Soleris® system evaluation of testing applications for UHT/aseptic packs

September 2012

Summary

Neogen Corporation (Lansing, Mich.) conducted internal studies to validate optimal test procedures for the Soleris ultrahigh temperature (UHT) and aseptic pack test system. The Soleris system used with NF-105 vials was shown to be more sensitive than traditional plating methods for UHT/sterility-based products. The increased sensitivity allows for positives to be detected an average of three days sooner as compared to plates. This allows for faster turnaround time and increased inventory turnover.

Soleris assay principles

Soleris technology monitors changes in the chemical characteristics of microbial liquid growth medium and detects microorganisms with carbon dioxide (CO_2) sensitive reagents. The reagents change their optical patterns as the metabolic process takes place. These changes are detected photometrically by an optical reader and monitored every six minutes.

Soleris nonfermenting total viable count (NF-105) medium vials allow for greater inclusivity with a shorter detection time in sterility testing. As organisms grow in the broth medium, the CO_2 produced diffuses through a membrane layer into a soft agar plug containing a dye indicator. The color change in the dye is read by the Soleris instrument. The membrane layer also serves as a barrier, eliminating product interference within the reading frame.

NF-105 vials are used as a semiquantitative method for detection of any heterotrophic bacterial microorganism. This makes Soleris a valuable diagnostic tool for real-time detection of a wide range of microorganisms in a variety of products.

Protocol

The study used an injury procedure to mimic the effects of UHT/aseptic processing on microorganisms. Samples of UHT/aseptically packaged products were inoculated with heat and peroxide-injured *Staphylococcus warneri, Pseudomonas aeruginosa* and *Bacillus circulans,* which historically have been associated with UHT/aseptic packaged product deviations. The heat and peroxide exposure was varied between each organism to achieve the desired sub-lethal injury measurement. [1]

[1] Applied and Environmental Microbiology, December 2005, p. 7661-7669, Vol. 71, No. 12

The goal was to achieve fractional positives, which then were adjusted for Poisson distribution. This allowed for the use of larger inoculation volume with a low amount of contamination.

Each organism underwent an injury protocol specific to the organism:

1. *Staphylococcus warneri* was tested as noninjured, heat injured and peroxide injured.
2. *Pseudomonas aeruginosa* was tested as noninjured, heat injured and peroxide injured.
3. *Bacillus circulans* was tested as noninjured and heat injured.

Calculations were performed as follows:

Log_{10} reduction (LR) = Mean Log_{10} Initial - Mean Log_{10} Surviving
Initial = Washed cells before heat kill
Percent reduction (%) = 100 X (1 - 10^-LR)
Percent sublethal injury (%) = (Cell count of nonselective - cell count selective) / (cell count nonselective) X 100%

Aseptic protocol testing

Following peroxide or heat injury, the organism was inoculated into the sample aseptic product cartons. At the appropriate holding time (2, 3, 6, or 10 days), each product sample was used to inoculate five Soleris NF-105 5 mL vials (a total of 50 vials). Each product sample (10 µL) also was streaked to TSA plates for confirmation at 30°C for three days. At each time interval, product samples that produced an above specification result were removed from the testing field.

Soleris

1. At each day interval (2, 3, 6 and 10), remove 5 mL from each product sample carton and add each 5 mL sample to a new NF-105 vial. Repeat this procedure four more times so five vials have been inoculated.
2. Test at 30°C for 48 hours in the Soleris unit.

Plating

1. At each day interval (2, 3, 6 and 10), remove five, 10 µL samples from each product sample carton and streak each 10 µL sample to TSA plates.
2. Incubate TSA plates at 30°C for 72 hours.

Results

The Soleris method was shown to provide quicker results and was found to be more sensitive than traditional plating methods for measuring microorganism growth in UHT products.

Staphylococcus

Staphylococcus was injured using a peroxide solution to achieve an injury rate of >50%. Sublethal injury was recorded at approximately 61%. After three days of incubation, 1 cfu/bottle was detected in all Soleris vials. Soleris NF-105 vials also consistently showed higher growth rates than plating methods. Therefore, it was found that Soleris vials are more sensitive and can detect growth earlier than plating methods.

