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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, FOOD AND DRUG ADMINISTRATION

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

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

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INTRODUCTION

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

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

I. BACKGROUND

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

II. LEGAL EFFECT OF THE GUIDELINE

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

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

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

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

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

B. Biological products for human use.

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

C. Drugs not subject to premarket approval.

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

D. Medical Devices.

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

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

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

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

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

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

IV. HUMAN AND ANIMAL DRUGS AND BIOLOGICAL PRODUCTS

GENERAL REQUIREMENT

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

A. VALIDATION OF THE LAL TEST

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

1. INITIAL QUALIFICATION OF THE LABORATORY

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

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

2. INHIBITION AND ENHANCEMENT TESTING

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

a) GEL-CLOT TECHNIQUE

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

water. Results of endotoxin determination in water and the drug product should fall within plus/minus a twofold dilution of the labeled sensitivity. If the undiluted drug product shows inhibition, the drug product can be diluted, not to exceed the MVD, with the same diluent that will be used in the release testing and the above procedure repeated. Negative controls (diluent plus lysate) should be included in all inhibition/enhancement testing.

b) CHROMOGENIC AND ENDPOINT-TURBIDIMETRIC TECHNIQUES

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

An alternate procedure may be performed as described above except the RSE/CSE standard curve is prepared in LAL negative drug product, i.e. no detectable endotoxin, instead of LAL negative water. The standard curve must meet the test for linearity, i.e. r equal to or greater than 0.980, and in addition the difference between the O.D. readings for the lowest and highest endotoxin concentrations must be greater than 0.4 and less than 1.5 O.D. units. If the standard curve does not meet these criteria, the drug product cannot be tested by the alternate procedure.

c) KINETIC-TURBIDIMETRIC TECHNIQUE

In inhibition/enhancement testing by this technique, a drug concentration containing 4 lambda concentration of the RSE or CSE (lambda is equal to the lowest endotoxin concentration used to generate the standard curve) is tested in duplicate according to the lysate manufacturer's methodology. The standard curve shall consist of at least four RSE or CSE concentrations. If the desired range is greater than one log, additional standard concentrations should be included. The standard curve must meet the criteria outlined in Appendix A(3). The calculated mean amount of endotoxin in the spiked drug product, when referenced to the standard curve, must be within plus or minus 25% to be considered to neither enhance nor inhibit the assay. If the undiluted drug product shows

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

An alternate procedure may be performed whereby the RSE/CSE standard curve is prepared in drug product or product dilution instead of water. The drug product cannot have a background endotoxin concentration of more than 10% (estimated by extrapolation of the regression line) of the lambda concentration (lambda equals the lowest concentration used to generate the standard curve). The standard curve must meet the test for linearity, i.e. r equal to or less than -0.980, and in addition the slope of the regression must be less than -0.1 and greater than -1.0. If the standard curve does not meet these criteria, the drug product cannot be tested by the alternate procedure.

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

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

B. Routine Testing of Drugs by the LAL Test.

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

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

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

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

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

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

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

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

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

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

Drugs exempted from the above endotoxin limits are:

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

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

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

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

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

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) <u>GEL-CLOT TECHNIQUE</u>

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) <u>CHROMOGENIC AND ENDPOINT-TURBIDIMETRIC TECHNIQUES</u>

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.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

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Interim Guidance for Human and Veterinary Drug Products and Biologicals

KINETIC LAL TECHNIQUES

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

INHIBITION/ENHANCEMENT TESTING

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

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

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

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

ROUTINE TESTING

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

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

APPENDIX A

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

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

VI. APPENDICES

APPENDIX A

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 LAL 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 of 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,▪1 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. ▪1 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 a 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.▪1 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 or 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 1 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▪1 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 ratio. ▪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.▪1 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. ▪▪1

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 determined 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 solutions, 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▪1 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-clot sensitivity is within 0.5λ to 2.0λ, where λ is the labeled sensitivity in Endotoxin Units per mL.▪▪1 The RSE (or CSE) concentrations 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 or Enhancement Test—Conduct assays with standard endotoxin, of untreated specimens in which there is no endogeneous 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 antilog $\Sigma e/f$, in which Σe is the sum of the log end-points of the dilution series used and

[1] LAL Reagent Water—Sterile Water for Injection or other water that shows no reaction with the specific LAL Reagent with which it is to be used, at the limit of sensitivity of such reagent.

[2] For a test for validity of procedure for inactivation of endotoxins, see "Dry-heat Sterilization" under *Sterilization and Sterility Assurance of Compendial Articles* ⟨1211⟩. Use a LAL Reagent having a sensitivity of not less than 0.15 Endotoxin Unit per mL.

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 endogeneous 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. ■■1 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°■1). 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■1 so that they correspond to a geometric series in which each step is greater than the next lower by a constant ratio.■■1 Do not store diluted 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 racks for incubation together, ■■1 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.■■1 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 or 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 or 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■1 is the limit dilution factor for the preparation for the test to be valid.

Procedure—To 10- × 75-mm test tubes add aliquots of the appropriately 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 block, accurately recording the time at which the tubes are so placed. Incubate each tube, undisturbed, for 60 ± 2 minutes at 37 ± 1°, 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 the 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 of 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:

RSE = 0.3 EU/mL
CSE = 0.018 ng/mL

The EUs per ng of CSE are calculated as follows:

$$\frac{\text{RSE}}{\text{CSE}} = \frac{0.3\ \text{EU/mL}}{0.018\ \text{ng/mL}} = 16.7\ \text{EU/ng}$$

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 RSE 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 (Log_{10} T versus Log_{10} 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.

CSE Endo. Conc. (ng/mL)	Log Mean T	EU/mL Equivalent (RSE Std. Line) Log	Antilog	EU/ng
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.

