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Report from the 2023 NIST-Hosted Workshop on Collaborative Efforts to Enable Adoption of Rapid Microbial Testing Methods for Advanced Therapy Products

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This publication is intended to capture external perspectives related to NIST standards, measurement, and testing-related efforts. These external perspectives can come from industry, academia, government, and other organizations. This report was prepared as an account of a workshop; it is intended to document external perspectives; and does not represent official NIST positions.

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Abstract

On April 25, 2023, the National Institute of Standards and Technology (NIST) hosted a one-day hybrid workshop focused on bringing together organizations and working groups with the common goal of enabling validation and implementation of rapid microbial testing methods (RMTMs). The purpose of this workshop was to identify opportunities to leverage and coordinate ongoing and future efforts on RMTMs to accelerate their adoption for advanced therapy products. Based on workshop feedback, future areas of focus for the RMTM community to consider addressing include the need for data sharing – either via existing or new (e.g., interlaboratory study) datasets; increased awareness of existing regulatory and compendial initiatives, best practices, reference materials, and resources; and tools and measurement assurance strategies to advance the use of molecular-based RMTMs (such as next generation sequencing (NGS) and polymerase chain reaction (PCR). Increased collaborative efforts in these and other areas will promote the use of RMTMs and support improved safety for advanced therapy products.

Keywords

Rapid microbial testing methods; Consortium; Sterility testing; Advanced therapy products; Cell and gene therapy; Regenerative medicine; Molecular methods; Microbial cell reference materials; Interlaboratory study; Next generation sequencing.

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1. Motivation

Enabled by scientific breakthroughs and anchored by the rigor of research, the field of regenerative medicine includes a special class of advanced therapy products comprising cellular therapies, gene therapies, and tissue-engineered medical products. The aspiration of these therapies is both to reinstate normative physical and functional integrity of damaged or declining tissues, and to offer cures for some of today's most debilitating and intractable diseases such as refractory blood cancers. As such, advanced therapies are well poised to usher in a new era of healthcare. As with any clinical therapeutic product, they must be free of microbial contamination to ensure product quality and patient safety and to deliver on their promise.

Sterility assurance of advanced therapies is typically achieved by testing these products for microbiological contamination via culture-based compendial methods. Although widely regarded as the "gold standard," compendial methods can take up to 14 d to acquire conclusive results. [1]. Cell therapies are composed of living cells that typically lose efficacy rapidly (over the course of a few days) making this time-to-result of classical methods less desirable for many advanced therapies. [2] The use of alternative rapid microbial testing methods (RMTMs) that obtain results in a shorter time (e.g. methods that detect metabolic activity or molecular based methods) have been proposed as a solution for sterility testing in these short shelf-life products; however, despite their clear benefits RMTMs have not been widely adopted across the advanced therapy industry.

Many organizations in the advanced therapy community have recognized the need for RMTMs. To that end, relevant organizations have begun activities to help support the use of rapid (also referred to as alternate or modern) detection methods for microbes, and new organizations have been established with this same goal in mind. For instance, the National Institute of Standards and Technology (NIST) RMTM Consortium was established in 2020 to develop standards and measurement tools to support the use of RMTMs for advanced therapy products. While there is some overlap in membership across these various organizations, they are for the most part operating independently. Intentional, systematic communication across organizations regarding ongoing and future activities does not exist. Sharing information among organizations can help prevent redundant activities, promote collaborative efforts, enable sharing of limited resources, and ultimately increase and accelerate the use of RMTMs.

2. Workshop Goal and Structure

NIST organized and hosted a hybrid workshop on April 25, 2023 to bring together organizations focusing on tools and solutions to support the use of RMTMs for sterility and adventitious agent testing with a focus on advanced therapy applications.

Workshop Goal:

- *To identify opportunities for the coordination of efforts to advance rapid microbial testing methods and accelerate the validation and adoption of RMTMs in advanced therapy products.*

Expected Outcomes:

- *An established line of communication among professional organizations supporting the use of RMTMs*
- *Identification of common themes, unique roles, areas of overlap, and gaps in activities to support RMTM adoption*

- *Follow-up meetings to discuss potential collaborations and how to leverage activities*
- *Workshop report*

In total, 161 individuals registered, representing industry (82), federal government (39), academia (12), contracting or testing labs (11), non-profits (9), government (non-federal) entities (3), consultants (3), and analytic/bioinformatic service providers (2). Of those registered, approximately 25 individuals joined in person, with the remainder joining virtually.

