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Application of an Environmental Phage-Based Assay (Sample6 Detect HT/L) for the Detection of *Listeria* spp. in Ice Cream

ELBA VERONICA ARIAS-RIOS,

Molecular Epidemiology, Inc., 15300 Bothell Way NE, Lake Forest Park, WA 98155

KRISTINA TENNEY,

IEH Laboratories and Consulting Group, 15300 Bothell Way NE, Lake Forest Park, WA 98155

TAM MAI,

Molecular Epidemiology, Inc., 15300 Bothell Way NE, Lake Forest Park, WA 98155

SAM ANDERSON,

IEH Laboratories and Consulting Group, 15300 Bothell Way NE, Lake Forest Park, WA 98155

RUTH MARIE CANTERA,

IEH Laboratories and Consulting Group, 15300 Bothell Way NE, Lake Forest Park, WA 98155

JASMINE M. PANDO,

Molecular Epidemiology, Inc., 15300 Bothell Way NE, Lake Forest Park, WA 98155

BRANDON SELOVER,

IEH Laboratories and Consulting Group, 15300 Bothell Way NE, Lake Forest Park, WA 98155

LOURDES M. NADALA,

Molecular Epidemiology, Inc., 15300 Bothell Way NE, Lake Forest Park, WA 98155;
Microbiologique, Inc., 8215 Lake City Way NE, Seattle, WA 98155

SEANA K. DAVIDSON, and

IEH Laboratories and Consulting Group, 15300 Bothell Way NE, Lake Forest Park, WA 98155

MANSOUR SAMADPOUR¹

Molecular Epidemiology, Inc., 15300 Bothell Way NE, Lake Forest Park, WA 98155; IEH
Laboratories and Consulting Group, 15300 Bothell Way NE, Lake Forest Park, WA 98155

Abstract

Background: Dairy products are common sources of *Listeria* outbreaks, and early detection of the pathogen is critical to prevent outbreaks of illnesses and financial losses for dairy producers.

Objective: This study aimed to evaluate Sample6 Detect HT/L for effective detection of *Listeria monocytogenes* and *L. innocua* in ice cream.

Methods: Performance of the Sample6 DETECT HT/L was compared with U.S. Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM) Chapter 10 method for detection of *Listeria* spp. in ice cream using an unpaired study design.

¹Corresponding author's ms@iehinc.com.

Results: R2-enriched samples tested with Sample6 Detect HT/L performed as well as the reference method at all time points tested from 15 to 24 h. R2 is a proprietary blend for use with the test kit that helps with early detection. All the dPOD_C values (Sample6 Detect HT/L presumptive and confirmed results) equaled zero, indicating 100% concordance between the methods. Both Sample6 Detect HT/L and FDA BAM results showed low dPOD_C values, with confidence intervals indicating no significant differences between Sample6 Detect HT/L and reference method results.

Conclusions: Sample6 Detect HT/L is suitable to detect *Listeria* spp. in ice cream, even with a 12 h enrichment. Sample6 Detect HT/L demonstrated equivalent detection of *L. monocytogenes* and *L. innocua* from R2-enriched samples as expected with 15 and 18 h enrichment when compared with the 24 h FDA BAM method for *L. monocytogenes*.

Highlights: These results indicate that Sample6 Detect HT/L, primarily developed for environmental samples, can be used to detect *Listeria* spp. in ice cream with less incubation time, resulting in faster detection.

Listeria monocytogenes is a Gram-positive bacterium that causes listeriosis, a potentially life-threatening illness. Foods identified as “high risk” for contamination with *L. monocytogenes* include products that are eaten without cooking or reheating, such as soft cheeses, ice cream, and other dairy products (1). Advances in technology and sanitary practices in the 1990s reduced the prevalence of *Listeria* spp.; however, the rate of listeriosis has remained constant in the last decade, with a more severe and systemic (invasive) form occurring more frequently than previously recognized (2). A very low cell number of *L. monocytogenes* is sufficient to cause illness and may be difficult to detect in dairy products.

