The Bacterial Endotoxins Specification – Points to Consider

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Learning Objectives

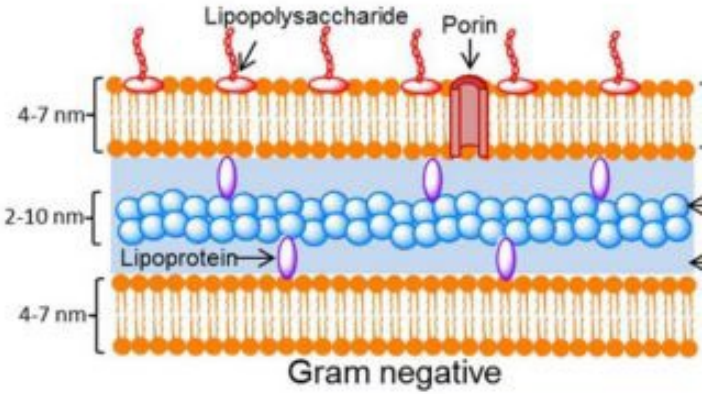
- What are bacterial endotoxins and why they are a patient risk?
- How do you control endotoxins in injectable drug products?
- How do you set acceptance criteria for release/stability endotoxins specifications?

Why am I here?

- People in my office assess the information submitted in Abbreviated New Drug Applications
  - Release/stability specification for bacterial endotoxins
- Too high endotoxins levels in drug products – patient risk
- The release/stability acceptance criterion for endotoxins can be hard to set!

What are endotoxins?

- Endotoxins are a component of the cell wall of Gram-negative bacteria (like *E. coli*)
- Interact with the human immune system to cause an inflammatory response
- Patient risk from endotoxins exposure
  - Fever
  - Shock
  - Death



Adapted from https://www.europeanpharmaceuticalreview.com/article/87191/endotoxin-definition-and-standardisation/

How do we control endotoxins in drugs?

- Endotoxins are not inactivated by most sterilization methods
- Quality by design
  - Control of API/excipients
  - Bioburden control
  - Depyrogenation of container closure system
  - **Release/stability testing**

The endotoxins specification

- Specification = test + method + acceptance criteria
- Test – for bacterial endotoxins
- Method – most typically USP <85> “Bacterial Endotoxins Test”
  - Gel clot
  - Turbidimetric
  - Chromogenic
  - Product-specific suitability testing should be performed
- Acceptance criteria

Setting the acceptance criteria

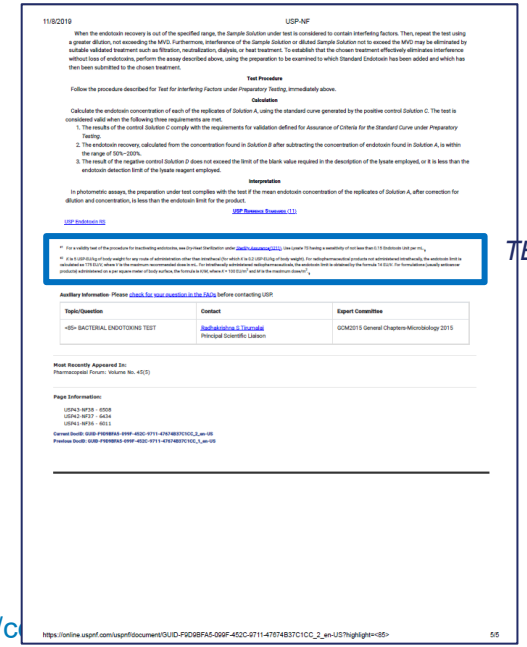
- USP <85> - Injectable products

$$\textit{Endotoxins Acceptance Criteria} = \frac{K\ (threshold\ pyrogenic\ dose\ of\ endotoxin\ per\ kg\ body\ weight)}{M\ (maximum\ recommended\ bolus\ dose\ of\ product\ per\ kg\ body\ weight)}$$

Okay so…what are K and M?

The secret to finding K

USP <85> - LAST PAGE!!!



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When the endotoxin recovery is out of the specified range, the *Sample Solution* under test is considered to contain interfering factors. Then, repeat the test using a greater dilution, not exceeding the MVD. Furthermore, interference of the *Sample Solution* or diluted *Sample Solution* not to exceed the MVD may be eliminated by suitable validated treatment such as filtration, neutralization, dialysis, or heat treatment. To establish that the chosen treatment effectively eliminates interference without loss of endotoxins, perform the assay described above, using the preparation to be examined to which Standard Endotoxin has been added and which has then been submitted to the chosen treatment.

Test Procedure

Follow the procedure described for *Test for Interfering Factors* under *Preparatory Testing*, immediately above.

Calculation

Calculate the endotoxin concentration of each of the replicates of *Solution A*, using the standard curve generated by the positive control *Solution C*. The test is considered valid when the following three requirements are met.

1. The results of the control *Solution C* comply with the requirements for validation defined for *Assurance of Criteria for the Standard Curve* under *Preparatory Testing*.
2. The endotoxin recovery, calculated from the concentration found in *Solution B* after subtracting the concentration of endotoxin found in *Solution A*, is within the range of 50%–200%.
3. The result of the negative control *Solution D* does not exceed the limit of the blank value required in the description of the lysate employed, or it is less than the endotoxin detection limit of the lysate reagent employed.

