Check for updates

NIST Special Publication 1500
NIST SP 1500-36

NIST 2025 Rapid Microbial Testing Methods Workshop Report

Jason G. Kralj
Kirsten H. Parratt
Tyler S. Laird
Stephanie L. Servetas
Nancy J. Lin
Dawn Henke
Scott A. Jackson

This publication is available free of charge from:
https://doi.org/10.6028/NIST.SP.1500-36

NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

NIST Special Publication 1500
NIST SP 1500-36

NIST 2025 Rapid Microbial Testing Methods Workshop Report

Jason G. Kralj
Kirsten H. Parratt
Tyler S. Laird
Stephanie L. Servetas
Nancy J. Lin
Biosystems and Biomaterials Division
Material Measurement Laboratory

Dawn Henke
Standards Coordinating Body

Scott A. Jackson*
**Former NIST employee; all work for this publication was done while at NIST.*

This publication is available free of charge from:
https://doi.org/10.6028/NIST.SP.1500-36

September 2025



U.S. Department of Commerce
Howard Lutnick, Secretary

National Institute of Standards and Technology
Craig Burkhardt, Acting NIST Director and Under Secretary of Commerce for Standards and Technology

NIST SP 1500-36
September 2025

Certain equipment, instruments, software, or materials, commercial or non-commercial, are identified in this paper in order to specify the experimental procedure adequately. Such identification does not imply recommendation or endorsement of any product or service by NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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

NIST Technical Series Policies
Copyright, Use, and Licensing Statements
NIST Technical Series Publication Identifier Syntax

Publication History
Approved by the NIST Editorial Review Board on 2025-09-23

How to Cite this NIST Technical Series Publication
Kralj JG, Parratt KH, Laird TS, Servatas SL, Henke D, Jackson SA, Lin NJ (2025) NIST 2025 Rapid Microbial Testing Methods Workshop Report. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Special Publication (SP) NIST SP 1500-36. https://doi.org/10.6028/NIST.SP.1500-36

Author ORCID iDs
Jason G. Kralj: 0000-0003-4565-1176
Kirsten H. Parratt: 0000-0002-2640-3276
Tyler S. Laird: 0000-0003-2317-2895
Stephanie L. Servetas: 0000-0002-4924-4511
Nancy J. Lin: 0000-0002-6980-3288
Dawn Henke: 0009-0006-6317-1765
Scott A. Jackson: 0000-0003-4891-8323

Contact Information
nancy.lin@nist.gov
100 Bureau Dr. MS 8543
Gaithersburg, MD 20899

Abstract

In 2020, the National Institute of Standards and Technology (NIST)-led Rapid Microbial Testing Methods (RMTM) Consortium was established to develop standards and measurement-based solutions to facilitate the adoption of RMTMs in advanced therapy products. To update the community on the Consortium's progress and work on its deliverables, NIST hosted its 5th annual workshop on RMTMs. This report summarizes the key findings from the workshop, which was held as a single-day virtual event featuring external speakers. The workshop brought together a diverse group of stakeholders, including advanced therapy producers, developers of RMTM assays and instruments, and producers of cell reference materials.

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.

Table of Contents

1. Consortium and Workshop Overview 1

1.1. NIST Rapid Microbial Testing Methods Consortium 1

1.2. Workshop Overview 1

2. Technical Advances in Rapid Sterility Testing 3

2.1. Application of Nanopore Long-read Sequencing to Sterility Testing for Cell Therapy Products 3

2.2. Detection of Pathogens in Complex Matrices via NomadX Platforms 3

3. NIST RMTM Consortium Working Group 1 (WG1) – Reference Materials: Toward Microbial Cell Reference Materials Characterized beyond CFU 4

4. NIST RMTM Consortium Working Group 3 (WG3)- Interlaboratory Studies: Microbial Contaminant Detection Across Rapid Sterility Testing Methods - Preliminary Interlaboratory Study Findings 5

5. NIST RMTM Consortium Working Group 2 (WG2) - Methods: Tools to Support Next Generation Sequencing as a Rapid Sterility Method 6

6. Workshop Summary and Future Consortium Directions 7

7. References 8

Appendix A. Comparison of 2020 and 2025 Survey Responses 9

List of Tables

Table 1. 2025 RMTM Workshop Agenda 2

List of Figures

Fig. 1. Demographics of workshop registrants 1

Acknowledgments

This manuscript was edited with the assistance of Llama-4-Maverick-17B128E-Instruct-FP8 (Llama), developed by Meta. Llama was used to refine language, improve clarity, and enhance readability in accordance with the authors' instructions. All content, scientific claims, and conclusions have been reviewed and verified by the authors to ensure accuracy and originality.

