must be free and clear from readily detectable foreign insoluble matter."
- World Health Organization
- British Pharmacopeia
- Korea, China, India

1.1. VISUAL METHOD

- *In visual inspection, each injectable is inspected visually against white and black backgrounds. The white background aids in detection of dark colored particles.*
- *The light or reflective particles will appear against the black back ground. Some visual-enhancing aids can increase the efficiency.*
- *A magnifying lens at 2.5 × magnification set at the eye level facilitates the inspection. Microscopic examination enhances detection of particulate matter in injectable. Visual inspection gives the qualitative estimation of the particulate matter.*
- *Acceptance Standards is that each container checked must not contain any visible particulate matter.*

Visual Inspection System



❖ SUBVISIBLE CONTENT AND DETERMINATION

Two Methods with 10µm and 25µm size thresholds for counting

- **1.2. Primary method is an optical particle counter - *LIGHT OBSCURATION (LO)* -** for moving fluid
  - What is being measured electronically?
    - ✓ Size is Equivalent Circular Diameter of suspended substance
    - ✓ Optical particle counter will register air and immiscible liquids as "particles"
- **1.3. Secondary method - *MEMBRANE MICROSCOPY (MM)* -** fluid filtration and membrane capture, with light microscopy count
  - What is being measured microscopically?
    - ✓ Particles retained on a membrane may appear different than in their "wet" state
    - ✓ Size is Longest Chord
- **Harmonized <788> Limits**
  - Two methods = "two-tiered approach"
  - *Light Obscuration* is the preferred first pass
  - Membrane Microscopic method is run if LO results are suspicious, or fail limits, or alone when LO cannot be run (emulsions)

	Method 1 – LO		Method 2 - Microscope	
Parenteral Volume	≥ 10µm	≥ 25µm	≥ 10µm	≥ 25µm
SVI 100 mL and lower	6000 per container	600 per container	3000 per container	300 per container
LVI above 100 mL	25 per mL	3 per mL	12 per mL	2 per mL

Should there be a Total Load limit for LVI's?

2. LEAKER TEST

- *Leaker test is employed to test the package integrity. Package integrity reflects its ability to keep the product in and to keep potential contamination out.*
- *It is because leakage occurs when a discontinuity exists in the wall of a package that can allow the passage of gas under pressure or concentration differential existing across the wall.*

- **LEAK TEST METHODS:**

2.1. VISUAL INSPECTION

- Visual inspection is the easiest leak test method to perform. But this method is least sensitive..
- The method is used for the evaluation of large volume parenteral. To increase the sensitivity of the method, the visual inspection of the sample container may be coupled with the application of vacuum to make leakage more readily observable.
- This method is simple and inexpensive. However, the method is insensitive, operator dependent, and qualitative.

Sometimes, the method is used in combination with pressure and /or temperature cycling to accelerate leakage to improve sensitivity

2.2. BUBBLE TEST

- The test package is submerged in liquids. A differential pressure is applied on the container. The container is observed for bubbles.
- Sometimes, surfactant added liquid is used for immersion of test package. Any leakage is evident after the application of differential pressure as the generation of foaming in immersion liquid. The method is simple and inexpensive.
- The location of the leaks can be observed in this method. However, it is relatively insensitive and the findings are operator dependent and are qualitative.

- The optimized conditions can be achieved using a surfactant immersion fluid along with the dark background and High intensity lighting. Generation of a differential positive pressure of 3 psi inside the vial and observation of any leakage using magnifying glass within a maximum test time of 15 minutes.

2.3. DYE TESTS

- The test container is immersed in a dye bath. Vacuum and pressure is applied for some time.
- The container is removed from the dye bath and washed. The container is then inspected for the presence of dye either visually or by means of UV spectroscopy.
- The dye used may be of blue, green, yellowish-green color.
- The dye test can be optimized by use of a surfactant and or a low viscosity fluid in the dye solution to increase the capillary migration through the pores.
- The dye test is widely accepted in industry and is approved in drug use. The test is inexpensive and is requires no special equipment required for visual dye detection.
- However, the test is qualitative, destructive and slow. The test is used for ampoules and vials.

