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NIST 2024 Rapid Microbial Testing Methods Workshop Report



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Abstract

The NIST-led Rapid Microbial Testing Methods (RMTM) Consortium launched in 2020 to develop standards and measurement-based solutions to advance the use of RMTMs in advanced therapy products. In April 2024, NIST hosted the 4th annual workshop on RMTMs to update the community on RMTM Consortium efforts and to advance Consortium deliverables. This report provides a summary of workshop activities and findings. The workshop consisted of several working sessions, 1 h to 2 h in length, focused on specific Working Group activities and deliverables. Day 1 was open to the public, and Day 2 was for Consortium members only. Parties that participated in the workshop included advanced therapy producers, RMTM assay/instrument developers, and cell reference material producers. Progress was made on multiple Consortium activities via the session discussions, and feedback was provided to help scope potential future Consortium activities. Overall, the workshop helped ensure relevance and impact of RMTM Consortium activities.

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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Author Contributions

Kralj JG: Conceptualization, Writing- Original draft preparation, Writing- Reviewing and Editing. **Parratt KH:** Conceptualization, Writing- Reviewing and Editing. **Servetas SL:** Conceptualization, Writing- Reviewing and Editing, Supervision. **Henke D:** Conceptualization, Writing- Reviewing and Editing. **Jackson SA:** Conceptualization, Writing- Reviewing and Editing, Supervision. **Lin NJ:** Conceptualization, Writing- Reviewing and Editing, Supervision.

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1. Workshop Overview

On April 11-12, 2024, NIST hosted a virtual workshop on Rapid Microbial Testing Methods (RMTMs) for Advanced Therapies. The goal of the workshop was to convene Consortium members and additional stakeholders and interested parties to share the latest activities of the Consortium, learn about advances in RMTMs, and discuss and receive feedback on current and potential future activities for the Consortium. Details of the workshop, including the workshop booklet, can be found on the workshop website.¹

The RMTM workshop had a total of 145 registrants (Fig. 1), with the greatest proportion coming from industry.

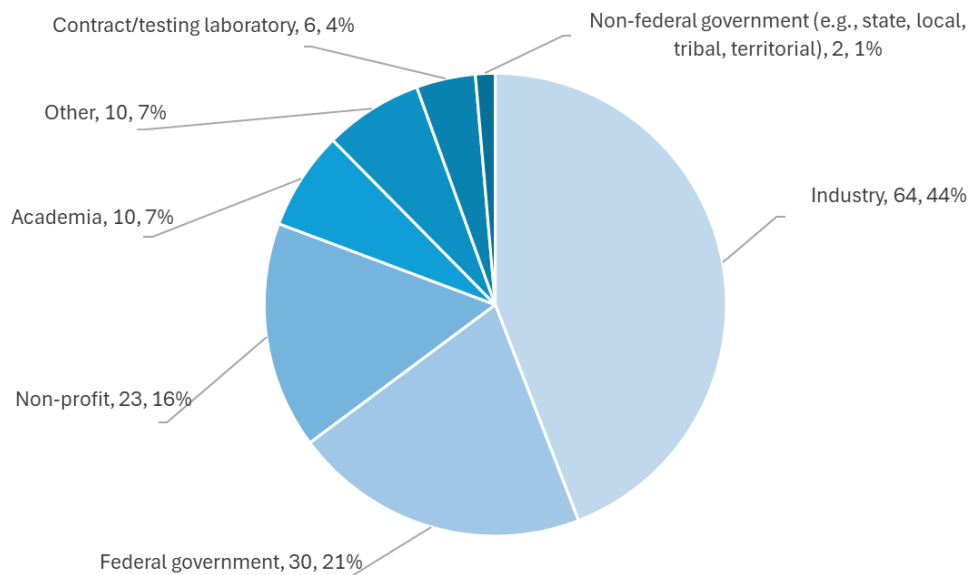


Fig. 1. Demographics of workshop attendees.

