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A New Combined Method for Simple and Rapid Detection of Listeria spp. in Environmental and Food Samples

Method Comparison Study

A New Combined Method for Simple and Rapid Detection of *Listeria* spp. in Environmental and Food Samples

The food safety regulations have tended to adopt a zero tolerance attitude for the presence of *Listeria monocytogenes* in ready-to-eat foods. Therefore, detection of *L. monocytogenes* in foods and food processing environment is one of the key elements to a food safety strategy. Furthermore, detection of any *Listeria* species in environmental samples is considered as an indication of a potential risk to find *L. monocytogenes* in manufactured food.

A collaboration between FoodChek Systems Inc. (Canada) and DuPont Nutrition & Health (USA) has resulted in the development of one of the fastest methods of *Listeria* detection available in the food industry. This method combines a 20-26 hour single-step enrichment of environmental and food samples in Actero™ Listeria Enrichment Media (Actero™ Listeria medium) followed by two hours of sample preparation and processing with the DuPont™ BAX® System Real-Time PCR assay for Genus *Listeria* (BAX® System assay).

The main advantage of the Actero™ Listeria medium is the ability to provide the ideal growing environment for sub lethally injured *Listeria* to allow for accelerated, reliable and faster detection. The BAX® System assay is a next-generation test, which combines shorter, simpler sample preparation and faster real-time processing without sacrificing accuracy or reliability. Combining the improved technology of the new BAX® System assay with the optimized Actero™ Listeria medium undoubtedly affords one of the best solutions for rapid and effective monitoring of *Listeria* in the food industry.

A series of internal laboratory validation studies were completed for submission to the AOAC-Research Institute that compared the new combined method of *Listeria* spp. detection to the USDA-FSIS MLG 8.09 and the US FDA BAM 10 reference methods. Environmental sponge samples collected from food contact (stainless steel and plastic) and non-food contact (sealed concrete) surfaces were tested in the presence of high levels of competing background flora. Food sample type tested in the studies included frankfurters, fresh bagged spinach, soft Mexican style cheese, frozen cooked shrimps and cold smoked salmon.

The independent laboratory validation studies were performed for stainless steel samples (Q Laboratories, Inc., Cincinnati, USA) and for soft Mexican style cheese samples (Food Microbiology, Agriculture and Food Laboratory, University of Guelph, Guelph, Canada).

The samples artificially inoculated with different *Listeria* spp. were enriched with the Actero™ Listeria followed by the detection of *Listeria* spp. using the BAX® System assay.

The results of the study demonstrated that performance of the candidate method is statistically equivalent or superior to the reference culture methods for detecting *Listeria* spp. in environmental and food samples.

The time to detect *Listeria* spp. was significantly reduced when single-step enrichment in Actero™ Listeria was combined with the detection using the BAX® System assay.

The high accuracy and reliability of the method were confirmed by the independent laboratory validation studies.

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Methodology

Equipment, reagents and supplies

- Actero™ Listeria Enrichment Media
  Dehydrated formats: ***FCM-011*** *– 500 g,* ***FCM-022*** *– 2 kg,* ***FCM-192*** *– 1 L MediaPouch,* ***FCM-193*** *– 10 liter MediaPouch.*
  Ready to use liquid formats: ***FCM-045 –*** *5 L MediaBox,* ***FCM-046****: 10 L MediaBox.*
- DuPont™ BAX® System Real-Time PCR Assay Genus *Listeria*. **Kit #D15131113**
- BAX® System standard equipment and supplies
- Buffered Listeria enrichment broth (BLEB)
- UVM enrichment broth
- MOPS – Buffered Listeria enrichment broth (MOPS-BLEB)
- Modified Oxoid (MOX) agar plates
- Trypticase Soy broth with 0.6% yeast extract (TSA-YE)
- Horse blood overlay agar (HL)
- Biochemical test panel Listeria API® system

Sample inoculation

The strains of *Listeria* spp. were selected from FoodChek Laboratories Inc. Collection to artificially inoculate environmental surfaces and food matrices. One strain of *Pseudomonas aeruginosa* and one strain of *Enterococcus faecalis* were also selected to represent competing flora. For independent laboratory validation study carried out with stainless steel samples, well-characterized ATCC target and non-target strains were used (see Table 1).