Confirmed *Staphylococcus* growth – peroxide injured

	5 mL from dilution 6		5 mL from dilution 7		5 mL from dilution 8	
Days of pre-inc.	**Plate growth**	**Vial growth**	**Plate growth**	**Vial growth**	**Plate growth**	**Vial growth**
2	96% (48/50)	100% (50/50)	32% (16/50)	56% (28/50)	10% (5/50)	30% (15/50)
3	NA	NA	96% (48/50)	100% (50/50)	28% (14/50)	42% (21/50)
6	NA	NA	NA	NA	48% (24/50)	50% (25/50)
10	NA	NA	NA	NA	48% (24/50)	50% (25/50)

Staphylococcus was heat injured, achieving a sublethal injury rate of 89.33%. The Soleris vial performed better than the traditional plating method in detecting *Staphylococcus*.

Confirmed *Staphylococcus* growth – heat injured

	Heat injured				Uninoculated control	
Days of pre-inc.	**Plate growth**	**Vial growth**	**Plate growth**	**Vial growth**	**Plate growth**	**Vial growth**
	5 mL from dilution 8		**5 mL from dilution 9**			
2	40% (20/50)	60% (30/50)	10% (5/50)	10% (5/50)	NA	NA
3	66% (30/45)	66% (30/45)	10% (5/50)	10% (5/50)	NA	NA
6	77% (35/45)	77% (35/45)	11% (5/45)	11% (5/45)	NA	NA
10	86% (39/45)	88% (40/45)	14% (5/35)	14% (5/35)	0% (0/50)	0% (0/50)

Pseudomonas aeruginosa

Pseudomonas' sublethal injury rate was 83%, with the bacteria naturally exhibiting a 32% sublethal injury. The Soleris system performed as well as, and in one instance, better than traditional plating methods.

Confirmed *Pseudomonas* growth – heat injured

	Noninjured		Heat injured		Uninoculated control	
Days of pre-inc.	Plate growth	Vial growth	Plate growth	Vial growth	Plate growth	Vial growth
2	25/50 (50%)	25/50 (50%)	5/50 (10%)	7/50 (14%)	NA	NA
3	20/50 (40%)	20/50 (40%)	10/50 (20%)	10/50 (20%)	NA	NA
6	15/50 (30%)	15/50 (30%)	5/50 (10%)	5/50 (10%)	NA	NA
10	10/50 (20%)	10/50 (20%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)

Pseudomonas achieved a sublethal injury rate of 69.7%. In this study, the Soleris system performed as well as the traditional plating method.

Confirmed *Pseudomonas* growth – peroxide injured

	10% injury				Uninoculated control	
	5 mL from dilution 8		5 mL from dilution 9			
Days of pre-inc.	Plate growth	Vial growth	Plate growth	Vial growth	Plate growth	Vial growth
2	5/50 (10%)	5/50 (10%)	0/50 (0%)	0/50 (0%)	NA	NA
3	5/50 (10%)	5/50 (10%)	0/50 (0%)	0/50 (0%)	NA	NA
6	5/50 (10%)	5/50 (10%)	0/50 (0%)	0/50 (0%)	NA	NA
10	5/50 (10%)	5/50 (10%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)

Bacillus circulans

Bacillus was injured using an exposure to solution of H_2O_2. The Soleris system performed better than traditional plating methods in detecting heat-injured *Bacillus* and the same as the traditional method in detecting peroxide-injured *Bacillus*. A 92% sublethal injury level was attained from exposure to H_2O_2, exceeding the goal of >50% sublethal injury. The Soleris vial detected *Bacillus* at the same sensitivity and time frame as the traditional plating method, making it an acceptable and accurate method for detecting *Bacillus*.