Author Contributions

Kralj JG: Conceptualization, Writing—original draft, Writing—review & editing. **Parratt KH**: Conceptualization, Writing—original draft. **Laird TS**: Conceptualization, Visualization. **Servetas SL**: Conceptualization, Visualization, Supervision. **Lin NJ**: Conceptualization, Visualization, Supervision. **Henke D**: Conceptualization, Visualization, Writing—original draft. **Jackson SA**: Conceptualization, Visualization, Supervision.

1. Consortium and Workshop Overview

1.1. NIST Rapid Microbial Testing Methods Consortium

The National Institute of Standards and Technology (NIST) Rapid Microbial Testing Methods (RMTM) Consortium was established in 2020 to unite experts from across the regenerative medicine field, including stakeholders from industry, academia, and government to address the need for measurement standards for rapid microbial testing of regenerative medicine and advanced therapy products.

1.2. Workshop Overview

On April 8, 2025, NIST hosted its 5th annual workshop on RMTMs. The goal of this open, half-day virtual workshop was to discuss recent technological advances in the field of rapid sterility testing for advanced therapy products and to provide an update on the NIST RMTM Consortium activities.

The workshop drew a diverse group of 308 registrants, with the largest group (36 %) coming from industry (Fig. 1). Other registrants represented academia, non-profits, federal and non-federal government agencies, and contract test laboratories. The event also attracted participants from industries outside of regenerative medicine, such as food and cosmetics, highlighting the broader relevance of RMTMs.

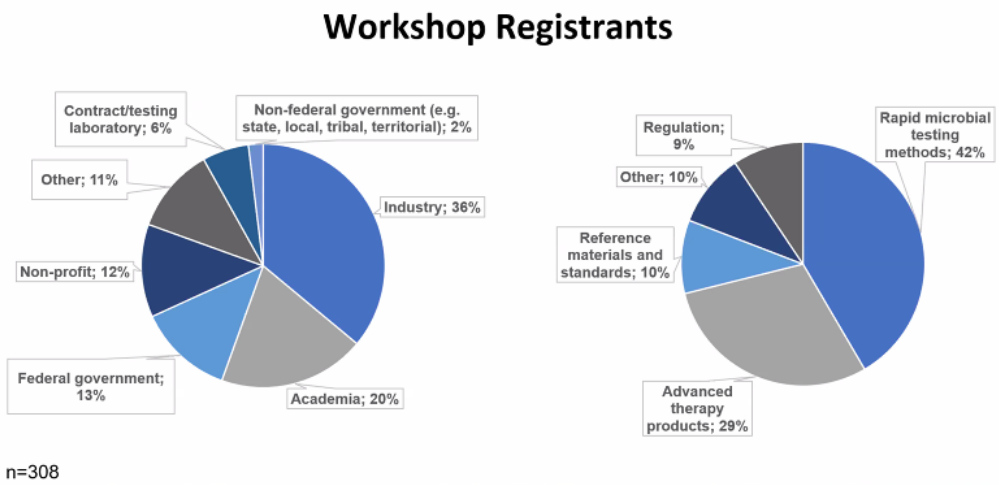


Fig. 1. Demographics of workshop registrants

Table 1 provides the workshop agenda, and the workshop website[1] includes additional details on the agenda and links to the presentation slides.

[1] https://www.nist.gov/news-events/events/2025/04/2025-nist-workshop-rapid-microbial-testing-methods

