Comparison of Standard Plate Counting to Alternative Quantitation Methods for *E. coli*

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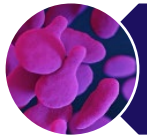
CAFPA-ASM D.C. Branch FALL MEETING 2025

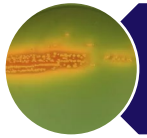
ATCC
More than a biorepository

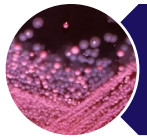
- Founded in 1925, ATCC is a private, nonprofit, global biological resource center and standards organization that provides scientists with the biomaterials and resources they need to conduct critical life science research.
- World's trusted, premier biological materials resource and standards development organization:
  - 4,000+ cell lines
  - 80,000+ microorganisms
  - Genomic and synthetic nucleic acids
  - Media, sera, and reagents
  - Advanced cell models
  - Standards

Learn more about ATCC's Standards and Controls


Pharmaceutical Testing

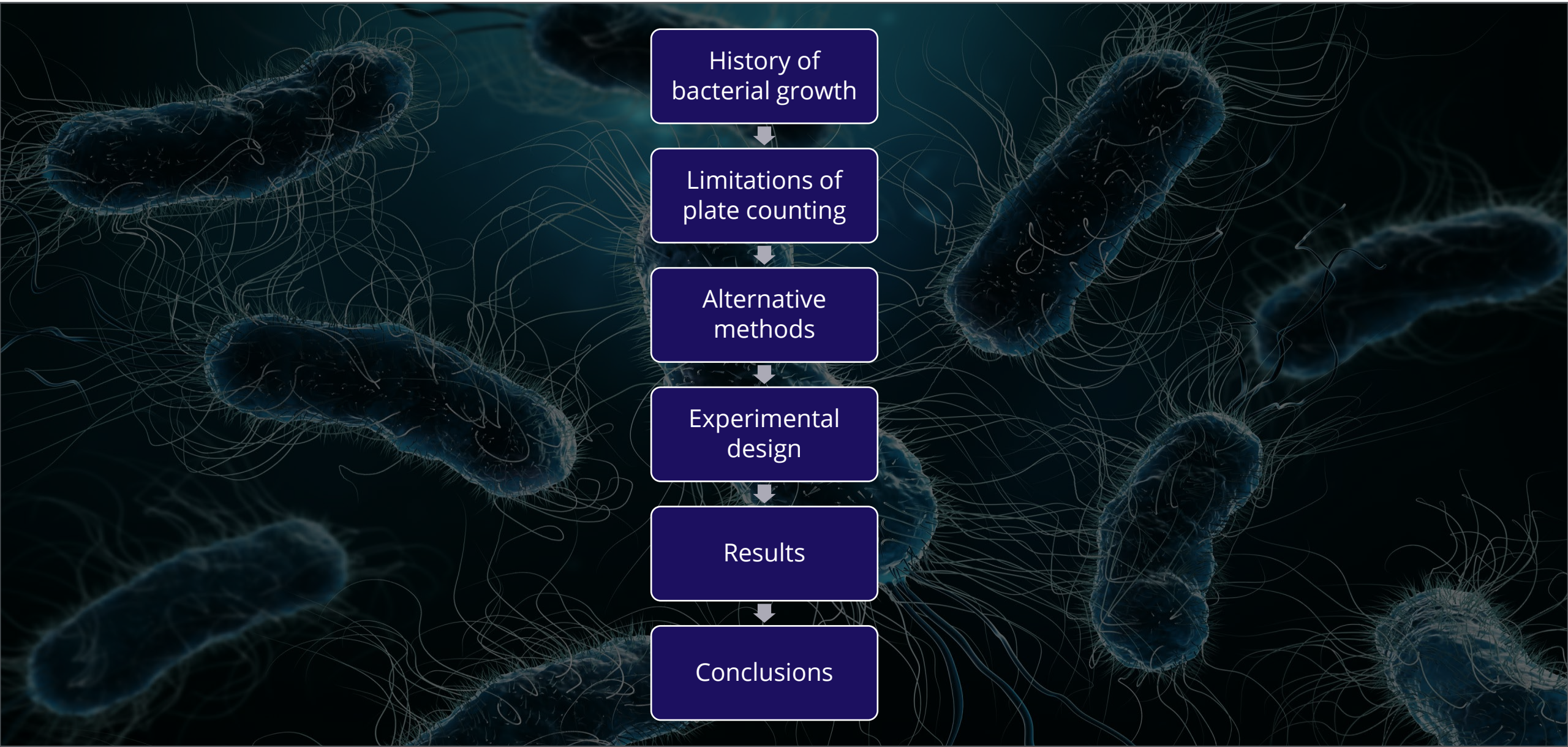

Mycoplasma Testing


Water Testing


Food Testing

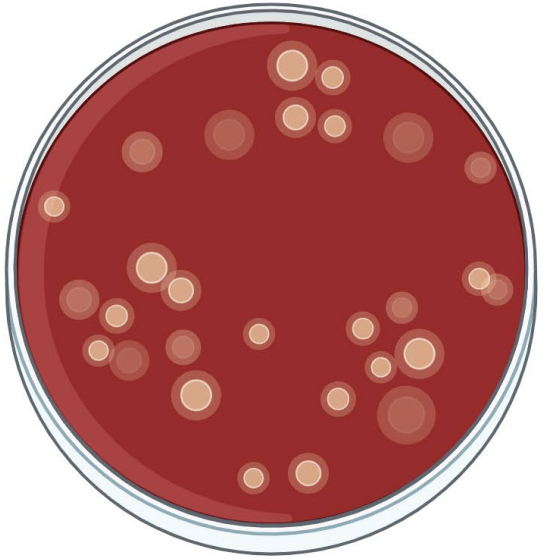

Standards Development

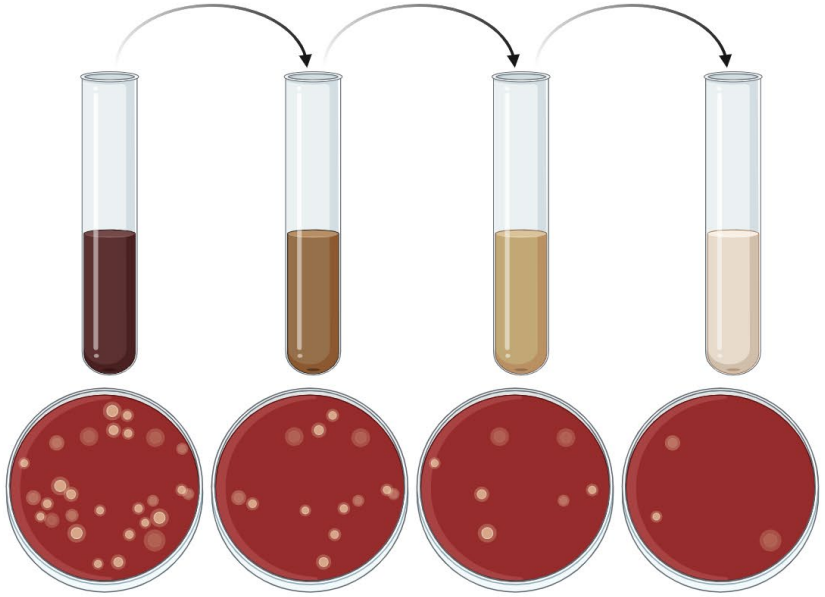
