

**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.

CONTENTS

I. BACKGROUND	4
II. LEGAL EFFECT OF THE GUIDELINE	6
III. REGULATORY PROVISIONS THAT PERMIT INITIATION OF END-PRODUCT TESTING WITH LAL	7
IV. HUMAN AND ANIMAL DRUGS AND BIOLOGICAL PRODUCTS	9
A. Validation of the LAL Test	9
B. Routine Testing of Drugs by the LAL Test	11
V. MEDICAL DEVICES	14
A. Validation of the <i>LAL</i> Test	14
B. ROUTINE TESTING OF DEVICES BY THE LAL TEST	17
VI. APPENDICES	19
APPENDIX A: INITIAL QUALITY CONTROL	20
APPENDIX B: BACTERIAL ENDOTOXINS TEST UNITED STATES PHARMACOPEIA XXI/NATIONAL FORMULARY XVI AND FIRST SUPPLEMENT TO USP XXI/NF XVI	21
APPENDIX C: DETERMINATION OF THE RELATIONSHIP BETWEEN THE CONTROL STANDARD ENDOTOXIN (CSE) AND THE REFERENCE STANDARD ENDOTOXIN (RSE)	26
APPENDIX D	29
APPENDIX E	32

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 LA 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 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 (CBER) 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, CBER. 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 pre-market approval.

A manufacturer of an injectable drug for human or animal use that is not subject to pre-market 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 pre-clearance 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 pre-market notification under section 510(k) of the Federal Food, Drug, and Cosmetic Act (the Act) or a Pre-market 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 pre-market 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 official 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'G (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 re-verify the validity of the LAL test for the product. Firms that are obtaining an ingredient from a new manufacturer are encouraged 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 lambda 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 R/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.

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.

c) KINETIC-TURBIDIMETRIC TECHNIQUE

In inhibition/enhancement testing by this technique, 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 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 eluate/extract, 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 device eluate/extract shows inhibition or enhancement, the device cannot be tested by this procedure.

An alternate procedure may be performed whereby the RSE/CSE standard curve is prepared in device eluate/extract instead of water. The eluate/extract 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 device cannot be tested by the alternate procedure.

Impurities present in different sources of raw materials may inhibit the LAL test and, therefore, inhibition testing would be necessary when the raw material source is changed.

For each group of devices, protocols and test results from endotoxin sensitivity and inhibition/enhancement studies, including the actual data from the standard series and inhibition studies should be compiled and kept on file and be available for FDA inspection. Data should be expressed in the format most meaningful for the product (usually tabular or graphics).

B. ROUTINE TESTING OF DEVICES BY THE LAL TEST

Testing should be done using rinsing/eluting and sampling techniques as used for inhibition/enhancement testing. As in inhibition/enhancement testing, sampling can be adjusted for special situations. After a suitable eluate/extract pool is obtained from a finished production lot, this pooled extract is kept under conditions appropriate for endotoxin stability until it is tested 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 lambda 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 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. The second repeat consists of an additional 10 units.

For each product group, protocols and test results should be compiled and kept on file to be available for FDA inspection.

VI. APPENDICES

APPENDIX A: INITIAL QUALITY CONTROL

QUALITY CONTROL PROCEDURE

The following procedures and criteria are used for initial qualification and requalification of analysts in the laboratory, and to test new lots of lysate before use.

1) GEL CLOT ENDPOINT TECHNIQUE

For the gel-clot technique the procedures in the USP Bacterial Endotoxins Test Monograph (see Appendix B) should be used for quality control testing.

2) CHROMOGENIC AND ENDPOINT-TURBIDIMETRIC TECHNIQUES

Each test should be conducted according to the specific manufacturer's methodology.

Using the RSE or CSE whose potency is known, assay 4 replicates of a set of endotoxin concentrations which extend over the labeled linear range. The standard concentrations must include the stated lower and upper limits of the range. Linear regression analysis is performed on the absorbance values of the standards (y-axis) versus their respective endotoxin concentrations (x-axis). The coefficient of correlation, r , shall be greater than or equal to 0.980. If r is less than 0.980 the cause of the non-linearity should be determined and the test repeated. This linearity limit is also used to judge the validity of standard curves used for inhibition/enhancement tests and sample tests. In addition to meeting these requirements, any other test or requirements specified by the lysate manufacturer should also be met.

3) KINETIC-TURBIDIMETRIC TECHNIQUE

Each test should be conducted according to the manufacturer's instructions.

Using the RSE or CSE whose potency, in endotoxin units (See Appendix C), is known, assay at least 6 concentrations in triplicate which extend over the range 0.03 - 1.0 EU/mL. If instrument configuration does not allow you to run all 6 concentrations at one time, the data can be obtained in multiple runs and combined. Perform regression-correlation analysis on the log of the Time of Reaction (T) versus the log of the endotoxin concentration (E). The coefficient of correlation, r , shall be less than or equal to -0.980. If r is greater than -0.980 the cause of the non-linearity should be determined and the test repeated. In addition to meeting these requirements, any other test or requirements specified by the lysate manufacturer should also be met.

APPENDIX B: BACTERIAL ENDOTOXINS TEST UNITED STATES PHARMACOPEIA XXI/NATIONAL FORMULARY XVI AND FIRST SUPPLEMENT TO USP XXI/NF XVI

<85> BACTERIAL ENDOTOXINS TEST

This chapter provides a test for estimating the concentration of bacterial endotoxins that may be present in or on the sample of the article(s) to which the test is applied using Limulus Amebocyte Lysate (LAL) which has been obtained from aqueous extracts of the circulating amebocytes of the horseshoe crab, *Limulus polyphemus*, and which has been prepared and characterized for use as a LA reagent for gel-clot formation.

Where the test is conducted as a limit test, the specimen is determined to be positive or negative to the test judged against the endotoxin concentration specified in the individual monograph. Where the test is conducted as an assay of the concentration of endotoxin, with calculation of confidence limits of the result obtained, the specimen is judged to comply with the requirements if the result does not exceed (a) the concentration limit specified in the individual monograph, and (b) the specified confidence limits for the assay. In either case the determination of the reaction end-point is made with dilutions from the material under test in direct comparison with parallel dilutions of a reference endotoxin and quantities of endotoxin are expressed in defined Endotoxin Units.

Since LAL reagents have also been formulated to be used for turbidimetric (including kinetic assays) or colorimetric readings, such tests may be used if shown to comply with the requirements for alternative methods. These tests require the establishment of a standard regression curve and the endotoxin content of the test material is determined by interpolation from the curve. The procedures include incubation for a pre-selected time of reacting endotoxin and control solutions with LAL Reagent and reading of the spectrophotometric light absorbance at suitable wavelengths. In the case of the turbidimetric procedure the reading is made immediately at the end of the incubation period or in the kinetic assays the absorbance is measured throughout the reaction period and rate values are determined from those readings. In the colorimetric procedure the reaction is arrested at the end of the pre-selected time by the addition of an appropriate amount of acetic acid solution prior to the readings. A possible advantage in the mathematical treatment of results if the test be otherwise validated and the assay suitably designed, could be the application of tests of assay validity and the calculation of the confidence interval and limits of potency from the internal evidence of each assay itself (see Design and Analysis of Biological Assays <111>).

Reference Standard and Control Standard Endotoxins

The reference standard endotoxin (RSE) is the USP Endotoxin Reference Standard which has a defined potency to 10,000 USP Endotoxin Units (EU) per vial. Constitute the entire contents of 1 vial of the RSE with 5 mL of LAL Reagent Water.¹

- Vortex for not less than 20 minutes
- And use this concentrate for making appropriate serial dilutions. Preserve the concentrate in a refrigerator, for making subsequent dilutions for not more than 14 days
- Allow it to reach room temperature, If applicable, and vortex it Vigorously for not less than 5 minutes before use. Vortex each dilution for not less than 1 minute before proceeding to make the next dilution
- Do not use stored dilutions. A control standard endotoxin (CSE) is an endotoxin preparation other than the RSE that has been standardized against the RSE. If a CSE is a preparation not already adequately characterized its evaluation should include characterizing parameters both for endotoxin quality and performance (such as reaction in the rabbit), and for suitability of the material to serve as reference (such as uniformity and stability).

