2025 NIST Workshop on Rapid Microbial Testing Methods

RMTM Consortium Working Group 1 Update

April 8, 2025

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National Institute of Standards and Technology
U.S. Department of Commerce

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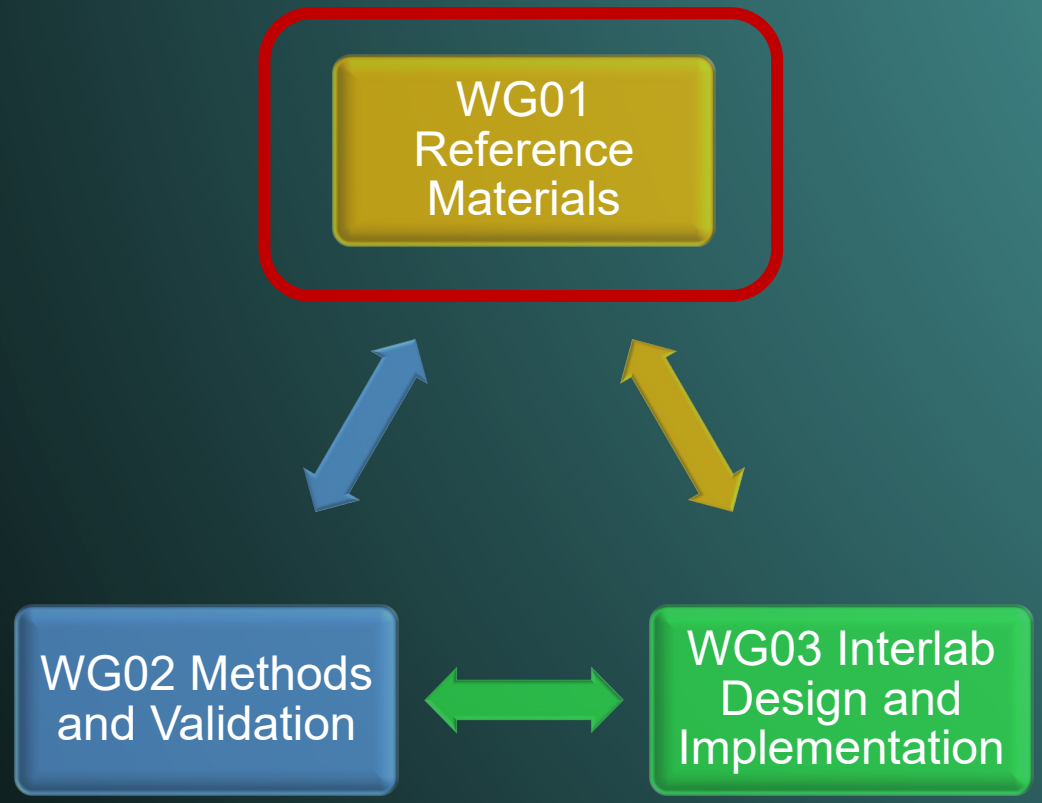
WORKING GROUP UPDATES



Acknowledgements and Disclaimer

Nancy Lin
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Kirsten Parratt
Co-chair

Jennifer Dootz
(Previously at NIST)

Sandra Da Silva

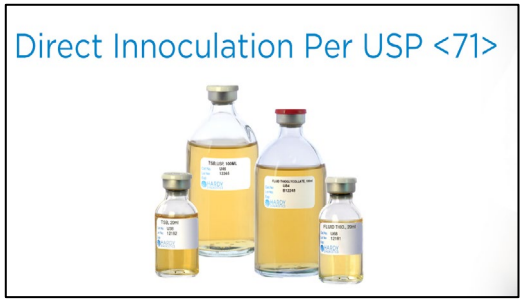
Joy Dunkers

Monique Hunter

Stephanie Servetas

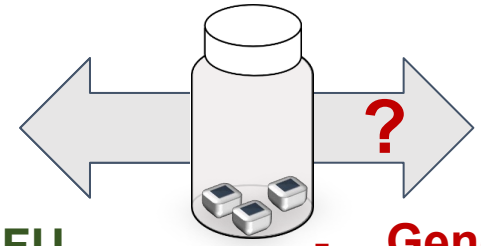
Available Reference Materials Fall Short of "Bridging" Compendial and Molecular Methods