The workshop agenda (Appendix A) was designed to facilitate information sharing from stakeholder organizations that support the use of RMTMs and discussion on advancing the use of RMTMs. Session 1 focused on efforts that support RMTMs for advanced therapies and comprised presentations from seven organizations (plus 1 slide deck from an 8th organization). Session 2 focused on feedback and discussion of RMTMs and consisted of breakout sessions and a final panel discussion. The workshop website contains additional details and recordings.[1]

3. Industry Perspective on Adoption of RMTMs

The workshop began with a keynote presentation from Dr. Veera Deehanadhayalan, AstraZeneca (AZ). In his role as Director of Biosafety and Bioassay Development, Dr. Deehanadhayalan is responsible for streamlining biosafety testing, which includes the introduction and implementation of modern technologies such as alternative microbial methods. To this end, he presented on AZ's pathway for implementation of a rapid microbial test method. First, he outlined critical points affecting rapid method selection for cell therapies, which included:

- Turn-around time for product release
- Sensitivity
- Coverage for a wide variety of microbial species
- Method considerations and in-house capabilities:
    - test sample handling
    - ability to reduce inadvertent contamination during testing
    - lab space requirements
    - scope and application
    - implementation timeline
    - method support (internal and external)
    - robust procedure for testing (i.e., easy to operate with respect to Good Manufacturing Practices (GMP) and transferability of method across locations)
    - ease of data interpretation and investigation procedure
    - differentiation of false positives and false negatives
- Method for identifying the microorganism
- Regulatory acceptance and guidance

Dr. Deehanadhayalan noted that while regulatory acceptance is listed last, it is one of the first aspects to be investigated. Related to this, he provided several resources they utilize, including the FDA's Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) published in January 2020 [3], The European Commission's Guidelines on Good Manufacturing Practice Specific to Advanced Therapy Medicinal Products [4], and PH Eur. 2.6.1 [5]. US

[1] https://www.nist.gov/news-events/events/2023/04/nist-hosted-workshop-collaborative-efforts-enable-adoption-rapid

Pharmacopeia (USP) chapters also include specific language on using alternative methods (**Table 2**). A recurring theme among these documents was language on qualifying and validating the selected method.

Following this high-level overview, Dr. Deehanadhayalan detailed the systematic approach AZ is following in their adoption of RMTMs. He shared their approach to project management and a five-step plan focused on bringing a quantitative polymerase chain reaction (qPCR) method online for AZ cell therapies. Additional details on AZ's approach can be found in a recording of the full presentation on the workshop website[2]. Highlights from the presentation include:

- Identifying the main justification for RMTM adoption will help determine an appropriate method—for AZ the emphasis was on a fast turn-around time.
- Evaluating DNA extraction efficiency is an important step in understanding sensitivity and limit of detection (LOD) for molecular methods.
- In addition to validation of RMTMs to fulfill regulatory requirements, AZ has internal requirements for adopting new assays such as routine use and training, documentation for data analysis and interpretation, life-cycle management (to keep up with evolving technologies/instrumentation), and sustainability (e.g., critical reagent qualification plans and back up reagent sources).

In launching the workshop, Dr. Deehanadhayalan provided a detail-oriented, first-hand account of how AZ is working towards the implementation of RMTMs for their cell therapy products. It was recognized that this is one example, and each manufacturer will ultimately follow a unique path to apply RMTMs to their specific applications and products.

4. Ongoing Efforts to Support RMTM Adoption

One of the primary goals for this workshop was to convene and have discussion among groups working towards a similar goal: adoption of RMTMs. The following stakeholder groups were represented at the workshop:

- Advanced Virus Detection Technologies Interest Group (AVDTIG)
- MIT Center for Biomedical Innovation (CBI)
- Modern Microbial Methods Collaboration (M^3)
- National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL)
- NIST Rapid Microbial Testing Methods (RMTM) Consortium
- Parenteral Drug Association (PDA)
- Standards Coordinating Body for Regenerative Medicine (SCB)
- United States Pharmacopeia (USP)

These groups represent different types of organizations ranging from large formal organizations such as USP and PDA to newly formed working groups such as the M^3 Collaboration. This section provides a brief overview of each organization, with a focus on their outputs and how they set and achieve goals. An overview of the organizations including contaminant(s) of interest, primary matrices, and key outputs can be found in Error! Reference source not found..