- Detailed procedures for its weighing and/or constitution and use to assure consistency in performance should also be included
- Standardization of a CSE against the RSE using a LAL Reagent for the gel-clot procedure may be effected by assaying a minimum of 4 vials of the CSE or 4 corresponding aliquots, where applicable, of the bulk CSE and 1 vial of the RSE, as directed under *Test Procedure*, but using 4 replicate reaction tubes at each level of the dilution series for the RSE and 4 replicate reaction tubes similarly for each vial of aliquot of the CSE. If all of the dilutions for the 4 vials or aliquots of the CSE cannot be accommodated with the dilutions for the 11 vial of the RSE on the same rack for incubation, additional racks may be used for accommodating some of the replicate dilutions for the CSE, but all of the racks containing the dilutions of the RSE and the CSE are incubated as a block. However, in such cases, the replicate dilution series from the 1 vial of the RSE are accommodated together on a single rack and the replicate dilution series from any one of the 4 vials or aliquots of the CSE are not divided between racks.
- The antilog of the difference between the mean \log_{10} end-point of the RSE and the mean \log_{10} end-point of the CSE is the standardized potency of the CSE which then is to be
- Converted to and expressed in Units per ng under stated drying conditions for the CSE, or in Units per container, whichever is appropriate. Standardize each new lot of CSE prior to use in the test. Calibration of a CSE in terms of the RSE must be with the specific lot of LAL Reagent and the test procedure with which it is to be used. Subsequent lots of LAL Reagent from the same source and with similar characteristics need only checking of the potency ration.
- The inclusion of one or more dilution series made from the RSE when the CSE is used for testing will enable observation of whether or not the relative potency shown by the latter remains within the determined confidence limits
- A large lot of a CSE may, however, be characterized by a collaborative assay of a suitable design to provide a representative relative potency and the within-laboratory and between-laboratory variance.

A suitable CSE has a potency of not less than 2 Endotoxin Units per ng and not more than 50 Endotoxin Units per ng, where in bulk form, under adopted uniform drying conditions, e.g., to a particular low moisture content and other specified conditions of use, and a potency within a corresponding range where filled in vials of a homogeneous lot.

Preparatory Testing

Use a LAL reagent of confirmed label or determine sensitivity. In addition, where there is to be a change in lot of CSE, LAL Reagent or another reagent, conduct tests of a prior satisfactory lot of CSE, LAL and/or other reagent in parallel on changeover. Treat any containers or utensils employed so as to destroy extraneous surface endotoxins that may be present, such as by heating in an oven at 250° or above for sufficient time.²

The validity of test results for bacterial endotoxins requires an adequate demonstration that specimens of the article, or of solution, washings, or extracts thereof to which the test is to be applied do not of themselves inhibit or enhance the reaction or otherwise interfere with the test. Validation is accomplished by testing untreated specimens or appropriate dilutions thereof, concomitantly with and without known and demonstrable added amounts of RSE or a CSE and comparing the results obtained. Appropriate negative controls are included. Validation must be repeated if the LAL Reagent source or the method of manufacture or formulation of the article is changed.

Test for confirmation of labeled LAL Reagent sensitivity –

- Confirm the labeled sensitivity of the particular LAL reagent with the RSE (or CSE) using not less than 4 replicate vials, under conditions shown to achieve an acceptable variability of the test, viz, the antilog of the geometric mean \log_{10} lysate gel-slot sensitivity is within 0.5λ to 2.0λ , where λ is the labeled sensitivity in

Endotoxin Units per mL. The RSE (or CSE) concentration selected in confirming the LAL reagent label potency should bracket the stated sensitivity of the LAL reagent. Confirm the labeled sensitivity of each new lot of LAL reagent prior to use in the test.

Inhibition of Enhancement Test – Conduct assays with standard endotoxin or untreated specimens in which there is no endogenous endotoxin detectable, and of the same specimens to which endotoxin has been added as directed under *Test Procedure*, but using not less than 4 replicate reaction tubes at each level of the dilution series for each untreated specimen and for each specimen to which endotoxin has been added. Record the end-points (E, in Units per mL) observed in the replicates. Take the logarithms (e) of the end-points and compute the geometric means of the log end-points for the RSE (or CSE), for the untreated specimens and for specimens containing endotoxin by the formula $\text{antilog } \Sigma e/f$, in which Σe is the sum of the log end-point of the dilution series used and f is the number of replicate end-points in each case. Compute the amount of endotoxin in the specimen to which endotoxin has been added. The test is valid for the article if this result is within twofold of the known added amount of endotoxin. Alternatively, if the test has been appropriately set up, the test is valid for the article if the geometric mean end-point dilution for the specimen to which endotoxin has been added is within one 2-fold dilution of the corresponding geometric mean end-point dilution of the standard endotoxin.

If the result obtained for the specimens to which endotoxin has been added is outside the specified limit, the article is unsuitable for the *Bacterial Endotoxins Test*, or, in the case of Injections or solutions for parenteral administration, it may be rendered suitable by diluting specimens appropriately.

Repeat the test for inhibition or enhancement using specimens diluted by a factor not exceeding that given by the formula x/λ (see *Maximum Valid Dilution*, below). Use the least dilution sufficient to overcome the inhibition or enhancement of the known added endotoxin for subsequent assays of endotoxin in test specimens.

If endogenous endotoxin is detectable in the untreated specimens under the conditions of the test, the article is unsuitable for the *Inhibition or Enhancement Test*, or, it may be rendered suitable by removing the endotoxin present by ultra-filtration, or by appropriate dilution. Dilute the untreated specimen (as constituted, where applicable, for administration or use), to a level not exceeding the maximum valid dilution, at which no endotoxin is detectable. Repeat the test for *Inhibition or Enhancement* using the specimens at those dilutions.

Test Procedure

In preparing for and applying the test, observe precautions in handling the specimens in order to avoid gross microbial contamination. Washings or rinsings of devices must be with LAL Reagent Water in volumes appropriate to their use and, where applicable, of the surface area which comes into contact with body tissues or fluids. Use such washings or rinsings if the extracting fluid has been in contact with the relevant pathway or surface for not less than 1 hour at controlled room temperature (15° to 30°) Such extracts may be combined, where appropriate. The ultimate rinse or wash volume is such as to result in possible dilution of any contained endotoxin to a level not less than that suitable for use in the *Pyrogen Test* <151> under *Transfusion and Infusion Assemblies* <161>.

For validating the test for an article, for endotoxin limit tests or assays, or for special purposes where so specified, testing of specimens is conducted quantitatively to determine response end-points for gel-clot readings. Usually graded strengths of the specimen and standard endotoxin are made by multifold dilutions.

- Select dilutions so that they correspond to a geometric series in which each step is greater than the next lower by a constant ratio
- Do not store dilutee endotoxin, because of loss of activity by adsorption. In the absence of supporting data to the contrary, negative and positive controls are incorporated in the test.

Use not less than 2 replicate reaction tubes at each level of the dilution series for each specimen under test. Whether the test is employed as a limit test or as a quantitative assay, a standard endotoxin dilution series involving not less than 2 replicate reaction tubes is conducted in parallel. A set of standard endotoxin dilution series is included for each block of tubes, which may consist of a number of tracks for incubation together, provided the environmental conditions within blocks are uniform.

Preparation – Since the form and amount per container of standard endotoxin and of LAL reagent may vary, constitution and/or dilution of contents should be as directed in the labeling. The pH of the test mixture of the specimen and the LAL Reagent is in the range 6.0 to 7.5 unless specifically directed otherwise in the individual monograph. The pH may be adjusted by the addition of sterile endotoxin-free sodium hydroxide of hydrochloric acid or suitable buffers to the specimen prior to testing.

Maximum Valid Dilution (MVD) – The Maximum Valid Dilution is appropriate to Injections or to solutions for parenteral administration in the form constituted of diluted for administration or where applicable to the amount of drug by weight if the volume of the dosage form for administration could be varied. Where the endotoxin limit concentration is specified in the individual monograph in terms of volume (in EU per mL), divide the limit by λ , which is the labeled sensitivity (in EU per mL) of the lysate employed in the assay, to obtain the MVD factor. Where the endotoxin limit concentration is specified in the individual monograph in terms of weight or of Units of active drug (in EU per mg or in EU per Unit), multiply the limit by the concentration (in mg per mL or in Units per mL) of the drug in the solution tested or of the drug constituted according to the label instructions, whichever is applicable, and divide the product of the multiplication by λ , to obtain the MVD factor. The MVD factor so obtained is the limit dilution factor for the preparation for the test to be valid.

