The Development of Metagenomics Control Materials for Pathogen Detection

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Background

- Metagenomics provides an opportunity to understand the microbial population present in a given environment.
- The development of high-throughput sequencing has made the study of microbiomes increasingly possible.
- However, with recent increased activity in metagenomics research, there is need for reference materials that enable data accuracy and quality to be assessed.
- Control materials could enable performance evaluation of sample processing, library preparation, sequencing methods, and data analysis, thus aiding in the comparison of different studies.

Materials and Methods

We developed a metagenomic control material (MCM) mock community comprising genomic DNA prepared from 10 pathogenic bacterial species mixed at varying concentrations (Table 1, Figure 1). The batch-to-batch production of this material was characterized using digital PCR (Figure 2). This reference material will be available from ATCC® MSA-4000™.

Using this material, we investigated various methods of microbial genome quantification, including absorbance, fluorescence, high-throughput qPCR (ht-qPCR), digital PCR (dPCR), different 16S rRNA amplicon sequencing strategies, and whole genome sequencing (WGS) (Figure 3).

Table 1. Individual bacterial strains within the MCM genomic DNA mock community

ATCC® No.	Species	Abundance	Gram Status	% GC	Genome Size (Mb)	GenBank ID	16S copies
17978™	*Acinetobacter baumannii*	0.10%	-	39	4	CP000521	5
700802™	*Enterococcus faecalis*	0.70%	+	37.3	3.34	AE016830	4
700928™	*Escherichia coli*	1.40%	-	50.6	5.23	AE014075	7
700721™	*Klebsiella pneumoniae*	14.40%	-	57.1	5.32	CP000647	8
700532™	*Neisseria meningitides*	28.90%	-	51.7	2.19	AM421808	4
47085™	*Pseudomonas aeruginosa*	0.30%	-	66.6	6.26	AE004091	4
BAA-1556™	*Staphylococcus aureus* (MRSA)	0.70%	+	32.8	2.87	CP000255	5
BAA-1718™	*Staphylococcus aureus* (MSSA)	14.40%	+	32.8	2.87	AASB02000000	5
BAA-611™	*Streptococcus agalactiae*	2.90%	+	35.6	2.16	AE009948	7
700669™	*Streptococcus pneumoniae*	28.90%	+	39.6	2.22	FM211187	4
700294™	*Streptococcus pyogenes*	7.20%	+	38.5	1.85	AE004092	6

