Yeast and Mold - Finished Herbal Products - In this case only two levels of inoculation were used; one below spec and one above the spec level. Two samples were positive by the Soleris system and negative by the plate count method. One sample had a false DT in the instrument while the other sample was inoculated and was part of an inoculated batch, therefore it was supposed to be positive by the plate count method, but was negative due to an operator error. Based upon the use of the USP method as a gold standard, the accuracy was 95%; the specificity was 91.3% and the sensitivity was 95%, however, based upon the resolution of all discrepancies, the accuracy was 97.5%, and the specificity was 95.5%. The USP method had similar values.

Enterobacteriaceae Raw Materials - All the samples tested were naturally contaminated at a level above the spec level.

Result Summary

- The Soleris system provides a rapid and convenient alternative to conventional USP plate count methodology.
- Samples were diluted so that only samples with counts above the specified level were detectable by the instrument.
- The samples that did not contain organisms above the specified level by USP plating methodology were not detected by the instrument.
- There was a high correlation between the results obtained by the standard USP method and the Soleris instrument, resulting in high accuracy, specificity and sensitivity.
- Overall, the system would work well to increase speed of results, decrease hands-on labor and reduce operator error.

References

1. ANSI/NSF Standard 173 – 2005. Dietary Supplements.

2. USP, United States Pharmacopeia, USP 28-NF, 23, 19.

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Investigation into the Efficacy of Utilizing the Soleris™ System as an Alternate Technology to USP Plating Methodology for Evaluating Microbiological Contamination in Nutraceutical Products

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NSF INTERNATIONAL NSF SINCE 1944™

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Abstract

In today's marketplace, quality in production is extremely important to the nutraceutical and food industries. Currently, USP plating methodology is being used to test for bacterial contamination in dietary supplement finished products and raw materials. Although accurate in assessing the microbial load of a given product, the methods are costly and time intensive. Throughout the food industry, rapid microbiological testing methods are being developed and implemented to validate "Hazard Analysis Critical Control Point" (HACCP) programs along with other quality management systems. One of these rapid testing systems is the new Soleris system which uses state-of-the-art automated optical technology to quickly and accurately obtain qualitative and/or quantitative results for bacterial contamination in various nutraceutical samples. Quantitative determination of CFU/g in a specific food type or testing against a specific organism threshold via the "dilute to spec" approach may be utilized. This spec level approach allows for accurate and faster results in the testing of a variety of nutraceutical samples.

NSF International conducted a validation study designed to compare the USP plating methods and the Soleris "dilute to spec" protocol. Side-by-side testing was performed on samples spiked with a natural existing organism found in representative sample types. Four assay types were evaluated: total aerobic bacterial count, total yeast, total mold, and Enterobacteriaceae. Several different matrixes were tested for the assays: Multi-vitamins; finished herbal product; soft-gel fish oil capsules; raw material. The study design allows evaluating the sensitivity of each method as well as the consistency among analysts. Each sample organism combination was tested 30 times by each method, by two different analysts (for a total of 60 samples at three levels of contamination). A total of 720 samples were tested, yielding an accuracy of 98.5%; specificity of 97.3%; and a sensitivity of 99.1%. Based on the data sets thus far, the results indicated good correlation between the methods for the assays tested.

Introduction

The FDA has proposed new regulations to require "current good manufacturing practices" (cGMP) in the industry to help reduce risks associated with adulterated or misbranded dietary supplement products. Microbial contamination is one of the issues that will be addressed by the new FDA regulations, as some recalls were associated with microbiological problems. Faster and more streamlined microbiological tests are required by this industry to meet the new challenges.

The Soleris system (previously BioSys), using its rapid optical technology can deliver microbiological test results in hours not days, providing significant benefit to the operation of manufacturing companies, including providing a risk management solution; internalizing the microbiological procedures; addressing an increase in production demand and increasing laboratory workload; and alerting personnel to contamination problems sooner.

Materials

Vials, culture media and reagents used:

- **Enterobacteriaceae Medium**
- **TVC Medium**
- **Mold and Yeast Medium**
- **Tryptic Soy Agar (TSA)**
- **Potato Dextrose Agar (PDA)**
- **Levine Eosin-Methylene Blue Agar (L-EMB)**
- **Butterfield's Phosphate Buffer (BPB)**
- **Sterile Buffer Dilution Water (SBDW)**

