# Evaluating DNA Recovery of Enteric Pathogens From Puritan® Fecal Opti-Swab Collection And Transport System Following Prolonged Storage

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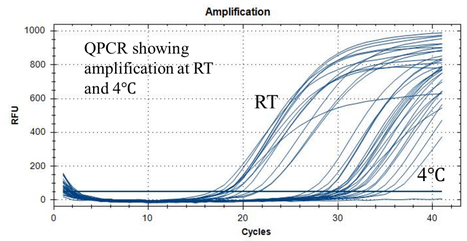
## Introduction

Optimization of antimicrobial use in today's healthcare systems that ensures the best clinical outcomes requires accurate and speedy characterization of the microbiome in biological samples. Often this characterization is based upon genetic analysis. Essential for such assays is reliable collection and transport systems for the preservation of genetic material. Specifically, collection and transport systems applicable to assessing enteric bacterial species within stool samples that do not inhibit later downstream molecular genetic procedures. The goal of this study was to assess the ability to detect enteric bacterial DNA following long-term storage in a commonly available Puritan® Fecal Opti-Swab collection and transport system.


## Methods

Three species-specific real-time quantitative PCR (QPCR) assays were used to measure concentrations of *Escherichia coli, Shigella sonnei* and *Salmonella enterica* stored for 0, 2, 7 and 30 days at room temperature (25°C, RT) and 4°C. Bacterial suspensions were prepared from each fresh culture (24 hours) in a separate vial containing 10 mL of 0.85 % sterile saline and verified by 0.5 McFarland Standard to obtain 1.5 x $10^8$ CFU/mL suspensions. From the starting suspensions, a series of five ten-fold dilutions were made resulting in final concentrations of 1.5×$10^7$, 1.5×$10^6$, 1.5×$10^5$, 1.5×$10^4$, and 1.5×$10^3$ CFU/mL. One mL aliquots were then transferred into sterile 1.5 mL centrifuge tubes. Synthetic stool samples were added to each centrifuge tube and allowed to incubate for 5 minutes. The fecal swabs (2 replicates each) were then immersed in the corresponding tubes and allowed to absorb for approximately 15-20 seconds. Following absorption, the swabs were removed from the wells and immediately placed in vials containing Puritan® Fecal Opti-Swab medium. The swabs were held in the transport medium for 2, 7 and 30 days at RT and 4°C. A small aliquot was taken from each tube and stored at -80°C as a time zero sample. For each subsequent time point a small aliquot (400 µL) was taken for DNA isolation using Qiagen QIAamp DNA Stool kit. Quantitative real-time PCR (Qiagen Microbial DNA qPCR Assay) was used to quantify *E. coli, S. sonnei* and *S. Enterica* concentrations (N=70 for each species).




## Results

Yields of DNA from all samples were sufficient for multiple PCR assays, even at the lowest concentrations. For all pathogens QPCR detected a linear positive relationship between the initial sample concentration and quantified relative copy numbers across all concentrations.