Primary focus areas for registrants were as follows:

- 52 % - Rapid microbial testing methods
- 20 % - advanced therapy products
- 13 % - reference materials and standards
- 10 % - other
- 5 % - regulation

In terms of interactions with the RMTM Consortium, the registrants were fairly evenly split:

¹ <https://www.nist.gov/news-events/events/2024/04/2024-nist-rapid-microbial-testing-methods-consortium-workshop>

- 37 % were members of the RMTM Consortium
- 35 % were not members of the Consortium
- 28 % selected "maybe" (e.g., their organization is a member but the registrant had not joined or participated in the Consortium, their organization had submitted a letter of interest but wasn't a Consortium member yet, or they were not sure if they were a member)

The workshop agenda (Appendix I) included a session on RMTM Consortium updates, a session on developments in RMTM technologies, and five sessions on current or potential future Consortium activities, with Day 1 open to the public and Day 2 closed for Consortium members only. The sessions were designed to share the progress and future directions of the consortium and gather feedback from participants. Overviews of the sessions are provided in this report.

2. Day 1: Open Workshop

2.1. NIST RMTM Consortium Update

The NIST RMTM Consortium provided an overview of its activities and progress updates for the three Working Groups.

2.1.1. Working Group 1 (WG1) - Progress

The Reference Material Working Group (WG1) is focused on measurements to enhance the suitability of commercially available reference materials for use in demonstrating and validating molecular (e.g., nucleic acid-based) detection methods as RMTMs. The approach is to help enable certification of values on microbial cell reference materials beyond traditional colony-forming unit (CFU) measurements. Specifically, WG1 has explored total cell count and genome copy number as additional certified values. NIST has been performing feasibility studies to evaluate the challenges in measuring these properties for commercially available microbial cell reference materials, and these studies will be disseminated as a NIST Technical Publication. The conclusions from this work will be summed up in a perspective article to highlight the need for suitable reference materials certified beyond CFU to support molecular sterility methods.

2.1.2. Working Group 2 (WG2) Progress

The Methods and Validation Schemes Working Group (WG2) is focused on tools and frameworks to support validation of RMTMs. The group has drafted a manuscript to emphasize the importance of reference materials being certified for attributes relevant to the specific RMTMs they are used to validate. Following that manuscript, they sought to identify where to focus their efforts. In a past survey where participants identified measurement technologies, they were most hopeful to be adopted as RMTMs, polymerase chain reaction (PCR) and NGS metagenomics were highly ranked, accounting for more than half of the responses. Consequently, WG2 decided to focus on developing customized analysis tools, reference databases, and workflows for NGS-based microbial detection. In addition to the bioinformatic tools, a key aspect of this effort will be the implementation of spike-in controls to assess data quality and normalization as well as a corresponding interlaboratory study (ILS). For the near future, WG2 will focus on these NGS-based activities including the ILS.

2.1.3. Working Group 3 (WG3) Progress

The Interlaboratory Study Design and Implementation Working Group (WG3) is focused on ILS efforts to establish publicly available datasets based on RMTMs and relevant reference materials. The first ILS was designed to evaluate the performance and suitability of commercially available materials, including microbial cell reference materials, extraction kits, and quantitative PCR (qPCR) kits, for detecting microbial contamination. Results across multiple laboratories showed that commercially available, high CFU *E. coli* materials appear suitable for

qPCR detection assays, with qPCR results being consistent across the ILS participants. A peer-reviewed manuscript describing these findings is in preparation. WG3 is also preparing to launch a second ILS involving compendial methods and RMTMs. ILS-2 will be a survey of RMTMs where participants will use RMTMs of their choice on a common sample set consisting of low levels of microbial cells added to a human cell background. NIST is currently developing the test material, which will be distributed to participating laboratories for detection on their respective platforms. At previous workshops, stakeholders have indicated the need for shared, relevant datasets for RMTMs. These ILS efforts will help to address this need and provide data on RMTMs for the community.

2.2. Next Generation Sequencing (NGS) – based Sterility Testing

The first workshop session centered on the development of next-generation sequencing (NGS)-based analytical tools for microbial sterility testing in biomanufacturing environments, particularly in the context of advanced therapy products. The discussion encompassed the need for customized reference genome/gene databases, bioinformatic pipelines, and standardized reporting formats to support the adoption of NGS technology. NGS offers several advantages over traditional methods, including faster turnaround times and the ability to detect a broad range of microorganisms without prior knowledge of their presence (i.e., "target-agnostic" detection).