Table 1. 2025 RMTM Workshop Agenda

April 8, 2025	
11:00 AM – 11:15 AM	Welcome, Consortium Overview, and Workshop Introduction *Nancy Lin (NIST)*
11:15 AM – 12:15 PM	Technical Advances in Rapid Sterility Testing *Multiple Speakers*
11:15 AM – 11:45 AM	Application of nanopore long-read sequencing to sterility testing for cell therapy products *James Strutt (Singapore-MIT Alliance for Research & Technology)*
11:45 AM – 12:15 PM	Detection of Pathogens in Complex Matrices via NomadX Platforms *Heidi Leonard (NomadX)*
LUNCH (on your own) **12:15 PM – 1:15 PM**	
1:15 PM – 1:45 PM	NIST RMTM Consortium Working Group 1 - Reference Materials: Toward Microbial Cell Reference Materials Characterized beyond CFU *Nancy Lin (NIST)/ Kirsten Parratt (NIST)*
1:45 PM – 2:45 PM	NIST RMTM Consortium Working Group 3 - Interlaboratory Studies: Microbial Contaminant Detection Across Rapid Sterility Testing Methods - Preliminary Interlaboratory Study Findings *Jason Kralj (NIST)/ Stephanie Servetas (NIST)*
Break **2:45 PM – 3:00 PM**	
3:00 PM – 4:00 PM	NIST RMTM Consortium Working Group 2 - Methods: Tools to Support Next Generation Sequencing as a Rapid Sterility Method *Scott Jackson (NIST) /Tyler Laird (NIST)*
4:00 PM – 4:30 PM	Closing Discussion *Scott Jackson (NIST)*

2. Technical Advances in Rapid Sterility Testing

2.1. Application of Nanopore Long-read Sequencing to Sterility Testing for Cell Therapy Products

James Strutt of the Singapore-MIT Alliance for Research & Technology (SMART) presented on the application of Nanopore sequencing for untargeted microbial detection, including preliminary insights into the baseline characterization of the "kitome" [1].

The need for sterility testing is widespread in the advanced therapy industry, as producers seek to detect adventitious agents that can be introduced during leukophoresis or manufacturing. Long-read sequencing technology is well-suited for this purpose, offering real-time, agnostic detection with a limit of detection that meets regulatory requirements. Compared to short-read sequencing, long-read sequencing provides easier genome assembly, more complete coverage, and detection of structural variants, resulting in rapid time-to-results with relatively small sample volumes.

To improve the limit of detection, Dr. Strutt's team targeted full-length 16S and 18S amplicon sequencing of ribosomal RNA, which enables species-level identification. Ribosomal RNA is an ideal target for long-read sequencing due to its high copy number in most genomes. Shotgun sequencing, on the other hand, has a limited detection threshold of approximately 1000 CFU.

Dr. Strutt developed a workflow to handle low-biomass samples, minimizing the impact of contaminant DNA and facilitating the detection of the actual sample. The results demonstrated that this workflow can detect aerobic and anaerobic species at 10 CFU (n=31/31) within 16 hours using less than 1 mL of sample. A machine learning model was trained to identify the kitome, filter human reads, and generate unbiased identification of contaminated samples. The recommendations and best practices derived from the development of this workflow are summarized in Dr. Strutt's presentation online.

2.2. Detection of Pathogens in Complex Matrices via NomadX Platforms

Heidi Leonard from NomadX presented on the application of Raman spectroscopy for detecting pathogens in complex matrices using rapid sample concentration and enrichment. The Concentrating Pipette, a novel product, enables efficient concentration of samples with adjustable volume and a user-friendly interface. Following concentration, surface-enhanced Raman spectroscopy (SERS) is employed to detect and identify contaminants. SERS relies on the enhancement of Raman signals from molecules adsorbed onto a nanostructured metallic surface, facilitated by plasmon resonance. NomadX's current technology utilizes electroplated silver dendrites as the SERS substrate, which is a crucial component of their workflow. The target time to detection is approximately 1 hour starting from a concentrated sample. The resulting spectra generate a unique "fingerprint" for each pathogen, enabling label-free detection using artificial intelligence and machine learning (AI/ML) algorithms. While this method shows promise, challenges remain, including managing background matrix interference, optimizing dendrite morphology for specific bacterial targets, ensuring substrate shelf life, and scaling up the process. Notably, the workflow has achieved recovery rates of up to 80 %.

3. NIST RMTM Consortium Working Group 1 (WG1) – Reference Materials: Toward Microbial Cell Reference Materials Characterized beyond CFU

Nancy Lin and Kirsten Parratt from NIST led a session discussing WG1's efforts to support the expanded certification of existing reference materials to help bridge the gap between traditional compendial sterility testing methods and new rapid methods, such as molecular techniques. Currently, most microbial cell reference materials are certified for colony-forming units (CFU), which may not accurately represent properties more relevant to molecular methods, such as total genome copy number or total cell count. Methods are needed to quantify total cells and genome copies across a range of microbial cell commercial reference materials, especially those based on compendial microorganisms listed in USP <71>. These methods would enable reference material producers to certify additional values on their reference materials to support testing and validation of molecular RMTMs.

