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Summary

This document describes a uniquely simple system for assuring that compounded sterile products are free of endotoxin (pyrogen) within limits set by the Pharmacopeia (USP) or consistent with current scientific opinion.

MICROBIAL SOLUTIONS

Simplified Endotoxin Test Method for Compounded Sterile Products

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Official Microbial Tests

The USP describes two tests for microbial contamination of a Compounded Sterile Product (CSP). The sterility test screens for live, infectious bacteria. The Bacterial Endotoxins Test (BET) detects unsafe levels of microbial cell wall debris, from live or dead gram-negative bacteria, that causes fever and symptoms of septic shock. Until now, the BET required skilled analysts and manipulation of cumbersome reagents. This discussion describes a uniquely simple system for assuring that CSPs are free of endotoxin (pyrogen) within limits set by the Pharmacopeia (USP) or consistent with current scientific opinion.

Why worry about endotoxin?

Endotoxin is extremely potent, is heat stable, passes sterilizing membrane filters and is present everywhere bacteria are or have been present. Of greatest concern are intraspinal infusion solutions because this route of administration is about 1000 times more potent for endotoxin than is IV injection.[1] Non-sterile powders, components and water are the most likely sources of endotoxin in the compounding pharmacy.

What are Endotoxin Limits?

An endotoxin limit of 5 EU/kg/hr or 350 EU per adult (70 kg) was scientifically established to avoid the fever and hypotension from IM or IV injection of endotoxin contamination.[2] Time is a factor because there are mechanisms in the liver and blood that neutralize endotoxin. However, there are no clearance mechanisms in intraspinal spaces, so the IT (intrathecal) endotoxin limit is much more stringent. An IT limit of 0.2 EU/kg/hr was arbitrarily set without supporting experimental data. A more scientifically sound IT endotoxin limit of 14 EU per day avoids the serious mortality and morbidity associated with the signs of meningitis that are induced by endotoxin in IT spaces.[1] An Endotoxin Unit (EU) is a unit of biological activity of the USP Reference Endotoxin Standard.

Endotoxin Test Methods

Limulus amebocyte lysate (LAL) reagent, FDA approved, is used for all USP endotoxin tests. Two types of endotoxin tests are described in the USP <85> BET. Photometric tests require a spectrophotometer, endotoxin-specific software and printout capability. The simplest photometric system is a handheld unit employing a single-use LAL cartridge that contains dried, pre-calibrated reagents; there is no need for liquid reagents or standards. The FDA-approved unit is marketed under the name of Endosafe®-PTS™. The device requires about 15 minutes to analyze small amounts of sample, a 25-μL aliquot from CSP diluted in a sterile tube, and to print out results. In contrast, gel-clot methods require a dry-heat block, calibrated pipettes and thermometer, vortex mixer, freeze-dried LAL reagents, LAL Reagent Water (LRW) for hydrating reagents and depyrogenated glassware. In this clot test, diluted sample and liquid reagents require about an hour for sample and positive-control preparation and an hour's incubation in a heat block; results are recorded manually.[1] Thus, the simplicity and speed of the automated system make it ideally suited to the pharmacy setting.

Sources of BET Interference

The LAL-endotoxin reaction is an enzymatic cascade that requires specific conditions for optimum reaction, including neutral pH, optimum concentrations of mono-valent and divalent cations and low concentrations of most drug substances. Fortunately, the BET has great sensitivity that allows substantial dilution of test materials without sacrificing safety with respect to endotoxin limits.

BET for CSPs Intended for Intraspinal Infusions

The challenge posed in testing a CSP is preparation of a test sample that is 1) diluted sufficiently to avoid inhibitory or interfering factors and 2) is tested in a method sufficiently sensitive to guarantee that test results are safely within an IT endotoxin limit of <14 EU per day. Validation data for testing intraspinal analgesics and antispasmodic agents by Endosafe® LAL reagents was published and apeer reviewed.[1] The study reported that dilution of intraspinal infusions to < 1 mg/mL and testing in a sensitive system gave results within the desired endotoxin limit. Validated test methods for individual as well as combinations of intraspinal medications were described.

