

**Table 28-2** Dose Dependency of Rabbit Pyrogen Test

<i>E. Coli</i> endotoxin concentration (ng/mL)	Volume solution injected (mL/kg)	USP total temperature (°C)	Mean temperature increase (°C) <sup>a</sup>	Standard deviation (°C) <sup>b</sup>	Coefficient of variation (%)
3.125	1.0	7.80 <sup>c</sup>	0.975	0.246	25.2
1.56	1.0	4.75 <sup>c</sup>	0.594	0.218	36.7
1.00	1.0	3.70 <sup>c</sup>	0.462	0.158	34.2
0.78	1.0	1.40	0.144	0.208	144.4
0.39	1.0	1.00	0.088	0.187	212.5
0.195	1.0	1.20	0.150	0.065	43.3

Eight rabbit pyrogen test results in saline with *E. coli* 055:BS using 3 to 5 kg rabbits.

<sup>a</sup>Negative rabbit temperature values were excluded from total temperature increase determinations according to USP.

<sup>b</sup>Negative rabbit temperature values were included in the determinations of means and standard deviations to properly reflect total variability.

<sup>c</sup>Failed USP test criteria of 3.7°C total increase.

Source: From Ref. 9.

No two rabbits will possess exactly the same body temperature or respond identically to the same pyrogenic sample. Rabbits are extremely sensitive and vulnerable to their environment. This translates into an expensive proposition in terms of facilities, control of the environment, and training of the animal. Pyrogen testing of rabbits is not only expensive but also laborious. Several hours are consumed in performing the pyrogen test including a great amount of preliminary effort in preparing the animals. Rabbits must be fed and watered properly, cages cleaned to prevent disease, and time spent in training the animals to adapt to the conditions of the pyrogen testing facility and the test itself.

#### *Rabbit Sensitivity to Pyrogens*

The pyrogenic response in rabbits is dose dependent. The greater the amount of pyrogen injected per kilogram body weight, the greater the temperature increases in rabbits (Table 28-2) (9).

A collaborative study initiated under the auspices of the Health Industry Manufacturers Association (HIMA) demonstrated that rabbits from 12 laboratories consistently failed (pyrogenic) the test at = 1.0 ng/mL doses (10 mL/kg of 10 ng/kg endotoxin) of *E. coli* 055:B5 endotoxin, and all colonies passed (no pyrogenicity) at the 0.156 ng/kg dose (or 0.156 ng/mL using a 10 mL/kg dose) (10). The same study reported that the "average" rabbit colony will attain a 50% pass/fail rate with 95% confidence at an endotoxin level above 0.098 ng/mL (10 mL/kg dose). The LAL test generally will detect endotoxin levels of 0.025 ng/mL or less. Thus, the rabbit test is less sensitive to endotoxin than the LAL test is.

Sensitivity of the rabbit bioassay for endotoxin appears to fall in the range of 1 to 10 ng/kg (11,12). Greisman and Hornick (11) found that the threshold pyrogenic dose of *E. coli* endotoxin for both rabbits and humans is 1.0 ng/kg of body weight. This holds true regardless of the volume of pyrogenic solution administered because of the dose (rather than concentration) dependency of the rabbit response to pyrogen.

#### **Interferences of the Rabbit Pyrogen Test**

Many products administered parentally cannot be tested for pyrogens with the rabbit test because of the interferences they create in the rabbit response to pyrogens, if they are present in the product. Any product having a pyretic side effect, such as cancer chemotherapeutic agents, will interfere with the rabbit response. Several products are inherently toxic to the rabbit (e.g., cytotoxic compounds) and must be diluted to concentrations far below the pharmacologically effective dose of the drug.

Despite these major limitations and the insurgence of the LAL test, it must not be forgotten that the USP rabbit pyrogen test for decades has nobly served as a sufficiently sensitive test for pyrogens and has helped to eliminate pyrogenic contamination from drugs reaching the marketplace, though most pharmaceutical and device manufacturers currently use the LAL