2007

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Q-409

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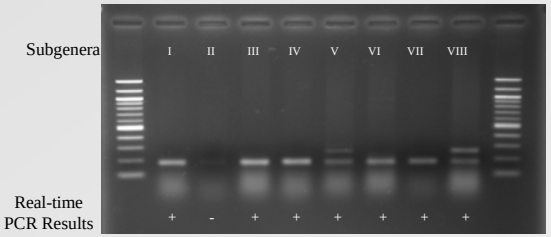
Abstract

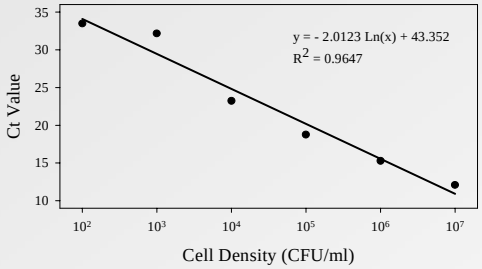
The U.S. Environmental Protection Agency water quality guidelines for assessing bacteriological recreational water quality is currently based on *Escherichia coli* and enterococci counts as indicators of the presence of fecal pollution. Although these indicator organisms have been widely used, detection of specific pathogens rather than their surrogates may be preferable in the future for assessing health risks. This necessitates development of rapid, sensitive and reliable methods for pathogen detection in environmental waters. A quantitative PCR-based assay for detecting *Salmonella* spp., an important enteric pathogen, in recreational water and beach sediment samples was developed in the present study. The assay is based on the amplification of a 172 bp fragment in the *invA* gene which is quantified using a molecular beacon specific for *Salmonella*. The assay tests for the presence of *Salmonella* in 50 ml of environmental water samples and relies on the use of a 6 hr culture enrichment procedure. It has a lower detection limit of one CFU per mL and works within the 1 to 10^6 CFU per mL concentration range tested thus far. Due to the possible presence of PCR inhibitors in environmental samples, an internal amplification control, using the same primers but a different molecular beacon target sequence, was created to be included in each amplification reaction to distinguish true from false negative results. Preliminary studies reveal the presence of *Salmonella* in several coastal water samples along the northern Gulf of Mexico.

The PCR assay detects 7 of 8 *Salmonella* subgenera*



*Univ Calgary Salmonella Genetic Stock Centre Salmonella Reference Collection C

Salmonella quantified using beacon-based q-PCR



Primer / Probe	Sequence (5' → 3')
SEN* (Fwd)	TTTCAATGGGAACTCTGC
SEN* (Rev)	AACGACGACCCTTCTTTT
Sal Beacon	FAM-cgacgcAGCGCCGCCAAACCTAAAACCAgcgtcg-Dabcyl
Control Beacon	HEX-ccagcGTTTGATACTAACCGTGCTATGCgctgg-Dabcyl

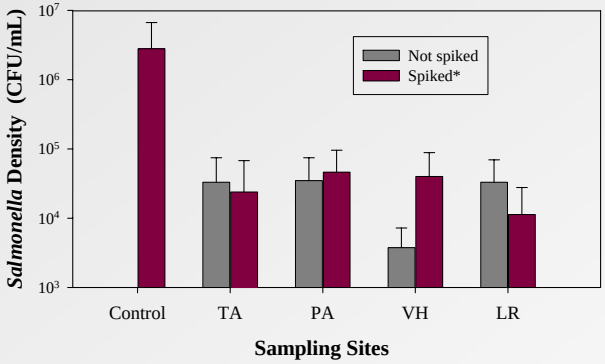
*Csordas AT, JD Barak & MJ Delwiche. 2004. Lett. Appl. Microbiol. 39:187-193.

Sampling sites are located along MS Gulf Coast

TA – Trautman Ave
PA – Pratt Ave

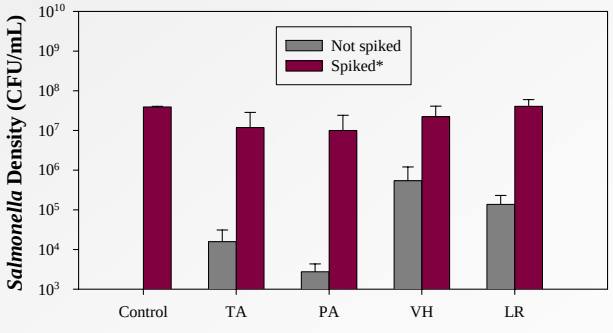
VH – VA Hospital
LR – Loraine Road

Boiled lysates are simple to prepare but prone to PCR inhibition



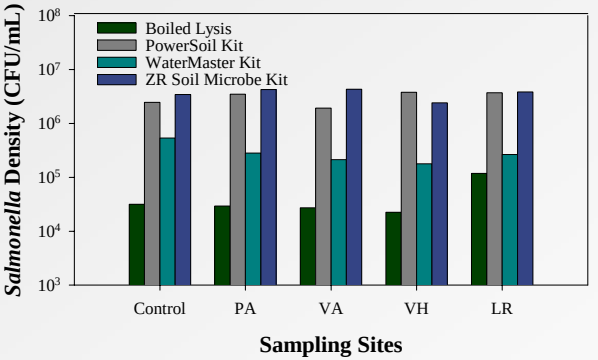
*Each 500 mL water sample spiked with 5 x 10^8 CFU *S. typhimurium.*
Pelleted cells resuspended in 300 uL TE and boiled. Lysate used in qPCR directly.

Sample enrichment enhances detection

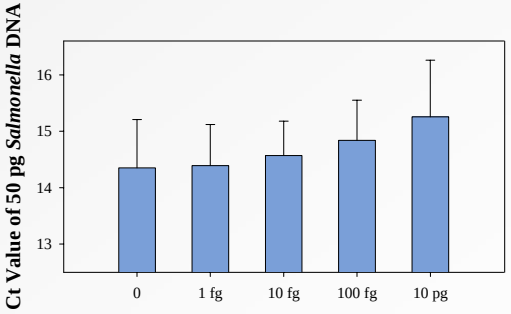


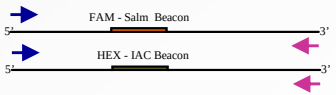
*Each 500 mL water sample spiked with 5 x 10^8 CFU *S. typhimurium.*
Pelleted cells resuspended in 5 mL BHI broth and incubated at 37ºC for 6 hr.
After centrifugation, cells resuspended in 300 uL TE and boiled.
Lysate used in qPCR directly.

DNA extraction method used affects quantitation



Internal Amplification Control (IAC) guards again false negatives but affects q-PCR





Conclusions

- A molecular beacon-based PCR assay was developed to quantify *Salmonella* spp. in environmental water samples.
- PCR inhibitors affect quantitation but sample enrichment and the use of a good DNA isolation method ameliorate the inhibitory effect.
- An internal amplification control is useful in guarding against false negatives but affects quantification.

Acknowledgements

Financial support from the U.S. Environmental Protection Agency (EPA MX-96401204 and MX-96429505) is gratefully acknowledged.