To facilitate the application of NGS in sterility testing for advanced therapy manufacturing, WG2 recognized the need for a well-curated reference database suitable for this application. Collaboration with a cloud analytics organization (EzBiome) is underway to develop a database specifically designed for analyzing metagenomics data in this context. Key considerations for database development that were discussed included:

- Incorporating diverse sequences, including those from contaminants, environmental organisms, and pathogens, to enable accurate differentiation between microorganisms
- Addressing the challenge of limited sequence availability for certain pathogens, which may be relevant contaminants in advanced therapy products
- Establishing criteria for data inclusion and curation to ensure database quality
- Utilizing background sequence data from environmental samples to inform database development
- Considering the use of commercially available NGS standards to understand how background noise and biases from the NGS workflow affect results

The main takeaways from the discussion were:

- WG2 will continue to focus on tools to support the adoption of NGS for sterility testing
- A reference genome database specifically for sterility testing of advanced therapy products, including cell and gene therapies, is under development and will help to support NGS analysis

- Benchmarking and spike-in studies will be necessary to validate NGS-based sterility testing methods, and may be a future focus of for WG2

2.3. Developments in RMTM Technology

This session centered on novel microbial detection technologies and their adoption for microbial sterility testing. The discussion aimed to evaluate both existing and emerging non-compendial methods for rapid microbial testing from the perspective of end-users, with a focus on identifying potential challenges to their adoption and assessing their future prospects.

2.3.1. Development of a Next Generation Sequencing Workflow for Rapid Microbial Testing: Neeraj Salathia (Resilience)

Resilience is developing a novel Next-Generation Sequencing (NGS)-based approach for rapid microbial quality control (QC) testing in biomanufacturing. The proposed workflow and analytical strategies aim to accelerate and comprehensively assess the sterility of drug products. The method uses a bulk NGS-based mycoplasma and sterility assay, which employs a multi-faceted approach, including detection of RNA instead of DNA, to enhance detection of contaminants while minimizing interference from the cell therapy product. Their approach results in a rapid turnaround time of two days, with high specificity and sensitivity and the ability to identify the contaminating organism.

The development of highly sensitive assays, such as this one, raises regulatory challenges regarding specificity and inclusivity. One must be ready to handle false positives, mainly misclassifying signals from organisms that are present. The second challenge is inclusivity and evaluating the workflow against a range of organisms to ensure the method's accuracy, which they found is dependent on the number of organisms present and the assay's ability to detect them without bias. Equivalency testing to compendial methods is also planned. Moving to automation of the assay, the team will aim to demonstrate robustness, repeatability, and ruggedness to cover sources of variability.

One of the challenges faced by Resilience is obtaining test samples that can reliably assess the assay's capabilities. Additionally, they emphasized the need for high-quality, curated databases to ensure the accuracy and reliability of their results. While their method was not initially designed for high-throughput applications, Resilience is exploring the possibility of expanding its use to environmental monitoring.

2.3.2. CalScreener+ Rapid Phenotypic Sterility Test Method for Direct Inoculation of Advanced Therapy Medicinal Products (ATMPs): Wilhelm Paulander (Symcel)

Symcel has developed a novel instrument based on isothermal microcalorimetry, which enables continuous monitoring of metabolic activity from a wide range of contaminating microorganisms in advanced therapy medicinal products (ATMPs). This method is non-destructive and independent of the product matrix, making it a versatile tool for detecting contaminants. The instrument requires a minimal sample volume of 0.05 mL and can detect

contaminants within 3 d, with a limit of detection of less than 5 CFU. It has the capability to resolve heat signal changes as small as 1 μ W, thereby allowing for the detection of low-level contaminants even in complex matrices with high eukaryotic cell backgrounds (e.g., $>10^6$ cells/mL).

