ENDOTOXIN TESTING OF ALLERGEN EXTRACTS

Rosa Codina Ph.D., Mathew D. Roby, B.S., and Robert E. Esch, Ph.D.
Greer Laboratories, Inc., Lenoir, NC

Abstract

Rationale: While endotoxins are ubiquitous in nature, their role in the immunomodulatory is controversial. This study was conducted to optimize an assay to measure endotoxins in allergenic extracts (AE) and determine their concentrations in various AE.

Objectives

- Optimize an LAL chromogenic kinetic assay to measure endotoxin concentrations in allergenic extracts
- Obtain preliminary data on endotoxin concentrations in various allergen extracts utilizing the optimized assay

Reagents and Supplies

- Pyrococcus spp. LAL reagent, control standard endotoxin, pyrogen-free supplies, Q-Q planar inhibiting buffer: Associates of Cape Cod, Inc., East Falmouth, MA.
- Pyrogen-free water and allergen extracts (N = 152): Greer Laboratories, Inc. (ubated below).

Methods

- Chromogenic kinetic assay was optimized to generate a log-log endotoxin standard curve (50 EU/mL - 0.05 EU/mL), which was utilized to calculate test concentrations, including positive and negative controls. Assay performance characteristics (linearity, precision, and accuracy) were evaluated. Spike recovery experiments were performed with components typically in AE (i.e., 0.05% and 0.2% LPS), and with solutions containing these combined components. The AE analyzed were derived from Dermatophagoides pteronyssinus (N=44), D. farinae (N=98), pollen (N=40), fungal (N=16); and foods (N=9). Non-parametric statistical analysis was performed.

Results: Negative control values were below 0.05 EU/mL. The standard curve correlation coefficient was >0.998. Assay precision and accuracy were 9.0% and 37.4%, respectively. 50% and 0.2% LPS interfered in the test (average endotoxin spike recovery: 0.01% and 0.2%, respectively). Interference was overcome by increasing sample dilutions. Endotoxins were selectively detected in most AE, with the greatest concentrations measured in D. farinae (AE median = 1.424 EU/mL) and the least in fungal AE (median = 0.125 EU/mL).

Conclusions: An assay for measuring endotoxins in AE has been optimized. Endotoxins are present in AE at various concentrations, generally at levels less than FDA proposed thresholds for parenteral exposure to endotoxins in humans (2800 - 5000 EU/mL/kg person).

Introduction

The role of endotoxin exposure in the immunomodulatory is controversial. While endotoxins can cause adverse health effects upon injection, determination of their presence in allergenic extracts is not currently required. Therefore, endotoxin concentrations in these products have not been defined.

Endotoxins are typically measured using the Limulus Ameoba lyase test (LAL test), which can be performed using gel-clot, chromogenic, and turbidimetric techniques. Gel-clot techniques are the simplest to perform, and were utilized by the FDA in a pilot study to measure endotoxin contents in allergenic extracts. However, these techniques are semi-quantitative, have a two-fold error, and cannot detect potential generation effects of sample matrix in test results. Chromogenic and turbidimetric techniques are quantitative, have a greater resolution than gel-clot techniques, and can detect both inhibition and enhancement effects.

Endotoxins are ubiquitously present, their role in the immunomodulatory is controversial. This study was conducted to optimize an assay to measure endotoxins in allergenic extracts (AE) and determine their concentrations in various AE.

Objectives

- Optimize an LAL chromogenic kinetic assay to measure endotoxin concentrations in allergenic extracts
- Obtain preliminary data on endotoxin concentrations in various allergen extracts utilizing the optimized assay

Reagents and Supplies

- Pyrococcus spp. LAL reagent, control standard endotoxin, pyrogen-free supplies, Q-Q planar inhibiting buffer: Associates of Cape Cod, Inc., East Falmouth, MA.
- Pyrogen-free water and allergen extracts (N = 152): Greer Laboratories, Inc. (ubated below).

Results (I)

- Endotoxin standard curves have a log-log fit
- Endotoxin concentrations in allergenic extracts have been measured and utilized to generate a log-log standard curve which was used to calculate test concentrations, including positive and negative controls. Assay performance characteristics (linearity, precision, and accuracy) were evaluated. Spike recovery experiments were performed with components typically in AE (i.e., 0.05% and 0.2% LPS), and with solutions containing these combined components. The AE analyzed were derived from Dermatophagoides pteronyssinus (N=44), D. farinae (N=98), pollen (N=40), fungal (N=16); and foods (N=9). Non-parametric statistical analysis was performed.

Results (II)

- Assay Validation:
  - Normality tests
  - Parameters analyzed: Positive control, spike recovery of the negative control, B-curve parameter (slope), and RI
- Allergen Extracts:
  - Non-parametric Mann Whitney U test

Results (III)

- Spike recoveries of Endotoxin Solutions

Clinical Relevance

Although extracts are typically diluted ten-fold to provide maintenance concentrations, injection of 0.5% or even a full-strength extract to a patient would generally deliver endotoxin doses ranging from 0.05 EU/mL (in 2800 EU/mL) to 720 EU/mL (in 1000 EU/mL). A mix of the five extract types tested in this study would deliver 172 EU.

The proposed FDA limit for intravenous endotoxin exposure in humans to elicit symptoms is 40-50 EU/mL (2800-5000 EU/mL for a 75 kg person), greater than the endotoxins detected in the allergenic extracts for testing/diagnostics tested in this study.