
AIM: TO STUDY THE PYROGEN TEST OF GIVEN SAMPLE.

REQUIREMENT:

Animal: Rabbits (of either sex weighing 1500g or more.)

Apparatus: Rabbit holder, Automatic temperature recording device calibrate to 0.1⁰ C with applicator, 24 Gauge needle and 30 ml syringe.

PRINCIPLE:

Rabbits and human being are equally responsive to the threshold level of pyrogen given intravenously, on a dose per kg basis. If a sample containing pyrogen is injected it produce rise in temp. Within 3 hr. according to official methods given in B.P., I.P. and U.S.P. etc. If maximum rise to temperature within 3 hours. After injection of sample exceeds 2.4⁰ C. the sample is considered to be pyrogenic and is discarded. This is rabbit fever response test.

THEORY:

- Chemically pyrogens are phospholipids attached to the polysaccharide carrier molecules.
- They are synthesized in the gram negative bacteria and gram positive bacteria.
- Pyrogens include increase in the body temperature if they are administered to human being or animals by parenteral route. To check the presence of pyrogens in the injectable formulation, different biological assay are used.

PROCEDURE:

Pyrogen test by rabbit methods:

- In this methods rise in the body temperature of the rabbits after intravenous administration of the test solution is studied.
- The rabbit used for pyrogen test:
- They have body weight more than 1.5 kg.
- It can be of either sex.
- They are maintained in quite place with constant room temperature and humidity.
- They are not used for the pyrogen testing more frequently than once every 48 hours.
- The rabbit which has been used in testing of a pyrogen and has shown more than 0.6⁰C rise in body temperature is not used for other pyrogen testing before two weeks.
- The rabbit are kept in the holders one hour before the actual commencement of test.
- They are given diet overnight before the test and are withheld from water during the testing.

A) Sham Test:

It is performed to select the rabbits for actual testing.

- The animals satisfying above state are selected at random and are administered with 10ml/kg pyrogen free saline solution (warmed at 38.5⁰C) through the marginal ear vein.
- The body temperature recording is started 90 minutes before this dosing and is continued for further 3 hrs at the interval of 30 minutes.
- The rabbit showing more than 0.6⁰C rise in the body temperature are not used for main test.

B) Main test:

- The test substance is dissolved in pyrogen free saline solution and warmed at 38.5⁰C.
- Initially, three rabbits are used for testing. The recording of body temperature is start 90 minutes before the injection of test materials.
- Initial body temperatures are recorded at the interval of 30 minutes, not preceding 40 minutes of the injection.
- If there is change in the initial body temperature of any individual rabbit by more than or equal to 0.2⁰C the rabbit is not used for further testing.
- The initial body temperature in individual rabbits should not be less than 38⁰C or greater than 39.8⁰C.
- Each rabbit selected for the test is injected the test solution (<0.5 ml/kg body weight and not > 10 ml/kg body weight) through marginal ear vain slowly and steadily within 4 minutes.
- The height body temperature reached in each rabbit after injection is considered to be the response. (Reading are taken for at least 3 hrs at the interval of 30 minutes).

RESULTS:

- **The drugs said to pass the test if:**
 - Sum of rise in the body temperature of three rabbits is not more than 1.4⁰C and in any individual rabbit the rise in the body temperature does not exceed 0.6⁰C.
- **The test sample fails if:**
 - Rise in body temperature in individual rabbits is more than or equal to 0.6⁰C and sum of the responses in the three rabbits is more than or equal to 1.4⁰C.
 - If the sample fails this initial test, the test is carried out in FIVE more rabbits and pooled observation from all eight rabbits is considered.

- The sample passes the pyrogen tests if:
 - Not more than three rabbits shows individual rise by more than or equal to 0.6°C and sum of rise in body temperature in all eight rabbits does not exceed 3.7°C .

PYROGEN TESTING



	Test:	Rabbit	LAL	WBT
Pyrogens				
Bacteria gram-negative		+	+	+
Bacteria gram-positive		+	-	+
Fungi		+	-	+
Applications				
Biologicals		+	-	+
Pharmaceuticals		+	+	+
Medical Devices		-	+	+
Air quality		-	(+)	(+)
Blood components		-	-	+

TEACHER'S SIGNATURE