Overview


History of bacterial growth
Limitations of plate counting
Alternative methods
Experimental design
Results
Conclusions

History of Plate Counting

From a new frontier to the golden standard





Mid-1800s → **1870s-1880s** → **Early 1900s** → **Recent decades**

Microbes cultured in liquid media

Use of solid media to distinguish different species

The birth of plate counting

CFU becomes the golden standard

The Limits of Plate Counting

6 Limited dynamic range

- 30-300 colonies per plate

1 Limited cultivability

- Culture condition dependent
- Misses viable but nonculturable cells

5 Clumping and aggregation

- Clumped cells look like 1 CFU
- Underestimation

2 Time consuming

- Usually 24-72 hours incubation (maybe longer)

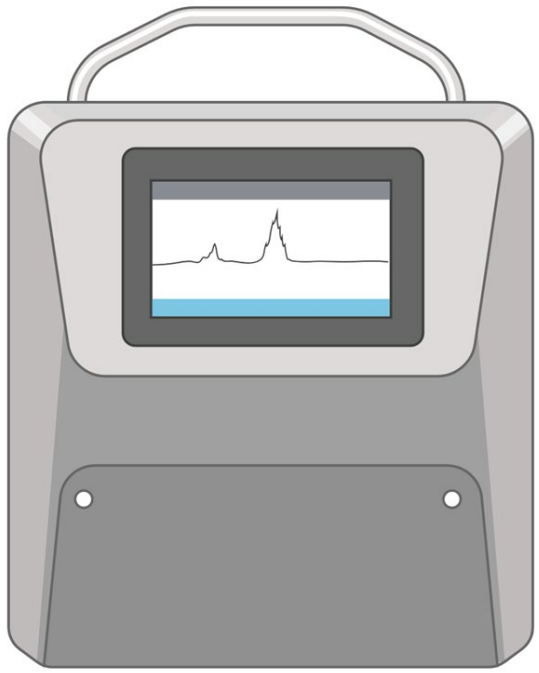
4 Reproducibility with mixed populations

- Species can grow at different rates
- Fast vs. slow growing

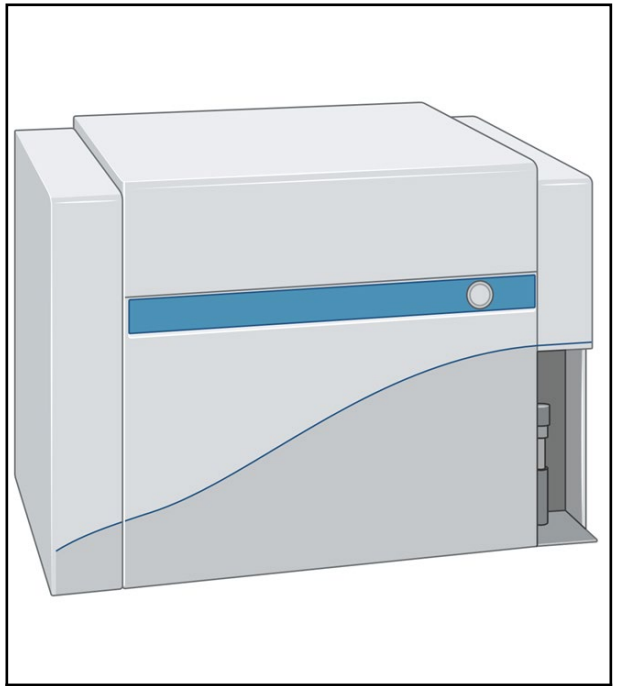
3 Labor intensive & human error

- Serial dilutions, plating, and manual colony counting
- Small technique variations

