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Recommended Citation
Chandrasekar, Keerthy V.; Ellender, R.D.; and Wang, Shiao Y., "Overcoming the Inhibitor Problem in PCR Detection of Salmonella in Recreational Waters: A Volume Based Approach" (2008). *Presentations*. 7.
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# Overcoming the Inhibitor Problem in PCR Detection of *Salmonella* in Recreational Waters: A Volume Based Approach

Q-551

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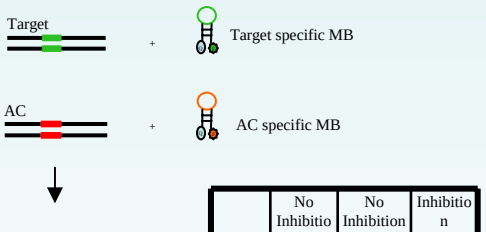
## Abstract

Fecal contamination of environmental or recreational waters is a world wide health concern. Methods currently endorsed by the U.S. EPA for monitoring fecal contamination are based on plate counts of fecal surrogates such as *E. coli* or *Enterococci* spp. Due to the sensitivity and specificity of PCR, there is growing interest in using PCR-based methods to detect and quantitate pathogens directly. However, a major impediment to more widespread use of PCR-based methods is the presence of inhibitors that frequently contaminate DNA isolated from environmental sources, especially water rich in organic matter. The major objectives of the present project are to ascertain the extent of this inhibitor problem and to develop alternative approaches. Using *Salmonella* as a model, real-time PCR and an Amplification Control (AC), we demonstrate that PCR inhibition occurs to varying degrees with all environmental water samples. Inhibition can be ameliorated by using less template DNA but the level of dilution required to permit amplification of the AC is often too severe to permit detection of the pathogen. Our results show that because the severity of the inhibitor problem is stochastic and there is not a reliable method to correct for the effect of the inhibitor, it is not feasible to convert real-time PCR data to bacterial counts. Instead, we propose a volume based approach to quantitate pathogens in recreational waters. The method is based on real-time PCR detection of bacteria, *Salmonella* in this case, in a specific volume of water. It includes a short enrichment step in a non-selective medium prior to DNA isolation and PCR. The enrichment step increases the amount of template DNA isolated without a concomitant increase in inhibitor level and solves the inhibitor problem because the ratio of inhibitors to template DNA used in PCR is greatly reduced. The detection limit of the method was 10 CFU/dL using a 6 hr enrichment period. Because the risk of exposure to a particular waterborne pathogen is directly related to its concentration, we believe this volume based approach provides a useful method to assess exposure risk in the future.

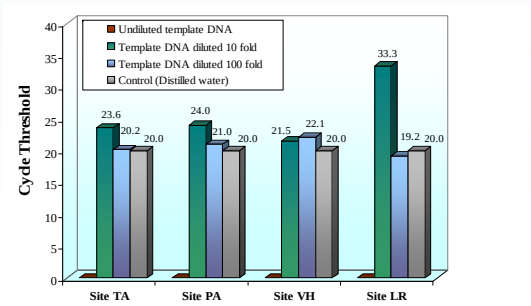
### Amplification Control (AC) used to ascertain the level of PCR inhibitors present in DNA extracted from beach waters




| | No Inhibition | No Inhibition | Inhibition |
|---|---|---|---|
| Target | + | – | – |
| AC | + | + | – |

+ Fluorescence detected; – No fluorescence

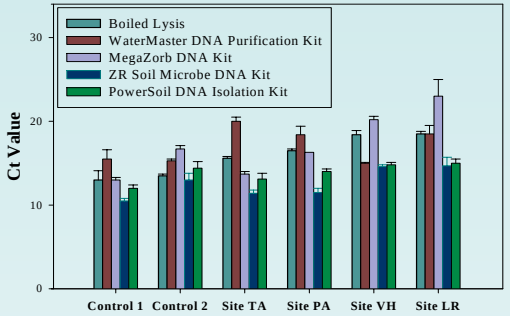
### PCR inhibitors prevent detection of *Salmonella* unless template DNA was diluted




* DNA extracted from environmental samples and spiked with known amounts of AC

Beach sampling sites located in Harrison County, Mississippi
TA – Trautman avenue; PA – Pratt avenue; VH – Veteran hospital; LH – Lorraine road

