GUIDELINE ON
VALIDATION OF THE LIMULUS AMEBOCYTE LYSATE TEST AS AN END-PRODUCT ENDOTOXIN TEST FOR HUMAN AND ANIMAL PARENTERAL DRUGS, BIOLOGICAL PRODUCTS, AND MEDICAL DEVICES

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INTRODUCTION

This guideline sets forth acceptable conditions for use of the Limulus Amebocyte Lysate test. It also describes procedures for using this methodology as an end-product endotoxin test for human injectable drugs (including biological products), animal injectable drugs, and medical devices. The procedures may be used in lieu of the rabbit pyrogen test.

For the purpose of this guideline, the terms "lysate" or "lysate reagent" refer only to Limulus Amebocyte Lysate licensed by the Center for Biologic Evaluation and Research. The term "official test" means that a test is referenced in a United States Pharmacopeia drug monograph, a New Drug Application, New Animal Drug Application or a Biological License.

I. BACKGROUND

In a notice of January 12, 1973 (38 FR 1404), FDA announced that Limulus Amebocyte Lysate (LAL), derived from circulating blood cells (amebocytes) of the horseshoe crab, (Limulus polyphemus), is a biological product. As such, it is subject to licensing requirements as provided in section 351 of the Public Health Service Act (42 U.S.C. 262). Since 1973, LAL has proved to be a sensitive indicator of the presence of bacterial endotoxins (pyrogens). Because of this demonstrated sensitivity, LAL can be of value in preventing the administration or use of products which may produce fever, shock, and death if administered to or used in humans or animals when bacterial endotoxins are present.

When the January 12, 1973 notice was published, available data and experience with LAL were not adequate to support its adoption as the final pyrogen test in place of the rabbit pyrogen test, which had been accepted and recognized for many years. In order to establish a data base and gain experience with the use of LAL, that notice permitted the introduction of LAL into the marketplace without a license. This was upon the condition that its use be limited to the in-process testing of drugs and other products, that the decision to use it be reached voluntarily by affected firms, and the labeling on LAL state that the test was not suitable as a replacement for the rabbit pyrogen test.

Since that time, production techniques have been greatly improved and standardized so that they consistently yield LAL with an endotoxin sensitivity over 100 times greater than originally obtained. Moreover, it is widely recognized that the LAL test is faster, more economical, and requires a smaller volume of product than does the rabbit pyrogen test. In addition, the procedure is less labor intensive than the rabbit test, making it possible to perform many tests in a single day.

In a notice published in the Federal Register of November 4, 1977 (42 FR 57749), FDA described conditions for the use of LAL as an end-product test for endotoxins in human biological products and medical devices. The notice stated further that the application of LAL testing to human drug products would be the subject of a future Federal Register publication.

The then Bureau of Medical Devices, now FDA's Center for Devices and Radiologic Health (CDRH), issued recommended procedures for the use of LAL testing as an end-product endotoxin test on March 26, 1979. These procedures were revised as a result of the comments received from interested parties .

As a direct result of CDRH's experience in approving petitions for the use of the LAL test in place of the rabbit pyrogen test, several procedures for using the LAL test have evolved and have been adopted for devices.

In the FEDERAL REGISTER of January 18, 1980 (45 FR 3668), FDA announced the availability of a draft guideline that set forth procedures for use of the LAL test as an end-product testing method for endotoxins in human and animal injectable drug products. This draft guideline was made

available to interested parties to permit manufacturers, especially those who had used the LAL test in parallel with the rabbit pyrogen test, to submit data that could be considered in the preparation of any final guideline.

In response to comments received on the January 18 draft guideline, FDA made several significant changes (i.e. Endotoxin limits changed and deletion of section on Absence of Non-endotoxin Pyrogenic Substances), and many minor editorial changes. The agency also determined that a single document should be made available covering all FDA regulated products that may be subject to LAL testing. Primarily because of the addition of biological products and medical devices to the guideline, the the agency made, in the FEDERAL REGISTER of March 29, 1983 (43 FR 13096), another draft of the guideline available for public comment.