Compendial Method



Output: Growth

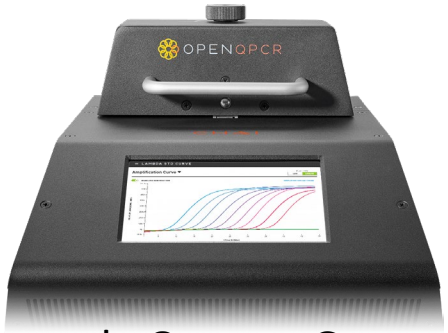
Commercial Microbial Cell RMs



✓ CFU

- Genome Copies
- Total Cells

Molecular Method



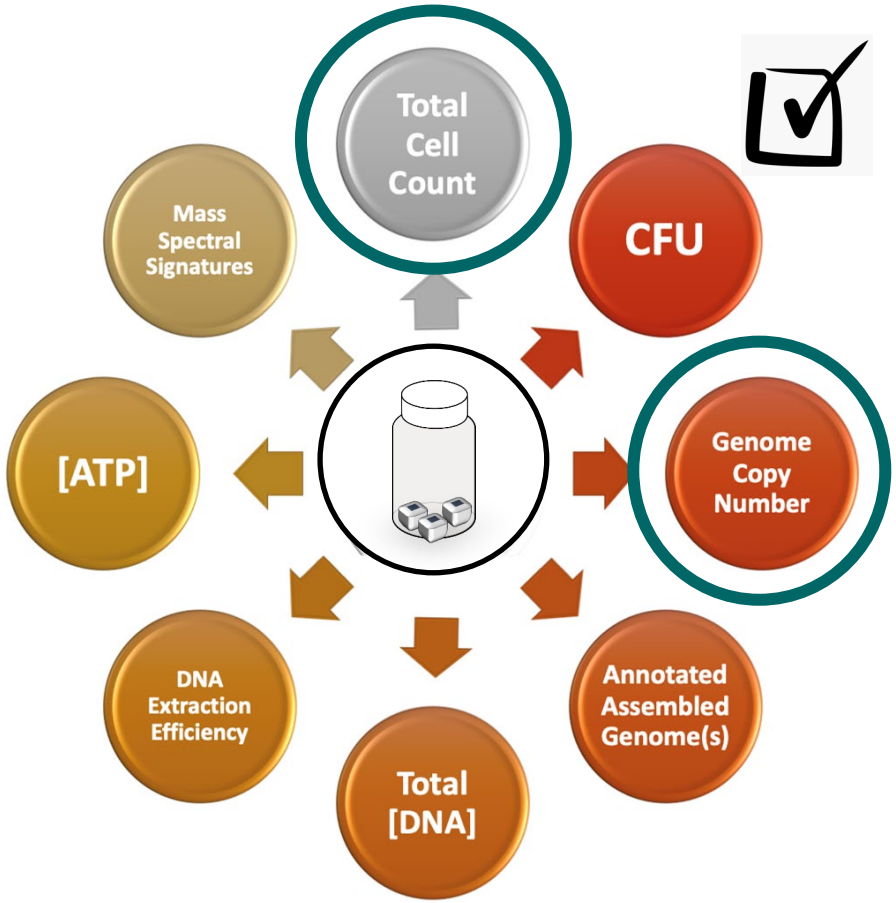
Example Output: Genome copies

Commercial materials are currently certified for CFU

CFU ≠ Genome Copies ≠ Total Cells

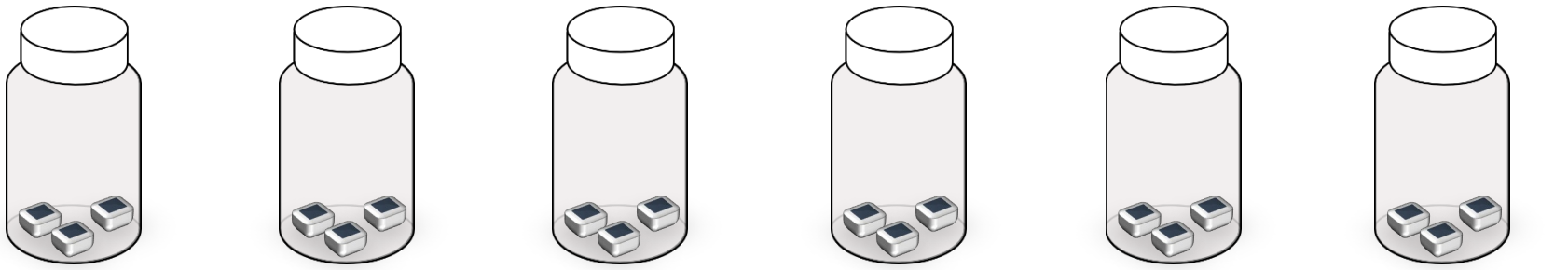
Consensus Need:
Microbial Cell RMs with Additional Certified Values

- RMTMs have measurands beyond growth
- Microbial cell RMs with additional certified values could help support RMTM validation and use



Idea: Suite of Compendial Organisms Certified Beyond CFU

USP <71> Compendial Organisms



Staphylococcus aureus	*Candida albicans*	*Aspergillus niger*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Clostridium sporogenes*
CFU = xx Total Cells = xx Genome Copies = xx	CFU = xx Total Cells = xx Genome Copies = xx	CFU = xx Total Cells = xx Genome Copies = xx	CFU = xx Total Cells = xx Genome Copies = xx	CFU = xx Total Cells = xx Genome Copies = xx	CFU = xx Total Cells = xx Genome Copies = xx

With methods, data, and best practices supporting their use for RMTMs

Objective: Develop methods for quantifying total cells and genome copies to enable expanded certification of commercially available reference materials

Proposed Translation Model

RMTM/NIST: Develop and demonstrate methods to quantify genome copies and total cells

- Develop methods using in-house (NIST) materials
- Confirm methods are promising for commercial materials
- Demonstrate methods on compendial strains (in house or commercial materials)

RMTM/NIST: Transfer methods to industry, contract labs, etc.