This study evaluates the Sample6 Detect HT/L method for effective detection of *Listeria* spp. in ice cream. Dairy products have been a common source of *L. monocytogenes* outbreaks, and from 2010 to 2015, ice cream contaminated with very low levels of *L. monocytogenes* (8 MPN/g) was linked to ten cases of listeriosis after patients were served ice cream in a hospital (3). Some of the ice cream was made into milkshakes in which *Listeria* spp. cell numbers may have increased, highlighting the need for efficient detection to avoid increases in cell numbers in products made with contaminated ice cream. In 2015, Blue Bell voluntarily recalled all products from all facilities in five states after ice cream contaminated with *L. monocytogenes* led to an outbreak that caused three fatalities and ten hospitalizations across four states (3). Early detection is critical for preventing outbreaks and financial losses for dairy product producers.

There are several procedures and commercially available assays developed for the detection of *L. monocytogenes* and other *Listeria* spp. (4–10). Additional confirmatory tests are subsequently performed on presumptive positive colonies, and the time to final confirmation can take about a week (11). The standard methods for detection of the organisms in ice cream involve adding samples to an enrichment medium and incubating for 48–96 h. This incubation can complicate detection of the targeted *Listeria* because other organisms in the food, either innate or imparted, increase during incubation (12). As a result, detection and estimation of *L. monocytogenes* CFU in different food samples may be masked by the growth of other organisms, or potentially antagonistic organisms can inhibit or outcompete

L. monocytogenes in the medium, preventing effective detection (12). For example, the *L. monocytogenes* signal in ice cream can be lost or decreased because bacilli relatives become enriched in the product. Ottesen et al. (2016) reported that during the first 8 h of ice cream enrichment, an increase, almost doubling, of *Anoxybacillus* and *Geobacillus* species may interfere with growth and detection of *L. monocytogenes* (12).

In a study on ice cream using molecular methods for detection, *Listeria* was detected after only a 24–28 h incubation, and sufficient DNA was obtained for sequencing a full *Listeria* sp. genome. This result suggested that using the 96 h incubation may not be necessary for the detection of *Listeria* spp. if alternative methods are used that do not rely on cell culture. These studies indicate that a method for rapid and accurate identification of *Listeria* spp. in food is needed, and the selected method should work for a range of food matrixes. Detection methods that minimize enrichment time required and increase specificity and sample throughput would improve detection of *L. monocytogenes* in dairy products. The Sample6 Detect HT/L Assay is a high-throughput phage-based detection system designed for the detection of *L. monocytogenes* and other *Listeria* species in environmental samples. The assay can detect *Listeria* spp. in up to 96 simultaneous samples using Sample6's proprietary BioIllumination technology and next-day pathogen detection. A swab sample may be enriched for only a few hours and then analyzed in a Sample6 instrument. This is the first report on its application in a food matrix, as we tested ice cream, which has been the subject of U.S. Food and Drug Administration (FDA) recalls because of potential contamination with *L. monocytogenes*. Sample6 Detect will provide an effective method to identify foods contaminated with *Listeria* spp. more rapidly than what is currently available (6).

Principle of the Method

The Sample6 Detect HT/L assay is a high-throughput phage-based detection system designed for the detection of *Listeria* spp. in environmental samples. A swab sample may be enriched in a proprietary R2 Medium (Microbiologique, Inc., Seattle, WA) for 12–18 h before analysis in a Sample6 instrument. The assay detects *Listeria* spp. in up to 96 simultaneous samples by specific phage binding to *Listeria* spp. Phage binding is detected using Sample6's proprietary BioIllumination™ technology and is for next-day pathogen detection.

Materials and Methods

Following the AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, Appendix J (2012; 13), the performance of the Sample6 Detect HT/L for detection of *Listeria* spp. in ice cream was compared with FDA reference culture method for *Listeria* using an unpaired study design (14). A proprietary R2 Medium (Microbiologique, Inc., Seattle, WA) was used for enrichments during the assessment, and all materials and reagents for the Sample6 Detect HT/L were obtained from Sample6, Woburn, MA.

Test Kit Information

All materials and reagents for the Sample6 Detect HT/L were obtained from Phage Diagnostics Inc., Kent, WA, following the package insert.

