LAL UPDATE®

Volume 9, No. 3 **September 1991**

Dear LAL User,

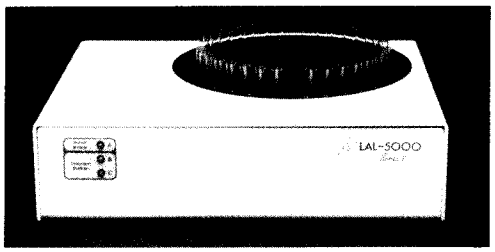
My readers should be aware that the FDA's guidelines on LAL testing are currently under revision. In the Kinetic Corner of this issue, the latest (July 15, 1991) "Interim Guidance for Human and Veterinary Drug Products and Biologicals, Kinetic LAL Techniques" is reprinted. Original copies may be obtained from Mr. Terry E. Munson, Sterile Drug Branch (HFD-322) CBER Office of Compliance, Tel: 301-295-8095.

Also included in this issue of the USP Says are the proposed changes for Chapter <85> of the USP Bacterial Endotoxins Test, and for Chapter <151>, Pyrogen Test. I strongly urge my readers to review the original text in the Pharmacopeial Forum and to send any comments to the USP.

We thank you for your patience with us in the aftermath of 'Hurricane Bob'.

Sincerely,

Thomas J. Novitsky, Ph.D.
Editor



Kinetic Corner

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 characteristics have to be established for each lot by performing the procedures outlined in Appendix A.

Inhibition/Enhancement Testing

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

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

The standard curve shall consist of at least three RSE or CSE concentrations. Additional standards should be included to bracket each log increase in the range of the standard curve so that there is at least one standard per log increment of the range. The standard curve must meet the criteria outlined in Appendix A. The calculated mean amount of endotoxin when referenced to the standard curve,

minus any measurable endogenous endotoxin in the spiked drug product, must be within plus or minus 50% of the known spike concentration to be considered to neither enhance or inhibit the assay. If there is no measurable endogenous endotoxin in the product the value will usually be equal to or less than plus or minus 25% of the standard curve value. If the undiluted drug product shows inhibition or enhancement, the drug product can be diluted, not exceeding the MVD, and test repeated.

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

Routine Testing

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

Appendix A

Using a RSE or CSE of known potency, in endotoxin units, assay at least 3 concentrations in triplicate that extend over the desired endotoxin range. Additional standards should be included to bracket each log increase in the range of the standard curve so that there 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.

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(Permission to reproduce this transcript was granted by Mr. Terry E. Munson.)

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CALENDAR

New England Chapter of the Parenteral Drug Association
Marriott Hotel, Newton, MA
October 2,1991

Center for Professional Advancement
East Brunswick, NJ, October 8-10,1991
LAL Testing: Drugs, Medical Devices, and Biotechnology
"Endotoxin Detection in QA/QC and Product Development"
Course Director - Michael E. Dawson, Ph.D.

Parenteral Drug Association Meeting
Wyndham Franklin Plaza Hotel,
Philadelphia, PA, October 23-25, 1991
Visit Associates of Cape Cod, Inc. at Booth #227

The American Society for Cell Biology 31st Annual Meeting
J.B.Hynes Veterans Memorial Convention Center
Boston, MA, Dec. 9-12, 1991
Visit Associates of Cape Cod, Inc. at Booth #1228

USP Says

The following proposed revisions concerning the *Bacterial Endotoxins Test* <85> and the *Pyrogen Test* <151> are included in the Pharmacopeial Forum, July-Aug. 1991, Vol. 17, No. 4. in IN-PROCESS REVISION. (Permission to reprint revisions was granted by the USP.)

Biological Tests and Assays

<85> Bacterial Endotoxins Test, USP XXII page 1493 and page 679 of *PF* 16(4) [July-Aug. 1990]. Based on public comments received at USP and discussions at the recent USP Open Conference on Microbiological and Sterilization Issues on the proposed changes published in *PF* 16(4), additional proposed changes are made. In particular, use of the test as an assay, and the reference to the calculation of confidence intervals under *Design and Analysis of Biological Assays* <111> are deleted. The *Test for confirmation of labeled LAL reagent sensitivity* and the *Inhibition or Enhancement Test* have been modified for ease of application and clarity. Under *Test Procedure* it is proposed to delete the reference to the procedure for *Transfusion and Infusion Assemblies* <161> and to propose instructions for these and other medical devices for inclusion under *Transfusion and Infusion Assemblies* to avoid contradictory instructions. Under *Calculation and Interpretation* it is proposed to simplify the calculation of endotoxin content.

