

Sterility Validation and Testing of a Liquid Sampling System

Frank S. Kohn

A study was designed to validate that the NovaSeptum liquid sampling unit (NovAseptic Equipment AB, www.novaseptic.se) does not inhibit the recovery of microorganisms in bioprocess applications. The intention is also to validate our ability to hold samples sterile and perform a sterility test using the Millipore (www.millipore.com) Steritest method and the NovaSeptum liquid sampling system. In this study, a sample analysis of one lot of sterile disposable sampling units for liquid sampling (NovaSeptum bags) was subjected to bacteriostasis and fungistasis testing, and three lots were tested for sterility using the Millipore Steritest method. All laboratory data generated for this study report are in compliance with good laboratory practices (21 CFR Part 58)

PRODUCT FOCUS: PRODUCTS OF FERMENTATION AND CELL CULTURE

PROCESS FOCUS: PRODUCTION AND PROCESSING

WHO SHOULD READ: QA/QC, MANUFACTURING, PROCESS DEVELOPERS, ANALYTICAL LABORATORY SCIENTISTS

KEYWORDS: SAMPLING, STERILITY TESTING, BIOBURDEN TESTING

LEVEL: BIOTECH BASICS

MATERIALS AND EQUIPMENT

MEDIA

Fluid A (Becton Dickinson and Company, www.bd.com)
 Fluid thioglycollate medium (BioMerieux, www.biomerieux.com)
 Tryptic soy broth (BioMerieux, www.biomerieux.com)
 Tryptic soy agar (MicroDiagnostics, www.microdiagnostics.de)
 Sabouraud dextrose agar (MicroDiagnostics, www.microdiagnostics.de)

ORGANISMS

Clostridium sporogenes ATCC #11437 (Chrisope Technologies, Lake Charles, LA)
Staphylococcus aureus ATCC #6538 (Chrisope Technologies, Lake Charles, LA)
Pseudomonas aeruginosa ATCC #9027 (Chrisope Technologies, Lake Charles, LA)
Bacillus subtilis ATCC #6633 (Chrisope Technologies, Lake Charles, LA)
Candida albicans ATCC #10231 (Chrisope Technologies, Lake Charles, LA)
Aspergillus niger ATCC #16404 (Chrisope Technologies, Lake Charles, LA)

EQUIPMENT

Steritest Compact System (Millipore Corporation, www.millipore.com)
 Biosafety cabinet model NU-425-600 (Nuair, www.nuair.com)
 30–35 °C incubator model 307A (Fisher Scientific International, www.fishersci.com)
 20–25 °C incubator model 307A (Fisher Scientific International, www.fishersci.com)
 35–37 °C incubator model 307A (Fisher Scientific International, www.fishersci.com)
 Waterbath model 101M (Fisher Scientific International, www.fishersci.com)
 Microwave model R-320EQ (Sharp Electronics Corp., www.sharp-usa.com)

MATERIALS

Steritest canister, Catalog #TTHA LAZ 10 (Millipore Corporation, www.millipore.com)
 BBL Gas Pak Pouch (Becton Dickinson and Company, www.bd.com)
 Water for Injection (Abbott Laboratories, abbot.com)

Method: USP 25 <71> Sterility Tests				
Organism	Reference Plates CFUs* (Plate 1/Plate 2)	Medium	Specification	Result
<i>Staphylococcus aureus</i>	12/11	FTM (with product) FTM (without product)	Growth	Growth
<i>Pseudomonas aeruginosa</i>	26/15	FTM (with product) FTM (without product)	Growth	Growth
<i>Bacillus subtilis</i>	9/12	FTM (with product) FTM (without product)	Growth	Growth
<i>Clostridium sporogenes</i>	8/13	FTM (with product) FTM (without product)	Growth	Growth
<i>Aspergillus niger</i>	46/50	FTM (with product) FTM (without product)	Growth	Growth
<i>Candida albicans</i>	13/20	FTM (with product) FTM (without product)	Growth	Growth

*CFU = colony-forming units



NovAseptic sample device and Millipore Steritest. MILLIPORE CORPORATION (WWW.MILLIPORE.COM)



Connecting the NovAseptic bag with the Steritest instrument. MILLIPORE CORPORATION (WWW.MILLIPORE.COM)

and good manufacturing practices (21 CFR 211).

EXPERIMENT

The sterility test was validated by performing the bacteriostasis/fungistasis test. This test is intended to demonstrate the reproducibility of a method in recovering microorganisms and to indicate whether a product or sampling container interferes with their recovery. Such testing demonstrates the ability of a sampling method in recovering each type of microorganism according to the USP (*United States Pharmacopeia*) sampling procedure (1). The product is

challenged with six organisms: *Bacillus subtilis*, *Candida albicans*, *Aspergillus niger*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Clostridium sporogenes*. If all six organisms can be recovered under all conditions of growth, then the sampling method is considered valid. The sample system used is a sterile disposable sampling unit for liquid sampling, consisting of a plastic sampling bag and septum unit, two tubes, a pinch pipe, and a Luer lock.