NIST feasibility studies have evaluated characterization methods for cell count and genome copy number across multiple commercial reference materials, with results indicating that some methods are more susceptible to matrix or background interference than others. Key findings and recommendations toward enabling additional quantification of existing reference materials include:

- Cell concentration should be sufficiently high and will depend on the characterization methods used as well as the microbial strain and formulation being characterized.
- Several total-cell-count methods show promise for suitability for most microbial cell reference materials.
- Genome copy measurements remain challenging due to issues such as debris/matrix interference, DNA staining, RNA interference, and extraction protocols.

Given the complexity of characterizing additional properties for microbial cell reference materials, a unified experimental protocol that works across all materials may not be feasible. Instead, a roadmap to optimize methods for each material of interest was proposed, including workflows with decision points and optimization criteria. This roadmap could be implemented by reference material manufacturers to enable additional certified values for their microbial cell products.

Taken together, NIST feasibility studies and the proposed roadmap have demonstrated a potential route to characterize microbial cell RMs beyond CFU values, supporting the validation and comparability testing of molecular sterility testing methods. A perspective manuscript and a technical paper describing results of the NIST feasibility studies are under development.

4. NIST RMTM Consortium Working Group 3 (WG3)- Interlaboratory Studies: Microbial Contaminant Detection Across Rapid Sterility Testing Methods - Preliminary Interlaboratory Study Findings

Jason Kralj and Stephanie Servetas from NIST presented on the accomplishments of the Consortium's Interlaboratory Studies Working Group, which has focused on collecting data on RMTMs via interlaboratory studies.

Interlaboratory Study 1

The first study aimed to determine whether off-the-shelf materials were suitable for qualifying or validating RMTMs. This proof-of-concept test had nine participating laboratories who all use the same materials: two commercial *E. coli* materials, commercial qPCR and extraction kits, and a NIST-provided DNA standard to generate a calibration curve for reporting genome copies. The study found that the *E. coli* materials were fit-for-purpose as qPCR controls, with a relative range of consensus consistent with CFU.

Interlaboratory Study 2

The second interlaboratory study surveyed existing and emerging sterility testing methods using a common sample set designed to mimic CAR-T product aliquots contaminated with microorganisms. The study generated a dataset that could be used to compare the features of each method against specific application needs. The sample set contained 0.9 % saline with 3 % human albumin, spiked with either 0, 10, or 100 CFU of live compendial microorganisms.

The study included 11 laboratories, 8 different methods, and 16 total datasets. The methods tested included BacT/ALERT, Bactec, Celsis, microcalorimetry, USP <71>, RNAseq, qPCR, and monocyte activity. The results demonstrated the suitability of the NIST-generated sample set for testing viability, with many viability-based methods performed comparably to USP <71>, with similar or faster times to result. Several methods also included an organism identification. RNAseq performed well among the molecular methods, with a good time-to-result. However, qPCR and MAT methods were affected by background DNA and LPS contamination, potentially due to the albumin used in the matrix. The working group is currently reviewing the results, examining reagent contamination, and drafting a manuscript.

5. NIST RMTM Consortium Working Group 2 (WG2) - Methods: Tools to Support Next Generation Sequencing as a Rapid Sterility Method

Scott Jackson and Tyler Laird from NIST presented on WG2 efforts to develop tools to support NGS-based sterility testing methods for advanced therapy products.

A key achievement is the development of a reference genome database and bioinformatics pipeline, termed the ENSure (**E**zBiome **N**IST **S**terility) database and pipeline, led by Consortium member EzBiome and NIST. The database contains a curated list of sterility relevant taxa with a target of one representative genome per species. The database was tested *in silico* using simulated sequencing data and showed good performance in detecting and differentiating microbial species. The testing also highlighted the importance of host read removal and filtering to reduce false positives.

WG2 is planning an interlaboratory study to provide an open dataset on NGS-based workflows for sterility testing. The study aims to address several outstanding topics, including:

- Lowering the barrier to entry for the technology
- Familiarization with the methods
- Assessing performance (i.e., sensitivity, specificity)
- Assessing variability across labs
- Demonstrating the ENSure database's utility
- Demonstrating the utility of spike-in controls for data normalization

Two approaches are being considered for the interlaboratory study: (a) testing the same method across labs to assess its reproducibility and performance, and (b) a survey testing different methods to assess the impact of methodological variables. Both approaches will use a common sample set and include spike-ins as reference controls.