Procedure – To 10 x 75 mm test tubes add aliquots of the appropriate constituted LAL Reagent, and the specified volumes of specimens, endotoxin standard, negative controls, and a positive product control consisting of the article, or of solutions, washings or extracts thereof to which the RSE (or a standardized CSE) has been added at a concentration of endotoxin of 2λ for that LAL reagent (see under *Test for confirmation of labeled LAL Reagent sensitivity*). Swirl each gently to mix, and place in an incubating device such as a water bath or heating clock, accurately recording the time at which the tubes are so placed. Incubate each tube undisturbed, for 60 ± 2 minutes at $31 \pm 1^\circ$, and carefully remove it for observation. A positive reaction is characterized by the formation of a firm gel that remains when inverted through 180° . Record such a result as positive (+). A negative result is characterized by the absence of such a gel or by the formation of a viscous gel that does not maintain its integrity. Record such a result as negative (-). Handle the tubes with care, and avoid subjecting them to unwanted vibrations, or false negative observations may result. The test is invalid if the positive product control or ht endotoxin standard does not show the end-point concentration to be within ± 1 twofold dilutions from the label claim sensitivity of the LAL Reagent or if any negative control shows a gel-clot end-point.

Calculation and Interpretation

Calculation – Calculate the concentration of endotoxin (in Units per mL or in Units per g or mg) in or on the article under test by the formula pS/U , in which S is the antilog of the geometric mean \log_{10} of the end-points, expressed in Endotoxin Units (EU) per mL for the Standard Endotoxin, U is the antilog of $\Sigma e/f$, where e is the \log_{10} of the end-point dilution factors, expressed in decimal fractions, f is the number of replicate reaction tubes read at the end-point level for the specimen under test, and p is the correction factor for those cases where a specimen of the article cannot be taken directly into test but is processed as an extract, solution, or washing.

Where the test is conducted as an assay with sufficient replication to provide a suitable number of independent results, calculate for each replicate assay the concentration of endotoxin in or on the article under test from the

antilog of the geometric mean log end-point ratios. Calculate the mean and the confidence limits from the replicate logarithmic values of all the obtained assay results by a suitable statistical method (see *Calculation of Potency from a Single Assay* <111>).

Interpretation – The article meets the requirements of the test if the concentration of endotoxin does not exceed that specified in the individual monograph, and where so specified in the individual monograph or in this chapter, the confidence limits of the assay do not exceed those specified.

APPENDIX C: DETERMINATION OF THE RELATIONSHIP BETWEEN THE CONTROL STANDARD ENDOTOXIN (CSE) AND THE REFERENCE STANDARD ENDOTOXIN (RSE)

If a manufacturer chooses to use an endotoxin preparation (CSE) other than the United States Pharmacopeia Reference Standard Endotoxin (RSE), the CSE will have to be standardized against the RSE. If the CSE is not a commercial preparation which has been adequately characterized, it should be studied and fully characterized as to uniformity, stability or the preparation, etc. The relationship of the CSE to the RSE should be determined prior to use of a new lot, sensitivity, or manufacturer of the LAL or a new lot source or manufacturer of the CSE.

A. GEL-CLOT TECHNIQUE

The following is an example of a procedure to determine the relationship of the CSE to the RSE:

At least 4 samples (vials) for the lot of CSE should be assayed. State in ng/mL the endpoint for the CSE and in EU/mL of the RSE. The values obtained should be the geometric mean of the endpoints using a minimum of 4 replicates.

Example: LAL endpoints for the RSE and CSE are as follows:

$$\begin{aligned} \text{RSE} &= 0.3 \text{ EU/mL} \\ \text{CSE} &= 0.018 \text{ ng/mL} \end{aligned}$$

The EUs per ng of CSE are calculated as follows:

$$\begin{aligned} \text{RSE} &= 0.3 \text{ EU/mL} = 16.7 \text{ EU/ng} \\ \text{CSE} &= 0.018 \text{ ng/mL} \end{aligned}$$

This indicates that 0.018 ng of the CSE is equal to 0.3 EU of the RSE. Thus, the CSE contains 16.7 EU/ng.

B. CHROMOGENIC AND ENDPOINT-TURBIDIMETRIC TECHNIQUES

At least 4 samples (vials) for the lot of CSE should be assayed. In addition to a water blank, assay dilutions of RSE which fall in the linear range and dilutions of the CSE. Linear regression analysis is performed on the absorbance values of the RSE standards (y-axis) versus their respective endotoxin concentrations (x-axis). Calculate the EU/ng of the CSE by inserting the average CSE O.D. readings for each concentration which falls in the RSE standard range into the SE straight line equation. The resulting CSE values (in EU) are then divided by their corresponding concentrations (in ng/mL). These values are then averaged to obtain the potency of the CSE lot.

EXAMPLE:

RSE Standard Curve

	Concentration	O.D.
RSE (EU/mL)	0.1	0.11
	0.25	0.26
	0.5	0.49
	1.0	1.06

y-intercept = -0.008 slope = 1.056 r = 0.999

Straight Line Equation (Y) = -0.008 + (1.056 * X)

CSE Standard Curve

CSE Conc. (ng/mL)	AVERAGE O.D.	Corresponding RSE (EU/mL)	EU/ng (RSE/CSE)
0.01	0.12	0.119	11.9
0.025	0.31	0.301	12.0
0.05	0.60	0.626	12.5
0.1	1.23	1.291	12.9

Mean EU/ng = 12.3

C. KINETIC-TURBIDIMETRIC TECHNIQUE

In order to assign EUs to a CSE, the following should be performed on 4 vials from the same CSE lot.

Twofold dilutions of the RSE should be made in the range of 1.0 EU/mL to 0.03 EU/mL. Determine the Time of Reaction (T) for at least duplicates of each standard concentration. Construct a standard curve (Log10 T versus Log10 endotoxin concentration (E)). Calculate the mean T for 1.0 and 0.03 EU/mL. These T's define the RSE standard range.

For each of the four vials of CSE make twofold dilutions such that the T values for at least 3 concentrations of the CSE are within the RSE standard range. Determine the T values for at least duplicates of each endotoxin concentration. Calculate the EU/ng of CSE by inserting the log mean CSE T values for each endotoxin concentration which falls in the RSE standard range into the RSE straight line equation. The resulting CSE values (in EU) are then divided by their corresponding concentrations (in ng/mL). These values are averaged to obtain the potency of the CSE lot.

EXAMPLE:

RSE Standard Curve

Straight Line Equation (Y) = 3.03 + (-0.181 * X)

RSE Standard Range = 1037 - 2235 seconds (17.3-37.3 minutes)

CSE Standard Curve

Vial	Endotoxin Concentration(ng/mL)					
	0.1	0.05	0.025	0.0125	0.006	0.003
1	1018.8	1114	1218.6	1402.7	1548.7	1740.7
2	990.7	1090.6	1249.8	1406.4	1586.0	1780.0
3	998.2	1116.8	1227.8	1411.0	1554.1	1800.9
4	1003.4	1086.1	1198.5	1415.6	1593.9	1781.0

Note: Each T in the above table is expressed in seconds and represents the mean of at least duplicate determinations.

Mean T (sec.)	1002.8*	1101.9	1223.7	1408.9	1570.7	1775.7
Log mean T	3.001	3.042	3.088	3.149	3.196	3.249

Calculations:

Solving for EU/mL equivalent by substituting onset times generated with CSE (ng/mL) into the above RSE standard line equation, $X = (Y - 3.03) / -0.181$ where Y = log mean onset time and X = log EU/ml equivalent.

<u>CSE Endo. Cone.</u> (ng/mL)	<u>Log Mean</u> T	EU/mL Equivalent (RSE Std. Line)		EU/ng
		<u>Log</u>	<u>Antilog</u>	
0.1*	3.001	0.16	1.45	14.5
0.05	3.042	-0.066	0.859	17.2
0.025	3.088	-0.32	0.479	19.2
0.0125	3.149	-0.657	0.22	17.6
0.006	3.196	-0.917	0.121	20.2
0.003	3.249	-1.210	0.062	20.6

Mean EU/ng = 19.0 (SD = 1.52)

* Outside the RSE standard range - not used in calculation of mean.

The values for the y-intercept and slope of the four CSE curves used for the EU/ng determination may be stored for use in routine testing (archived standard curve) instead of running a series of standards each day. Using the EU/ng conversion factor, CSE standards within the range of the RSE curve can be made up in endotoxin units. Standards outside this range require the use, of RSE and a new RSE standard curve. If CSE standards outside the RSE standard range are required the EU/ng conversion factor must be determined for the new range as described above.

APPENDIX D

MAXIMUM VALID DILUTION

To determine how much the product can be diluted and still be able to detect the limit endotoxin concentration, the following two methods will determine the Maximum Valid Dilution:

METHOD I

This method is used when there is an official USP limit or when the limits listed in Appendix E are used.

$$\text{MVD} = \frac{\text{Endotoxin Limit} \times \text{Potency of Product}}{\text{Potency of Product}}$$

For drugs administered on a weight-per-kilogram basis, the potency is expressed as mg or units/mL and for drugs administered on a volume-per-kilogram basis, the potency is equal to 1.0 mL/mL.

