



PERFORMANCE OF A NEW MOLECULAR PLATFORM FOR THE RECOVERY AND DETECTION OF *Salmonella* spp. FROM FRESH RASPBERRIES



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Introduction

Consumption of minimally-processed fruits has increased rapidly in recent years. The claim of health benefits from some fruits, such as antioxidant properties in Raspberries, has increased the demand of such fruit all year. Thereby, fruit imports and exports from all world around have been extended, and correspond to increased outbreaks associated with the consumption of these products (2). In North America and Europe, Raspberries are required in great amounts due its wide variety in uses, including dish decorations, which imply berries are used with no processing between harvest and serving. Fruit growers, transporters, distributors, brokers and vendors are responsible of the quality and safety of their products. Implementation and verification of Good Agricultural Practices reduce the risk of fruit contamination. Furthermore, each country will require inspections of fruit entering, and *Salmonella* analysis is involved in most cases. Obtaining reliable results in a short time period is a "must have" characteristic of the analysis (3).

Molecular methods are of interest for the food industry because the benefit of obtaining results in a relatively short time compared with traditional culture methods. The 3M™ Molecular Detection System combines two technologies: Isothermal DNA Amplification, which uses multiple, specific primers targeting distinct regions of the genome (4), and Bioluminescence real-time detection. However, the wide variety of food commodities requires proper validation of alternative methods for *Salmonella* detection.

The purpose of this study was to compare the recovery of *Salmonella* from inoculated fresh raspberries with the 3M Molecular Detection System and traditional culture method.

Materials and methods

Salmonella enterica strains isolated from berries farm environments (*S. Branderup*, *S. Montevideo*, *S. Anatum*, *S. Infantis*, *S. Agona* and *S. Poona*) were used to inoculate raspberries at low level (1-5 CFU/25g). Additionally, uninoculated fruit portions were tested. Samples were analyzed according the Food and Drug Administration's Bacteriological Analytical Manual (5) and 3M Molecular Detection System (MDS) methods. Sample preparation (soak and stomaching) and pre-enrichment media (Buffered Peptone Water (BPW) and 3M Buffered Peptone Water ISO) for pre-enrichment step were also evaluated.

Ten replicates for each inoculum level (low and uninoculated), sample preparation and pre-enrichment media were analyzed. Data between recovery methods were compared using McNemar's test for paired data at significant level of 0.05.

Results and discussion

Salmonella spp. was recovered from 9 and 8 soak samples with the 3M Molecular Detection System and traditional culture method respectively when BPW ISO was used as pre-enrichment media. Pre-enrichment using BPW yield a 50% and 40% of recovery respectively (Figure 1).

Sample homogenization (stomaching) resulted in 60 to 90% false negative results by both methods (BAM and MDS) being the higher incidence among samples pre-enriched with BPW. No statistical differences were observed among BAM method and Molecular Detection System ($P>0.05$). On every pre-enrichment media, pH was measured after 24 h of incubation at 35°C. Homogenization of pre-enrichment broth/sample inhibits *Salmonella* spp. recovery, producing false negative results with either traditional culture methods or 3M Molecular Detection System. This may be attributed to the release of phenolic compounds active against pathogenic bacteria (1) rather than pH decrease. Table 1 shows average data, which supports that pH of homogenized samples was not the inhibitory factor of cells growth on inoculated samples.

In order to recovery *Salmonella* spp cells by means of the traditional culture method is necessary to reach aprox. 10^6 cells/ml after incubation of pre-enrichment broth, whereas only 10^4 cells/ml are required by the or 3M Molecular Detection System (3). Additionally, an estimation of *Salmonella* cells on pre-enrichment media was obtained by the Most Probable Number Technique. Samples that were determined as positive by the BAM method, yielded counts of ≥ 5.9 log MPN cells/ml in the pre-enrichment media, whereas positive samples detected by the 3M Molecular Detection System included samples with counts below 5.4 log MPN/ml.

Matrix control analysis were run for each inoculated and uninoculated samples. Eight of 40 samples resulted on invalid reaction, which after diluting pre-enrichment broth with buffer (1:1) yield valid results. Further analysis are required to establish inhibitory compounds present on raspberries, which may inhibit the growth of *Salmonella* cells during pre-enrichment of stomached samples.

None uninoculated sample yielded positive results with any method. This study was an intralaboratory evaluation. Further research should be done by other laboratories to perform interlaboratory validation and establishment of the detection limit for recovery of *Salmonella* from this type of samples.

Table 1. pH values* of pre-enrichment broth for *Salmonella* inoculated raspberries samples, as influenced by sample preparation and pre-enrichment media.

