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. Samples that do not inhibit later downstream molecular genetic procedures. For all pathogens qPCR detected a linear positive relationship between the initial sample concentration and quantified relative copy numbers across all concentrations.

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

Results
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.

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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 2, 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.