The working group is also exploring the development of methods for microbial signal enrichment, including amplicon-based sequencing approaches. Target enrichment and host-depletion methods are considered essential for any method to be successful. Further development of methodologies is needed, including the selection of specific samples to be analyzed.

During the discussion, there was some support for employing and testing a prescribed workflow with the ENSure database. However, some potential participants have already developed their own workflows and custom databases. There was broad agreement on the need for target enrichment and host-depletion methods, and further development of methodologies is planned. Study planning will continue in the WG2 meetings.

6. Workshop Summary and Future Consortium Directions

The 2025 NIST RMTM Workshop was held to highlight recent advances in sterility testing technology and share the progress of the NIST RMTM Consortium. With over 300 registrants and over 120 active participants, the workshop showcased future directions of novel RMTM technologies, including long-read sequencing and Raman Spectroscopy-based methods, which are being developed to address challenges in analysis time, application-specific needs, and microbial identification.

The Consortium has made significant progress, with multiple publications, three interlaboratory studies, and a reference genome database and bioinformatic pipelines in development across the three working groups. In addition, a new interlaboratory study (#3) has been proposed to survey NGS-based sterility testing and identify best practices. NGS-based sterility testing has shown promise but also faces challenges, including the need for appropriate signal enrichment (or host depletion).

The workshop also shared the results of a survey (re)taken five years apart, first in 2020 and again in 2025. Comparison of the results (with the caveat that different groups of people took the two surveys) show that the need for RMTMs remains prevalent, molecular methods are still the highest ranked in terms of being “hopeful to be adopted,” and there has been increased adoption (and interest in adopting) rapid methods. The perception of the necessary time to result for an RMTM has increased, reflecting a growing awareness of the capabilities of new technologies (e.g., results within 1 h or 3 h may be too lofty a goal, and 8 h to 48 h may be more realistic). In addition, the need for methods to detect viability has increased as well. Detailed results comparison is provided in Appendix A.

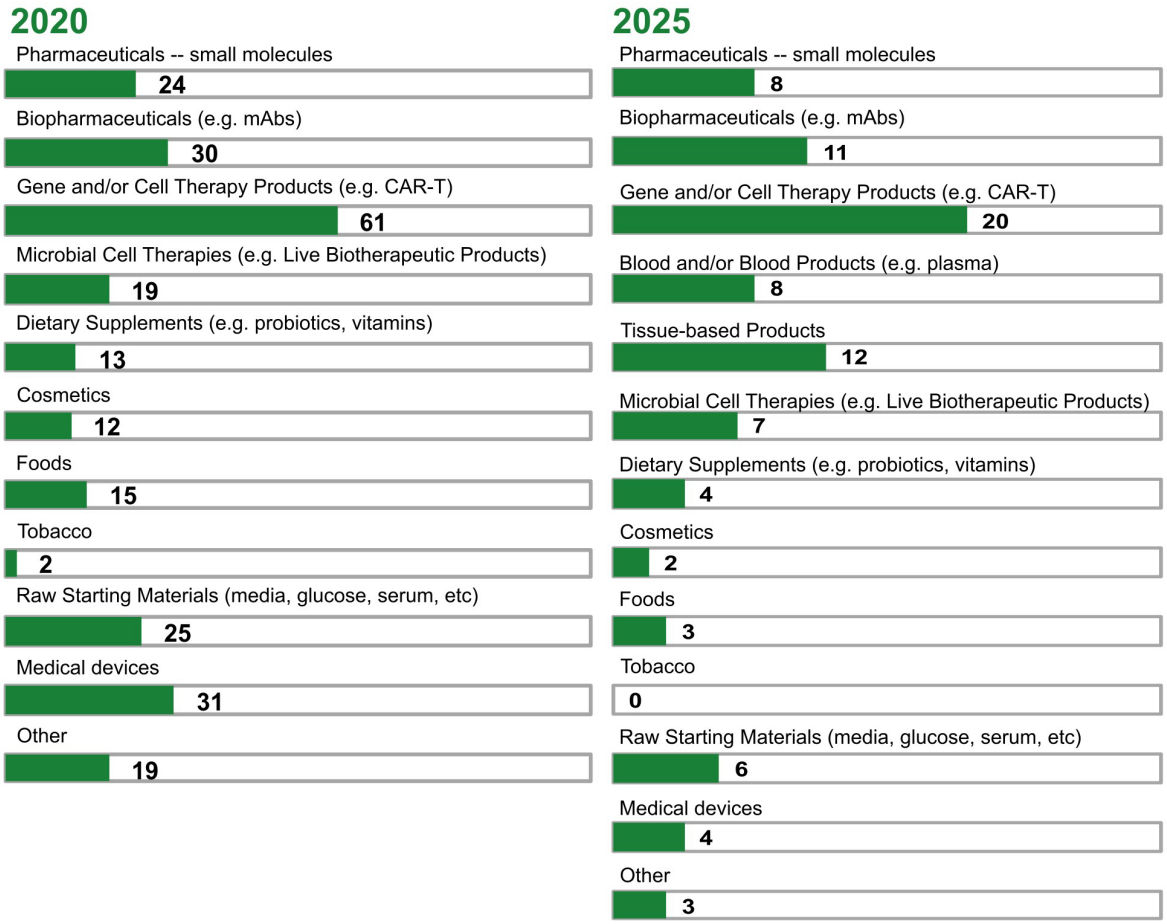
The participants discussed the future of the NIST RMTM Consortium, as the original 5-year collaborative agreement is ending in August 2025. The possibility of extending the CRADA for another year (or more) to complete ongoing work was considered, along with the potential for some efforts to shift to other stakeholder groups focused on RMTMs.

