A Comparative Analysis of Standard Plate Counting and Alternative Quantitation Methods Using ATCC® 8739-MINI-PACK™

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Abstract

Traditional microbiological plating and colony-forming unit (CFU) counting is the standard for enumerating bacterial cells in a culture as it is effective and inexpensive. However, the drawbacks of this method are that CFUs only account for cells that grow under specific laboratory conditions and don't account for cell clumping—both factors leading to an underestimation of the total number of cells. Additionally, obtaining CFU counts can be time-consuming as the incubation period can be anywhere from days to weeks depending on the organism. Alternative methods to plate counting have been developed to allow for quicker and more accurate results. Here, we used *Escherichia coli* (ATCC® 8739-MINI-PACK™) formulated as a ready-to-use glycerol stock to compare several of these alternative methods (impedance and fluorescent flow cytometry, including live/dead staining) to plate counting.

Our data demonstrated consistent CFU values for *E. coli* on all three days for the plate count method; the alternative quantitation methods identified higher concentrations of cells in comparison to plate counting. This is most likely due to the ability of these methods to detect non-culturable but intact cells. The greatest difference in the methods was the precision and amount of time to results. The alternative methods provided quicker and more precise results when compared to CFU counting. With proper optimization, these techniques could be better suited to estimate CFU counting. Further, the use of these alternative methods also gives scientists a better understanding of the overall physiology of the cells.

Materials and Methods

Six vials of *E. coli* were thawed and diluted in appropriate media to the appropriate dynamic range for each method. For plate counting, material was diluted in 1x PBS 1/1,000,000 and 100 µL was spread onto tryptic soy agar, dried, and incubated at 37°C for 24 hours prior to counting. For BactoBox measurements, material was diluted 1/10,000 in 0.1x PBS and each vial was measured in duplicate using the "default" setting on the instrument. For the CytoFLEX, material was diluted 1/1,000 and stained using the ThermoFisher LIVE/DEAD BacLight Bacterial Viability and Counting Kit for flow cytometry.

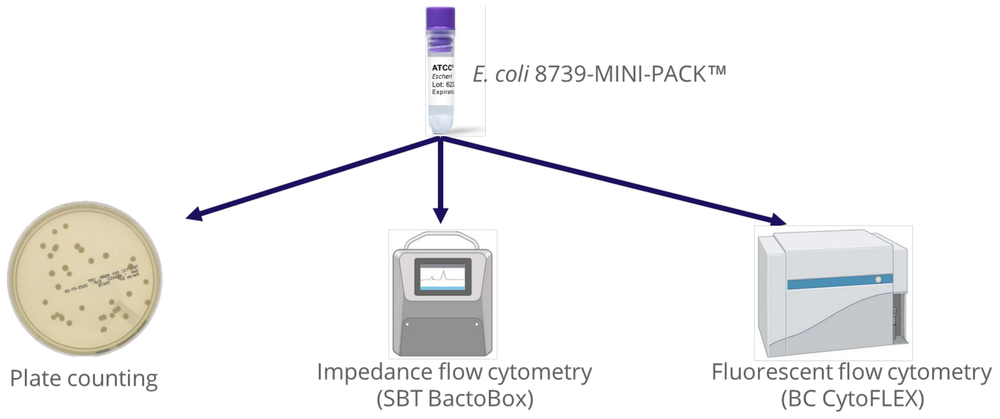

Figure 1: Quantitation methods. The schematic illustrates three distinct approaches for quantifying ATCC® 8739-MINI-PACK™: plate counting, impedance flow cytometry, and fluorescent flow cytometry. Flow cytometry images created using BioRender.com.

Results

8739-MINI-PACK™ vials produced an average of 6.5x10^8 CFU/mL, alternative methods produced slightly higher concentrations

The 8739-MINI-PACK™ vials measured an average of 6.5x10^8 CFU/mL; however, both the BactoBox and CytoFLEX measured a ~2-fold higher concentration of cells/mL in comparison to plate counting. The BactoBox and CytoFLEX were more precise in comparison to plate counting.