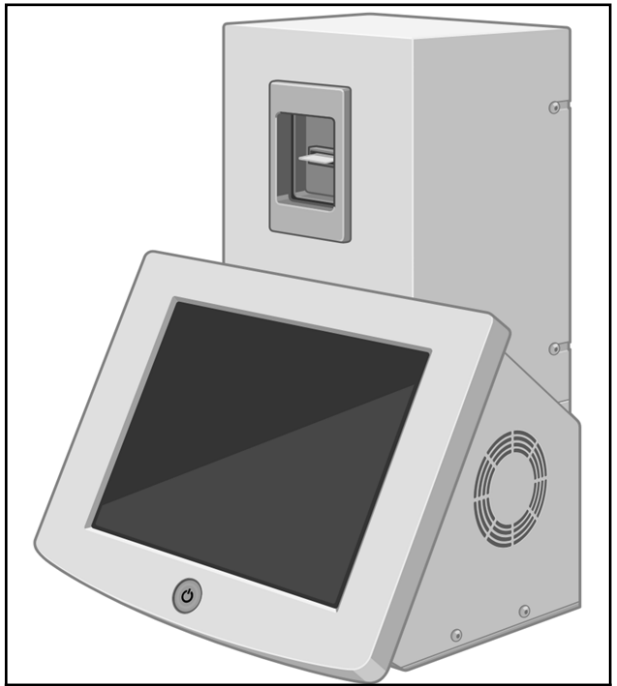
Alternative Quantitation Methods



Impedance Flow Cytometry



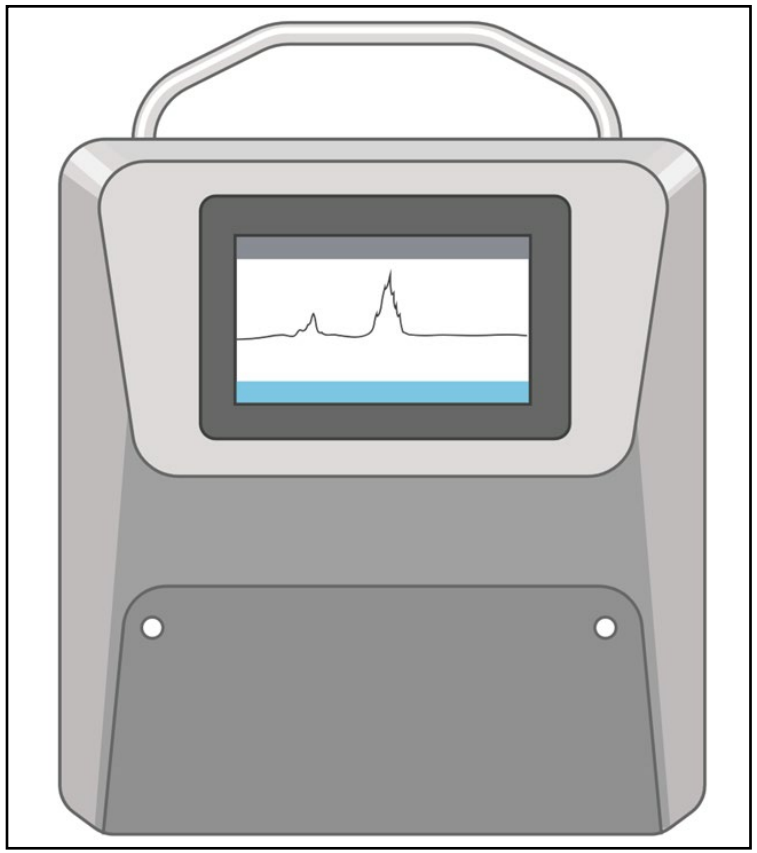
Fluorescent Flow Cytometry



Imaging

dPCR

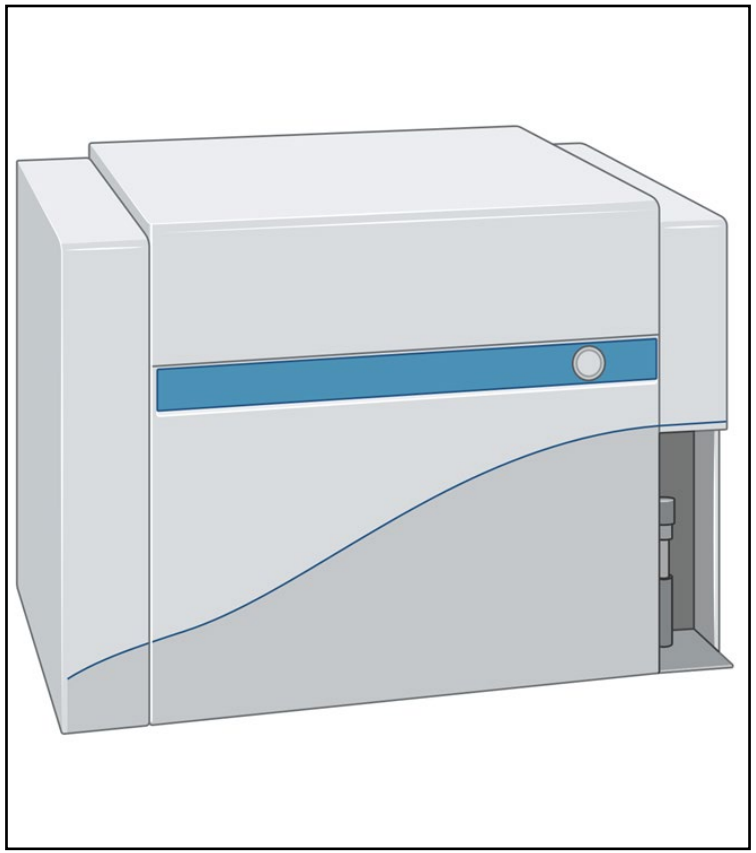
Alternative Quantitation Methods



Impedance Flow Cytometry

Pros	Cons
• Cost • Mobile and small • Fast • No media necessary	• Cannot discern between live and dead • Cannot discern between different organisms

