Pharma&Biotech

Endotoxin Testing and Regulatory Requirements

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60-Minute Agenda

- Regulatory agency and major compendia
- Meeting the requirements
  - Endotoxin limits
  - Validation
  - Routine testing
  - Medical devices
- Questions and answers

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Regulatory Agency – FDA

- US Food and Drug Administration (FDA)
  - Department of Public Health Service and one of the USA's oldest customer protection agencies
- Documents published affecting the Limulus Amebocyte Lysate (LAL) test:
  - Withdrawn in July 2011:
    - Guideline on Validation of the LAL Test as an End-product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products, and Medical Devices. December 1987
    - Interim Guidance for human and veterinary drug products and Biologicals. July 1991
  - New Q&A guidance document published June 2012
    - Guidance for Industry – Pyrogen and Endotoxins Testing: Questions and Answers

Major Compendia

- U.S. Pharmacopeia (USP)
  - Private company, not a U.S. government agency
  - Jointly responsible with the EP for the provision of the Reference Standard Endotoxin (RSE)
  - Does not have the power to enforce the monographs contained in the pharmacopeia (this is the responsibility of the FDA)
  - Bacterial Endotoxins Test (BET) is described in section <85>
- European Pharmacopeia (EP)
  - Part of the European Department for the Quality of Medicines (EDQM)
  - BET is described in chapter 2.6.14
- Japanese Pharmacopeia (JP)
  - BET is described in chapter 4.01

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Meeting the Requirements

- Sterile parenteral drugs and medical devices must be tested with the BET and must be shown to contain less than their Endotoxin Release Limit (ERL)
- The performance of this test and endotoxin limits that are allowed in a product are defined in regulatory documents
- In common with many other Quality Control tests, validation is a key element in preparing for testing and product release

Recognized Methodologies

- Recognized LAL methodologies in the pharmacopeia:
  - Gel clot (limit) test
  - Chromogenic endpoint test
  - (Turbidimetric endpoint test)
  - Kinetic chromogenic test
  - Kinetic turbidimetric test

Arbitration

- The gel clot limit test, conducted at the Maximum Valid Dilution (MVD) serves in the case of dispute, unless indicated in the drug monograph
- Where other LAL techniques are referenced in a drug monograph, this acknowledges that the test used in arbitration would be the one which is indicated

The Three Essentials for Endotoxin Testing

- Establish endotoxin limits for pharmaceutical and medical devices
- Establish procedures for validating the use of the BET in your laboratory
- Establish procedures for conducting routine testing

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Endotoxin Limits

- Sources
  - Pharmacopeial monograph
  - Calculation (for Pharmaceuticals)

Endotoxin limit = (K / D) x Potency

K= max. allowable endotoxin exposure

5 EU/Kg/Hour (or 350 EU/Total Body/Hour in USA & Europe, 300 EU/Total Body/Hour in Japan)

0.2 EU/Kg/Hour (Intrathecals)

2.5 EU/Kg/Hour (Radiopharmaceuticals)

D= max. human dose

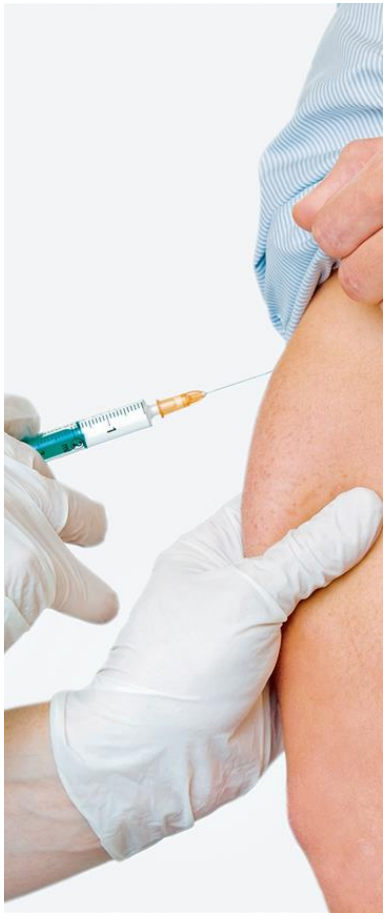
Potency = drug concentration (not required if dose is expressed in ml)

Endotoxin Limit Calculations

Example 1

- Product: Insulin
- Dose: 2 Units/kg
- Potency: 100 Units/ml
- Endotoxin limit calculation:

$$\frac{5\ \text{EU}/\text{kg}}{2\ \text{Units}/\text{kg}} \times 100\ \text{Units}/\text{ml} = 250\ \text{EU}/\text{ml}$$