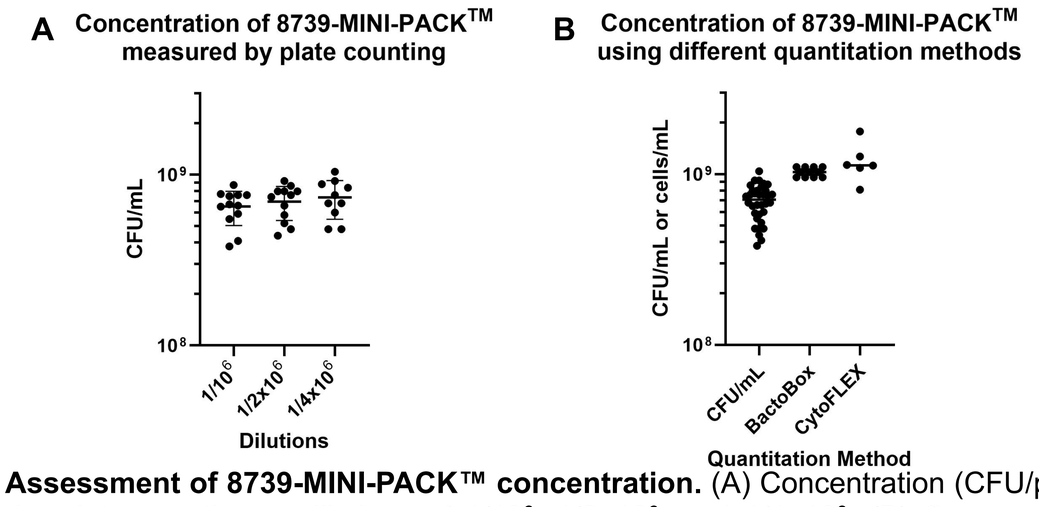

Figure 2: Assessment of 8739-MINI-PACK™ concentration. (A) Concentration (CFU/plate) measured by plate counting at dilutions of 1/10^6, 1/2×10^6, and 1/4×10^6. (B) Concentration (CFU/mL or cells/mL) measured via three quantitation methods.

Results Continued

Fluorescent flow cytometry allows for the distinction between live and dead cells. 8739-MINI-PACK™ contains mostly live cells.

Fluorescent flow cytometry revealed that there were more live cells than dead cells by roughly 10:1. The material was diluted 1/1,000 for staining and this dilution was not inhibited by the ingredients found in the material for preservation.

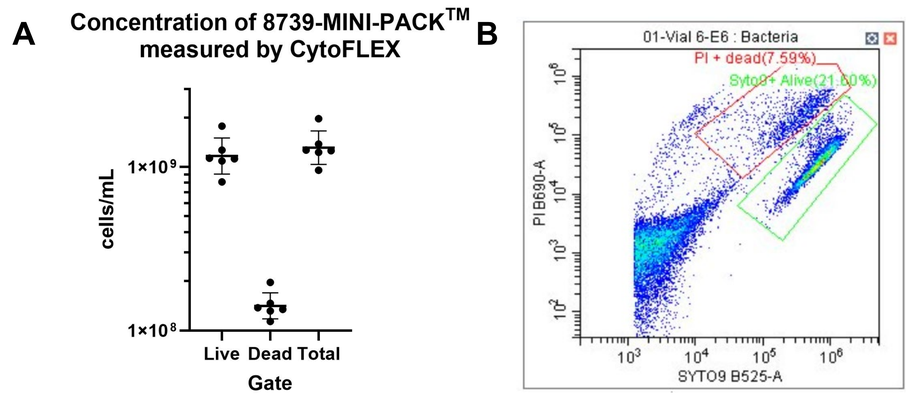

Figure 3: Quantification via fluorescent flow cytometry. (A) Live, dead, and total cells/mL measured on the CytoFLEX. (B) Distribution of live and dead stained cells within the gate on the CytoFLEX.

Impedance flow cytometry does not allow for the distinction between live and dead cells but can be optimizable to reflect CFU

The BactoBox measured a similar number of live cells compared to the CytoFLEX. The instrument does not distinguish between live and dead cells though. The cells that were measured showed consistency in conductivity and size between replicates. The material was diluted 1/10,000 for staining and was not inhibited by the ingredients found in the material for preservation, nor did these preservatives interfere with the conductivity of the samples.

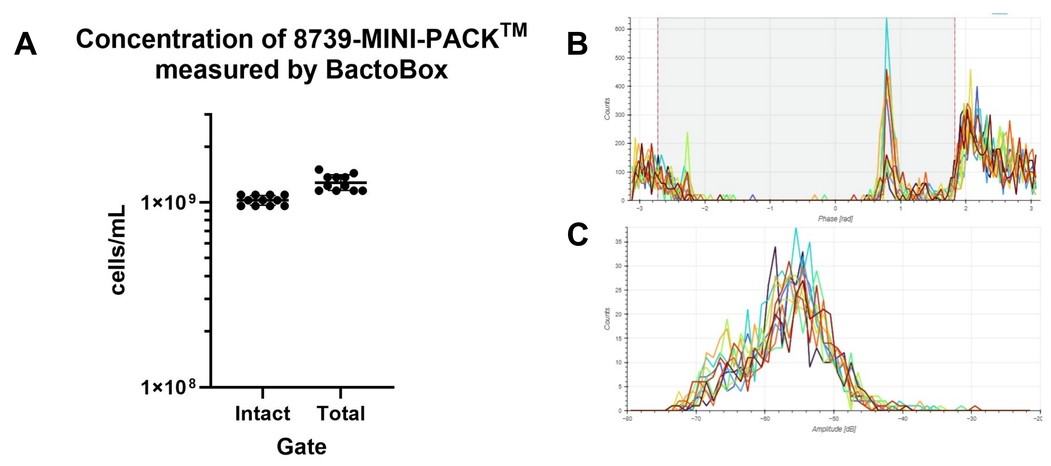

Figure 4: Quantification via impedance flow cytometry. (A) Intact and total cells/mL measured on the BactoBox. (B) Distribution of particle conductivity and (C) size in the BactoBox.

Conclusions

- 8739-MINI-PACK™ vials were suitable for use for the alternative methods without significant washing or manipulation.
- Plate counting is the gold standard for bacterial quantitation, but the method was less precise than the alternative methods.
- The alternative methods produced higher cell counts but offer expanded capabilities for measuring microbial physiology and can be optimized for plate counting depending on use.

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