The NIST Rapid Microbial Testing Methods (RMTM) Consortium Update

April 19th, 2022

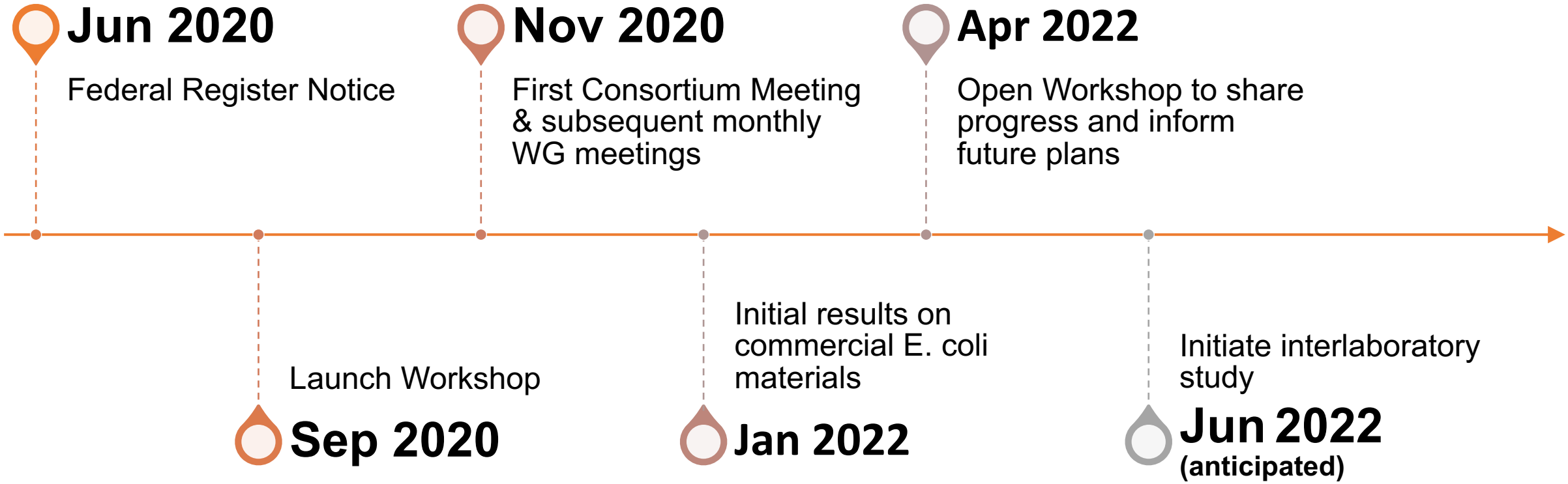
Scott Jackson

- Leader: Complex Microbial Systems Group
- Co-Lead: NIST RMTM Consortium

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

Graphic Credit: Natasha Hanacek

RMTM Consortium Timeline



30 Consortium Members

- Agilent Technologies
- Allele Biotechnology and Pharmaceuticals, Inc.
- AlloSource
- American Type Culture Collection (ATCC)
- Apsis Healthcare Systems, LLC
- bioMérieux
- Bionique Testing Laboratories, Inc.
- Bristol Myers Squibb
- Defense Biological Product Assurance Office, CBRND-EB, JPEO, DoD
- EMD Millipore Corporation (MERCK Kommanditgesellschaft auf Aktien)
- EzBiome Inc
- Gentech Biosciences
- George Washington University - Computational Biology Institute
- Independent (Spencer Hoover)
- Independent (Vicki Barbur)
- Latham BioPharm Group
- Microbiological Consulting, LLC
- Microbiologics, Inc.
- Microbiology Consultants, LLC
- NIH Clinical Center - Center for Cellular Engineering
- National Institute for Biological Standards and Control (NIBSC)
- National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL)
- Sartorius Aktiengesellschaft
- Siolta Therapeutics
- SmartGene GmbH
- United States Pharmacopeial Convention
- University of Delaware (Udel)
- U.S. Food and Drug Administration
- Vericel Corporation
- Weill Medical College of Cornell University

RMTM Consortium Goal

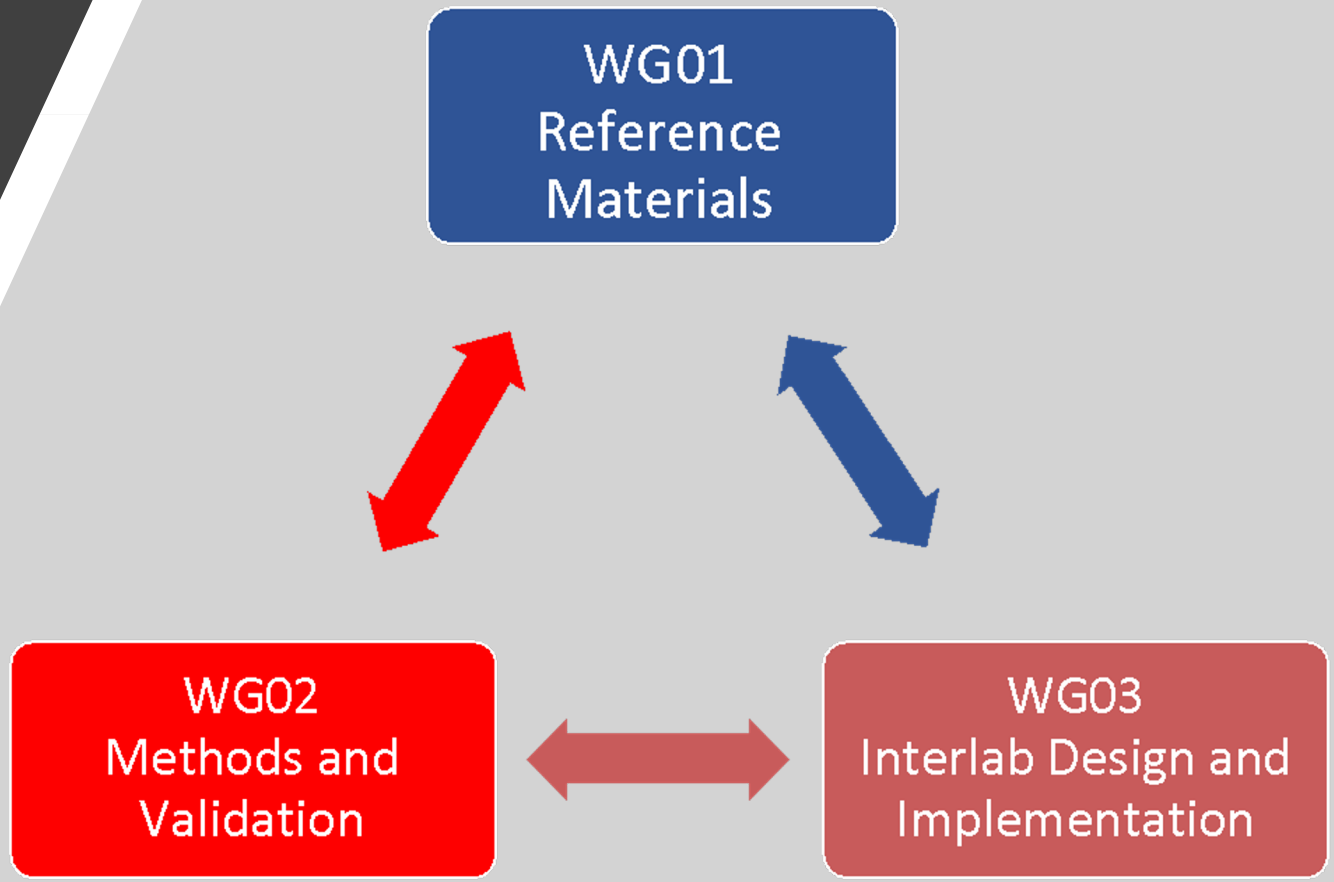
Goal

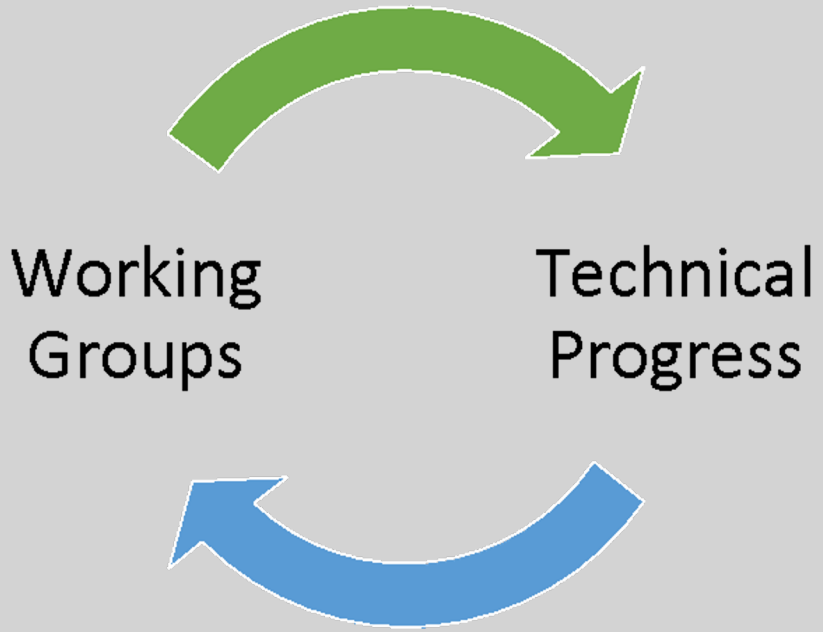
Facilitate validation and adoption of RMTMs in regenerative medicine and advanced therapy products

Approach

Convene stakeholders in the pre-competitive space to develop measurement solutions and standards that increase confidence in RMTM results

RMTM Working Groups





WG01: Reference Materials

WG01 MISSION: The mission of the Reference Material Working Group (WG01) is to identify and facilitate the development, characterization, and qualification of reference materials (RMs) to support the wide adoption of new and existing Rapid Microbiology Test Methods (RMTMs) within the Advanced Therapy Industry.

WG02: Methods and Validation

WG02 MISSION: The mission of the Methods and Validation Schemes Working Group (WG02) is to develop a framework for the validation of methods to support the wide adoption of new and existing Rapid Microbiology Test Methods (RMTMs) by the Advanced Therapy Industry.

