

# Guidance for Industry and FDA Staff

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## Class II Special Controls Guidance Document: Endotoxin Assay

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**U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Devices and Radiological Health**

**Division of Microbiology Devices  
Office of In Vitro Diagnostic Device Evaluation and Safety**

# **Preface**

## **Public Comment**

Comments and suggestions may be submitted at any time for Agency consideration to Dockets Management Branch, Division of Management Systems and Policy, Office of Human Resources and Management Services, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD, 20852. When submitting comments, please refer to Docket No. 2003D-0221. Comments may not be acted upon by the Agency until the document is next revised or updated.

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*This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.*

### 1. Introduction

This document was developed as a special control to support the classification of the endotoxin assay into class II (special controls). An endotoxin assay is a device that uses serological techniques in whole blood. The device is intended for use in conjunction with other laboratory findings and clinical assessment of the patient to aid in the risk assessment of critically ill patients for progression to severe sepsis.

This guidance is issued in conjunction with a *Federal Register* notice announcing the classification of the endotoxin assay.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in Agency guidances means that something is suggested or recommended, but not required.

### The Least Burdensome Approach

The issues identified in this guidance document represent those that we believe need to be addressed before your device can be marketed. In developing the guidance, we carefully considered the relevant statutory criteria for Agency decision-making. We also considered the burden that may be incurred in your attempt to comply with the guidance and address the issues we have identified. We believe that we have considered the least burdensome approach to resolving the issues presented in the guidance document. If, however, you believe that

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there is a less burdensome way to address the issues, you should follow the procedures outlined in the “A Suggested Approach to Resolving Least Burdensome Issues” document. It is available on our Center web page at:  
<http://www.fda.gov/cdrh/modact/leastburdensome.html>.

## **2. Background**

A manufacturer who intends to market a device of this generic type should (1) conform to the general controls of the Federal Food, Drug & Cosmetic Act (the Act), including the premarket notification requirements described in 21 CFR 807 Subpart E, (2) address the specific risks to health associated with the endotoxin assay identified in this guidance, and (3) obtain a substantial equivalence determination from FDA prior to marketing the device, unless exempt from the premarket notification requirements of the Act (refer to 21 CFR 807.85).

This guidance document identifies the classification regulation and product code for the endotoxin assay (Refer to Section 4 – **Scope**). In addition, other sections of this guidance document identify the risk to health and describe measures that, if followed by manufacturers and combined with the general controls, will generally address the risk associated with these endotoxin assays and lead to a timely premarket notification [510(k)] review and clearance. This document supplements other FDA documents regarding the specific content requirements of a premarket notification submission. You should also refer to 21 CFR 807.87 and other FDA documents on this topic, such as the **510(k) Manual - Premarket Notification: 510(k) - Regulatory Requirements for Medical Devices**, <http://www.fda.gov/cdrh/manual/510kprt1.html>.

Under “**The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; Final Guidance**<sup>1</sup>,” a manufacturer may submit either a Traditional 510(k) or an Abbreviated 510(k). FDA believes an Abbreviated 510(k) provides the least burdensome means of demonstrating substantial equivalence for a new device, particularly when FDA has issued a guidance document that provides recommendations on what should be addressed in a submission for the device. Alternatively, manufacturers considering modifications to their own cleared devices may lessen the regulatory burden by submitting a Special 510(k).

## **3. The Content and Format of an Abbreviated 510(k) Submission**

An Abbreviated 510(k) submission must include the required elements identified in 21 CFR 807.87, including the proposed labeling for the device sufficient to describe the device, its intended use, and the directions for its use. In an Abbreviated 510(k), FDA may consider the contents of a summary report to be appropriate supporting data within the meaning of 21 CFR 807.87(f) or (g); therefore, we recommend that you include a summary report. The

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<sup>1</sup> <http://www.fda.gov/cdrh/ode/parad510.html>

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report should describe how this guidance document was used during device development and testing and the methods or tests used. The report should also include a summary of the test data or description of the acceptance criteria applied to address the risks identified in this document, as well as any additional risks specific to your device. This section suggests information to fulfill some of the requirements of 21 CFR 807.87, as well as some other items that we recommend you include in an Abbreviated 510(k).

### **Coversheet**

The coversheet should prominently identify the submission as an Abbreviated 510(k) and cite the title of this guidance document.

### **Proposed labeling**

Proposed labeling should be sufficient to describe the device, its intended use, and the directions for its use. (Refer to Section 8 for specific information that we recommend you include in the labeling for this type of device.)