Based on the comments received on the March 29 draft guideline, FDA has made several changes in this final guideline. The comments used in support of these changes may be viewed at FDA's Dockets Management Branch, Room 4-62, 5600 Fishers Lane, Rockville, MD between 9 am and 4 pm Monday through Friday. Briefly, the significant changes made are:

A. Inclusion of validation criteria for the chromogenic, endpoint-turbidimetric and kinetic-turbidimetric LAL techniques.

B. Any technique (gel-clot, chromogenic or turbidimetric) can be used in testing a product for endotoxin. However, if a gel-clot lysate is used in a different technique the results must be interpreted using the criteria for the technique being used.

C. Elimination of the requirement to test the sensitivity of a rabbit pyrogen testing colony.

D. The Center for Devices and Radiological Health (CDRH) has adopted the USP Endotoxin Reference Standard and revised the limit expressions from ng/mL to EU/mL. The new limit for medical devices is 0.5 EU/mL except for devices in contact with cerebrospinal fluid for which the limit is 0.06 EU/mL. These limits for devices are equivalent to those for drugs for a 70 Kg man when consideration is given to the following:

    1. In the worst case situation, all endotoxin present in the combined rinsings of 10 devices could have come from just one device. A wide variation in bioburden is common to some devices.

    2. Published FDA studies indicate that less than half of added endotoxin is recovered from devices using a non-pyrogenic water rinse.

E. The Center for Drug Evaluation and Research (CDER) has added a listing of the maximum doses per Kg per hour and the corresponding endotoxin limits for most of the aqueous injectable drugs and biologics currently on the market. This listing was added to promote uniformity among companies making the same product.

II. LEGAL EFFECT OF THE GUIDELINE

This guideline is issued under section 10.90(b) (21 CFR 10.90(b)) of FDA's administrative regulations, which provides for use of guidelines to outline procedures or standards of general applicability that are acceptable to FDA for a subject matter within its statutory authority. Although guidelines are not legal requirements, a person who follows an agency guideline may be assured that the procedures or standards will be acceptable to FDA. The following guideline has been developed to inform manufacturers of human drugs (including biologicals), animal drugs, and medical devices of procedures FDA considers necessary to validate the use of LAL as an end-product endotoxin test. A manufacturer who adheres to the guideline would be considered in compliance with relevant provisions of the applicable FDA current good manufacturing practice regulations (CGMP) for drugs and devices and other applicable requirements. As provided in 21 CFR 10.90(b), persons who use methods and techniques not provided in the guideline should be able to adequately assure, through validation, that the method or technique they use is adequate to detect the endotoxin limit for the product.

III. REGULATORY PROVISIONS THAT PERMIT INITIATION OF END-PRODUCT TESTING WITH LAL

The regulatory provisions that a firm must meet before using the LAL test as an end-product test are not the same for all categories of products because of the different applicable statutory provisions and regulations. These provisions are as follows:

A. Human Drugs subject to New Drug Applications (NDAs) or Abbreviated New Drug Applications (ANDAs), Antibiotic Drug Applications, and animal drugs subject to New Animal Drug Applications (NADAs), and Abbreviated New Animal Drug Application.

For these classes of drugs, manufacturers are to submit a supplemental application to provide for LAL testing. However, under 21 CFR 314.70(c) for drugs for human use and 21 CFR 514.8(d)(3) for drugs for animal use various changes may be made before FDA approval. Under these sections changes in testing of a human or animal drug that give increased assurance that the drug will have the characteristics of purity it purports or is represented to possess should be placed into effect at the earliest possible time. Therefore, if a firm validates the LAL test for a particular drug product covered by a new drug application by the procedures in this guideline using a LAL reagent licensed by the Center for Biologic Evaluation and Research (OBER) for the technique being used, the change may be made concurrently with the submission of the supplement providing for it. The supplement should contain initial quality control data, inhibition/enhancement data and the endotoxin limit for the drug product.

