Pyrogen Test

- Qualitative biologic test based on fever response of rabbits.
- Rabbits show a physiologic response to pyrogens similar to that of human beings.
- Greater danger exists from the injection of LVP containing pyrogens than from SVP.
- Pyrogenic effect is less with IM injection than IV injection.

Pyrogen Test

- The test involves **measurement of the rise in body temperature of rabbits following the intravenous injection of a sterile solution** of the substance under examination.
- 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.**

Preliminary Test (SHAM test)

1. If animals are used for first time in pyrogen test or have not been used during the two previous weeks, condition them one to three days before testing the substance being examined, by injecting I.V. into them 10 ml per kg of body weight of a pyrogen free saline solution.

2. Carry out the test in a room where there is no risk of disturbance of exciting the animals and in which the room temperature is within 3 of that of the area where the animals are housed or in which the animals have been kept for at least 18 hrs before the test.

3. With hold food from the animals overnight and until the test is completed; withhold water during the test.

4. Record the temperature of the animals beginning at least 90 min. before injection and continuing for 3 hrs after injection of the solution being examined.

5. Any animal showing a temp. variation of 0.6 or more must not be used in the main test.

Main test

- Carry out the test using a group of three rabbits.
- **Preparation of the sample:**

  Dissolve the substance under examination in, or dilute with, pyrogen-free saline solution or other solution prescribed in the monograph. Warm the liquid under examination to approximately 38.5ºC before injection.

Procedure

- Inject the solution(38.5ºC) under examination slowly into the marginal vein of the ear of each rabbit over a period not exceeding 4 minutes, unless otherwise prescribed in the monograph.
- Record the temperature of each animal at half-hourly intervals for 3 hours after the 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.

- The amount of sample to be injected varies according to the preparation under examination and is prescribed in the individual monograph.
- The volume of injection is not less than 0.5 ml per kg and not more than 10 ml per kg of body weight.

	IP	BP	USP
Animal	Healthy, adult rabbits of either sex	Healthy, adult rabbits of either sex	Healthy, adult rabbits of either sex
Body wt.	NLT 1.5 kg	NLT 1.5 kg	NLT 2-4 kg
Condition before test	± 2°C	Within 3°C	NMT ± 3°C
Condition for animal to use	Do not use animals for pyrogen tests more frequently than once every 48 hours	Not used (a) during the preceding 3 days or (b) during the preceding 3 weeks unless the material being examined passed the test	Do not use animals for pyrogen tests more frequently than once every 48 hours
Fail test	0.6°C or more	Mean rise exceed 1.2	0.6°C or more
Apparatus preparation	Hot air oven 250 for 30 min. or at 200 for 1hr.	Hot air oven 250 for 30 min. or at 200 for 1hr.	Hot air oven 250 for NLT 30 min
Animal in instrument	1 hr before the test	1 hr before the test	Not mentioned

Interpretation of results (IP)

- If the sum of the responses of the group of **three rabbits does not exceed 1.4°C** and if the response of **individual rabbit is less than 0.6°C**, the preparation under examination **passes** the test.
- If exceeds continue the test using five other rabbits
- If not more than **three of the eight rabbits show individual responses of 0.6°C** or more, and if the sum of responses of the group of **eight rabbits does not exceed 3.7°C**, the preparation under examination **passes** the test.