WG03: Interlaboratory Studies

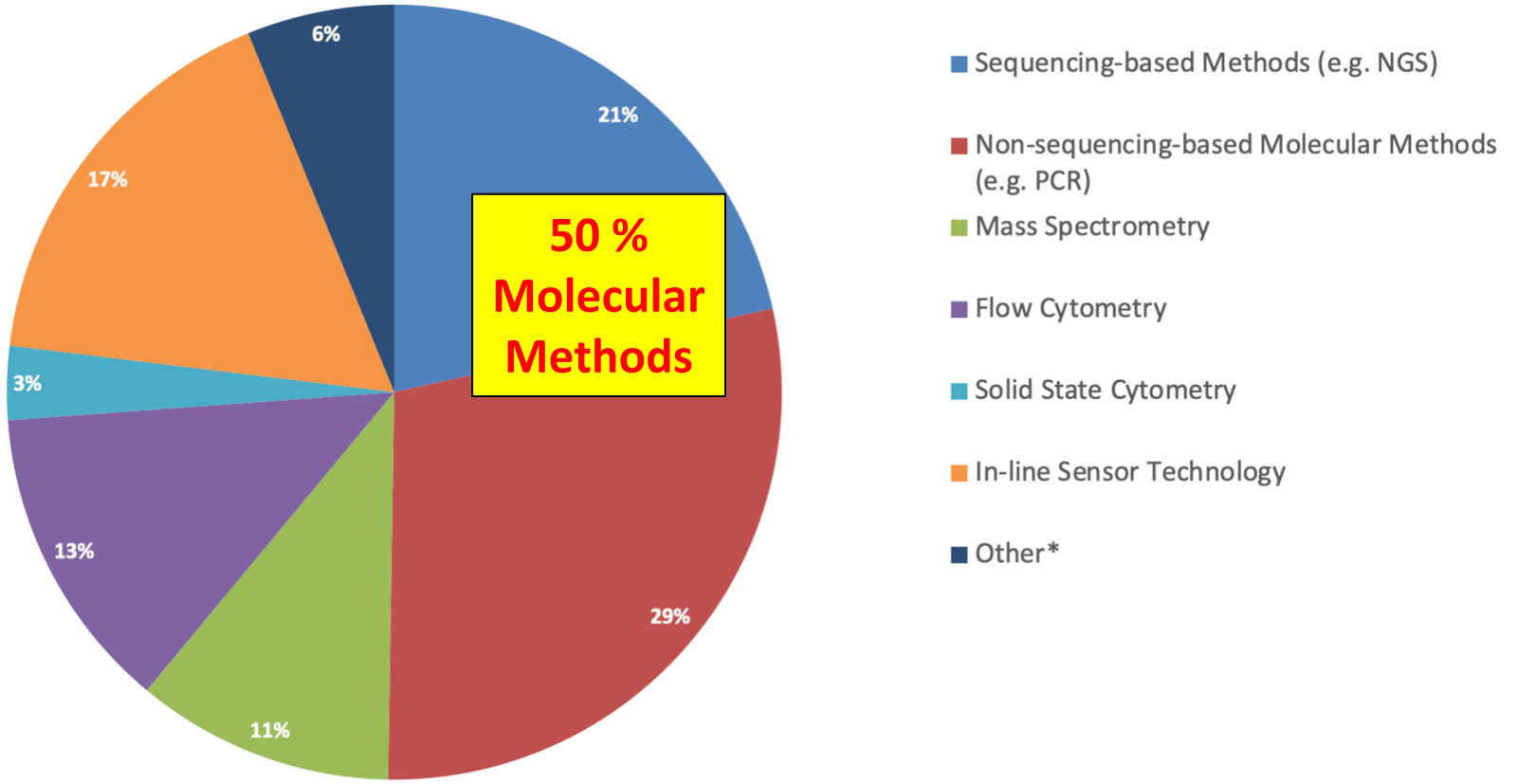
WG03 MISSION: The Interlaboratory Study Design and Implementation Working Group (WG03) mission is to design and implement interlaboratory studies to assess the analytical performance of various RMTMs while also evaluating the performance and fitness for purpose of candidate reference materials.

Polling

- The consortium is a powerful tool for gathering information
- We've run several (many) polls inquiring about gaps and hurdles for adopting RMTMs
- Your input is critical to our success

Poll Question from RMTM 2020 Launch Workshop:

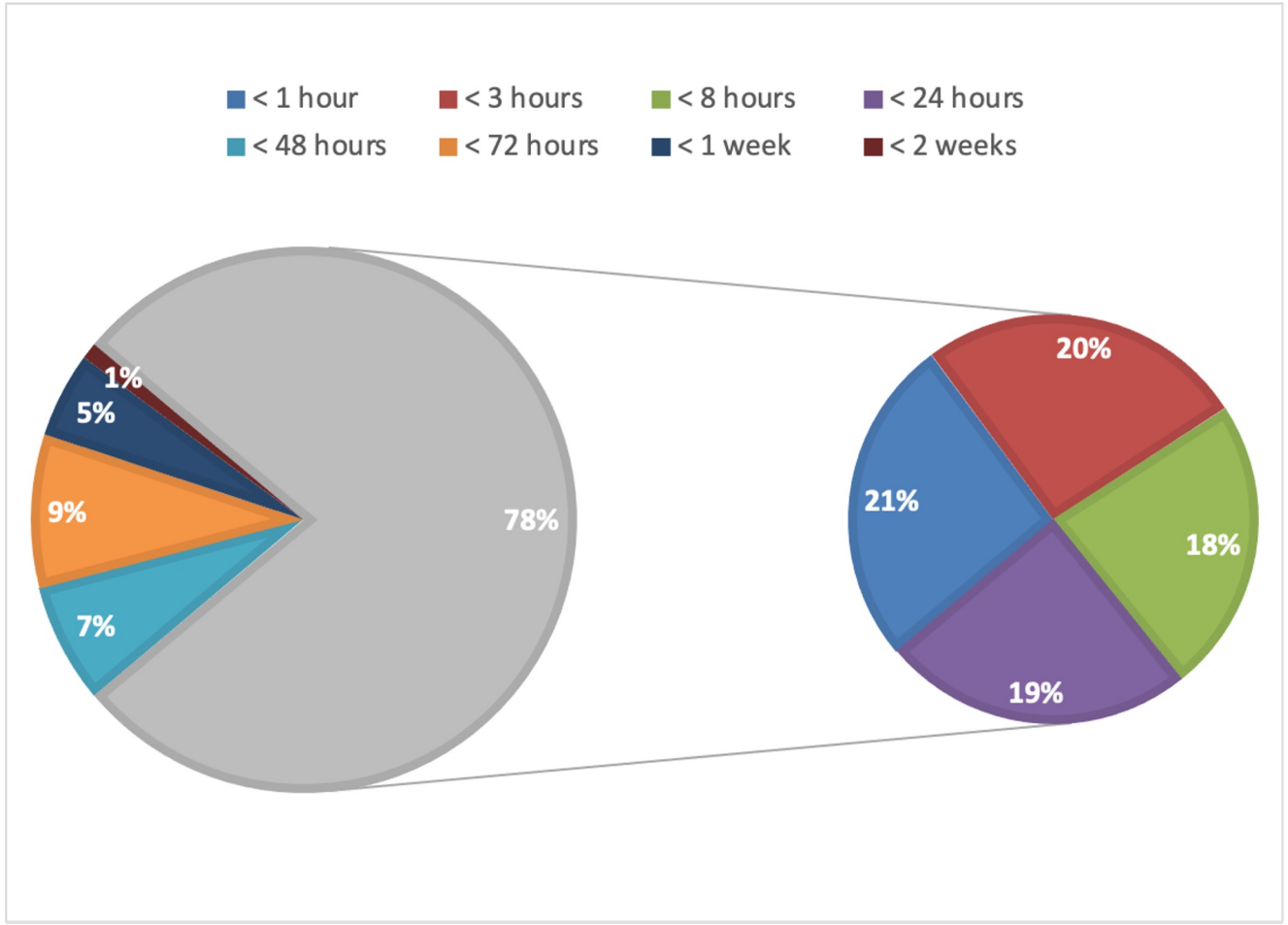
What rapid microbial measurement technologies are you most hopeful to be adopted in your industry?



Other:
CE
Live bacteriology
ATP bioluminescence. Raman spectroscopy coupled with viability staining; intrinsic fluorescence for real-time detection.
Reviewing all
ATP Bioluminescence
CO2 detection and ATP bioluminescence
Raman
metabolic and toxin/anti-toxin, programmed cell death
Raman Spectroscopy

Poll Question from RMTM 2020 Launch Workshop:

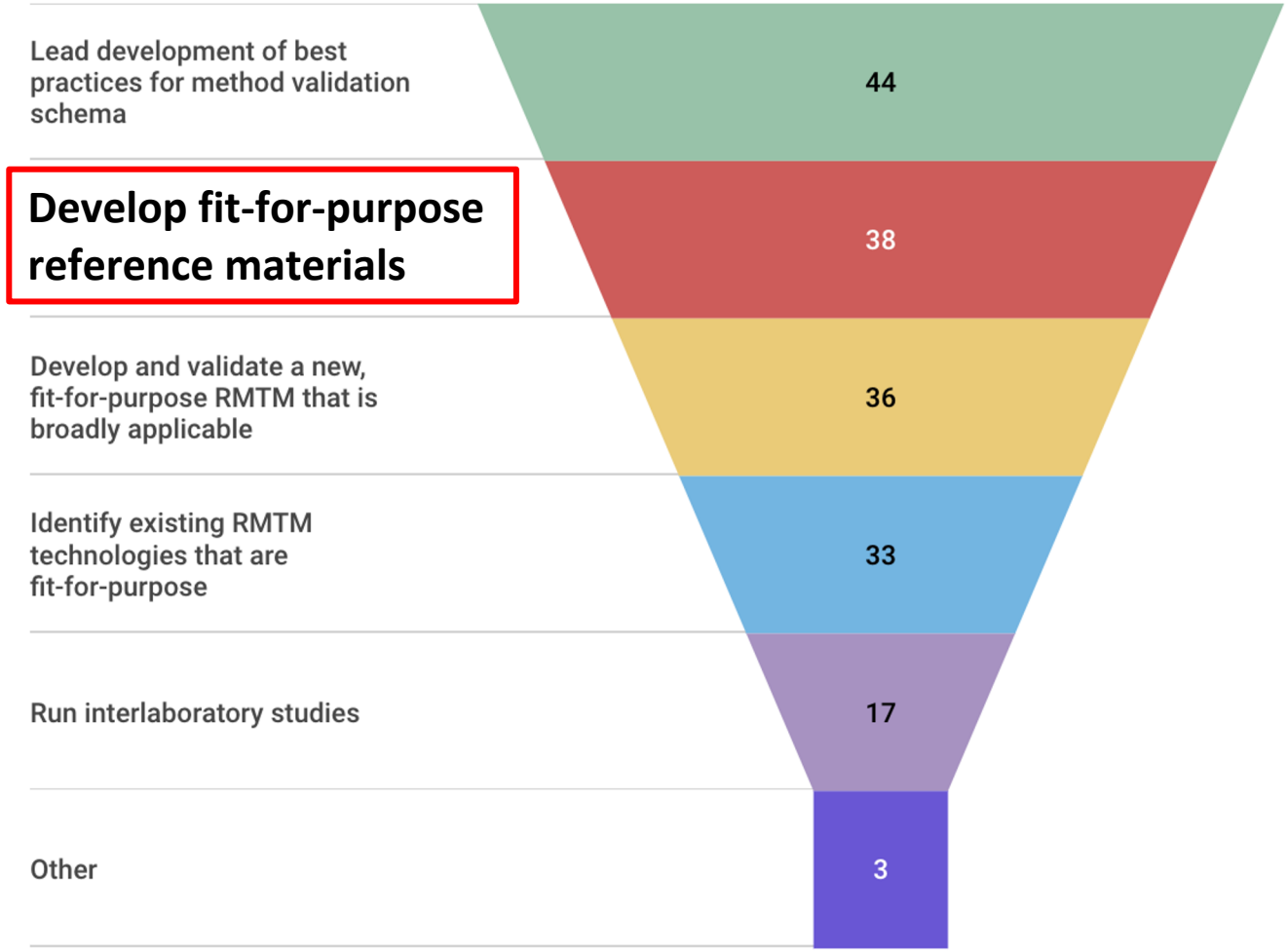
What does rapid mean to your process? How rapid is necessary?



