VI. APPENDICES

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

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*■ 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.■■ 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. ■■ Repeat the test for *Inhibition or Enhancement* using the specimens at those dilutions.

Test Procedure

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

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

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

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 $\rho S/U$, 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 ρ 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

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

Endotoxin Limit Expressed by Volume:

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

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

METHOD II EXAMPLES

PARENTERAL DRUGS EXCEPT INTRATHECAL

Drug Administered on a Weight-per-Kilogram Basis

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

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

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

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

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

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

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

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

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

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

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

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