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

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)

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

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 execpt those administered intrathecally; 0.2 EU/Kg for intrathecal drugs

Step 2. Maximum Valid Dilution (MVD)

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

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

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

Endotoxin Limit Expressed by Volume:

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

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

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

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

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

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 (λ): 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

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

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

APPENDIX E

April ,1992

MAXIMUM DOSE AND ENDOTOXIN LIMIT TABLE

Drug Name	Dose (M)	Endotoxin Limit (EU/mg,ml,units of product)
-A-		
Acetic Acid Irrigation	10.00 mL	0.50 +
Acetazolamide Sodium	10.00 mg	0.50 +
Acetylcysteine Injection	150.00 mg	0.03
Acyclovir Sodium	30.00 mg	0.17
Adenosine Phosphate	0.71 mg	7.04
Albumin,Normal Human Serum (25%)	3.00 mL	1.67
Albumin,Normal Human Serum (20%)	3.75 mL	1.33
Albumin,Normal Human Serum (5%)	10.00 mL	0.50
Albuterol Sulfate	0.008 mg	625.00
Alcohol and Dextrose Injection	1.79 mL	2.70
Alfentanil Hydrochloride	250.00 mcg	0.02
Alkaloids of Belladonna	0.007 mg	714.29
$Alpha_1$-Proteinase Inhibitor	60.00 mg	0.08
Alphaprodine HCl Injection	0.60 mg	8.33
Alprostadil (Postaglandin)	6.00 mcg	0.83
Alteplase	1.25 mg	4.00
Amdinocillin	10.00 mg	0.50 +
Amikacin Sulfate Injection	15.00 mg	0.33 +
Amino Acid Injection	25.00 mg	0.20
Amino Acids and Electrolytes	25.00 mg	0.20
Essential Amino Acids and Dextrose	25.00 mg	0.20
Aminocaproic Acid Injection	100.00 mg	0.05
Aminohippurate Sodium Injection	125.00 mg	0.04 +
Aminophylline Injection	5.00 mg	1.00
Amitriptyline HCl Injection	0.42 mg	12.00
Ammonia N 13 Injection	7.00 mL	25.00 +
Ammonium Chloride Injection	2.90 mEq Cl	1.72
Amobarbital Sodium	14.30 mg	0.40 +
Amoxicillin, Sterile and Suspension	20.00 mg	0.25 +

Amphotericin B for Injection	1.00 mg	5.00
*Amphotericin B for Injection	0.01 mg	20.00
Ampicillin Sodium	33.30 mg	0.15 +
Ampicillin and Sulbactam	28.60 mg	0.17
Amrinone Lactate	10.00 mg	0.50
Anileridine	0.70 mg	7.20 +
Anticoagulant Heparin Solution	2.00 mL	2.50
Anticoagulant, Citrate Dextrose Sol.	-.-- mL	5.56
Anticoagulant, Citrate Phosphate Dextrose	-.-- mL	5.56
Anticoagulant, Citrate Phosphate Dextrose Adenine Solution	-.-- mL	5.56
Antihemophilic Factor	10.00 units	0.50
Antihemophilic Plasma(1 hr. at 56-57oC)	3.00 mL	1.67
Antirabies Serum	3.00 mL	1.67
Antitoxin (Gas Gangrene)	3.00 mL	1.67
Antivenom	3.00 mL	1.67
Apommorphine HCl Tablets for Injection	0.09 mg	55.56
Arginine HCl Injection	500.00 mg	0.01
Ascorbic Acid	4.20 mg	1.20 +
Asparaginase for Injection	1000.00 IU	0.01 #
Atracurium Besylate	0.50 mg	10.00
Atropine Sulfate	0.09 mg	55.60 +
Aurothioglucose Suspension	0.70 mg	7.14
Azathioprine Sodium for Injection	5.00 mg	1.00 +
Azlocillin	75.00 mg	0.07 +
Aztreonam for Injection	28.60 mg	0.17 +

-B-

Bacitracin	500.00 units	0.01 +
Bacitracin Zinc	500.00 units	0.01
Benzquinamide HCl	1.00 mg	5.00
Benztropine Mesylate Injection	0.09 mg	55.60 +
Benzylpenicilloyl Polylysine	0.004 mL	1250.00
Betamethasone Acetate and Betamethasone Sodium Phosphate	0.17 mg	29.20 +
Betamethasone Sodium Phosphate	0.17 mg	29.20 +
Betazole HCl Injection	2.86 mg	1.75
Bethanechol Chloride	0.20 mg	25.00
Biperiden Lactate Injection	0.06 mg	83.30 +

Bleomycin Sulfate	0.50 unit	10.00 #+
Bretylium Tosylate Injection	25.00 mg	0.20
Bretylium Tosylate in Dextrose	25.00 mg	0.20
Brompheniramine Maleate Injection	0.14 mg	35.71
Bumetanide	0.01 mg	500.00 +
Bupivacaine Hydrochloride Injection	2.50 mg	2.50 +
Bupivacaine Hydrochloride and Epinephrine Injection	3.20 mg	1.60 +
Bupivacaine HCl and Dextrose	0.11 mg	1.80 +
Buprenorphine HCl	0.004 mg	1250.00
Butorphanol Tartrate	0.057 mg	88.00 +

-C-

Caffeine Citrated	20.00 mg	0.25
Caffeine and Sodium Benzoate	7.14 mg	0.70 +
Calcitonin - Human	0.007 mg	714.30
Calcitonin - Salmon	4.00 IU	1.25
Calcitriol	0.05 mcg	100.00
Calcium Ascorbate	14.30 mg	0.35
Calcium Chloride	25.00 mg	0.20 +
Calcium Disodium Edetate	35.00 mg	0.143
Calcium Gluceptate Injection	15.70 mg	0.32 +
Calcium Gluconate	28.60 mg	0.17 +
Calcium Glycerophosphate and Calcium lactate	1.43 mg	3.50
Calcium Levulinate	0.14 mL	35.70 +
Capreomycin Sulfate	14.30 mg	0.35 +
Carbazochrome Salicylate	0.14 mg	34.96
Carbenicillin Disodium	100.00 mg	0.05 +
Carboplatin	9.26 mg	0.54 #
Carboprost Tromethamine	0.007 mcg	714.30 +
Carmustine for Injection	5.14 mg	1.00 #
Cefamandole Nafate and Sodium	33.30 mg	0.15 +
Cefazolin Sodium	33.30 mg	0.15 +
Cefmetazole Sodium	-.-- mg	0.20 +
Cefonicid Sodium	14.30 mg	0.35 +
Cefoperazone Sodium	28.57 mg	0.20 +
Ceforanide	20.00 mg	0.25 +
Cefotaxime Sodium	28.50 mg	0.20 +
Cefotetan Disodium	28.60 mg	0.17