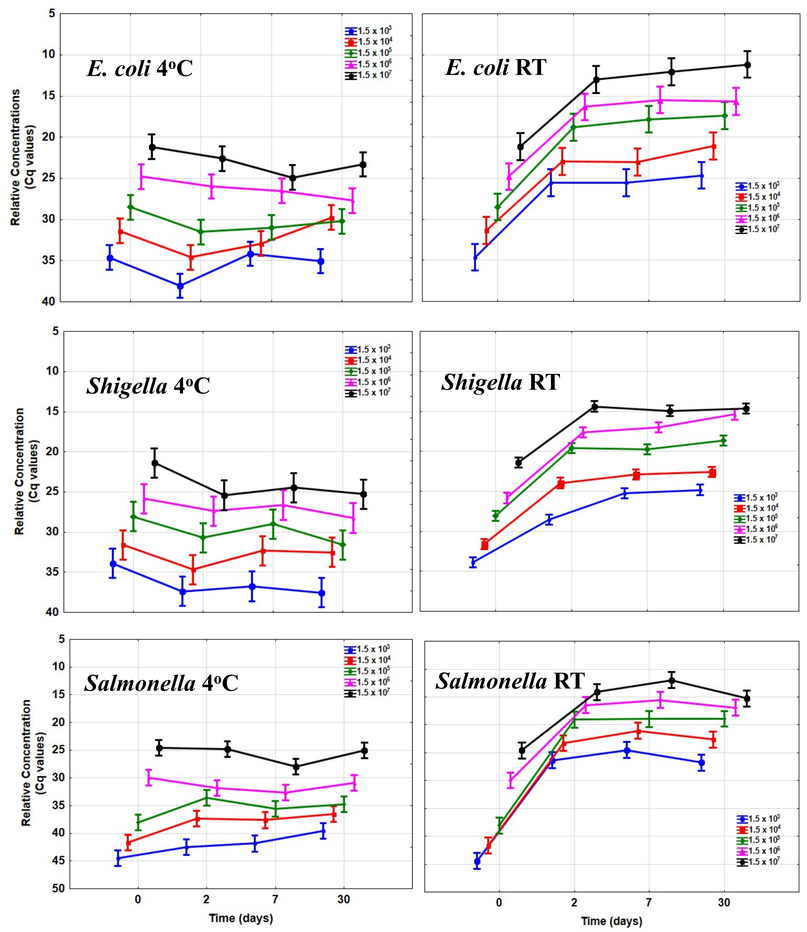



**Figure 1. Relative concentrations of *E. coli, S. sonnei* and *S. enterica* at 4°C and RT. Low Cq values denote increased concentrations.**

Table 1. ANOVA Analysis of each species room temperature and 4°C. Post-hoc analysis (Scheffe test) indicates significant differences are from day 0 to day 2.

Room Temperature

| Effect | df | *E coli* F | *E coli* p | *S. sonnei* F | *S. sonnei* p | *S. enterica* F | *S. enterica* p |
|---|---|---|---|---|---|---|---|
| Conc | 4 | 179.18 | 0.00 | 955.9 | 0.00 | 271.47 | 0.00 |
| Time | 3 | 182.58 | 0.00 | 908.0 | 0.00 | 717.88 | 0.00 |
| Conc*Time | 12 | 0.38 | 0.96 | 8.2 | 0.00 | 8.63 | 0.00 |

4°C

| Effect | df | *E coli* F | *E coli* p | *S. sonnei* F | *S. sonnei* p | *S. enterica* F | *S. enterica* p |
|---|---|---|---|---|---|---|---|
| Conc | 4 | 189.20 | 0.00 | 119.18 | 0.000 | 350.13 | 0.00 |
| Time | 3 | 10.82 | 0.00 | 12.39 | 0.000 | 12.64 | 0.00 |
| Conc*Time | 12 | 3.67 | 0.01 | 0.73 | 0.711 | 5.98 | 0.00 |

## Results (continued)

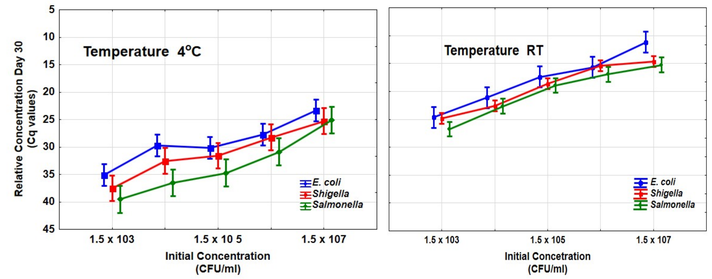



**Figure 2. Relative final concentrations of *E. coli, S. sonnei* and *S. enterica* for RT and 4°C following incubation for 30 days.**

## Conclusions

•For all three pathogens the yields of DNA isolated from 400 µL of media was more than sufficient for multiple QPCR assays at all concentrations.

•Quality of the isolated DNA was sufficient for successful use in a standard quantitative polymerase chain reaction (QPCR) assay verifying that DNA quality and quantity is adequate for molecular genetic assays.

•For all three pathogens detected concentrations increased significantly from Day 0 to Day 2 (Figure 1, P<0.0001) though the relationship with initial concentrations was maintained. After day 2, with the exception of *Shigella* at room temperature, there was not significant growth of pathogens in media.

•For all three pathogens QPCR detected a linear positive relationship between the initial sample concentration and detected concentration after 30 days of storage regardless of storage temperature or storage time (Figure 2).

•These results indicate that the Puritan Fecal Opti-Swab collection and transport system allows for the detection and relative quantification of the enteric pathogens *E. coli, S. sonnei and S. enterica* following long-term storage without special handling.

## Acknowledgements

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