METHOD II

This method is used when there is no official USP limit and the limits listed in Appendix E are not used.

Step 1. Minimum Valid Concentration (MVC)

$$\text{MVC} = \frac{\lambda M}{K}$$

Where:

λ = GEL CLOT: Labeled sensitivity-EU/mL.

CHROMOGENIC, TURBIDIMETRIC and KINETIC-TURBIDIMETRIC:

The lowest point used in the standard curve.

M = Rabbit Dose or Maximum Human Dose/Kg of body weight that would be administered in a single one hour period, whichever is larger. For radiopharmaceuticals, M equals the rabbit dose or maximum human dose/Kg at the product expiration date or time. Use 70 Kg as the weight of the average human when calculating the maximum human dose per Kg. Also, if the pediatric dose/Kg is higher than the adult dose then it shall be the dose used in the formula.

K = 5.0 EU/Kg for parenteral drugs except those administered intrathecally; 0.2 EU/Kg for intrathecal drugs

APPENDIX D (cont.)

Step 2. Maximum Valid Dilution (MVD)

$$\text{MVD} = \frac{\text{Potency of Product}}{\text{MVC}}$$

For drugs administered on a weight-per-kilogram basis, the potency is expressed as mg or units/mL and for drugs administered on a volume-per-kilogram, the potency is equal to 1.0 mL/mL.

METHOD I EXAMPLES

Endotoxin Limit Expressed by Weight

Product: Cyclophosphamide Injection
Potency: 20 mg/mL
Lysate Sensitivity (λ): 0.065 EU/mL
Endotoxin Limit (Appendix E): 0.17 EU/mg

$$\text{MVD} = \frac{0.17 \text{ EU/mR} \times 20 \text{ mR/ml}}{0.065 \text{ EU/mL}} = \frac{3.4}{0.065} = 1:52.3 \text{ or } 1:52$$

Endotoxin Limit Expressed by Volume:

Product: 5% Dextrose Injection
Lysate Sensitivity (X): 0.065 EU/mL
Endotoxin Limit (Appendix E): 0.5 EU/mL

$$\text{MVD} = \frac{0.5 \text{ EU/mL} \times 1 \text{ mL/mL}}{0.065 \text{ EU/mL}} = \frac{0.5}{0.065} = 1:7.7$$

METHOD II EXAMPLES

PARENTERAL DRUGS EXCEPT INTRATHECAL

Drug Administered on a Weight-per-Kilogram Basis

Product: Cyclophosphamide Injection
Potency: 20 mg/mL
Maximum Dose/Kg (M): 30 mg/Kg
Lysate Sensitivity (λ): 0.065 EU/mL

$$\text{MVC} = \frac{\lambda M}{K} = \frac{0.065 \text{ EU/mL} \times 30 \text{ mg/Kg}}{5.0 \text{ EU/Kg}} = 0.390 \text{ mg/mL}$$

$$\text{MVD} = \frac{\text{Potency of Product}}{\text{MVC}} = \frac{20 \text{ mg/mL}}{0.390 \text{ mg/mL}} = 1:51.2 \text{ or } 1:51$$

APPENDIX D (cont.)

Drug Administered on a Volume-per-Kilogram Basis

Product: 5% Dextrose in Water
Maximum Dose/Kg (M): 10.0 mL/Kg
Lysate Sensitivity (λ): 0.065 EU/mL

$$MVC = \frac{\lambda M}{K} = \frac{0.065 \text{ EU/mL} \times 10.0 \text{ mL/Kg}}{5.0 \text{ EU/Kg}} = 0.13 \text{ mL/mL}$$

$$MVD = \frac{\text{Potency of Product}}{MVC} = \frac{1.0 \text{ mL/mL}}{0.13 \text{ mL/mL}} = 1:7.7$$

INTRATHECAL DRUGS

Drug Administered on a Weight-per-Kilogram Basis

Product: Gentamicin Sulfate
Potency: 2.0 mg/mL
Maximum Dose/Kg (M): 0.11 mg/Kg
Lysate Sensitivity (A): 0.1 EU/mL

$$MVC = \frac{\lambda M}{K} = \frac{0.1 \text{ EU/mL} \times 0.11 \text{ mg/Kg}}{0.2 \text{ EU/Kg}} = 0.055 \text{ mg/mL}$$

$$MVD = \frac{\text{Potency of Product}}{MVC} = \frac{2.0 \text{ mg/mL}}{0.055 \text{ mg/mL}} = 1:36.4$$

Drug Administered on a Volume-per-Kilogram Basis

Product: Lidocaine Hydrochloride Injection
Maximum Dose/Kg (M): 0.057 mL/Kg
Lysate Sensitivity (λ) 0.1 EU/mL

$$MVC = \frac{\lambda M}{K} = \frac{0.1 \text{ EU/mL} \times 0.057 \text{ mL/Kg}}{0.2 \text{ EU/Kg}} = 0.0285 \text{ mL/mL}$$

$$MVD = \frac{\text{Potency of Product}}{C} = \frac{1.0 \text{ mL/mL}}{0.0285 \text{ mL/mL}} = 1:35.0$$

APPENDIX E
December ,1987

MAXIMUM DOSE AND ENDOTOXIN LIMIT TABLE

<u>Drug Name</u>	<u>Dose (M)</u> (R)= Rabbit Dose	<u>Endotoxin Limit</u> (EU/mg,ml,units of product)
-A-		
Acetazolamide Sodium	10.0 mg	0.50
Acetylcysteine Injection	150 mg	0.03
Acyclovir Sodium	30 mg	0.17
Adenosine Phosphate	0.71 mg	7.04
Albumin,Normal Human Serum (25X)	3.0 ml(R)	1.67
Albumin,Normal Human Serum (20X)	3.75 ml(R)	1.33
Albumin,Normal Human Serum (5X)	10.0 ml(R)	0.50
Alcohol and Dextrose Injection	10.0 ml(R)	0.50
Alfentanil Hydrochloride	250 mcg	0.02
Alkaloids of Belladonna	0.007 mg	714.29
Alphaprodine HU Injection	1 mg(R)	5.00
Alprostadil (Postaglandin)	100 mcg	0.05 +
Amdinocillin	10 mg	0.50
Amikacin Sulfate Injection	25 mg(R)	0.20
Amino Acid Injection	25 mg	0.20
Amino Acids and Electrolytes	25 mg	— 0.20
Essential Amino Acids and Dextrose	25 mg	— 0.20
Aminocaproic Acid Injection	250 mg(R)	0.02
Aminohippurate Sodium Injection	125.0 mg	0.04
Aminophylline Injection	5.0 mg	1.00
Amitriptyline HCl Injection	0.42 mg	12.0
Ammonium Chloride Injection	50 mg(R)	0.10
Amobarbital sodium	14.3 mg	0.35
Amoxicillin	20.0 mg	0.25
Amphotericin B for Injection	2.0 mg(R)	2.50
*Amphotericin B for Injection	0.007 mg	28.57
Ampicillin Sodium	20.0 mg(R)	0.25
Amrinone Lactate	1.50 mg	3.35
Anileridine	0.7 mg	7.20
Anticoagulant Heparin Solution	2.0 ml(R)	2.50
Antihemophilic Factor	10 units(R)	0.50
Antihemophilic Plasma(1 hr. at 56-57oC)	3.0 ml(R)	1.67
Antirabies Serum	3.0 ml(R)	1.67
Antitoxin (Gas Gangrene)	3.0 ml(R)	1.67
Antivenom	3.0 ml(R)	1.67
Apomorphine HCl Tablets for Injection	0.09 mg	55.56
Arginine HU Injection	500 mg	0.01
Ascorbic Acid	25 mg	0.2

Asparaginase for Injection	200 IU	2.9
Atracurium Besylate	0.6 mg	8.35
Atropine Sulfate	0.029 mg	172.0
Aurothioglucose Suspension	0.7 mg	7.14
Azathioprine Sodium for Injection	5.0 mg	1.00
Azlocillin	75.0 mg	0.07
Aztreonam for Injection	50.0 mg(R)	0.10