A pure culture of each *Listeria* strain was grown overnight in TSB-YE at 35 °C, then diluted in 10% non-fat dry milk (for environmental samples) or in PBS (for food samples) to levels expected to produce fractional positive results. The selected competing flora strains were diluted to a level approximately 10-fold higher than the *Listeria* strain dilutions.

Artificially contaminated food samples were stabilized at 2-8 °C for 48-72 hours (for frankfurters, fresh bagged spinach, soft Mexican style cheese and cold smoked salmon) or at -20 °C for 14 days (for frozen cooked shrimps) prior to testing.

For each type of environmental samples, decontaminated surfaces of 100 cm^2 were spread evenly with 250 µL of mixed culture of the target and competitor bacteria. The surfaces were dried at room temperature for 18-20 hours to stress the target bacterium, and then swabbed using non-bactericidal sampling sponges pre-hydrated with 10 mL D/E neutralizing broth by performing five vertical swabs (up and down) and five horizontal swabs (side to side). Sponges were held at room temperature for at least two hours before testing.

Table 1. Bacterial strains selected for environmental surface and food matrix contamination

Matrix type	Inoculating organism	Strain ID	Strain source
Stainless steel	*L. monocytogenes* 1/2c *P. aeruginosa*	MSR0119 MSR0132	Food-ready-to-eat Field isolate
Stainless steel[1]	*L. monocytogenes* 1/2c *P. aeruginosa*	ATCC 7644 ATCC 15442	Human Animal room water bottle
Plastic	*L. seeligeri* *P. aeruginosa*	MSR0460 MSR0132	Raw milk Field isolate
Sealed concrete	*L. welshimeri* *E. faecalis*	MSR0461 MSR0118	Chicken drip N/D
Frankfurters	*L. monocytogenes* 1/2a	MSR0453	Milk
Fresh bagged spinach	*L. monocytogenes* 1/2a	MSR0437	Lettuce
Soft Mexican style cheese[2]	*L. innocua*	MSR0127	Manure (soil)
Frozen cooked shrimps	*L. seeligeri*	MSR0458	Field water
Cold smoked salmon	*L. innocua*	MSR0433	Turkey/ham/cheese stick

Notes: [1]Independent laboratory validation study. [2]The same *L. innocua* strain was used for internal and independent laboratory validation studies.

Sample Enrichment

BAX® System method – Environmental sponge samples were stomached for 30 seconds with 90 mL of pre-warmed to 35°C Actero™ Listeria and incubated at 35°C for 20 hours.

Frankfurter samples (125 g) were stomached for 30 seconds with 750 mL of pre-warmed to 35°C Actero™ Listeria and incubated at 35°C for 26 hours.

Fresh bagged spinach, soft Mexican style cheese, frozen cooked shrimp and cold smoked salmon samples (25 g) were stomached for 30 seconds with 150 mL of pre-warmed to 35°C Actero™ Listeria and incubated at 35°C for 22 hours.

USDA-FSIS MLG 8.09 method – Environmental sponge samples were stomached for two minutes with 225 mL of pre-warmed to 30°C UVM and incubated at 30°C for 24 hours. Frankfurter samples (125 g) were stomached for two minutes with 1125 mL of pre-warmed to 30°C UVM and incubated at 30°C for 24 hours. For each sample, 100 µL aliquot of the primary UVM enrichment was transferred to 9.9 mL of MOPS-BLEB and then incubated at 35°C for an additional 24 hours.

US FDA BAM 10 method – Fresh bagged spinach, soft Mexican style cheese, frozen cooked shrimp and cold smoked salmon samples (25 g) were stomached for two minutes with 225 mL of pre-warmed to 30°C BLEB without selective supplements and incubated at 30°C for 4 hours. After the pre-enrichment phase, the selective supplements acriflavin, nalidixic acid and cycloheximide were added, and the samples were incubated at 30°C for a total time of 48 hours.

