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Aseptic Sampling Best Practices

Endotoxin Binding Affinity

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Abstract

This technical report describes a study to evaluate binding affinity of Control Standard Endotoxin (CSE) to different plastic sample collection containers. This scope of this study is limited to four different plastic containers and a glass control. Each was inoculated with a 0.5 endotoxin units per mL (EU/mL) CSE solution. Test articles were incubated between 18 ° and 25 ° Celsius. Samples from each container were pooled and measured for endotoxin using the Kinetic Turbidimetric test method with sensitivity of 0.005 EU/mL.

Introduction

The U.S. Food and Drug Association (FDA) issued Inspection Technical Guide 32, "Pyrogens, Still a Danger" in January of 1979. The Technical Guide described the novel risk of "attendant infections, adverse drug reactions, fevers of unknown etiology, and even deaths from shock" after injection of sterile solutions (U.S. Food and Drug Association, 1979). It was known when Technical Guide 32 was published that these maladies are caused by bacterial endotoxins (also named bacterial pyrogens). Endotoxin safety limits for intravenous and intramuscular delivery of drugs is set at 5EU/kg/hr.

Bacterial endotoxins are lipopolysaccharides (LPS) found in the outer wall of gram negative bacteria. LPS are released when bacterial cells divide (Association for the Advancement of Medical Instrumentation, 2011), die and lyse (U.S. Food and Drug Association, 1985). Heating, filtration or adsorption techniques do not eliminate pyrogens from parenteral solutions (U.S. Food and Drug Association, 1979). To demonstrate process control and monitor endotoxin levels samples collected at critical process steps and assayed for endotoxin (Arbesser-Rastburg, et al., 2015).

Best practices for collection of samples for endotoxin assay have been proposed. Containers should be sterile, disposable systems that are aseptically closed from the environment. The FDA's Guidance for Industry; Pyrogen and Endotoxins: Questions and Answers concludes that the ability to detect endotoxins can be affected by sample storage and handling.

The preferred sample collection container should not interfere with endotoxin recovery (Arbesser-Rastburg, et al., 2015) but endotoxin has been shown to adsorb to surfaces (Twohy, 1986). Thus, we suggest containers where the fluid-contact surface is predictable and controllable so that incidence of endotoxin binding is limited.

This study measured recovery of CSE from rigid containers constructed of polystyrene, polycarbonate, polypropylene and polyethylene terephthalate (PETG) at four different time points across 24 hours. The goal of this study is to expand on the knowledge base of endotoxin recovery from sample containers and inform best practices for the most accurate results.

Endotoxin Monitoring in Mammalian Monoclonal Antibody Process

A monoclonal antibody (mAb) production process is divided into upstream and downstream operations. Upstream operations include production of the protein and initial protein recovery steps. Downstream operations culminate with the final drug product after chromatography, viral clearance, ultrafiltration, diafiltration and formulation processes. Buffers and media and other additives are required throughout the process.

In-process monitoring of endotoxin is required to demonstrate process control and react to conditions which could cause the final dosage to exceed the endotoxin safety limits. A generic sampling plan is illustrated. The plan includes suggested practices at each process step.

Example Endotox in Sampling Plan

Media
- Sample and assay from a statistically appropriate number of batches to establish maximum hold-times of bioreactor media
- Sample and assay each batch if complex or multi-preparation processes
- Sample and assay periodically or after extend shutdowns

Bioreactor | Cell Culture
- Endotoxin is not typically monitored

Chromatography and UF | DF
- Sample and assay from a statistically appropriate number of batches
- Sample and assay WFI rinse after removal of storage solution, after pre-use sanitization and prior to storage
- Sample and assay equilibration buffer prior to concentration
- Sample and assay each pooled protein batch

Buffer

Filtered Buffer
- Sample and assay from a statistically appropriate number of batches

Non- filtered, Diafiltration & Formulation Buffer
- Sample and assay each batch prior to use

Cell Harvest
- Sample and assay each batch after clarification steps

Final Drug Product
- Sample and assay each filtered drug substance batch

(adapted from Arbesser-Rastburg, et al., 2015)

Materials and Methods

The following describes the collection containers included in this study.