-B-

Bacitracin	500 units	0.01
Bacitracin Zinc	500 units	0.01
Benzquinamide HU	1.0 mg	5.0
Benztropine Mesylate Injection	0.086 mg	58.00
Benzylpenicilloyl Polylysine	0.03 ml(R)	167.00
Betamethasone Acetate and Betametasone Sodium Phosphate Injection	0.17 mg	29.2
Betamethasone Sodium <i>Phosphate Injection</i>	0.17 mg	29.2
Betazole HCl Injection	2.86 mg	1.75
Bethanechol Chloride	0.2 mg	25.00
Biperiden Lactate Injection	0.06 mg	83.30
Bleomycin Sulfate	0.5 unit(R)	10.00
Bretylum Tosylate Injection	25.0 mg	0.20
Bretylum Tosylate in Dextrose	25.0 mg	0.20
Brompheniramine Maleate Injection	0.29 mg	17.00
Bumetanide	0.25 mg(R)	20.00
Bupivacaine Hydrochloride Injection	2.5 mg	2.5
Bupivacaine Hydrochloride and Epinephrine Injection	0.26 mg	19.2
Bupivacaine HU and Deztose	0.71 mg	7.0
Buprenorphine HU	0.009 mg	556.0
Butorphanol Tartrate	0.057 mg	88.0

-C-

Caffeine	8.0 mg	0.63
Caffeine and Sodium Benzoate	8.0 mg	0.63
Calcitonin - Salmon	4 USP unit	1.25
Calcium Ascorbate	14.3 mg	0.35
Calcium Chloride	25 mg	0.20
Calcium Disodium Edetate	35.0 mg	0.143
Calcium Gluceptate Injection	25.7 mg Ca	0.2
Calcium Gluconate	300 mg(R)	0.02
Calcium Levulinate	200 mg(R)	0.03
Capreomycin Sulfate	20.0 mg	0.25
Carbazochrome Salicylate	0.14 mg	34.96
Carbenicillin Disodium	200 mg(R)	0.025
Carboprost Tromethamine	10.0 mcg(R)	0.5
Carmustine for Injection	5.3 mg	0.95

Cefamandole Nafate	50 mg(R)	0.10
Cefazolin Sodium	50 mg(R)	0.10
Cefonicid Sodium	14.3 mg	0.35
Cefoperazone Sodium	28.57 mg	0.2
Ceforanide	14.3 mg	0.35
Cefotaxime Sodium	28.5 mg	0.20
Cefotetan Disodium	50.0 mg(R)	0.10
Cefoxitin Sodium	50 mg(R)	0.10
Ceftazidime	50 mg(R)	0.10
Ceftizozime Sodium	50 mg(R)	0.10
Ceftriaxone Sodium	28.6 mg	0.20
Cefurozime Sodium	21.4 mg	0.23
Cephacetrile Sodium for Infection	80 mg(R)	0.06
Cephaloridine	50 mg(R)	0.10
Cephalothin Sodium Injection	50 mg(R)	0.10
Cephapirin Sodium	100 mg(R)	0.06
Cephradine for Infection	80 mg(R)	0.06
Cerulitide diethylamine	0.3 mcg	16.67
Chloramphenicol Sodium Succinate	25 mg	0.2
Chlordiazepozide HCl	- 4 mg(R)	1.25
Chlorprocaine HCl	11.43 mg	0.45
Cholecystokinin	1.0 IDU	5.0
Chorionic Gonadotropin	1000 uaits(R)	0.005
Chlormerodrin Hg197 Injection	7 ml	25.00 +
Chlormerodrin Hg203 Infection	7 ml	25.00 +
Chlormerodrine	1.4 mg	3.57
Chloroquine HCl Injection	7.5 mg	0.70
Chlorothiazide Sodium	15 mg	0.30
Chlorpheniramine Maleate	0.57 mg	8.80
Chlorpromazine HCl	0.72 mg	6.90
Chlorprothixene Infection	0.72 mg	6.90
Chlortetracycline HCl	5 mg	1.00
Chormate Sodium Cr51 Injection	7 ml	25.00 +
Chromic Chloride Injection	1.0 ug(R)	5.00
Chromic Phosphate P32 Suspension	7 ml	25.00 +
Chymopapain	57.14 units	0.09
Chymotrypain	4.3 units	1.16
Cimetidine HCl Injection	10.0 mg	0.5
Cisplatin for Injection	2.7 mg	1.90
Citrate,Phosphate,Dextrose,Adenine Sol.	0.9 ml	5.56
Cliadamycin Phosphate Infection	24 mg(R)	0.20
Cloxacillin	20.0 mg(R)	0.25
Codeine Phosphate Infection	0.86 mg	5.80
Colchicine Infection	0.04 mg	125.00
Colistimethate Sodium	10 mg(R)	0.50
Conjugated Estrogens	0.36 mg	13.89
Corticotropin, Gel, Zinc & Repository	1.1 units	4.60
Cortisone Acetate	5.0 mg	1.00
Cosyatropin	3.57 mcg	1.40

Cryptenamine Acetate	1.86 CSR units	2.69
Cupric Chloride Injection	0.2 mg(R)	25.00
Cupric Sulfate Injection	0.2 mg(R)	25.00
Cyanocobalamine and Repository	14.3 mcg	0.35
Cyclizine Lactate	1.00 mg	5.00
Cyclophosphamide	30.0 mg	0.20
Cyclosporine Injection and Concentrate	0.12 mL	42.00 +
Cysteine HCl	7.14 mg	0.70
*Cytarabine	3.00 mg	0.07

-D-

Dacarbazine for Injection		5.00 mg(R)	1.00
Dactinomycin for Injection		0.2 mg(R)	25.0
Dantrolene Sodium		10 mg	0.50
Daunorubicin HU		2.25 mg(R)	2.20
Decamethonium Bromide		0.043 mg	116.3
Deferoxamine Mesylate		30 mg(R)	0.17
Dehydrocholate Sodium Injection		150 mg	0.04
Deslanoside		0.03 mg	167.0
Desmopressin Acetate		0.5 Meg	10.00
Desoxycorticosterone Acetate Injection		0.07 mg	71.40
Desoxycorticosterone Pivalate Suspension		1.8 mg	2•78
Dexamethasone Acetate Suspension		0.23 mg	21.74
Dexamethasone Sodium Phosphate Injection		0.16 mg	31.30
Dexpanthenol		7.1 mg	0.70
Dextran 40		5.0 ml	1.00
Dextran 40 in Sodium Chloride		5.0 ml	1.00
Dextran 70		10.0 ml	0.50
Dextrose- 5%-70%		10.0 ml(R)	0.50
Dextrose and Sodium Chloride		10.0 ml(R)	0.50
Diatrizoate Meglumine Injection		60% 1.0 ml	5.0
		30% 4.4 ml	1.10
Diatrizoate Meglumine and Diatrizoate Sodium	66% - 10%	2.3 ml	2.17
	60% - 30%	1.4 ml	3.57
	52% - 8%	2.8 ml	1.80
	50%	- 25%	2.8 ml 1.80
	34.3%	- 35%	2.8 ml 1.80
	28.5% - 29.1%	2.8 ml	1.80
Diatrizoate Sodium	50%	1 ml	5.0
	25%	4 ml	1.25
	20%	0.9 ml	5.56
Diazepam Injection		0.43 mg	11.60
Diazoxide Injection		10.0 mg	0.50
*Dibucaine		0.14 mg	35.70
Dibucaine HU and Dextrose		0.07 mg	71.43
Dicloxacillin Sodium		20 mg(R)	0.25
Dicyclomine HCl Injection		0.29 mg	17.20

Diethylstilbestrol Injection	7.14 mg	0.70
Diethylstilbestrol Diphosphate	7.14 mg	0.70
Digitoxin Injection	0.045 mg	111.00
Digoxin Injection	0.025 mg	200.0
Dihydroergotamine Mesylate	0.014 mg	357.00
Dihydroergotamine Mesylate, Heparin Sodium & Lidocaine HCl	1667 units	0.003 (Heparin)
Dihydrostreptomycin Sulfate	10.0 mg(R)	0.50
Dihydrotachysterol	0.03 mg	166.67
Diluent for Meningococcal Vaccine	5.0 ml(R)	1.00
Dimenhydrinate Injection	1.25 mg	4.00
Dimercaprol	5.0 mg	1.00
Dinoprost Tromethamine	0.57 mg	8.77
Diphenhydramine HCl Injection	1.5 mg	3.35
Diphenidol	0.3 mg	16.67
Diphtheria Antitoxin,Pur.Conc.(equine)	3.0 ml(R)	1.67
Dobutamine HCl	5.0 mg(R)	1.00
Dopamine HU	3.0 mg	1.70
Dopamine HU in Dextrose	3.0 mg	1.70
Doxapram HCl Injection	4.00 mg	1.25
Doxorubicin HCl for Injection	2.25 mg(R)	2.20
Doxycycline Hyclate for Injection	7.5 mg(R)	0.67
Dromostanolone Propionate	1.4 mg	3.57
Droperidol	0.14 mg	35.70
Dyphylline Injection	7.1 mg	0.70

