

the animal model for detecting the presence of pyrogens in injectables. Seibert also demonstrated conclusively that pyrogens originate from water-borne organisms are heat resistant, filterable, and can be eliminated from water by distillation. The pyrogen test became an official United States Pharmacopeia (USP) quality control test for parenterals in 1942. The rabbit pyrogen test methodology officially recognized in compendial standards has remained essentially unchanged. The LAL test for endotoxin became an official USP test in 1985. Today the LAL test, or more commonly called the Bacterial Endotoxin Test, has pre-empted the rabbit test as the USP method of choice for detection of endotoxin in parenteral products. Also, the more sensitive and accurate LAL assay for testing raw materials, in-process pyrogen control or pharmaceuticals and medical devices, and end-product evaluation of devices, is small and large-volume parenteral products (7). The LAL test also is widely used in the validation of depyrogenation of dry-heat sterilization processes.

### GENERAL DESCRIPTION OF THE USP PYROGEN TEST

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 not more than 10 minutes. 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.

All apparatuses—glassware, containers, syringes, needles, etc.—and all diluents used in performing the pyrogen test must themselves be free from pyrogenic contamination. Heat-durable items such as glass and stainless steel can be depyrogenated by exposure to dry-heat cycles at temperatures greater than 250°C for at least 30 minutes. Negative controls utilize the diluent rather than the product sample as the injection, with the diluent being exposed to the same procedure and materials as the product sample. The use of negative controls with each pyrogen test is not standard practice because of prior knowledge and assurance that materials used in the test are nonpyrogenic.

Rabbits are used as pyrogen test models because they physiologically respond similarly to pyrogens as do human beings. Rabbits and humans respond identically on a nanogram per kilogram basis to pyrogenic quantities of endotoxin. Rabbits for pyrogen testing are not used more frequently than once every 48 hours, nor prior to two weeks following a maximum rise of its temperature of 0.6°C or more while being subjected to the pyrogen test or following its having been given a test specimen that was adjudged pyrogenic.

The albino rabbit is the most widely used rabbit, particularly strains from New Zealand and Belgium. It is essential that the rabbit colony be treated with utmost care. The environment in which the rabbits are housed must be strictly controlled with respect to temperature, humidity, lighting, and potential contamination of air, surfaces, and feed. Any new shipment of rabbits should be quarantined and monitored for one to two weeks following receipt of the shipment for the presence of illness and/or disease.

Rabbits must become accustomed to being restrained in their cages and being handled both in the rectal insertion of the thermocouple and the injection of the test product. The normal basal body temperature of rabbits ranges between 38.9°C and 39.8°C (102.0–103.6°F). Rabbit baseline temperature is established by measuring rectal temperature during the conductance of several “sham” tests (following the entire pyrogen test procedure using pyrogen-free sodium chloride solution as the injection sample). Rabbits may become tolerant to pyrogenic activity after repeated injections of endotoxin. It is for this reason that a rabbit showing a rise of its body temperature of 0.6°C or more during a pyrogen test cannot be used again as a pyrogen test animal for at least two weeks.

### USP PYROGEN TEST PROCEDURE

The USP procedure (8) requires the test to be performed 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