Table 28-4 Example of Geometric Mean Determination for a Small-Volume Injectable Product Undergoing LAL Testing for Endotoxin[a] (18)

	Gel end point results for specimen dilutions				
Replicates (*f*)	**Unity**	**0.5**	**0.25**	**0.125**	**End point dilution factors (*E*)**
1	+	+	+	−	0.25
2	+	+	−	−	0.5
3	+	+	−	−	0.5
4	+	+	+	−	0.25
5	+	+	−	−	0.5
					$\Sigma E = 2.0$

[a]Geometric mean $= \frac{\sum E}{f} = \frac{2.0}{5} = 0.4$.

to cause inhibition or enhancement of the LAL test, the following courses of action can be taken (20):

- If the drug product is amenable to rabbit testing, then the rabbit test will still be the appropriate pyrogen test for that drug.
- If the interfering substances can be neutralized without affecting the sensitivity of the test or if the LAL test is more sensitive than the rabbit pyrogen test, then the LAL test can still be used.
- For those drugs not amenable to rabbit pyrogen testing, the manufacturer should demonstrate that the LAL test can detect the endotoxin limit established for the particular drug. If the limit cannot be met, the smallest quantity of endotoxin that can be detected must be determined.

There are various miscellaneous requirements in the procedures for validating the LAL test:

- Use positive and negative controls in all tests.
- Use the highest and lowest drug concentrations for drug products marketed in three or more concentrations.
- Use three lots of each drug concentration for the validation tests.
- If the lysate manufacturer is changed, the validation test must be repeated on at least one unit of product.
- The LAL reagent should have a sensitivity of at least 0.25 EU/mL.
- The endotoxin control must always be referenced to the RSE.
- Any change in the product formulation, manufacturing process, source of formulation ingredients, or lot of lysate necessitates a revalidation of the LAL test for the product.

The possibility of a device inhibiting or enhancing the LAL–endotoxin reaction is determined by extraction testing of each of three device production lots. The extract solution must be pyrogen-free water or saline to which known amounts of standard endotoxin, bracketing the sensitivity of the lysate, have been added. Depending on the type of device, extracts may be obtained by flushing, immersing, or disassembling, then immersing the device with the endotoxin-spiked solution. The LAL test results of the extract should not be different than the results of testing standard solutions containing endotoxin that have not been exposed to the device.

Endotoxin highly adsorbs to container surfaces. Recovery of endotoxin occurs with polystyrene containers while the worst for recovering endotoxin were polypropylene containers. In fact, regardless of extraction method, less than 1% endotoxin was ever recovered from polypropylene containers. Bonosilicate glass allowed higher recovery than flint glass (21).

Manual LAL Test Procedure

Four or more replicate samples at each level of the dilution series for the test samples are used in most cases. The pH of the reaction mixture must be between 6.0 and 7.5 unless