-E-

Edetate Calcium Disodium	400 mg(R)	0.01
Edetate Disodium	250 mg(R)	0.02
Edrophonium Chloride Injection	1 mg(R)	5.00
Electrolyte Solutions- LVP	10 ml(R)	0.50
Emetine HCl	0.93 mg	5.40
Ephedrine Sulfate Injection	0.75 mg	6.70
Epinephrine Injection	0.014 mg	357.00
Epinephrine Suspension	0.025 mg	200.00
Ergocalciferol (D2)	142.8 units	0.035
Ergoloid Mesylates	0.004 mg	1250.00
Ergonovine Mateate	6.00 mcg	0.8
Ergotamine Tartrate	0.014 mg	357.00
Erythromycin Gluceptate and Lactobionate	30 mg(R)	0.17
Estradiol (aqueous)	0.02 mg	250.00
Estrogens (Combined) Aqueous	0.026 mg	
	Estrone	192.31
Estrogens Conjugated	0.36 mg	14.00
Estrogenic Substances or Estrogens	0.057 mg	88.00
Estrone Aqueous Suspension	0.057 mg	88.00
Ethacrynate Sodium	1.4 mg	3.60
Ethamivan Injection	5 mg(R)	1.0

Ethylnorepinephrine HU Injection	0.029 mg	172.40
Etidocaine HCl	5.50 mg	0.90
Etidocaine HU and Epinephrine	5.5 mg	0.08
Etomidate Injection	0.6 mg	8.35
Etoposide Injection	2.64 mg	1.90
Evans Blue Injection	0.36 mg	14.00

-F-

Factor IX	50.0 ml(R)	0.10
Fat Emulsion	(10X) 3.2 ml	1.56
	(20X) 1.6 ml	3.13
Fentanyl Citrate	0.15 mg	33.3
Fentanyl Citrate and Droperidol	0.004 mg	
	Fentanyl	1250.00
Ferrous Citrate Fe59 Infection	7 ml	25.00 +
Fibrinogen	30.0 mg(R)	0.17
Fibrinogen, Dried	30.0 mg(R)	0.17
Fibrinolysin and Desoxyribonuclease	1.0 units(R)	5.00
Floxuridine	50 mg(R)	0.10
Fluorescein Sodium Injection	250 mg(R)	0.02
Fluorouracil Infection	12 mg	0.40
Fluphenazine HM	0.05 mg	100.00
Fluphenazine Enanthate or Decanoate	1.43 mg	3.5
Folate Sodium	0.01 mg	500.00
Fructose	10.0 ml(R)	0.50
Fructose and Sodium Chloride	10.0 ml(R)	0.50
Furosemide Injection	1.4 mg	3.60

-G-

Gallamine Triethiodide	1.4 mg	3.60
Gallium Citrate Ga67 Infection	7 ml	25.00 +
Gelatin 6%	1.0 ml(R)	5.00
Gentamicin Sulfate	10 mg(R)	0.50
*Gentamicin Sulfate	0.11mg	45.46
Globulins (Humans)	1.0 ml(R)	5.00
Glucagon for Infection	0.11 mg	45.50
Glycopyrrolate	0.009 mg	555.50
Gold Au198 Infection	7 ml	25.00 +
Gold Sodium Thiomalate Injection	1.0 mg	5.00
Gonadorelin HCl	1.4 mcg	3.60

-H-

Haloperidol, Decanoate and Lactate	0.07 mg	71.4
Hemin for Injection	4.0 mg	1.25
Heparin Sodium and Calcium	2000	USP units(R) 0.003
Heparin Sodium Infection	2000	USP units(R) 0.003

Heparin Lock Flush Solution	10.0 ml(R)	0.50
Heparin and Sodium Chloride	10.0 ml	0.50
Hetacillin Potassium	18 mg(R)	0.30
Hetastarch	20 ml	0.25
Hexafluorenum Bromide Injection	0.6 mg	8.35
Histamine Phosphate	0.04 mg	125.00
Hyaluronate Sodium	0.071 mg	70.42
Hyaluronidase Injection and for Infection	75 USP units(R)	0.07
Hydralazine HCl Injection	3.5 mg	1.45
Hydrocortisone Suspension	4.0 mg	1.25
Hydrocortisone Acetate	1.07 mg	4.67
Hydrocortisone Sodium Phosphate	4.0 mg	1.25
Hydrocortisone Sodium succinate	4.0 mg	1.25
Hydromorphone HCL	0.057 mg	88.00
Hydroxocobalamin	14.30 mcg	0.35
Hydroxyprogesterone Caproate	14.3 mg	0.35
Hydroxystilbamidine Isethionate	4.5 mg	1.10
Hydroxyzine HCl Injection	1.4 mg	3.60
Hyocyanine Sulfate	0.007 mg	714.29
Hyocyanine Sulfate and Scopolamine	0.007 mg	714.29

-I-

Imipenem and Cilastatin	7.14 mg	0.7
Imipramine HCl Injection	1.0 mg	5.00
Immune Serum Globulin	5.5 ml(R)	0.91
Indigotindisulfonate Sodium Injection	1 ml(R)	5.00
*Indium Pentetate InIII Injection	0.5 ml	28.00
Indium Chlorides In113m Injection	2.0 ml	87.50
Indocyanine Green	0.7 mg	7.10
Indomethacin Sodium	0.25 mg	20.00
Insulin	2 units	2.50
Insulin Human	- - -	0.80
Inulin	50 mg	0.10
Invert Sugar	10 ml	0.50
Iodamide meglumine - 24%	4.3 ml	1.20
Iodide Sodium 1123 Solution	7.0 ml	25.00
Iodinated 1125 Albumin Injection	7.0 ml	25.00
Iodide Sodium 1125 Solution	7.0 ml	25.00
Iodinated 1131 Albumin Injection	7.0 ml	25.00
Iodinated 1131 Albumin Aggregated Injection	7.0 ml	25.00
Iodohippurate Sodium 1131 Injection	7.0 ml	25.00
Rose Bengal Sodium 1131 Injection	7.0 ml	25.00
Iodide Sodium 1131 Solution	7.0 ml	25.00
Iodipamide Meglumine Injection – 52%	0.6 ml	8.33
10.5%	1.4 ml	3.60
Iodipamide Meglumine – Diatrizoate meglumine	0.14 ml	35.71

Iohexol	5.0 mg	0.1
Iopamidol	8.34 mg	0.6
*Iophendylate Injection	0.22 ml	0.90
Iothalamate Meglumine Injection	80%- 1.4 ml	3.57
	60%- 2 ml	2.50
	43%- 5.7 ml	0.90
	30%- 4.3 ml	1.16
	17.2% - 5.7 ml	0.90
Iothalamate Meglumine -		
Iothalamate Sodium	52% - 26% 1.5 ml	3.35
Iothalamate Sodium	66.8% - 1.5 ml	3.35
	54.3% - 0.9 ml	5.56
Ioxaglate Meglumine	3.6 ml	1.40
Ioxaglate Sodium	3.6 ml	1.40
Iron Dextran Injection	25 mg(R)	0.20
Iron Sorbitex	0.5 ml(R)	10.00
Isobucaine HU and Epinephrine	0.14 ml	35.70
Isoniazid	30 mg	0.20
Isoproterenol HU Injection	0.029 mg	172.00
Isosulfan Sulfate	0.71 mg	7.00
Isoxsupine HCl Injection	5 mg(R)	1.00

-K-

Kanamycin Sulfate Injection	10 mg(R)	0.50
Ketamine HU	13.0 mg	0.40

-L-

Labetalol HU	4.3 mg	1.20
Leucovorin Calcium Injection	0.071 mg	70.40
Leuprolide Acetate	0.014 mg	357.00
Levallorphan Tartrate Injection	0.04 mg	125.00
Levarterenol(norepinephrine bitartrate)	0.06 mg	83.33
Levorphanol Tartrate Injection	0.20 mg(R)	25.00
Levothyroxine Sodium for Injection	0.007 mg	714.00
Lidocaine HCl Injection (and with D5W)	4.5 mg	1.10
Lidocaine HCl with Epinephrine	1.43 mg	3.50
Lincomycin HU	10 mg	0.50
Liver Derivative Complex	0.03 mg	166.67
Lorazepam	0.05 mg	100.00
Loxapine	0.71 mg	7.00