Cefoxitin Sodium	40.00 mg	0.13 +
Ceftazidime	50.00 mg	0.10 +
Ceftizoxime Sodium	50.00 mg	0.10 +
Ceftriaxone Sodium	28.60 mg	0.20 +
Cefuroxime Sodium	50.00 mg	0.10 +
Cephacetrile Sodium for Injection	80.00 mg	0.06
Cephaloridine	14.30 mg	0.35
Cephalothin Sodium Injection	60.00 mg	0.08 +
Cephapirin Sodium	28.60 mg	0.17 +
Cephradine for Injection	25.00 mg	0.20 +
Cerulitide diethylamine	0.30 mcg	16.67
Chloramphenicol Sodium Succinate	25.00 mg	0.20 +
Chlordiazepoxide HCl	1.40 mg	3.57 +
Chloroprocaine HCl	11.43 mg	0.45
Cholecystokinin	1.00 IDU	5.00
Chorionic Gonadotropin	142.90 units	0.03 +
Chlormerodrin Hg197 Injection	7.00 mL	25.00 +
Chlormerodrin Hg203 Injection	7.00 mL	25.00 +
Chlormerodrine	1.40 mg	3.57
Chloroquine HCl Injection	7.50 mg	0.70 +
Chloroprocaine HCl	20.00 mg	0.25
Chloroprocaine HCl - Epinephrine	20.00 mg	0.25
Chlorothiazide Sodium	15.00 mg	0.30 +
Chlorpheniramine Maleate	0.57 mg	8.80 +
Chlorpromazine HCl	0.72 mg	6.90 +
Chlorprothixene Injection	0.72 mg	6.90 +
Chlortetracycline HCl	5.00 mg	1.00 +
Chromate Sodium Cr51 Injection	7.00 mL	25.00 +
Chromic Chloride Injection	0.30 ug	16.70
Chromic Phosphate P32 Suspension	7.00 mL	25.00 +
Chymopapain	42.90 pKat	0.12
Chymotrypsin	4.30 units	1.16
Sterile Cilastatin Sodium	-.-- mg	0.23 +
Cimetidine HCl Injection	10.00 mg	0.50
Cisplatin for Injection	2.57 mg	1.90 #
Citric Acid, Magnesium Oxide, & Sodium Carbonate Irrigation	-.-- mL	0.50 +
Citrate,Phosphate,Dextrose,Adenine	0.90 mL	5.56
Clindamycin Phosphate Injection	8.60 mg	0.58 +
Cloxacillin	12.50 mg	0.40 +
Codeine Phosphate Injection	0.86 mg	5.80 +

Colchicine Injection	0.03 mg	166.70 +
Colistimethate Sodium	2.50 mg	2.00 +
Conjugated Estrogens	0.36 mg	13.89
Corticotropin, Gel, Zinc & Re.	1.60 units	3.10 +
Cortisone Acetate	5.00 mg	1.00
Cosyntropin	3.57 mcg	1.40
Cryptenamine Acetate	1.86 CSR units	2.69
Cupric Chloride Injection	0.02 mg	250.00 +
Cupric Sulfate Injection	0.02 mg	250.00 +
Cyanocobalamine and Repository	14.30 mcg	0.40 +
Cyclizine Lactate	1.00 mg	5.00
Cyclophosphamide	30.00 mg	0.20 #+
Cyclosporine Injection and Conc.	0.12 mL	42.00 +
Cysteine HCl	7.14 mg	0.70 +
Cytarabine	3.00 mg	0.07 +
*Cytarabine	1.93 mg	0.10 #

-D-

Dacarbazine for Injection	9.60 mg	0.52 #
Dactinomycin for Injection	0.05 mg	100.00 #+
Dantrolene Sodium	10.00 mg	0.50
Daunorubicin HCl	1.16 mg	4.30 #
Decamethonium Bromide	0.043 mg	116.30
Deferoxamine Mesylate	15.00 mg	0.33
Dehydrocholate Sodium Injection	150.00 mg	0.04
Deslanoside	0.03 mg	167.00
Desmopressin Acetate	0.30 mcg	16.70
Desoxycorticosterone Acetate	0.07 mg	71.40 +
Desoxycorticosterone Pivalate Sus.	1.80 mg	2.78 +
Dexamethasone Acetate Suspension	0.23 mg	21.74 +
Dexamethasone Sodium Phosphate	0.16 mg	31.30 +
Dexpanthenol	7.10 mg	0.70
Dextran 40	5.00 mL	1.00
Dextran 40 in Sodium Chloride	5.00 mL	1.00
Dextran 70	10.00 mL	0.50
Dextrose <5%	10.00 mL	0.50
Dextrose- 5%-70%	0.50 gm	10.00
Dextrose and Sodium Chloride	0.50 gm	10.00
Dezocine	0.29 mg	17.24