Description	Quantity
15 mL Polystyrene Centrifuge Tube	4 lots, 3 units from each lot
60 mL Polycarbonate Bottle	4 lots, 3 units from each lot
50 mL Polypropylene Centrifuge Tube	4 lots, 3 units from each lot
60 mL PETG Bottle	4 lots, 3 units from each lot

Baseline Testing

3 units from one lot of collection containers were filled with WFI heated to 36 °–38 °C and extracted for 60 minutes at 18 °–25 °C. Containers were inverted 5–10 times at the end of the extraction time. The extraction liquid was pooled for testing using Kinetic Turbidimetric test method with an assay sensitivity of 0.005 EU/mL.

Description	Extraction Volume	Detected Endotoxin Level	PPC Recovery
15 mL Polystyrene Centrifuge Tube	15 mL	<0.005 EU/mL	112%
60 mL Polycarbonate Bottle	60 mL	<0.005 EU/mL	107%
50 mL Polypropylene Centrifuge Tube	50 mL	<0.005 EU/mL	119%
60 mL PETG Bottle	60 mL	<0.005 EU/mL	108%

All units from baseline testing have positive product control (PPC) within 50%–200% indicating the test solution is free of endotoxin inhibiting or enhancing factors and the study is valid.

Endotoxin levels in baseline testing are below detection limits indicating that the sample containers are endotoxin (pyrogen) free.

Inoculum Preparation

1 vial of CSE (lot number EM54512) was reconstituted with 7 mL water for injection (WFI) and vortexed for 5 minutes to create a solution with a concentration of 1,000 EU/mL.

1 mL of the concentrated CSE solution was added to 1,999 mL of WFI in a depyrogenated glass container to create the 0.5 EU/mL inoculum solution. The solution was mixed with a depyrogenated magnetic stir bar and stored at 18 °C–25 °C. 2 replicates of the solution were prepared.

Inoculation Testing

Test articles were inoculated with the 0.5 EU/mL inoculum solution at the prescribed extraction volume and incubated at 18 °C–25 °C.

Generally recommended practices suggest samples collected during a production process be assayed within 24 hours of sample collection (Arbesser-Rastburg, et al., 2015). At 1 hr, 6 hr, 12 hr and 24 hr after inoculation, each container was inverted 5–10 times and a 1 mL sample was collected. 3 units from each lot of each test article were pooled for testing. The pooled samples were tested using Kinetic Turbidimetric test method with an assay sensitivity of 0.005 EU/mL.

As a control reference, the glass inoculum preparation container was shaken for 60 seconds and a sample was tested using Kinetic Turbidimetric test method with an assay sensitivity of 0.005 EU/mL at each time point.

The Takeone® is a pre-assembled and single-use aseptic sampling system. Samples collected using Takeone® are perfectly representative and aseptically closed from the environment.
Takeone® may be configured with a variety of sample collection containers including; polystyrene or polypropylene centrifuge tubes, PETG or PC bottles and Flexboy® bags.

Description	Extraction Volume	1 hr EU/mL	1 hr PPC	6 hr EU/mL	6 hr PPC	12 hr EU/mL	12 hr PPC	24 hr EU/mL	24 hr PPC
15 mL Polystyrene Centrifuge Tube	15 mL	0.462	100%	0.418	104%	0.393	90%	0.468	83%
15 mL Polystyrene Centrifuge Tube	15 mL	0.467	131%	0.412	98%	0.397	88%	0.364	87%
15 mL Polystyrene Centrifuge Tube	15 mL	0.507	95%	0.399	119%	0.425	95%	0.374	92%
60 mL Polycarbonate Bottle	60 mL	0.505	92%	0.436	121%	0.329	87%	0.379	95%
60 mL Polycarbonate Bottle	60 mL	0.477	91%	0.411	82%	0.339	77%	0.338	87%
60 mL Polycarbonate Bottle	60 mL	0.475	109%	0.385	97%	0.366	86%	0.373	93%
50 mL Polypropylene Centrifuge Tube	50 mL	0.420	105%	0.391	122%	0.332	77%	0.310	72%
50 mL Polypropylene Centrifuge Tube	50 mL	0.508	97%	0.448	74%	0.425	96%	0.350	90%
50 mL Polypropylene Centrifuge Tube	50 mL	0.407	86%	0.369	90%	0.386	87%	0.371	83%
60 mL PETG Bottle	60 mL	0.405	84%	0.406	96%	0.467	98%	0.515	93%
60 mL PETG Bottle	60 mL	0.407	93%	0.472	99%	0.386	88%	0.459	103%
60 mL PETG Bottle	60 mL	0.493	105%	0.415	84%	0.384	72%	0.423	107%
Glassware Control (inoculum bottle)		0.464	97%	0.407	82%	0.398	88%	0.440	88%

All units from inoculum testing have PPC within 50% – 200% indicating the test solution is free of endotoxin inhibiting or enhancing factors and the study is valid.