[2] https://www.nist.gov/news-events/events/2023/04/nist-hosted-workshop-collaborative-efforts-enable-adoption-rapid

Table 1. Organizations that Presented at the Workshop

Organization (with link if applicable)	Microbial Contaminants (e.g. Fungi, Bacteria)	Adventitious Agents (e.g. Viruses)	Type(s) of Matrix	Outputs
ADVTIG		X	Biologics (Cell substrate, viral seeds, bulk harvest etc).	Publications, Collaborative studies
M[3] Collaboration[3]	X		Pure samples (e.g., air, water)	Articles, tools (e.g., templates, checklists), presentations
MIT CBI[4]	X	X	Advanced Therapies	White papers, peer-reviewed publications, technology innovations, educational training
NIST-led RMTM Consortium[5]	X		Advanced Therapies	White papers, interlaboratory studies, datasets, methods for quantifying cell reference materials
NIIMBL[6]	X	X	Advanced Therapies, process samples, monoclonal antibodies	Case studies, peer-reviewed publications
PDA [7]	X	X	Numerous (Parental, sterile, and biotechnology)	Technical Reports, PDA journal, Guidelines and recommendations, Training and Education programs, commentaries on standards and regulations
SCB [8]	X	X	Advanced Therapies	standards, workshops, peer-reviewed publications, educational training
USP [9]	X	<1050.1> for Viral Clearance	Diverse Matrices including Advanced Therapies	Compendial analytical methods, informational documentary standards and physical reference materials

4.1. Historical Organizations Broadly Supporting Quality and Safety of Pharmaceuticals

Representatives from the USP and PDA presented on the how these organizations support RMTMs and their adoption. Both organizations are internationally recognized and focused on the pharmaceutical and biopharmaceutical industry.

4.1.1. USP

The USP was established in 1820 by a small group of physicians that wanted to protect patients from poor quality medicines. Keeping in line with the founders' goal, the mission of the USP is to improve global health and help ensure quality, safety, and the benefit of medicines and foods through public

[3] https://www.modernmicrobialmethods.com/
[4] https://cbi.mit.edu/
[5] https://www.nist.gov/programs-projects/nist-rapid-microbial-testing-methods-consortium
[6] https://www.niimbl.org/
[7] https://www.pda.org/
[8] https://www.standardscoordinatingbody.org/
[9] https://www.usp.org/

standards and related programs. The USP is divided into six collaborative groups: Biologicals, Small Molecules, Excipients, Healthcare Quality and Safety, Dietary Supplements, Herbal Medicines and Food Ingredients, and General Chapters (GC). The GC is unique in that it supports all areas including Microbiology. USP efforts on RMTMs are largely within the Microbiology Expert Committee (MEC) under the GC group. This committee is composed of expert volunteers, government liaisons from FDA and NIH, and USP liaisons. With respect to quality control and microbial testing, USP compendial methods USP <71> and USP <63> are often cited; however, the USP recognizes that traditional methods take up to 14 days (28 days for mycoplasma) and are thus not suitable for short shelf-life products, limited supply products, or products prepared for immediate and/or urgent use. [6, 7] Rapid microbial methods not only offer reduced time to testing but also the potential for automation, along with increased sensitivity and accuracy. The USP perspective on alternative methods (i.e., RMTMs) is stated in General Notice 6.30 *Alternative and Harmonized Methods and Procedures*. General Notice 6.30 states one must validate these methods following USP <1225> *Validation of Compendial Methods*, and the alternative method must produce comparable results to the compendial method. [8] More specific guidance on RMTMs can be found in USP <1223> and USP <1071>. [9, 10] Additionally, under the <1071> parent chapter, the MEC is considering a series of new chapters on specific rapid microbial methods. These methods must be broad in application (e.g., suitable for the majority of monograph[10] products), open source (e.g., not single source or patented technology), and available to any laboratory. Validation data to support these method chapters can come from sponsored submissions, peer reviewed literature, and/or collaborative proof-of concept and validation studies. There are several new chapters either recently approve or being considered by the USP GC MEC (Table 2).

Table 2. Additional USP Chapters Approved or under Consideration

Chapter	Topic
<72>	Respiration-Based Microbiological Methods for the Detection of Contamination in Short-Life Products[11]
<73>	ATP Bioluminescence-Based Microbiological Methods for the Detection of Contamination in Short-Life Products[12]
<74>	Solid Phase Cytometry-Based Rapid Microbial Methods for the Detection of Contamination in Short Shelf-Life Products[13]
<77>	Mycoplasma Nucleic Acid Amplification Tests in Short-Life Products[14]
<1071>	Rapid Microbiological Methods for the Detection of Contamination in Short-Life Products A Risk-Based Approach [15]
<1114>	Microbial Contamination Control Strategies for Cell Therapy Products[16]
	In plans for development in MEC 2022-2025 cycle
<65>	Alternative method to general chapter 60, Nucleic Acid Amplification Test for *Burkholderia cepacia* Complex
<75>	Nucleic Acid Amplification-Based Rapid Microbial Methods for the Detection of Contamination in Short Shelf-Life Products