78 % of respondents indicated RAPID meant results were back in <24 hours

Poll Question from RMTM 2020 Launch Workshop:

What are the top two priorities that this Consortium should seek to address first? (select up to 2)



NIST Reference Materials

Credit: J. Stoughton/NIST

Lyophilized Whole-Cell Microbial Reference Materials

Microbiologics “Pellets”

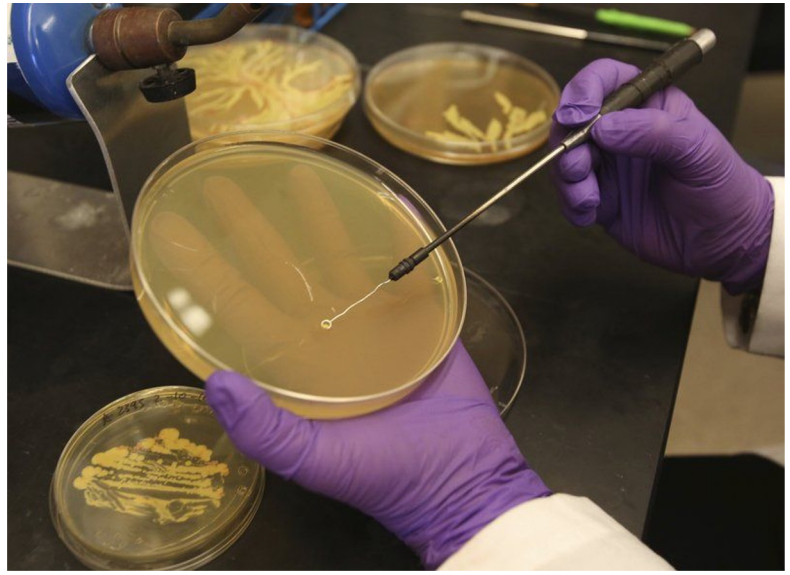
MilliporeSigma “Vitroid”

Biomerieux “Bioballs®”

- **Certified for CFU (only) from the manufacturer**
- **Ideally suited for culture-based methods (e.g. USP <71>)**

How Do We Transition from Culture-Based Methods to Rapid Molecular Methods?

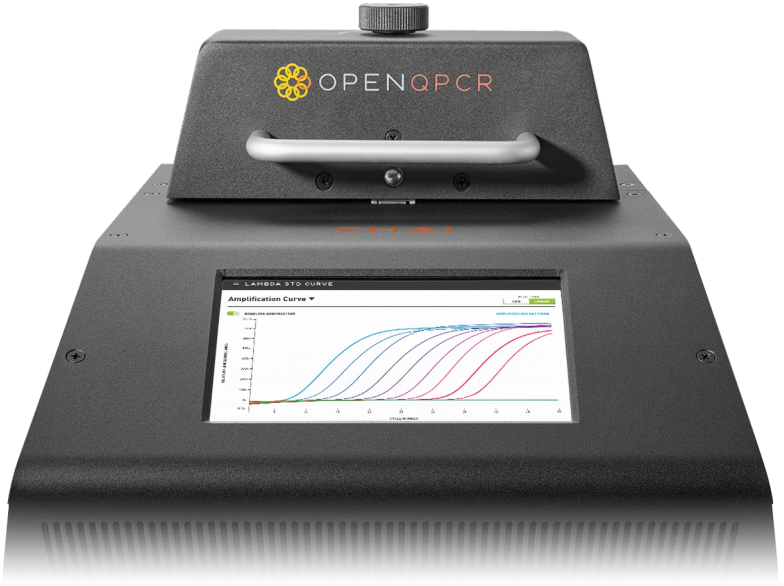
Compendial Method



Culture: Measures CFU

?

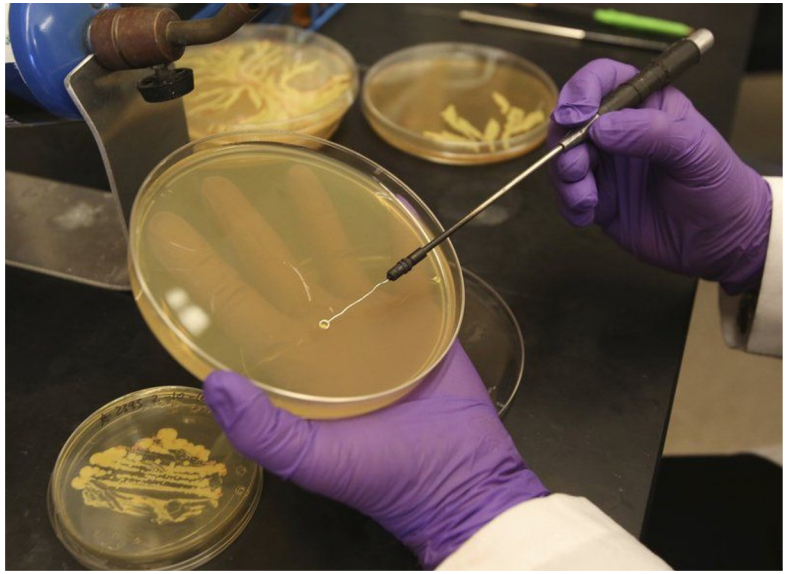
RMTM



PCR: Measures Genome Copies

Needed: Reference materials certified for both CFU ***and genome copies*** to compare outputs across methods

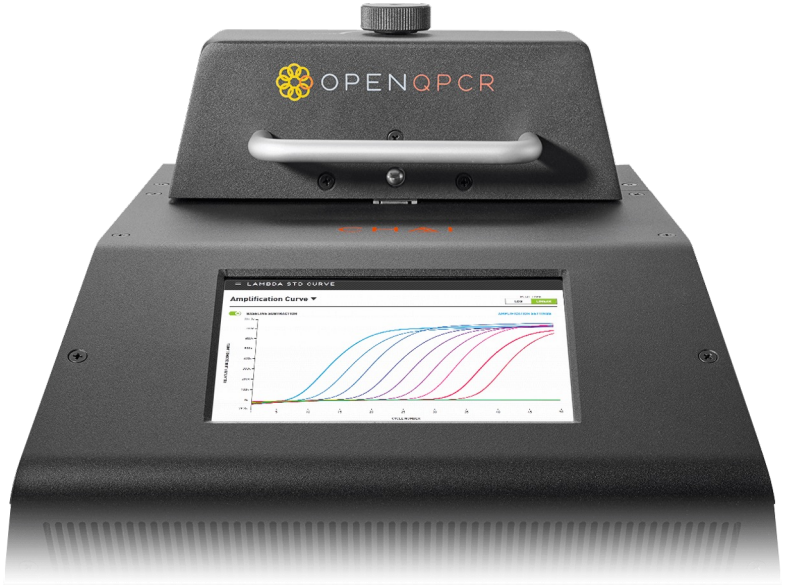
Compendial Method



Culture: Measures CFU

”Bridging the Gap”

RMTM



PCR: Measures Genome Copies

Need to re-certify these materials for genome copy number

Microbiologics “Pellets”

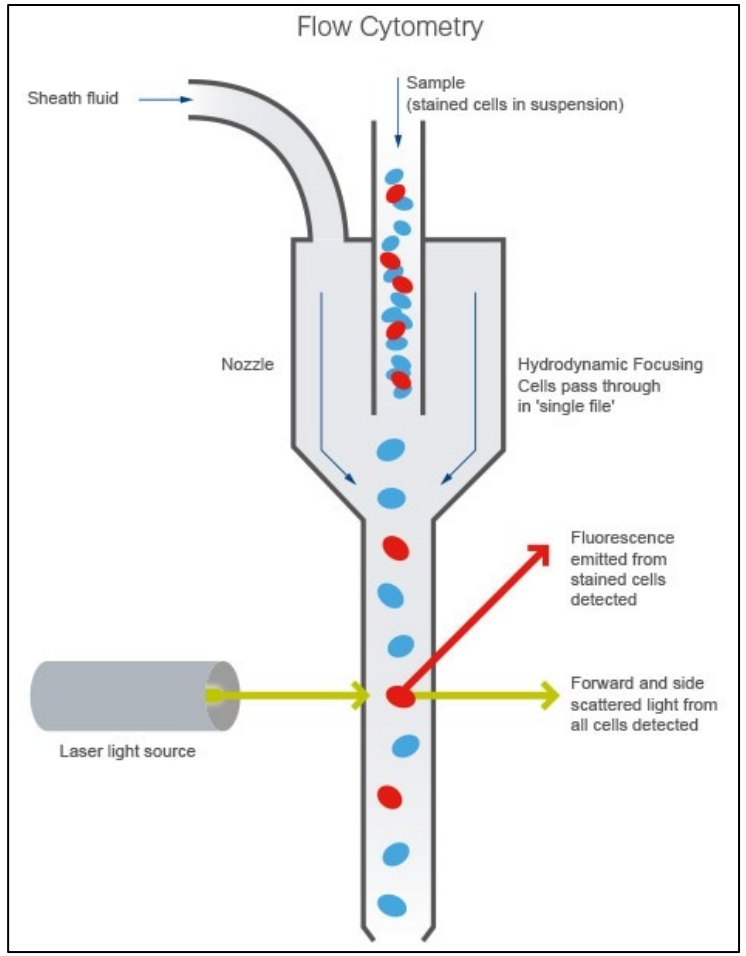
MilliporeSigma “Vitroid”

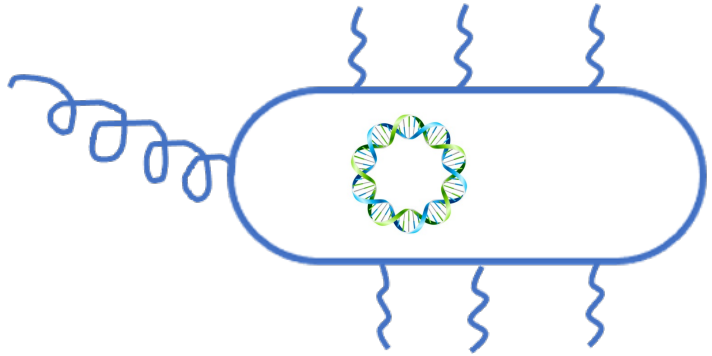
Biomerieux “Bioballs®”

- Commercial materials are currently certified for CFU only
- CFU ≠ Genome Copies ≠ Total Cells

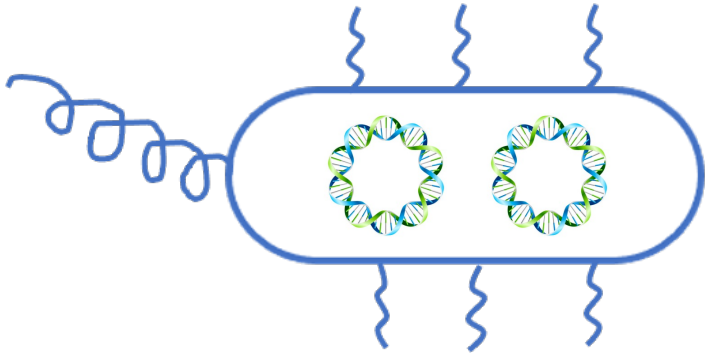
Objective: Develop methods to quantify genome copies (GC) and total cells
Enable reference materials (RMs) with expanded certifications