7. References

[1] Strutt JPB, Natarajan M, Lee E, Teo DBL, Sin W, Cheung K, Chew M, Thazin K, Barone PW, Wolfrum JM, Williams RBH, Rice SA, Springs SL (2023) Machine learning-based detection of adventitious microbes in T-cell therapy cultures using long-read sequencing. *Microbiol Spectr* 11(5):e01350-23. https://doi.org/10.1128/spectrum.01350-23

Appendix A. Comparison of 2020 and 2025 Survey Responses

2. What types of products do you test for microbial contamination? (multiple answers permitted)



3. What type of microbial testing do you currently perform? (multiple answers permitted)

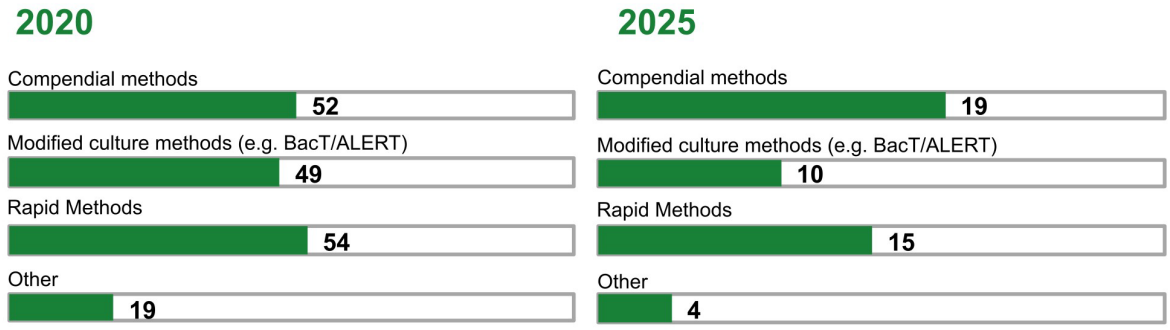
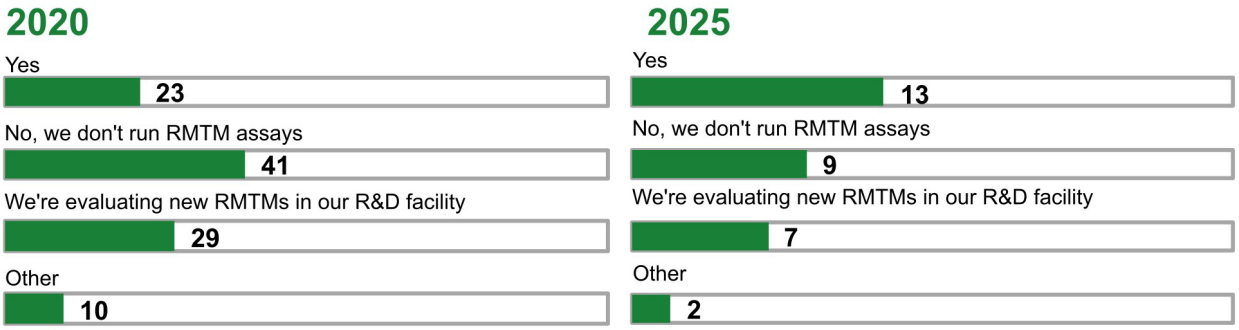
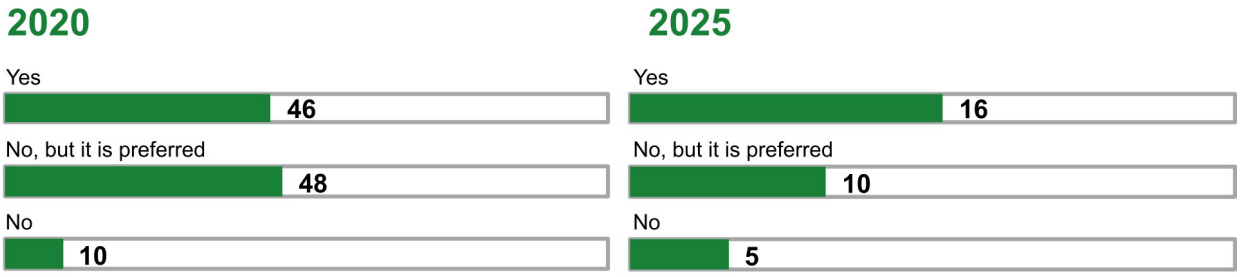