Closure Re-seal Method Parameters	USP 31 <381> Ph.Eur. 3.2.9	ISO 8362-5 Annex C	Modified ISO
Dye	0.1% aq. Methylene Blue		
Vacuum	-27 KPa	-25 KPa	-37 KPa
Time at Vacuum	10 min	30 min	30 min
Time at Ambient	30 min	30 min	30 min
Detection method	Visual inspection		

2.4. VACUUM IONIZATION TEST

- Vacuum ionization test is useful for testing leakage in the vials or bottles sealed under vacuum.
- This test is used for online testing of the lyophilized products. High voltage, high frequency field is applied to vials which to cause residual gas, if present to glow.
- The blue glow is the indicative of vacuum which indicates the absence of leak while the purple glow indicative of no vacuum.
- The sensitivity of the method is not documented. This test is on-line, rapid and is non destructive test.
- However, the proteins present in the test sample may be decomposed. This method is used for the lyophilized vials of biopharmaceuticals.

❖ **High Voltage Leak Detector (HVLD)**

High Voltage Leak Detection is a preferred in-line test method for continuous leak testing on head and/or bottom areas of hermetically sealed plastic containers and ampoules containing sterile liquids.

HVLD High Voltage Leak Detectors have enormous measuring accuracy. Leaky containers with holes of µm sizes can be detected with a sufficiently high degree of reliability, even with liquids of low conductivity. HVLD systems meet the pharmaceutical industry's and the authority's current quality standards. In addition, the system's high operational capacity, product and process safety and its low production cost further speak for its high quality.

3. CHECK FOR FILL VOLUME:

- After filling the containers they are checked for the

correct volume filling.

- Check for fill volume is by visual inspection.
- This is important for small volume parenterals where the volume is less .
- By keeping the container against a bright background we can check for the fill volume.

- After conforming for the correct fill volume then they are lacked and labelled and sent to the ware house.

4. pH TEST

- Checking the bulk solution, before filling for drug content,

pH, color, clarity and completeness of solution

- The pH of a formulation must be considered from following standpoint:

1) The effect on the body when the solution is administered

2) The effect on stability of the product

3) The effect on container-closure system

- **pH measurement:**

- pH is measured by using a pH meter .
- pH meter is initially calibrated with respective buffer capsule then the pH of the preparation is measured.

5. PYROGENS

- The term pyrogen frequently refers to gram negative bacterial endotoxin.
- Pyrogen is a general term for any substance that causes fever after IV administration / inhalation .
- Microbial substances which act as pyrogen includes bacteria , fungi , plasmodia , viruses, staphylococcal endotoxin.
- The amount of USP reference standard endotoxin needed to initiate pyrogenicity in humans and rabbits is about 1ng/kg.
- Naturally occurring bacterial endotoxin contain the lipid, carbohydrate and protein makeup of the outer cell membrane of gram negative bacteria.
- Test for pyrogens can be carried out by in-vitro and in-vivo methods.

5.1. Rabbit test (in-vivo)

5.2. LAL test (Limulus amoebocyte lysate) (in-vitro)

5.1. RABBIT TEST:

- **PRINCIPLE:**

  - The test involves measurement of rise in body temperature of the rabbits following the intravenous injection of a sterile solution of the substance to be tested.
  - The body temperature of the rabbits increases if pyrogens are present in the injected test solution.

- **SELECTION OF TEST ANIMALS:**

  Healthy , adult rabbits of either sex, each weighing not less than 1.5kg.

  Do not use any rabbit for main test if:

  1) It shows a temperature variation greater than 0.2^0 C between two successive readings noted during the determination of initial temperature

  2) And it's temperature is higher than 39.8^0 c or lower than 38^0 c

- **EQUIPMENTS REQUIRED FOR THE TEST:**

  - All glass ware, syringes, 20 – 30 gauge needle is used and thermometer must be thoroughly washed with water for injection and heated in a hot air oven at 250^0c for NLT 30 min.
  - Retaining boxes for rabbits.

- **TEST:**

The test is carried out on a group of three rabbits.

Procedure:

i. Preparation of the sample:

- Warm the solution to about $37 \pm 2\ ^o$c before the injection. In the case of lyophilized products dissolve it in normal saline solution.

ii. Determination of initial temperature of rabbits:

- Insert a clinical thermometer into the rectum of each rabbit and normal readings of body temperature are taken prior to the injection of test solution.
- Two such readings are taken at an interval of 30 minutes and the mean is calculated.
- This mean reading is taken as the initial temperature of the rabbits or as a control.