Interpretation

In photometric assays, the preparation under test complies with the test if the mean endotoxin concentration of the replicates of *Solution A*, after correction for dilution and concentration, is less than the endotoxin limit for the product.

USP Reagent Specs (11)

USP Endotoxin RS

* For a validity test of the procedure for inactivating endotoxins, see Dry-Heat Sterilization under Sterility Assurance (1211). Use Lysate TS having a sensitivity of not less than 0.15 Endotoxin Unit per mL.

** If a 5 USP-EU/kg of body weight for any route of administration other than intrathecal (for which it is 0.2 USP-EU/kg of body weight). For radiopharmaceutical products not administered intrathecally, the endotoxin limit is calculated as 175 EU/*K*, where *V* is the maximum recommended dose in mL. For intrathecally administered radiopharmaceuticals, the endotoxin limit is obtained by the formula 14 EU/*V*. For formulations (usually anticancer products) administered on a per square meter of body surface, the formula is *K*/*M*, where *K* = 100 EU/m^2 and *M* is the maximum dose/m^2.

Auxiliary Information Please check for your question in the FAQs before contacting USP.

Topic/Question	Contact	Expert Committee
<85> BACTERIAL ENDOTOXINS TEST	Radhakrishna S Tirumalai Principal Scientific Liaison	GCM2015 General Chapters-Microbiology 2015

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https://online.usp.org/usp/nf/document/GUID-F9D9BFA5-099F-452C-9711-47674B37C1CC_2_en-US?highlight=<85> 5/5

TEENY TINY writing

K – as listed in USP <85>

- 0.2 EU (endotoxin units)/kg body weight/hour (intrathecal)
- 5 EU/kg body weight/hour (any route other than intrathecal)
- 100 EU/m^2 body surface area/hour (products administered per meter of body surface area)
- Additional limits for radiopharmaceuticals

Setting the acceptance criteria

$$\textit{Endotoxins Acceptance Criteria} = \frac{K\ (threshold\ pyrogenic\ dose\ of\ endotoxin\ per\ kg\ body\ weight)}{M\ (maximum\ recommended\ bolus\ dose\ of\ product\ per\ kg\ body\ weight)}$$

- When considering M:
  - Consider the product label
  - Co-packaged products should not collectively exceed the limit

Setting the acceptance criteria

$$\textit{Endotoxins Acceptance Criteria} = \frac{K\ (threshold\ pyrogenic\ dose\ of\ endotoxin\ per\ kg\ body\ weight)}{M\ (maximum\ recommended\ bolus\ dose\ of\ product\ per\ kg\ body\ weight)}$$

- What weight?
  - OPMA considers 70 kg as the standard adult weight for endotoxins calculations
  - Pediatric weight should be considered – recommend CDC Weight-for-Age Charts

Setting the acceptance criteria

- Consider justifying your endotoxins specification in eCTD section 3.2.P.5.6.
- Discuss your rationale for your K and your M.

Case study 1 (Simple)

- Leuprolide acetate injection
  - Treatment for prostate cancer

- “The recommended dose is 1 mg (0.2 mL) administered as a single daily subcutaneous injection”

- Subcutaneous injection – not intrathecal
- No pediatric indications listed in label
- No kit – just drug product

Case study 1 (Simple)

- Leuprolide acetate injection

$$\textit{Endotoxins Acceptance Criteria} = \frac{K\ (\textit{threshold pyrogenic dose of endotoxin per kg body weight})}{M\ (\textit{maximum recommended bolus dose of product per kg body weight})}$$

$$\textit{Endotoxins Acceptance Criteria} = \frac{5\ \frac{EU}{kg}}{\frac{1}{70}\ \frac{mg}{kg}}$$

Taken directly from USP <85> - this product is not administered intrathecally

1 mg dose for a 70 kg adult patient

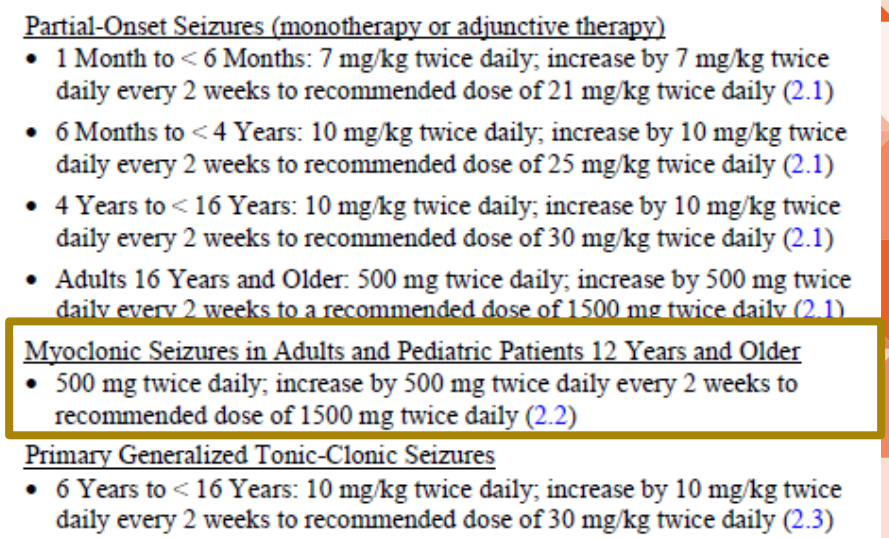
$$\textit{Endotoxins Acceptance Criteria} = 5\ \frac{EU}{kg}\ x\ \frac{70}{1}\ \frac{kg}{mg}$$