Confirmed *Bacillus* growth – peroxide injured

	5 mL from dilution 7		5 mL from dilution 8	
Day	**Plate growth**	**Vial growth**	**Plate growth**	**Vial growth**
2	70% (35/50)	70% (35/50)	10% (5/50)	10% (5/50)
3	70% (35/50)	70% (35/50)	10% (5/50)	10% (5/50)
6	70% (35/50)	70% (35/50)	30% (15/50)	30% (15/50)
10	70% (35/50)	70% (35/50)	30% (15/50)	30% (15/50)

Bacillus achieved a heat injury of 67%, exceeding the goal of >50% sublethal injury. The Soleris vial detected *Bacillus* at the same sensitivity and time frame as the traditional plating method, making it an acceptable and accurate method for detecting *Bacillus*.

Confirmed *Bacillus* growth – heat injured

	5 mL from dilution 5		5 mL from dilution 6	
Day	**Plate growth**	**Vial growth**	**Plate growth**	**Vial growth**
2	20/45 (44%)	20/45 (44%)	50% (25/50)	60% (30/50)
3	30/45 (67%)	30/45 (67%)	50% (25/50)	60% (30/50)
6	30/45 (67%)	30/45 (67%)	60% (30/50)	70% (35/50)
10	30/45 (67%)	30/45 (67%)	60% (30/50)	70% (35/50)

Uninoculated

After 10 days of incubation, no contaminant was found with either method.

Summary results

The Soleris testing method was shown to have overall higher sensitivity in a shorter time frame than traditional plating methods.

Total *Staphylococcus*	
Total tests	1105
Positives with plates	688
Positives with Soleris	740
Difference	52
% of total	4.71%

Total *Pseudomonas*	
Total tests	1800
Positives with plates	425
Positives with Soleris	427
Difference	2
% of total	0.11%

Total *Bacillus*	
Total tests	1050
Positives with plates	432
Positives with Soleris	450
Difference	18
% of total	1.71%

Days of pre-inc.	Total positives with Soleris		Total positives with plates	
2	331	87%	289	76%
3	361	95%	337	89%
6	375	99%	375	99%
10	380	100%	378	99%

Statistical summary using analysis of variance (ANOVA)

Test	F value	F_{crit} value	Significantly different	Comments
ANOVA complete data set Plates vs. Soleris	12.7877	6.5914	Yes	More confirmed positives show the Soleris test is more sensitive than plates.
ANOVA plate results for day 3 Pre-inc. vs. day 2 pre-inc. results for Soleris	0.1446	3.9042	No	High sensitivity of Soleris means the same results as plates can be obtained days earlier.
ANOVA plate results for day 6 Pre-inc. vs. day 3 pre-inc. results for Soleris	0.0768	4.1300	No	High sensitivity of Soleris means the same results as plates can be obtained days earlier.

NOTE: If the F value < F_{crit} value, results are not significantly different at the 0.95 confidence interval.

Total time savings

Incubation for Soleris	Total time for Soleris	Incubation for plates	Total time for plates	Time saved using Soleris
2 days	3 days	3 days	5 days	2 days
3 days	4 days	6 days	8 days	4 days

Conclusion

The production of fractional positives show the Soleris system handled the challenge to its protocol well. In many cases, the Soleris system not only was more sensitive than the traditional plating method, but also detected bacteria in a shorter time frame. The average detection time in the Soleris vial was approximately nine hours in more than 1,400 positive results compared to the 48 hour plate.

Statistical analysis shows the Soleris method is significantly more sensitive than the traditional method. Additionally, there was not a significant difference in the three day incubation results using plates as compared to Soleris at two day incubation. Based on Soleris' average detection time of nine hours, the Soleris method provided positive results at an average time of 47 hours versus the time to positive results on plates of 120 hours.

The data generated shows the Soleris NF-105 methodologies produce more rapid results than traditional plating methods, which allow for the quicker release of products, faster turnaround time and earlier real-time detections if products demonstrate above specification requirements.

NEOGEN CORPORATION

E-mail foodsafety@neogen.com for more information on all of our testing solutions.
800/234-5333 or **517/372-9200** • www.neogen.com