- Disseminate methods
- If needed, produce relevant NIST RM(s)

INDUSTRY: Certify genome copies and/or total cells for microbial cell RMs

- Optimize methods for specific products (eg proprietary matrix, culture SOP, etc.)
- If needed, use NIST RM to demonstrate capabiltiies
- Apply methods to expand certification for cell RM(s)

RMTM: Establish best practices to apply RMs with expanded certification to validate RMTMs

Plan for Microorganisms for Expanded Characterization

	E. coli	Compendials	Environmental Isolates
Materials	• Several commercial materials available • NIST methods already in progress	• Select a subset from USP <71> • Primarily for ease of characterization	• Expand to common contaminants, if desired • Strains chosen per input from industry

	Total Cell Number	Total Genome Copies	Total Viable Cell Number
Methods*	• Flow Cytometry • Coulter Counter • Microscopy • BactoBox	• dPCR (with DNA extraction) • Flow Cytometry • Microscopy	• Plate counting (CFU) • Flow Cytometry • Microscopy

**Use of orthogonal methods supports robust characterization of the materials*

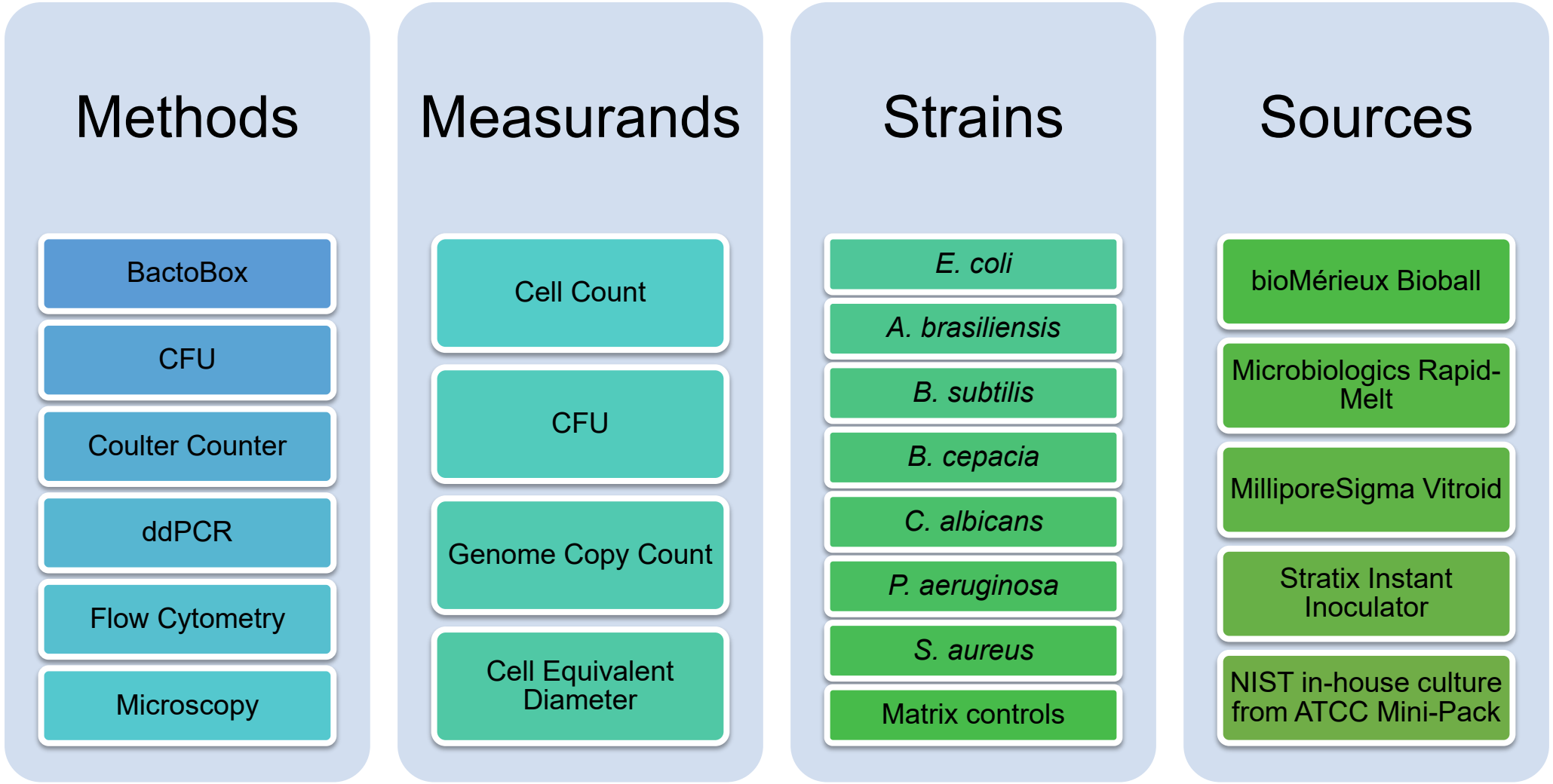
Methods used herein are for whole cell RM characterization –
we are not indicating that these methods are or should be used as RMTMs