[10] https://www.usp.org/about/public-policy/overview-of-monographs
[11] https://doi.org/10.31003/USPNF_M16055_03_01
[12] https://doi.org/10.31003/USPNF_M98813_03_01
[13] https://doi.org/10.31003/USPNF_M17295_010201_01
[14] https://doi.org/10.31003/USPNF_M17296_0101010201_01
[15] https://doi.org/10.31003/USPNF_M12457_02_01
[16] https://doi.org/10.31003/USPNF_M17035_101010201_01

4.1.2. PDA

Serving an overlapping stakeholder community with USP, the vision of the PDA is to maximize product quality, availability, and value by connecting people, science, and regulation within the pharmaceutical/ biopharmaceutical community. The PDA was established in 1946 by pharmaceutical manufacturers that recognized the need for an organization to disseminate technical information within the industry. Within the PDA, rapid microbial methods (i.e., RMTMs) are seen as a tool to help support the PDA mission of advancing pharmaceutical/ biopharmaceutical manufacturing science and regulation so members can better serve patients. The PDA has over 10,000 members and is organized into different topic areas, sub-committees, interest groups, and task forces. The PDA has several mechanisms in place that can support the adoption of RMTMs. The PDA Journal of Pharmaceutical Science and Technology provides a medium for dissemination of key scientific discoveries in pharmaceutical and biopharmaceutical quality and regulatory best practices; between January 2023 to June 2023 there were at least 9 publications related to Rapid Microbial Methods [Table 3]. The PDA also publishes Technical Reports that are peer reviewed global consensus documents written by subject matter experts. PDA Technical Report No. 33, Revised 2013 Evaluation, Validation, and Implementation of Alternative Rapid Microbial Methods is currently undergoing revision with an anticipated update release date in early 2024[17]. The PDA also plays an important convening role by hosting the annual PDA Pharmaceutical Microbiology Conference and a biennial Rapid Microbiological Methods Workshop.

Table 3. List of PDA Journal of Pharmaceutical Science and Technology Publications evaluating specific Rapid Microbial Methods (January 2023 to June 2023)

Document Title	Method/Focus
The Use of Amplified ATP Bioluminescence for Rapid Sterility Testing of Drug Product Formulations[18]	ATP detection
Challenges Encountered in the Implementation of Bio-Fluorescent Particle Counting Systems as a Routine Microbial Monitoring Tool[19]	Bio-fluorescent particle counting (BFPC)
Rapid Sterility Test Systems in the Pharmaceutical Industry: Applying a Structured Approach to Their Evaluation, Validation and Global Implementation[20]	Validation strategy
Performance Equivalence and Validation of a Rapid Microbiological Method for Detection and Quantification of Yeast and Mold in an Antacid Oral Suspension[21]	Metabolic indicator dye
Design, Development, and Validation of a Culture-Independent Nucleic Acid Diagnostics Method for the Rapid Detection and Quantification of the *Burkholderia cepacia* Complex in Water with an Equivalence to ISO/TS 12869:2019[22]	qPCR
Understanding the Non-Equivalency of Bio-Fluorescent Particle Counts versus the Colony Forming Unit[23]	BFPC
Multisite Qualification of an Automated Incubator and Colony Counter for Environmental and Bioburden Applications in Pharmaceutical Microbiology[24]	CFU
Identification of *Burkholderia* cepacia Complex by PCR: A Simple Way[25]	PCR

[17] As of the publication of this report this document is still under revision.
[18] https://doi.org/10.5731/pdajpst.2022.012762
[19] https://doi.org/10.5731/pdajpst.2021.012726
[20] https://doi.org/10.5731/pdajpst.2021.012672
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[24] https://doi.org/10.5731/pdajpst.2022.012742
[25] https://doi.org/10.5731/pdajpst.2021.012720

4.2. Organized Efforts Focused on Biopharmaceuticals and Advanced Therapies

In addition to large organizations like the PDA and USP that have branches catering to the many sectors of pharmaceutical and biopharmaceutical industry, there are also organized efforts that have arisen more recently to focus specifically on the unique challenges presented by the biotherapeutic sector, including SCB, NIIMBL, the NIST RMTM Consortium, and the MIT CBI.