Listeria spp. Detection and Confirmation

BAX® System method – Lysis reagent was prepared by adding 150 µL protease and 200 µL of lysing agent 2 to 12 ml of lysis buffer. For each sample, 5 µL enriched sample was added to 200 µL prepared lysis reagent in cluster tubes. Tubes were heated for 30 minutes at 55°C and 10 minutes at 95°C, and then cooled for at least 5 minutes at 4°C. PCR tablets were hydrated with 30 µL lysate and a full process was run in the BAX® System Q7 instrument.

All results obtained with the BAX® System method were confirmed according to the USDA-FSIS MLG 8.09 or US FDA BAM 10 reference methods.

USDA-FSIS MLG 8.09 method – After secondary enrichment, 10 µL aliquot of each sample was streaked onto MOX agar plate. The plates were checked for typical *Listeria* colonies after 24-48 hours of incubation at 35°C. Typical colonies were then picked and confirmed using biochemical test panel Listeria API® system.

US FDA BAM 10 method – At the end of the each enrichment period (after 24 and 48 hours of incubation), the samples were directly streaked onto MOX agar plates. The plates were incubated at 35 °C for 24 to 48 hours. Typical colonies were then picked and confirmed using biochemical test panel Listeria API® system.

Data Analysis

Probability of detection (POD) statistical model was used to evaluate the differences between presumptive and confirmed results as well as between the alternative and the reference methods. All the data are presented in summary tables of POD values, dPOD values, and confidence intervals by matrix and concentration.

Results

No interference effects of the Actero™ Listeria medium on detection of *Listeria* spp. by RT-PCR BAX® assay in environmental and food samples were found.

The results for environmental samples tested are summarized in Tables 2-3 below. For plastic and stainless steel (independent laboratory validation study) samples, POD analysis didn't show any significant differences (the 95% confidence interval of the dPODs contains zero) in the method performance between the BAX® System and the USDA-FSIS MLG 8.09 methods. However, according to the POD statistical model,

significantly superior method performance was observed for fractionally inoculated stainless steel (internal validation study) and sealed concrete samples tested using the BAX® System method as compared to the USDA-FSIS MLG 8.09 method.

No false positive results have been observed. No more than one false negative outcome (stainless steel) was detected for the all environmental samples tested.

The results for food samples tested are summarized in Tables 4-5. For fresh bagged spinach, soft Mexican style cheese (independent laboratory validation study), frozen cooked shrimps and cold smoked salmon samples, POD analysis didn't show any significant differences (the 95% confidence interval of the dPODs contains zero) in the method performance between the BAX® System and the US FDA BAM 10 methods. Frankfurter and soft Mexican style cheese (internal validation study) samples analysed according to the BAX® System method demonstrated significantly superior method performance as compared to the USDA-FSIS MLG 8.09 (for frankfurters) or US FDA BAM 10 (for soft Mexican style cheese) reference methods.

No false positive or false negative results have been observed for the all food samples tested.

Conclusions

The results of the study demonstrated that performance of the BAX® System method to detect *Listeria* spp. in environmental and food samples enriched with Actero™ Listeria medium is equivalent or superior to the reference methods.

Table 2. BAX® System Method Presumptive *vs* Confirmed Results (environmental samples)										
Matrix	**CFU / 100 cm²**	**N**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$**	**95%CI**
			X	**POD$_{CP}$**	**95%CI**	**X**	**POD$_{CC}$**	**95%CI**		
Stainless steel	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	5.0	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
	63.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Stainless steel[1]	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	90.0	20	8	0.40	(0.22, 0.61)	9	0.45	(0.26, 0.66)	-0.05	(-0.33, 0.24)
	220.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Plastic	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	4.8	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
	58.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Sealed concrete	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	4.3	20	11	0.55	(0.34, 0.74)	11	0.55	(0.34, 0.74)	0.00	(-0.28, 0.28)
	22.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

Notes: [1]Independent laboratory validation study.

