V. MEDICAL DEVICES

General Requirements

The CDRH has reviewed the results of the "HIMA Collaborative Study for the Pyrogenicity Evaluation of a Reference Endotoxin by the USP Rabbit Test." This study recommends 0.1 ng/mL (10 mL/kg) of E. coli 055:B5 endotoxin from Difco Laboratories as the level of endotoxin which should be detectable in the LAL test when used for end-product testing of medical devices. This sensitivity (0.1 ng/mL given 10 mL/kg) is sufficient for LAL testing and for retest of devices in rabbits. According to recent collaborative studies in the rabbit pyrogen and LAL tests, one nanogram of E. coli 055:B5 endotoxin is similar in potency to 5 EU of the USP Endotoxin Reference Standard. The endotoxin limit for medical devices has been converted to EU and is now 0.5 EU/mL using the rinse volume recommended in Section 2 below. Liquid devices should be more appropriately validated and tested according to the requirements for drugs by taking the maximum human dose per kilogram of body weight per hour into consideration (See Section IV,B).

Manufacturers may retest LAL test failures with the LAL test or a USP rabbit pyrogen test. If the endotoxin level in a device eluate has been quantitated by LAL at 0.5 EU/mL endotoxin or greater, then retest in rabbits is not appropriate. Medical devices that contact cerebrospinal fluid should have less than 0.06 EU/mL of endotoxin. These values correspond to those set by the CDER for intrathecal drugs.

Manufacturers shall use an LAL reagent licensed by OBRR in all validation, in-process, and end-product LAL tests.

A. Validation of the LAL Test

1. Sensitivity: Data demonstrating the sensitivity and reproducibility of the LAL test.

2. Inhibition/Enhancement Testing: Each product line of devices utilizing different materials or methods of manufacture should be checked for inhibition or enhancement of the LAL test.

Further explanation of the above points is given as follows:

1. SENSITIVITY

A manufacturer must be able to demonstrate a sensitivity of at least 0.5 EU/mL. The level of endotoxin selected as the pass/fail point for evaluating pyrogenicity of products using the LAL test must be equivalent to or below this level. Manufacturers may use another endotoxin if a reproducible correlation between it and the USP Reference Endotoxin Standard has been demonstrated in their laboratory (see appendix C).

The sensitivity of the LAL technique used should be determined by the procedures and criteria in Appendix A. Routine performance of the LAL test should include standards (run in duplicate) and a negative control. An endotoxin standard series is useful for checking lysate sensitivity and the competence of the technician, and for identifying other problems such as the contamination of glassware.

The stability of the endotoxin standards and appropriate storage conditions should also be considered; dilute endotoxin solutions are not as stable as more concentrated solutions under certain conditions.

2. INHIBITION AND ENHANCEMENT TESTING

Lack of product inhibition or enhancement of the LAL test should be shown for each type of device before use of the LAL test. Possible inhibition of different chemical components of similar devices should be considered. A manufacturer may logically divide its device products into groups of products according to common chemical formulation; and may then qualify only a representative product from each such group. Ideally, the product chosen from each group would be the one with the largest surface area contacting body or fluid for administration to a patient.

At least three production lots of each product type should be tested for inhibition. In general, use of the sampling technique selected should result in a random sampling of a finished production lot. CDRH recommends testing 2 devices for lot sizes under 30, 3 devices for lot sizes 30-100, and 3 percent of lots above size 100, up to a maximum of 10 devices per lot.

The process of preparing an eluate/extract for pyrogen or inhibition/enhancement testing may vary for each device. Some medical devices can be flushed, some may have to be immersed in the non-pyrogenic rinse solution, while others may be tested by disassembling or by cutting the device into pieces prior to extraction by immersion. In general, for devices being flushed, the non-pyrogenic rinse solution should be held in the fluid pathway for one hour at room temperature (above 18° C); effluents should be combined. If a device is to undergo extraction, a minimum extraction time should be 15 minutes at 37° C, one hour at room temperature (above 18° C) or other demonstrated equivalent conditions.