- a. *Kit name.*—Sample6 Detect HT/L High Throughput Pathogen Detection System for *Listeria* Environmental.
- b. *Cat. No.*—55-100-00 (96 tests), 55-101-00 (72 tests), 55-102-00 (48 tests), 55-103-00 (24 tests).
- c. *Ordering information.*—Sample6 is located at 12 Gill St, Suite 4200, Woburn, MA 01801. Customer Assistance and Technical Service can be reached between 8:00 AM and 6:00 PM Eastern Time via phone, website, or E-mail. Tel: 844-SAMPLE6; website: <https://support.sample6.com>; e-mail: support@sample6.com.
- d. *Test kit components.*—Contents of the Detect HT/L Reagent Kit includes Detect HT family of Reagent Kits containing various single-use cartridges. Reagents needed for the reactions include the following: one cartridge of *Listeria* Detection Solution, one cartridge of Quench Buffer Q, one cartridge of Read Reagent R, one syringe of Read Supplement, one blunt needle, one white 96well HT assay plate, one clear plate cover.
- e. *Additional supplies and reagents.*—*L. monocytogenes* American Type Culture Collection (ATCC) 19115 and *L. innocua* ATCC 51742. Required parts of the Detect HT System include Detect HT Instrument, Detect HT Tablet Interface loaded with Detect HT Tablet Application, Detect HT Web Application accessible from any Internet-connected computer at <https://sample6control.com/>. Other required material (not provided) includes Modified University of Vermont (UVM) Broth, incubator capable of maintaining 35° (±2°) C, refrigerator capable of maintaining 2–8°C, and a micropipettor and pipet tips. Materials required for confirmation include UVM Modified *Listeria* Enrichment Broth (Becton Dickinson 222310 or equivalent) and Modified Oxford (MOX) agar plate.

Apparatus

A Sample6 Detect HT/L instrument, with Detect HT Tablet Interface loaded with Detect HT Tablet Application, and the Detect HT Web Application accessible from any Internet-connected computer at <https://sample6control.com/> are needed.

Standard Solutions

- a. *R2 Medium.*—Microbiologique, Inc., Seattle, WA.
- b. *MOX.*—Hardy Diagnostics Oxford Medium, modified for selective isolation of *L. monocytogenes* in food.
- c. *Sterile 0.1% buffered peptone water (BPW).*—Fisher Scientific, Houston, TX.
- d. Buffered *Listeria* enrichment broth (BLEB) with pyruvate.

- e. Filter sterilized 10 mg/L acriflavin, 40 mg/L cycloheximide, and 50 mg/L sodium nalidixic acid in BLEB with pyruvate.
- f. *Tryptic soy broth (TSB)*.—Becton Dickinson, Sparks, MD, with 20% glycerol.
- g. *Tryptic soy agar with 5% sheep blood agar (TSASB)*.—Becton Dickinson.

Safety Precautions

All work should be conducted in properly equipped facilities utilizing appropriate safety equipment (for example, a Biosafety Level 2 (BLS-2) biosafety cabinet or a contained area). Individuals should be trained in accordance with applicable regulatory and company/institution requirements before working with potentially infectious materials. Always wear personal protective gear when handling biological samples. Personal protective gear includes, but is not limited to, lab coats, eyewear, and examination gloves (either nitrile or latex).

General Preparations

Following the AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces (13), a matrix study was performed on ice cream using the new R2 Medium to compare the performance of the Sample6 Detect HT/L with FDA BAM Chapter 10 (*L. monocytogenes*) as the reference method. All materials and reagents for the Sample6 Detect HT/L were obtained from Phage Diagnostics, Inc., Kent, WA.

- a. *Application of the new R2 medium for Sample6 Detect HT/L kit*.—The current Sample6 Detect HT/L kit recommends a 24 h enrichment as the standard procedure. This study evaluated the use of a new R2 medium, a proprietary formulation, to detect *Listeria* spp. in 12 h enrichments.
- b. *Preparation of inocula*.—*L. monocytogenes* ATCC 19115 and *L. innocua* ATCC 51742 were prepared as individual inocula of each species. Bacterial strains were available as frozen ($-80 \pm 2^{\circ}\text{C}$) stock cultures in TSB (Becton Dickinson) with 20% glycerol and activated by streaking onto TSA-SB (Becton Dickinson) and incubated at $35 \pm 2^{\circ}\text{C}$ for 24 h. Following incubation, a pure colony was picked from TSASB, inoculated into TSB, and incubated at $35 \pm 2^{\circ}\text{C}$ for 24 h. Following incubation, the culture was refrigerated at $5 \pm 2^{\circ}\text{C}$ for up to 96 h. The refrigerated cell cultures were plated every 24 h for up to 96 h using TSA and MOX to determine the bacterial concentration.
- c. *Preparation of ice cream sample*.—Approximately 3 kg of ice cream was used to perform comparative tests and enumeration by the most probable number (MPN) procedure. Ice cream (vanilla flavor) was purchased from a local retail store and stored at -20°C prior to use. Total mesophilic aerobic count and total coliform/*Escherichia coli* were enumerated to establish background microflora levels.
- d. *Inoculation of ice cream samples*.—Refrigerated and stabilized broth cultures were used as inoculum for the study. Serial dilutions from each culture were

performed by sequentially transferring 1 mL of the cell suspension into 9 mL of sterile 0.1% BPW (Fisher Scientific).