Fig. A-1. Select responses from a RMTM questionnaire in 2020 and 2025, questions 2 & 3. For question 2, we see that the types of products for testing have not changed significantly. Similarly for question 3, the testing methods being used have not changed significantly.

5. Do you currently have a validated RMTM assay that you're using?



6. Do microbial detection methods need to indicate viability for your purpose?



7. Do detection methods also need to provide taxonomic identification (e.g. *Salmonella enterica*) of microbial contaminants?

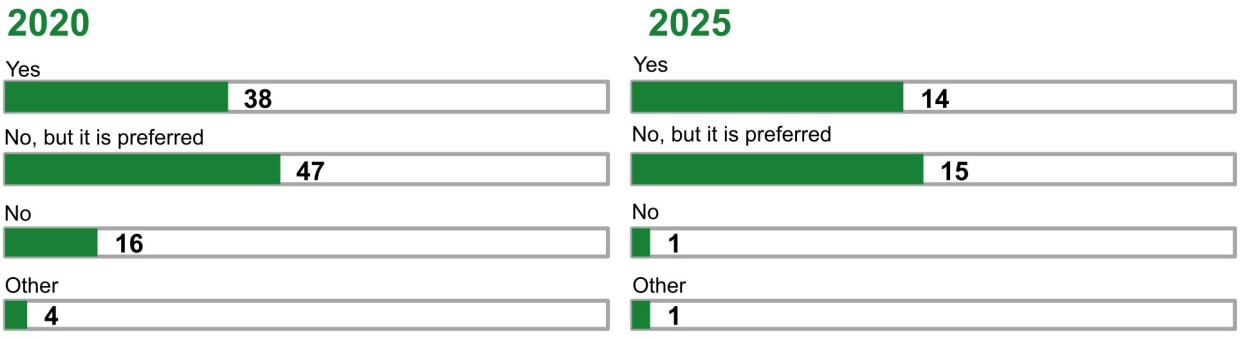
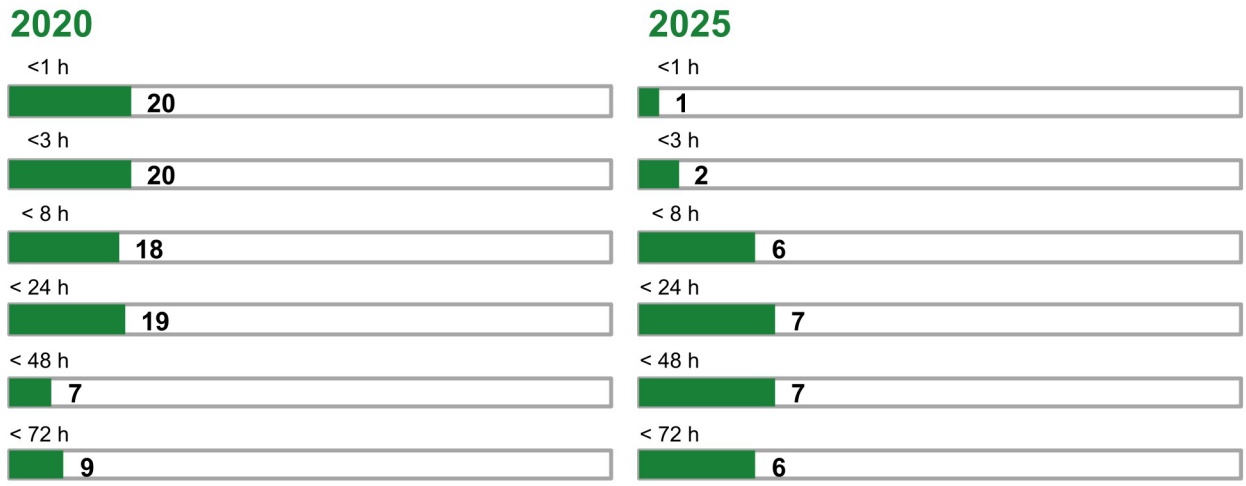