The presentation also shared how the instrument has been shown to detect various microorganisms, including *S. aureus*, *P. aeruginosa*, *B. subtilis*, *C. sporogenes*, and *C. albicans*, in Jurkat cells at concentrations as low as 5 CFU/mL within 24 h. Similarly, *A. brasiliensis* was detected in approximately 30 h, and slow-grower *C. acnes* in 72 h. These results demonstrate the instrument's ability to rapidly detect a range of contaminants in complex matrices.

2.3.3. Commercial Cell Therapy Experience Implementing Rapid Microbial Testing Methods: John Duguid (Vericel Corporation)

The rapid detection of contaminants is crucial for cell therapy products with short shelf lives. To enable timely final product lot release prior to product expiration and surgical implantation, it is essential to have final product release tests that require minimal sampling, produce rapid and valid results, and are cost-effective. Vericel was a pioneer in implementing rapid sterility testing with the first implementation occurring in 2004, followed by the introduction of a rapid mycoplasma test using real-time PCR in 2013.

Initially, Vericel used a growth-based respiration method that utilized a CO₂ sensor, which they automated to improve efficiency. The validation process for this methodology took 10 y to complete. The rapid sterility test proved to be effective, detecting contaminants in four shipped products prior to implantation. Notably, these contaminants would likely have gone undetected if compendial methods had been used.

In addition to sterility testing, Vericel also implemented a rapid mycoplasma test using PCR-based technology. This test was designed to detect multiple mycoplasma species at concentrations as low as 10 CFU, identify viable organisms, and be compatible with antibiotics used in manufacturing. Since the launch of MACI in 2017, Vericel has tested tens of thousands of samples using this method.

Although the test had a manageable false positive rate initially, an increase in false positives was observed between 2018 and 2020, leading to product lot rejections. Investigations revealed that these false positives were artifacts of the test. Vericel collaborated with the vendor to resolve the issue, and the test is now back to its baseline performance. The automation of both tests has significantly improved their speed and efficiency.

2.3.4. Discussion

The discussion centered on several key topics related to the use of NGS for microbial detection. These topics included varying levels of RNA present in different organisms, handling contamination from multiple organisms using NGS, the ability of NGS technology to detect a wide range of genetic sequences, and unique metabolic patterns in each organism to

complement NGS. Moreover, it was emphasized that a comprehensive reference database is required for comparison to support interpretation of NGS data

All assays that were discussed follow the recommendations and requirements outlined in USP <1223>, a standard for the validation of microbial detection methods. Challenges raised during the discussion primarily revolved around the validation of assays and the mitigation of false positives. These challenges highlight the need for robust validation protocols and strategies to minimize false positives, ensuring the accuracy and reliability of NGS-based microbial detection assays.

2.4. Roadmap for Microbial Cell Reference Material Characterization

NIST presented a draft roadmap prepared with input from WG1 aimed at optimizing methods for characterizing total cell count and total genome count in commercial microbial cell reference materials. The goal of this session was to gather input and refine the technical considerations outlined in the roadmap.

To bridge the gap between molecular methods and compendial methods, one potential solution is for reference material manufacturers to expand their certified values from only CFU to include other properties relevant to molecular methods and facilitate comparability studies across compendial and rapid sterility methods. To do this, characterization methods are needed to support total cell count and/or total genome copy count of whole cell microbial materials, while also minimizing material manipulation during the measurement process. Feasibility studies conducted by NIST indicated that variability in microbial strains and existing commercial products necessitates individualized approaches, as no single protocol is expected to be effective on all microbial cell reference materials. The draft roadmap provides an approach for reference material manufacturers to follow a series of steps to optimize measurements for their product lines and improve suitability of existing reference materials for molecular-based RMTMs.

2.5. Day 1 Summary

The first day of the workshop was open to the public and centered on the ongoing efforts of the RMTM Consortium and technology updates from the field. The Consortium's future focus includes developing strategies and tools to help support the development and adoption of molecular-based RMTMs and corresponding microbial cell reference materials. The workshop also featured discussions on emerging technologies, including NGS- and metabolic activity-based assays that are currently under development or have been implemented in manufacturing settings. These new approaches aim to enhance the detection and characterization of microorganisms, and their potential applications in the field were explored during the workshop.