B. Biological products for human use.

Under 21 CFR 601.12 significant changes in the manufacturing methods of biological products are required to be reported to the agency and may not become effective until approved by the Director, OBER. Therefore, a manufacturer of a biological product shall obtain an approved amendment to its product license before changing to the use of LAL in an end-product test, irrespective of the validation procedure used.

C. Drugs not subject to premarket approval.

A manufacturer of an injectable drug for human or animal use that is not subject to premarket approval would be able to use the LAL test as an end-product test for endotoxins without submitting any information to the agency. CGMPs require the manufacturer to have data on file to validate the use of the LAL test for each product for which it is being used.

D. Medical Devices.

On the basis of extensive experience in review of LAL data on devices since November 1977, CDRH believes that the LAL test,

when validated according to this guideline, is at least equivalent to the rabbit pyrogen test as an end-product test for medical devices. A manufacturer labeling a device as non-pyrogenic must validate the LAL test for that device in the test laboratory to be used for end-product testing before using the LAL test as an end-product endotoxin test for any device.

The data discussed under Section V of this guideline may be expressed graphically or in tabular form and should be on file at the manufacturing site; no preclearance prior to use of the LAL test as an end-product test is required if it is used according to this FDA guideline. Voluntary submission of LAL validation and inhibition data obtained following issuance of this guideline will be accepted for CDRH review and comment.

When a manufacturer plans to use LAL test procedures that deviate significantly from the LAL guideline, a premarket notification under section 510(k) of the Federal Food, Drug, and Cosmetic Act (the Act) or a Premarket Approval Application (PMA) supplement under section 515 of the Act should be submitted. Significant deviations would include-- but not necessarily be limited to-- higher endotoxin concentration release criteria, sampling from fewer than three lots for inhibition/enhancement testing, lesser sensitivity to endotoxin, rabbit retest when the LAL method shows endotoxin above the recommended allowable endotoxin dose, and a device rinsing protocol resulting in greater dilution of endotoxin than that recommended in this guideline.

CDRH will also consider submissions in the form of a premarket notification or PMA supplement for another deviation from this draft guideline; process control of endotoxin contamination with reduced end-product testing, i.e., a decrease in the number of devices per lot undergoing end-product testing. The manufacturer must demonstrate adequate control of the production process by the use of routine checks for endotoxin at key stages of production except where it has been shown that no possibility of contamination exists.

To facilitate subsequent PMA review, providers of investigational devices subject to 21 CFR part 812 or 813 are encouraged to use this guideline when a non-pyrogenic device is to be manufactured.

IV. HUMAN AND ANIMAL DRUGS AND BIOLOGICAL PRODUCTS

GENERAL REQUIREMENT

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

A. VALIDATION OF THE LAL TEST

Validation of the LAL test as an endotoxin test for the release of human and animal drugs includes the following: (1) initial qualification of the laboratory, and (2) inhibition and enhancement tests.

1. INITIAL QUALIFICATION OF THE LABORATORY

Various methodologies have been described for the detection of endotoxin, using limulus amebocyte lysate. Currently, commercially available licensed lysates use the gel clot, chromogenic, endpoint-turbidimetric or kinetic-turbidimetric techniques. Other methods which have been reported show potential for increasing further the sensitivity of the LAL method.

Manufacturers should assess the variability of the testing laboratory before any offical tests are performed. Each analyst using a single lot of LAL and a single lot of endotoxin should perform the test for confirmation of labeled LAL reagent sensitivity or of performance criteria. Appendix A gives the procedures and test criteria for the current licensed techniques.