Twentyfive gram portions of ice cream were used as test samples. The ice cream was thawed at 4°C, and then two sets of samples, one each of *L. monocytogenes* and *L. innocua*, were inoculated at the following levels: 5 replicates of uninoculated sample (0 CFU/25 g), 20 replicates inoculated with low inoculum level (0.2–2 CFU/25 g), and 5 replicates inoculated with a high inoculum level (5–10 CFU/25 g). The ice cream portions were dispensed into sterile WhirlPak bags (Nasco, Fort Atkinson, WI) and inoculated with the appropriate dilution of the cell cultures to obtain approximately the targeted low, high, and negative control levels. Then, they were hand massaged for 60 s to disperse the inoculum throughout the product. Both inoculated and uninoculated samples were held at $-20 \pm -2^{\circ}\text{C}$ for 2 days for the cells to stabilize. A parallel set of samples was also prepared for FDA BAM analysis according to Chapter 10 (14).

- e. *Enrichment and incubation of ice cream samples.*—The stabilized samples were then added to prewarmed ($42 \pm 2^{\circ}\text{C}$) R2 medium at 1:4 (sample:medium) ratio, hand massaged for 60 ± 6 s to thoroughly mix the sample with the media, and incubated at 35°C for 12–24 h. All reference test portions were enriched and incubated at 30°C for 24–48 h following the U.S. Food and Drug Administration (14). Aliquots were obtained at specific time points for analyses.
- f. *Analysis using the Sample6 Detect HT/L.*—A portion (75 g) of the sample enrichment was removed from the WhirlPak bag and tested for *Listeria* spp. following the package insert of Sample6 Detect HT/L. All samples were carried through confirmation using the reference method (14).
- g. *FDA BAM samples.*—The stabilized samples were added with 225 mL of BLEB with pyruvate and incubated at 30°C for 4 h. Three filter sterilized selective agents were added to achieve final concentrations of 10 mg/L acriflavin, 40 mg/L cycloheximide, and 50 mg/L sodium nalidixic acid in the BLEB with pyruvate preenrichments. The samples were then further incubated at 30°C for the remainder of the 24–48 h enrichment period and analyzed according to Vazquez-Boland et al. (1).
- h. *MPN analysis for determining level of inoculum.*—A fivetube, threelevel MPN was performed following the reference method and analyzed following the AOAC guideline (13).
- i. *Data analysis.*—Data from the Sample6 Detect HT/L and FDA BAM reference method were analyzed according to Annex C, Calculation of POD and dPOD Values from a Qualitative Method Single Laboratory Data (13).

Results and Discussion

In this performance assessment of Sample6 Detect HT/L for the detection of *L. monocytogenes* and *L. innocua* in ice cream, the artificially spiked samples with low (0.2–2 CFU/25 g) and high (5–10 CFU/25 g) levels of *L. monocytogenes* or *L. innocua* provided

equivalent detection of the organisms as the FDA BAM Chapter 10 method (*L. monocytogenes*), confirming the Sample6 Detect HT/L is an effective method of *Listeria* spp. detection for ice cream. Results are summarized in Tables 1–4. The background microflora of the ice cream sample used in the study were as follows: 10 CFU/g, <10 CFU/g, and <10 CFU/g for total aerobic bacteria count, total coliform, and *E. coli* counts, respectively.

Table 1 presents the presumptive results for *L. monocytogenes* obtained using the Sample6 Detect HT/L Assay and corresponding confirmation by the reference method (FDA BAM Chapter 10). Table 2 shows the confirmed Sample6 Detect HT/L *L. monocytogenes* results and FDA results. Table 3 shows the presumptive *L. innocua* results obtained using the Sample6 Detect HT/L assay and corresponding confirmation by the reference method (FDA BAM Chapter 10). Table 4 gives a summary of the confirmed Sample6 Detect HT/L *L. innocua* results and FDA BAM results.