TEMPERATURE RECORDING:

USP <85> states the following:

- Use accurate temperature sensing device such as clinical thermometer, or thermistor probes or similar probes that have calibrated to assure an accuracy of $\pm 0.1^0$c.
- Insert the temperature sensing probe into the rectum of the test rabbit to a depth of not less than 7.5 cm .
- Thermocouple contains two dissimilar electric conductor wires joined at one end to form measuring junction that produce thermal EMF.
- Thermocouples must be calibrated against National institute of standards and technology.

DETERMINATION OF THE RESPONSE OF RABBITS:

- The test solution is injected into the ear vein of each rabbit.
- The volume of injection is 10 ml/kg of the body weight.
- This volume varies according to the test substance and is prescribed in the individual monograph.
- Record the temperature of each rabbit at an interval of 30 minutes for 3 hours after the injection.
- This is the maximum temperature recorded for that rabbit.
- The difference between maximum temperature and initial temperature is taken as its response.
- If this difference is negative, it is taken as a zero response.

- **INTERPRETATION OF THE RESULTS:**
  - Consider any temperature decrease as zero rise. If no rabbit shows an individual rise in temperature of $0.5\ ^{0}$c / more above its respective control temperature , the product meets the requirements for absence of pyrogens.
  - If any rabbit shows an individual temperature rise of $0.5\ ^{0}$c or more continue test using five other rabbits .
  - If not more than three of eight rabbits show individual rise in temperature of $0.5\ ^{0}$c / more if sum of eight individual maximum temperature does not exceed $3.3\ ^{0}$c the material under examination meets the requirement for absence of pyrogens.

- **LIMITATIONS OF RABBIT PYROGEN TEST:**

i. In vivo model

  - A test method that uses a living animal as model will suffer from number of problems offered by biological systems.
  - No two rabbits will possess exactly the same body temperature or respond identically to same pyrogenic sample.
  - Rabbits are sensitive and vulnerable to their environment.

ii. Rabbit sensitivity to pyrogens

  - The pyrogenic response in rabbits is dose dependent.
  - The greater the amount of pyrogen injected per kilogram body weight the greater the temperature increase in rabbits.
  - The rabbit test is less sensitive to endotoxin.
  - It reports 50% pass/fail rate with 95% confidence at endotoxin level above 0.098ng/m (9/10ml/kg) dose
  - The greatest rise in temperature for any given dose of endotoxin occurred in the afternoon , while the least raise occurred at midnight.

- **INTERFERENCES OF THE RABBIT PYROGEN TESTS**
  - Many products administered parenteral cannot be tested for pyrogens with the rabbit test because of the interferences they create in the rabbit response to pyrogens if they are present in the product

Any products having a pyretic side effect such as the prostaglandins and the cancer chemotherauptic agents will interfere with rabbit response.

5.2. LIMULUS AMOEBOCYTE LYSATE TEST (LAL)

In this test the presence of endotoxin is detected by formation of solid gel.

- **REACTION MECHANISM:**
  - Endotoxin or a suitably prepared lipid A derivative of endotoxin activates a proenzyme of LAL having a molecular weight of 150,000.
  - Activation also depends on the presence of divalent metal cations such as calcium, manganese or magnesium. It has been shown that the sensitivity of the LAL assay for endotoxin detection can be increased 10 to 30 times by using LAL reagent containing 50mM magnesium.

The lower molecular fraction called coagulogen is cleaved into soluble and insoluble subunit .The insoluble subunit appears as a solid clot , or a turbid solution depending on coagulogen.