Endotoxin Limit Calculations

Example 2

- Product: 5% Dextrose
- Dose: 10 ml/kg
- Potency: Not applicable
- Endotoxin limit calculation:

$$\frac{5\ \text{EU} / \text{kg}}{10\ \text{ml} / \text{kg}} = 0.5\ \text{EU} / \text{ml}$$

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Validation

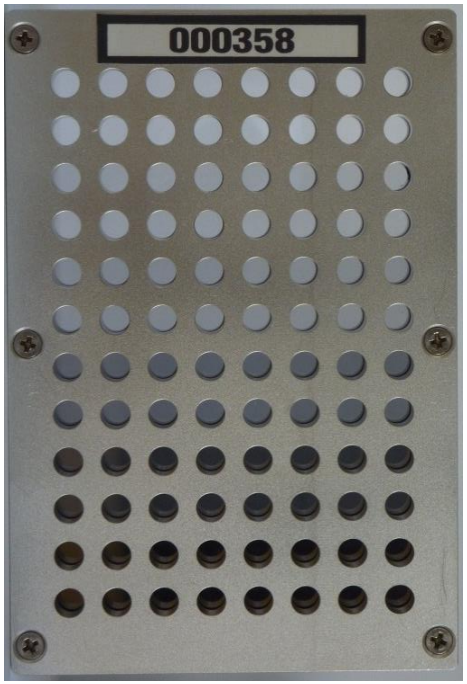
- Why do we undertake validation?
  - To confirm performance
  - To assure reproducibility
- What resources are available to the user when undertaking validation?
  - USP, EP & JP
  - Association for the Advancement of Medical Instrumentation (AAMI) ST72:2011 – Test methodologies, monitoring and alternatives to batch testing
  - LAL manufacturers documentation
  - FDA Q&A document

BET Validation Requirements

- Product independent
- Product dependent

Product Independent Validation – Confirmation of Equipment Performance

- Validation of heating block or water bath
- Validation of optical linearity and temperature uniformity for kinetic readers
  - Part of IOPQ (Installation/Operational/Performance Qualification)
  - WinKQCL™ Software: Other Tests – Validation
  - Lonza products
    - WinKQCL™ Software Qualification Manual
    - Stepped Neutral Density Plate / Absorbance Test Plate
- Validation of software for kinetic systems
  - Lonza product: WinKQCL™ Software Validation Package



Product Independent Validation – Confirmation of Equipment Performance

- Validation of storage containers
- Validation of plastics and accessories
  - Pharmacopeia: “If employing plastic apparatus such as microplates and pipette tips for automatic pipetters, use only that which has been shown to be free of detectable endotoxin and not to interfere with the test”
  - “Apyrogenic” is not necessarily suitable
  - Lonza offers pre-screened accessories (tips, tubes, plates, etc.)

Product Independent Validation – Confirmation of Equipment Performance

- Validation of depyrogenation ovens
  - Demonstrate 3-log reduction of endotoxin content
  - Lonza products
    - Endotoxin Challenge Vials™ (ECVs)
    - High Potency Endotoxin

Product Independent Validation – Confirmation of Lysate Sensitivity/Linearity

- Confirmation of the laboratory/analyst
  - Confirmation of lysate sensitivity or lysate linearity for a new analyst
- Confirmation of reagent performance
  - Confirmation of lysate sensitivity (label claim) or lysate linearity when changing reagent lot number

These two requirements can be jointly satisfied by the same assay.

Gel Clot – Confirmation of Label Claim

- The confirmation of the label claim requires the operator to run the standard series in quadruplicate
- The geometric mean of the series must then be calculated
- The result must be within a two-fold dilution of the label claim (i.e. between 0.5λ and 2λ)

Calculating the Geometric Mean

- The geometric mean is calculated by adding together the log of the endotoxin concentration at which the last clot occurred for each set of replicates
- The four log values are added and divided by four
- The antilog of the result gives the geometric mean value

Calculating the Geometric Mean – Example

Replicate	0.25 EU/ml	0.125 EU/ml	0.06 EU/ml	0.03 EU/ml	0.015 EU/ml	Neg. Control
1	+	+	+	-	-	-
2	+	+	+	-	-	-
3	+	+	+	+	-	-
4	+	+	+	-	-	-

- Example: Sensitivity λ = 0.06 EU/ml, results see above
  - Log 0.06 + log 0.06 + log 0.03 + log 0.06
  - Add four log values and divide by 4
  - The antilog of the results gives **0.05** EU/ml
  - Permitted range 0.03 to 0.12 EU/ml
- Result: Reagents/analyst meets the requirements