WG1 Ongoing Outputs

- NIST Technical Publication "*Microbial Whole Cell Characterizations*"
  - A broad overview of studies performed
  - Conclusions related to fit-for-purpose reference material selection

- Consortium Perspective "*Toward Microbial Cell Reference Materials with Certified Attributes that Support Nucleic Acid-based Rapid Microbial Methods for Sterility Testing*"
  - Perspective from consortium members related to conclusions from technical publication
  - Co-authors: Tony Cundell, Stefan Emler, Sam Forry, Mina Izadjoo, Nancy J. Lin, Kirsten Parratt, Tricia Vail

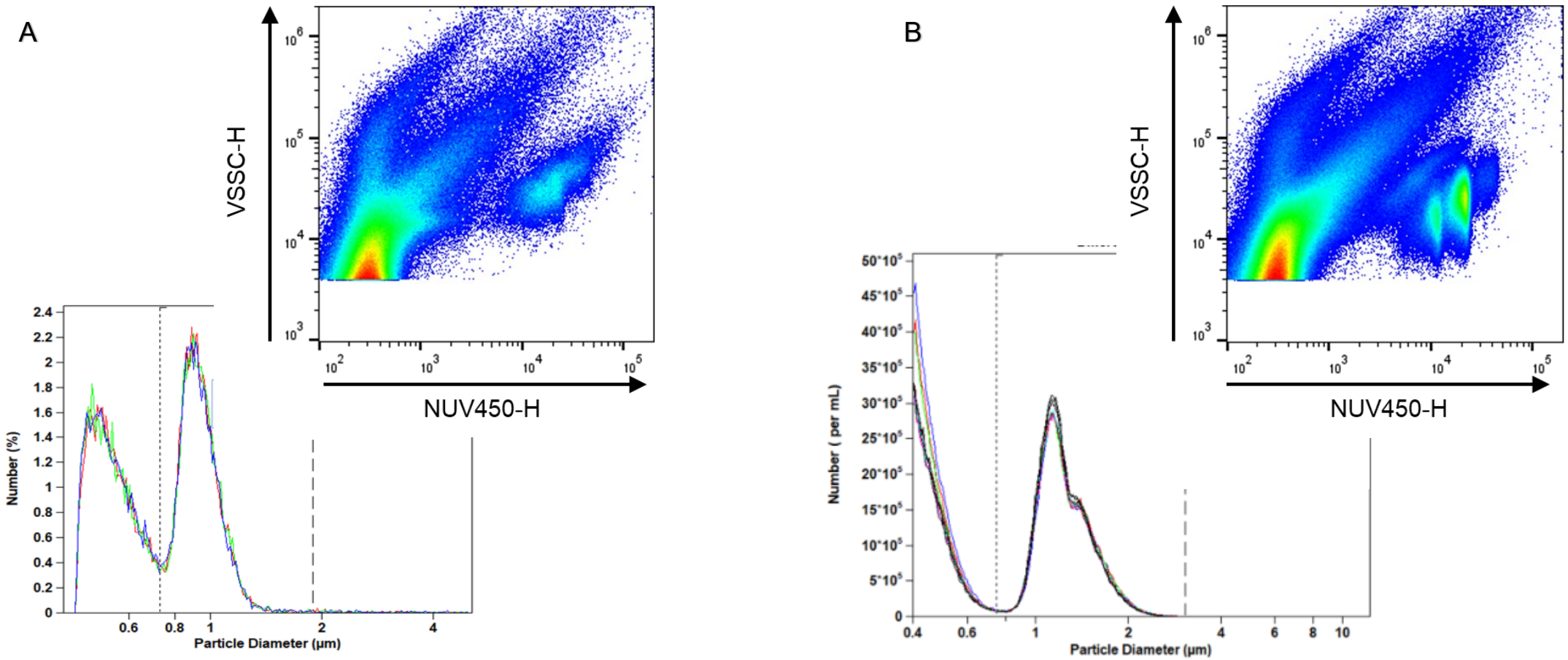
NIST WG1 Feasibility Studies: Overview



Parratt, NIST team et al, In preparation

Disclaimer: These studies were not comprehensive. Studies were performed *ad hoc* as materials became available and depending on the availability of NIST staff, and specific methods used varied between the studies.

