Optimizing DNA Extraction from Pediatric Stool for Diagnosing Tuberculosis and Use in Next Generation Sequencing Applications

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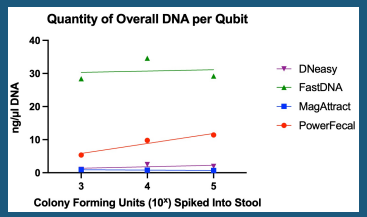
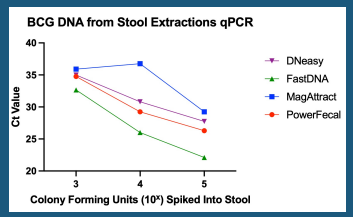
Background

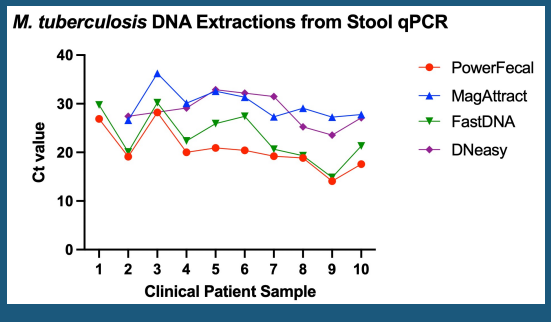
- WHO has endorsed stool for diagnosis of TB in children
- Targeted Next-Generation-Sequencing (tNGS) of stool has been shown to support diagnosis and provide information about drug-susceptibility (DS).
- Optimizing extraction of DNA from stool for sequencing is paramount to ensure high diagnostic sensitivity and accurate DS.

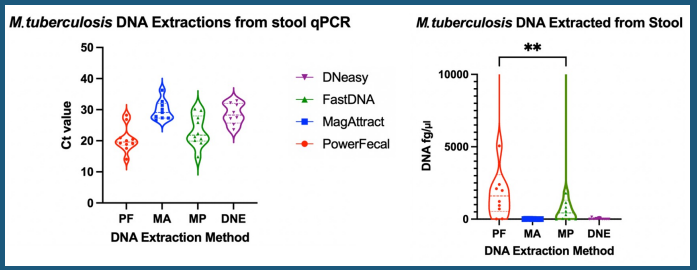
Methods

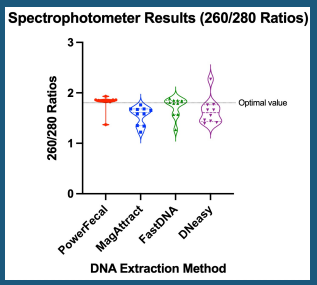
- Stool samples were spiked with varying known concentrations of BCG and extracted with four different DNA extraction kits.
- Each sample underwent qPCR and analysis to assess overall DNA yield, fragment length, and purity.
- 10 pediatric clinical samples diagnosed with pulmonary TB (culture or GeneXpert positive sputum) underwent the same process (extraction and analysis) to provide replication of findings in a clinical setting

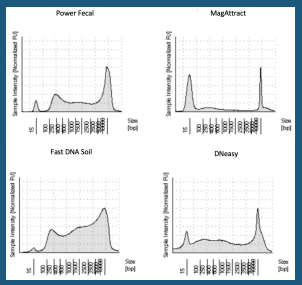












Results

- The FastDNA Spin Kit for Soil showed the most optimal results on model samples spiked with known quantities of BCG.
- On clinical samples, the FastDNA and PowerFecal Pro DNA kit both showed an increase in overall DNA quantity, M tuberculosis specific DNA quantity, and successful targeted sequencing when performed on stool samples, when compared to the two other kits.
- Three samples extracted via PowerFecal and three samples extracted via FastDNA (different patients) provided successful sequencing data with average depth of coverage of the rpoB region for FastDNA being 298 (range 107-550) and PowerFecal being 310 (182-474) which was comparable to one another (P value 0.946).

Conclusions

- The PowerFecal Pro and FastDNA Spin Kit were superior for extracting DNA from pediatric stool samples for tNGS.

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