Diatrizoate Meglumine Injection 60%	1.00 mL	5.00
30%	4.40 mL	1.10
Diatrizoate Meglumine and Diatrizoate Sodium 66% - 10%	2.30 mL	2.17
60% - 30%	1.40 mL	3.57
52% - 8%	2.80 mL	1.80
50% - 25%	2.80 mL	1.80
34.3% - 35%	2.80 mL	1.80
28.5% - 29.1%	2.80 mL	1.80
Diatrizoate Meglumine and Iodipamide Meglumine	0.11 mL	45.45
Diatrizoate Sodium 50%	1.00 mL	5.00
25%	4.00 mL	1.25
20%	0.90 mL	5.56
Diazepam Injection	0.43 mg	11.60 +
Diazoxide Injection	10.00 mg	0.50 +
*Dibucaine	0.14 mg	35.70 +
Dibucaine HCl and Dextrose	0.07 mg	71.43
Dicloxacillin Sodium	0.29 mg	16.70 +
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	200.00 +
Digoxin Immune Fab	5.00 mg	1.00
Dihydroergotamine Mesylate	0.014 mg	357.00
Dihydroergotamine Mesylate, Heparin Sodium & Lidocaine HCl	1667.00 units (Heparin)	0.003 +
Dihydrostreptomycin Sulfate	10.00 mg	0.50 +
Dihydrotachysterol	0.03 mg	166.67
Diluent for Meningococcal Vaccine	5.00 mL	1.00
Dimenhydrinate Injection	1.25 mg	4.00
Dimethyl Sulfoxide Irrigation	10.00 mL	0.50 +
Dimercaprol	5.00 mg	1.00
Dinoprost Tromethamine	0.57 mg	8.77
Diphenhydramine HCl Injection	1.50 mg	3.40 +
Diphenidol	0.30 mg	16.67
Diphtheria Antitoxin,Pur.Conc.(equine)	3.00 mL	1.67
Dipyridamole	0.14 mg	37.70
Dobutamine HCl	0.90 mg	5.56

Dopamine HCl	0.30 mg	16.67 +
Dopamine HCl in Dextrose	0.30 mg	16.67 +
Doxapram HCl Injection	1.50 mg	3.30 +
Doxorubicin HCl for Injection	1.93 mg	2.20 #+
Doxycycline Hyclate for Injection	4.40 mg	1.14 +
Dromostanolone Propionate	1.40 mg	3.57
Droperidol	0.14 mg	35.70
Dyphylline Injection	7.10 mg	0.70 +

-E-

Edetate Calcium Disodium	50.00 mg	0.10 +
Edetate Disodium	25.00 mg	0.20
Edrophonium Chloride Injection	0.60 mg	8.33 +
Electrolyte Solutions- LVP	10.00 mL	0.50
Multiple Electrolytes Type 1 & 2	--.-- mL	0.50
Multiple Electrolytes and Invert Sugar Type 1,2, and 3	--.-- mL	0.50
Multiple Electrolytes and Dextrose Type 1,2,3,and 4	--.-- mL	0.50
Emetine HCl	0.93 mg	5.40 +
Enalaprilat	0.018 mg	280.00
Ephedrine Sulfate Injection	3.00 mg	1.70 +
Epinephrine Injection	0.014 mg	357.00 +
Epinephrine Suspension	0.025 mg	200.00
Ergocalciferol (D2)	142.80 units	0.035
Ergoloid Mesylates	0.004 mg	1250.00
Ergonovine Maleate	6.00 mcg	0.80
Ergotamine Tartrate	0.014 mg	357.00 +
Erythromycin Gluceptate/Lactobionate	5.00 mg	1.00 +
Esmolol	0.50 mg	10.00
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	1.00 mg	5.00 +
Ethamivan Injection	1.40 mg	3.60
Ethylnorepinephrine HCl Injection	0.029 mg	172.40 +

Etidocaine HCl	5.50 mg	0.90
Etidocaine HCl and Epinephrine	5.50 mg	0.08
Etidronate Disodium	7.50 mg	0.67
Etomidate Injection	0.60 mg	8.35
Etoposide Injection	2.57 mg	1.95 #
Evans Blue Injection	0.36 mg	14.00 +

-F-

Factor IX	50.00 units	0.10
Famotidine	0.30 mg	16.67
Fat Emulsion	(10%) 3.20 mL	1.56
	(20%) 1.60 mL	3.13
Fentanyl Citrate	0.10 mg	50.00
Fentanyl Citrate and Droperidol	0.004 mg Fentanyl	1250.00
Ferrous Citrate Fe59 Injection	7.00 mL	25.00 +
Fibrinogen	30.00 mg	0.17
Fibrinogen, Dried	30.00 mg	0.17
Fibrinolysin and Desoxyribonuclease	1.00 units	5.00
Flurodopa F 18 Injection	7.00 mL	25.00 +
Floxuridine	0.60 mg	8.33 #
Fluorodeoxyglucose F18	7.00 mL	25.00
Fluorescein Sodium Injection	10.70 mg	0.47
Fluorouracil Injection	15.00 mg	0.33 #
Fluphenazine HCl	0.03 mg	166.67
Flupenthixol Decanoate	0.57 mg	8.77
Folate Sodium	0.01 mg	500.00
Folic Acid Injection	0.014 mg	357.10 +
Fructose	10.00 mL	0.50 +
Fructose and Sodium Chloride	10.00 mL	0.50 +
Furosemide Injection	1.40 mg	3.60 +

-G-

Gallamine Triethiodide	1.00 mg	5.00 +
Gallium Citrate Ga67 Injection	7.00 mL	25.00 +
Gentamicin Sulfate	3.00 mg	1.70 +
*Gentamicin Sulfate	0.11 mg	45.46
Globulins (Humans)	1.00 mL	5.00
Glucagon for Injection	0.04 units	125.00

Glycine Irrigation	--.-- mL	0.50 +
Glycopyrrolate	0.009 mg	555.50 +
Gold Au198 Injection	7.00 mL	25.00 +
Gold Sodium Thiomalate Injection	1.00 mg	5.00
Gonadorelin HCl	1.40 mcg	3.60