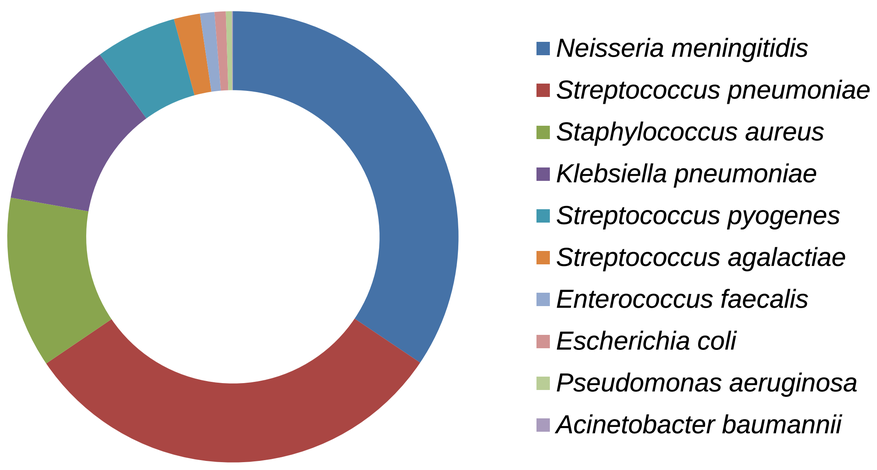

Figure 1. Mass-based composition of MCM

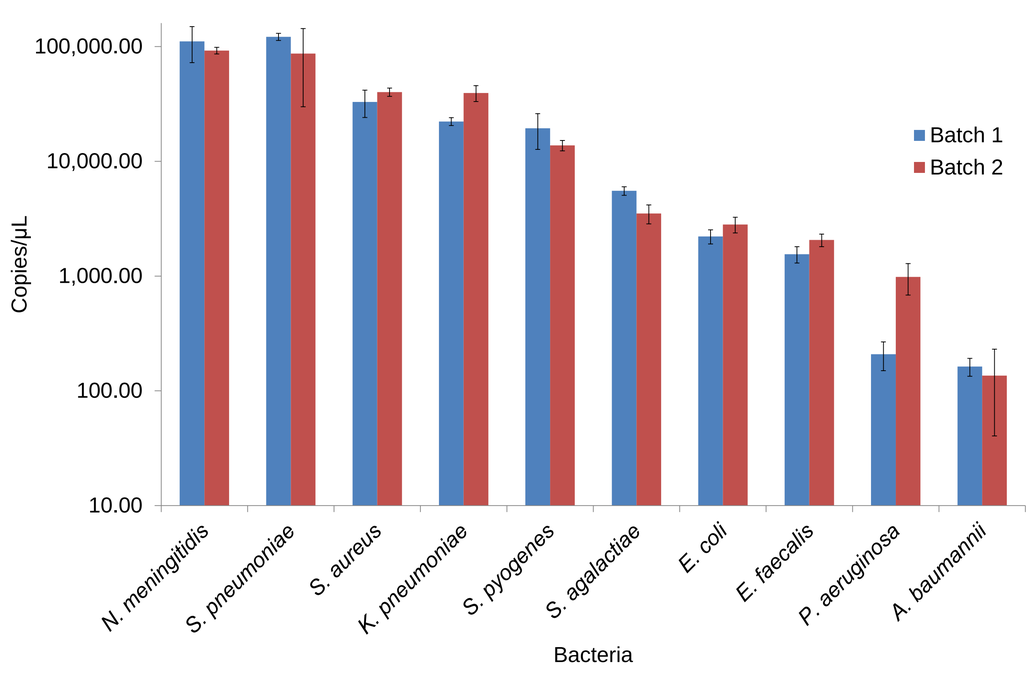

Figure 2. Batch-to-batch production. Evaluation of the composition of two different MCM mock community batches using dPCR (error bars: 95% confidence interval). dPCR enabled the calibration-free value assignment and comparison of different batches.

Results

- Control materials enabled different measurement approaches to be assessed.
- All methods gave comparable results; however, the MCM mock community was able to demonstrate where and by what magnitude the methods differed.
- The ht-qPCR results agreed with the Qubit® (Thermo Fisher Scientific) results; the standard curves were generated using calibrators initially quantified by fluorescence.
- The most significant difference observed between the various methods was the relative abundance of *Pseudomonas aeruginosa*.

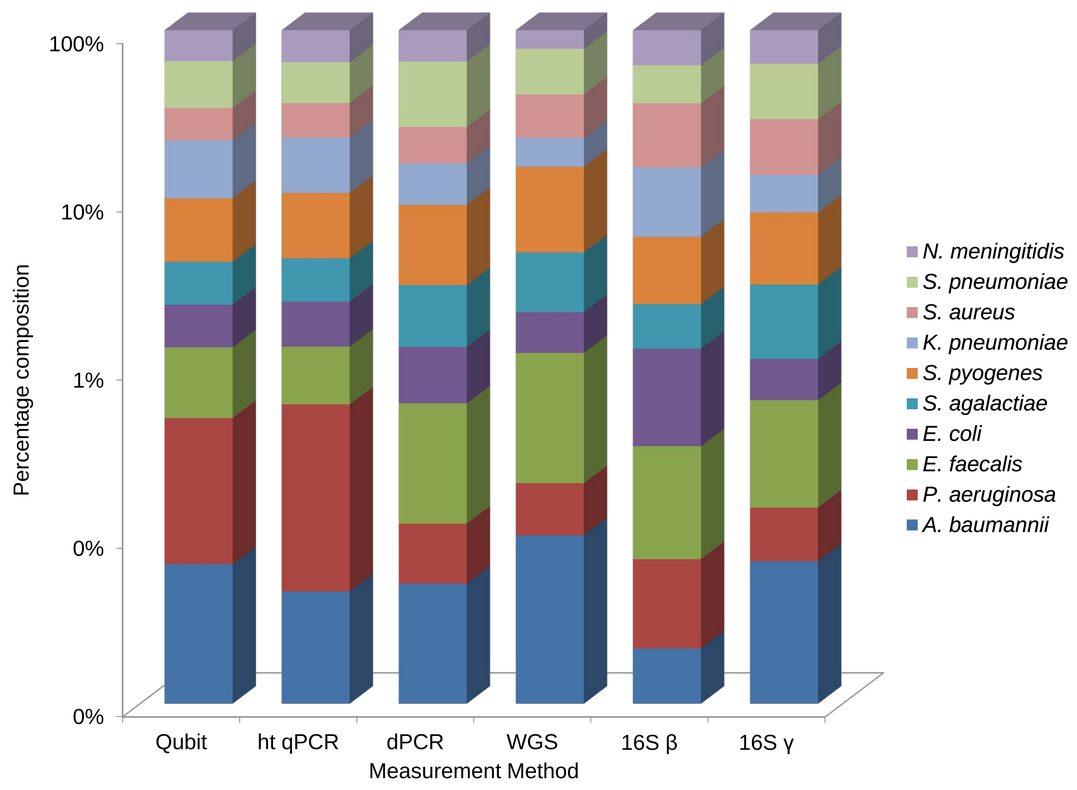

Figure 3. Assessment of the MCM mock community using different methods

Conclusions

- We demonstrated reproducible production of the MCM mock community.
- There was good agreement between the methods investigated with < 2.5 fold difference in the MCM mock community composition.
- These findings demonstrated that the MCM mock community can assist in evaluating the technical performance of different molecular quantification methods frequently used in microbiome analyses.
- Further application would allow laboratories to monitor results and compare technical performance with other laboratories, enabling them to identify sources of error that will improve accuracy and comparability when performing microbiome analysis as well as pathogen detection using molecular methods.

References

1. O'Sullivan DM, *et al*. Assessing the Accuracy of Quantitative Molecular Microbial Profiling. Int J Mol Sci 15(11): 21476-21491, 2014.
2. Huggett JF, *et al*. Considerations for the Development and Application of Control Materials to Improve Metagenomic Microbial Community Profiling. Accred Qual Assur 18(2): 77-83, 2013.

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Disclaimers

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