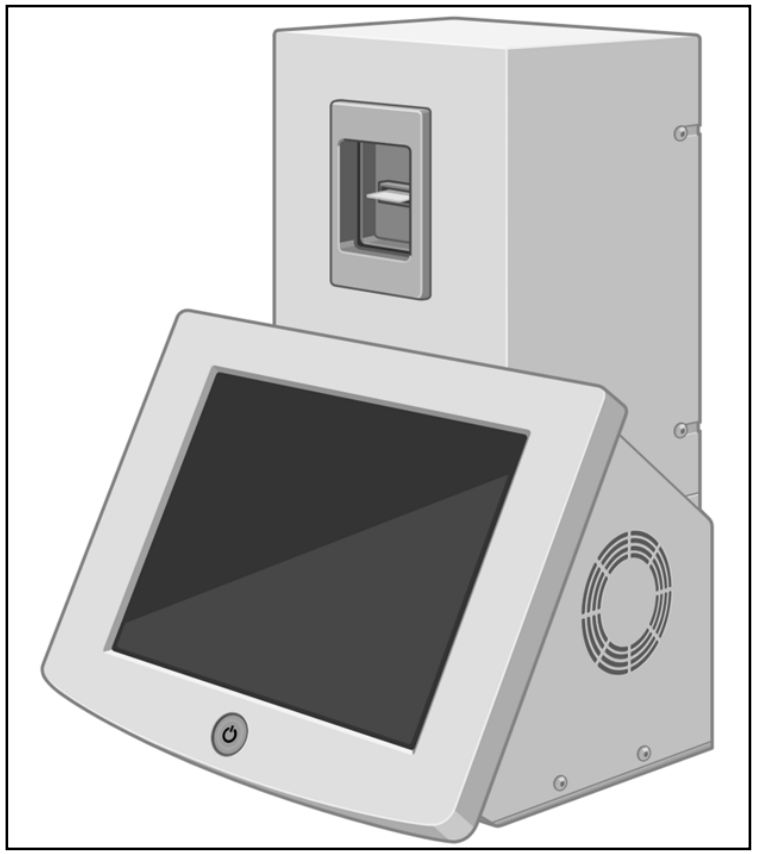
Alternative Quantitation Methods



Fluorescent Flow Cytometry

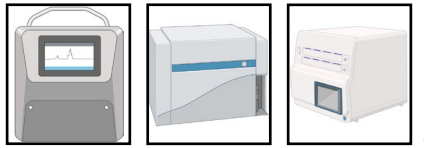
Pros	Cons
• Can differentiate live and dead cells (with staining) • Can differentiate between different organisms (sometimes) • Optimizable • Fast • No media necessary	• Cost • Non-mobile and size • Training

Alternative Quantitation Methods



Imaging

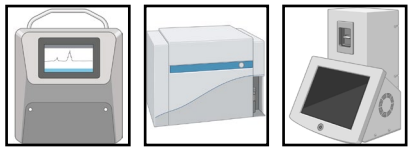
Pros	Cons
• Fast • No media necessary • Can differentiate live and dead cells (with staining) • Small sample volume	• Sensitive to clumps • Cannot discern between different organisms/particulates



Alternative Quantitation Methods

dPCR

Pros	Cons
• Faster than plate counts • High sensitivity • Absolute quantification • Detects VBNC • Can differentiate between different organisms	• Cost • Complex workflow • Potential inhibitor sensitivity



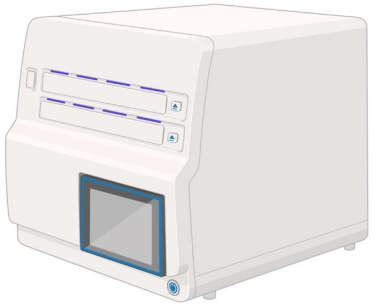
Experimental Design

Objectives

- Determine extended applications for the use of ATCC® Mini format
- Compare different quantitation technologies to CFU

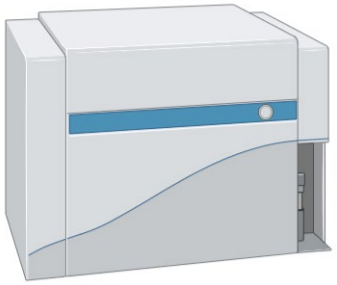
Plate counting

E. coli
8739-MINI-PACK™



Digital PCR
(QIAcuity)

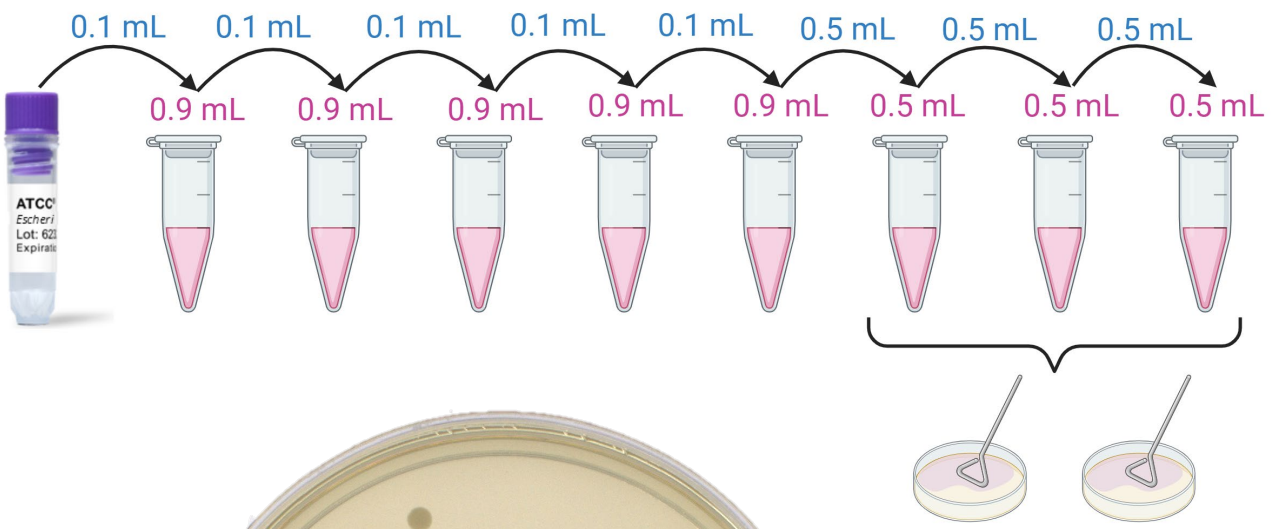
Impedance flow cytometry
(SBT BactoBox)



