

TEST FOR PYROGENS / ENDOTOXINS

PYROGEN: In Greek Pyro = fire, gen = beginning

A Pyrogen is a substance i.e. products of the growth of micro organisms or may be parts of dead cells or metabolic products which cause febrile reactions like fever, chills, back pain etc.

Sources of Pyrogens and its elimination methods:

Sl.no.	Source		Elimination method
1	Equipment	Glass	Heating to 250°C for 45 min / 650°C for 1 min/ washed with dil.acid or dil. Alkali.
		Metal	Heating to 250°C for 45 min / 650°C for 1 min/ washing with dil.acid or dil. Alkali.
		Plastic	Washing with detergent.
2	solvent	Volatile	Distillation
		Non-volatile	Adsorption
3	Solute		Re-crystallization

TEST FOR PYROGENS:

The Pyrogen test is designed to limit the risk of febrile reaction following parenteral administration of drugs. It includes both In vitro and In vivo tests.

1. In Vitro Test / LAL Test
2. In Vivo Test / Rabbit Test.

1. LAL Test: Limulus Amoebocyte Lysate Test. (In Vitro Test)

In Vitro assay used to detect the presence and concentration of bacterial endotoxins in drugs and biological products.

Limulus Amoebocyte Lysate (LAL) is an aqueous extract of blood cells (amoebocytes) from the horseshoe crab, *Limulus polyphemus*.



LAL reacts with bacterial endotoxin or lipopolysaccharide (LPS), which is a membrane component of **Gram negative bacteria** and forms gel which is then used for the detection and quantification of bacterial endotoxins.

Sample + LAL (mL) → **Gel formation (+)**
Incubation at 37 °C for 1 hr.

Limitations of LAL Test:

1. Disturbed by endotoxin binding components like lipids, blood components etc.
2. Difficult to correlate with rabbit test.
3. False positive for cellulose and many herbal preparations.

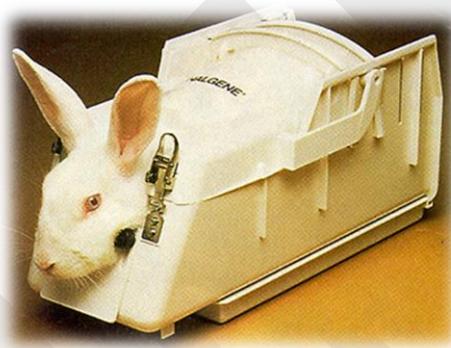
2. Rabbit Test: SHAM TEST (In Vivo)

Principle:

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

Animals used:

Select same variety of healthy mature rabbits weighing less than 1.5Kg and should maintain balanced diet. They should not show any loss of body weight during the preceding week of test.



Temperature measurement:

Accurate temperature sensing devices such as Clinical Thermometer graduated in 0.1°C / Thermister / Probes are used to measure the temperature of rabbit. Insert the thermometer into the rectum of rabbit to a depth of not less than 6 cm and record the temperature.

Materials used:

Glass ware and syringes must be washed with water for injection and heated to 250°C for 30 minutes / 200°C for 1 hour in hot air oven.

Procedure:

It includes

1. Preliminary test.
2. Main test.

Preliminary test:

Select fresh animals / Animals not been used during 2 previous weeks.



Conditioned them for 1 to 3 days.



With hold the food from animal before 2hours of starting the test and access to water may be allowed.



Record the temperature of animals using thermometer.



After 90 min, give IV injection 10mL/Kg (Pyrogen free saline solution)



Record the temperature of animals after IV injection at an interval of 30min and continued for 3 hrs after injection.



Animals show temperature variance of 0.6°C should not be used for main test.

Note: It is carried out in room without disturbances and temperature variance must be $\pm 3^{\circ}\text{C}$.

Main test:

Preparation of Sample: Dissolve test substances in Pyrogen free saline water and warm the liquid to 38°C before giving injection.

3 groups of selected rabbits (preliminary test passed rabbits)



With held food for 2 hrs before experiment and during the experiment



Record initial temperatures (mean of two measurements at an interval of 30 minutes)



Rabbits showing a temperature variance ≥ 0.2 °C between two successive readings should not be used.



Use only those rabbits that do not deviate a temperature variance 1°C between two successive readings.



Inject sample into the marginal vein of the ear of 3 rabbits

Not less than 0.5mL/ Kg and not greater than 10mL/Kg body wt.



Record the temperature of animals during a period of 3 hours at intervals of 30 minutes.



Interpretation of Results

Case: 1

No rabbit shows individual raise in temperature of 0.6 °C (or)

Sum of three rabbits raise in temperature does not exceed 1.4 °C



Absence of Pyrogens in the test sample.

Case: 2

If 2 or 3 rabbits show increase in temperature $\geq 0.6\text{ }^{\circ}\text{C}$ or

Sum of three rabbits raise in temperature exceeds $1.4\text{ }^{\circ}\text{C}$



Continue or Repeat the experiment using additional 5 rabbits.



Not more than 3 rabbits shows individual raise in temperature of $0.6\text{ }^{\circ}\text{C}$ (or)

Sum of eight rabbits raise in temperature does not exceed $3.7\text{ }^{\circ}\text{C}$



Absence of Pyrogens in the test sample

Number of rabbits	Product passes if summed response does not exceed	Product fails if summed response exceeds
3	$1.15\text{ }^{\circ}\text{C}$	$2.65\text{ }^{\circ}\text{C}$
6	$2.80\text{ }^{\circ}\text{C}$	$4.30\text{ }^{\circ}\text{C}$
9	$4.45\text{ }^{\circ}\text{C}$	$5.95\text{ }^{\circ}\text{C}$
12	$6.60\text{ }^{\circ}\text{C}$	$6.60\text{ }^{\circ}\text{C}$

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

References:

1. <http://apps.who.int/phint/en/p/docf/>
2. https://en.wikipedia.org/wiki/Limulus_amebocyte_lysozyme