2. INHIBITION AND ENHANCEMENT TESTING

The degree of product inhibition or enhancement of the LAL procedure should be determined for each drug formulation before the LAL test is used to assess the endotoxin content of any drug. All validation tests should be performed on undiluted drug product or on an appropriate dilution. Dilutions should not exceed the Maximum Valid Dilution (MVD) (see Appendix D). At least three production batches of each finished product should be tested for inhibition and enhancement.

a) GEL-CLOT TECHNIQUE

Inhibition/enhancement testing should be conducted according to the directions in the preparatory section of the USP Bacterial Endotoxins Test (see Appendix B). Briefly, the method involves taking a drug concentration containing varying concentrations of a standard endotoxin that bracket the sensitivity of the lysate and comparing it to a series of the same endotoxin concentrations in water alone. The drug product is "spiked" with endotoxin and then diluted with additional drug product (so that the drug concentration remains constant) to the same endotoxin concentrations in

water. Results of endotoxin determination in water and the drug product should fall within plus/minus a twofold dilution of the labeled sensitivity. If the undiluted drug product shows inhibition, the drug product can be diluted, not to exceed the MVD, with the same diluent that will be used in the release testing and the above procedure repeated. 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 drug concentration 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 standards 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 drug must be within plus or minus 25% of the 4 lambda concentration for the drug concentration to be considered to neither enhance nor inhibit the assay. If the undiluted drug product shows inhibition, the drug product can be diluted, not to exceed the MVD, and the test repeated.

An alternate procedure may be performed as described above except the RSE/CSE standard curve is prepared in LAL negative drug product, 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 drug product cannot be tested by the alternate procedure.

c) KINETIC-TURBIDIMETRIC TECHNIQUE

In inhibition/enhancement testing by this technique, a drug concentration 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 shall consist of at least four RSE or CSE concentrations. If the desired range is greater than one log, additional standard concentrations should be included. The standard curve must meet the criteria outlined in Appendix A(3). The calculated mean amount of endotoxin in the spiked drug product, when referenced to the standard curve, must be within plus or minus 25% to be considered to neither enhance nor inhibit the assay. If the undiluted drug product shows

inhibition or enhancement, the drug product can be diluted, not to exceed the MVD, and the test repeated.

An alternate procedure may be performed whereby the RSE/CSE standard curve is prepared in drug product or product dilution instead of water. The drug product cannot have a background endotoxin concentration of more than 10% (estimated by extrapolation of the regression line) of the lambda concentration (lambda equals the lowest concentration used to generate the standard curve). The standard curve must meet the test for linearity, i.e. r equal to or less than -0.980, and in addition the slope of the regression 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.

In those instances when the drug is manufactured in various concentrations of active ingredient while the other components of the formulation remain constant, only the highest and lowest concentration need be tested. If there is a significant difference, i.e. greater than twofold, between the inhibition endpoints or if the drug concentration, per mL, in the test solutions is different, then each remaining concentrations should be tested. If the drug product shows inhibition or enhancement at the MVD, when tested by the procedures in the above sections, and is amenable to rabbit testing, then the rabbit test will still be the appropriate test for that drug. If the inhibiting or enhancing substances can be neutralized without affecting the sensitivity of the test or if the LAL test is more sensitive than the rabbit pyrogen test the LAL test can be used. For those drugs not amenable to rabbit pyrogen testing, the manufacturer should determine the smallest quantity of endotoxin that can be detected. This data should be submitted to the appropriate FDA Office for review.

The inhibition/enhancement tests must be repeated on one unit of the product if the lysate manufacturer is changed. If the lysate technique is changed, the inhibition and enhancement tests must be repeated using three batches. When the manufacturing process, the product formulation, the source of a particular ingredient of the drug formulation, or lysate lot is changed, the positive product control can be used to reverify the validity of the LAL test for the product. Firms that are obtaining an ingredient from a new manufacturer are encourged to include as part of their vendor qualification the rabbit pyrogen test to determine that the ingredient does not contain non-endotoxin pyrogens.