Table 3. BAX® System Method *vs* Reference Method Results (environmental samples)										
Matrix	**CFU / 100 cm²**	**N**	**BAX® System Method**			**USDA FSIS MLG 8.09 Method**			**dPOD$_C$**	**95%CI**
			X	**POD$_C$**	**95%CI**	**X**	**POD$_R$**	**95%CI**		
Stainless steel	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	5.0	20	16	0.80	(0.58, 0.92)	10	0.50	(0.30, 0.70)	0.30	(0.00, 0.53)
	63.0	5	5	1.00	(0.57, 1.00)	4	0.80	(0.38, 0.96)	0.20	(-0.26, 0.62)
Stainless steel[1]	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	90.0	20	8	0.40	(0.22, 0.61)	5	0.25	(0.11, 0.47)	0.15	(-0.13, 0.40)
	220.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Plastic	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	4.8	20	10	0.50	(0.3, 0.7)	5	0.25	(0.11, 0.47)	0.25	(-0.05, 0.49)
	58.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Sealed concrete	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	4.3	20	11	0.55	(0.34, 0.74)	4	0.20	(0.08, 0.42)	0.35	(0.05, 0.58)
	22.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

Notes: [1]Independent laboratory validation study.

Table 4. BAX® System Method Presumptive *vs* Confirmed Results (food samples)										
Matrix	**MPN, CFU/sample**	**N**	**Presumptive**			**Confirmed**			**dPOD$_{CP}$**	**95%CI**
			X	**POD$_{CP}$**	**95%CI**	**X**	**POD$_{CC}$**	**95%CI**		
Frankfurters	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.0	(-0.43, 0.43)
	0.3	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)
	3.9	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Fresh bagged spinach	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	1.6	20	16	0.80	(0.58, 0.92)	16	0.80	(0.58, 0.92)	0.00	(-0.25, 0.25)
	1000.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Soft Mexican style cheese	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.4	20	20	1.00	(0.84, 1.00)	20	1.00	(0.84, 1.00)	0.00	(-0.16, 0.16)
	13.9	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Soft Mexican style cheese[1]	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.6	20	10	0.50	(0.30, 0.70)	10	0.50	(0.30, 0.70)	0.00	(-0.28, 0.28)
	3.6	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Frozen cooked shrimps	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.7	20	12	0.60	(0.39, 0.78)	12	0.60	(0.39, 0.78)	0.00	(-0.28, 0.28)
	5.3	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cold smoked salmon	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	1.3	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)
	13.9	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)

Notes: [1]Independent laboratory validation study.

Table 5. BAX® System Method Presumptive *vs* Confirmed Results (food samples)										
Matrix	**MPN, CFU/sample**	**N**	**BAX® System Method**			**USDA FSIS MLG 8.09 / US FDA BAM 10 Methods[2]**			**$dPOD_C$**	**95%CI**
			X	**POD_C**	**95%CI**	**X**	**POD_R**	**95%CI**		
Frankfurters	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.3	20	14	0.70	(0.48, 0.85)	7	0.35	(0.18, 0.57)	0.35	(0.04, 0.58)
	3.9	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Fresh bagged spinach	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	1.6	20	16	0.80	(0.58, 0.92)	15	0.75	(0.53, 0.89)	0.05	(-0.21, 0.30)
	1000.0	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Soft Mexican style cheese	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.4	20	20	1.00	(0.84, 1.00)	7	0.35	(0.18, 0.57)	0.65	(0.38, 0.82)
	13.9	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Soft Mexican style cheese[1]	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.6	20	10	0.50	(0.30, 0.70)	8	0.40	(0.22, 0.61)	0.10	(-0.19, 0.37)
	3.6	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Frozen cooked shrimp	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	0.7	20	12	0.60	(0.39, 0.78)	11	0.55	(0.34, 0.74)	0.05	(-0.24, 0.33)
	5.3	5	5	1.00	(0.57, 1.00)	5	1.00	(0.57, 1.00)	0.00	(-0.43, 0.43)
Cold smoked salmon	0.0	5	0	0.00	(0.00, 0.43)	0	0.00	(0.00, 0.43)	0.00	(-0.43, 0.43)
	1.3	20	14	0.70	(0.48, 0.85)	14	0.70	(0.48, 0.85)	0.00	(-0.27, 0.27)
	13.9	5	5	1.00	(0.57, 1.00)	5	1.0	(0.57, 1.00)	0.00	(-0.43, 0.43)

Notes: [1]Independent laboratory validation study.

[2]Frankfurters were analysed according to the USDA FSIS MLG 8.09 method, fresh bagged spinach, soft Mexican cheese, frozen cooked shrimps and cold smoked salmon were tested according to the US FDA BAM 10 method.