per dose, and did not permit sufficient dilution of small-volume parenterals known to inhibit the LAL test reaction.

The Parenteral Drug Association proposed an alternative endotoxin limit based on rabbit or human dose (24) that FDA accepted and became part of the new FDA draft guideline for end product testing published in December 1987 (25). The new endotoxin limit is:

$$\frac{K}{M} = \frac{\text{Threshold pyrogen dose (TPD)}}{\text{Maximum rabbit or human dose}}$$

where the TPD has been defined as 5 EU/kg, the lower 95% confidence limit of the average dose found to produce a pyrogenic response in rabbits and humans (26). For drugs administered intrathecally, where pyrogenic contamination can be much more dangerous, the TPD is 0.2 EU/kg.

The maximum rabbit or human dose is that dose administered per kilogram of body weight of rabbit or man[4] in a single hour period, whichever is larger. For example, if a drug of a concentration of 1 mg/mL has a maximum human loading of 25 mg/kg while the rabbit pyrogen test dose is 10 mg/kg, the maximum dose used in the denominator of the endotoxin limit equation would be the human dose of 25 mg. On the other hand, were the above human dose only 2.5 mg/kg, then the rabbit dose of 10 mg would be the larger of the two doses. The endotoxin limit for the two examples would be:

$$\text{EU} = \frac{5\,\text{EU/kg}}{25\,\text{mg/kg}} = 0.2\,\text{EU/mg}$$

$$\text{EU} = \frac{5\,\text{EU/kg}}{10\,\text{mg/kg}} = 0.5\,\text{EU/mg}$$

For devices, the endotoxin limit is 0.1 ng/mL of extract solution.

Four classes of drugs are exempted from the endotoxin limit defined by K/M:

- Compendial drugs for which other endotoxin limits have been established.
- Drugs covered by new drug applications, antibiotic Form 5 and Form 6 applications, new animal drug applications, and biological product license where different limits have been approved by the Agency.
- Investigational drugs or biologics for which an investigational new drug application (IND) or investigational new animal drug application (INAD) exemption has been filed and approved.
- Drugs or biologics that cannot be tested by the LAL method example.

Maximum doses per kilogram and the corresponding endotoxin limits for a large number of aqueous injectable drugs and biologics on the market are listed in Ref. 25.

LAL Sensitivity

LAL sensitivity is defined as the lowest concentration of a purified endotoxin that will produce a firm gel, which will remain intact when inverted carefully after one hour of incubation at 37°C. (LAL sensitivity is also expressed as how many times its sensitivity is greater than the rabbit test.) In general, it seems to be well established that the LAL test is sensitive to picogram quantities of endotoxin and that LAL is from 5 to 50 times more sensitive than the rabbit to the presence of endotoxin, depending on the type of comparative study conducted.

In earlier years, the LAL test is at least five times more sensitive to purified endotoxin than the rabbit test (28). Improvements in LAL production and formulation methodology increased the sensitivity of LAL 10 to 50 times greater than the rabbit test (29). These numbers were based on a gel time of one hour and a rabbit test dose of 1 mL/kg. The ability of LAL to detect *E. coli* endotoxin in pyrogen-free distilled water was found to be 100 times more sensitive than the rabbit test (30).

[4] Body weight of average human considered to be 70 kg. For pediatric indications, body weight needs to be adjusted for age of child, for example, average weight of 3-year-old child is 15 kg, 6-year-old child 20 kg, and 8-year-old child 25 kg). For preclinical animal studies, endotoxin limits for injections are based on the following body weights: mouse 0.03 kg, gerbal 0.09 kg, rat 0.45 kg, rabbit 4 kg, monkey 8 kg, baboon 12 kg (27).