L. Monocytogenes

As shown in Tables 1 and 2, for samples inoculated with low level of *L. monocytogenes* (estimated by the MPN analysis as <0.04 MPN/g), the Sample6 Detect HT/L detected 11 presumptive positive samples out of 20 test portions at 15 and 18 h, all of which were confirmed positive by the reference method. Of the five highinoculum test portions, four were detected as positive for *L. monocytogenes* (estimated at 0.485 MPN/g) by the Sample6 Detect HT/L Assay and confirmed by the FDA BAM reference method, including the negative sample. All five uninoculated test portions resulted in negative tests by Sample6 Detect HT/L assay and the reference method.

The dPOD_{CP} calculations for the presumptive and confirmed Sample6 Detect HT/L results for *L. monocytogenes* are shown in Table 1. All presumptive results from the Sample6 Detect HT/L Assay were confirmed by the reference method showing 100% concordance between both methods. All dPOD_{CP} values equaled zero, and the 95% confidence intervals around the relevant dPOD_{CP} calculations included zero, indicating no significant differences between the Sample6 Detect HT/L Assay and the FDA BAM method results.

The dPOD_C calculations for the method comparison of the Sample6 Detect HT/L Assay and the FDA BAM reference method for *L. monocytogenes* are presented in Table 2. The dPOD_C values at low inoculum level were calculated as 0.00 for both 15 and 18 h time points. The remaining dPOD_C values for uninoculated and inoculated materials at high levels were all zero and –0.20, respectively. In all cases, the confidence intervals around the relevant dPOD_C calculations included zero, and the confidence intervals around the relevant dPOD_{CP} calculations included zero.

L. Innocua

With *L. innocua* inoculated at low level (estimated by the MPN analysis as <0.044 MPN/g), the Sample6 Detect HT/L Assay detected 12, 14, and 14 presumptive positive samples out of 20 test portions at 12, 15, and 18 h, respectively. All these presumptive results were later confirmed positive by the reference method. All five highinoculum test portions (with *L. innocua* estimated at 0.475 MPN/g) detected positive by the Sample6 Detect HT/L Assay

and were confirmed by the reference method, while all five uninoculated test portions gave negative results for the Sample6 Detect HT/L Assay and the reference method.

The dPOD_{CP} calculations for the presumptive and confirmed Sample6 Detect HT/L Assay results for *L. innocua* confirmed all presumptive results from the Sample6 Detect HT/L by the reference method, showing 100% concordance between the two methods (Table 3). All dPOD_{CP} values equaled zero, and the confidence intervals around the relevant dPOD_{CP} calculations included zero, indicating no significant differences between the Sample6 Detect HT/L and the FDA BAM method and confirming that Sample6 Detect is as reliable as the FDA BAM method but more efficient, detecting *Listeria* spp. in 15–18 h.

Samples enriched and tested following the FDA BAM method gave 16 confirmed positive samples out of 20 inoculated at the low inoculum level (Table 4). Confirmed positive results were obtained for all five of the high inoculum test portions and negative results for all five uninoculated test portions.

When results from both methods were compared, the dPOD_C values for low inoculum level were calculated as –0.20, –0.10, and –0.10 for 12, 15, and 18 h, respectively. In all cases, the confidence intervals around the relevant dPOD_C calculations included zero. The remaining dPOD_C values for uninoculated and inoculated materials at high levels were all zero, indicating concordance between the two methods.

Conclusions

Results from the comparative study between Sample6 Detect HT/L and FDA BAM Chapter 10 in artificially inoculated ice cream showed that R2-enriched samples tested with the Sample6 Detect HT/L Assay gave equivalent performance at 15 and 18 h compared with the 24 h FDA BAM Chapter 10 method for *L. monocytogenes*. With *L. innocua*, the 12 h time point detection by Sample6 Detect HT/L Assay was equivalent to the 24 h FDA BAM method. All the dPOD_C values equaled zero, and the confidence intervals around the relevant dPOD_{CP} calculations included zero, indicating no significant differences between the two methods. These results indicate that Sample6 Detect HT/L, which was primarily developed for environmental samples, can also be used for the detection of *Listeria* species in ice cream and may detect *Listeria* species earlier than the standard reference methods. However, this study was conducted using vanilla ice cream, as recommended by the AOAC testing guidelines, which may allow easier detection of the fluorescent signal than a darker matrix, such as chocolate ice cream. Additional testing of this method is currently underway to determine the performance and applicability of Sample6 Detect HT/L on other food matrices, including dark chocolate.

