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Comparison of the BAC*Gene Listeria* spp. Real Time PCR and BAX® System 24E Genus *Listeria* PCR Methods for the Detection of Genus *Listeria* in Food and Environmental Samples from Two Dairy Production Facilities

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ABSTRACT

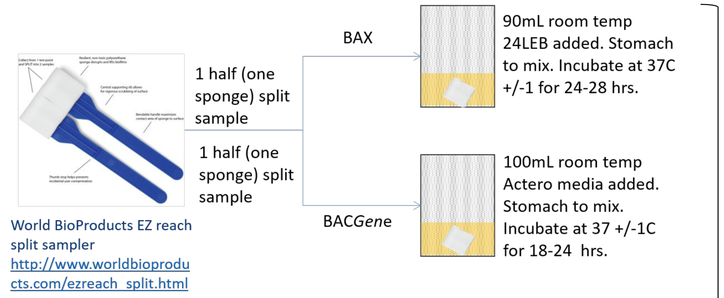
The purpose of this study was to compare the performance of two PCR based methods for the detection of Genus *Listeria* in food and environmental samples from dairy production facilities. The BAX® System 24E method for Genus *Listeria* and the BAC*Gene Listeria* spp. methods were used to test 244 environmental samples and 33 product samples collected from two working production plants in different locations. Environmental samples were collected using a novel split sampling device in an attempt to provide as close to equivalent input samples as possible for both methods. Culture confirmation of presumptive positives was performed if either of two sister samples was presumptive positive by either test method. For some negative sister samples additional culture work was performed (secondary enrichments and additional platings) in an attempt to recover any *Listeria* present. No presumptive positives were seen for any product samples by either method. For environmental samples BAX gave 50 presumptive positives of which 48 were confirmed by culture. The BAC*Gene* method gave 40 presumptive positives all of which were confirmed by culture. Out of 56 positive samples, 34 samples agreed between methods. Of the 20 samples that disagreed, 14 were BAX positive/BAC*Gene* negative and 6 were the reverse. Chi-square analysis of the total number of positives by each method showed no statistically significant difference in performance at (p <0.05, p-value = 0.05876).

INTRODUCTION

A large dairy product manufacturer expressed interest in an alternate PCR method for the detection of Genus Listeria in their environmental and product testing program due to recurring false-positive detections and high levels of environmental Genus Listeria positives in the recent past. Two active production facilities (one in the northeast and the other in the southeast United States) participated in the study. To ensure that study results would closely match what would be expected under real world conditions, collection and shipping of the samples for the study was performed by personnel at each participating production facility in exactly the same fashion as their existing and ongoing monitoring programs. The samples were processed and tested by each method in a third-party testing lab (Eurofins Microbiology Laboratories, Louisville, KY) in the same manner as regularly submitted monitoring samples.

METHODS

- Environmental samples were collected using a dual sampling device (Figure 1) that allowed simultaneous collection of two swabs from each sampling site (Plant A & Plant B) for duplicate testing by each method (World BioProducts EZ reach split sampler, Bothell, WA) hydrated in Letheen broth. At each plant, the devices were used to collect surface samples according to regular procedures for environmental sample collection. After sample collection, each device was spilt and the resultant paired swabs were placed into separate sterile sampling bags. Swabs were chilled and cold-shipped to EML for testing by both BAX Genus *Listeria* 24E (Hygienia, Wilmington, DE) and BAC*Gene* Real-time *Listeria spp*. (Eurofins Technologies, Freiberg, Germany) methods. Figure 1 shows the sample processing and enrichment scheme for both test methods for environmental samples
- Product samples were collected at each site using routine company sampling protocols and then chill-shipped to EML. Upon receipt, product samples were individually homogenized and split into two 25-g paired aliquots for testing by each method. Figure 2 shows the sample processing and enrichment scheme for both methods.
- Enrichment, sample preparation, and PCR testing were conducted exactly as specified by the kit manufacturers. For the BAC*Gene* method an additional enzymatic digestion of exogenous nucleic acids (PREraser) was performed on all initial presumptive positive enrichments followed by PCR. Samples that were presumptive positive by either method (and their corresponding paired sample) were taken to culture confirmation by two different Listeria culture methods based on the *Listeria monocytogenes* culture method of FDA-BAM. Samples were direct plated to BCMLM and Palcam, and/or MOX, Palcam, and Brilliance Listeria agar (BLA) and incubated for 48h at 37°C. Following incubation, plates were examined for typical colonies. Typical colonies were confirmed as *Listeria* by gram stain, wet mount, and catalase testing. Some paired samples that were negative by one test but positive by another test were taken into secondary enrichment in Fraser broth followed by plating as above.



World BioProducts EZ reach split sampler
http://www.worldbioproducts.com/ezreach_split.html

Figure 1. Sample processing and enrichment scheme for environmental samples for both test methods.