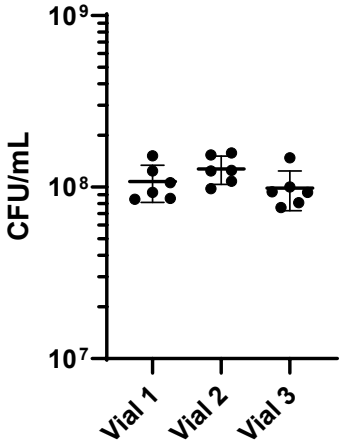
Fluorescent flow cytometry
(BC CytoFLEX)

Imaging
(Cellometer)

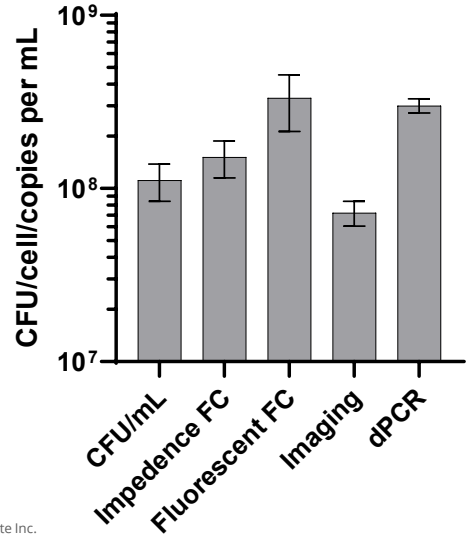
Plate Counting - CFU



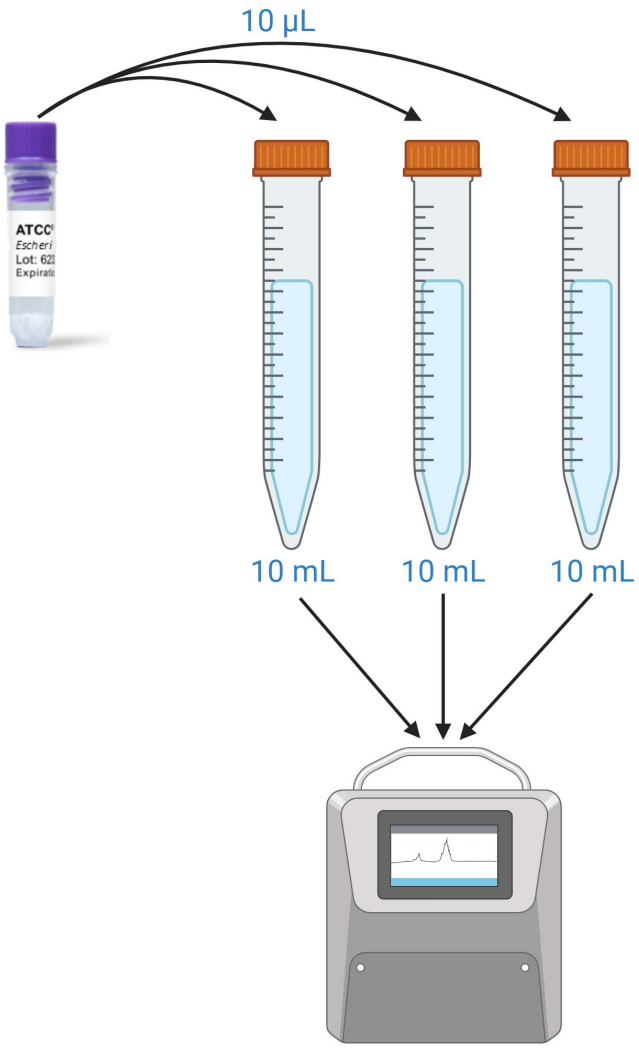
Concentration of *E. coli* (ATCC 8739-MINI-PACK™) measured by plate counting



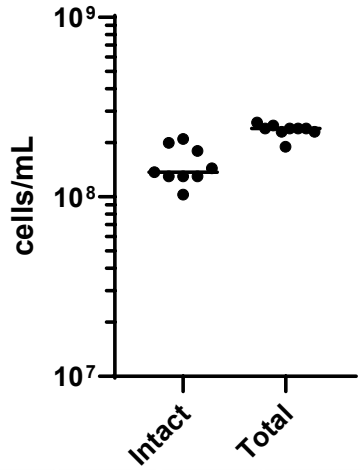
Quantitation of *E. coli* (ATCC 8739-MINI™) using different quantitation methods