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Table 1.

Sample6 Detect HT/L test method, presumptive versus confirmed, *Listeria monocytogenes* on ice cream

Time point, h	MPN ^b /g	n ^c	Sample6 Detect HT/L test method presumptive			Sample6 Detect HT/L test confirmation by reference method ^a			
			X ^d	POD _{CP} ^e	95% CI	X ^d	POD _{CC} ^f	95% CI	dPOD _{CP} ^g 95% CI ^h
15	NA ⁱ	5	0	0.00	0.00 to 0.43	0	0.00	0.00 to 0.43	0.00 -0.43 to 0.43
	<0.04	20	11	0.55	0.34 to 0.74	11	0.55	0.34 to 0.74	0.00 -0.28 to 0.28
	0.485	5	4	0.80	0.38 to 0.96	4	0.80	0.38 to 0.96	0.00 -0.45 to 0.45
18	NA	5	0	0.00	0.00 to 0.43	0	0.00	0.00 to 0.43	0.00 -0.43 to 0.43
	<0.04	20	11	0.55	0.34 to 0.74	11	0.55	0.34 to 0.74	0.00 -0.28 to 0.28
	0.485	5	4	0.80	0.38 to 0.96	4	0.80	0.38 to 0.96	0.00 -0.45 to 0.45

^aReference method: FDA BAM Chapter 10, *Listeria monocytogenes*.^bMPN = Most Probable Number calculated using the AOAC recommended MPN calculator, with 95% confidence intervals.^cn = Number of test portions.^dX = Number of positive test portions.^ePODCP = Sample6 Detect HT/L method presumptive positive outcomes divided by the total number of trials.^fPODCC = Sample6 Detect HT/L method confirmed positive outcomes divided by the total number of trials.^gdPODCP = Difference between the Sample6 Detect HT/L method presumptive result and confirmed result POD values.^h95% CI = Confidence interval. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.ⁱNA = Not applicable.

Table 2.

Method comparison results for *Listeria monocytogenes*, Sample6 Detect HT/L Assay versus reference method^a

Sample6 Detect HT/L test method confirmed						FDA BAM Chapter 10					
Time point, h	MPN ^b /g	n ^c	X ^d	POD _C ^e	95% CI	Time point, h	X ^d	POD _R ^f	dPOD _C ^g	95% CI ^h	
15	NA ⁱ	5	0	0.00	0.00 to 0.43	24 ^j	0	0.00	0.00 to 0.43	0.00	-0.43 to 0.43
	<0.04	20	11	0.55	0.34 to 0.74		11	0.55	0.34 to 0.74	0.00	-0.28 to 0.28
	0.485	5	4	1.00	0.57 to 1.00		5	1.00	0.57 to 1.00	-0.20	-0.62 to 0.26
18	NA	5	0	0.00	0.00 to 0.43		0	0.00	0.00 to 0.43	0.00	-0.43 to 0.43
	<0.04	20	11	0.55	0.34 to 0.74		11	0.55	0.34 to 0.74	0.00	-0.28 to 0.28
	0.485	5	4	1.00	0.57 to 1.00		5	1.00	0.57 to 1.00	-0.20	-0.62 to 0.26

^aReference method: FDA BAM Chapter 10 for *Listeria monocytogenes*.

^bMPN = Most Probable Number calculated using the AOAC recommended MPN calculator, with 95% confidence intervals.

^cn = Number of test portions.

^dX = Number of positive test portions.

^ePODC = Confirmed Sample6 Detect HT/L method positive outcomes divided by the total number of trials.

^fPODR = Confirmed reference method positive outcomes divided by the total number of trials.

^gdPODC = Difference between the Sample6 Detect HT/L method and reference method POD values.

^h95% CI = Confidence interval. If the confidence interval of a dPODC does not contain zero, then the difference is statistically significant at the 5% level.

ⁱNA = Not applicable.

^jSame results were obtained at 48 h.