4.2.1. SCB

The Standards Coordinating Body for Gene, Cell, and Regenerative Medicines and Cell-Based Drug Discovery (SCB), launched in January of 2017. SCB was established to enhance the standards development process by engaging with stakeholders in the regenerative medicine field. The organization's goal is to ensure that new and revised standards benefit the broader regenerative medicine community. To achieve this, SCB collaborates with the community to identify, prioritize, and advance standards; coordinates standards-related activities; and provides education on available standards, their benefits, and implementation. By doing so, SCB bridges the gap between the regenerative medicine community and the standards development process, occupying a unique position within the regenerative medicine landscape. As an impartial organization, SCB focuses on facilitating the development and use of standards in response to stakeholder needs.

Generally, the use of voluntary consensus standards is preferred from a regulatory perspective as it streamlines the approval process. To support this, SCB has developed various resources, including a regenerative medicine portal, a "needed standards" portal, sector calls, and a newsletter. The organization is also conducting feasibility studies on DNA/RNA templates and flow cytometry, in collaboration with the FDA, and invites experts to participate. Current opportunities for involvement in standards development include advancement of cryopreservation, RMTM framework, sterility testing for Tissue Engineered Medical Products (TEMPs), RMTM method inventory, cell viability, and USP <71>. Additionally, SCB has identified the need for documentary standards related to RMTMs, such as standardized language and testing practices for sterility testing, and methods to detect sterility, Mycoplasma, and adventitious agents. A comprehensive list of relevant microbial standards is available on the SCB website[26] providing further information and resources.

Table 4. List of Relevant Standards for RMTMs compiled by SCB

ORGANIZATION	ID	TITLE	STATUS
ASTM International	ASTM E3251-23	Standard Test Method for Microbial Ingress Testing on Single-Use Systems	Published 2023
ASTM International	ASTM E3251-23	Standard Test Method for Microbial Ingress Testing on Single-Use Systems	Published 2023
ASTM International	ASTM WK70143	New Guide for Sampling Methods of Tissue Engineered Medical Products (TEMPs) for Sterility Assurance	In Development October 2019
ASTM International	ASTM WK78574	New Guide for Best Practices for Microbial Control for Cell Therapeutics	In Development October 2021

[26] www.standardscoordinatingbody.org

ORGANIZATION	ID	TITLE	STATUS
European Directorate for the Quality of Medicines — EDQM	EP 2.6.27	Microbiological examination of cell-based preparations	Published 2017
International Organization for Standardization — ISO	ISO/TS 22456:2021	Sterilization of Healthcare Products — Microbiological Methods— Guidance on Conducting Bioburden Determinations and Tests of Sterility for Biologics and Tissue-Based Products	Published 2021
International Organization for Standardization — ISO	ISO 18362:2016/Amd 1:2022	Manufacture of cell-based health care products — Control of microbial risks during processing — Amendment 1	Published 2022

4.2.2. NIIMBL

NIIMBL is a public-private partnership established in 2017, funded through a cooperative agreement with NIST and support from its members, and part of Manufacturing USA. The NIIMBL mission is to "I don't accelerate biopharmaceutical innovation, support the development of standards that enable more efficient and rapid manufacturing capabilities, and educate and train a world-leading biopharmaceutical manufacturing workforce, fundamentally advancing U.S. competitiveness in this industry"[27]. NIIMBL works with partners from industry, academia, non-profits, and government agencies to accelerate manufacturing innovations that can reduce time and cost to bring advanced therapies to market.

The NIIMBL representative introduced the current public-private partnership and the ongoing RMTM related efforts. NIIMBL is focused on addressing the scale-up gap and helping advance the field rapidly. Current NIIMBL-funded efforts are intended to help de-risk new RMTM technologies through collaboration. NIIMBL funds community-led projects that require collaboration through open project calls. Of the 120+ projects that have been launched, seven funded projects relate to rapid microbial testing. These collaborative projects focused on RMTM technologies are more heavily industry led. Currently there is an end-to-end control strategy for rapid microbial testing publication forthcoming from these efforts. [11] Another project includes the open access publication of a case studies intended to generate and share learning and understanding through a control strategy for a hypothetical product. In this publication, a chapter is dedicated to adventitious agent control strategy considerations. NIIMBL also participates in multiple consortia and interlaboratory studies related to RMTMs.

4.2.3. NIST RMTM Consortium

The NIST Rapid Microbial Testing Methods Consortium launched in 2020 to convene stakeholders to collaboratively address the need for measurements and standards to increase confidence in the use of rapid testing for microbial contaminants in regenerative medicine and advanced therapy products. The Consortium is working to enable fit for purpose microbial cell reference materials, develop guidance and tools to support the use of appropriate reference materials and the validation of RMTMs, and design and run interlaboratory studies that support the development of reference materials, best practices and validated methods.