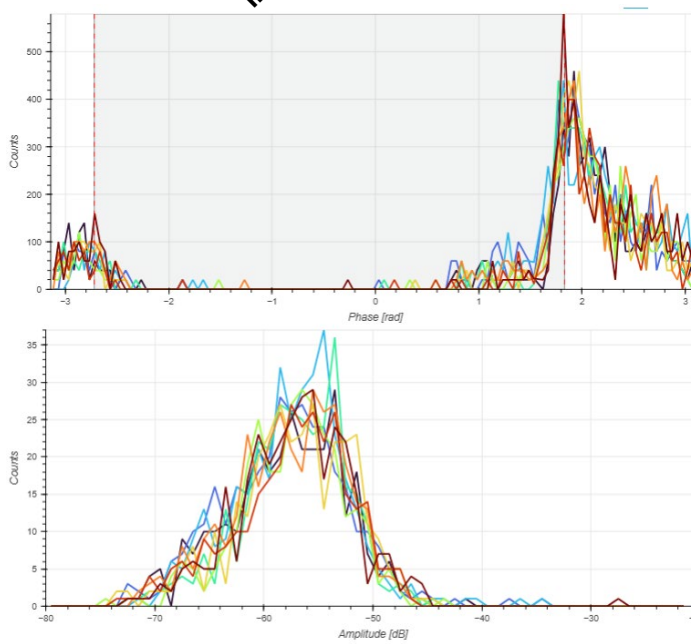


Impedance Flow Cytometry (BactoBox)

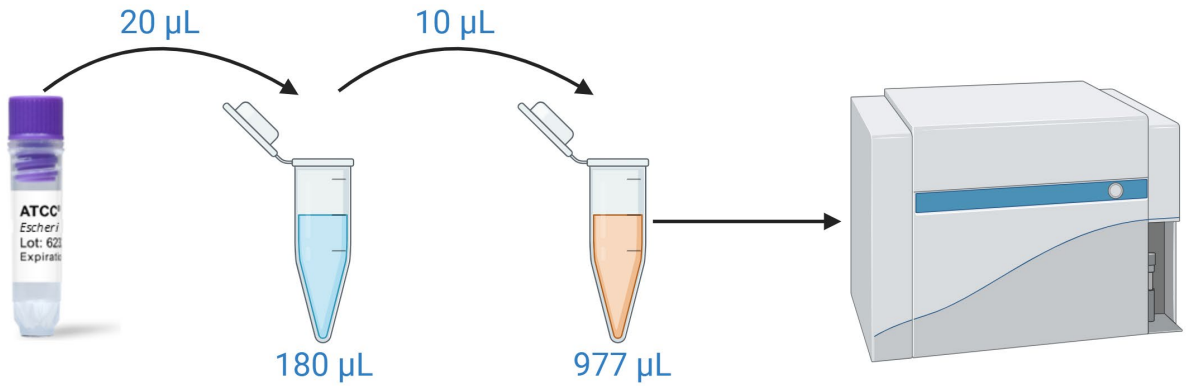


Concentration of *E. coli* (ATCC 8739-MINI™) measured by impedance flow cytometry

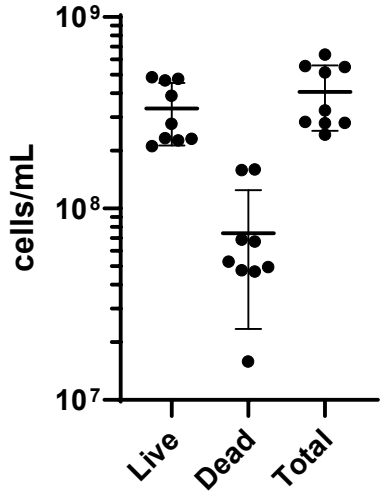


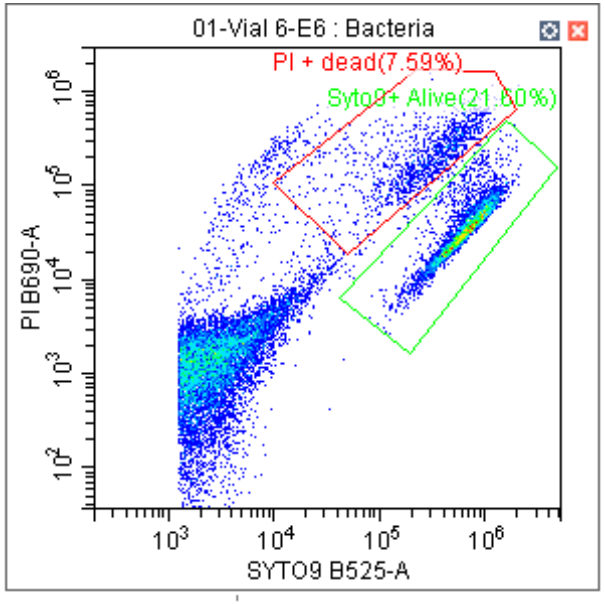


Fluorescent Flow Cytometry (CytoFLEX)

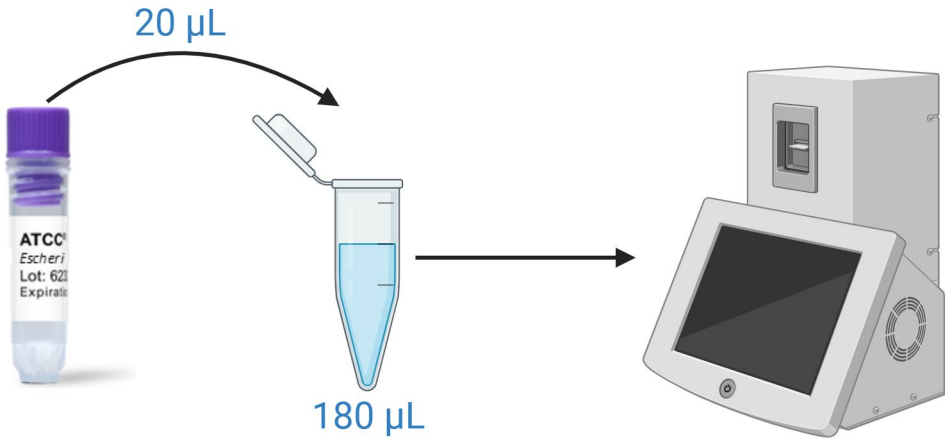


Concentration of *E. coli* (ATCC 8739-MINI™) measured by fluorescent flow cytometry