### **Summary report**

We recommend that the summary report contain a:

- Description of the device and its intended use. We recommend that the description include a complete discussion of the performance specifications and, when appropriate, detailed, labeled drawings of the device. You should also submit an "indications for use" enclosure.<sup>2</sup>
- Description of the device design.
- Identification of the Risk Analysis method(s) used to assess the risk profile in general as well as the specific device's design and the results of this analysis. (Refer to Section 5 for the risks to health generally associated with the use of this device.)
- Discussion of the device characteristics that address the risks identified in this guidance document, as well as any additional risks identified in your risk analysis.
- Brief description of the test method(s) you have used or intend to use to address each performance aspect identified in Sections 6 and 7 of this guidance document. If you follow a suggested test method, you may cite the method rather than describing it. If you modify a suggested test method, you may cite the method, but you should provide sufficient information to explain the nature of and reason for the modification. For each test, you may either (1) briefly present the data resulting from the test in clear and concise form, such as a table, **or** (2) describe

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<sup>2</sup> Refer to <http://www.fda.gov/cdrh/ode/indicate.html> for the recommended format.

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the acceptance criteria that you will apply to your test results.<sup>3</sup> (See also 21 CFR 820.30, Subpart C - Design Controls under the Quality System Regulation.)

- If any part of the device design or testing relies on a recognized standard, (1) a statement that testing will be conducted and meet specified acceptance criteria before the product is marketed, or (2) a declaration of conformity to the standard.<sup>4</sup> Please note that you must certify that the device is in conformity with the standard. (Section 514(c)(1)(B) of the Act.); this means that testing must be completed before submitting a declaration of conformity. For more information, refer to the FDA guidance, **Use of Standards in Substantial Equivalence Determinations; Final Guidance for Industry and FDA**, <http://www.fda.gov/cdrh/ode/guidance/1131.html>.

If it is not clear how you have addressed the risks identified by FDA or additional risks identified through your risk analysis, we may request additional information about aspects of the device's performance characteristics. We may also request additional information if we need it to assess the adequacy of your acceptance criteria. (Under 21 CFR 807.87(l), we may request any additional information that is necessary to reach a determination regarding substantial equivalence.)

As an alternative to submitting an Abbreviated 510(k), you can submit a Traditional 510(k) that provides all of the information and data required under 21 CFR 807.87 and described in this guidance. A Traditional 510(k) should include all of your methods, data, acceptance criteria, and conclusions. Manufacturers considering modifications to their own cleared devices should consider submitting Special 510(k)s.

## **4. Scope**

The scope of this document is limited to the following devices as described in 21 CFR 866.3610 (product code NGS):

“An endotoxin assay is a device that uses serological techniques in whole blood. The device is intended for use in conjunction with other laboratory findings and clinical assessment of

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<sup>3</sup> If FDA makes a substantial equivalence determination based on acceptance criteria, the subject device should be tested and shown to meet these acceptance criteria before being introduced into interstate commerce. If the finished device does not meet the acceptance criteria and, thus, differs from the device described in the cleared 510(k), FDA recommends that submitters apply the same criteria used to assess modifications to legally marketed devices (21 CFR 807.81(a)(3)) to determine whether marketing of the finished device requires clearance of a new 510(k).

<sup>4</sup> See Required Elements for a Declaration of Conformity to a Recognized Standard (Screening Checklist for All Premarket Notification [510(K)] Submissions), <http://www.fda.gov/cdrh/ode/reqrecstand.html>.

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the patient to aid in the risk assessment of critically ill patients for progression to severe sepsis.”

The classification identification above identifies the device as it existed at the time of classification.

### **5. Risks to Health**

There are no known *direct* risks to patient health. However, failure of the test to perform as indicated or error in interpretation of results may lead to improper patient management. Therefore, use of assay results to adjust a treatment regimen, without consideration of other clinical factors, could pose a risk. A falsely low endotoxin measurement could result in a determination that the patient is at a lower risk for sepsis, which could delay appropriate treatment. A falsely high endotoxin measurement could result in a determination that the patient is at a higher risk for sepsis, which could lead to unnecessary monitoring or potentially toxic therapy.

In the table below, FDA has identified the risk to health generally associated with the use of the endotoxin assay addressed in this document. The measures recommended to mitigate the identified risk are given in this guidance document, as shown in the table below. We recommend that you conduct a risk analysis, before submitting your premarket notification, to identify any other risks specific to your device. The premarket notification should describe the risk analysis method. If you elect to use an alternative approach to address the risk identified in this document, or have identified risks additional to those in this document, you should provide sufficient detail to support the approach you have used to address that risk.