Results

The baseline testing finds that each container is endotoxin free. This supports that any of the sampling containers are suitable for endotoxin sample collection (Association for the Advancement of Medical Instrumentation, 2011).

Recovery nearer to the 0.5 EU/mL inoculum solution concentration suggests it is a preferred sample collection container. To better visualize and interpret the data the percent endotoxin recovered was calculated using the average of detected endotoxin for each container type at each time point.

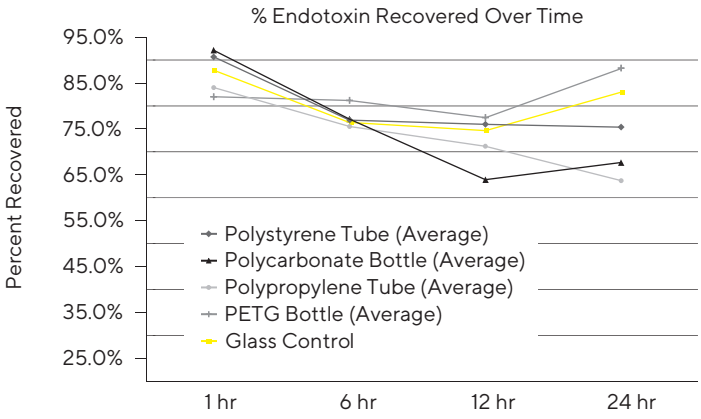

Figure 1: *Average Endotoxin Recovered*
Containers were spiked with 0.5 EU/mL endotoxin solution.

Polycarbonate bottles and polystyrene tubes had the best recovery up to 1 hour after sampling, even compared to glass control.

Polystyrene tubes and PETG bottles exhibited better recovery than polycarbonate beyond 6 hours.

Polypropylene tubes and polycarbonate bottles showed significant drops in recovery over time.

The data reports that PETG had the most stable recovery levels over time. The standard deviation of the average of the endotoxin recovery across all time points for PETG is 0.019 compared to 0.032 for polystyrene and 0.054 for polycarbonate.

Description	1 hr EU/mL	6 hr EU/mL	12 hr EU/mL	24 hr EU/mL	St. Dev.
15 mL Polystyrene Centrifuge Tube (Average)	0.479	0.410	0.405	0.402	0.032
60 mL Polycarbonate Bottle (Average)	0.486	0.411	0.345	0.363	0.054
60 mL PETG Bottle (Average)	0.435	0.431	0.412	0.466	0.019

However, the consistency of results between different samples of the same container type is best with polystyrene. The standard deviation of polystyrene tubes assayed at each time point was the lower than PETG (except at 24 hr) and lower than polycarbonate (except at 1 hr).

Description	1 hr EU/mL	6 hr EU/mL	12 hr EU/mL	24 hr EU/mL
15 mL Polystyrene Centrifuge Tube (St. Dev.)	0.025	0.010	0.017	0.057
60 mL Polycarbonate Bottle (St. Dev.)	0.017	0.026	0.019	0.022
60 mL PETG Bottle (St. Dev.)	0.050	0.036	0.047	0.046

Conclusion

Recovery efficiency of endotoxin varies upon on a number of factors, including but not limited to; plastic resin manufacturer, sample container manufacturer, sample container manufacturing lot and endotoxin species (Associates of Cape Cod, Inc., 1988). Adequate safety factors established by the FDA mitigate the risk to patients when achieving less than 100% recovery in a bacterial endotoxins test (Association for the Advancement of Medical Instrumentation, 2011).

Polycarbonate or polystyrene collection containers are suggested for the most accurate endotoxin assay results when the assay is conducted within 6 hours of sampling event.

PETG or polystyrene collection containers are suggested for the most consistent endotoxin assay results when assay is conducted at variable times up to 24 hours of the sampling event.

Because of its relatively high recovery and stability over time, polystyrene is suggested as the preferred sample collection container for endotoxin assay. Indeed, polystyrene has been noted in other publications as a preferred sample container for endotoxin assays (Arbesser-Rastburg, et al., 2015) (Associates of Cape Cod, Inc., 1988).

There is a significant change in recovered endotoxin across most materials between 1 and 6 hours after inoculation. Thus, an assay for endotoxin should be conducted as soon as possible, preferably within 1 hour of sample collection.

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