-H-

Haloperidol, Decanoate and Lactate	0.07 mg	71.40 +
Hemin for Injection	4.00 mg	1.25
Heparin Sodium and Calcium	143.00 units	0.03
Heparin Sodium Injection	143.00 units	0.03
Heparin Lock Flush Solution	10.00 mL	0.50
Heparin and Sodium Chloride	10.00 mL	0.50
Hetacillin Potassium	--.-- mg	0.30 +
Hetastarch	20.00 mL	0.25
Hexafluorenium Bromide Injection	0.60 mg	8.35
Histamine Phosphate	0.04 mg	125.00 +
Hyaluronate Sodium	0.071 mg	70.42
Hyaluronidase Injection and for Injection	2.14 units	2.30 +
Hydralazine HCl Injection	3.50 mg	1.45 +
Hydrocortisone Suspension	4.00 mg	1.25 +
Hydrocortisone Acetate	1.07 mg	4.67
Hydrocortisone Sodium Phosphate	4.00 mg	1.25 +
Hydrocortisone Sodium succinate	4.00 mg	1.25 +
Hydromorphone HCL	0.057 mg	88.00 +
Hydroxocobalamin	14.30 mcg	0.40 +
Hydroxyprogesterone Caproate	14.30 mg	0.35
Hydroxystilbamidine Isethionate	4.50 mg	1.10 +
Hydroxyzine HCl Injection	1.40 mg	3.60 +
Hyocyamine Sulfate	0.007 mg	714.30 +
Hyocyamine Sulfate and Scopolamine	0.007 mg	714.29

-I-

Idarubicin HCl Injection	0.31 mg	16.13 #
Ifosfamide	30.86 mg	0.16 #+
Imipenem	-.-- mg	0.23 +
Imipenem and Cilastatin	7.14 mg	0.70
Imipramine HCl Injection	1.00 mg	5.00 +

Immune Serum Globulin	5.50 mL	0.91
Indigotindisulfonate Sodium Injection	1.00 mL	5.00 +
Indium In111 Oxyquinoline	7.00 mL	25.00 +
*Indium Pentetate In111 Injection	0.50 mL	28.00 +
Indium Chlorides In113m Injection	2.00 mL	87.50 +
Indocyanine Green	0.70 mg	7.10 +
Indomethacin Sodium	0.20 mg	25.00
Insulin	2.00 units	2.50
Insulin Human	- - -	0.80 +
Interferon Alfa-n1	77142.00 units	0.65/10,000 #
Interferon Alfa-n3	3571.00 units	0.14/100 #
Interferon Alfa - 2a	428571.00 units	0.10/10,000 #
Interferon Alfa - 2b	514285.00 units	0.10/10,000 #
Inulin	50.00 mg	0.10 +
Invert Sugar	2.38 mL	2.10
Iodamide meglumine - 24%	4.30 mL	1.20
Iodide Sodium I123 Solution	7.00 mL	25.00 +
Iodinated I125 Albumin Injection	7.00 mL	25.00 +
Iodide Sodium I125 Solution	7.00 mL	25.00 +
Iodinated I131 Albumin Injection	7.00 mL	25.00 +
Iodinated I131 Albumin Aggregated Injection	7.00 mL	25.00 +
Iodohippurate Sodium I131 Injection	7.00 mL	25.00 +
Rose Bengal Sodium I131 Injection	7.00 mL	25.00 +
Iodide Sodium I131 Solution	7.00 mL	25.00 +
Iodipamide Meglumine Injection - 52%	0.60 mL	8.33
- 10.5%	1.40 mL	3.60 +
Iodipamide Meglumine - Diatrizoate meglumine	0.14 mL	35.71
*Iohexol	43.70 mg I	0.11
*Iopamidol	40.00 mg I	0.6 +
*Iophendylate Injection	0.22 mL	0.90 +
Iothalamate Meglumine Injection	80%- 1.40 mL	3.57
	60%- 2.00 mL	2.50
	43%- 5.70 mL	0.90 +
	30%- 4.30 mL	1.16
	17.2% -5.70 mL	0.90
Iothalamate Meglumine - Iothalamate Sodium 52%-26%	1.50 mL	3.35 +
Iothalamate Sodium 66.8% -	1.50 mL	3.35 +
54.3% -	0.90 mL	5.56
Ioxaglate Meglumine	3.00 mL	1.67

Ioxaglate Sodium	3.00 mL	1.67
Iron Dextran Injection	0.90 mg	5.60
Iron Sorbitex	0.50 mL	10.00 +
Isobucaine HCl and Epinephrine	0.14 mL	35.70
Isoniazid	20.00 mg	0.30 +
Isoproterenol HCl Injection	0.014 mg	350.00
Isosulfan Sulfate	0.71 mg	7.00
Isoxsupine HCl Injection	0.14 mg	35.70 +

-K-

Kanamycin Sulfate Injection	7.50 mg	0.67 +
Ketamine HCl	13.00 mg	0.40 +

-L-

Labetalol HCl	4.30 mg	1.20 +
Leucovorin Calcium Injection	2.57 mg	1.95
Leuprolide Acetate	0.03 mg	16.67
Levallorphan Tartrate Injection	0.04 mg	125.00
Levarterenol	0.06 mg	83.33
Levorphanol Tartrate Injection	0.04 mg	125.00 +
Levothyroxine Sodium for Injection	0.007 mg	714.00
Lidocaine HCl Injection (with D5W)	4.50 mg	1.10 +
Lidocaine HCl with Epinephrine	7.00 mg	0.70 +
Lincomycin HCl	10.00 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	57.10 mg	0.09 +
Manganese Chloride Injection	11.00 ug	0.45 +
Manganese Sulfate	11.00 ug	0.45 +
Mannitol <= 10%	120.00 mg	0.04
Mannitol > 10%	2.00 g	2.50
Mannitol and Sodium Chloride	120.00 mg	0.04 +
Mechlorethamine HCl for Injection	0.40 mg	12.50 #+
Medroxyprogesterone Acetate	14.30 mg	0.35
Menadiol Sodium Diphosphate (K-4)	0.20 mg	25.00 +