Sample preparation	Buffered Peptone Water		Buffered Peptone Water ISO	
	Initial pH	Final pH	Initial pH	Final pH
Soak (50 g fruit/50 ml preenrichment media)	6.76 ±0.13	3.56 ± 0.17	6.75 ± 0.07	3.95 ± 0.31
Stomacher (50 g fruit/450 ml preenrichment media)	6.74 ± 0.14	5.07 ± 0.66	6.71 ± 0.08	5.42 ± 0.28

* Mean value of 10 replicates ± standard deviation. Initial pH was measured after 1 h pre-enrichment, and final pH after 24 h incubation for pre-enrichment at 35°C.

Conclusion

The 3M™ Molecular Detection Assay *Salmonella* can be implemented for the suitable recovery of *Salmonella* spp. from raspberries. The 3M Buffered Peptone Water ISO yields better recovery of *Salmonella* spp. than BPW. Homogenization of pre-enrichment broth/sample produces false-negative results with either traditional culture methods or 3M™ Molecular Detection System.

References

1. Puupponen-Pimiä R, Nohynek L, Hanna-Leena A, Kirsi- Marka O-C. 2005. Bioactive berry compounds-novel tools against human pathogens. *Appl. Microbiol. Biotechnol.* 67:8-18.
2. Sivapalasingam S, Friendman CR, Cohen L, Tauxe RV. 2004. Fresh Produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot.* 67:2343-2353
3. U.S. Food and Drug Administration. [Updated 04 20 2012]. Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce. Chapter IV. Outbreaks Associated with Fresh and Fresh-Cut Produce. Incidence, Growth, and Survival of Pathogens in Fresh and Fresh-Cut Produce. [Internet]. [Accessed 12 May 2012]. Available at: <http://www.fda.gov/Food/ScienceResearch/ResearchAreas/SafePracticesforFoodProcesses/ucm091265>.
4. Gill P, Ghaemi A., Nucleic Acid Isothermal Amplification Technologies-A Review, Nucleosides, Nucleotides, and Nucleic Acids, 27:224-243, 2008.
5. US Food and Drug Administration. Bacteriological Analytical Manual. Chapter 5. Salmonella. Available at <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethod/s/BacteriologicalAnalyticalManualBAM/UCM070149> Accessed 3 July 2011cvv

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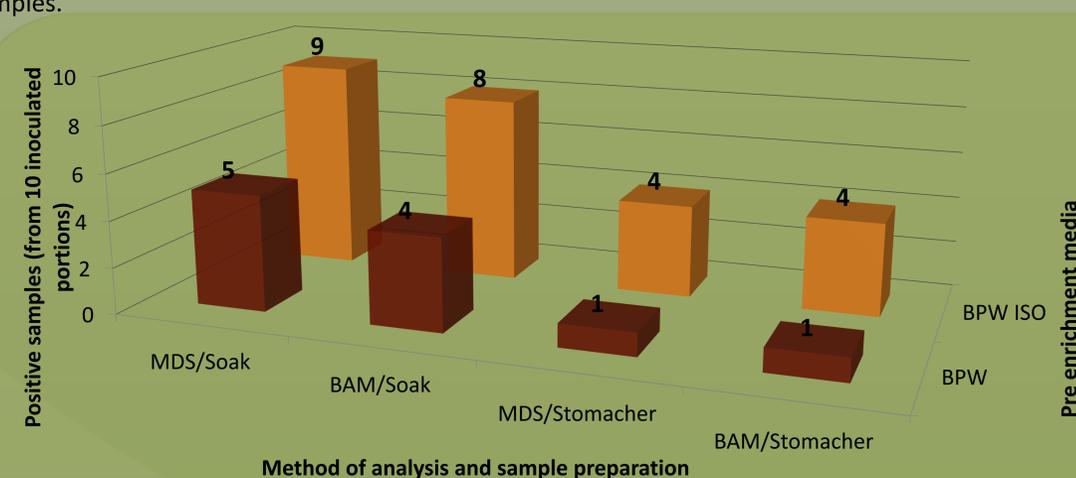


Figure 1. Efficacy of 3M Molecular Detection System (MDS) and the Food and Drug Administration's Bacteriological Analytical Manual (BAM) methods on the recovery of *Salmonella* spp. from inoculated raspberries as influenced by sample preparation (soak and stomached) and pre-enrichment media (Buffered Peptone Water (BPW) and 3M Buffered Peptone Water ISO (BPW ISO)). Inoculation level: 1-5 CFU/25 g