3H00300 (MCB; B&M) RTS-8452-01; 7274-01

Change to read:

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 an 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 endpoint 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 or colorimetric readings ~~(including kinetic assays)~~, 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 preselected time of reacting endotoxin and control solutions with LAL reagent and reading of the spectrophotometric light absorbance at suitable wave-lengths. In the case of the endpoint turbidimetric procedure the reading is made immediately at the end of the incubation period. In the kinetic assays (turbidimetric and colorimetric), the absorbance is measured throughout the reaction period and rate values are determined from those readings. In the endpoint colorimetric procedure the reaction is arrested at the end of the preselected time by the addition of ~~an appropriate amount of acetic acid solution,~~ an enzyme-reaction-terminating agent 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

Change to read:

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~~, mix intermittently for 30 minutes, using a vortex mixer, 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~~ Mix vigorously, using a vortex mixer, for not less than ~~5~~ 3 minutes before use. ~~Vortex~~ Mix each dilution for not less than 1 minute before proceeding to make the next dilution Do not ~~use stored~~ store 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. Standardization of a CSE against the RSE using an LAL reagent for the gel-clot procedure may be effected by assaying a minimum of ~~4~~ 1 vial of the CSE ~~or 4 corresponding aliquots, where appli-~~

[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.

~~cable, of the bulk CSE~~ and 1 vial of the RSE, as directed under *Test Procedure*, but using 4 replicate reaction tubes at each level of the dilution series for the RSE and 4 replicate reaction tubes similarly for each vial of aliquot of the CSE. ~~If all of the dilutions for the 4 vials or aliquots of the CSE cannot be accommodated with the dilutions for the 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 $\log_{10}$ endpoint of the RSE and the mean $\log_{10}$ endpoint of the CSE is the standardized potency of the CSE, which then is to be converted to and expressed in Units per ng under stated drying conditions for the CSE, or in Units per container, whichever is appropriate. Standardize each new lot of CSE prior to use in the test. Calibration of a CSE in terms of the RSE must be with the specific lot of LAL reagent and the test procedure with which it is to be used. ~~Subsequent lots of LAL reagent from the same source and with similar characteristics need only checking of the potency 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. A large lot of a CSE may, however, be characterized by a collaborative assay of a suitable design to provide a representative relative potency and the within laboratory and between laboratory variance.~~

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

Preparatory Testing
Change to read:

Use an 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.~~ performing the inhibition or enhancement test as described below.

Appropriate negative controls are included. Validation must be repeated if the LAL reagent source or the method of manufacture or formulation of the article is changed.

Test for confirmation of labeled LAL reagent sensitivity - Confirm the labeled sensitivity ~~of the particular LAL reagent with the RSE (or CSE) using not less than 4 replicate vials, one vial tested in quadruplicate, 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. The RSE (or CSE) concentrations selected in confirming the LAL reagent label potency should bracket the stated sensitivity of the LAL reagent.~~ using a single vial of the LAL reagent lot. Prepare a series of two-fold dilutions of the RSE (or CSE) to give concentrations of 2λ,λ,0.5λ and 0.25λ where λ is the labeled sensitivity of the LAL reagent in Endotoxin Units per mL. Perform the test on the four standard concentrations in quadruplicate and include negative controls. The geometric mean endpoint concentration (See *Calculations and Interpretation*) must be greater than or equal to 0.5λ and less than or equal to 2.0λ.

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 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.~~

Perform the test on aliquots of the specimen, or a dilution not to exceed the *Maximum Valid Dilution*, in which there is no detectable endotoxin. Perform the test on the specimen without added endotoxin and with endotoxin added to give final concentrations of 2.0λ,λ,0.5λ,and 0.25λ, as directed under *Test Procedure*, but using not less than 4 replicate tubes for the untreated specimen and for each specimen to which endotoxin has been

[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 an LAL Reagent having a sensitivity of not less than 0.15 Endotoxin Unit per mL.

added. In parallel with the above, test in duplicate the same endotoxin concentrations in water and untreated negative controls. Calculate the geometric mean endpoint endotoxin concentration for the specimen as described under *Calculations and Interpretation*. The test is valid for the article if the geometric mean endpoint concentration in the specimen is greater than or equal to 0.5λ and less than or equal to 2.0λ.

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~~ the *Maximum Valid Dilution*. Use a dilution, not to exceed the *Maximum Valid 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 ultrafiltration, or by appropriate dilution. Dilute the untreated specimen (as constituted, where applicable, for administration or use), to a level not exceeding the maximum valid dilution, at which no endotoxin is detectable. Repeat the test for *Inhibition or Enhancement* using the specimens at those dilutions.

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 is the limit dilution factor for the preparation for the test to be valid.