Quantitative Assays – Confirmation of Linearity

- The operator must run a standard curve in at least triplicate
- The full operating range of the assay should be used (i.e. 0.005 – 50 EU/ml for the Kinetic-QCL™ Assay)
- To verify the standard curve, assay a minimum of three concentrations. When standard curve is >2 log range an extra standard must be added for each log increment

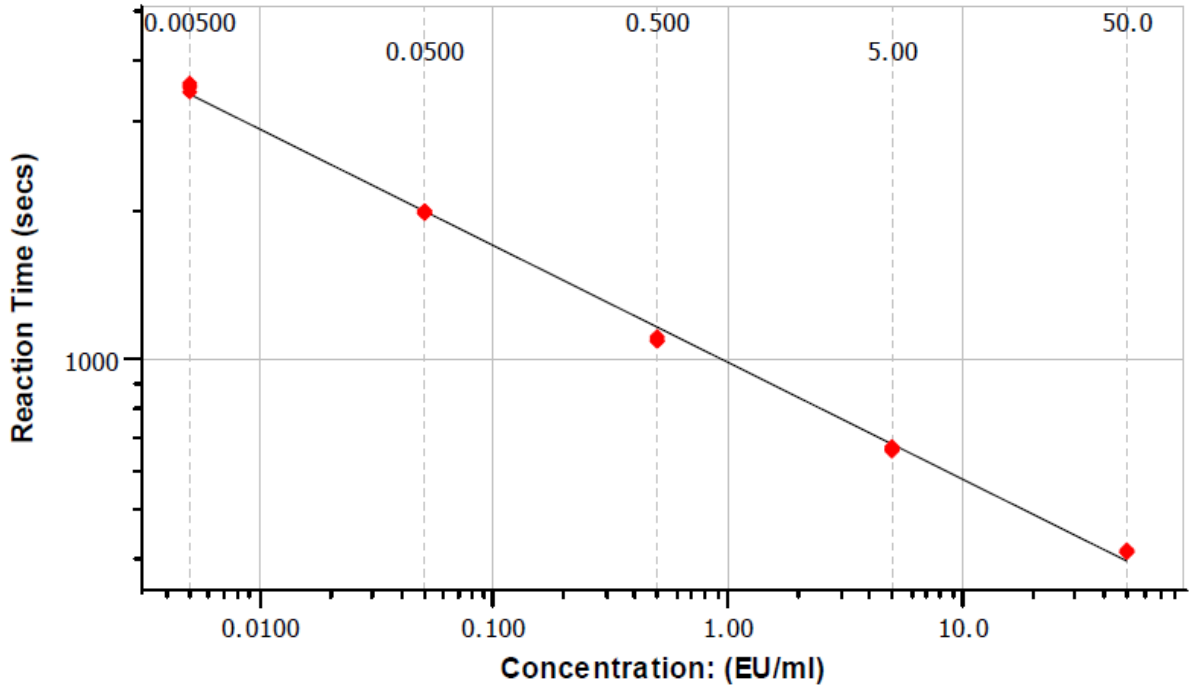
Quantitative Assays – Confirmation of Linearity

- Linear regression must be used for analysis
- The correlation coefficient must be $|R| \geq 0.980$
- The results must not be averaged

Confirmation of Linearity – Example

Lonza - Scientific Support
SST BioTek ELx-808 Reader A
Lonza - WinKQCL Version 5.1.2

Performed By : SHAUBER (VA)	LAL Lot No.	: ML062LVM17	Exp : 3/6/2015	Time : 9:22:30AM
KQCL - Initial Qualification Test	Water Lot No.	: 0000375767	Exp : 4/2/2016	Date : 1/22/2014
Template : Today IQ	Endotoxin Lot No.	: 0000373630	Exp : 9/4/2017	ELx808
Temperature : 36.9°C - 37.0°C				S/N 1305225
Linear Regression :	Correlation Coefficient = -0.999	Slope = -0.234	Y-Intercept = 2.995	



Product Dependent Validation

- Determination of the conditions under which the product does not interfere with the LAL test
- Dilution with LAL Reagent Water (LRW) is the most common method used to overcome interference but other techniques are allowed
- This requires the laboratory to undertake assays to ascertain the correct dilution/treatment

Calculating the Degree of Dilution

- As the sample is diluted to overcome interference, any endotoxin present is also diluted
- Eventually, the concentration of endotoxin, if present at the ERL, will be reduced to a level which is beyond the capability of the assay to detect. It is therefore necessary to calculate:
  - Maximum Valid Dilution (MVD)

    or
  - Minimum Valid Concentration (MVC)

Maximum Valid Dilution (MVD)

$$MVD = \frac{\text{Endotoxin Limit}}{\lambda}$$

- λ is equal to
  - Label claim for gel clot lysate
  - Lowest standard for quantitative assays

