WEEK-6

Sterile Purified Water (USP)

- ✓ This is the purified water that is **sterilized and suitably packaged.**
- ✓ It contains no antimicrobial agent.
- ✓ It is used in the preparation of non-parenteral dosage forms where sterile form of purified water is required.

- ✓ **Do not use this type of water for parenterally administered dosage forms**

Highly purified water (EP)

- Combination of **double pass reverse osmosis and ultrafiltration** can be used for preparing this water.

- This is the water type which injectable water is not needed but a high quality of water must be used.
- It must be stored in suitable conditions to avoid contamination .

- ✓TOC value must be less than 0,5 mg/L.
- ✓**Conductivity value is: 1.1 µS/cm (20°C)**

Water for Injection (USP)

- Must carry the same properties with purified water but but **is also pyrogen-free**
- Intended to be used in the manufacture of injectable products which are to be sterilized after their preparation.
- Intended to be used for 24-hours after its collection. It must be protected from microbial contamination.
- **Maximum endotoxin limit is 0.25 IU/mL**
- **Re-distillation or reverse osmosis** methods should be applied for final purification.

Sterile Water for Injection (USP)

- **sterile**, **non-pyrogenic** preparation of water for injection which contains no bacteriostatic, antimicrobial agent or added buffer
- is supplied only in **single-dose containers** with **less than 1L**
- It is used to dilute or dissolve drugs for injection.
- **Maximum endotoxin limit is 0.25 IU/mL**

Precautions:

- ✓ For I.V. injection, add sufficient solute to make an approximately isotonic solution,
- ✓ pH 5.0 to 7.0
- ✓ Do not use unless water is clear, seal is intact and container is undamaged.

Bacteriostatic Water for Injection (USP)

- is sterile water **containing suitable antimicrobic preservatives**
- **Bacterial endotoxin limit must not exceed 0.5 IU/mL**
- can be used in diluting drugs that can subsequently be administered by intravenous, intramuscular, or subcutaneous injection.
- İt is supplied in plastic containers and not pressurized.
  Single dose or 30 mL multiple-dose
- Their container can be re-entered multiple times (usually by a sterile needle)
- It is not used for neonatal medications because of possible blood pressure changes and toxicity of preservatives
- If it is injected i.v. without any diluted compound, it may cause some red blood cell lysis because it is not isotonic.

Sterile Water for Irrigation (USP)

- is a **sterile, distilled, non-pyrogenic water** for injection **intended only for sterile irrigation**, washing, rinsing and dilution purposes.
- pH 5.5 (5.0 to 7.0).
- It contains no bacteriostatic, antimicrobial agent or added buffer
- is intended for use only as a **single-dose (containers larger than1litre)**or short procedure irrigation.
- When smaller volumes are required the unused portion should be discarded.
- Sterile Water for Irrigation may be classified as a sterile irrigant, wash, rinse, diluent and pharmaceutical vehicle.
- NOT FOR INJECTION BY USUAL PARENTERAL ROUTES
- Do not heat container over 66 C (150 F).

Sterile Water for Inhalation (USP)

- is Water for Injection that is packaged and rendered sterile and is intended for use in inhalators and in the preparation of inhalation solutions.
- It carries a less stringent specification for bacterial endotoxins than Sterile Water for Injection, and therefore, is not suitable for parenteral applications.
- Bacterial endotoxin limit must not exceed 0.5 IU/mL

Water for injection (EP)

In EP water for injection is divided into 2 subgroups;

- **Water for injection in bulk**
- **Sterilized water for injection**

- ✓ **Bakterial endotoxin limit: 0.25 IU/mL**
- ✓ TOC value: 0,5 mg/L
- ✓ **Conductivity value: 1.1 µS/cm (20°C)**

Water for injection in bulk is used as vehicle in parenteral dosage forms

Sterilized water for injection is used for dissolving or diluting of parenteral dosage forms. It is injectable water and is sterilized after sealing the container.

Conductivity:
✓25 $\mu S \cdot cm^{-1}$ for volume less than 10mL
✓5 $\mu S \cdot cm^{-1}$ for volume higher than 10 mL

Total Organic Carbon (TOC) (EP 5)

TOC determination is an indirect measure of organic substances present in water for pharmaceutical use. This test also can monitor the performance of various operations in preparation of medicines.