An endotoxins test by the Endosafe®- PTS™ requires precalibrated LAL cartridges, a PTS™ reader, sterile dilution tubes, fixed-volume pipette with sterile pipette tips, and a test sample that is diluted to a BET-compatible level. A suitable tube for dilutions is a 14-mL Polystyrene sterile tube, product # 2057 by Becton Dickinson. The most convenient time to sample a CSP is just prior to sterilizing filtration of the final product. The following guideline for dilution-prior-to-testing applies to opioids and antispasmodic drugs commonly used for intraspinal infusions:

Intraspinal infusions ≤ 20 mg/mL:
Make a 1:80 dilution by transferring 25 μL of CSP to 2 mL SWI (Sterile Water for Injection or Irrigation used in compounding) or LRW in a sterile 14-mL tube. The sensitivity (LOD, limit of detection) of an assay will be 4 EU/mL, when using a cartridge with a standard curve of 0.05-to-5 EU/mL.

Intraspinal infusions > 20 mg/mL:
Make a 1:280 dilution by transferring 25 μL of CSP to 7 mL SWI or LRW in a sterile 14-mL tube. The sensitivity (LOD, limit of detection) of an assay will be 14 EU/mL, when using a cartridge with a standard curve of 0.05-to-5 EU/mL.

Intraspinal mixtures:
As directed above, make a 1:80 dilution for infusions that contain ≤20 mg/mL of total drug components and make a 1:280 dilution for > 20 mg/mL, total. The intraspinal endotoxin limit is always met when the test result is less than 14 EU/mL

Table 1 lists the maximum daily dose published by a panel of experts for long-term intraspinal administration.[3] The recommended intrathecal limit of 14 EU/day was divided by the panel's maximum dose, in mg/day, to generate the drug-specific endotoxin limit. The calculations were done on a daily basis to be compatible with daily dosage, to increase the BET sensitivity and to better reflect the kinetics of cerebrospinal fluid flow and resorption.

Table 1: Endotoxin Limits for Intraspinal Infusions

IT Medication	Maximum Daily Dose* (mg/day)	Endotoxin Limit∞ (EU/mg)
Morphine	20	0.7
Baclofen	2	7
Bupivicaine	25	0.6
Clonidine	1	14
Fentanyl	5	2.8
Hydromorphone	12	1.2

* Maximum daily dosage published by an expert panel for long-term intraspinal infusion.

∞ EL for IT based on 14 EU/mL/Day divided by the maximum daily dose.

Table 2 indicates how IT infusion mixtures would be prepared for endotoxin testing by the PTS™ system. When no endotoxin is detected, the results are reported as <4 or <14 EU/mL, depending upon the extent of initial dilution of an aliquot from an intraspinal infusion.

Table 2: Summary of CSP Dilution and Results for Representative IT Infusion Mixtures

Medications*	Dilution Factor	Result (EU/ml)∞
MS 30/CL 0.1	280	< 14
MS 10/BP 10	80	< 4
HM 30/BP 20	280	< 14
FN 4/BP 15	80	< 4

* Data for coded IT medications and concentration in mg/mL; where, **MS** = morphine sulfate; **CL** = clonidine HCL; **BP** = bupivicaine HCL; **HM** = hydromorphone HCL; **FN** = fentanyl citrate.

∞ Sensitivity for LAL cartridge = 0.05 EU/mL (PTS™ System)

BET for CSPs Intended for IV or IM injection

A great variety of CSP medications are prepared, from sodium chloride injection to steroidal or antibiotic suspensions, so that the development of a simplistic dilution scheme is challenging. Simple solutions or very dilute drugs may be tested with little or no dilution. The dilutions recommended below should avoid test inhibition, as evidenced by recovery of the positive control, and give results that are within the endotoxin limit (see below). The validated test dilutions detailed in Table 3 and discussed throughout this document apply to Endosafe® LAL reagents and may not apply to reagents from other sources. The dilution required for a therapeutic CSP is related to the concentration of the active ingredient, as detailed below:

Intravenous or IM solutions ≤ 20 mg/mL:
Make a 1:100 dilution by transferring 25 µL of CSP to 2.5 mL SWI or LRW in a sterile 14-mL tube. The specific drug or mixture endotoxin limit may be determined by applying the calculations suggested below or existing methods.