$$\textit{Endotoxins Acceptance Criteria} = 350\ EU/mg$$

Case Study 2 (Complex)

- Levetiracetam
  - Anti-epileptic drug, many formats including injectable (intravenous)

This tells us that
K will be 5 EU/kg



Partial-Onset Seizures (monotherapy or adjunctive therapy)

- 1 Month to < 6 Months: 7 mg/kg twice daily; increase by 7 mg/kg twice daily every 2 weeks to recommended dose of 21 mg/kg twice daily (2.1)
- 6 Months to < 4 Years: 10 mg/kg twice daily; increase by 10 mg/kg twice daily every 2 weeks to recommended dose of 25 mg/kg twice daily (2.1)
- 4 Years to < 16 Years: 10 mg/kg twice daily; increase by 10 mg/kg twice daily every 2 weeks to recommended dose of 30 mg/kg twice daily (2.1)
- Adults 16 Years and Older: 500 mg twice daily; increase by 500 mg twice daily every 2 weeks to a recommended dose of 1500 mg twice daily (2.1)

Myoclonic Seizures in Adults and Pediatric Patients 12 Years and Older

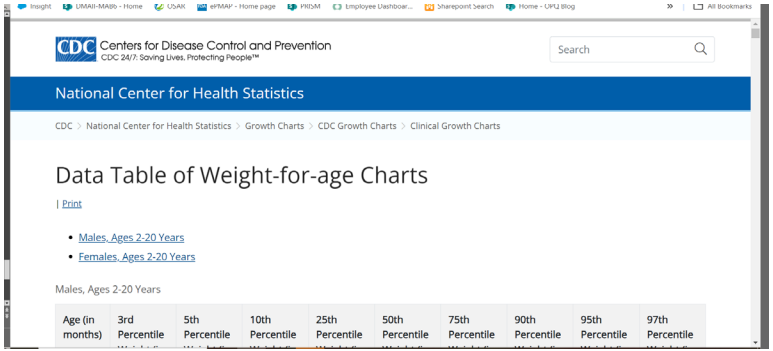
- 500 mg twice daily; increase by 500 mg twice daily every 2 weeks to recommended dose of 1500 mg twice daily (2.2)

Primary Generalized Tonic-Clonic Seizures

- 6 Years to < 16 Years: 10 mg/kg twice daily; increase by 10 mg/kg twice daily every 2 weeks to recommended dose of 30 mg/kg twice daily (2.3)

Case Study 2 (Complex)

- Levetiracetam
  - Worst-case exposure – 1500 mg in 12 yo patient



  - CDC – 144.5 month female, 50th percentile weight = 41.8 kg
  - 1500 mg/41.8 kg = 36 mg/kg

Case Study 2 (Complex)

Yes – 36 mg/kg is our highest dose. Now we have our M!

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$$\textit{Endotoxins Acceptance Criteria} = \frac{K\ (\textit{threshold pyrogenic dose of endotoxin per kg body weight})}{M\ (\textit{maximum recommended bolus dose of product per kg body weight})}$$

$$\textit{Endotoxins Acceptance Criteria} = \frac{5\ \text{EU/kg}}{36\ \text{mg/kg}} = \mathbf{0.14\ EU/mg}$$

Resources

- United States Pharmacopeia Chapter <85> - Bacterial Endotoxins
  - Remember – K is on the last page in teeny tiny writing
- United States Pharmacopeia Chapter <771> - Ophthalmics Products – Quality Tests
- CDC Weight for Age charts
  - https://www.cdc.gov/growthcharts/html_charts/wtage.htm
- FDA Guidance for Industry: Pyrogen and Endotoxins Testing: Questions and Answers (June 2012)
- FDA Guidance for Industry: Controlled Correspondence Related to Generic Drug Development (March 2024)
  - Explain your rationale

Recap

- Exposure to excessive endotoxins is a risk to patients.
- Process design AND end-product testing are important for the control of endotoxins.
- The United States Pharmacopeia chapter <85> contains information on appropriate endotoxins acceptance criteria.
- The release/stability acceptance criteria should be set using information from the product label.

Challenge Question 1

- The acceptance criterion for the endotoxins specification for a subcutaneous injection should be calculated from the formula provided in USP <85>.
  1. True
  2. False

Challenge Question 2

- K is the same for all injectable products
  1. True
  2. False

U.S. FOOD & DRUG ADMINISTRATION

Thank you!

Thanks to:
John Arigo, Ph.D.
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