[27] https://niimbl.my.site.com/s/about-niimbl

The NIST RMTM Consortium representative provided an overview of the Consortium and its three Working Groups, which focus on reference materials, methods and validation, and interlaboratory studies. Via general consensus, the Consortium chose to focus their activities on the characterization and use of microbial cell reference materials that are fit for purpose for RMTMs. Commercially available microbial cell reference materials are typically certified for colony forming units (CFU). While CFU is relevant for growth-based compendial microbial detection assays, other measurements such as total genome copies and total cells per sample are more appropriate for validation and use of RMTMs, particularly non-culture based methods. The Consortium is developing methods to quantify total genomes and total cells in microbial cell materials, with the goal of enabling reference material manufacturers to expand the certified values for their existing microbial cell reference materials. The methods, which include flow cytometry, microscopy, impedance-based measurements, optimized DNA extraction, and digital droplet PCR, have been demonstrated on in-house and commercial *Escherichia coli* (*E. coli*) materials. Ongoing work is focused on evaluating the methods for use with compendial microorganisms listed in USP <71>. [7] To support the use of reference materials, a white paper on developing and adopting appropriate reference materials for RMTMs was submitted to a peer-reviewed journal. In addition, the first Consortium interlaboratory study was designed and run to evaluate suitability and reproducibility of commercially available products by assessing detection of commercially available *E. coli* reference materials using commercial DNA extraction and qPCR kits. NIST also launched the NIST Microbial Strain Collection[28] as a resource for the broader community. The collection can be used for depositing strains relevant to contamination of advanced therapy products as well as developing cell-based reference materials. The expected impact of Consortium activities is the improved capability to validate and adopt RMTMs.

4.2.4. MIT CBI

The Massachusetts Institute of Technology's (MIT's) Center of Biomedical Innovation (CBI) has the mission of improving global health care by overcoming obstacles to the development and implementation for biomedical innovation. Three pillars of CBI are collaboration, education, and research. MIT CBI is home to two pre-competitive programs: the Consortium on Adventitious Agent Contamination in Biomanufacturing (CAACB) and the Biomanufacturing Consortium (BioMAN) providing a venue for industry, academia and government to work collaboratively to address challenges in manufacturing. Although the representative from MIT CBI was unable to attend the workshop, they did provide a slide deck summarizing their projects to share as part of the workshop materials. A major strength of the CAACB has been their ability to survey the stakeholder community, then analyze and disseminate the results to their consortium members through white papers and to the broader community through peer-reviewed publications. As one example, CAACB has completed a survey of viral contamination in biomanufacturing that resulted in a Nature Biotechnology publication. [12] In addition to helping elucidate the scope and magnitude of the problem, CAACB has also been active in evaluating new technologies to improve detection methods. They recently published a paper on the ability of next generation sequencing (NGS) to replace in vivo adventitious agent virus testing. [13] A full list of publications from CAACB can be found on their website[29]. In addition to a convening role, research is another pillar of MIT's CBI. For example, cell therapy manufacturing research carried out under the SMART CAMP (Singapore-MIT Alliance for Research and Technology – Critical Analytics for Manufacturing Personalized Medicine) has led to identification of a new rapid method for detecting microbial contamination through identification of unique microbial metabolites as well as a machine

[28] https://www.nist.gov/programs-projects/nist-microbial-strain-collection
[29] https://cbi.mit.edu/about-cbi/collaboration/caacb/caacb-publications/

learning-based-approach for rapid detection of adventitious microbes in cell therapies using long read sequencing. [14, 15] In a joint effort, MIT CBI and SMART CAMP are undertaking a contamination survey project to assess adventitious agent contamination in manufacturing of cell and gene therapy products On a final note, there are several ways to engage with MIT CBI including: joining the industry consortia, participating in offered online classes, participating in class development, joining in sponsored research activities, or sponsoring new projects.

4.3. Organized Efforts with Specialized Scopes

4.3.1. AVDTIG

Initiated as a task force organized by the PDA, the Advanced Virus Detection Technologies Interest Group (AVDTIG) was formed in 2012 by regulatory and industry scientists as a forum for discussing and sharing data and experiences with advanced virus detection technologies, primarily high throughput sequencing technologies. With a focus on using new technologies for detection of adventitious viruses, the group has over 200 members representing 60 different organization.