3. Day 2 – Closed Workshop for RMTM Consortium Members Only

The second day of the workshop built upon the discussions from the first day, with a focus on advancing the efforts of the RMTM Consortium. The primary objective was to solicit feedback from Consortium members on current initiatives and drive progress on Consortium activities. The main topics of discussion centered around two planned interlaboratory study efforts. Additionally, one session explored a potential new initiative to develop a tool to openly communicate a typical validation strategy for implementation of RMTMs, thus providing an example to help stakeholders work through the validation process, a critical step in ensuring the accuracy and reliability of these methods.

3.1. Proposal for the Second RMTM Consortium Interlaboratory Study

WG3 presented a plan for ILS-2, including a draft experimental protocol and timeline (Fig. 2), which was followed by open discussion and plan revisions.

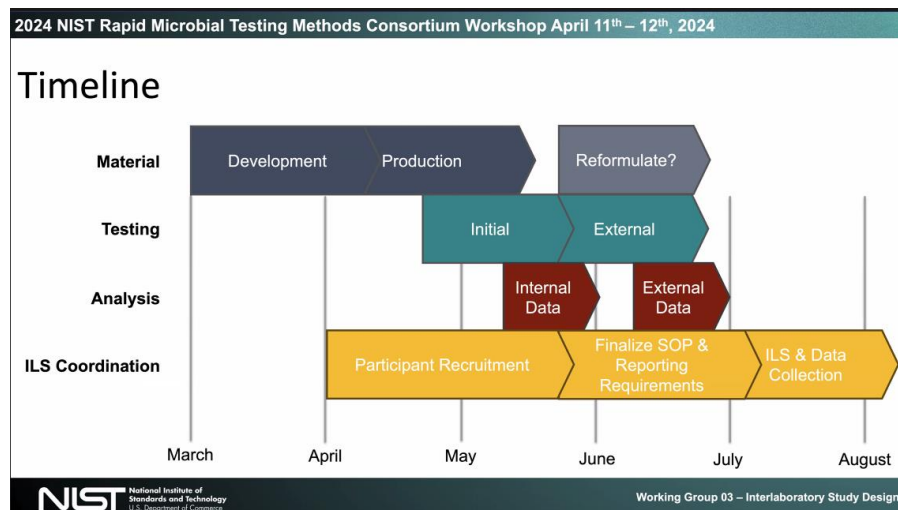


Fig. 2. Estimated timeline for the second ILS.

The goals of the second ILS are to:

- Develop and use a relevant set of test samples
- Survey the breadth of sterility testing methods currently in use
- Assess the comparability between compendial methods and RMTMs
- Demonstrate the fitness-for-purpose of RMTMs

When completed, the resulting dataset from this study will help to demonstrate capabilities, as well as any potential pitfalls, of RMTMs. The study design was developed with a focus on balancing the capabilities of the participating laboratories with the need to gather meaningful data that would address the outlined goals. Much of the discussion was focused on sample matrix or background and whether a human cell background (typical of a cell therapy product) should be included in the test samples. As opposed to providing microbes in a sterile buffer, microbes provided with a human cell component would present a more real-world mimic and

better capture some of the challenges faced during microbial testing. By including a human cell component, study participants can evaluate the performance of assays where at least part of the signal may come from the human cells. For example, respiration and activity-based tests have shown that the microbial levels can be sufficient to distinguish contaminant signals from that of host cells, potentially eliminating the need for enrichment or filtration steps. In fact, for mycoplasma testing, the removal of host cells is not permitted as some mycoplasma species are cell-associated. Based on the discussion, a human cell background was preferred. With the remainder of the time, formulation and distribution of samples were discussed, including fresh versus frozen samples and microbial cells being provided separately or combined with human cells in a single sample. The discussion also touched on the selection of strains for inclusion in the study.

The data obtained during the session provided valuable input for the upcoming ILS. Next steps include optimizing the study protocol with WG3 members, as well as recruiting laboratories to participate. In parallel NIST will begin developing and evaluating potential materials to be used for the study.