5.2.1. Gel clot bacterial endotoxin test:

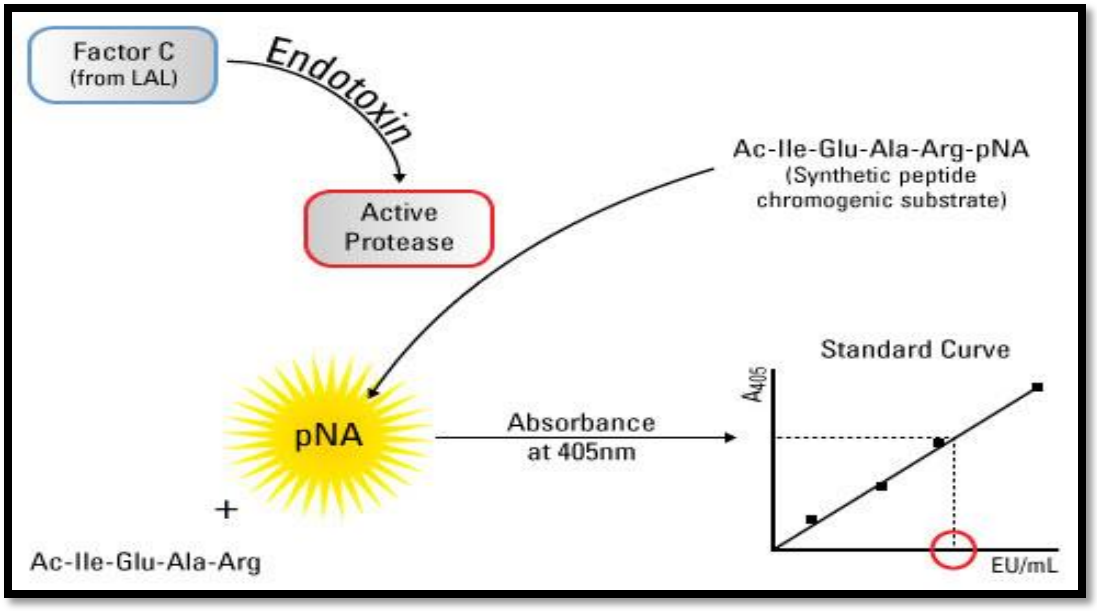
- The gel clot end point is the most commonly used endotoxin test
- It is simple and requires minimal laboratory equipment
- Equal volumes of test solution and LAL reagent (usually 0.1 ml of each) are mixed in glass test tubes .
- After incubation at 37 degrees C for one hour and tubes are observed for clot formation after inverting them.
- Formation of solid gel clot that withstands inversion of the tube constitutes a positive test.
- Each lot of gel clot reagent licensed by the FDA must be labeled with sensitivity(lambda) to reference standard endotoxin(RSE).

5.2.2. Kinetic turbidimetric assay

- During LAL endotoxin reaction the solution mixture becomes increasingly turbid
- The kinetic turbidimetric assay (KTA) requires the incubating micro plate or tube reader driven by an endotoxin specific software.
- The reaction mixture in a KTA system are continuously monitored for changes in optical density in each sample that are caused by scattering and absorption of light.
- Generally the KTA measures the onset time needed to reach a predetermined absorbance by each reaction mixture .
- The onset times for sample are compared with those of endotoxin studies to yield quantitative values for each sample / control that contain endotoxin

5.2.3. Kinetic and endpoint chromogenic assay

- Chromogenic LAL test do not utilize the coagulogen proteins from LAL reagent to produce an endpoint
- Although endotoxin activates the same enzymatic cascade from the reagent as described above the clotting enzyme reacts with synthetic substrate that has been added to the reaction mixture.
- The substrate consists of a colorless amino acid chain attached to a chromophore.
- The activated clotting enzyme cleaves the bond that holds the chromophore to the amino acid.
- The amount released is proportion to the concentration of endotoxin
- The chromophore that is released changes the color of the reaction mixture thereby increasing the optical density
- After reaction is determined the absorbance is read over one log range.



- **<u>ADVANTAGES COMPARED TO THE RABBIT TEST</u>**

  Proponents of the LAL test claim that the test offers at least seven advantages over the rabbit test for detecting pyrogens.

  - Greater sensitivity
  - Greater reliability
  - Better specificity
  - Less variation
  - Wider application
  - Use as a problem solving tool
  - Less expense.

  Researchers summarized the superiority of LAL test over the rabbit test by following:

  - ✓ After tens of thousands of tests an unexplained negative LAL test result –rabbit positive test result was never recorded.
  - ✓ Sometimes the rabbit test initially fails to detect pyrogens that were sometimes confirmed by initial LAL tests.

- **LIMITATIONS OF THE LAL TESTS:**
  - The greatest limitation of the LAL test is the problem of interference of the lysate –endotoxin interaction that is caused by a variety of drugs and
  - The reaction is mediated by clotting enzyme that is heat labile, pH sensitive and chemically related to Trypsin .
  - Inhibition caused by any material known to denature protein or to inhibit enzyme action .
  - Inhibition can be overcome by dilution or pH adjustment.
  - Dilution reduces the concentration of endotoxin and places greater demand on the sensitivity of the LAL regent to detect diluted amounts of endotoxin.