The AVDTIG representative focused on the group's work to advance NGS for viral risk evaluation through collaboration. AVDTIG is working to publish best practices and position papers on rapid microbial methods, specifically focused on viral detection. One primary focus involves challenges with standardization of NGS, as NGS has multiple sequencing platforms and multiple sample handling methods that have variable needs. In response to a lack of studies and reference materials on this topic, AVDTIG has formed various subgroups:

- Sample selection/prep/processing and viral reference materials (hoping to publish in 2023),
- Reference Virus Database (annotation of current version and revisions to data), and
- Bioinformatics pipeline and follow up investigations (continuing discussions and collaboration).

AVDTIG has been very active over the past decade running several interlaboratory studies[16], hosting workshops, and publishing extensively. [16-23] Currently, AVDTIG has multiple collaborative spike-in studies underway. A future focus of this group is determining how these adventitious agent tests can be applicable to microbial testing.

4.3.2. M^3 Collaboration

The Modern Microbial Methods Collaboration (M^3 Collaboration) was formed in 2021 to establish a formal collaboration across four existing groups with scope overlap. The original four working groups were the BioPhorum Fill Finish Alternative and Rapid Micro Methods BFPC team, the Kilmer Community Rapid Microbiology Methods group, the Online Water Bioburden Analyzer (OWBA) working group, and the Process and Environmental Monitoring Methods (PEMM) working group. M^3 pursues shared goals to promote excellence, international harmonization and continuity, as well as support of the implementation and use of technologies commonly referred to as Modern Microbial Methods or Alternative and Rapid Microbial Methods (i.e., RMTMs) within the industry.

The M^3 collaboration representative introduced M^3 as a collaboration that supports implementation and use of modern microbial method technologies through facilitating discussions across experts and organizations. M^3 has 3 sub-teams focused on

- challenges associated with Bio-Fluorescent Particle Counter (BFPC) implementation,

- establishing baseline count and alert and/or action levels, and
- a communication toolbox and roadmap.

M^3 has published an umbrella article and is working on sub-articles to provide further detail on these topics. The future directions of the collaboration include increased outreach and communication over the next year to make people more aware of the efforts that M^3 has been undertaking.

5. Panel and Breakout Discussions Help Identify Gaps and Needs

The final two sessions of the workshop were discussion-based, with the goal of identifying potential collaborative efforts relevant to standards and measurements that could help advance adoption of RMTMs. Three breakout sessions were held concurrently to facilitate small group discussions, and then participants reconvened as a large group. Upon reconvening, each small group leader provided a report back with highlights of their discussion. Then four subject-matter experts occupying different roles in cell and gene therapy sector served as a panel to continue dialogue on the breakout session theme. Three main questions were used to guide the breakout and panel discussions:

a) What gaps/challenges remain to be addressed for RMTM adoption?
b) Would shared data on RMTM validation studies help advance the field? Please explain.
c) Are RMTMs useful (e.g., compared to CFU) if they can't differentiate viable cells? What are the benefits/drawbacks of RMTMs if they detect all microbial cells and not just viable/culturable cells??

Several key takeaways and recommendations emerged from the discussion sessions and are summarized below.

Key Takeaways

- Collaborative efforts are needed and can have an impact by generating sharable data and helping present a unified voice.
- Relevant reference materials and knowledge on how to incorporate them are lacking.
- RMTMs generate new types of data necessitating best practices for
    - analyzing and managing data,
    - making results actionable, and
    - implementing results.
- There is added value from RMTMs even if they are unable to ascertain viability.

Recommendations for the NIST RMTM Consortium and other collaborative efforts to consider:

- Design and run interlaboratory studies to collect new datasets.
- Establish a data repository to facilitate sharing of existing data and/or open-access case studies.
- Promote existing regulatory and compendial initiatives, best practices, and resources.

- Enable quantification of properties beyond CFU (such as total cell count, total DNA content) for microbial cell reference materials at low (10 cells/mL to 100 cells/mL) cell concentrations.
- Demonstrate detection of viable microorganisms using existing RMTMs.
- Develop tools and strategies to support next generation sequencing (NGS)-based RMTMs.

6. Summary

The 2023 NIST-Hosted Workshop on Collaborative Efforts to Enable Adoption of Rapid Microbial Testing Methods (RMTMs) for Advanced Therapy Products brought together 8 different organized efforts with the shared goal of enabling the adoption of RMTMs. Through presentations and discussions, key areas where the NIST-led RMTM Consortium can contribute were identified. These included: (1) coordinating interlaboratory study(s) to generate sharable data; (2) disseminating and raising awareness of existing regulatory and compendial initiatives, best practices, reference materials, and resources; (3) developing tools and strategies with a focus on reference materials to support molecular-based RMTMs. The workshop helped establish a network of groups that will be leveraged going forward to better support the adoption of RMTMs across the advanced therapy community.

