MINISTRY OF HEALTH OF THE RUSSIAN FEDERATION

GENERAL PHARMACOPOEIA MONOGRAPH

Pyrogenicity **GPM**.1.2.4.0005.15

Replaces the State Pharmacopoeia of the Russian Federation XII, Part 1 Monograph, GPM 42-0061-07

The present General Pharmacopoeia Monograph applies to testing the pyrogenicity of injectable solutions and drug substances used for preparation of such solutions. The test is based on measuring body temperature in rabbits prior to and after an injection.

Housing and pre-test preparation of experimental animals

Each rabbit should be kept in a separate cell, on an appropriate food ration, away from irritating influences (acoustic, optical, and others). A constant air temperature (mean, 20 ± 3 ºC) should be maintained in the animals housing and testing area. Before testing, the animals should be examined with the aim to select healthy non-albino rabbits of the same gender, with a body weight between 2.0 and 3.5 kg, which have not lost any of the body weight within the preceding week.

At 18 hours before the test, the rabbits should be deprived of fodder, with no water restriction imposed. During the experiment, the animals should receive neither fodder nor water. Rabbits that are either naïve to experiments or have not been used in experiments within the preceding four weeks should be prepared for the testing procedure in advance by carrying out all testing routine (visual examination, weighing, body temperature measurement), with the exception of the injection.

Rabbits previously used in the experiment can be taken into the experiment

again after three days, provided that the medicinal product they were administered proved non-pyrogenic. If an animal develops a body temperature elevation of 0.6 ºC or more, such rabbit may be used for further experiments after an interval of at least two weeks.

If the tested drug product possesses antigenic properties, the procedure of re-using animals for experiments should be specified in the Pharmacopoeia Monograph.

Materials and equipment

Laboratory ware for dilution, syringes, and needles for injections should be sterile and non-pyrogenic, which is ensured by processing at temperature 250 ºC over 30 minutes or at 200 ºC over 60 minutes.

Unless a different solvent is specified in the Pharmacopoeia Monograph, 0.9 % sodium chloride solution for injections should be used for dilution of tested medicinal products. All solvents should be sterile and non-pyrogenic.

Rectal temperature should be measured in rabbits within the accuracy of 0.1 ºC using a medical maximum mercury thermometer or an electronic thermometer equipped with a heat sensor. The thermometer or sensor should be introduced into the rectum of the animal to a depth of 5 to 7.5 cm, depending on the animal's body weight.

Administration of the tested medicinal product

The tested medicinal product should be administered into the rabbit's auricular vein, unless a different route of administration is specified in the Pharmacopoeia Monograph. The volume of injected solution should be not less than 0.2 mL and not more than 10 mL per 1.0 kg of the animal's body weight. Before administration, the solution should be heated to a temperature of 37.0 ± 2 ºC.

The test dose of the tested medicinal product, the volume of administered solution, and, if necessary, the administration rate should be specified in the Pharmacopoeia Monograph.

Procedure description

The medicinal product should be tested in a group of three rabbits with a baseline body temperature in the range of 38.5 to 39.5 ºC.

Body temperature should be measured in every experimental rabbit twice before the test, allowing an interval of at least 30 minutes between the measurements. The difference between temperature readings in the same animal should not exceed 0.2 ºC. If the difference is greater, such rabbit should be excluded from the experiment. The last obtained measurement result is considered the baseline temperature.

The tested medicinal product solution should be administered to experimental animals immediately after the second temperature measurement.

Temperature measurements after an intravenous dose of the tested medicinal product should be carried out over three hours at an interval not exceeding 30 minutes. The measurement period should be five hours for any other parenteral routes of administration.

Obtained results

A medicinal product may be tested in a number of phases. Three rabbits should be used for every phase. The maximum number of phases should not exceed four.

After completion of each of the testing phases, the maximum body temperature change (Δt) compared to baseline is calculated for each rabbit. A body temperature change going below the baseline value is considered equal to zero and disregarded.

The sum of individual maximum temperature elevations ($\Sigma \Delta t$) is calculated for three rabbits. The $\Sigma \Delta t$ values obtained in different test phases are summed up consecutively, and obtained results are compared to the levels presented in Table 1.

A medicinal product is considered non-pyrogenic after the first test phase if the obtained result is less than or equal to 1.2 ºC (Table 1, Column 3) and the individual temperature elevation does not exceed 0.5 ºC in any of the three rabbits (Column 4).

If the result obtained in the first test phase exceeds 1.2 ºC (Column 5) or the

registered individual temperature elevation exceeds 0.5 ºC in at least one of the three experimental rabbits (Column 6), the next phase of testing should be initiated.

A drug product is considered non-pyrogenic after the second test phase if the obtained result is less than or equal to 2.8 ºC (Column 3) and the individual temperature elevation does not exceed 0.5 ºC in more than one out of the six rabbits (Column 4).

If the result obtained in the second test phase is more than 2.8 ºC but less than 4.3 ºC (Column 5) or the registered individual temperature elevation exceeds 0.5 ºC in more than one animal (Column 6), the next phase of testing should be initiated.

A drug product is considered non-pyrogenic after the third test phase if the obtained result is below or equal to 4.5 ºC (Column 3) and the individual temperature elevation does not exceed 0.5 ºC in more than two out of the nine rabbits (Column 4).

If the result obtained in the third test phase is more than 4.5 ºC but less than 6.0 ºC (Column 5) or the registered individual temperature elevation exceeds 0.5 ºC in more than two animals (Column 6), the next phase of testing should be initiated.

A drug product is considered non-pyrogenic after the fourth test phase if the obtained result is below or equal to 6.6 ºC (Column 3) and the individual temperature elevation does not exceed 0.5 ºC in more than three out of the twelve rabbits (Column 4).

A drug product is considered pyrogenic if the result obtained in the second or any subsequent phase of testing exceeds the values indicated in Column 7. A drug product is also considered pyrogenic if the registered individual temperature elevation exceeds 0.5 ºC in more than three out of the twelve rabbits after the four completed test phases.

Table 1 – Interpretation of test results

Phase	Total number of animals	Interpretation of test results (ΣΔt)				
		Medicinal product considered non-pyrogenic		Re-test (rearrangement) conducted		Medicinal product considered pyrogenic if the ΣΔt is
		if the Σ Δt is	if the number of animals with temperature elevation Δt > 0.5 °C does not exceed	if the Σ Δt is	if the number of animals with temperature elevation Δt > 0.5 °C is	
1	*2*	*3*	*4*	*5*	*6*	*7*
I	3	≤ 1.2	–	> 1.2	≥ 1	–
II	6	≤ 2.8	1	> 2.8 and < 4.3	> 1	> 4.3
III	9	≤ 4.5	2	> 4.5 and < 6.0	> 2	> 6.0
IV	12	≤ 6.6	3	–	–	> 6.6*

* A medicinal product is considered pyrogenic if the individual temperature elevation exceeds 0.5 ºC in more than three out of the twelve rabbits.