

ENVIRONMENTAL ENDOTOXIN (Limulus Amebocyte Lysate Assay)

Introduction. Endotoxin (ET) is a generic name for a heterogeneous group of biologically active substances, consisting of lipopolysaccharide (LPS) aggregates derived from the cell walls of gram negative bacteria. These substances have extraordinary proinflammatory effects which may result in fever, malaise, alterations in WBC counts, respiratory distress, shock, headache and death. In addition, they have proven effects as an adjuvant for IgE responses and may augment antigen induced histamine release.

Endotoxin exposure has been shown to be an important factor in occupational disease, especially with agricultural workers. Of additional interest, recent studies with non-occupational exposures have shown it to be significant in residential dust where high levels correlated with symptoms of asthma and allergy. The following substances may be significant sources of endotoxin:

Water-based industrial fluids (e.g. cutting fluids)
Agricultural bioaerosols (cotton, grain & feed dust)
 Textile industry *Sewage facility*
 Carpet dust *Contaminated water*
 Forced recirculated & contaminated air
Aqueous solutions used in patient management

Description of Test Method. The quantitative kinetic version of the *Limulus* amebocyte lysate (LAL) test is the method used.

[Background: Levin and Bang made the observation that very small amounts of endotoxin could activate a coagulation pathway in the horseshoe crab, Limulus polyphemus. The proteins involved are isolated from a lysate of the Limulus amebocytes. Upon contact with endotoxin, proteases are activated and release a chromophore from a synthetic peptide. A log/log correlation between the time required for the appearance of color and the endotoxin concentration is linear from 0.005 to 50 EU/mL. All samples are tested for the presence of enhancing or inhibiting factors by spike recovery studies.]

Units Reported: Endotoxin Units per mL (EU/mL) are provided for aqueous samples. A conversion to mass units (ng/mL) is used if the sample is dust or air. One EU is defined as the potency of 0.10 ng of the EC5 reference (U.S. Pharmacopoeia).

Sample Collection & Handling Requirement

- ❑ **Dust/Bulk Samples-** 500 mg of dry dust collected in suitable device. Ship by courier at ambient temperature.
- ❑ **Air Samples-** Sterile 0.45 µm filter cassettes are usually recommended. The filter types that we recommend for most collections are MCE or polycarbonate. Note that special projects may require specific collection media. Also submit an unexposed filter as a reference. Store dry and ship by courier at ambient temperatures.
- ❑ **Water Sample-** 5.0 mL of water or aqueous sample collected in **sterile, pyrogen-free glass tube**. Liquid samples should be shipped cold with ice packs by overnight courier. **Do not freeze.**

Results Reported:

Test Code	Sample	Reference Range
3047	Air*	< 2.0 ng / m ³
3045	Household Dust	< 5 µg / g of dust
3046	Water	By report

**Although there is no permissible exposure level (PEL) for this agent, the 2.0 ng/m³ threshold is a useful guideline for air. Exposures above 10 ng/m³ result in acute adverse effects. See ACI Int. 1999;11: 109 - 111.*

References:

- ❑ Milton DK et al. Environmental endotoxin measurement: interference and sources of variation in the Limulus assay of house dust. AIHA Journal 1997; 58: 861-867.
- ❑ Douwes J et al Influence of various dust sampling and extraction methods on the measurement of airborne endotoxin. Appl Environ Microbiol 1995; 61: 1763 - 1769.
- ❑ Reynolds SJ and Milton DK. Comparison of methods for analysis of airborne endotoxin. Appl Occup Environ Hyg 1993; 8: 761-767.

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