Humic acid (high MW; colloid structure) and Fulvic acid (low MW) are the main reasons of TOC in water. These organic colloidal complexes cause poor negative electric flow in water and thus resulted with an increase in conductivity which is the indicator of poor purification in water.
Cl^- ions in water can react with humic acid and this resulted with trihalomethane

TOC value must be less than 0,5 mg/L in purified water.

TOC test (EP 5)

The objective of TOC test is, completely oxidizing the organic substances in sample water to produce carbon dioxide followed by measuring the amount of CO_2 produced.

Amount of CO_2 gives the carbon concentration in water which is indicator of organic substances.

TOC water must comply with the following specifications,

- ✓ **Conductivity:**
  not greater than 1.0 µS/cm at 25°C
- ✓ **Total organic carbon:**
  not greater than 0.1 mg/L

Endotoxin:

- Endotoxin is a toxin that is found on the outer membrane of various **Gram negative bacteria and are lipopolysaccharide (LPS)** structured.
- LPS consists of a polysaccharide (sugar) chain and a lipid moiety, which is responsible for the toxic effects
- water is the main source for endotoxins
- **They are heat stable** to 250°C and and cannot be inactivated by autoclaving.

Pyrogenic

- Any substance which on injection causes a rise of temperature can be called as pyrogenic.
- Endotoxins are pyrogenic materials.
- They can easily pass through the normal bacterial filters. Special filters which act by adsorption and ion exchange can be used rather than simple filtration.
- Official method for preparing non- pyrogenic water is distillation rather than ion exchange resins, because the pyrogenic materials are non-volatile.

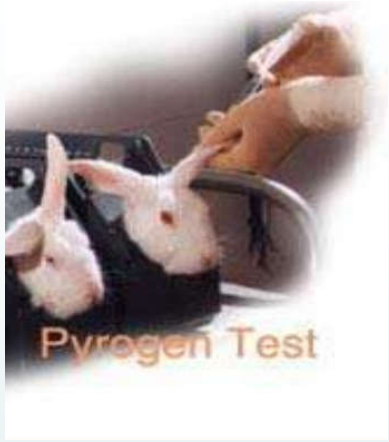
Tests for pyrogen

1- rabbit test → in vivo

2- bacterial endotoxin test (LAL test) → in vitro

3-Monosit Activation test (in vitro)

Rabbit test (USP 27, EP 2002)



The test involves measurement of the rise in body temperature of rabbits following the intravenous injection of a sterile solution of the substance being examined.

- **Measures the pyrogenic level**

- Rabbits are suitable test animals as they show similar physiological response to pyrogens as human beings.
- It is designed for products that can be tolerated by the test rabbit in a dose not exceeding 10 ml per kg injected intravenously within a period of not more than 10 minutes.

Before the test,

- All diluents, solutions and equipment should be sterile and pyrogen-free.
- Temperature of rabbits must be recorded 90 minutes before the test by inserting the thermometer into the rectum.

For the test,

- Pyrogenic-free saline solution must be injected to the ear of rabbit at 38.5°C.
- Temperature recorded for 3 hours after injection of the solution being examined.
- Any animal showing a temperature variation of 0.6°C or more must not be used in the main test.

Table 2.6.8.-1

Number of rabbits	Product passes if summed response does not exceed	Product fails if summed response exceeds
3	1.15 °C	2.65 °C
6	2.80 °C	4.30 °C
9	4.45 °C	5.95 °C
12	6.60 °C	6.60 °C

Rabbits used in a test for pyrogens where the mean rise in the rabbits' temperature has exceeded 1.2 °C are permanently excluded.

1. If the individual response is less than 0.6 °C for 3 group of rabbits

→ the preparation being examined passes the test.

2. If the response of any rabbit is over 0.6 °C, or
3. if the sum of the response of the three rabbits exceeds 1.4°C

→ continue test using five other rabbits.

Advantages

- It measures the fever
- Detects all kinds of injectable pyrogen unlike LAL test.

Disadvantages

- Time consuming
- Expensive Procedure
- It is pass-fail test than assay
- It cannot be used to test certain drugs that depresses the fever
- Tolerance to certain class of drugs can develop in rabbits & also biological variations are observed

Bacterial endotoxin test (LAL)

is used to detect or quantify endotoxin of gram negative bacterial origin using amoebocyte lysate from horseshoe crab (Limulus polyphemus).

- The test is based on the observation that when a endotoxin contacts clot protein from circulating amoebocyte of horse shoe crab (Limulus) a gel clot forms.
- There are three general technique for this test

Gel clot technique: based on gel formation.