6. ASSAY FOR DRUG CONTENT

- Assay is performed according to method given In the monograph

of that parental preparation in the pharmacopeia

- Assay is done to check the quantity of medicament present in the parenteral preparation.
- So we can know the exact amount of medicament present such that it can perform its action.
- We should follow the official mon ograph IP/BP/USP for performing the assay.

7. STERILITY TEST

Sterility can be defined as the freedom from the presence of viable microorganisms.

- It is done for detecting the presence of viable forms of bacteria, fungi and yeast in parenteral products.
- The test for Sterility must be carried out under strict aseptic conditions in order to avoid accidental contamination of the product during test.

- All glassware's required for the test must be Sterile.
- Sterility testing attempts to reveal the presence or absence of viable micro-organisms in a sample number of containers taken from batch of product.
- Based on results obtained from testing the sample a decision is made as to the sterility of the batch.

MAJOR FACTORS OF IMPORTANCE IN STERILITY TESTING:

i. the environment in which the test is conducted
ii. the quality of the culture conditions provided
iii. the test method
iv. the sample size
v. the sampling procedure

i. ENVIRONMENTAL CONDITIONS:

- Environmental conditions avoid accidental contamination of the product during the test.
- The test is carried out under aseptic conditions regular microbiological monitoring should be carried out.

ii. CULTURE CONDITIONS:

- Appropriate conditions for the growth of any surviving organism should be provided by the culture media selection.
- Factors affecting growth of bacteria :
    - Nutrition
    - Moisture
    - Air

- Temperature
- pH
- Light
- Osmotic pressure
- Growth inhibitors

➢ Culture media used for sterility testing:

a) *Fluid thioglycolate medium*

b) *Soybean casein digest medium*

a. Fluid thioglycolate medium(FTM):

➢ FTM provides both aerobic and anaerobic environments within the same medium.FTM is an excellent medium for the detection of bacterial contamination.

➢ Thioglycolate has the advantage of neutralizing the bacteriostatic properties of mercurial preservatives.

Composition :

- L-cysteine , trypticase peptone, dextrose , yeast extract, sodium chloride, sodium thioglycolate,resazurin,agar,purified water ,final pH 7.1
- specific role of some ingredients primarily intended for the culture of anaerobic bacteria.
- incubation of the media: 14 days at 30 -35°C

b. Soybean casein digest medium:

Soya-bean casein digest medium primarily intended for the culture of both fungi and aerobic bacteria specific role of some ingredients.

incubation of the media: 14 days at 20 -25°C

composition:

Trypticase soya broth, trypticase peptone, phytone peptone, sodium chloride , dipotassium phosphate, dextrose, purified water , final pH 7.3

iii. STERILITY TEST METHODS

- ✓ Membrane filtration method
- ✓ Direct inoculation method

7.1. Membrane filtration methods

Selection of filters for membrane filtration:

- pore size of 0.45µm effectiveness established in the retention of micro-organisms appropriate composition the size of filter discs is about 50 mm in diameter

The procedure of membrane filtration:

- sterilization of filtration system and membrane filtration of examined solution under aseptic conditions.
- Filtration of the sample through a membrane filter having the nominal size of 0.45µm and a diameter of 47mm.
- After filtration the membrane is removed aseptically from the metallic holder and divided into two halves.
- The first half is transferred into 100 ml of culture media meant for fungi and incubated at 20˚ to 25 ˚c for not less than seven days.
- The other half is transferred into 100ml of fluid thioglycolate medium and incubated at 30 to 35 ˚C for not less than 7 days.

Advantages of membrane filtration over direct inoculation method

- Greater sensitivity

- The entire contents can be tested providing an advantage in the sterility testing of LVP and increasing the ability to detect contamination.
- The antimicrobial agent and antimicrobial solutes in the product sample can be eliminated by rinsing prior transferring the filter into test tubes of media thereby minimizing the incidence of false-negative test results.
- Organisms present in an oleaginous product can be separated from the product during filtration and cultured in a more desirable aqueous medium.

Disadvantages:

- This method cannot differentiate the extent of contamination between units if present because all product contents are combined and filtered through a single filter and cultured in single test tube.
- There exists a higher probability of inadvertent contamination in manual operations.