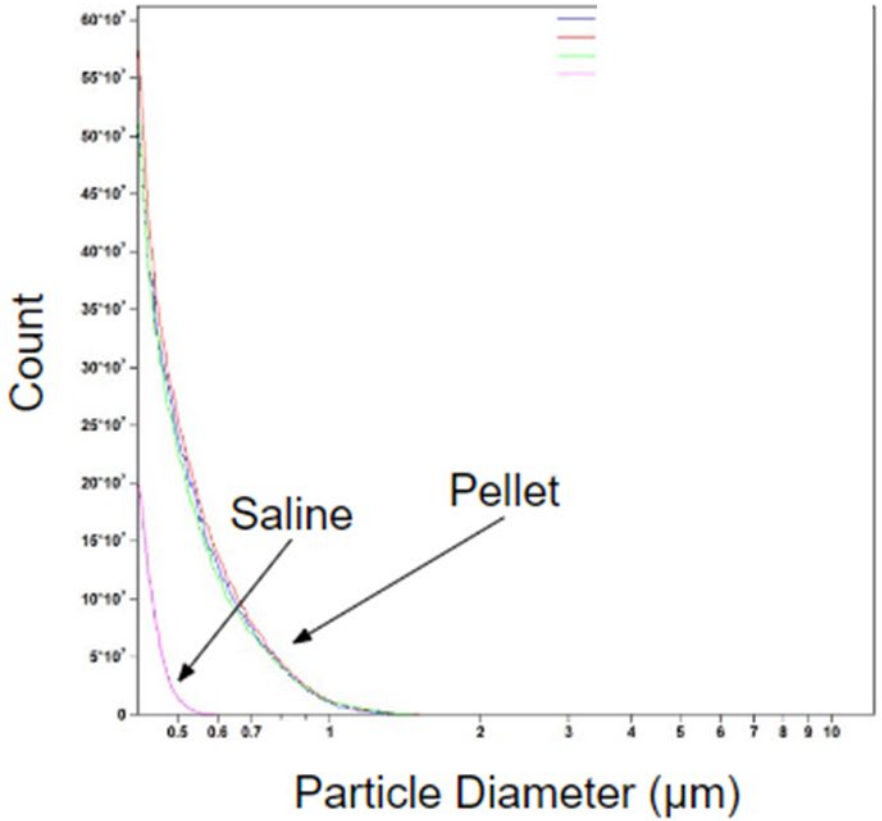
Strain-matched Materials Vary Across Manufacturer

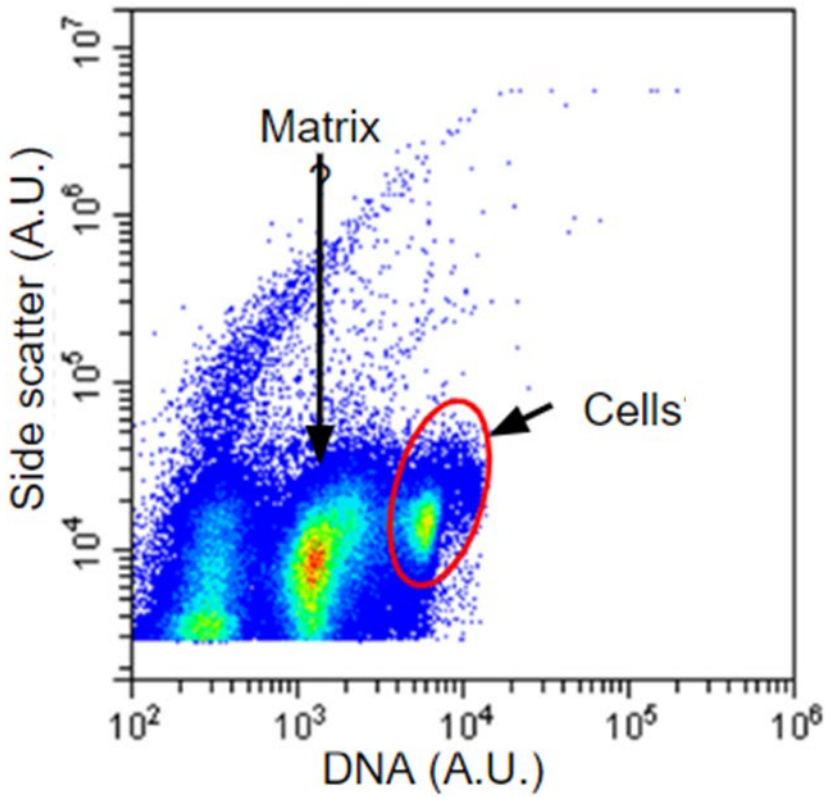


It is not unexpected that the same strain may exhibit different characteristics based on handling. However, this could be an important consideration for material selection.

