Aim: Pyrogen Testing by Rabbit Method

References:

1. European Pharmacopoeia (EP). (2021). Section 2.6.8. Pyrogens.

2. United States Pharmacopeia (USP). (2021). Chapter <151> Pyrogen Test.

3. Vogel, H. G. (2008). Drug Discovery and Evaluation: Pharmacological Assays. Springer.

Introduction:

Pyrogen testing is essential to ensure that pharmaceutical products are free from substances that can cause fever when administered. The rabbit pyrogen test is a traditional method used to detect the presence of pyrogens in injectable products. This test involves monitoring the body temperature of rabbits following the administration of the test substance.

Objective:

To determine the presence of pyrogens in a pharmaceutical product by measuring the increase in body temperature of rabbits after intravenous injection of the test substance.

Materials and Reagents:

- Healthy rabbits (2.0-3.0 kg, either sex)
- Test substance (pharmaceutical product)
- Control substance (sterile, pyrogen-free saline)
- Thermometer (rectal or electronic)
- Intravenous injection apparatus (syringes, needles, etc.)
- Animal cages with suitable restraint devices
- Disposable gloves
- Laboratory coat
- Anesthetic (if necessary for restraint)

Procedure:

Animal Preparation

1. Acclimatize the rabbits to the laboratory environment for at least one week before the experiment.

2. Fast the rabbits overnight, providing only water.

Initial Health Check:

1. Conduct a thorough health check on each rabbit to ensure they are healthy and free from any signs of infection or illness.

2. Record the baseline body temperature of each rabbit.

Group Division:

Divide the rabbits into the following groups, with a minimum of three rabbits per group:

1. Control group: Receive sterile, pyrogen-free saline

2. Test group: Receive the pharmaceutical product

Administration of Substances:

1. Warm the test and control substances to body temperature (37°C) before administration.

2. Administer 10 mL/kg of the test substance or control substance intravenously into the marginal ear vein of each rabbit.

Monitoring and Recording:

1. Measure and record the rectal temperature of each rabbit at 1 hour, 2 hours, and 3 hours post-injection.

2. Ensure that the rabbits are adequately restrained but not stressed during temperature measurement.

Interpretation of Results:

1. Calculate the temperature change (ΔT) for each rabbit at each time point by subtracting the baseline temperature from the post-injection temperatures.

2. A rise in temperature of 0.5°C or more in any rabbit indicates the presence of pyrogens.

3. If the sum of temperature rises in the three rabbits exceeds 1.15°C, the test substance is considered pyrogenic.

Rejection Criteria:

1. If one rabbit shows a temperature rise of 0.6°C or more, continue the test with an additional three rabbits.

2. If two or more rabbits show a temperature rise of 0.5°C or more, the test substance fails the pyrogen test.

Results and Discussion:

1. Present the temperature changes in a table for each rabbit in the control and test groups.

2. Compare the mean temperature changes between the control and test groups.

3. Discuss the results, indicating whether the test substance contains pyrogens based on the temperature changes observed.

Safety and Ethical Considerations:

1. Ensure all experimental procedures involving animals comply with institutional and national ethical guidelines for the care and use of laboratory animals.

2. Handle all animals with care to minimize their distress.

3. Ensure proper disposal of all biological waste according to safety guidelines.

Conclusion:

Summarize the findings, stating whether the test substance demonstrated the presence of pyrogens based on the rabbit pyrogen test.

Sample Data Table

Rabbit ID	Baseline Temp (°C)	Temp at 1 hr (°C)	Temp at 2 hr (°C)	Temp at 3 hr (°C)	ΔT at 1 hr (°C)	ΔT at 2 hr (°C)	ΔT at 3 hr (°C)
Control 1	38.5	38.6	38.5	38.6	+0.1	0.0	+0.1
Control 2	38.6	38.6	38.7	38.6	0.0	+0.1	0.0
Control 3	38.4	38.5	38.4	38.5	+0.1	0.0	+0.1
Test 1	38.5	39.1	39.2	39.3	+0.6	+0.7	+0.8
Test 2	38.6	39.0	39.2	39.3	+0.4	+0.6	+0.7
Test 3	38.4	38.9	39.1	39.2	+0.5	+0.7	+0.8