7.2. DIRECT INOCULATION METHOD:

- Required quantities of liquid is removed from the test containers with a sterile pipette / sterile syringe.
- Aseptically transfer the specified volume of the material from each container to vessel of culture medium
- Mix the liquid with medium but not aerate excessively
- Incubate the inoculated media for not less than 14 days , unless otherwise specified in the monograph at 30^0C - 35^0C in the case of fluid thioglycolate medium and 20^0C - 25^0 C for soybean casein digest medium.
- When materials examined renders the medium turbid so presence / absence of microbial growth cannot be determined readily by visual examination transfer suitable portions of medium to fresh vessels of the same medium between 3^{rd} and 7^{th} day after test is started.

- Continue incubation of the transfer vessel for not less than 7 additional days after transfer and total of NLT 14 days.

Interpretation of results:

At the end of the incubation period the following observations are possible:

- No evidence of growth; hence the preparation being examined passes the test for sterility.
- If there is evidence of growth, retesting is performed using the same number of samples, volumes to be tested and the media as in the original test. If no evidence of microbial growth is then found, the preparation being examined passes the test for sterility.
- If there is again evidence of the microbial growth then isolate and identify the organisms. If they are not readily distinguishable from those growing in the containers of the first test then the preparation being examined fails the test for sterility.
- If they are distinguishable from the organisms of the first test then again do the test using twice the number of samples. The preparation being examined passes the test for sterility in case there is no evidence of microbial growth. In case there is evidence of growth of any micro organisms in second re –test, the preparation being examined fails the tests for sterility.

iv. SAMPLES SIZE TO BE TAKEN

Number of articles in batch (injectables)	Number of articles to be tested
Not more than 100 articles	10%/ 4 articles whichever is greater
More than 100 but not more than 500	10 articles
For more than 500	2%/ 20 articles whichever is less
For large volume parenterals	2%/10 containers whichever is less.

8. TEST FOR PACKAGING CONTAINERS

8.1. Tests for Glass Containers:

- The Glass Grains Test combined with the Surface Glass Test for hydrolytic resistance determines the glass type. The hydrolytic resistance is determined by the quantity of alkali released from the glass under the conditions specified. This quantity of alkali is extremely small in the case of the more resistant glasses, thus calling for particular attention to all details of the tests and the use of apparatus of high quality and precision.
- Glass containers must comply with their respective specifications for identity and surface hydrolytic resistance to be as described in classified as Type I, II, or III glass.

Type	General Description[1]
I	Highly resistant, borosilicate glass
II	Treated soda-lime glass
III	Soda-lime glass
NP	General-purpose soda-lime glass

✓ 8.1.1. SURFACE GLASS TEST (Water Attack Test)

- Rinse 3 or more containers with high purity water.
- Fill each container to 90% of its capacity or to fill volume with high purity water (Carbon Dioxide Free) .
- Cap all the flasks with borosilicate glass beaker, place in the autoclave at 121 ± 1° C for 60 ± 1 minutes.
- The cooling time does not exceed 30 minutes.

- extraction solutions are analyzed by titration
- Carry out the titration within 1 hour of removal
- Add Methyl Red Solution and titrate with 0.01 M hydrochloric acid.

Table 5. Limit Values for the Surface Glass Test

	Maximum Volume of 0.01 M HCl per 100 mL of Test Liquid (mL)	
Filling Volume (mL)	Types I and II	Type III
Up to 1	2.0	20.0
Above 1 and up to 2	1.8	17.6
Above 2 and up to 5	1.3	13.2
Above 5 and up to 10	1.0	10.2
Above 10 and up to 20	0.80	8.1
Above 20 and up to 50	0.60	6.1
Above 50 and up to 100	0.50	4.8
Above 100 and up to 200	0.40	3.8
Above 200 and up to 500	0.30	2.9
Above 500	0.20	2.2

✓ 8.1.2 POWDERED GLASS TEST (GLASS GRAINS TEST)

Procedure

- Use crushed glass containers in 250-ml conical flask, add 50 ml high purity water, cap the flask with borosilicate glass beaker
- Place the containers in the autoclave and close it securely hold temperature at 121^0C $\pm 2^0$C for 30 min., counting from the time this temperature is reached.
- cool the flask, decant the water from the flask into a clean vessel, and wash the residual powdered glass with high purity water, add 5 drops methyl red solution, titrate immediately with 0.02 N sulfuric acid .
- Record the volume of 0.02 M hydrochloric acid used to neutralize the extract from 10 g of the prepared specimen of glass.
- Record reading with blank and thrice with test.