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Appendix A. AGENDA

NIST-Hosted Workshop on Collaborative Efforts to Enable Adoption of Rapid Microbial Testing Methods for Advanced Therapy Products

Tuesday, April 25, 2023, 9:00 AM – 5:00 PM (all times are EDT)

AGENDA

(updated 4/24/2023)

OPENING	
9:00 AM – 9:25 AM	Welcome and Opening Remarks *Scott Jackson, PhD, Leader, Complex Microbial Systems Group, Biosystems and Biomaterials Division (BBD), Material Measurement Laboratory (MML) at the National Institute of Standards and Technology (NIST)* *Stephanie Hooker, PhD, Acting Director, MML at NIST*
9:25 AM – 10:00 AM	KEYNOTE PRESENTATION A Pathway for Implementing Rapid Microbial Test Method *Veera Dheenadhayalan, PhD, Director of Biosafety at AstraZeneca*

SESSION 1: Efforts that Support Rapid Microbial Testing Methods for Advanced Therapies *Moderator: Kirsten Parratt, BBD, MML, NIST*	
10:00 AM – 10:15 AM	USP Evolving Position on Use of Rapid Microbial Methods *Huiping Tu, PhD, Senior Principal Scientist at USP*
10:15 AM – 10:30 AM	PDA Activities Related to Rapid Microbial Methods *Fred Ayers, Advisor – Global Quality Systems at Eli Lilly and Company*
10:30 AM – 10:45 AM	Accelerating Adoption of Rapid Microbial Detection Technologies *Jennifer Mantle, PhD, Regulatory Committee Coordinator/Technical Project Manager at the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL)*
10:45 AM – 11:00 AM	**BREAK**
11:00 AM – 11:15 AM	Advanced Virus Detection Technologies Interest Group (AVDTIG) - Advancing the Adoption of NGS for Adventitious Agent Detection *Siemon Ng, PhD, Senior Director of Analytical Development and Quality at Notch Therapeutics*
11:15 AM – 11:30 AM	An Overview of the Modern Microbial Methods Collaboration *Allison Scott, PhD, Principal Scientist at MicronView LLC*
11:30 AM – 11:45 AM	NIST Rapid Microbial Testing Methods (RMTM) Consortium: Activities and Directions *Nancy Lin, PhD, Leader, Biomaterials Group, BBD, MML at NIST*
11:45 AM – 12:00 PM	Current State of Standards for RMTMs in Advanced Therapies *Dawn Henke, PhD, Senior Technical Program Manager, Standards Coordinating Body for Regenerative Medicine (SCB)*

LUNCH (on your own) **12:00 PM – 1:30 PM**

SESSION 2: Feedback and Discussion on Rapid Microbial Testing Methods *Moderator: Nadratun Chowdhury, BBD, MML, NIST*	
1:30 PM – 1:45 PM	Introduction to Breakout Sessions *Nadratun Chowdhury, PhD, National Research Council Postdoctoral Fellow, BBD, MML at NIST*
1:45 PM – 2:55 PM	Breakout Sessions
2:55 PM – 3:25 PM	Breakout Session Report-out and Discussion *Breakout Session Moderators, NIST*
3:25 PM – 3:45 PM	**BREAK**
3:45 PM – 4:45 PM	Panel Discussion *Moderator: Scott Jackson, NIST* *Panelists:* • *Guo-Chiuan Hung, PhD, Chemistry, Manufacturing, and Control (CMC) Reviewer at FDA/CBER/Office of Advanced Therapies (OTAT)/Division of Cellular and Gene Therapies (DCGT)/Gene Therapy Branch* • *Michael Miller, PhD, President at Microbiology Consultants, LLC* • *Jennifer Robbins, Senior Principal Scientist in Cell Therapy Development at Bristol Myers Squibb* • *Tricia Vail, Regional Segment Marketing Manager – Applied Research Markets at Sartorius Corporation*

CLOSING	
4:45 PM – 5:00 PM	Concluding Remarks *Scott Jackson, NIST*

SLIDES ONLY (No presentation)
Activities of the MIT Center for Biomedical Innovation Related to Rapid Microbial Methods *Stacy Springs, PhD, Executive Director, MIT Center for Biomedical Innovation*