Procedures: All testing was performed in a biosafety cabinet. All materials were disinfected with 10%

sodium hypochloride before being moved into that cabinet. The photos to the right show the NovaSeptum sampling device and the Millipore Steritest unit. In preparation for the bacteriostasis/fungistasis test, six bags were filled with 25 mL of sterile water for injection (WFI) through the tubing with the green end (the second photo illustrates the connection of a NovaSeptum bag with the Steritest unit). All bags were incubated at 20–25 °C for 16–24 hours.

Bacteriostasis/fungistasis testing was performed following USP 25 <71> sterility tests (2). For each test organism, the liquid in one bag was filtered using the septum outlet of the bag through one Steritest canister filter. The filters were rinsed with three 100-mL rinses of Difco Fluid A (Becton Dickinson and Company, www.bd.com), the last rinse containing the test organism. With fluid thioglycollate medium (FTM), the following organisms were used:

Clostridium sporogenes, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. With tryptic soy broth (TSB), the following organisms were used: *Bacillus subtilis*, *Candida albicans*, and *Aspergillus niger*. FTM canisters were incubated at 30–35 °C for no more than seven days, and TSB was incubated at 20–25 °C for no more than seven days. Growth of each organism was compared to that of a positive control (containing no sample). Plate counts for each organism were performed in duplicate to confirm inoculation levels at <100 cfu (colony-forming units).

Before testing, each plastic bag was filled with approximately 25 mL of sterile WFI. The bags were filled through the tubing with the green end. Filled bags were placed at 20–25 °C for 16–24 hours before testing. Testing was then performed using the membrane filtration method and the Millipore Steritest system. For testing, the fluid in 10 bags was removed through the septum outlet and filtered through two Steritest canister filters, which were rinsed with 300 mL of Fluid A. Then one canister was filled with 100 mL of FTM, and the other was filled with 100 mL of TSB. Negative controls were prepared by performing the testing without the sample. FTM canisters were

Table 2: Sterility testing

Product: Sterile disposable sampling unit for liquid sampling (NovAseptum Bags) **Storage:** Ambient

Packaging: Plastic container with a paper peel-off closure, 5 sampling units per container

Method: USP 25<71> Sterility Tests

Lot Number: 011017-02

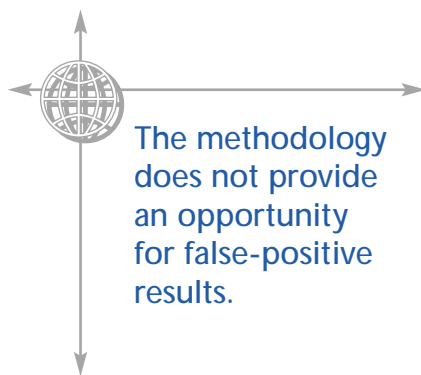
Media	Specification	Result
Tryptic Soy Broth (with product)	Report Results	No growth
Fluid Thioglycollate (with product)	Report Results	No Growth

Lot Number: 011113-01

Media	Specification	Result
Tryptic Soy Broth (with product)	Report Results	No growth
Fluid Thioglycollate (with product)	Report Results	No Growth

Lot Number: 01113-02

Media	Specification	Result
Tryptic Soy Broth (with product)	Report Results	No growth
Fluid Thioglycollate (with product)	Report Results	No Growth



The methodology does not provide an opportunity for false-positive results.

incubated at 30–35 °C for at least 14 days, and the TSB canisters were incubated at 20–25 °C for at least 14 days. After incubation, all canisters were examined for signs of microorganism growth in the media.

RESULTS AND DISCUSSION

Table 1 lists the results of bacteriostasis/fungistasis testing, and Table 2 lists the results of sterility testing. As shown, this study documents that microbial recovery from inoculated product is comparable throughout the incubation period. Our testing method demonstrated that the methodology

does not provide an opportunity for a false-positive result caused by growth inhibitor in the product (WFI) or in the sampling device. Lot 011017-02 of the sterile, disposable sampling unit (NovaSeptum Bags) for liquid sampling passed bacteriostasis/fungistasis testing. Lots 011017-02, 011113-01, and 011113-02 passed sterility testing. The study validated that the NovAseptum sampling unit does not inhibit growth of various microorganisms. Also, the study validated our ability to keep samples sterile and test sample solutions for sterility using the Steritest method.

REFERENCES

- 1 *United States Pharmacopeia–National Formulary* (USP 26–NF 21); Rockville, MD.
- 2 USP 25 <71> Sterility Tests; in the *United States Pharmacopeia–National Formulary*.

Frank S. Kohn, PhD, is president of FSK Associates, Inc., Manson, Iowa, an independent consulting company serving the biotechnology industry; fsk@iowatelecom.net.