Fig. A-2. Select responses from a RMTM questionnaire in 2020 and 2025, questions 5 to 7. In question 5, we see that the fraction of respondents who have a validated RMTM assay has increased. Question 6 indicates a slight shift in favor of a need for viability-based detection. In question 7, respondents were divided on the need for taxonomic identification of contaminating microbes.

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



12. What rapid microbial measurement technologies are you most hopeful to be adopted in yoru industry (multiple answers permitted)

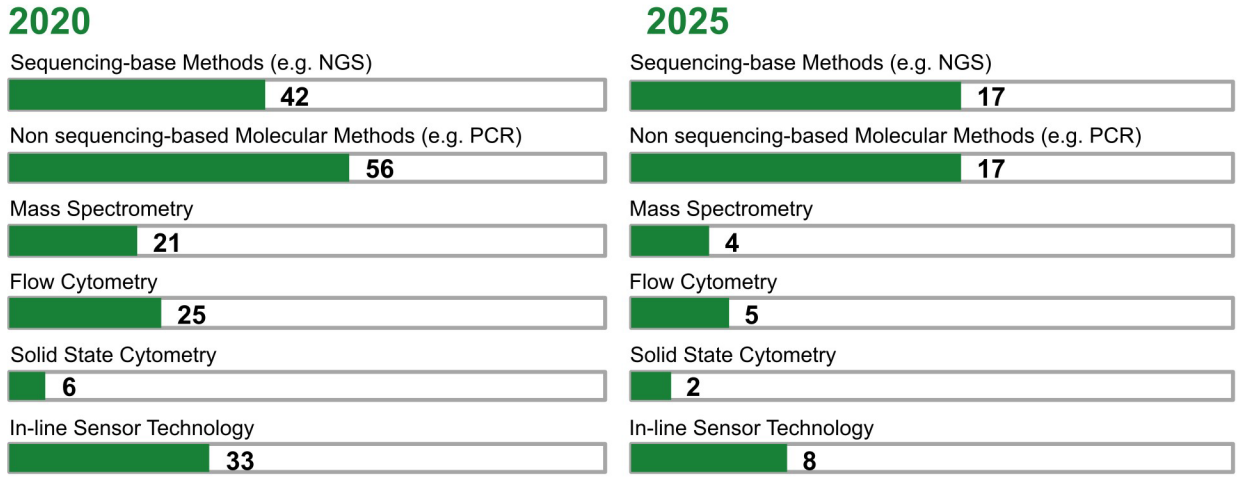


Fig. A-3 Select responses from a RMTM questionnaire in 2020 and 2025, questions 10 & 12. There was a clear shift in opinion regarding what "rapid" means in this context, from generally < 24 h in 2020 to < 3 d in 2025. This shift may indicate greater awareness of the latter respondents on product testing needs and acknowledgement of testing capabilities and limitations. The overall consensus remained hopeful that molecular methods (sequencing- and non-sequencing-based) would be adopted, with a strong minority looking for in-line sensing as well.