Flow Cytometry for Measuring Genome Copies





1x Fluorescence

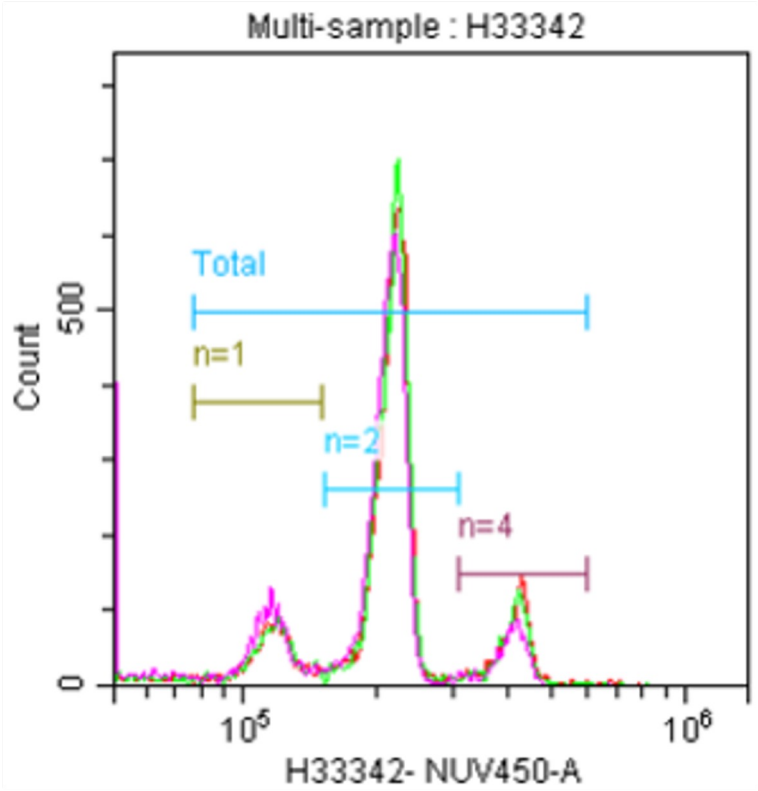


2x Fluorescence

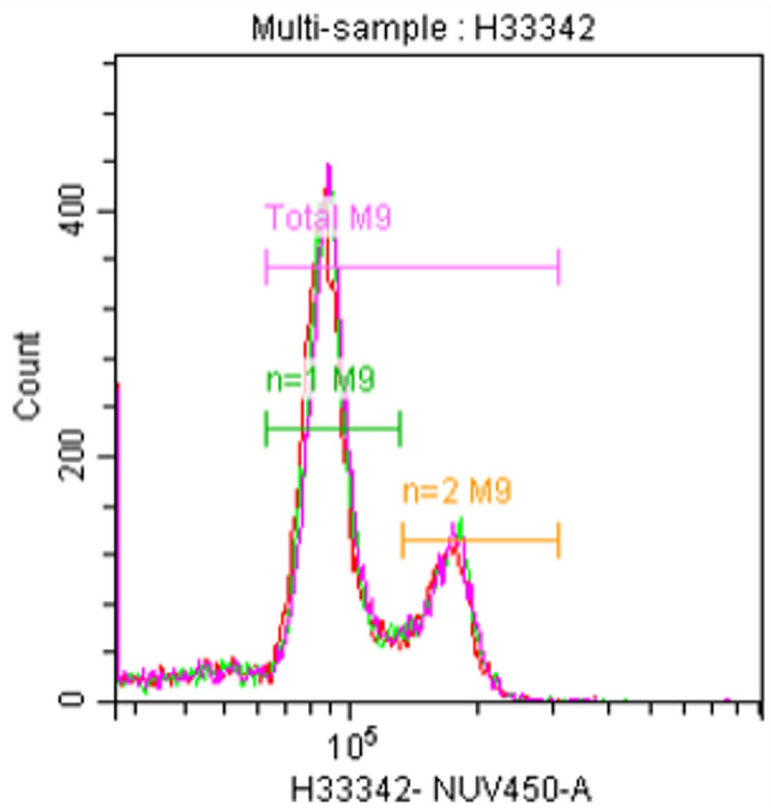
Flow Cytometry to Enumerate Genome Copies per Cell

Sandra Da Silva - NIST

Circa 2019



***E. coli* grown in Rich Media**



***E. coli* grown in Minimal Media**

Manuscript in preparation

NIST Pilot Study v1

❖ Commercially Available Products

Microbiologics "Pellets"


MilliporeSigma "Vitroid"

Biomerieux "Bioballs®"

Manufacturer	Product	Organism	Strain	CFU	Compendial	BSL
BioMerieux	BioBall SingleShot;Multishot 550/10^8	*E. coli*	ATCC8739	30; 500-600; 10^8	USP 62	BSL-1
BioMerieux	BioBall SingleShot; HighDose 10K	*E. coli*	ATCC11775	30, 8K-12K	USP 62	BSL-2
MilliporeSigma	Vitroids	*E. coli*	ATCC8739	80	USP 62	BSL-1
MilliporeSigma	Vitroids	*E. coli*	ATCC11775	50,200,1K,10K	USP 62	BSL-2
Microbiologics	EZ-AccuShot	*E. coli*	ATCC8739	1000	USP 62	BSL-1

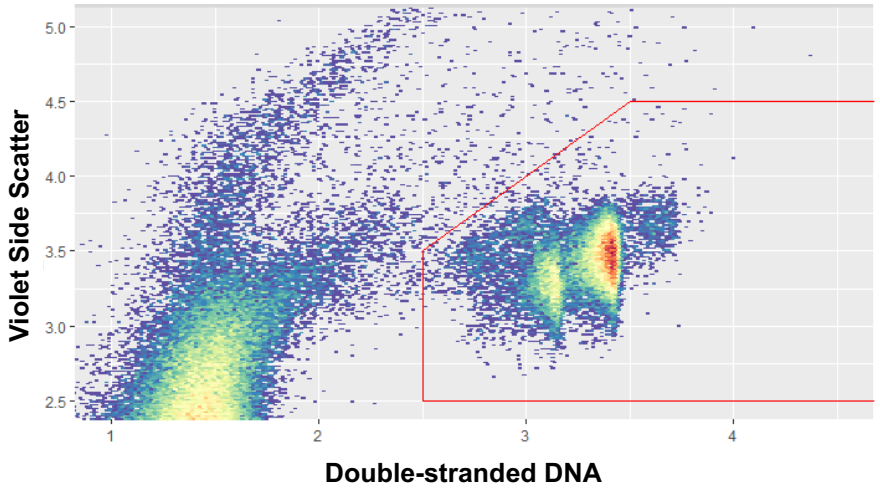
❖ Selected Method/Properties for characterization

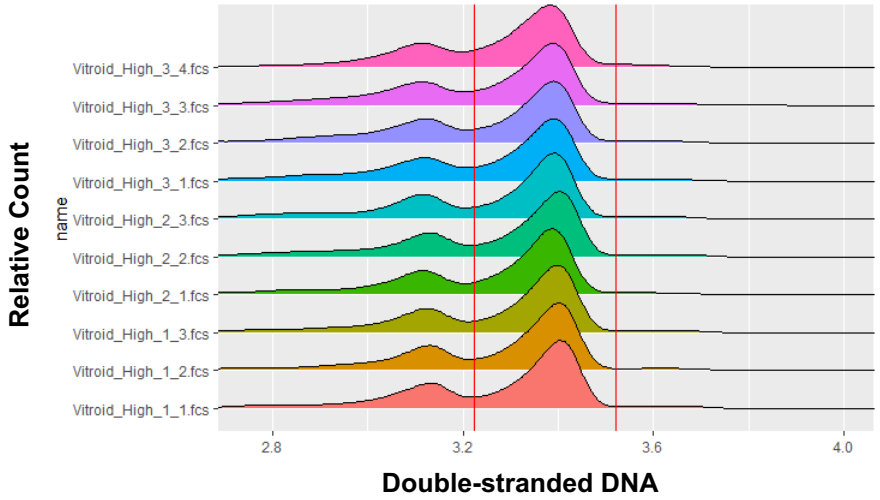
- ➢ Flow cytometry: genome copy number & total cell count
- ➢ Agar plating: CFU

Vitroid Data

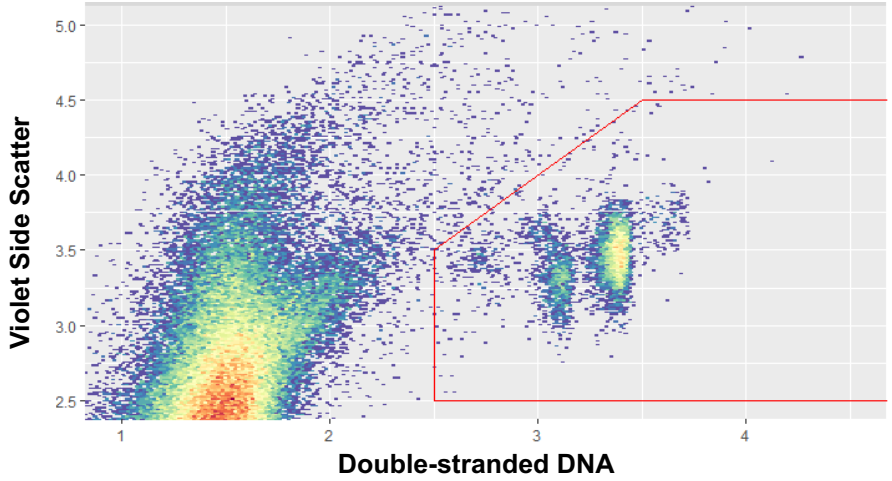
Kirsten Parratt

General Protocol





Refined Protocol



Percent cells in each dsDNA Peak

dsDNA Peak #	% of Cells (RSD %)
1	36.2 (5.66)
2	60.9 (2.83)
3	2.89 (9.58)

USP <71> Compendial Organisms

Table 1. Strains of the Test Microorganisms Suitable for Use in the Growth Promotion Test and the Method Suitability Test

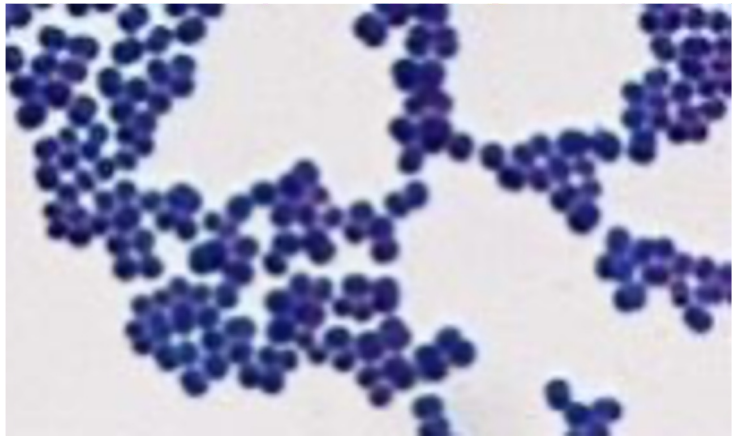
Aerobic bacteria	
Staphylococcus aureus	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276
Bacillus subtilis	ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134
Pseudomonas aeruginosa [1]	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275
Anaerobic bacterium	
Clostridium sporogenes [2]	ATCC 19404, CIP 79.3, NCTC 532 or ATCC 11437, NBRC 14293
Fungi	
Candida albicans	ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594
Aspergillus niger	ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455

[1] An alternative microorganism is *Micrococcus luteus* (*Kocuria rhizophila*), ATCC 9341.

