3.5 Test for pyrogens

The pyrogen test is designed to limit the risk of a febrile reaction following parenteral administration of drugs. It is intended to be used for liquid products that can be tolerated by the test rabbit in a dose of 10 mL per kg, injected intravenously, generally within a period of not more than 4 minutes. For products that require preliminary preparation or are subject to special conditions of administration, additional directions given in the monograph should be followed.

Test animal

Use healthy, adult rabbits, preferably of the same variety. House the animals individually in an area of uniform temperature (±2 °C), possibly with uniform humidity, and free from disturbances likely to excite them. The animals are given *ad libitum* water and food, commonly used for laboratory animals. One to 3 days before using an animal that has not previously been used for a pyrogen test, condition it by conducting a training exercise as described under the recommended procedure, omitting the injection.

Do not use animals for pyrogen tests more frequently than once every 48 hours. After a pyrogen test in the course of which a rabbit's temperature has risen by 0.5 °C or more, or after a rabbit has been given a test substance that was adjudged pyrogenic, at least 2 weeks must be allowed to elapse before the animal is used again.

Temperature recording

Use an accurate thermometer graduated in 0.1 °C that has been tested to determine the time necessary to reach the maximum reading, or any other temperature-recording device of equal sensitivity. Insert the temperature-sensing device into the rectum of the test animal to a depth of about 6 cm. If the temperature-sensing device is to remain inserted throughout the sensing period, restrain the rabbit with a lightly-fitting neck stock that allows it to assume a natural resting posture. When a thermometer is used, allow sufficient time for it to reach a maximum temperature, as previously determined, before taking the reading.

Recommended procedure

Perform the test in the area where the animals are housed or under similar environmental conditions. For 2 hours before the test and during the test, withhold all food from the animals being used. Access to water may be allowed. The animals should be placed under the conditions of the test at least 1 hour before the injection.

Prior to the test, 40 minutes before the injection of the test material, determine the temperature of each animal by taking 2 measurements at an interval of 30 minutes. The mean of the 2 temperatures serves as the "control temperature" of the animal. The control temperature recorded for each rabbit constitutes the temperature from which any subsequent rise following the injection of the material is calculated.

In any one test, use only those animals the control temperatures of which do not deviate by more than 1.0 °C from each other. Those animals for which the 2 temperatures used to determine the control temperature have deviated by more than ±0.2 °C from the mean should not be used in the test, nor should any animal with a control temperature below 38.0 °C or above 39.8 °C.

Render the syringes, needles, and glassware free from pyrogens by heating at 250 °C for not less than 30 minutes or by any other suitable method. Warm the solution to be tested to approximately 38 °C.

Inject into a marginal vein of the ear of each of 3 rabbits 10 mL of the solution per kg of body weight or the amount specified in the monograph. The injection should last not longer than 4 minutes, unless otherwise specified in the monograph.

When the injection has been completed, record the temperature of the animal during a period of 3 hours, taking the measurements continuously or every 30 minutes. The maximum temperature recorded for each rabbit is considered to be its response; if the temperature readings taken after the injection are all below the control temperature, the response is treated as a zero temperature rise.

If no rabbit shows an individual rise in temperature of 0.6 °C or more above its respective control temperature, and if the sum of the 3 temperature rises does not exceed 1.4 °C, the tested material meets the requirements for the absence of pyrogens. If 1 or 2 rabbits show a temperature rise of 0.6 °C or more, or if the sum of the temperature rises exceeds 1.4 °C, continue the test using 5 other rabbits. If not more than 3 of the 8 rabbits show individual rises in temperature of 0.6 °C or more, and if the sum of the 8 temperature rises does not exceed 3.7 °C, the tested material meets the requirements for the absence of pyrogens.