Parratt, NIST team et al, In preparation

Matrix Material is Apparent in Characterization Measurements



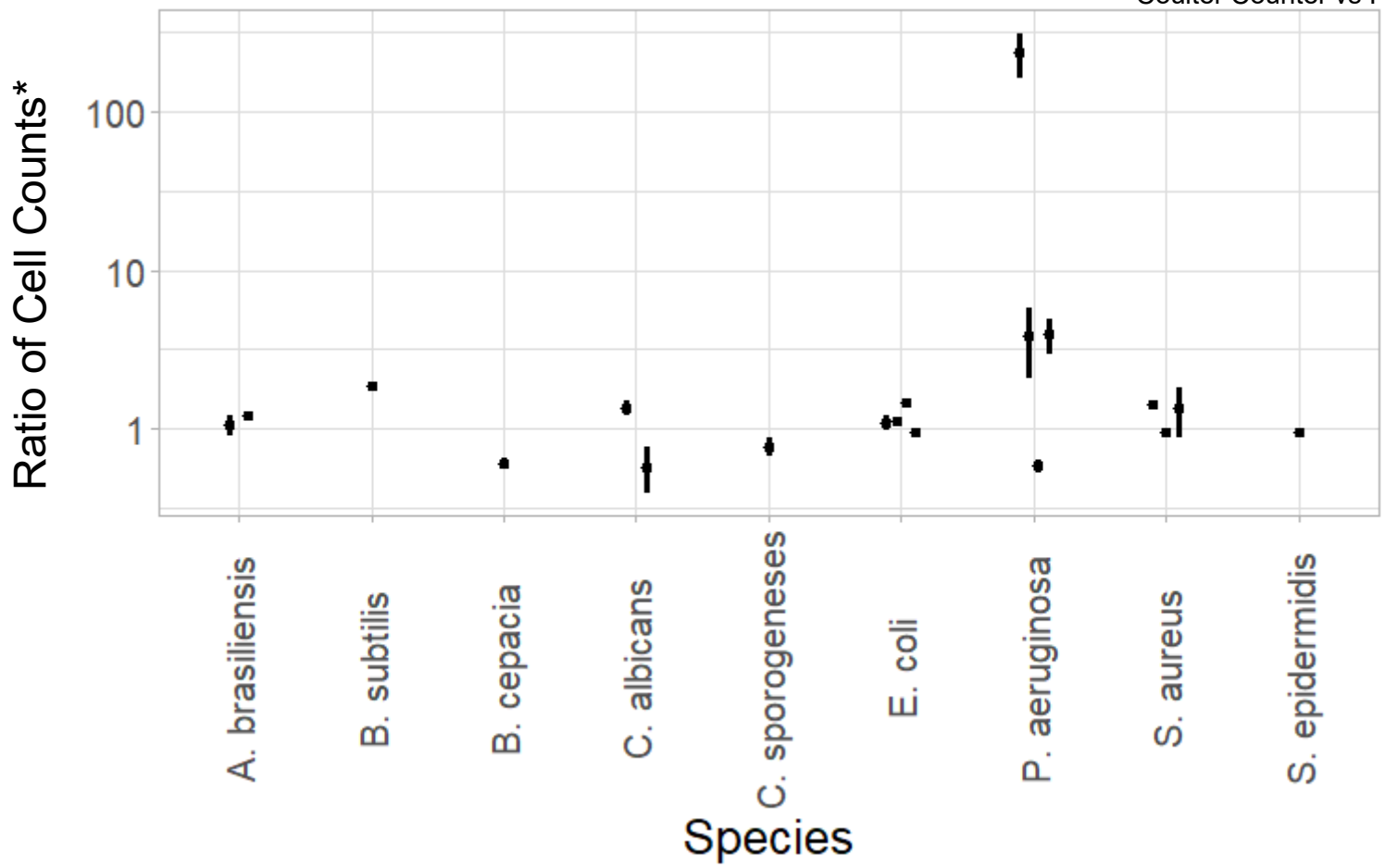


Matrix material could be natural (extracellular matrix) or artificial (preservation media). Depending on the source and cell type, these matrices can potentially interfere with characterization measurements.

Parratt, NIST team et al, In preparation

Orthogonal Cell Count Measurements Agree for Certain Materials

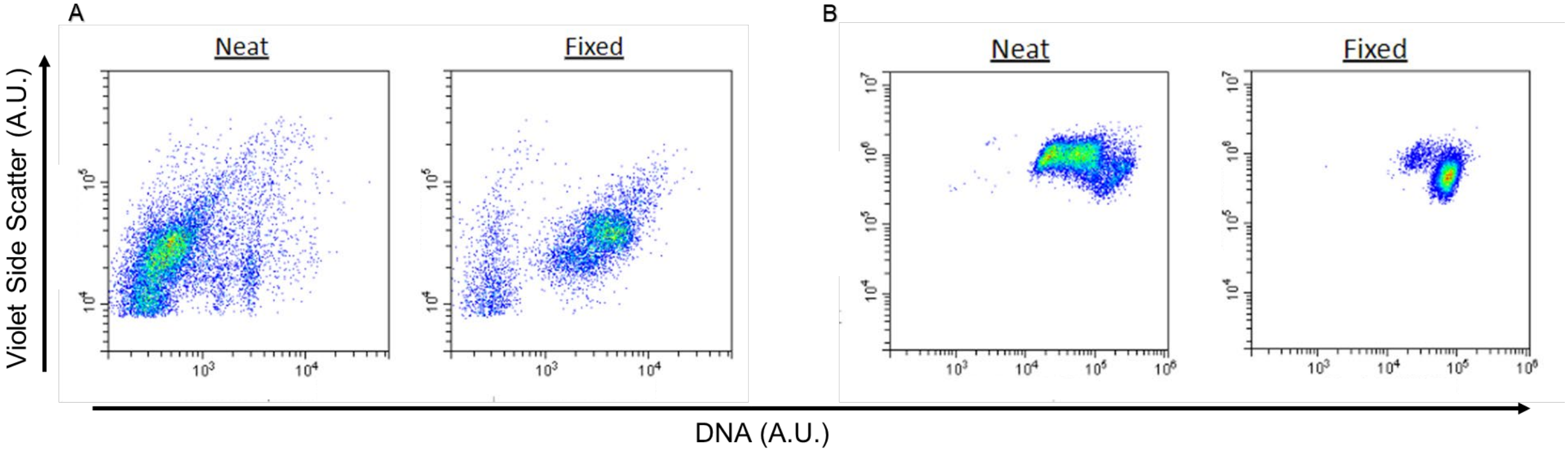
*Coulter Counter vs Fluorescence Flow Cytometry Counts