[2] An alternative to *Clostridium sporogenes*, when a nonspore-forming microorganism is desired, is *Bacetroides vulgatus* (ATCC 8482).

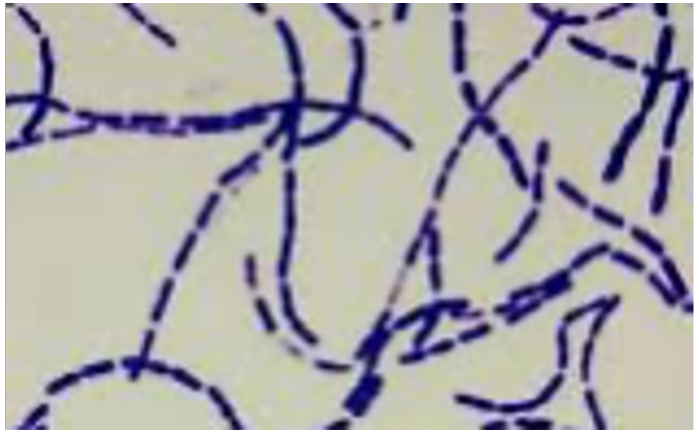
❖ Additional Strain Selection- USP 71 compendial Strains

Staphylococcus aureus



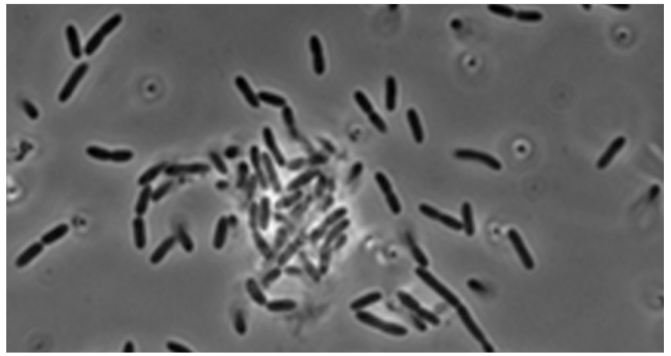
Aerobic bacteria

Bacillus subtilis



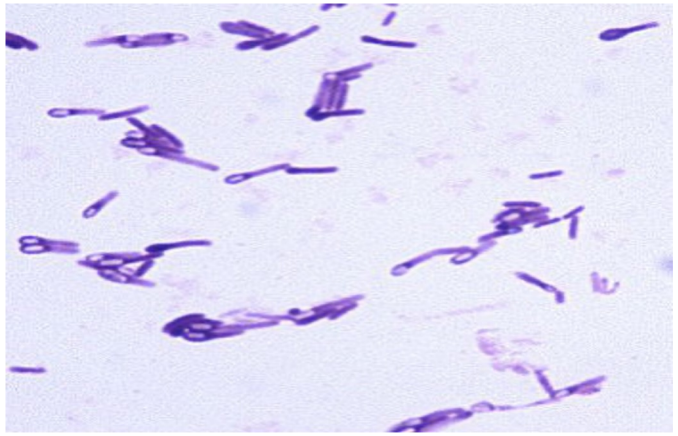
Aerobic bacteria

Pseudomonas aeruginosa



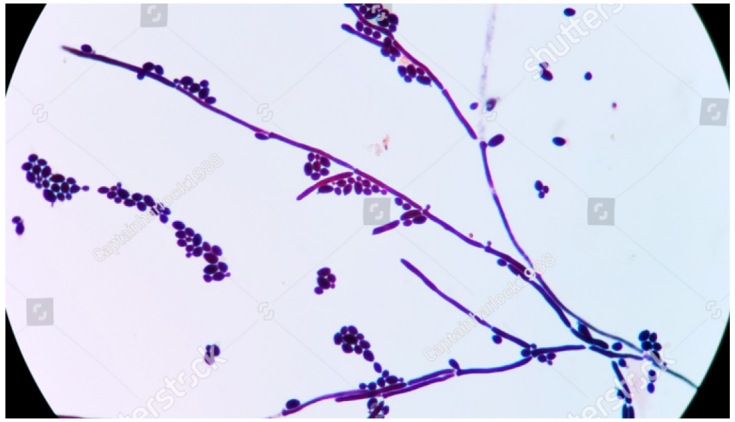
Aerobic bacteria

Clostridium sporogenes



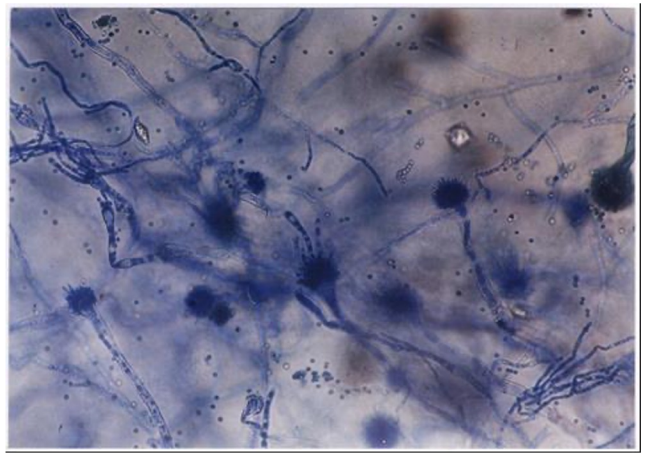
Anaerobic bacterium

Candida albicans



Fungi

Aspergillus niger

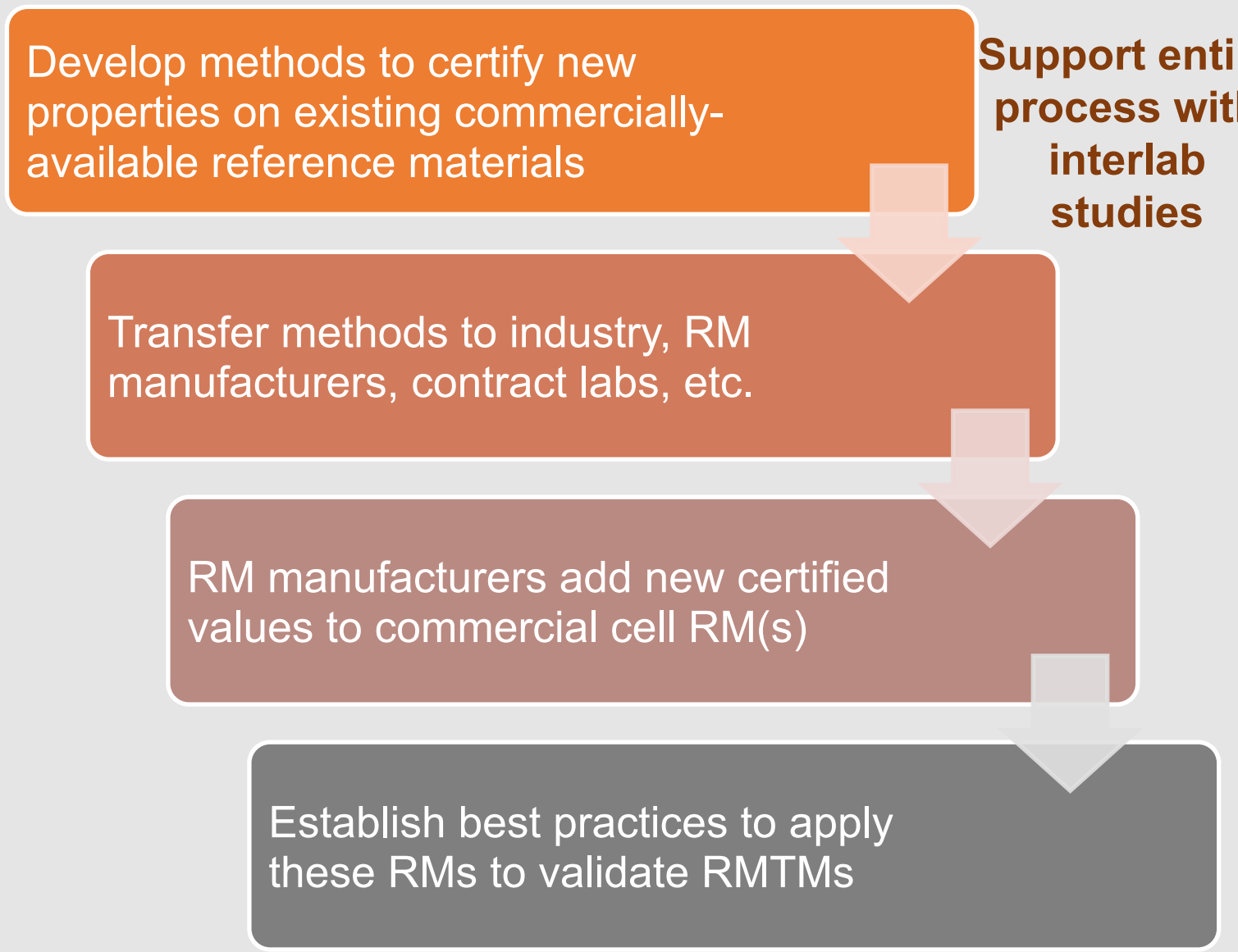


Fungi

Other properties can be certified in existing whole cell reference materials

Proposed Translation Model

(beyond the prototype material)



NIST

Search NIST

Menu

PROJECTS/PROGRAMS

NIST Microbial Strain Collection

Summary

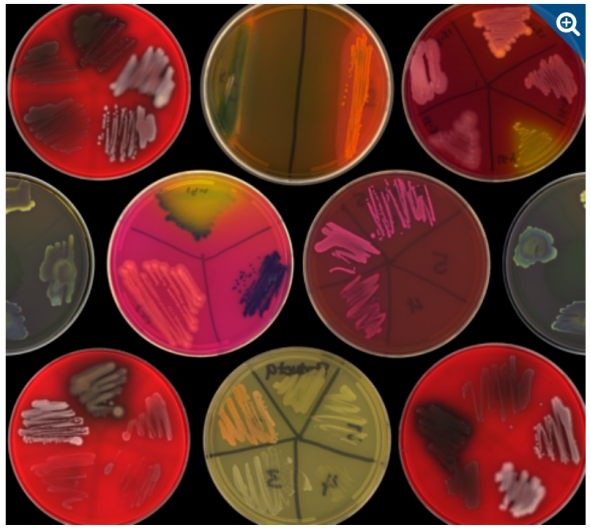
NIST established the NIST Microbial Strain Collection (NMSC) to advance microbial research and support standards development. The NMSC will accept deposits of microbial isolates (strains) following a screening and approval process that is further described below.

To submit strains to the NMSC, please complete the Strain Deposit Form .