<b>Identified risk</b>	<b>Recommended mitigation measures</b>
Improper patient management	Section 6, 7, and 8

### **6. Performance Characteristics**

#### **General Study Recommendations**

We recommend that you include a description of the method used to detect endotoxin. You should also include a description of the reagent components in the kit. Whenever possible, we recommend that you include patient sample s derived from the intended use population (e.g., critically ill patients) in the analytical protocols described below.

We also recommend that you evaluate your assay in at least two external sites in addition to that of the manufacturer. Generally, we recommend that performance be assessed in the testing environment where the device will ultimately be used (i.e., central laboratory or point of care, such as the intensive care unit (ICU)) by individuals who will use the test in clinical

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practice (e.g., trained technologists). We recommend that you initially analyze data separately to evaluate any inter-site variation and include results of the analysis in the 510(k) summary report. You can pool clinical study results from the individual sites in the package insert if you demonstrate that there are no significant differences in the results among sites. Before initiating any clinical study, you may contact the Division of Microbiology Devices.

We recommend that you provide specific information concerning protocols so that acceptance criteria or data summaries can be interpreted during the review of your submission. For example, when referring to NCCLS protocols or guidelines, we recommend that you indicate which specific aspects of the protocols or guidelines you followed.

### **Specific Performance Characteristics**

#### **Precision and Reproducibility**

We recommend that you characterize within-run and total precision using whole blood samples according to guidelines provided in “Evaluation of Precision Performance of Clinical Chemistry Devices,” Approved Guideline (1999), National Committee for Clinical Laboratory Standards (NCCLS), Document EP5-A. That document includes guidelines for experimental design, computations, and a format for stating performance claims. We recommend that you evaluate precision at relevant endotoxin measurements, including levels near medical decision points and measurements near the limits of the reportable range.

We recommend that you include in your 510(k) the items listed below:

- point estimates of the concentration
- standard deviations of within-run and total precision
- sites at which precision protocol was run
- number of days, runs, and observations
- number of sites and/or operators

We recommend that you identify which factors (e.g., instrument calibration, reagent lots, operators) were held constant and which were varied during the evaluation, and describe the computational methods, if they are different from that described in NCCLS EP5-A.

#### **Interference**

We recommend that you characterize the effects of potential interferents on assay performance. Examples of experimental designs, including guidelines for selecting interferents for testing, are described in detail in “Interference Testing in Clinical Chemistry; Proposed Guideline” (1986), National Committee for Clinical Laboratory Standards, Document EP7-P. Potential sources of interference can include compounds normally found in whole blood, such as triglycerides, hemoglobin, bilirubin, albumin, and anti-oxidants, such as vitamins C and E.

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We also recommend that you test blood components to determine the effect of different neutrophil counts on the endotoxin values. We recommend that you include patients with neutropenia in your study.

Typically, interference studies involve adding the potential interferent to the whole blood sample and determining any bias in the recovery of endotoxin relative to a control sample (to which no interferent has been added).

We recommend that you include in your 510(k) the following items:

- types and levels of interferents tested
- endotoxin level or activity in the sample
- number of replicates tested
- definition or method of computing interference.

We recommend that you identify any observed trends in bias (i.e., negative or positive) and indicate the range of observed recoveries in the presence of the particular interferent. This approach is more informative than listing average recoveries alone. We recommend that you state the criteria or level on which non-interference is determined.

You may not need to perform additional interference testing with potential interferents identified in literature or by other sources. However, we recommend that you identify the potential interferents in the labeling.

### **Cross reactivity**

We recommend that you include data on the assay specificity by measuring the cross reactivity of your device with other bacterial and fungal cell wall components, such as: lipoteichoic acid (LTA) from gram positive bacteria, cell wall extracts from gram positive bacteria, yeast mannan, and yeast cell wall extracts. We recommend that you describe the purity of the components used to evaluate cross reactivity.

### **Analytical Sensitivity**

We recommend that you calculate the analytical sensitivity. This is often defined as the lowest level of endotoxin that can be reliably measured by the test.

- We recommend that you describe the sample type, define your measures of sensitivity, provide your acceptance criteria, or provide a data summary that clarifies how measurements below the level of sensitivity are reported to the user.