Menadione	0.09 mg	58.30 +
N. Meningococcal Polysaccharide Pur. Bulk, Group A	0.25 ug	20.00
N.Meningococcal Polysaccharide Pur. Bulk, Group C	0.25 ug	20.00
Meningococcal Polysaccharide Vaccine Group A	0.025 ug	200.00
Meningococcal Polysaccharide Vaccine Group C	0.025 ug	200.00
Meningococcal Polysaccharide Vaccine Group A and C	0.05 ug	100.00
Menotropin	2.00 units	2.50 +
Meperidine HCl Injection	2.14 mg	2.40 +
Mephentermine Sulfate	0.64 mg	7.80 +
Mepivacaine HCl	6.60 mg	0.80 +
Mepivacaine HCl and Levonordefrin	6.60 mg	0.80 +
Meprobamate Injection	1.00 mg	5.00
Meprylcaine HCl and Epinephrine	6.60 mg	0.80 +
Mercaptomerin Sodium	3.57 mg	1.40
Mersalyl with theophylline	2.90 mg	1.72
Merethoxylline Procaine	2.90 mg	1.72
Mesoridazine Besylate Injection	0.71 mg	7.00 +
Metaraminol Bitartrate	1.43 mg	3.50 +
Methadone HCl	0.57 mg	8.80 +
Methandroil	1.43 mg	3.50
Methapyrilene HCl	0.60 mg	8.33
Methicillin Sodium	50.00 mg	0.10 +
Methiodal Sodium Injection	1300.00 mg	0.004
Methocarbamol Injection	28.60 mg	0.20 +
Methohexital Sodium	10.00 mg	0.50 +
Methotrexate Sodium Injection	-.-- mg	2.00 +
Methotrimerprazine	0.28 mg	17.90 +
Methoxamine HCl	0.25 mg	20.00 +
Methyldopate HCl	10.00 mg	0.50 +
Methylene Blue Injection	2.00 mL	2.50 +
Methylergonovine Maleate	2.90 mcg	1.70 +
Methylprednisolone Acetate Sus	0.80 mg	6.25
Methylprednisolone Sodium Succinate for Injection	30.00 mg	0.17 +
Metoclopramide	2.00 mg	2.50 +
Metocurine Iodide	0.40 mg	12.50 +

Metoprolol Tartrate		0.20 mg	25.00 +
*Metrizamide		4.29 mg I	1.17
Metrizamide		634.00 mg I	0.008
Metrizoic	73% -	2.90 mL	1.72
	46.18% -	1.00 mL	5.00
Metronidazole HCl		15.00 mg	0.35 +
Metyrapone Tartrate Injection		15.00 mg	0.35
Mezlocillin Sodium		87.50 mg	0.06 +
Miconazole Injection		40.00 mg	0.10 +
*Miconazole Injection		0.29 mg	0.69
Midazolam HCl		0.35 mg	14.30
Minocycline HCl		4.00 mg	1.25 +
Mithramycin for Injection		0.05 mg	100.00
Mitomycin for Injection		0.51 mg	9.80 # +
Mitoxantrone HCl		0.36 mg	13.90 #
Molybdenum		2.30 ug	2.17
Morphine Sulfate		0.29 mg	17.00
*Morphine Sulfate		0.014 mg	14.29
Morrhuate Sodium		3.60 mg	1.40 +
Moxalactam		100.00 mg	0.05 +
Muromonab-CD3		0.10 mg	50.00

-N-

Nafcillin	40.00 mg	0.13 +
Nalbuphine HCl	3.00 mg	1.67
Nalorphine HCl	0.43 mg	11.60
Naloxone HCl Injection	0.01 mg	500.00
Neomycin Sulfate	3.80 mg	1.30 +
Neostigmine Methylsulfate	0.04 mg	125.00
Netilmicin	4.00 mg	1.25 +
Niacin	1.43 mg	3.50 +
Niacinamide Injection	1.43 mg	3.50 +
Nicotinamide	0.70 mg	7.14
Nikethamide	0.90 ml:25% sol	5.56
Nine Vitamin Injection	1.00 mL	5.00
Nitrofurantoin	2.50 mg	2.00
Nitroglycerin	50.00 ug	0.10 +
Nitroprusside Sodium	1.40 mcg	3.67
Norepinephrine bitartrate	0.06 mg	83.40 +
Novobiocin for Injection	7.10 mg	0.70

-O-

Opium Alkaloids HCl	0.28 mg	17.86
Orphenadrine Citrate Injection	0.86 mg	5.80 +
Ouabain	0.007 mg	714.29
Oxacillin Sodium	25.00 mg	0.20 +
Oxymorphone HCl	0.021 mg	238.10 +
Oxytetracycline	12.50 mg	0.40 +
Oxytocin	0.14 units	35.70

-P-

Pancuronium Bromide	0.10 mg	50.00
Papaverine HCl	1.70 mg	2.90 +
Paraldehyde	0.15 mL	33.33
Parathyroid Hormone	0.57 units	8.80
Penicillin G Benzathine Suspension	50,000.00 units	0.01/100 +
Penicillin G Potassium	50,000.00 units	0.01/100 +
Penicillin G Procaine and Suspension	50,000.00 units	0.01/100 +
Penicillin G Procaine, Dihydrostreptomycin Sulfate, Chlorpheniramine Maleate and Dexamethasone Suspension	--.-- units	0.01/100 +
Penicillin G Procaine, Dihydrostreptomycin Sulfate, Prednisolone Suspension	--.-- units	0.01/100 +
Penicillin G Sodium	50,000.00 units	0.01/100 +
Pentagastrin	0.006 mg	833.00
Pentamidine Isethionate	4.00 mg	1.25
Pentobarbital Sodium Injection	6.00 mg	0.83 +
Pentazocine Lactate Injection	0.86 mg	5.80 +
Perphenazine	0.14 mg	35.70 +
Phenobarbital Sodium Injection	20.00 mg	0.30 +
Phenolsulfonphthalein	0.09 mg	55.60
Phentolamine Mesylate	0.86 mg	5.80 +
Pentylenetetrazol	7.14 mg	0.70
Phenylephrine HCl	0.20 mg	25.00 +
Phenytoin Sodium Injection	20.00 mg	0.30 +
Physostigmine Salicylate	0.06 mg	83.40 +
Phytonadione	0.36 mg	14.00 +
Piperacillin Sodium	75.00 mg	0.07 +
Piperocaine HCl	4.30 mg	1.16