DESCRIPTION

Microorganisms of interest include but are not limited to those relevant to: biomanufacturing of advanced therapy products; rapid microbial testing; microbial therapeutics (e.g., live biotherapeutic products, probiotics, and fecal microbiota transplantations (FMTs)); infectious disease identification and surveillance; engineering biology; and animal, human, agricultural, or environmental microbiome research. NIST will use microbial strains or sets of microbial strains from the NMSC to address stakeholder needs. These applications may include but are not limited to:

- Interlaboratory studies
- Incorporation into reference materials



Credit: Jennifer Dootz and Jason Kralj

ORGANIZATIONS

Material Measurement Laboratory

Biosystems and Biomaterials Division

Complex Microbial Systems Group

NIST STAFF

Stephanie Servetas
Scott Jackson
Nancy Lin
Jennifer Dootz
Monique Hunter
Jason Kralj
Samuel P. Forry
Tara Eskandari

CONTACT

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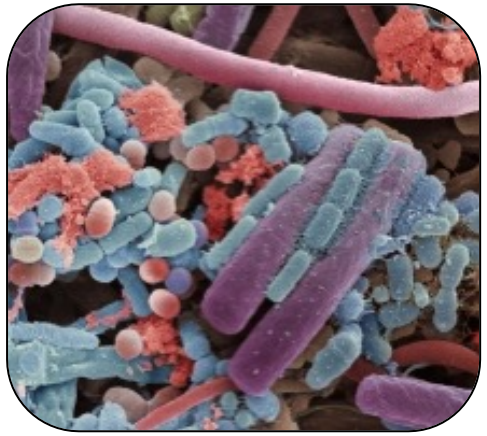
PROJECT STATUS

ONGOING

Tools/Approaches Can Translate to Other Sectors



Live Biotherapeutic Products (LBPs)

Food Safety

Biothreat Detection

Environmental Biosurveillance

We Want You to Join The RMTM Consortium

Become A Member

- Complete the Letter of Interest Form
- Participants will sign a Cooperative Research and Development Agreement (CRADA); Federal Agencies may join under Letter Agreement
- No cost to join the Consortium

Member Benefits

- Access to a neutral forum to address pre-competitive needs
- Participation in the development of reference materials, methods, and protocols, and interlaboratory studies
- Access to tools developed by the Consortium ahead of public release
- Institutional representation on Consortium steering committee

Next Steps

- Host an interlab study using commercially-available *E. coli* materials
  - Assess the utility ("fitness for purpose") of newly-certified reference materials
  - Assess the analytical performance of RMTMs
- Continue to develop methods to certify new properties in existing commercially-available whole-cell reference materials (e.g., compendial organisms)
- As we gain confidence in our ability to certify new properties in existing commercially-available reference materials, we'll host additional interlab studies.
- A NIST whole-cell microbial reference material with certified properties?
  - TBD

Thanks!

Questions?

RM 8376 IS CURRENTLY AVAILABLE!

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

Date of Issue:
02 June 2021

Reference Material 8376

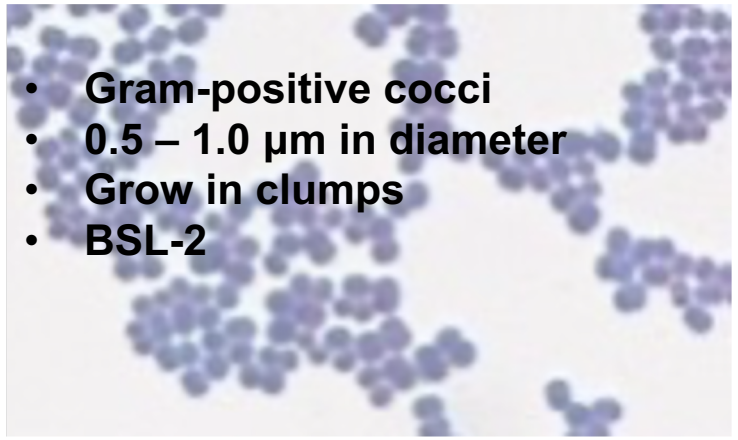
Microbial Pathogen DNA Standards for Detection and Identification

REFERENCE MATERIAL INFORMATION SHEET

Purpose: This reference material (RM) is intended for harmonizing measurements of abundance and identity using next-generation sequencing-based metagenomics.

https://tinyurl.com/rm8376

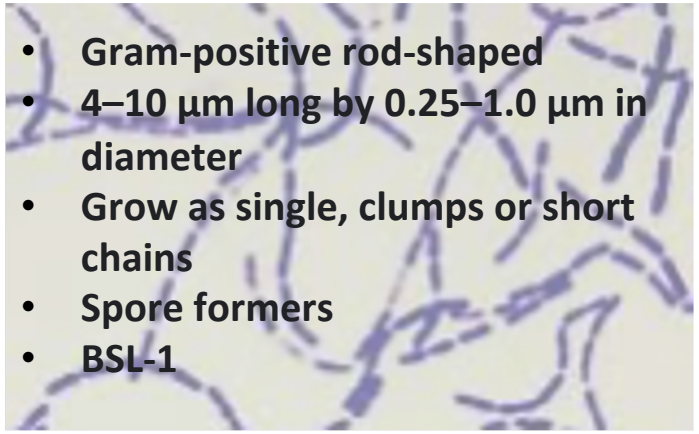
Staphylococcus aureus



- **Gram-positive cocci**
- **0.5 – 1.0 µm in diameter**
- **Grow in clumps**
- **BSL-2**

Aerobic bacteria

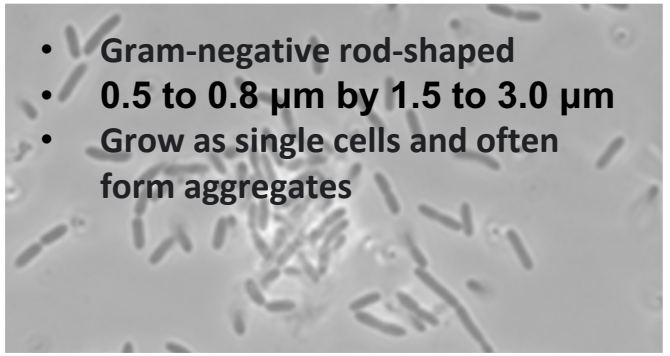
Bacillus subtilis



- **Gram-positive rod-shaped**
- **4–10 µm long by 0.25–1.0 µm in diameter**
- **Grow as single, clumps or short chains**
- **Spore formers**
- **BSL-1**

Aerobic bacteria

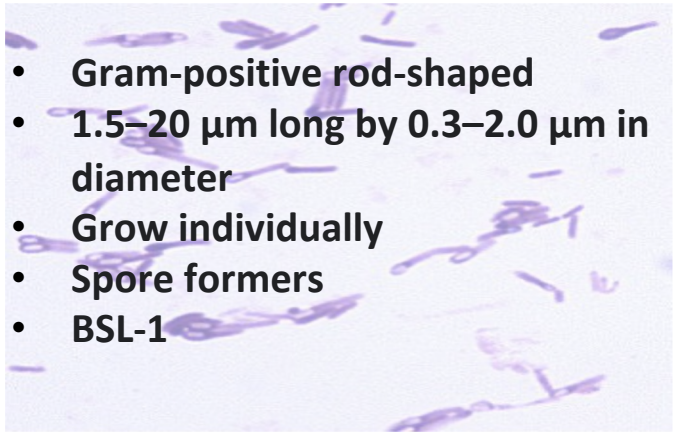
Pseudomonas aeruginosa



- **Gram-negative rod-shaped**
- **0.5 to 0.8 µm by 1.5 to 3.0 µm**
- **Grow as single cells and often form aggregates**

Aerobic bacteria

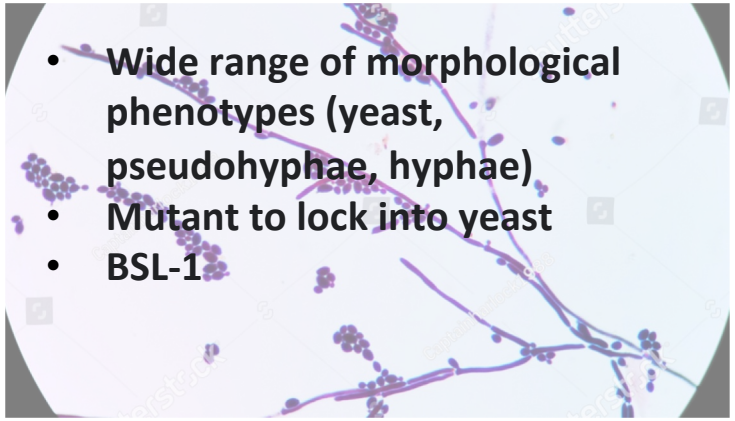
Clostridium sporogenes



- **Gram-positive rod-shaped**
- **1.5–20 µm long by 0.3–2.0 µm in diameter**
- **Grow individually**
- **Spore formers**
- **BSL-1**

Anaerobic bacterium

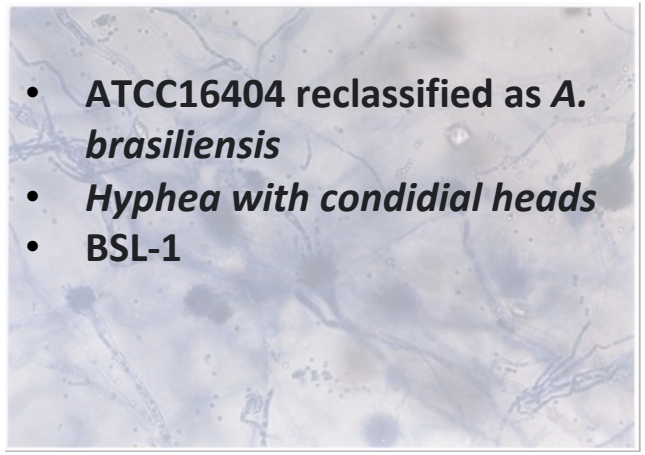
Candida albicans



- **Wide range of morphological phenotypes (yeast, pseudohyphae, hyphae)**
- **Mutant to lock into yeast**
- **BSL-1**

Fungi

Aspergillus niger



- **ATCC16404 reclassified as *A. brasiliensis***
- ***Hyphea with condidial heads***
- **BSL-1**

Fungi

Additional Materials Available

Manufacturer	Product	Organism	Strain	CFU	Compendial	BSL
BioMerieux	BioBall SingleShot;Multishot 550/10^8; HighDose 10K	*P. aeruginosa*	ATCC9027	30; 500-600; 10^8; 8K-12K	USP 71	BSL-2
MilliporeSigma	Vitroids	*P. aeruginosa*	ATCC9027	30,50,80,100,200, 1K	USP 71	BSL-2
Microbiologics	EZ-AccuShot	*P. aeruginosa*	ATCC9027	1000	USP 71	BSL-2
MilliporeSigma	Vitroids	*A. brasiliensis*	ATCC16404	80	USP 71	BSL-1
Microbiologics	EZ-AccuShot	*A. brasiliensis*	ATCC16404	1000	USP 71	BSL-1
BioMerieux	BioBall SingleShot;Multishot 550/10^8; HighDose 10K	*A. brasiliensis* (spore)	ATCC16404	30; 500-600; 10^8; 8K-12K	USP 71	BSL-1
MilliporeSigma	Vitroids	*B. subtilis*	ATCC6633	80, 10K	USP 71	BSL-1
Microbiologics	EZ-AccuShot	*B. subtilis*	ATCC6633	1000	USP 71	BSL-1
BioMerieux	BioBall SingleShot;Multishot 550/10^8; HighDose 10K	*B. subtilis* (spore)	ATCC6633	30; 500-600; 10^8; 8K-12K	USP 71	BSL-1
MilliporeSigma	Vitroids	*C. albicans*	ATCC10231	80,1K,10K	USP 71	BSL-1
BioMerieux	BioBall SingleShot;Multishot 550/10^8; HighDose 10K	*C. albicans* (cell)	ATCC10231	30; 500-600; 10^8; 8K-12K	USP 71	BSL-1
Microbiologics	EZ-AccuShot	*C. albicans* (yeast cell)	ATCC10231/26790	1000	USP 71	BSL-1
MilliporeSigma	Vitroids	*C. sporogenes*	ATCC19404	80	USP 71	BSL-2
Microbiologics	EZ-AccuShot	*C. sporogenes*	ATCC19404/11437	1000	USP 71	BSL-2
BioMerieux	BioBall SingleShot;Multishot 550	*C. sporogenes* (spore)	ATCC11437	30; 500-600	USP 71	BSL-2
BioMerieux	BioBall SingleShot;Multishot 550/10^8; HighDose 10K	*S. aureus*	ATCC6538	30; 500-600; 10^8; 8K-12K	USP 71	BSL-2
MilliporeSigma	Vitroids	*S. aureus*	ATCC6538	50, 80, 200, 1K	USP 71	BSL-2
Microbiologics	EZ-AccuShot	*S. aureus*	ATCC6538	1000	USP 71	BSL-2