Table 3.
SAMPLE6 Detect HT/L test method, presumptive versus confirmed, *Listeria innocua* on ice cream

Time point, h	Sample6 Detect HT/L test method presumptive				Sample6 Detect HT/L test confirmation by reference method ^a			
	MPN/g ^b	n ^c	X ^d	POD _{CP} ^e	95% CI	X ^d	POD _{CC} ^f	95% CI
12	NA ⁱ	5	0	0.00	0.00 to 0.43	0	0.00	0.00 to 0.43
	<0.044	20	12	0.60	0.39 to 0.78	12	0.60	0.39 to 0.78
	0.475	5	5	1.00	0.57 to 1.00	5	1.00	0.57 to 1.00
15	NA	5	0	0.00	0.00 to 0.43	0	0.00	0.00 to 0.43
	<0.044	20	14	0.70	0.48 to 0.85	14	0.70	0.48 to 0.85
	0.475	5	5	1.00	0.57 to 1.00	5	1.00	0.57 to 1.00
18	NA	5	0	0.00	0.00 to 0.43	0	0.00	0.00 to 0.43
	<0.044	20	14	0.70	0.48 to 0.85	14	0.70	0.48 to 0.85
	0.475	5	5	1.00	0.57 to 1.00	5	1.00	0.57 to 1.00

^aReference method: FDA BAM Chapter 10, *Listeria monocytogenes*.

^bMPN = Most Probable Number calculated using the AOAC recommended MPN calculator, with 95% confidence intervals.

^cn = Number of test portions.

^dX = Number of positive test portions.

^ePODCP = Sample6 Detect HT/L method presumptive positive outcomes divided by the total number of trials.

^fPODCC = Sample6 Detect HT/L method confirmed positive outcomes divided by the total number of trials.

^gdPODCP = Difference between the Sample6 Detect HT/L method presumptive result and confirmed result POD values.

^h95% CI = Confidence interval. If the confidence interval of a dPOD does not contain zero, then the difference is statistically significant at the 5% level.

ⁱNA = Not applicable.

Table 4.

Method comparison results for *Listeria innocua* Sample6 Detect HT/L Assay versus reference method^a

Sample6 Detect HT/L test method confirmed						FDA BAM Chapter 10					
Time point, h	MPN ^b /g	n ^c	X ^d	POD ^e	95% CI	Time point, h	X ^d	POD ^f	95% CI	dPOD ^g	95% CI ^h
12	NA ⁱ	5	0	0.00	0.00 to 0.43	24 ^j	0	0.00	0.00 to 0.43	0.00	-0.43 to 0.43
	<0.044	20	12	0.60	0.39 to 0.78		16	0.80	0.58 to 0.92	-0.20	-0.44 to 0.08
	0.475	5	5	1.00	0.57 to 1.00		5	1.00	0.57 to 1.00	0.00	-0.43 to 0.43
15	NA	5	0	0.00	0.00 to 0.43	16	0	0.00	0.00 to 0.43	0.00	-0.43 to 0.43
	<0.044	20	14	0.70	0.48 to 0.85		16	0.80	0.58 to 0.92	-0.10	-0.35 to 0.17
	0.475	5	5	1.00	0.57 to 1.00		5	1.00	0.57 to 1.00	0.00	-0.43 to 0.43
18	NA	5	0	0.00	0.00 to 0.43	16	0	0.00	0.00 to 0.43	0.00	-0.43 to 0.43
	<0.044	20	14	0.70	0.48 to 0.85		16	0.80	0.58 to 0.92	-0.10	-0.35 to 0.17
	0.475	5	5	1.00	0.57 to 1.00		5	1.00	0.57 to 1.00	0.00	-0.43 to 0.43

^aReference method: FDA BAM Chapter 10 for *Listeria monocytogenes*.^bMPN = Most Probable Number calculated using the AOAC recommended MPN calculator, with 95% confidence intervals.^cn = Number of test portions.^dX = Number of positive test portions.^ePODC = Confirmed Sample6 Detect HT/L method positive outcomes divided by the total number of trials.^fPODR = Confirmed reference method positive outcomes divided by the total number of trials.^gdPODC = Difference between the Sample6 Detect HT/L method and reference method POD values.^h95% CI = Confidence interval. If the confidence interval of a dPODC does not contain zero, then the difference is statistically significant at the 5% level.ⁱNA = Not applicable.^jSame results were obtained at 48 h.