Plasma Protein Fraction (5%)	10.00 mL	0.50
Plicamycin for Injection	0.05 mg	100.00 #
Polyestradiol Phosphate	1.10 mg	4.55
Polymyxin B Sulfate	1666.70 units	0.003
*Polymyxin B Sulfate	714.00 units	0.03/100 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 Chloride,Lactated Ringers and Dextrose Injection	-.-- mL	0.50 +
Potassium Phosphate Injection	4.43 mg	1.10 +
Potassium Phosphate in Dextrose	10.00 mL	0.50
Potassium Phos., Lactated Ringers	10.00 mL	0.50
Pralidoxine Chloride	40.00 mg	0.10 +
Prednisolone Acetate Suspension	0.16 mg	31.25
Prednisolone Acetate and Prednisolone Sodium Phosphate Suspension	1.14 mg Pred. Acet.	4.39
Prednisolone Sodium Phosphate	1.00 mg	5.00 +
Prednisolone Sodium Succinate	0.86 mg	5.80 +
Prednisolone Tebutate Suspension	0.57 mg	8.80 +
Prilocaine HCl	5.70 mg	0.90 +
Prilocaine HCl and Epinephrine	5.70 mg	0.90 +
Procaine HCl	8.60 mg	0.60 +
Procaine HCl & Epinephrine	-.-- mg	0.60 +
Procaine & Phenylephrine HCl	-.-- mg	0.60 +
Procaine, Tetracaine & Levonordefrin Injection	-.-- mg	0.60 +
Propofol	12.00 mg	0.42
Procainamide HCl Injection	14.30 mg	0.35 +
Prochlorperazine Edisylate & Mesylate	0.28 mg	17.90 +
Progesterone Aqueous & Suspension	1.43 mg	3.50
Promazine HCl Injection	2.80 mg	1.80 +
Promethazine HCl	1.00 mg	5.00 +
Propantheline Bromide	0.43 mg	11.60 +
Propiomazine HCl Injection	1.10 mg	4.60 +
Propoxycaine,Procaine HCl & Levonordefrin	6.60 mg	0.80 +
Propoxycaine,Procaine HCl & Norepinephrine Bitartrate	6.60 mg	0.80 +

Propranolol HCl Injection	0.09 mg	55.60 +
Protamine Sulfate Injection	0.71 mg	7.04 +
Protein Hydrolysate Injection	10.00 mL	0.50 +
Prothrombin Complex	50.00 units	0.10
Protirelin	7.00 mcg	0.70
Pyridostigmine Bromide	0.29 mg	17.00 +
Pyridoxine HCl	14.29 mg	0.40 +

-Q-

Quinidine Sulfate	8.60 mg	0.60 +
Quinidine Gluconate	8.60 mg	0.60

-R-

Ranitidine HCl	-.--	7.00
Reserpine	0.07 mg	71.50 +
Riboflavin	0.70 mg	7.10 +
Rifampin for Injection	-.-- mg	0.20 +
Ringer's Injection	10.00 mL	0.50 +
Ringer's Irrigation	10.00 mL	0.50 +
Ringer's in Dextrose	10.00 mL	0.50
Ringer's - Lactated Injection	10.00 mL	0.50 +
Ringer's - Lactated in Dextrose	10.00 mL	0.50
Ritodrine HCl	-.--	0.50 +
Rolitetracycline for Injection	5.00 mg	1.00 +
Rolitetracycline Nitrate	5.00 mg	1.00

-S-

Saralasin Acetate	0.26 mg	19.20
Secretin	1.00 unit	5.00
Scopolamine Butylbromide	0.29 mg	17.24
Scopolamine HBr	0.009 mg	555.60 +
Secobarbital Sodium Injection	5.50 mg	0.90 +
Selenious Acid (Selenium)	1.43 ug	3.50 +
Selenomethionine Se75 Injection	7.00 mL	25.00 +
Sincalide	0.02 mcg	250.00
Sisomicin Sulfate	10.00 mg	0.50 +
Sodium Acetate	1.29 mEq	3.90 +
Sodium Ascorbate	3.57 mg	1.40

Sodium Bicarbonate	1.00 mEq	5.00 +
Sodium Chloride 0.45-0.9%	10.00 mL	0.50 +
Sodium Chloride 3- 24.3%	1.40 mL	3.57 +
Sodium Chloride - Bacteriostatic	5.00 mL	1.00 +
Sodium Chloride 4.5%- Lactose 3%	10.00 mL	0.50
Sodium Chloride Irrigation	10.00 mL	0.50 +
Sodium Citrate	2.50 mEq	2.00
Sodium Iodide	14.30 mg	0.35
Sodium Lactate	2.40 mEq	2.00 +
Sodium Phosphate Injection	4.00 mg	1.10 +
Sodium Phosphate P32 Solution	7.00 mL	25.00 +
Sodium Salicylate	9.30 mg	0.54
Sodium Tetradecyl Sulfate	0.14 mL	35.71
Sodium Thiosalicylate	2.10 mg	2.38
Sodium Thiosulfate	167.00 mg	0.03 +
Somatrem for Injection	0.25 IU	20.00
Somatropin - Pituitary & Recombinant	0.20 IU	25.00
Soybean Oil Emulsion	3.13 mL	1.60
Spectinomycin HCl	57.00 mg	0.09 +
Streptokinase	21428.00 IU	0.02/100
Streptokinase-Streptodornase (Local)	3000.00 units	0.002
Streptokinase-Streptodornase (IM)	1000.00 units	0.005
Streptomycin Sulfate	20.00 mg	0.25 +
Streptozocin	38.60 mg	0.13 #
Succinylcholine Chloride	2.50 mg	2.00 +
Sufentanil citrate	0.05 mg	100.00
Invert Sugar	10.00 mL	0.50 +
Invert Sugar in Sodium Chloride	10.00 mL	0.50
Sulbactam Sodium	14.30 mg	0.35
Sulfadiazine Sodium	50.00 mg	0.10 +
Sulfamethoxazole & Trimethoprim	25.00 mg(sulf)	0.20
Sulfisoxazole Diolamine Injection	50.00 mg	0.10
Sulfobromophthalein	5.00 mg	1.00 +