For certain materials, total cell count estimates were similar across orthogonal measurements (Coulter Principle and Fluorescence Flow Cytometry). This suggests that a Total Cell Certified Value is feasible in the near-term.

Parratt, NIST team et al, In preparation

Genome Copy Measurements Require More Work



Genome Copy Certified Values are likely to be more difficult to measure with two orthogonal methods. Molecular methods exhibit material-dependent extraction biases and fluorescence measurements exhibit incomplete staining of some materials

Parratt, NIST team et al, In preparation

Characterization of Total Cells and Genome Copies – Summary from Commercial Microbial Cell RMs

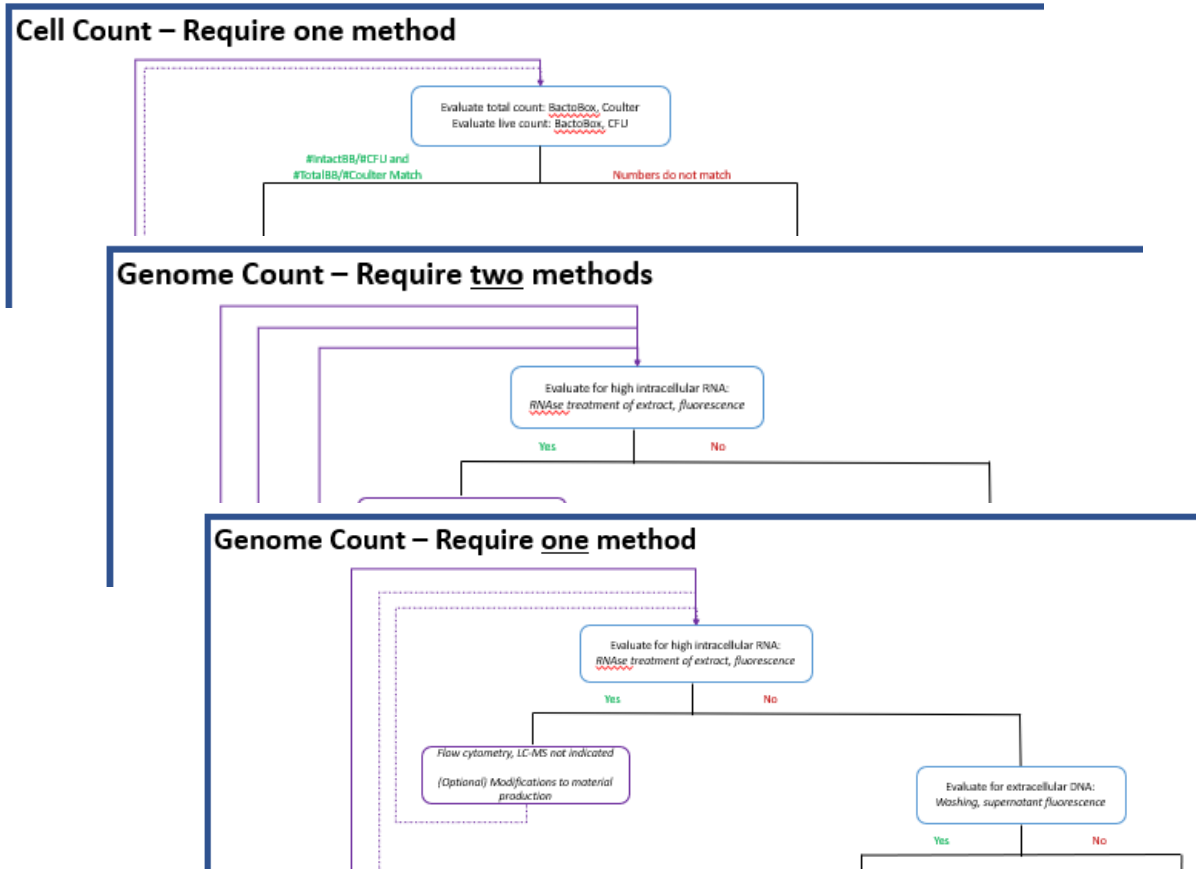
- Cell concentration should be sufficiently high (e.g., $>10^5$)
- Optimal characterization protocol will depend on strain AND formulation

- **Cell count measurements are promising** for most materials tested thus far
  - Cell debris/matrix can interfere
- **Genome copy measurements are more challenging**
  - Cell debris/matrix can interfere
  - Lack of DNA staining with fluorescent dyes (additional steps in some cases)
  - RNA can contribute to DNA measurements (some require RNAse treatment)
  - DNA extraction protocols need optimization