Table 3. Test Limits for Glass Grains Test

	Maximum Volume of 0.02 M HCl per g of Test Glass (mL)	
Filling Volume (mL)	Type I	Types II and III
All	0.1	0.85

8.2. Test for plastic Containers

8.2.1. Leakage test

10 containers are filled with the parenteral fluid and inverted

for 24 hrs. and checked for any leakage.

8.2.2. Transparency

Dilute the preparations and compare the cloudiness with the control that is water.

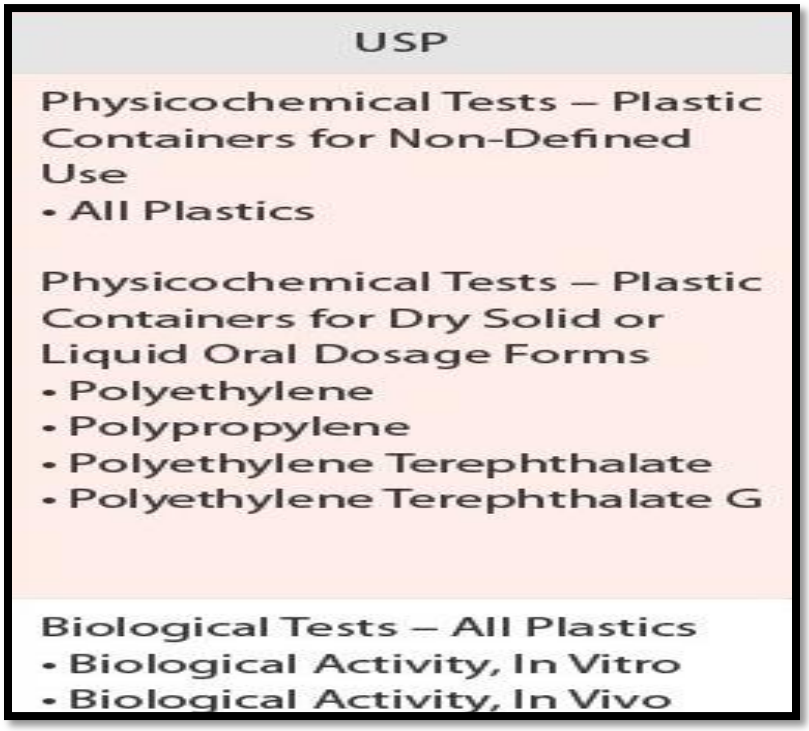
8.2.3. Water vapor permeability

Containers stored at 20-25^{o} c at 60±5% Rh for 14 days and check for water vapor permeability.

8.2.4. Rubber closure tests

- Sterilization
- Fragmentation
- Self sealability
- Clarity and color

8.2.5. Physicochemical and Biological Tests on Plastic Materials



9. Labels and labeling

- The label states the name of the preparation (in case of a liquid preparation)
- The percentage content of drug in a specified volume (in case of dry preparation)
- The route of administration
- Storage condition
- Expiration date
- Name of the manufacturer
- The lot number
- Containers for injection that are intended for use as dialysis, or irrigation solution are labeled to indicate that the contents are not intended for use by iv infusion
- Injection intended for veterinary use are labeled to that effect

- The containers are so labeled that a sufficient area of the container remains uncovered for its full length to permit inspection of the contents

10. UNIFORMITY OF CONTENTS:

- Unless otherwise stated in the individual monograph, suspensions for injection that are presented in single dose containers and that contain less than 10 mg or less than 10 % of active ingredient comply with the following test.
- For suspensions for injection containing more than one active ingredient carry out the test for each active ingredient that corresponds to above conditions.
- The test for uniformity of contents should be carried out only after the content of active ingredient in a pooled sample of the preparation has been shown to be within accepted limits of the stated content.
- Determine the content of active ingredient of each of 10 containers taken at random, using the suitable analytical method of equivalent accuracy and precision.
- The preparation under examination complies with the test if the individual values thus obtained are all between 85 and 115 % of the average value.
- The preparation under examination fails to comply with the test if more than one individual value is outside the limits 85 to 115% of the average value or if any one individual value is outside the limits 75 to 125% of the average value.
- If one individual value is outside the limits 85 to 115% but with in the limits 75 to 125% of average value, repeat the determination using another 20 containers taken at random.
- The preparation under examination complies with the test if in the total sample of 30 containers not more than one individual value is outside the limits 85 to 115% and none is out side the limits 75 to 125% of the average value.