⟨151⟩ PYROGEN TEST

INTRODUCTION

The pyrogen test is designed to limit to an acceptable level the risks of febrile reaction in the patient to the administration, by injection, of the product concerned. The test involves measuring the rise in temperature of rabbits following the intravenous injection of a test solution and is designed for products that can be tolerated by the test rabbit in a dose not to exceed 10 mL/kg injected intravenously within a period of NMT 10 min. For products that require preliminary preparation or are subject to special conditions of administration, follow the additional directions given in the individual monograph or, in the case of antibiotics or biologics, the additional directions given in the federal regulations (see *Biologics* ⟨1041⟩). A validated, equivalent in vitro pyrogen or bacterial endotoxin test may be used in place of the in vivo rabbit pyrogen test,[1] where appropriate.

APPARATUS AND DILUENTS

Render the syringes, needles, and glassware free from pyrogens by heating at 250° for NLT 30 min or by any other suitable method. Treat all diluents and solutions for washing and rinsing of devices or parenteral injection assemblies in a manner that will assure that they are sterile and pyrogen-free. Periodically perform control pyrogen tests on representative portions of the diluents and solutions for washing or rinsing of the apparatus. Where *Sodium Chloride Injection* is specified as a diluent, use Injection containing 0.9% of sodium chloride (NaCl).

TEMPERATURE RECORDING

Use an accurate temperature-sensing device such as a clinical thermometer, or thermistor probes or similar probes that have been calibrated to assure an accuracy of ±0.1° and have been tested to determine that a maximum reading is reached in less than 5 min. Insert the temperature-sensing probe into the rectum of the test rabbit to a depth of NLT 7.5 cm, and, after a period of time NLT that previously determined as sufficient, record the rabbit's body temperature.

TEST ANIMALS

Use healthy, mature rabbits. House the rabbits individually in an area of uniform temperature between 20° and 23° and free from disturbances likely to excite them. The temperature varies NMT ±3° from the selected temperature. Before using a rabbit for the first time in a pyrogen test, condition it NMT 7 days before use by a sham test that includes all of the steps as directed in *Procedure* except injection. Do not use a rabbit for pyrogen testing more frequently than once every 48 h, nor prior to 2 weeks following a maximum rise of its temperature of 0.6° or more while being subjected to the pyrogen test, or following its having been given a test specimen that was adjudged pyrogenic.

PROCEDURE

Perform the test in a separate area designated solely for pyrogen testing and under environmental conditions similar to those under which the animals are housed and free from disturbances likely to excite them. Withhold all food from the rabbits used during the period of the test. Access to water is allowed at all times, but may be restricted during the test. If rectal temperature-measuring probes remain inserted throughout the testing period, restrain the rabbits with light-fitting neck stocks that allow the rabbits to assume a natural resting posture. NMT 30 min prior to the injection of the test dose, determine the "control temperature" of each rabbit: this is the base for the determination of any temperature increase resulting from the injection of a test solution. In any one group of test rabbits, use only those rabbits whose control temperatures do not vary by more than 1° from each other, and do not use any rabbit with a temperature exceeding 39.8°.

Unless otherwise specified in the individual monograph, inject into an ear vein of each of three rabbits 10 mL of the test solution per kg of body weight, completing each injection within 10 min after start of administration. The test solution is *either* the product, constituted if necessary as directed in the labeling, *or* the material under test treated as directed in the individual monograph and injected in the dose specified therein. For pyrogen testing of devices or injection assemblies, use washings or rinsings of the surfaces that come in contact with the parenterally administered material or with the injection site or internal tissues of the patient. Assure that all test solutions are protected from contamination. Perform the injection after warming the test solution to a temperature of 37 ± 2°. Record the temperature at 30-min intervals between 1 and 3 h subsequent to the injection.

TEST INTERPRETATION AND CONTINUATION

Consider any temperature decreases as zero rise. If no rabbit shows an individual rise in temperature of 0.5° or more above its respective control temperature, the product meets the requirements for the absence of pyrogens. If any rabbit shows an individual temperature rise of 0.5° or more, continue the test using five other rabbits. If NMT three of the eight rabbits show

[1] United States Food and Drug Administration. Guidance for industry. Pyrogen and endotoxins testing: questions and answers. Rockville, MD: Food and Drug Administration; June 2012. http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm310098.pdf.

individual rises in temperature of 0.5° or more and if the sum of the eight individual maximum temperature rises does not exceed 3.3°, the material under examination meets the requirements for the absence of pyrogens.

RADIOACTIVE PHARMACEUTICALS

Test Dose for Preformulated, Ready-to-Use Products Labeled with Radioactivity

AGGREGATED ALBUMIN AND OTHER PARTICLE-CONTAINING PRODUCTS

For the rabbit pyrogen test, dilute the product with *Sodium Chloride Injection* to NLT 100 µCi/mL, and inject a dose of 3 mL/kg of body weight into each rabbit.

OTHER PRODUCTS

Where physical half-life of radionuclide is greater than 1 day: Calculate the maximum volume of the product that might be injected into a human subject. This calculation takes into account the maximum recommended radioactive dose of the product, in µCi, and the radioactive assay, in µCi/mL, of the product at its expiration date or time. Using this information, calculate the maximum volume dose per kg to a 70-kg human subject.

For the rabbit pyrogen test, inject a minimum of 10 times this dose per kg of body weight into each rabbit. If necessary, dilute with *Sodium Chloride Injection*. The total injected volume per rabbit is NLT 1 mL and NMT 10 mL of solution.

Where physical half-life of radionuclide is less than 1 day: For products labeled with radionuclides with a half-life of less than 1 day, the dosage calculations are identical to those described in the first paragraph in *Other Products*. These products may be released for distribution prior to completion of the rabbit pyrogen test, but such test must be initiated at NMT 36 h after release.

Test Dose for Pharmaceutical Constituents or Reagents to Be Labeled

The following test dose requirements pertain to reagents that are to be labeled or constituted prior to use by the direct addition of radioactive solutions such as *Sodium Pertechnetate Tc 99m Injection*, i.e., "cold kits".

Assume that the entire contents of the vial of nonradioactive reagent will be injected into a 70-kg human subject, or that $^1/_{70}$ of the total contents per kg will be injected. If the contents are dry, constitute with a measured volume of *Sodium Chloride Injection*.

For the rabbit pyrogen test, inject ($^1/_7$) of the vial contents per kg of body weight into each rabbit. The maximum dose per rabbit is the entire contents of a single vial. The total injected volume per rabbit is NLT 1 mL and NMT 10 mL of solution.