There will not be a unified characterization experimental protocol to transfer to industry partners

Alternate Measurement Approach: Method Optimization Roadmap

- Separate roadmaps for cell count and genome count
- Decision points and criteria to optimize a method for the cell material of interest
- The roadmap, rather than the specified method, can be transferred to RM manufacturers to apply to their materials



Credit: Stephanie Servetas, Kirsten Parratt

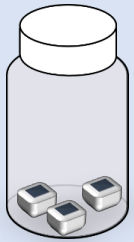
Perspective: Proposed Roles in Implementation of Microbial Cell RMs with Additional Certified Values

Group	Role	Benefits
NIST, NIST RMTM Consortium	• Develop and demonstrate a measurement approach to quantify total cells and/or genome copies for microbial cell RMs	• Consensus-based development • Measurement approach can be applied to commercial products
Standards-related organizations (eg USP, NIST)	• Provide primary reference standards (RMs), quantified for multiple properties using the measurement approach	• End users can demonstrate measurement capabilities using the RMs • Measurement approach demonstrated across multiple RMs
RM manufacturers (possibly via contract labs)	• Commercialize microbial cell RM products with multiple certified values	• Add value to existing products through new certified values • Provide RMs for new customer base • Support validation of RMTMs
Assay developers	• Develop/test/optimize molecular sterility tests using the available RMs • Develop in-house cell materials to support assay development	• Datasets can be generated on assay performance, so customers do not need to perform costly equivalence testing • Assays with performance demonstrated by RMs could fetch higher prices
RM users (advanced therapy producers, etc)	• Qualify the commercial RM (eg Vendor Qualification Test, plausibility testing) • Implement RMTMs, as supported by the RMs	• Enhanced ability to perform equivalence testing • Rapid and conclusive sterility testing enabled for a range of future high-value products
Regulatory organizations	• Review submission data based on RMs with certified values relevant for molecular methods • Could develop guidance based on RM usage to reduce variability in methods and facilitate review	• Common experimental approach and data reporting based on use of updated RMs will reduce burden on reviewers

Consortium team et al, In preparation

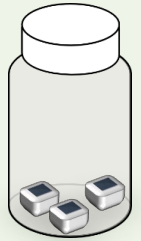
Current Overall Workflow *(for CFU certifications)*

RM Manufacturer



CoA:
- CFU = X CFU/tube

End-User

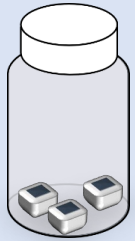


$CFU_{CoA} \approx CFU_{User}$

CoA – Certificate of Analysis

NEW Overall Workflows Enabled

RM Manufacturer



CoA:
- CFU = X CFU/tube
- **Total cells = Y cells/tube**
- **Genome copies = Z GC/tube**

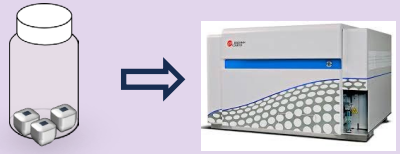
CFU

End-User

$CFU_{CoA} \approx CFU_{User}$

Total Cells

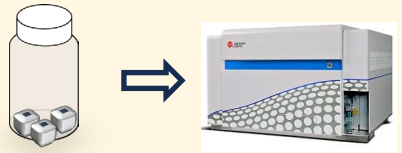
End-User



$Cells_{CoA} \approx Cells_{User}$

Genome Copies

End-User

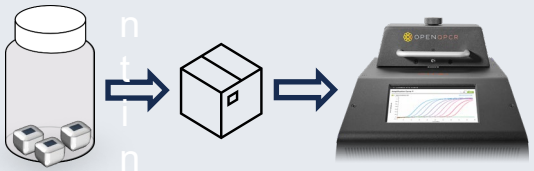


$GC_{CoA} \approx GC_{User}$

Extraction Efficiency

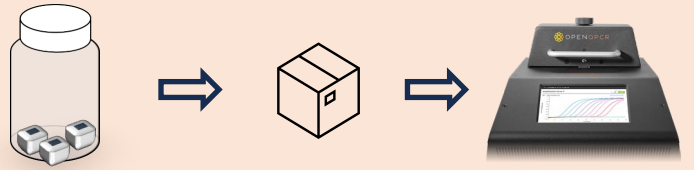
laboratory instrument

Extraction Kit (EK) Manufacturer



CoA_{EK}:
- Extraction efficiency ≈ E %/RM####
  OR
- Genome copies ≈ Z' GC/tube

End-User



$GenomeCopies_{CoA\ EK} \approx GenomeCopies_{User}$

Summary

- Molecular RMTMs are promising for timely evaluation of short shelf-life materials, such as cell and gene therapies
- Microbial cell RMs can be characterized beyond CFU to better support molecular sterility methods
- A measurement approach to optimize characterization methods for commercial products can be applied to certify additional properties, first total cell count and then genome copy count
- Cell RMs with expanded characterization can serve as fit-for-purpose materials to support molecular-based RMTMs
  - These RMs will also have broad applicability for molecular methods used across microbiology

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