Turbidimetric method: based on development of turbidity after cleavage of an endogenous substrate.

Chromogenic method: based on the development of color after cleavage of a synthetic peptide-chromo-gen complex

LAL test

- **Dissolve LAL in 1-2 mL non-pyrogenic steril water,**
- **Add 0.1 mL of this solution is added (n=3) to tubes**

Tube 1: **0.1 mL LAL + 1 mL non-pyrogenic steril water**

Tube 2: **0.1 mL LAL + 0.1 mL Endotoxin solution (1-5 mg/mL E.Coli)**

Tube 3: **0.1 mL LAL + 0.1 mL sample**

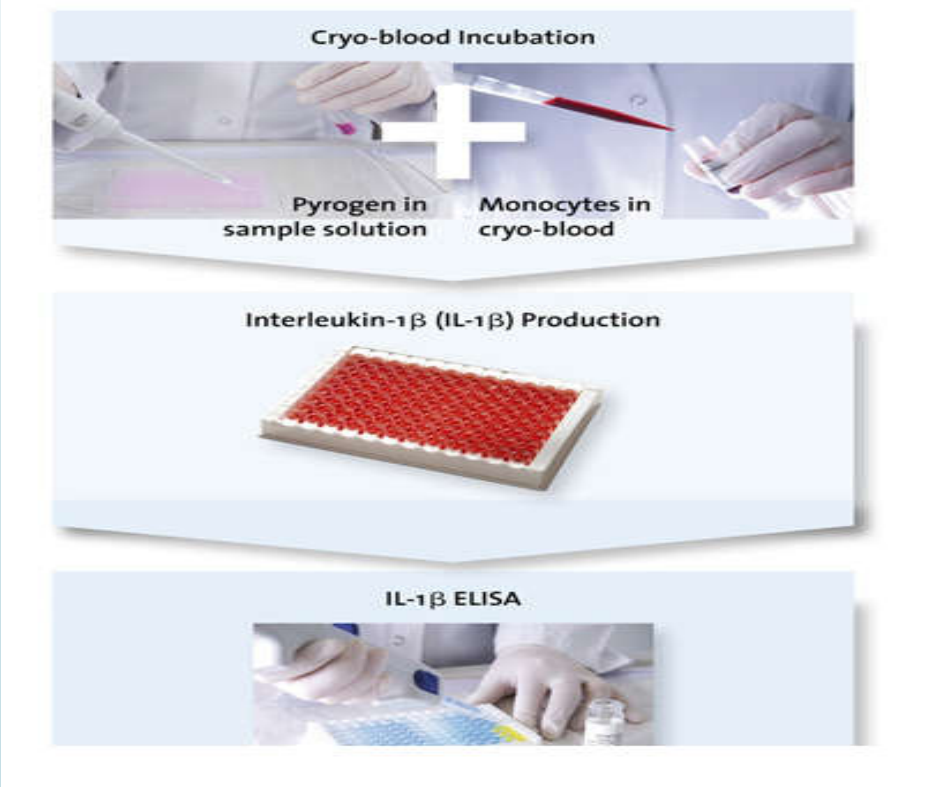
- ✓ Incubate tubes 37±1°C'de for 60 min
- ✓ Consider as (+) if there is gelation,
- ✓ Consider as (-) if there is not ant gelation inside the tubes.

Bacterial endotoxin test is 5-10 times more sensitive than rabbit test and because of the serial dilutions used in this test, it is considered as semiquantitative.

However it has some limitations,

- Only gram(-) bacteria sourced pyrogen can be examined
- Sensitivity affected from source of microorganism
- This is an in vitro test; you cannot determine the potential of fever for the tested endotoxin

İn vitro Pyrogen Test (Human blood model)



Comparison of Pyrogen Test Methods

		Rabbit	LAL	PyroDetect
	Test principle	Fever reaction in mammal	Defence mechanism	Fever reaction in human
Contamination	Gram-negative (LPS)	+	+	+
	Gram-positive (LTA)	+	-	+
	Yeasts & Molds	+	-	+
	Virus	+/-[1]	-	+
Application	Pharmaceuticals	+	+	+
	Biologicals (e.g. gene therapy, recombinant therapeutic proteins)	+	+/-[2]	+
	Medical devices	+[3]	+/-[3]	+
	Cell therapeutics (e.g. monoclonale antibody)	-	+/-	+

[1] Variable pyrogen results, [2] Rabbit test often required, [3] Indirect test with solution in pyrogen-free water