Table 1. *Sample types and test spec levels as defined by ANSI/NSF 173.*

Product	TPC	Yeast and Mold	Enterobacteriaceae
Multi-Vitamins	$<3.0 \times 10^3$	$<3.0 \times 10^2$	$<1.0 \times 10^2$
Soft-Gel Fish Oil Capsules	$<1.0 \times 10^5$	$<1.0 \times 10^4$	$<1.0 \times 10^2$
Finished Herbal Products	$<1.0 \times 10^5$	$<1.0 \times 10^4$	$<1.0 \times 10^2$
Raw Material	$<1.0 \times 10^7$	$<1.0 \times 10^5$	$<1.0 \times 10^3$

Methods

Instrument Description

The Soleris system (**Figure 1**) measures microbial growth by monitoring changes in pH or other biochemical reactions, which result in a color change as the microorganisms in the broth grow and metabolize. Samples are inoculated in ready-for-use vials that contain broth specific for the desired assay. The changes in color in the agar mirror, or reflect the changes in color of the broth, without letting the sample particles or turbidity influence the measurements.

Light from light-emitting diodes passes through the agar and a photo diode on the other side of the vial reads the color change as microbial growth occurs (**Figure 2**). A measurement is taken every six minutes. As soon as a color change is detected, the time of such detection is recorded (**Figure 3**). Detection time (DT) readings are inversely related to the number of organisms in the sample. The Soleris 128 is capable of monitoring 128 samples simultaneously, divided into four separate incubators, each capable of maintaining temperatures in the range of 15–60°C.

Figure 1. *Soleris 128 unit, 128 samples, four independent incubators.*

Figure 2. *Mechanism for assaying microbial growth via a light emitting diode detection system.*

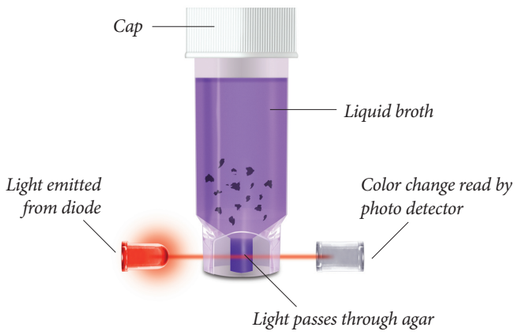
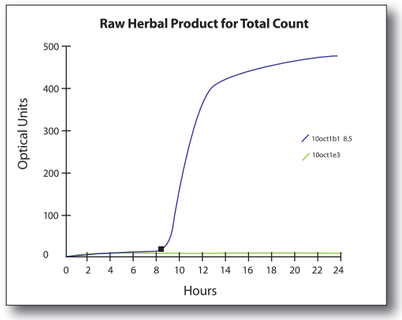


Figure 3. *Example growth curve on Soleris system.*



Protocol

A. Sample Preparation / Vial Inoculation

A 1:10 dilution of each sample is prepared using 10 g of sample in 90 mL of SBDW.

B. Dilute to Spec Approach

The sample amount that is inoculated into each vial is adjusted so that only samples with CFU (colony forming units) counts above the specification level will detect. This dilution scheme can be adjusted for the detection of lower levels of contamination than the specified level. By doing so, the new dilution scheme allows for a "caution" detection of samples that are not yet at the specified level but are approaching such a level. The dilution scheme and sample volume added per media vial is detailed in **Table 2**.

Table 2 (A-C). *Dilution scheme and sample spike volumes for the "dilute to spec" approach following an initial 1:10 dilution. The initial 1:10 dilution of the samples was prepared by adding 10 g of sample to 90 mL of SBDW. Further dilutions were made using BPB.*

2A: Total Viable Count Vial

Product	Dilution	Volume
Multi-Vitamins	1:1,000	200 µL
Soft-Gel Fish Oil Capsules	1:1,000	10 µL
Finished Herbal Products	1:1,000	10 µL
Raw Material	1:100,000	10 µL

2B: Enterobacteriaceae Vials

Product	Dilution	Volume
Multi-Vitamins	1:10	100 µL
Soft-Gel Fish Oil Capsules	1:10	100 µL
Finished Herbal Products	1:10	100 µL
Raw Material	1:1,000	1,000 µL

2C: Yeast and Mold Vials

Product	Dilution	Volume
Multi-Vitamins	1:100	200 µL
Soft-Gel Fish Oil Capsules	1:1,000	100 µL
Finished Herbal Products	1:1,000	100 µL
Raw Material	1:100,000	1,000 µL

Experimental Design

For each sample assay combination, 60 samples were tested by both the USP plating methodology and the Soleris instrument methodology using the dilute to spec approach. Twenty samples were uninoculated; 20 were inoculated at a level that ideally was just above the specified level for the product; and the other 20 samples were inoculated at least 10 times the specified level. All organisms used for inoculation were originally isolated from nutraceutical products. The 20 samples of each level were analyzed by two analysts. Therefore, each analyst analyzed 30 of the 60 samples. All samples were randomized after their inoculation and presented to the analysts in random order.