B. Routine Testing of Drugs by the LAL Test.

End-product testing is to be based on data from the inhibition/enhancement testing as outlined in Section A(2). Samples, standards, positive product controls and negative controls should be tested at least in duplicate.

For the gel-clot technique, an endotoxin standard series does not have to be run with each set of tests if consistency of standard endpoints has been demonstrated in the test laboratory. It should be run at least once a day with the first set of tests and repeated if there is any change in lysate lot, endotoxin lot or test conditions during the day. An endotoxin standard series should be run when confirming end-product contamination. Positive product controls (two lamda concentration of standard endotoxin in product) must be positive. If your test protocols state that you are using the USP Bacterial Endotoxin Test, remember that it requires a standard series to be run with each test. The above deviation must be noted in your test protocol.

For the chromogenic and endpoint-turbidimetric techniques, an endotoxin standard series does not have to be run with each set of tests if consistency of standard curves has been demonstrated in the test laboratory. It should be run at least once a day with the first set of tests and repeated if there is any change in lysate lot, endotoxin lot or test conditions during the day. However, at least duplicates of a 4 lambda standard concentration in water and in each product (positive product control) must be included with each run of samples. The mean endotoxin concentration of the standard must be within plus/minus 25% of the actual concentration and the positive product control must meet the same criteria after subtraction of any endogenous endotoxin. An endotoxin standard series should be run when confirming end-product contamination. If the alternate procedure is used, a standard in product series must be conducted each time the product is tested.

For the kinetic-turbidimetric test, it is not necessary to run a standard curve each day or when confirming end product contamination if consistency of standard curves has been demonstrated in the test laboratory.. However, at least duplicates of a 4 lambda standard concentration in water and in each product (positive product control) must be included with each run of samples. The mean endotoxin concentration of the standard when calculated using an archived standard curve (See Appendix C), must be within plus/minus 25% of the actual concentration and the positive product control must meet the same criteria after subtraction of any endogenous endotoxin. If the alternate procedure is used, a standard in product series must be conducted each time the product is tested.

Before a new lot of lysate is used, the labeled sensitivity of the lysate or the performance criteria should be confirmed by the laboratory, using the procedures in Appendix A.

The sampling technique selected and the number of units to be tested should be based on the manufacturing procedures and the batch size. A minimum of three units, representing the beginning, middle, and end, should be tested from a lot. These units can be run individually or pooled. If the units are pooled and any endotoxin is detected, repeat testing can be performed. The LAL test may be repeated no more than twice. The first repeat consists of twice the initial number of replicates of the sample in question to examine the possibility that extrinsic contamination occurred in the initial

assay procedure. On pooled samples, if any endotoxin is detected in the first repeat, proceed to second repeat. The second repeat consists of an additional 10 units tested individually. None of the 10 units tested in the second repeat may contain endotoxin in excess of the limit concentration for the drug product.

The following should be considered the endotoxin limit for all parenteral drugs to meet if the LAL test is to be used as an end-product endotoxin test:

1. K/M: For any parenteral drug except those administered intrathecally, the endotoxin limit for endotoxin is defined as K/M, which equals the amount of endotoxin (EU) allowed per ng or mL of product. K is equal to 5.0 EU/Kg. (SEE appendix D for definition of M).

   For parenteral drugs that have an intrathecal route of administration, K is equal to 0.2 EU/Kg.

Drugs exempted from the above endotoxin limits are:

1. Compendial drugs for which other endotoxin limits have been established.

2. Non-compendial drugs covered by new drug applications, antibiotic drug applications, new animal drug applications, and biological product licenses where different limits have been approved by the agency.

3. Investigational drugs or biologicals for which an IND or INAD exemption has been filed and approved.

4. Drugs or biologicals which cannot be tested by the LAL method.

A batch which fails a validated LAL release test should not be retested by the rabbit test and released if it passes. Due to the high variability and lack of reproducibility of the rabbit test as an endotoxin assay procedure, we do not consider it an appropriate retest procedure for LAL failures.