Roka Bioscience 2017 IAFP Poster Abstract

Validation of Individual and Wet Pooled Environmental Sample Analyses in Buffered Peptone Water and Lactose Broth by the Atlas Salmonella SEN Detection Assay

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Introduction: Wet compositing or "pooling" of analytical samples post-enrichment for rapid pathogen detection is a cost-effective and common practice, however, many methods have not been validated for this practice, which may increase false-negative risk via dilution and loss of analytical sensitivity.

Purpose: To validate the Atlas Salmonella SEN Detection Assay for the analyses of individual or 5 sample wet composited environmental sponge samples enriched in BPW or Lactose broth.

Methods: Sixty, 4" x 4" stainless steel coupons were utilized for surface inoculation. Ten replicates were non-inoculated, 40 were inoculated at a low-level and 10 at a high-level to approach POD=1. Samples were inoculated with *Salmonella* Derby (ATCC #6960) and 10x *Citrobacter freundii* (ATCC #6879) and allowed to dry overnight. Surfaces were sampled with sponge swabs pre-hydrated with D/E neutralizing broth. Half of the test samples were enriched with 190 mL BPW for 20 h at 42°C and half with 225 mL Lactose broth for 24 h at 35°C. Post enrichment, 1 mL of each sample was combined with 4 mL of non-inoculated sample enrichment to form a 5 sample wet composite. Both individual and pooled samples were assayed by the SEN method in duplicate using 12 and 400 µL analytical volumes as well as by FDA BAM 5 cultural analysis.

Results: For individual and wet pooled samples in BPW, SEN detected 9 and 5 presumptive positives from the low- and high-level replicates, respectively, and for the 12 µL and 400 µL analytical volumes. For individual and wet pooled samples in Lactose broth, SEN detected 8 and 5 presumptive positives across the study parameters. All presumptive results correlated 100% with FDA BAM 5 reference culture results.

Significance: These data support the application of the SEN assay for the accurate analyses of individual or 5 sample wet composited environmental samples enriched in BPW or Lactose broth.

For more information or to download the full poster scan here.

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Independent Matrix Validations for the Detection of *Salmonella enterica* in 375-g Samples across Various Product Categories by the Atlas Salmonella SEN Detection Assay

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Introduction: Diagnostic performance and accuracy of rapid pathogen detection methods may be adversely impacted by matrix type or other analytical parameters. Rapid method adoption and implementation should require validation for matrices pertinent to the stakeholder.

Purpose: To conduct matrix validation for the Atlas Salmonella SEN Detection Assay across various analytical parameters and food categories.

Methods: Eight matrices, each representing a category of finished product, were selected for matrix validation with the SEN assay. Each study consisted of 30, 375-g *Salmonella* inoculated portions of which 20 targeted fractional levels (0.2 to 2 CFU/portion) and 10 were inoculated to approach POD = 1 (2 to 10 CFU/portion). Inoculation serovars were stressed according to product type, and after a stabilization period, sample replicates enriched with BPW for 24 to 28 h at 42°C. SEN assay consisted of two analytical volumes at both 24 and 28 h for comparison to results confirmed by the appropriate paired USDA MLG or FDA BAM reference culture method.

Results: For each matrix (low moisture cheese, hot dogs, tomato, liquid whole egg, peanut butter cream pie, buttermilk biscuit, caramel popcorn, and sunflower seeds) fractional positive results were obtained in the low inoculated replicates. For high-level replicates, all were presumptive positive, except tomatoes, where 8 of 10 were presumptive. All presumptive results at 24 and 28 h for both 40 and 400 µL analytical volumes aligned with culture results with the exception of 1 false positive in tomato and popcorn for a single assay across replicate iterations. There were no significant differences by paired POD analysis for the SEN assay as compared to the cultural results.

Significance: These data support the application of the SEN assay for the accurate analyses of matrices within the specified food categories and types evaluated.

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Development and Validation of a Novel, Enzyme Based Sample Preparation Step as a Workflow Modification for the Atlas Listeria Environmental Detection Assay to Mitigate Free Nucleic Acid Detection Originating in Phage Based Processing Aids

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Introduction: Bacteriophage based processing aids for pathogen reduction on food or in food processing environments contain residual analyte that can cause false positive results when using rapid pathogen detection methods.

Purpose: To develop and validate a sample preparation solution for mitigating environmental free nucleic acid detection using the Atlas Listeria Environmental Detection Assay.

Methods: A phage based processing aid was serially diluted and assayed with the Atlas Listeria LSP Detection Assay to estimate load of residual analyte in the product. An enzyme treatment to degrade free RNA was investigated and application parameters (concentration, temperature, time, and environment) optimized to evaluate direct and indirect treatment of post-enriched environmental samples by the Atlas Listeria Environmental Detection Assay in the presence of the processing aid. To validate the optimized solution, 60 environmental swabs were collected from a facility, 30 of which were used to swab 4" x 4" coupons treated with the processing aid and another 30 without treatment for inoculation with *Listeria* spp. All swabs were enriched and assayed by the Atlas method with and without the novel sample preparation and by cultural analysis.

Results: The processing aid product alone contained greater than 10^{10} CFU equivalents of free, residual analyte. Enzyme efficacy at 50 U/reaction was markedly improved when samples were prepared with a wash step and optimized reconstitution buffer as compared to direct treatment of enrichment sample. For validation with Atlas, all 30 non-inoculated samples from processing aid treated surfaces were falsely positive by standard assay and 29 were resolved when assayed with the novel sample preparation treatment. From inoculated samples, 22 culture confirmed samples were detected by both the standard and modified assays.

Significance: The described sample preparation solution effectively mitigates free nucleic acid detection by the Atlas Listeria Environmental Detection Assay while providing an additional tool for troubleshooting environmental positive samples.

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Digging Deep: Making the Case for Molecular Based Detection with Real-World Performance and Discrepant Evaluation

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Introduction: Alternative molecular based detection technologies are rapidly evolving, yet results from such methods are often questioned or dismissed based upon follow up with imperfect culture based methodologies.

Purpose: To evaluate performance of the Atlas Listeria Environmental Detection Assay for *Listeria* spp. detection in real-world environmental samples with investigation of initially discrepant results.

Methods: Environmental sponge swabs (N = >700) were collected from zones 3 & 4 of multiple processing environments. Swab samples were enriched with 90 mL of FoodChek™ Actero™ Listeria Enrichment Media (ALEM) for 24 h at 35°C prior to assaying with the LE assay using two analytical volumes, 200 µL and 2000 µL. Screen positive results were initially struck to Modified Oxford Agar and transferred to Fraser secondary enrichment with subsequent plating to MOX and *Listeria* Chromogenic agars. Absence of typical morphology resulted in further analytical and culture analyses including PCR, IMS, and Filtration methodologies. Purified isolates were identified with biochemical or sequencing methodologies.

Results: Of n = 750 samples, the LE assay reported 24 presumptive results for both the 200 and 2000 µL analytical volumes. Of the 24 screen positive samples, *Listeria* spp. was isolated from 23 with standard or follow up culture analyses. Two discrepant samples were resolved utilizing a modified sample preparation for removal of free nucleic acid. In 2 cases, a biochemical method failed to identify typical isolates, yet were identified as *L. mono* and *L. innocua* by 16s based identification. Other samples required extensive culture attempts to obtain isolates for microbial identification, for which would be impractical under current industry testing scenarios and turnaround times.

Significance: These real-world data support the molecular based detection of *Listeria* spp. while highlighting potential pitfalls and shortcomings of downstream confirmation processes.

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