-M-

Magnesium Sulfate	57.1 mg	0.09
Manganese Chloride Injection	40.0 ug(R)	0.125
Manganese Sulfate	40.0 ug(R)	0.125
Mannitol	10.0 ml(R)	0.50

Mannitol and Sodium Chloride	10.0 ml(R)	0.50
Mechlorethamine HU for Injection	0.4 mg	12.50
Medroxyprogesterone Acetate	14.3 mg	0.35
Menadiol Sodium Diphosphate (K-4)	0.2 mg	25.00
Menadione	0.09 mg	58.30
N. Meningococcal Polysaccharide Pur. Bulk, Group A	0.25 ug(R)	20.00
N.Meningococcal Polysaccharide Pur. Buld, Group C	0.25 ug(R)	20.00
Meningococcal Polysaccharide Vaccine Group A	0.025 ug(R)	200.00
Meningococcal Polysaccharide Vaccine Group C	0.025 ug(R)	200.00
Meningococcal Polysaccharide Vaccine Group A and C	0.05 ug(R)	100.00
Menotropin	2 units (R)	2.50
Meperidine HC1 Injection	2.14 mg	2.35
Mephentermine Sulfate	0.64 mg	7.80
Mepivacaine HU	6.6 mg	0.80
Mepivacaine HC1 and Levonordefrin	6.6 mg	0.80
Meprobamate Injection	1.0 mg	5.0
Meprylcaine HU and Epinephrine	6.6 mg	0.80
Mercaptomerin Sodium	3.57 mg	1.40
Mersalyl with theophylline	2.9 mg	1.72
ldrethoxylline Procaine	2.9 mg	1.72
Mesoridazine Besylate Injection	0.71 mg	7.00
Metaraminol	1.43 mg	3.50
Methadone	0.57 mg	8.80
Methandroil	1.43 mg	3.50
Methapyrilene HC1	0.6 mg	8.33
Methicillin Sodium	60 mg(R)	0.08
Methiodal Sodium Injection	1300 mg(R)	0.004
Methocarbamol Injection	28.6 mg	0.20
Methohexital Sodium	2.0 mg	2.50
Methotrexate Sodium Injection	2.5 mg	2.00
*Methotrexate Sodium Injection	0.5 mg	. 0.40
Methotrimerprazine	0.28 mg	17.90
Methoxamine HC1	0.25 mg	20.00
Methyldopa HC1	10 mg	0.50
Methylene Blue Injection	2 ml	2.50
Methylergonovine Maleate	2.9 mcg	1.70
Methylprednisolone Acetate Suspension	1.7 mg	2.94
Methylprednisolone Sodium Succinate for Injection	30.0 mg	0.17
Metoclopramide	2.0 mg	2.50
Metocurine Iodide	0.4 mg	12.50
Metoprolol Tartrate	0.2 mg	25.00
*Metrizamide	4.29 mg	I 1.17
Metrizamide	634.0 mg	I 0.008

Metrizoic	73% - 2.9 ml	1.72
	46.18% -1.0 ml	5.0
Metronidazole H;1	15.0 mg(R)	0.35
Metyrapone Tartrate Injection	100 mg(R)	0.06
Mezlocillin Sodium	100 mg(R)	0.05
Miconazole Infection	40.0 mg	0.10
*Miconazole Infection	0.29 mg	0.69
Midazolam HU	0.4 mg	12.5
Minocycline HC1	5 mg(R)	1.0
Mithramycin for Injection (Plicamycin)	0.05 mg(R)	100.00
Mitomycin for Injection	0.5 mg(R)	10.00
Molybdenum	2.3 ug	2.17
Morphine Sulfate	0.29 mg	17.00
*Morphine Sulfate	0.06 mg	3.33
Morrhuate Sodium	3.6 mg	1.40
Moxalactam	100.0 mg	0.05
Muromonab-CD3	0.07 mg	71.50

-N-

Nafcillin	80.0 mg(R)	0.06
Nalbuphine HU	0.14 mg	35.7
Nalorphine HU	0.43 mg	11.60
Naloxone HU Injection	0.01 mg	500.00
Nandrolone Decanoate	2.9 mg	1.70
Nandrolone Phenpropionate	1.4 mg	3.60
Neomycin Sulfate	3.8 mg	1.30
Neostigmine Methylsulfate	0.04 mg	125.00
Netilmicin	10 mg(R)	0.50
Niacin	1.43 mg	3.5
Niacinamide Injection	1.43 mg	3.50
Nicotinamide	0.7 mg	7.14
Niketamide	0.9 ml:25% sol	5.56
Nine Vitamin Injection	1.0 ml	5.0
Nitrofurantoin	2.5 mg	2.00
Nitroglycerin	50.0 ug(R)	0.1
Nitroprusside Sodium	1.0 mg(R)	5.00
Norepinephrine bitartrate	0.06 mg	83.35
Novobiocin for Injection	10 mg(R)	0.50

-O-

Opium Alkaloids HU	0.28 mg	17.86
Orphenadrine Citrate Injection	0.86 mg	5.80
Ouabain	0.007 mg	714.2
Oxacillin Sodium	25.0 mg	0.20
Oxymorphone HC1	0.021 mg	238.10
Oxytetracycline	12.5 mg	0.40
Oxytocin	0.14 units	35.70

-P-

Pancuronium Bromide	0.10 mg	50.00
Papaverine HCl	1.7 mg	2.90
Paraldehyde	0.3 ml	17.00
Parathyroid Hormone	0.57 units	8.80
Penicillin G Benzathine Suspension	50,000 units	0.01/100 units
Penicillin G Potassium	50,000 units	0.01/100 units
Penicillin G Procaine and Suspension	68,500 units	0.007/100 units
Penicillin G Sodium	50,000 units	0.01/100 units
Pentagastrin	0.006 mg	833.00
Pentamidine Isethionate	4.0 mg	1.25
Pentobarbital Sodium Injection	7.14 mg	0.70
Pentazocaine	0.29 mg	17.24
Pentazocine Lactate Injection	0.86 mg	5.80
Perphenazine	0.14 mg	35.70
Phenobarbital Sodium Injection	20.0 mg	0.25
Phenolsulfophthalein	0.09 mg	55.60
Phentolamine	0.10 mg	50.00
Phentylenetetrazol	7.14 mg	0.70
Phenylephrine HCl	0.20 mg	25.00
Penytoin Sodium Injection	15.0 mg	0.35
Physostigmine Salicylate	0.06 mg	83.40
Phytonadione	.36 mg	14.00
Piperacillin Sodium	75.0 mg	0.07
Piperocaine HCl	4.3 mg	1.16
Plasma Protein Fraction (5X)	10.0 ml(R)	0.5
Plicamycin for Injection	0.05 mg(R)	100.00
Polyestradiol Phosphate	1.10 mg	4.55
Polymyrtin B Sulfate	20,000 units(R)	.03/100 units
*Polymyxin B Sulfate	714 units(R)	.03/100 units
Posterior Pituitary Injection	0.29 units	17.00
Potassium Acetate Injection	0.57 mEq	8.80
Potassium Chloride	0.57 mEq	8.80
Potassium Phosphate Injection	4.43 mg	1.10
Potassium Phosphate in Dextrose	10.0 ml	0.5
Potassium Phosphate in Lactated Ringers	10.0 ml	0.5
Pralidoxine Chloride	40 mg	0.10
Prednisolone Acetate Suspension	0.86 mg	5.81
Prednisolone Acetate and Predaisolone Sodium Phosphate Suspension	1.14 mg	4.39
Prednisolone Sodium Phosphate Injection	0.86 mg	5.80
Predaisolone Sodium Succinate Injection	0.86 mg	5.80
Predaisolone Tebutate Suspension	0.57 mg	8.77
Prilocaine HCl	08.6 mg	0.60

Prilocaine HCl and Epinephrine	2.8 mg	1.80
Procaine HCl	8.6 mg	0.60
Procainamide HCl Injection	14.3 mg	0.35
Prochlorperazine Edisylate Injection	0.28 mg	17.90
Progesterone Aqueous	1.43 mg	3.50
Promazine HU Injection	2.8 mg	1.80
Promethazine HU	1 mg	5.00
Propantheline Bromide	0.43 mg	11.60
Propiomazine HCl Injection	1.10 mg	4.60
Propoxycaïne,Procaine HCl & Levonordefrin	6.6 mg	0.80
Propoxycaïne,Procaine HCl & Norepinephrine Bitartrate	6.6 mg	0.80
Propranolol HU Injection	0.09 mg	55.60
Protamine Sulfate Injection	5 mg(R)	1.00
Protein Hydrolysate Injection	10.0 ml	0.50
Prothrombin Complex	50.0 units(R)	0.10
Protirelia	7 mcg	0.70
Pyridostigmine Bromide	0.29 mg	17.00
Pyridoxine HCl	25mg	0.20