Guidelines for rinse volumes include the following:

a. Each of the 10 test units should be rinsed with 40 mL of non-pyrogenic water.

b. For unusually small or large devices, the surface area of the device which comes in contact with the patient may be used as an adjustment factor in selecting the rinsing or extracting volume. The endotoxin limit can be adjusted accordingly.

The rinsing scheme should not result in a greater dilution of endotoxin than used in USP rabbit pyrogen testing of transfusion and infusion assemblies. For inhibition/enhancement testing, both the rinsing/extraction solution and the device eluate/extract should be tested as prescribed below under the specific technique being used.

a) GEL-CLOT TECHNIQUE

In inhibition/enhancement testing, a device eluate/extract containing varying concentrations of a standard endotoxin that bracket the sensitivity of the lysate is compared with a series of the same endotoxin concentrations in water alone. The device eluate/extract is "spiked" with endotoxin and then diluted with additional eluate/extract to the same endotoxin concentrations as in the water series. Results of endotoxin determination in water and the device product eluate/extract should fall within plus/minus a twofold dilution of the labeled sensitivity. If the device eluate/extract shows inhibition, the gel-clot technique cannot be used to test the device. Negative controls (diluent plus lysate) should be included in all inhibition/enhancement testing.

b) CHROMOGENIC AND ENDPOINT-TURBIDIMETRIC TECHNIQUES

In inhibition/enhancement testing by these techniques, a device eluate/extract containing 4 lambda concentration of the RSE or CSE (lambda is equal to the lowest endotoxin concentration used to generate the standard curve) is tested in duplicate according to the lysate manufacturer's methodology. The standard curve for these techniques shall consist of at least four RSE or CSE concentrations in water that extend over the desired range. If the desired range is greater than one log, additional standard concentrations should be included. The standard curve must meet the criteria for linearity as outlined in Appendix A(2). The detected amount of endotoxin in the spiked eluate/extract must be within plus or minus 25% of the 4 lambda concentration for the device to be considered to neither enhance nor inhibit the assay. If the device eluate/extract shows inhibition, the device cannot be tested by this technique.

An alternate procedure may be performed as described above except the RSE/CSE standard curve is prepared in LAL negative device eluate/extract, i.e. no detectable endotoxin, instead of LAL negative water. The standard curve must meet the test for linearity, i.e. r equal to or greater than 0.980, and in addition the difference between

the O.D. readings for the lowest and highest endotoxin concentrations must be greater than 0.4 and less than 1.5 O.D. units. If the standard curve does not meet these criteria the device cannot be tested by the alternate procedure.

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Food and Drug Administration
Rockville MD 20857

Interim Guidance for Human and Veterinary Drug Products and Biologicals

KINETIC LAL TECHNIQUES

Until we update the guideline the following guidance and the lysate manufacturers approved procedures can be used. The kinetic LAL techniques should be done according to the lysate manufacturers recommended procedures, i.e., sample/lysate ratio, incubation temperature and times, measurement wavelength, etc. Instrumentation other than the one recommended by lysate manufacturer can be used. The performance characteristics (slope, y-intercept and correlation coefficient), for the lysate lot, sent by the manufacturer will not be valid. New performance characteristic have to be established for each lot by performing the procedures outlined in Appendix A.

INHIBITION/ENHANCEMENT TESTING

In inhibition/enhancement testing of a product by kinetic techniques, test a drug concentration containing a quantity of the RSE or CSE between 0.1 and 0.5 EU/mL or 1.0 and 5.0 EU/mL depending on its Pass/ Fail Cutoff (PFC) in duplicate according to the lysate manufacturer's methodology. The 4 lambda spike procedure, in the current guideline, is still valid and can be used in the kinetic techniques. This procedure should be used with caution if lambda is less than 0.01 EU/mL.