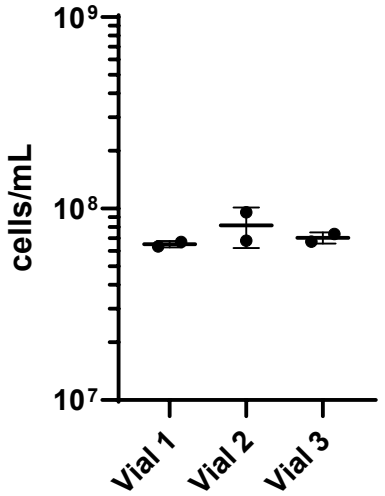


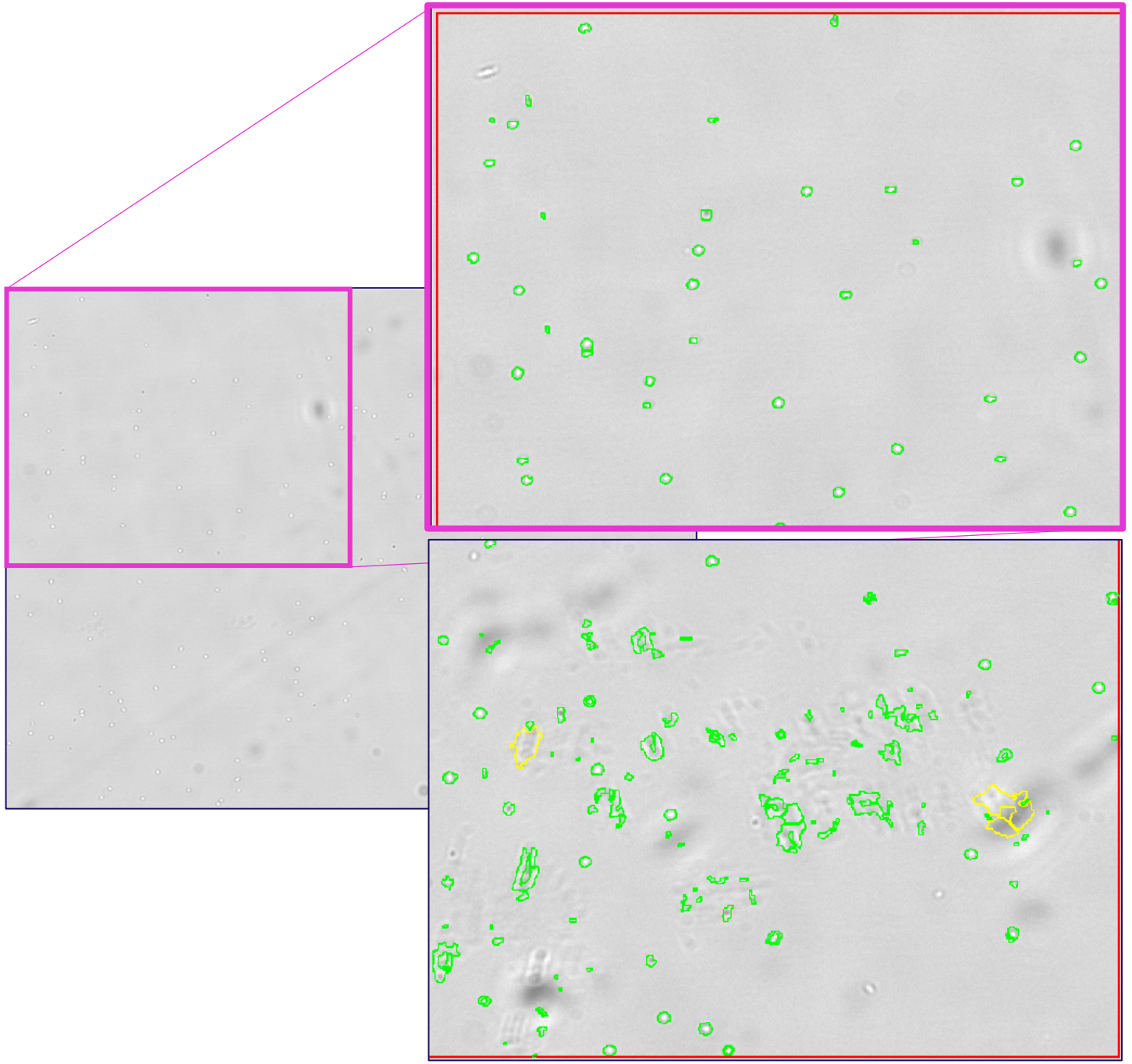


Imaging (Cellometer)