Data Analysis

The results of the data obtained were divided into four quadrants as shown in **Table 3**.

Table 3. *Quadrant scheme for comparative analysis of Soleris and USP data.*

	Reference +	Reference -
Alternative +	+/+ Positive agreement (PA)	-/+ Positive deviation (PD)
Alternative -	+/- Negative deviation (ND)	-/- Negative agreement (NA)

Accuracy (AC) defined as the degree of correspondence obtained by reference method (USP plating methodology) and the response obtained by alternative method (Soleris) on identical samples, is defined as follows: AC = (PA+NA)/N * 100%.

Specificity (SP) defined as the ability of alternative method to not detect analyte when it is not detected by reference method, relative to the reference method, is defined as: SP = NA/(NA+PD) * 100%.

Sensitivity (SE) defined as the ability of alternative method to detect analyte when it is detected by reference method, is defined as: SE = PA/(PA+ND) * 100%.

Results and Observations

The results for the comparative analysis for all three assay types are presented in **Tables 4–6**. A statistical summary of the comparison study is presented in **Table 7**.

Table 4. *TVC data comparative analysis.*

Finished Herbal Products Spec Level 1.0E+05			Fish Body Oil Spec Level 1.0E+05		
	USP +	USP -		USP +	USP -
Soleris +	42	0	Soleris +	42	1
Soleris -	0	18	Soleris -	0	17
Accuracy 100%	Specificity 100%	Sensitivity 100%	Accuracy 98.3%	Specificity 94.4%	Sensitivity 100%
Multi-Vitamins Spec Level 3.0E+03			**Raw Materials - Echinacea Spec Level 1.0E+07**		
	USP +	USP -		USP +	USP -
Soleris +	40	0	Soleris +	44	2
Soleris -	0	20	Soleris -	0	14
Accuracy 100%	Specificity 100%	Sensitivity 100%	Accuracy 96.7%	Specificity 87.5%	Sensitivity 100%

Table 5. *Yeast and Mold data comparative analysis.*

Finished Herbal Products Spec Level 1.0E+04			Fish Body Oil Spec Level 1.0E+05		
	USP +	USP -		USP +	USP -
Soleris +	17	2	Soleris +	39	2
Soleris -	0	21	Soleris -	0	19
Accuracy 97.5%	Specificity 95.5%	Sensitivity 100%	Accuracy 98.3%	Specificity 95.0%	Sensitivity 100%
Multi-Vitamins Spec Level 3.0E+03			**Raw Materials - Echinacea Spec Level 1.0E+07**		
	USP +	USP -		USP +	USP -
Soleris +	40	0	Soleris +	20	0
Soleris -	0	20	Soleris -	0	40
Accuracy 100%	Specificity 100%	Sensitivity 100%	Accuracy 100%	Specificity 100%	Sensitivity 100%

Table 6. *Enterobacteriaceae data comparative analysis.*

Finished Herbal Products Spec Level 1.0E+04			Fish Body Oil Spec Level 1.0E+05		
	USP +	USP -		USP +	USP -
Soleris +	40	0	Soleris +	30	0
Soleris -	0	20	Soleris -	1	20
Accuracy 100%	Specificity 100%	Sensitivity 100%	Accuracy 98.0%	Specificity 100%	Sensitivity 96.8%
Multi-Vitamins Spec Level 3.0E+03			**Raw Materials - Echinacea Spec Level 1.0E+07**		
	USP +	USP -		USP +	USP -
Soleris +	36	0	Soleris +	60	0
Soleris -	3	21	Soleris -	0	0
Accuracy 100%	Specificity 100%	Sensitivity 100%	Accuracy 100%	Specificity 100%	Sensitivity 100%

Table 7. *Summary of total comparative analysis between USP and Soleris.*

Assay Type	Accuracy	Specificity	Sensitivity
TVC	98.8%	95.8%	100%
Yeast and Mold	98.3%	96.8%	100%
Enterobacteriaceae	98.3%	100%	97.8%

The following are observations pertaining to each applicable study:

TVC Finished Herbal Products - Two naturally contaminated samples that were supposed to be below the spec level were found to be above the spec level by both methods.

TVC Fish Body Oil - Two samples were contaminated during processing, and they were above spec by both methods. One uninoculated sample had counts below spec but had a detection time (DT) in the vial. The vial did contain organisms. This was probably due to an operator error.

TVC Multi-Vitamins - Four of the natural samples were contaminated by both methods. Two of the samples were contaminated with the inoculated mixture whereas the second two were contaminated with *Bacillus*. Two additional samples had the vials contaminated, however, the plates were clean.