Intravenous or IM solutions > 20 mg/mL:
Make a 1:400 dilution by transferring 25 µL of CSP to 10 mL SWI or LRW in a sterile tube.

Suspensions:
Make a 1:200 dilution by transferring 25 µL of CSP to 5 mL of SWI or LRW in a sterile tube. A glucans blocker may be needed for certain suspending agents.

Table 3: Summary of CSP Dilution and Results for Representative CSPs

Medications*	Dilution Factor	Result (EU/ml)∞
Betameth Phos Acetate Susp. 6 mg/mL	100	< 5
Bromphen Maleate Inj. 10 mg/mL	100	< 5
Prednisolone Susp. 40 mg/mL	200	< 10
Sodium Chloride for Injection, USP	1	< 0.05

* Data for IM or IV medications in mg/mL.

∞ Sensitivity for LAL cartridge = 0.05 EU/mL (PTS™ System)

Understanding and Calculating Endotoxin Limits

The adverse effects of endotoxin are dose-dependent, so the objective of meeting endotoxin limits is to assure that any potential contamination is within safe limits, that is, below the threshold for pyrogenic reactions. These adverse effects are also dependent on route and rate of administration. If an IV drug is infused over a period of time that exceeds one hour, the dose may be divided by the number of hours to determine the dose-per-hour. Validation of BET methods, including extent of pre-test dilution, is done after endotoxin limits are established, based on factors such as dose, infusion rate and route of administration. Endotoxin may be expressed in EU/mg of a specific medication or EU/mL of a mixture of medications in solution. The term pyrogen-free may be applied to a CSP that contains endotoxin less than the endotoxin limit

Manufactured injectable products are made in specific concentrations, which simplify the calculation of endotoxin limits. However, CSPs vary in drug concentration and composition of mixtures, as required by prescription, which complicates the calculation of limits. Therefore, this discussion presents a method of determining the safety of a medication by grouping them by concentration, knowing that interfering conditions are concentration dependent. The objective is to assure that an adult patient (specified as 70 kg.) receives less than 350 EU per dose per hour of contamination in an IV or IM CSP or less than 14 EU/mL/day in an intraspinal infusion. Pediatric doses would have to meet a limit of 5 EU/kg of medication. The dilutions recommended here apply validations done with Endosafe® LAL reagents, which are buffered and otherwise highly interference resistant; reagents from other LAL suppliers may not give valid results under these test conditions.

To exemplify how this approach works, let us consider a CSP mixture that has a maximum dose of 10 mL, injected as a bolus of medication. The mixture contained >20 mg/mL of drug substances, so a dilution of 1:400 was made to avoid test interference. The PTS™ result was no-detectable-endotoxin and valid recovery of the positive control, giving a report of <20 EU/mL. Therefore, a maximum dose 10 mL exposes the patient to <200 EU per 10 mL of medication, which is less than the USP's allowable 350 EU per adult dose.

BET Enhancement

The dilutions specified herein were recommended to avoid inhibitory test conditions, where recovery of the positive control is < 50%. There is one known non-specific activator of LAL reagent that produces enhancement or endotoxin-like reactivity, usually accompanied by enhanced recovery of the positive control of < 200%. This agent is a β-D-glucan that is found in yeast cell wall and in cellulosic materials.[4] Therefore, suspending agents that contain CMC or other cellulosic materials may give a falsely high endotoxin value. If not solved by dilution, this enhancement interference may be avoided by using an endotoxin-specific reagent supplied by Charles River.

References

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