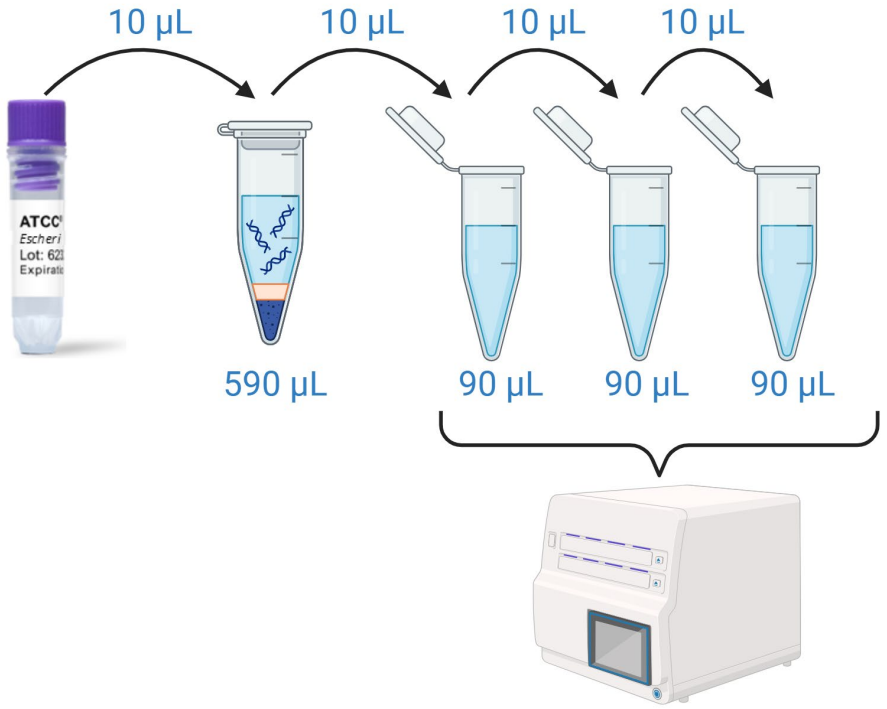


Concentration of *E. coli* (ATCC 8739-MINI™) measured by imaging

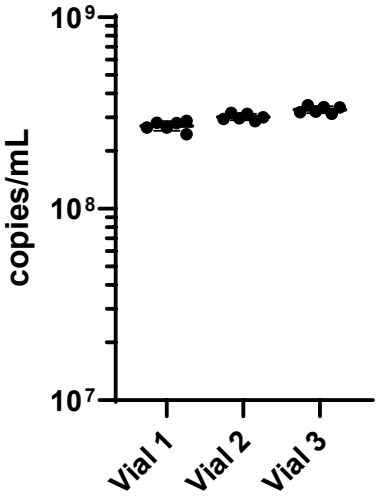


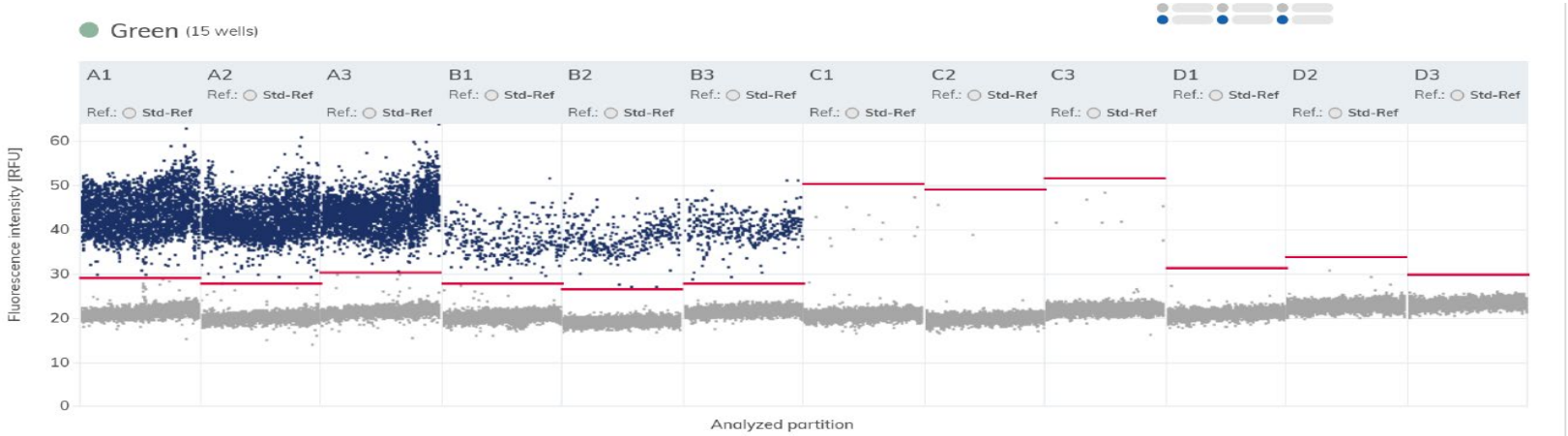


BIO-RAD ddPCR



Concentration of *E. coli* (ATCC 8739-MINI™) measured by ddPCR

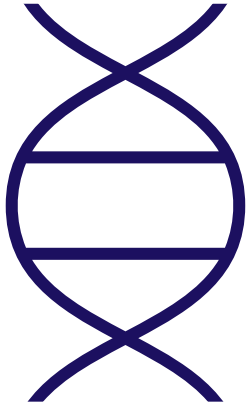




Conclusions

Versatility of ATCC® 8739-MINI-PACK™

Beyond CFU



Method trade-offs

Application-driven choice

ATCC® Minis

- Six pack of ready-to-use strains in glycerol stock
- Glass-free mini-cryovials with 2-D barcode for easy storage and tracking
- Most strains stable up to 1 year at -20°C
- ISO certified & ISO/IEC accredited

- Designed for quality control
- Testing for viability to minimum concentration
- No culturing or revitalization required

BIG QUALITY
in a **TINY TUBE**
Just open, plate, and go!

Acknowledgements

Thank you!

- Microbiology Product Development
  - Victoria Knight-Connoni, PhD
  - James Budnick, PhD
  - Leka Papazisi, DVM, PhD
  - Katherine Morin, MS

Questions?