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To flow or not to flow: Considerations for quantitative microbiome profiling

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Abstract

Culture-independent methods are changing the way scientists interrogate microbes in complex environments such as the human body. Specifically, targeted sequencing of the conserved 16S rRNA gene is relatively simple, inexpensive, and high-throughput. However, the analysis of such data quantifies each microbe as a fraction of the total read count, which can lead to misinterpretations of microbes that differ between communities associated with disease. To address this limitation, quantitative microbiome profiling (QMP) methods have been developed. QMP methods complement 16S rRNA gene sequencing with estimates of microbial load by quantifying bacterial cells or 16S rRNA copy number. Several QMP methods have been proposed, but their performance on biological samples have not been thoroughly evaluated. Here, we present our results benchmarking QMP methods to investigate the strengths and limitations of each on samples originating from high and low microbial biomass environments.

Methods

We compared three modalities for estimating microbial load: quantitative PCR (qPCR), droplet-digital PCR (ddPCR), and cell counting using SYTO BC staining with flow cytometry (flow). For each method of estimating microbial load on a mock microbial community dilution series, we evaluated variation as percent error and accuracy as log₂ fold change from expected.

Conclusions

The method of estimating microbial load must be carefully considered and evaluated for QMP. We found that ddPCR was more precise but less accurate than flow, and flow was highly accurate but not precise. ddPCR had the least variation in repeated measures of the same sample, indicating that the reproducibility and error introduced with this method is minimal compared to qPCR and flow. The high variability of flow cytometry is likely due to complications in sample preparation which is simpler for ddPCR and qPCR. Both ddPCR and flow appear to have a larger dynamic range where the accuracy is within reason.