FEASIBILITY STUDY OF COMMERCIALLY AVAILABLE *E. COLI* WHOLE CELL MATERIALS

E. coli Materials Received

Manufacturer	Material	Catalog #	Lot #	Strain (BSL)	Lot-specific CFU (uncertainty)
Microbiologics	AccuShot	0483A	483-1115-3	ATCC 8739 (1)	624 (52 per 0.1 mL)
MilliporeSigma	Vitroid (low CFU)	VT000906	BCCF4113	ATCC 11775 (2)	4.9 e3 (2.6 e3 – 9.3 e3)
MilliporeSigma	Vitroid (high CFU)	VT000127	BCCF1120	ATCC 8739 (1)	9.2 e4 (4.6 e4 – 1.9 e5)
bioMérieux	Bioball (low CFU)	56016	B6415	ATCC 8739 (1)	543.6 (sd = 30.7)
bioMérieux	Bioball (mid CFU)	56053	B6593	ATCC 11775 (2)	1.051 e4 (sd = 4.8 e2)
bioMérieux	Bioball (high CFU)	56146	B6634	ATCC 8739 (1)	1.235 e8 (sd = 4.6 e6)

sd – standard deviation

Two of the Six Materials Show Promise for Quantification of Total Cells and Genome Copies Using Flow Cytometry

Suitable?	Manufacturer	Material	Catalog #	Lot #	Strain (BSL)	Lot-specific CFU (uncertainty)
☒	Microbiologics	AccuShot	0483A	483-1115-3	ATCC 8739 (1)	624 (52 per 0.1 mL)
☒	MilliporeSigma	Vitroid (low CFU)	VT000906	BCCF4113	ATCC 11775 (2)	4.9 e3 (2.6 e3 – 9.3 e3)
☑	**MilliporeSigma**	**Vitroid (high CFU)**	**VT000127**	**BCCF1120**	**ATCC 8739 (1)**	**9.2 e4 (4.6 e4 – 1.9 e5)**
☒	bioMérieux	Bioball (low CFU)	56016	B6415	ATCC 8739 (1)	543.6 (sd = 30.7)
☒	bioMérieux	Bioball (mid CFU)	56053	B6593	ATCC 11775 (2)	1.051 e4 (sd = 4.8 e2)
☑	**bioMérieux**	**Bioball (high CFU)**	**56146**	**B6634**	**ATCC 8739 (1)**	**1.235 e8 (sd = 4.6 e6)**

sd – standard deviation

ddPCR-Based Assignment of GC per dsDNA Peak:
Genomes/Cell from ddPCR and Flow Cytometry Agree

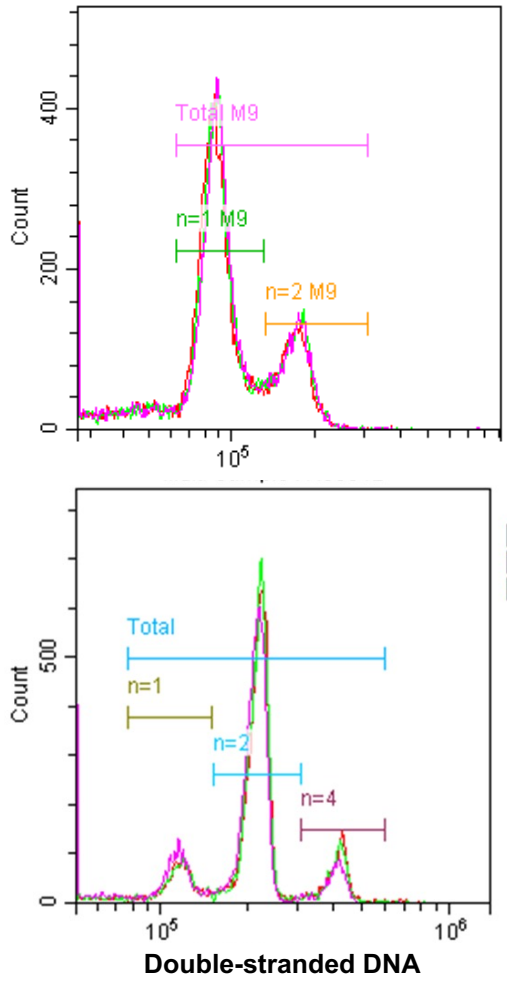
ddPCR Results

M9 (minimal) Media

M9	ycjM-2	23S	Mean
mean	1.21	1.21	1.2
STDEV	0.04	0.06	0.05
CV%	3.1	4.9	3.9
N	2	2	
n	3	3	

TSB (rich) Media

TSB	ycjM-2	23S	Mean
mean	2.16	1.98	2.1
STDEV	0.04	0.05	0.1
CV%	1.9	2.4	5.1
N	2	2	
n	3	3	



Flow Cytometry Results

chromosome (%)	M9			
	mean	SD	relsD%	n (data points)
n=1	70.4	2.7	3.8	9
n=2	29.4	2.7	9.2	9

$$Genomes\ per\ cell = \frac{(70.4\ x\ \mathbf{1}) + (29.4\ x\ \mathbf{2})}{100}$$

$$Genomes\ per\ cell = 1.29\ (\mathbf{M9})$$

chromosome (%)	TSB			
	mean	SD	relsD%	n (data points)
n=1	14.9	1.4	9.3	9
n=2	70.7	1.6	2.3	9
n=4	13.6	1.7	12.8	9

$$Genomes\ per\ cell = \frac{(14.9\ x\ \mathbf{1}) + (70.7\ x\ \mathbf{2}) + (13.6\ x\ \mathbf{4})}{100}$$

$$Genome\ per\ cell = 2.11\ (\mathbf{TSB})$$

Manuscript in preparation

Scope for Feasibility Study

Evaluate suitability of commercially available *E. coli* cell reference materials for existing NIST characterization methods

- Enumerate colony forming units (CFU) using plate counting
- Enumerate total cells using flow cytometry and Coulter counter
- Evaluate DNA content in cells using flow cytometry, toward quantifying genome copies (GC)
- Consider handleability, material properties, etc.

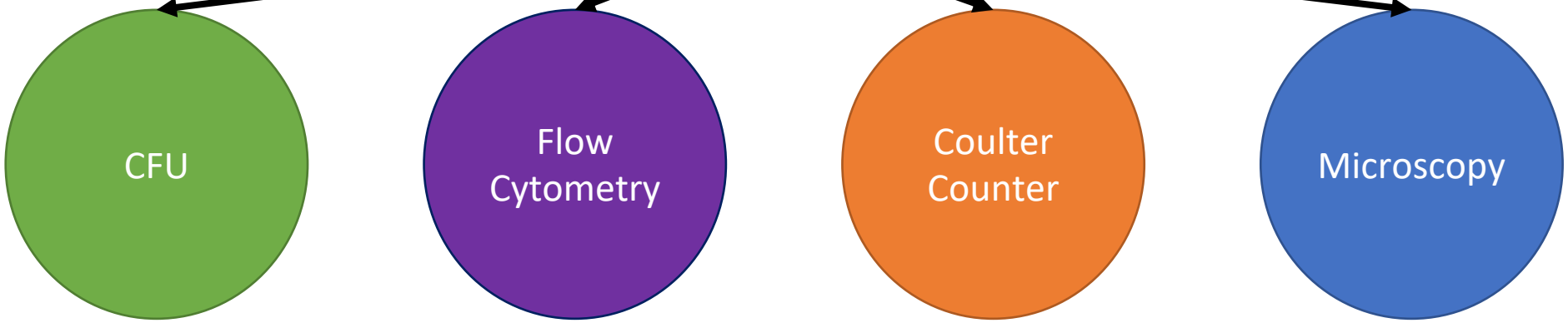
Out of scope:

- Optimized protocols
- Definitive values

Methods

Data Contributions

Sandra Da Silva
Jennifer Dootz
Joy Dunkers
Monique Hunter
Kirsten Parratt



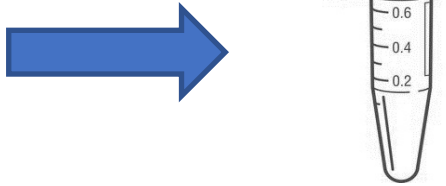
Flow Cytometry for Genome Copy Enumeration

1. Rehydrate
2. Dilutions (if appropriate)

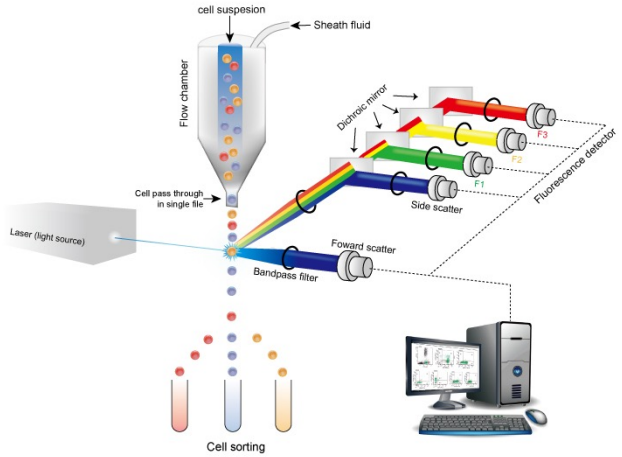
3. Hoechst incubation

4. Dilutions (if appropriate)

5. Analysis

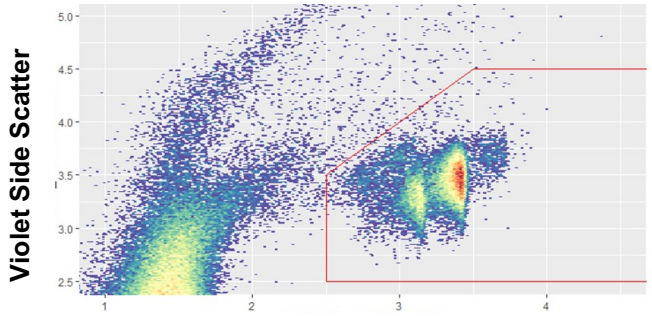


Beckman Coulter CytoFLEX LX



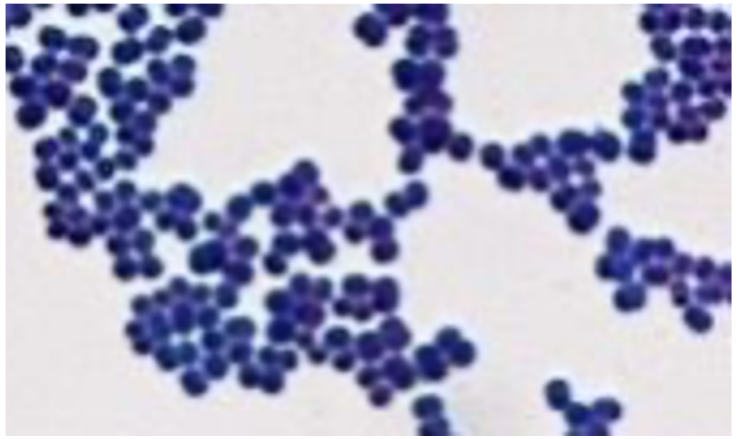
https://www.creative-diagnostics.com/flow-cytometry-guide.htm