3.2. New Study: Quantification of Total Cell Count for Compendial Organisms

WG1 is considering a new study to demonstrate a potential approach based on the roadmap to quantify total cell count and assess method suitability for characterizing commercially available reference materials using USP <71> compendial microorganisms. Cell count methods can be influenced by various factors, including matrix and cell characteristics. To address these challenges, WG1 proposed a collaborative approach, encouraging members to work together to evaluate potential methods and design experiments to answer key questions.

3.3. Developing Validation Strategies for RMTMs

The RMTM Consortium is considering developing a document that describes validation strategies to support stakeholders seeking to demonstrate and implement RMTMs in the current Good Manufacturing Practice (cGMP) biomanufacturing environment, with a focus on making the process less intimidating. A hypothetical validation study was used as an example, and the group provided feedback on the design and calculation of analytical performance. A potential goal would be to create a one-page guide or "cheat sheet" to help users navigate validation studies. The discussion centered on various topics related to validation, including what to sample, reference materials, experimental design, statistical analysis, and assessing analytical performance.

The overall goal of a validation strategy is to demonstrate the analytical performance of a method in the proper context. There is no single "right way" to validate a method, as it depends on the specific context. A validation study should employ an experimental design that assesses the analytical performance of a method in terms of sensitivity, specificity, precision, robustness, recall, and other relevant parameters.

The discussion highlighted the importance of balancing ruggedness and robustness in validation studies. Ruggedness refers to the ability of a method to withstand operator variability or lot-to-

lot variability of test articles, while robustness refers to the ability of a method to withstand subtle changes that could affect its performance. The Consortium emphasized that a validation study should be designed to assess both ruggedness and robustness.

Several key considerations for validation were discussed, including:

- The impact of lot-to-lot variability of the matrix itself on the validation study
- The need to account for potential interference and variability in cell therapy products
- The importance of determining the source of contamination in the event of a positive result
- The design of replicates and the control of variables in the validation study
- The use of true positives and true negatives to compare the performance of different methods

The discussion also highlighted some of the challenges associated with validation, including the need to demonstrate that a method is not prone to losing low-level contamination during the cleanup or separation process. To address these challenges, the Consortium suggested that a validation strategy should be developed in consultation with a statistician to ensure that the experimental design is properly guided.

The discussion referenced several resources, including PDA Report #33² and a paper published by BioPhorum³, that provide guidance on validation strategies. The next steps will involve reassessing the validation approach to determine if Consortium members are interested in contributing to a validation guide.

3.4. Day 2 Summary

The second day of the workshop for Consortium members centered on advancing ongoing initiatives and potential activities of the RMTM Consortium. The discussions covered three key areas: a second ILS involving a relevant sample and multiple RMTMs, the systematic characterization of total cell count for available microbial cell reference materials, and the potential development of a tool to describe validation strategies. These sessions provided valuable feedback on the planning and development of these initiatives, helping the Consortium decide activities to prioritize in the future.

² PDA Technical Report No. 33, Revised 2013 (TR 33) Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods.

³ BioPhorum, 2020, A framework for the evaluation, validation and implementation of alternative and rapid microbiological testing methods.

4. Workshop Summary and Future Consortium Directions

The two-day workshop was a significant step forward for the RMTM Consortium, providing a platform for discussion, feedback, and guidance on the Consortium's current and future efforts in developing measurement focused approaches and tools to facilitate the adoption of RMTMs for sterility testing in advanced therapies. The workshop achieved its goal of advancing multiple projects and moving the Consortium activities forward.

Future Directions

In particular, the workshop enabled progress on multiple Consortium activities. WG1 focused on approaches to enable certification of microbial cell reference materials beyond CFU for relevance to molecular RMTMs. The sessions highlighted the characterization of total cell count for available materials, with a discussion on the needs of stakeholders for quantification of cell counts. These efforts aim to provide strategies useful for reference material manufacturers to develop their own protocol to quantify values on their specific materials. WG2 sessions focused on ongoing development of databases and analytical tools to support NGS-based RMTMs, aiming to support a future NGS-centered interlaboratory study. WG3 discussed the design of an interlaboratory study with the inclusion of multiple RMTM technologies and a common sample representing a contaminated cell therapy product to produce a dataset spanning RMTM technologies across multiple laboratories. These Consortium activities will provide key deliverables for the stakeholder community to demonstrate and advance the use of RMTMs for sterility testing.