### Quantitation of *Salmonella* affected by the DNA extraction method used

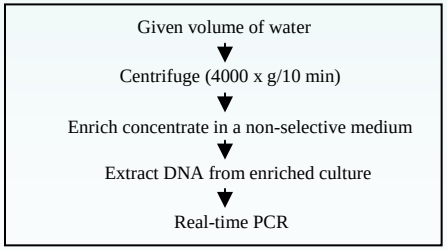



* Ct values from replicate runs using 50 ng template (*Salmonella*) DNA per reaction

### Quantifying pathogens in environmental waters by real-time PCR is problematic

- Sample dilution reduces PCR inhibition but reduces assay sensitivity.
- The level of PCR inhibition is highly variable among samples and is unpredictable.
- There is not a reliable method to correct Ct values to compensate for PCR inhibition.
- Converting real-time PCR data to bacterial counts is not reliable.

### Sample enrichment procedure to overcome the PCR inhibition problem




### Sample enrichment enables pathogen detection at low concentrations

| Sample | Ct Values from *Salmonella* Real time PCR | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 50 CFU/dL | | | 10 CFU/dL | | | 2CFU/dL | | |
| | 0h | 6h | 12h | 0h | 6h | 12h | 0h | 6h | 12h |
| Site TA | - | 17.3 ± 0.4 | 12.6 ± 0.4 | - | 19.5 ± 0.5 | 13.7 ± 0.2 | - | 21.8 ± 0.7 | 14.7 ± 0.3 |
| Site PA | - | 18.7 ± 0.1 | 12.4 ± 0.5 | - | 21.6 ± 0.3 | 14.4 ± 0.1 | - | 27.2 ± 0.6 | 14.5 ± 0.2 |
| Site VH | - | 17.2 ± 0.4 | 14.6 ± 0.6 | - | 19.2 ± 0.7 | 15.3 ± 0.7 | - | 20. 8 ± 0.6 | 13.8 ± 0.4 |

"-" no Ct value.

### Enrichment facilitated detection of *Salmonella* in environmental water samples

| Sampling Dates | Ct Value | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Site-TA | | Site-PA | | Site-VH | | Site-LR | |
| | 0 hr | 6 hr | 0 hr | 6 hr | 0 hr | 6 hr | 0 hr | 6 hr |
| 06-30-06 | - | 29.5 | - | 25.7 | 29.4 | 29.6 | 21.5 | 16.5 |
| 07-11-06 | 28.8 | 22.6 | 28.3 | 22.0 | 25.6 | 23.4 | - | 21.9 |
| 07-21-06 | - | 28.2 | - | 27.8 | - | 26.7 | - | 27.0 |
| 07-28-06 | - | 27.9 | - | 27.5 | - | 26.8 | 35.8 | 28.4 |
| 07-16-07 | - | - | - | - | - | - | - | - |
| 08-14-07 | - | - | - | - | - | - | - | - |
| 08-21-07 | - | - | - | - | - | - | - | - |
| 09-24-07 | - | - | - | - | - | - | - | - |
| 10-01-07 | - | 23.1 | - | 24.0 | - | 23.6 | - | 22.8 |
| 10-22-07 | - | - | - | - | - | - | - | - |
| 10-29-07 | - | - | - | - | - | - | - | - |

### Pathogen quantification based on sample enrichment is a possible solution to the inhibitor problem

- Enrichment increases ratio of template DNA to inhibitors and overcomes the PCR inhibitor problem.
- Pathogen concentration is inversely related to volume of water sample needed to get a positive PCR result thus proportional to exposure risk.

### PCR result in relation to water volume used reflects pathogen concentration

| Volume of water | qPCR result | Interpretation |
|---|---|---|
| 1 mL | Neg | ≤ 1000 CFU/L |
| 10 mL | Pos | ≥ 100 CFU/L |
| 100 mL | Pos | ≥ 10 CFU/L |

## Summary

- PCR inhibition is a persistent problem with DNA extracted from beach waters along the Northern Gulf of Mexico.
- We could not find a way to extract DNA completely free of inhibitors thus real-time PCR data could not be used to obtain reliable information on levels of *Salmonella* present.
- A volume based approach relying on sample enrichment overcomes the PCR inhibitor problem and can be used to assess whether pathogen level exceeds a predetermined threshold.

### Acknowledgements

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

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