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### **Linearity**

We recommend that you characterize the linear range of the assay by evaluating samples whose concentration levels are known to be relative to each other. “Evaluation of the Linearity of Quantitative Analytical Methods,” Proposed Guideline NCCLS Document EP6-P, describes a protocol for sample preparation and value assignment and a format for stating performance characteristics.

We recommend that you describe the sample types and preparation, concentrations, and number of replicates. When describing your acceptance criteria or summary data, we recommend that you include the slope, intercept, confidence intervals of the estimated line, the range of linearity, and the degree of deviations (biases) from the estimated line that were observed, or that are considered acceptable for the various concentration levels. Often these deviations can be best described by listing observed or acceptable values relative to the expected values for each level evaluated.

## **7. Method Comparison**

### **Clinical Sensitivity**

We recommend that you compare the endotoxin assay to the clinical diagnosis of sepsis according to accepted medical practices (e.g., guidelines from the American College of Chest Physicians/Society of Critical Care Medical Consensus Conference<sup>5</sup>). As with studies to evaluate performance characteristics, you may contact the Division of Microbiology Devices for input on your study plan before initiating clinical studies.

### **Clinical Specificity**

We recommend that you evaluate the clinical specificity of your endotoxin assay in hospitalized patients who are not critically ill, but who have similar signs and symptoms of sepsis. We prefer that you select control groups that have an age and gender distribution similar to the patients selected for the sensitivity study because such demographic factors may bias the assay results.

### **Specimen collection and handling conditions**

We recommend that you support statements in your labeling about specimen storage and transport by assessing whether the device can maintain acceptable performance (e.g., precision) over the storage times and temperatures recommended to users. For example, an appropriate study may include an analysis of aliquots stored under the conditions of time, temperature, or specified number of freeze/thaw cycles. We recommend that you state the criteria for an acceptable range of recoveries under the recommended storage and handling conditions.

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<sup>5</sup> Definition for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med* 1992; **20** : 864-874.

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### **Sample selection, inclusion and exclusion criteria**

We recommend that you evaluate patient samples with endotoxin levels distributed across the reportable range of the assay in your sensitivity study. We suggest that you provide a clear description of how the samples were selected, including the reasons that samples were excluded. We recommend that you include samples from the target population (i.e., whole blood samples from critically ill patients).

Appropriate sample size depends on factors such as precision, interference, range, and other performance characteristics of the test. We recommend that you provide a statistical justification to support the study sample size.

### **Expected values**

We recommend that you evaluate asymptomatic patients to determine the prevalence of endotoxin in a healthy population, such as non-diseased individuals under stress conditions (e.g., smokers). We also suggest that you collect data from other hospitalized patients who are critically ill, but who are not considered at risk for sepsis. We prefer that you select control groups that have an age and gender distribution similar to the patients selected for the sensitivity study because such demographic factors may bias the assay results.

### **Presentation of results**

When providing the results of your study, we recommend that you demonstrate the association between endotoxin values and the risk profile for sepsis. Data should indicate the prevalence of severe sepsis syndrome by each endotoxin activity level (high, intermediate, low). We recommend that you stratify data by important demographic factors (e.g., age and gender) if the factors have the potential to bias the results.

## **8. Labeling**

The premarket notification should include labeling in sufficient detail to satisfy the requirements of 21 CFR 807.87(e). The following suggestions are aimed at assisting you in preparing labeling that satisfies the requirements of 21 CFR 807.87(e).<sup>6</sup>

### **Directions for use**

We recommend clear and concise instructions that delineate the technological features of the specific device and how the device is to be used on patients. Instructions should

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<sup>6</sup> Although final labeling is not required for 510(k) clearance, final labeling must comply with the requirements of 21 CFR 801 or 21 CFR 809.10 before a medical device is introduced into interstate commerce.

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encourage local/institutional training programs designed to familiarize users with the features of the device and how to use it in a safe and effective manner.

### **Quality Control**

To mitigate the risk of inaccurate results due to a patient's neutrophil profile, we recommend that you provide quality control recommendations in the labeling.

### **Limitations**

We recommend that you address the limitations of your assay with statements in the labeling, such as:

- Endotoxin activity may be detected in the blood stream of patients with liver disease, leukemia, lymphoma, hypertension, major thermal injury, or victims of multiple trauma, or of patients undergoing major vascular procedures (such as cardiopulmonary bypass).
- Translocation of endotoxin may occur from gut or lung tissue into the bloodstream.
- Use the assay only at specific times in the course of evaluating a patient (e.g., use only on the first day of admission to the ICU).
- Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.