Evaluations :-

(1) Sterility test
(2) Clarity test
(3) Leakage test
(4) pyrogen test
(5) Assay.

(1) Sterility test :-

It is done by detecting the presence of viable ~~forms~~ of bacteria, fungi and yeast in parentral products.

+ The test for Sterility must be carried out under aseptic condations in order to avoid accidental contamination of the product during test

:- All glassware required for the test must be sterile.

→ The test for sterility may be carried out either by:-
(a) Membrane filtration method
(b) Direct inoculation method

Principle:- If bacteria or fungi are placed in a medium which provide nutrition & water, & kept at a favourable temperature, organism will grow & their presence can be indicated by their turbidity in the clear ~~st~~ solution.

→ Steps Involved in Sterility testing:-

I) Selection of the sample
II) Selection of the quantity of product to be used
III) method of testing
IV) observation & results.

:→ Selection of the Sample:-

Sample must be representative of the whole of bulk material & lot of final containers.

:- Random Sampling is taken from final containers

No of items in the batch	Minimum no. of items recommended to be tested
→ not more than 100 Containers	10% or 4 containers whichever is greater
→ between 100 to 500 "	10 Containers
→ more than 500 containers.	2% or 20 Container whichever is less

:→ Selection of the quantity of products to be used:

: Depends mainly on the Volume or weight of the containes.

: Minimum Samples to be used in each culture medium in the test for sterility are:

→ Quantity of each containe	Minimum quantity to be used in each culture medium
I ≤ 1 ml	whole contents of containes.
L = 1 ml or ≤ 40 ml	½ content of "
+ 100 ml or more	10% content of "

:→ methods of testing:

(a) Membrane filtration method
(b) Direct inoculation method.

Membrane filtration method (method-1):

: This method is suitable for:
→ Filterable aqueous preparations.
→ Alcoholic preparations
→ oily preparations.
→

Media used for membrane filtration method:
(A) Fluid Thioglycolate Media:
primarily intended for the culture of aerobic & anaerobic bacteria

→ Incubation of medin : 14 days at 30-35°C

(B) Soyabean Casein digest medium
primarily intended for the culture of fungi.

:- Incubation of media : 14 days at 20-25°C

:- Steps :-

membrane filter 0.45 μ porosity

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Filter the test solution

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After filtration remove the filter

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Cut the filter in two halves.

First halves (for Bacteria) → Transfer in 100 ml culture media (Fluid thioglycolate medium) → Incubate at 30-35°C for not less than 7 days. → observe the growth in the media

Second halves (for fungi) → Transfer in 100 ml culture media (Soyabean-casein digest media) → Incubate at 20-25°C for not less than 7 days → observe the growth in media.

→ Direct inoculation method (method-2) :-

:- Suitable for Samples with Small volumes.

:- Volume of the product is not more than 10% of the volume of media

:- Suitable method for aqueous Solutions, oily liquids, ointments an creams.

:- Direct inoculation of the culture medium Suitable quantity of the preparation to be examined is transferred directly into the appropriate Culture medium & incubate for not less than 14 days.

Observation & Results :-

:- culture media is examined during and after at the end of incubation. The following observations are possible.

→ No evidence of growth → Passed the test for Sterility

→ There is evidence of growth → Re-testing is performed for Same no. of Sample, Volume & media as in original test → no evidance of growth → Pass the test

⇒ There is evidance of growth → to isolate & identify the organism.

→ Re-testing is performed with twice no. of Sample.

(2) Clarity test:-

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

:- Each parenteral preparation in it's final container is subjected individually to a visual inspection to exclude the possibility of foreign particles.

:- The contents of the container are ~~held by the neck against strongly illto~~ slowly inverted & rotated, then examined. It may be dangerous when the particle size is larger than R.B.C & may block the blood ~~the~~ vessel. This type of products are immediatly rejected from the batch.

Applicable for :- 100 ml or more Volume contained of single dose IV given by IV infusion

Not applicable for :- multi-dose injections Single dose SVP Injectable Solutions constituted from Sterile Solids.

:→ Methods of monitoring particulate matter contamination :-

1. visual method
2. Coulter counter method
3. Filtration method
4. Light blockage method.

(3) Seal Packaging / Leaking test :-

:- The Sealed ampoules are Subjected to Small cracks which occur due to rapid temperature changes or due to mechanical Shocks.

Filled & Sealed ampoules.

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Dipped in 1% methylene blue Solution Under negative pressure in Vacuum Chamber

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Vacuum released coloured Solution enter in to the ampoule.

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Defective Sealing. [* vials & bottles are not suitable for this test because the Sealing material used is not rigid]

(4) Pyrogen testing :-

:- Pyrogen = Pyro (fire) + gen (beginning)

:- Fever producing, metabolic by-products of microbial growth and death.

⇒ (A) Rabbit test :-

Rabbits are used to perform this test because their body temp. increases when pyrogen are introduced

in to their bodies by Parental 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°c
→ Showing temp. variation > 0.2°c b/w two Successive reading

⇒ <u>method ∴</u>

Dissolve the Substance being examined in, or dilute it with a pyrogen free Saline Solution.

∴ warm the liquid being examined to approx 38.5°c temp before injection.

∴ The volume of injection is NLT 0.5 ml/Kg & NMT 10 ml/Kg of body weight.

∴ with hold water during test

∴ Clinical thermometer is insented in to the rectum of rabbit to record body temp.

∴ 2 normal reading of rectal temp are Should be taken prior to the test injection at an interval of ½ hr (30 min) & it's mean is calculated initial temp.

:- The Sol.n under test is injected through an ear vein.

:- Record the temp. of each rabbit in an interval of 30 min for 3 hrs.

:- The difference b/w initial temp & maximum temp. is recorded -

Result :-

Rabbits.	Individual Temp. (°C)	Temp. Group (°C)	Test
:- 3 Rabbits	0.6	1.4	Passed
:- If above test not passed 3+5 Rabbit taken	6.6	3.7	Passed

(B) LAL (Limulus Amebocyte Lysate) test.

(5) Assay :-

Assay is performed according to method in monograph.

:- Assay is done to check the quantity of medicament present in the preparation