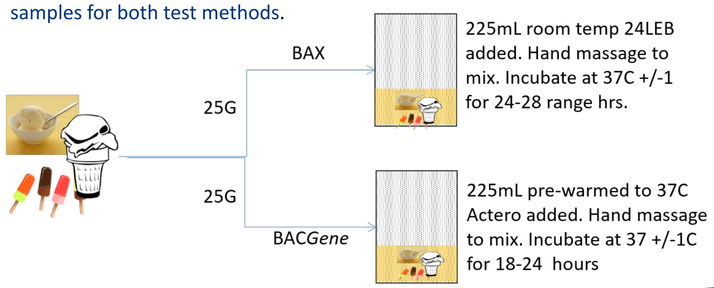


Figure 2. Sample processing and enrichment scheme for product samples for both test methods.

- All presumptive positives by either PCR method taken to culture confirmation.
- Negatives by either PCR method not taken through culture confirmation except in cases of results discordance
- For BAC*Gene* method all presumptive positives taken through PREraser protocol and retested by PCR.

	BAX	BAC*Gene*	Totals
# samples tested	277	277	554
# from Location A	131	131	262
# from Location B	146	146	292
# environmental	244	244	488
# product	33	33	66
# presumptive positives (total)	50	40	90
# true positives (total confirmed)	48	40	88
# presumptive positives (Location A)	5	3	8
# true positives (Location A)	5	3	8
# presumptive positives (Location B)	45	37	82
# true positives (Location B)	43	37	80
# true negatives	229	237	466
# false positives	2	0	2
# false negatives*	0	0	0
confirmation rate	96%	100%	na
FP rate	4%	0	na
sensitivity (TP/TP+FN)	100%	100%	na
specificity (TN/TN+FP)	99.10%	100%	na

Table 1. Summary of results for all samples tested.

	Method Disagreements*		Method Agreements
	BAX pos/BAC*Gene* neg	BAC*Gene* pos/BAX neg	
Location A	3	1	
Location B	11	5	
Total	14	6	34

*Out of n=56 samples with at least one sister sample presumptive positive

Table 2. Summary of results for all samples tested.

Figure 3. BAX Q7 system (ABI7500 Fast real time PCR thermal cycler).

Figure 4. BAC*GEne* system (Agilent AriaMX real time PCR thermal cycler). The BAC*Gene* method can be run on either the Agilent system shown or the Bio-Rad CFX96. The AriaMX was used for this study.

RESULTS

- Table 1 is a summary of environmental and product results for both processing locations.
  - No presumptive positives for any product samples were seen with either method
  - For environmental samples BAX gave 50 presumptive positives of which 48 were confirmed by culture (100% sensitivity, 99.1% specificity). The BAC*Gene* method gave 40 environmental positives all of which were confirmed by culture (100% sensitivity, 100% specificity).
- Table 2 is a summary of the discordant results.
  - Out of 56 positive samples, 34 paired samples agreed between methods. Of the 20 samples that disagreed, 14 were BAX positive/BAC*Gene* negative and 6 were the reverse.
  - By McNemar chi-square analysis (p-value, 1 tail =0.05876) the results were not statistically different ($p < 0.05$).
- Only one presumptive BACGene sample had a result changed as a result of PREraser treatment. It initially tested as presumptive with a Cq value of 39.1 but tested negative following PREraser treatment, and it culture confirmed negative. The corresponding BAX paired sample was presumptive positive and confirmed.

CONCLUSIONS AND DISCUSSSION

- These results suggest no significant difference in performance between the methods.
- Two false positives were observed with BAX and none with BAC*Gene* however there were a greater (though not statistically different) number of positives overall by the BAX method.
- While no statistical differenced was shown by McNemar chi-square other analyses are possible. All statistical analyses in this case were complicated by the fact that culture confirmation of the negative samples was not possible due to constraints inherent in the structure of the production testing lab where the work was carried out. For the chi-square calculation used here all samples for which both pairs tested negative by PCR were assumed to be culture negative and in agreement.
- Both methods have existing validations from external validation bodies (BAX, AOAC-RI and BAC*Gene* AOAC-RI and AFNOR) which demonstrates equivalent or better performance to existing reference methods and would suggest very similar performance overall.
- Multiple additional attempts were made to recover viable *Listeria* from the one BAC*Gene* sample that tested initially presumptive, and was then negative following PREraser treatment.. This included secondary enrichments in Fraser broth, Demi Fraser broth, 24LEB and fresh Actero media (1ml primary into 9ml media) with plating onto MOX, Palcam, BCMLM, and OCLA/BLA but all were negative with no growth observed on any of the media. PREraser is used to degrade free DNA in samples that may cause false-positive results (presumptive detection, not culture confirmed). Thus, this sample likely contained dead-cell DNA that was detected by PCR initially.
- Similar attempts were made to recover *Listeria* from 3 of the discordant BAC*Gene* enrichments but no *Listeria* was observed in the negative enrichments. It is not surprising to see discordant results with post-sanitation environmental samples taken at the same location, where *Listeria* population levels are expected to be very small. Therefore, care should be taken when using a split-sampling device as duplicate swabs may not yield the same result despite being taken from the same sampling site.

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