Test Procedure

Change to read:

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

For Validating the test for an article, for endotoxin limit tests ~~or assays~~, or for special purposes where so specified, testing of specimens is conducted quantitatively to determine response endpoints for gel-clot readings. Usually graded strengths of the specimen and standard endotoxin are made by multifold dilutions. Select dilutions so that they correspond to a geometric series in which each step is greater than the next lower by a constant ratio. Do not store 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, providing the environmental conditions within blocks are uniform.

Preparation - Since the form and amount per container of standard endotoxin and of LAL reagent may vary, constitution and/or dilution of contents should be as directed in the labeling. The pH of the test mixture of the specimen and the LAL reagent is in the range 6.0 to 7.5 unless specifically directed otherwise in the individual monograph. The pH may be adjusted by the addition of sterile, endotoxin-free sodium hydroxide 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 is the limit dilution factor for the preparation for the test to be valid.~~

Procedure - To 10- X 75-mm test tubes add aliquots of the 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 ex-

tracts 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 is negative or the endotoxin standard does not show the endpoint concentration to be within ± 1 two-fold dilutions from the label claim sensitivity of the LAL reagent or if any negative control ~~shows a gel clot end point~~. is positive.

Calculation and Interpretation

Change to read:

Geometric Mean Calculation - The endpoint is the last positive test in a series of decreasing concentrations of endotoxin, specimen, or specimen to which endotoxin has been added. Record the endpoint concentration, E, for each replicate series of dilutions. Determine the log endpoint concentrations, e, and calculate the geometric mean endpoint concentration using the following formula:

Geometric Mean Endpoint Concentration = antilog ($\Sigma e/f$), Where Σe is the sum of the log endpoints of the dilution series used and f is the number of replicates.

~~*Calculation*~~

Endotoxin Content 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,~~

~~$p\lambda/U$~~

λU,

in which ~~S is the antilog of the geometric mean $\log_{10}$ of the endpoints,~~ λ is the labeled sensitivity, expressed in Endotoxin Units (EU) per mL ~~for the Standard Endotoxin,~~ of the lysate employed in the test, U is the antilog of $\Sigma e/f$, where e is the $\log_{10}$ of the endpoint dilution factors, expressed in decimal fractions, f is the number of replicate reaction tubes read at the endpoint 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~~ is less than 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.~~

<151>Pyrogen Test, USP XXII page 1515. The proposed revisions of the general chapter are based on the recommendations published in *PF* 16(2) [Mar.-Apr.1990] and on the recommendations and discussions at the USP Open Conference on Microbiology and Sterilization Issues.

3H01100 (MCB) RTS-8492-01

Change to read:

Procedure - Perform the test in a separate area designated solely for pyrogen testing and under environmental conditions similar to those under which the animals are housed and free from disturbances likely to excite them. Withhold all food from the rabbits used during the period of the test. Access to water is allowed at all times, but may be restricted during the test. If rectal temperature-measuring probes remain inserted throughout the testing period, restrain the rabbits with light-fitting neck stocks that allow the rabbits to assume a natural resting posture. Not more than 30 minutes prior to the injection of the test dose, determine the "control temperature" of each rabbit: this is the base for the determination of any temperature increase resulting from the injection of a test solution. In any one group of test rabbits, use only those rabbits whose control temperatures do not vary by more than 1° from each other, and do not use any rabbit having a temperature exceeding 39.8°.

Unless otherwise specified in the individual monograph, inject into an ear vein of each of three rabbits 10 mL of the test solution per kg of body weight, completing each injection within 10 minutes after start of administration. The test solution is *either* the product, constituted if necessary as directed in the labeling, *or* the material under test treated as directed in the individual monograph and injected in the dose specified therein. For pyrogen testing of devices or injection assemblies, use washings or rinsings of the surfaces that come in contact with the parenterally administered material or with the injection site or internal tissues of the patient. Assure that all test solutions are protected from contamination. Perform the injection after warming the test solution to a temperature of 37 ± 2°. Record the temperature at ~~1,2, and 3 hours~~ 30-minute intervals between 1 and 3 hours subsequent to the injection.

Change to read:

Test Interpretation and Continuation- Consider any temperature decreases as zero rise. If no rabbit shows an individual rise in temperature of ~~0.6°~~ 0.5° or more above its respective control temperature, ~~and if the sum of the three individual maximum temperature rises does not exceed 1.4°,~~ the product meets the requirements for the absence of pyrogens. If any rabbit shows an individual temperature rise of ~~0.6°~~ 0.5° or more, ~~or if the sum of the three individual maximum temperature rises exceeds 1.4°,~~ continue the test using five other rabbits. If not more than three of the eight rabbits show individual rises in temperature of ~~0.6° or more, and if the sum of the eight individual maximum temperature rises does not exceed 3.7°,~~ 0.5°, the material under examination meets the requirements for the absence of pyrogens.