-T-

Technetium Tc99m Albumin Aggregated	7.00 mL	25.00 +
Technetium Tc99m Antimony Trisulfate	7.00 mL	25.00
Technetium Tc99m Dsofenin	7.00 mL	25.00 +
Technetium Tc99m Etidronate	7.00 mL	25.00 +
Technetium Tc99m Ferpentetate	7.00 mL	25.00 +

Technetium Tc99m Gluceptate	7.00 mL	25.00 +
Technetium Tc99m Human Serum Albumin	7.00 mL	25.00 +
Technetium Tc99m Lidofenin	7.00 mL	25.00
Technetium Tc99m Mebrofenin	7.00 mL	25.00
Technetium Tc99m Medronate	7.00 mL	25.00 +
Technetium Tc99m Oxidronate	7.00 mL	25.00 +
Technetium Tc99m Pentetate	7.00 mL	25.00 +
Technetium Tc99m Sodium Pertechnetate	7.00 mL	25.00 +
Technetium Tc99m Pyrophosphate	7.00 mL	25.00 +
Technetium Tc99m (Pyro- and trimeta-) Phosphates	7.00 mL	25.00 +
Technetium Tc99m Succimer	7.00 mL	25.00 +
Technetium Tc99m Sulfur Colloid	7.00 mL	25.00 +
Terbutaline Sulfate	0.004 mg	1250.00 +
Teriparatide Acetate	5.00 units	1.00
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	0.20 mg	1.00 +
Tetracycline HCl	10.00 mg	0.50 +
Tetracycline Phosphate Complex	5.00 mg	1.00 +
Thallus Chloride Tl201 Injection	7.00 mL	25.00 +
Theophylline and Dextrose	5.00 mg	1.00
Thiamine HCl	1.43 mg	3.50
Thiamylal Sodium	5.00 mg	1.00 +
Thiethylperazine Maleate	0.14 mg	35.80 +
Thiopental Sodium	5.00 mg	1.00 +
Thiotepa for Injection	0.80 mg	6.20 #
Thiothixene HCl Injection	0.057 mg	88.00 +
Thyrotropin for Injection	0.14 IU	36.00
Ticarcillin Disodium	100.00 mg	0.05 +
Ticarcillin Disodium and Clavulanate	75.00 mg	0.07 +
Tobramycin Sulfate	2.50 mg	2.00 +
Tolazoline HCl	6.00 mg	0.80 +
Tolbutamide Sodium	14.20 mg	0.35 +
Tranexamic Acid	10.00 mg	0.50
Triamcinolone Acetate Suspension	1.14 mg	4.40 +
Triamcinolone Acetonide	1.14 mg	4.39
Triamcinolone Diacetate Suspension	0.70 mg	7.10 +
Triamcinolone Hexacetonide Suspension	0.29 mg	17.20 +
Tridihexethyl Chloride	3.00 mg	1.70 +

Triethylenethiophosphoramide	0.80 mg	6.25
Triethylperazine Maleate	0.43 mg	11.63
Trifluoperazine HCl Injection	0.029 mg	172.00 +
Triflupromazine HCl Injection	0.86 mg	5.80 +
Trimethaphan Camsylate	5.00 mg	1.00 +
Trimethobenzamide HCl	2.80 mg	1.80 +
Tromethamine	154.00 mg	0.03 +
Tubocurarine Chloride	0.50 mg	10.00 +

-U-

Urea	1500.00 mg	0.003 +
Urofollitropine	1.06 units	4.70
Urokinase	4,400.00 IU	0.002

-V-

Vancomycin HCl	15.00 mg	0.33 +
Vasopressin	0.14 units	35.70
Vecuronium Bromide	0.10 mg	50.00
Verapamil Hydrochloride	0.30 mg	16.70 +
Vidarabine for Injection	10.00 mg	0.50 +
Vinblastine Sulfate for Injection	0.50 mg	10.00 #+
Vincristine Sulfate for Injection	0.05 mg	100.00 #
Viomycin Sulfate	14.30 mg	0.35
Vitamin A	714.30 IU	0.007

-W-

Warfarin Sodium for Injection	0.21 mg	24.00 +
Water for Injection and Sterile WFI	-.--	0.25 +
Bacteriostatic WFI	-.--	0.50 +
Sterile Water for Inhalation	-.--	0.50 +
Sterile Water for Irrigation	-.--	0.25 +

-XYZ-

Xenon Xe133 Injection	2.00 mL	87.50 +
*Ytterbium Yb169 Pentetate Injection	2.50 mL	5.60 +
Zinc Chloride Injection	0.20 mg Zn	25.00 +
Zinc Sulfate Injection	0.20 mg Zn	25.00 +

(*) - 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.

(#) - Drug Administered on a per Square Meter of Body Surface

Limit calculated according to the following formula:

5 EU/Kg / ((dose * 1.80 sq. m.)/70 Kg)

References:

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

United States Pharmacopeia Dispensing Information 1990, United States Pharmacopeia Convention, Inc.