Future of the Consortium

In the closed session, Consortium members also discussed the future of the NIST RMTM Consortium, as the five-year duration of the Consortium ends in Fall 2025. Possible options included renewing the Consortium with all three working groups, reducing the work scope for a more focused Consortium, transitioning to a paid consortium model, or wrapping up the RMTM Consortium in 2025. The discussion also touched on potential outside funding and collaboration opportunities. After additional discussions, it was decided to renew the RMTM Consortium for another five years to complete ongoing activities and support the RMTM community.

Appendix A. Workshop Agenda

Day 1: Thursday, April 11, 2024 (Sessions OPEN to all registrants, times listed in ET)

Time (ET, Duration)	Session
9:45 am - 10:00 am	Welcome Remarks and Workshop Overview
10:00 am - 12:00 pm	Next Generation Sequencing (NGS)-Based Sterility Testing <ul style="list-style-type: none"> Discuss the development of NGS-based analysis tools for microbial sterility testing in the biomanufacturing environment. Includes the development of custom reference genome/gene databases, bioinformatic pipelines, and standardized reporting formats.
12:00 pm - 1:00 pm	Lunch
1:00 pm - 2:00 pm	NIST RMTM Consortium Update <ul style="list-style-type: none"> Overview of the NIST RMTM Consortium and updates on Working Group activities.
2:00 pm - 2:15 pm	Break
2:15 pm - 3:30 pm	Developments in RMTM Technology This session will focus on new technologies and adoption of non-compendial methods for Microbial Testing <ul style="list-style-type: none"> Development of a Next Generation Sequencing Workflow for Rapid Microbial Testing Neeraj Salathia (Resilience) CalScreener+ for rapid phenotypic sterility test method for direct inoculation of ATMPs Wilhelm Paulander (Symcel) Commercial Cell Therapy Experience implementing Rapid Microbial Testing Methods John Duguid (Vericel Corporation)
3:30 pm - 3:45 pm	Break
3:45 pm - 4:50 pm	Roadmap for Microbial Cell Reference Material Characterization (Nancy Lin, Kirsten Parratt) <ul style="list-style-type: none"> Presentation: Overview of a draft roadmap to optimize methods for total cell count and total genome count of microbial cell reference materials. Discussion: <ul style="list-style-type: none"> Gather input to refine technical considerations in the roadmap. What level of agreement is needed between methods? How could this roadmap be transferred to manufacturers? What portions are infeasible?
4:50 pm - 5:00 pm	Day 1 Wrap-up
5:00 pm	Adjourn

Day 2: Friday April 12, 2024 (Sessions are for Consortium members only, times listed in ET)

Time (ET, Duration)	Session
9:00 am - 10:30 am	RMTM Interlaboratory Study Discussion (closed) <ul style="list-style-type: none"> • Interlaboratory Studies Working Group will present the plan (including draft experimental protocol and timeline) for the second RMTM interlaboratory study followed by open discussion and revisions.
10:30 am - 10:45 am	Break
10:45 am - 12:15 pm	New Study: Quantification of Total Cell Count for Compendial Organisms (closed) (Monique Hunter, Nancy Lin, Kirsten Parratt) <ul style="list-style-type: none"> • Presentation: Preliminary results on multi-method quantification of compendial organisms. • Discussion: Technical plan for quantifying total cell count in USP <71> compendial microorganisms. <ul style="list-style-type: none"> • Application of roadmap, technical plan design. • Identification of appropriate strains, potential inclusion of commercial materials, and selection of measurement methods.
12:15 pm - 1:15 pm	Lunch
1:15 pm - 3:15 pm	Validation Strategies Workflow (closed) <ul style="list-style-type: none"> • Discussions on how to develop validation strategies for adopting new RMTMs in the current Good Manufacturing Practice (cGMP) biomanufacturing environment. Includes topics related to: what to sample, reference materials, experimental design, statistical analysis, and assessing analytical performance.
3:15 pm - 4:00 pm	Workshop Summary, Open Dialogue, and Next Steps
4:00 pm	Adjourn