The Pass/Fail Cutoff equals the endotoxin limit of the product solution (EU/mL) times the potency of the product divided by the product dilution used for the test. For PFCs less than or equal to 1.0 EU/mL the endotoxin spike should be between 0.1 and 0.5 EU/mL, otherwise the endotoxin spike should be between 1.0 and 5.0 EU/mL.

The standard curve shall consist of at least three RSE or CSE concentrations. Additional standards should be included to bracket each log increase in the range of the standard curve so that there is at least one standard per log increment of the range. The standard curve must meet the criteria outlined in Appendix A. The calculated mean amount of endotoxin when referenced to the standard curve, minus any measurable endogenous endotoxin in the spiked drug product, must be within plus or minus 50% of the known spike concentration to be considered to neither enhance or inhibit the assay. If there is no measurable endogenous endotoxin in the product the value will usually be equal to or less than plus or minus 25% of the standard curve value. If the undiluted drug product shows inhibition or enhancement, the drug product can be diluted, not exceeding the MVD, and test repeated.

An alternate procedure may be used, in which the RSE/CSE standard is prepared in drug product or product dilution instead of water. The drug product (at the concentration used to prepare the standard curve), cannot have an endotoxin concentration greater than the lowest concentration used to generate the product standard curve, when referenced against a standard curve prepared in water. The product standard curve must meet the test for linearity, i.e., r equal to or greater than the absolute value of 0.980, and slope of the regression line must be less than -0.1 and greater than -1.0. If the standard curve does not meet these criteria, the drug product cannot be tested by the alternate procedure.

ROUTINE TESTING

The standard curve shall consist of at least three RSE or CSE concentrations in duplicate. Additional standards should be included to bracket each log increase in the range of the standard curve so that there is at least one standard per log increment of the range. The standard curve must meet the criteria outlined in Appendix A. For the kinetic techniques, it is not necessary to run a standard

curve each day if consistency of standard curves is shown in your test laboratory. Determine consistency by regression analysis of the data points from the standard curves generated over three consecutive test days (minimum of three curves). If the coefficient of correlation, r, meets the criteria in Appendix A then consistency is proven and the curve becomes the "archived curve." If r does not meet the criteria then consistency in your laboratory has not been shown and you cannot use an archived curve in routine testing. The archived curve is only valid for a lysate/endotoxin lot combination. If you use an archived standard curve , at least duplicates of a standard endotoxin concentration, equal to the mid-point on a log basis, between the endotoxin concentration of the highest and lowest standards in the standard curve, in water must be included with each run of samples. The mean endotoxin concentration of this standard control must be within plus/minus 25% of the standard curve concentration when calculated using the archived standard curve. Independent of using an endotoxin standard curve, at least duplicates of a standard endotoxin in each product or product dilution (positive product control), equal to either 0.1 - 0.5 or 1.0 - 5.0 EU/mL depending on its PFC or 4 lambda, must be included with each run of samples. The mean endotoxin concentration of the positive product control when referenced to the standard curve must be within plus/ minus 50% of the known concentration after subtraction of any endogenous endotoxin. An endotoxin standard series should be run when retesting to determine if end-product endotoxin contamination exceeds product limit. If you use the alternate procedure, a standard curve prepared in product must be conducted with each product test.

APPENDIX A

Using a RSE or CSE of known potency, in endotoxin units, assay at least 3 concentrations in triplicate that extend over the desired endotoxin range. Additional standards should be included to bracket each log increase in the range of the standard curve so that their is at least one standard per log increment of the range. Do regression - correlation analysis on the log Reaction Time versus the log of the endotoxin concentration for each replicate. DO NOT AVERAGE THE REACTION TIMES OF REPLICATES OF EACH STANDARD BEFORE PERFORMING REGRESSION-CORRELATION ANALYSIS.

The coefficient of correlation, r, shall be greater than or equal to the absolute value of 0.980. If r is less than the absolute value of 0.980 the cause of the non-linearity should be determined and test repeated.