Examples of MVD Calculations

- Gel Clot
- Lysate sensitivity = 0.06 EU/ml
- Endotoxin limit = 3.0 EU/ml

$$MVD = \frac{3.0 \text{ EU/ml}}{0.06 \text{ EU/ml}}$$

- MVD = 1:50

Examples of MVD Calculations

- Kinetic chromogenic
- Lowest standard = 0.005 EU/ml
- Endotoxin limit = 3.0 EU/ml

$$MVD = \frac{3.0 \text{ EU/ml}}{0.005 \text{ EU/ml}}$$

- MVD = 1:600

Testing for Interference

- The next step in validation is to test for the interference between the product and the test
- Interference may result in inhibition or enhancement

Gel Clot Interference Testing

- Screening assay
  - Dilution series within the MVD, in duplicate with positive product controls (PPCs)
  - Dilutions giving positive PPC results (clot) can be used in the interference test
  - Select a dilution where the interference has been effectively eliminated

Gel Clot Interference Testing

- Interference test
  - Preparation of two standard series: in water (duplicate) and in optimal test article dilution (quadruplicate)
  - Water and test article standard series geometric means are calculated and should both be within 0.5λ to 2λ range for the test article dilution to be confirmed as non-interfering

Gel Clot Interference Test

Replicate	Endotoxin Concentration Sensitivity 0.125 EU/ml Recovery 0.25 - 0.06 0.25	0.125	0.06	0.03	
LPS in LRW	1	+	+		Geometric mean = 0.125
	2	+	+		
LPS in Product	1	+			Geometric mean = 0.21
	2	+			
	3	+	+		
	4	+	+		

Inhibition/Enhancement Test for Kinetic Methods

- Simple single-stage process
- A dilution series of the test article is prepared within MVD and each dilution is tested in duplicate, with a PPC also in duplicate
- From the results, the lowest dilution that is closest to 100% PPC recovery is used for validations and most likely for routine testing

Product Validation

- Three lots of each product must be tested at the determined optimal conditions to show repeatability
- For some products (research, clinical, preclinical) testing single lots for validation purposes is adequate, but usually three-lot testing is done to show robustness and repeatability of the assay
- If appropriate, test samples from the beginning, middle and end of each production lot to show batch consistency

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Routine Testing

- Typical test done in the QC lab to determine the endotoxin content of a product (raw material, in-process or final release)
- Test typically includes:
  - Negative control
  - Set of standards
  - Samples
  - PPCs

Pass Fail Cutoff – To Determine PPC

$$PFC = \frac{\text{Endotoxin Limit}}{\text{Dilution}}$$

- PFC less than or equal to 1.0 → spike with
  - 0.5 EU/ml (Kinetic-QCL™ Assay)
  - 0.1 EU/ml (PYROGENT™-5000 Assay)
- PFC greater than 1.0 → spike with
  - 5.0 EU/ml (Kinetic-QCL™ Assay)
  - 1.0 EU/ml (PYROGENT™-5000 Assay)

Example – Pass Fail Cutoff Calculation

- Product endotoxin release limit = 5 EU/ml
- Dilution tested = 1:20
- Pass fail cutoff = 5/20 = 0.25

This result is <1, so:

- For Kinetic-QCL™ Assay use 0.5 EU/ml PPC
- For PYROGENT™-5000 Assay use 0.1 EU/ml PPC

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Medical Devices

- Special case
- Described in USP Chapter 161 and AAMI guideline
- Extraction/rinsing to wash off endotoxin
  - Eluant: LRW
  - Usually 1 hour at room temperature or shorter at 37°C
- Limits
  - 20.0 EU/device
  - 2.15 EU/device (for cerebrospinal contact)
- Monograph provides for the calculation of an MVD for the eluate from an extraction process

Medical Devices

- Required to test not less than three and not more than ten devices depending on batch size
  - 3 devices for batches <100
  - 3% to a maximum of 10 devices in batches >100
- Adjust the volume for rinsing or extraction fluid according to the size and configuration of the device

Medical Devices

- Endotoxin limit for extraction solution

$$\frac{K \times N}{V}$$

K = allowable endotoxin per device

N = number of devices tested

V = total volume of extraction solution

- Example
  - 10 devices extracted in 1,000 ml LRW
  - Endotoxin limit = (20 x 10) / 1000 = 0.5 EU/ml extraction solution

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Do You Have More Questions?

- Contact our Scientific Support team

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  scientific.support@lonza.com

- Join our upcoming webinar on 5 August:

  Enhancing Your LIMS Portfolio: How to Meet the Unique Automation Requirements of QC Microbiology

- Register at:

  http://www.lonza.com/qcwebinars

Thank You for Your Attention