1. Enumeration



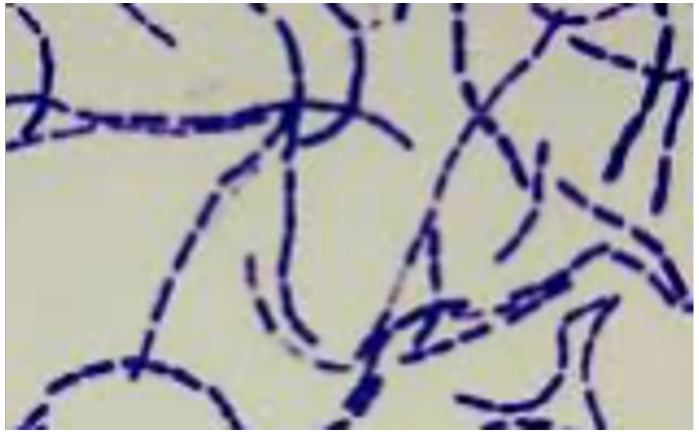
❖ Additional Strain Selection- USP 71 compendial Strains

Staphylococcus aureus



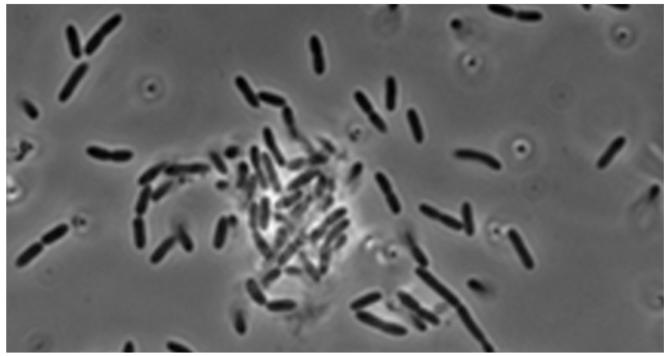
Aerobic bacteria

Bacillus subtilis



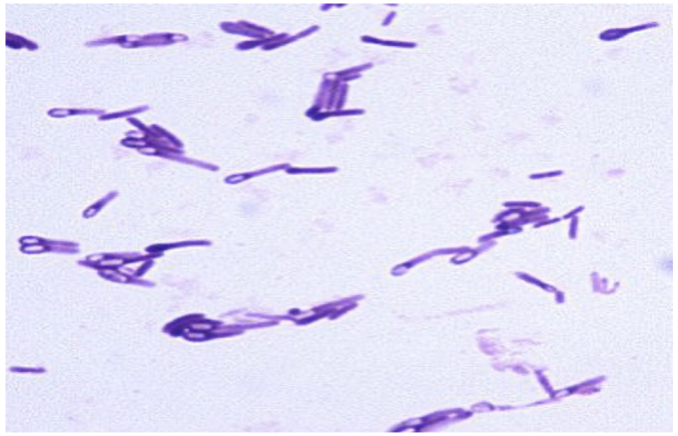
Aerobic bacteria

Pseudomonas aeruginosa



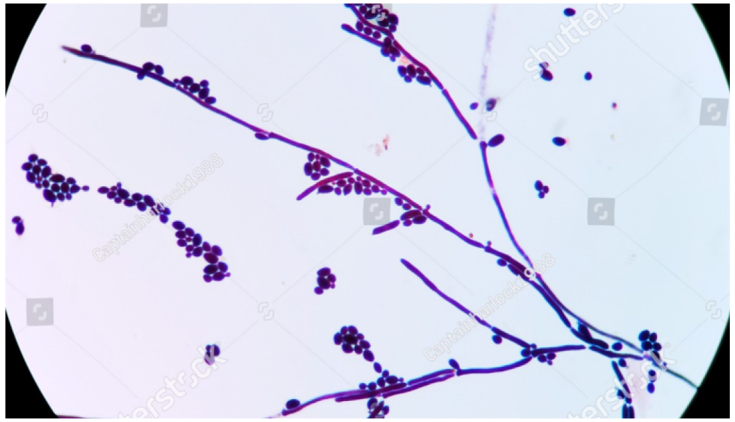
Aerobic bacteria

Clostridium sporogenes



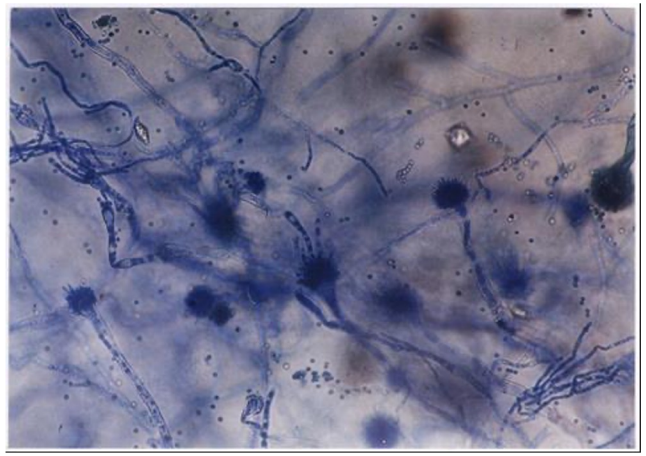
Anaerobic bacterium

Candida albicans



Fungi

Aspergillus niger



Fungi

Lyophilized Whole-Cell Microbial Reference Materials

Microbiologics “Pellets”

MilliporeSigma “Vitroid”

Biomerieux “Bioballs®”

Certified for CFU (only) from the manufacturer

Proposed Timeline

- NIST acquires whole-cell ***E. coli*** materials (3 manufacturers), assess genome copy/cell-pellet
- RMTM WG-03 Team met with NIST statistician Dr. Blaza Toman to discuss statistical sufficiency of the Study Design
- RMTM WG-03 Team met with Sartorius to discuss suitability of their kit for Interlab Study purposes
- RMTM WG-03 Team met internally to finalize Study Design

NIST presents data on whole-cell material, *E. coli*

WG03 coordinates interlaboratory study using newly-certified materials

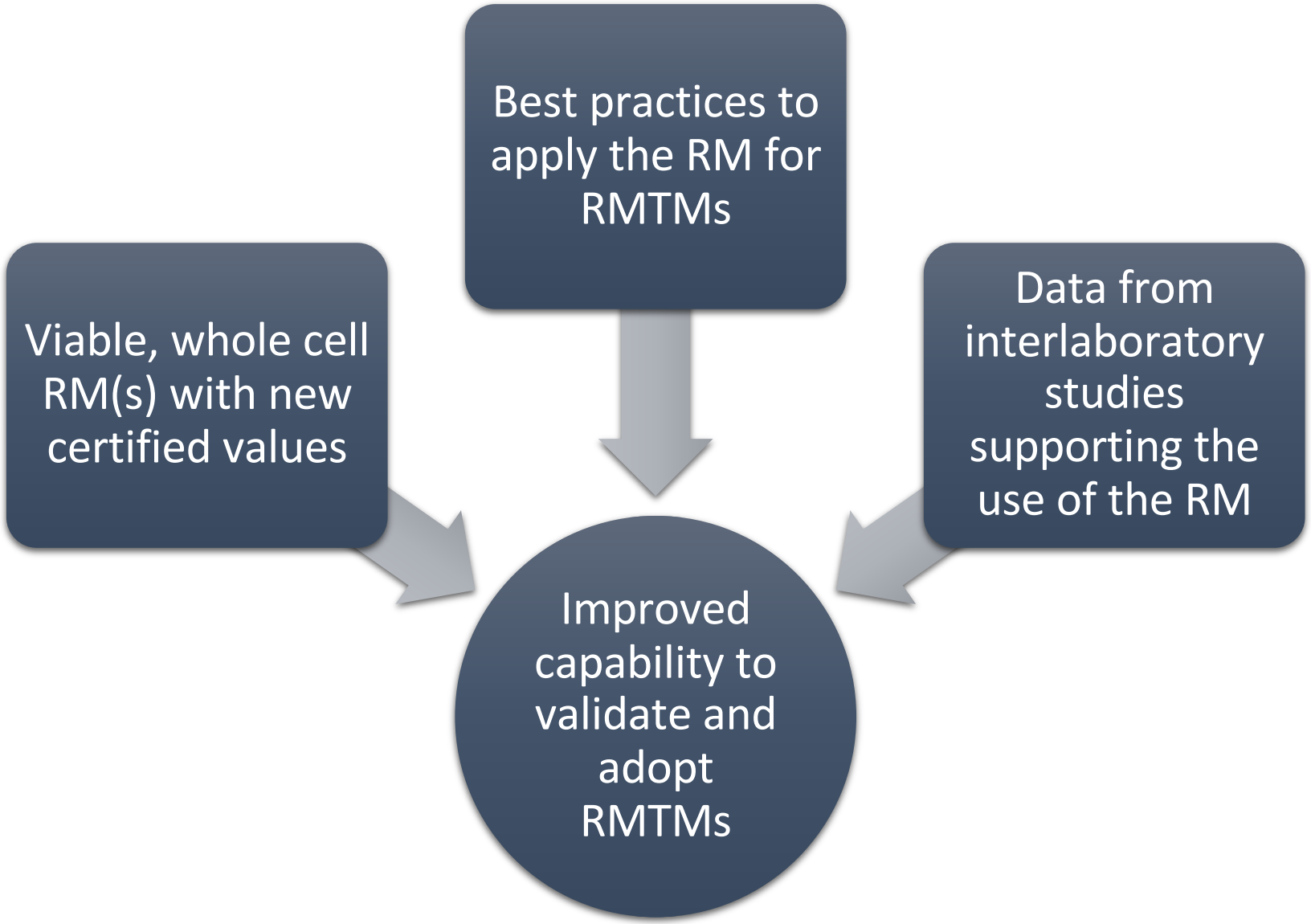
Start of Interlab Study# 1

Upcoming Meetings
(all on Tuesdays at 11 AM ET)

- 1st Tuesday of the month – WG01
- 2nd Tuesday of the month – WG02
- 3rd Tuesday of the month – WG03

- Next Full Consortium Meeting or Possible Workshop – TBD

Current Vision for Consortium Deliverables



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Nancy Lin – NIST
WG01 Lead

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WG02 Lead

Jason Kralj– NIST
WG03 Lead

Dawn Henke - SCB
SCB Liaison

Tara Eskandari - NIST
Partnership Manager