-Q-

Quiaidine Sulfate	8.6 mg	0.60
Quinidine Gluconate	11.4 mg	0.45

-R-

Ranitidine HU	0.71 mg	7.00
Reserpine	0.07 mg	71.50
riboflavin	0.7 mg	7.10
ringer's Injection	10.0 ml	0.50
Ringer's in Dextrose	10.0 ml	0.50
ringer's - Lactated Injection	10.0 ml	0.50
Ringer's - Lactated in Dextrose	10.0 ml	0.50
Ritodrine HU	10.0 mg(R)	0.50
Rolitetracycline for Injection	5 mg(R)	1.00
Rolitetracycline Nitrate	5 mg(R)	1.00

-S-

Saralasin Acetate	0.26 mg	19.2
Secretin	1.0 unit	5.0
Scopolamine HBr	0.009 mg	555.60
Secobarbital Sodium Injection	5.5 mg	0.90
Selenious Acid (Selenium)	1.43 ug	3.5
Selenomethionine Se75 Injection	7.0 ml	25.00 +
Siacalide	0.02 mcg	250.00
Sisomicin Sulfate	10.0 mg	0.50
Sodium Acetate	1.29 mEq	3.90

Sodium Ascorbate	3.57 mg	1.40
Sodium Bicarbonate	4.3 mEq	1.20
Sodium Chloride 0.45-0.9%	10.0 ml(R)	0.50
Sodium Chloride 3- 24.3%	1.4 ml	3.57
Sodium Chloride - Bacteriostatic	5.0 ml(R)	1.0
Sodium Chloride 4.5%- Lactose 3%	10.0 ml(R)	0.50
Sodium Citrate	2.5 mEq	2.0
Sodium Iodide	14.3 mg	0.35
Sodium Lactate	2.4 mEq	2.00
Sodium Phosphate Injection	40 mg P ₀₄	0.13
Sodium Phosphate P32 Solution	7.0 ml	25.00
Sodium Salicylate	9.3 mg	0.54
Sodium Tetradecyl Sulfate	0.14 ml	35.71
Sodium Thiosalicylate	2.1 mg	2.38
Sodium Thioaulfate	167 mg	0.03
Somatrem for <i>Injection</i>	0.25 IU	20.00
Somatropin	0.20 IU	25.00
Soybean 011 Emulsion	3.13 ml	1.60
Spectinomycin HU	50 mg(R)	0.10
Streptokinase	3571.43 IU	0.001
Streptokinase-Streptodornase (Local)	3000.0 units(R)	0.002
Streptokinase-Streptodornase (IM)	1000.0 units(R)	0.005
Streptomycin Sulfate	20 mg	0.25
Streptozocin	40.5 mg	0.12
Succinylcholine Chloride	2.5 mg	2.00
Sufentanil citrate	0.04 mg	125.00
Invert Sugar	10.0 ml	0.5
Invert Sugar in Sodium Chloride	10.0 ml	0.5
Sulbactam Sodium	14.3 mg	0.35
Sulfadiazine Sodium	50 mg	0.10
Sulfamethoxazole & Trimethoprim	25 mg(sulf)	0.20
Sulfisozazole Diolamine Injection	80 mg(R)	0.06
Sulfobromophthalein	5 mg	1.00

-T-

Technetium Tc99m Albumin Aggregated Injection	7 ml	25.00
Technetium Tc99m Dsofenin	7.0 ml	25.00
Technetium Tc99m Etidronate Injection	7.0 ml	25.00
Technetium Tc99m Ferpentetate Injection	7.0 ml	25.00
Technetium Tc99m Gluceptate Injection	7.0 ml	25.00
Technetium Tc99m Human Serum Albumin	7.0 ml	25.00
Technetium Tc99m Medronate Injection	7.0 ml	25.00
Technetium Tc99m Ozidronate Injection	7.0 ml	25.00
Technetium Tc99m Pentetate Injection	7.0 ml	25.00
Technetium Tc99m Sodium Pertechnetate	7.0 ml	25.00
Technetium Tc99m Pyrophosphate	7.0 ml	25.00

Techneium Tc99m (Pyro- and trimeta-)		
Phosphates Injection	7.0 ml	25.00
Techneium Tc99m Succimer	7.0 ml	25.00
Techneium Tc99m Sulfur Colloid Injection	7.0 ml	25.00
Terbutaline Sulfate	0.017 mg	50.00
Testolactone Suspension	1.43 mg	3.50
Testosterone (aqueous suspension)	1.43 mg	3.50
*Tetracaine Hydrochloride	0.29 mg	0.70
*Tetracaine HCl and Dextrose	0.2 mg	1.00
Tetracycline HCl	50 mg(R)	1.00
Tetracycline Phosphate Complex	5.0 mg(R)	1.00
Thallous Chloride T1201 Injection	7.0 ml	25.00 +
Theophylline and Dextrose	5.0 mg	1.00
Thiamine HCl	1.43 mg	3.50
Thiamylal Sodium	14.3 mg	0.35
Thiethylperazine Maleate	0.14 mg	35.80
Thiopental Sodium	5.0 mg	1.00
Thiotepa for Infection	0.8 mg	6.20
Thiothizene HU Injection	0.057 mg	88.00
Thyrotropin for Infection	0.14 IU	36.0
Ticarcillin Disodium	100 mg(R)	0.05
Ticarcillin Disodium and Clavulanate	75 mg	0.07
Tobramycin Sulfate	10 mg(R)	0.50
Tolazoline HCl	2 mg	2.60
Tolbulamide Sodium	100 mg(R)	0.05
Triamcinolone Acetate Suspension	1.14 mg	4.39
Triamcinolone Acetonide	1.14 mg	4.39
Triamcinolone Diacetate Suspension	0.7 mg	7.14
Triamcinolone Hexacetate Suspension	0.29 mg	17.24
Tridihexethyl Chloride	3.00 mg(R)	1.70
Triethylenethiophosphoramidate	0.80 mg	6.25
Triethylperazine Maleate	0.43 mg	11.63
Trifluoperazine HCl Infection	0.029 mg	172.00
Triflupromazine HCl Inejction	0.86 mg	5.80
Trimethaphan Camsylate	5.0 mg(R)	1.00
Trimethobenzamide HCl	20.0 mg(R)	0.25
Tromethamine	500 mg(R)	0.02
Tubocurarine Chloride	0.5 mg	10.00
	-U-	
Urea	1500 mg	0.003
Urofollitropine	1.06 units	4.70
Urokinase	4,400 IU	0.002
	-V-	
Vancomycin HCl	15 mg	0.33
Vassopressin	0.29 units	17.00

Vecuronium Bromide	0.12 mg	44.00
Verapamil Hydrochloride	0.3 mg	16.70
Vidarabine for Infection	10 mg(R)	0.50
Vinblastin Sulfate for Infection	0.5 mg	10.00
Vincristine Sulfate for Infection	0.038 mg	132.00
Viomycin Sulfate	14.3 mg	0.35
Vitamin A	714.3 IU	0.007

-W-

Warfarin Sodium for Infection	0.21 mg	24.00
Water for Infection and Sterile WFI	10.0 ml(R)	0.25 +
Bacteriostatic WFI	5.0 ml	0.5 +
Sterile Water for Inhalation	10.0 ml(R)	0.5 +
Sterile Water for Irrigation	10.0 ml(R)	0.25 +

-XYZ-

Xenon Xe133 Infection	2.0 ml	87.50 +
*Ytterbium Yb169 Pentetate Injection	2.5 ml	5.60 +
Zinc Chloride Infection	0.2 mg Zn	25.0
Zinc Sulfate Infection	0.2 mg Zn	25.0

(*) - Intrathecal Injections

(+) - USP Limit NOTE: The limit formula for radiopharmaceuticals is 175/V except for intrathecally administered products 14/V for intrathecal products. V equals the maximum recommended dose (listed in the dose column), in mL, at the expiration date or time.

References:

Facts and Comparisons, Editors E. Kastrup and J. Boyd, Facts and Comparisons, Inc.

United States Pharmacopeia Dispensing Information, Volume 1, 1985, United States Pharmacopeia Convention, Inc.

21 - Code of Federal Regulations