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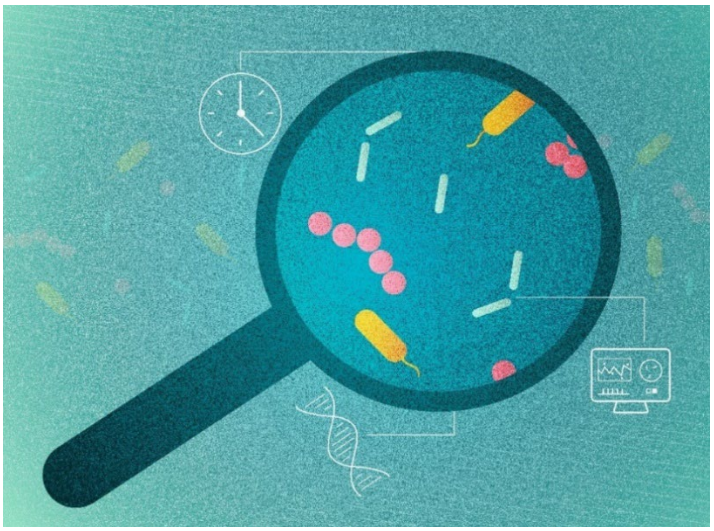
NIST SP 1272

Report from the NIST Workshop: Launch of the Rapid Microbial Testing Methods Consortium



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Abstract

On September 17, 2020, NIST hosted a virtual workshop to launch the Rapid Microbial Testing Methods (RMTM) Consortium. The RMTM Consortium aims to 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 purpose of the workshop was to publicize the launch of the Consortium, recruit new members, and obtain feedback from stakeholders on both the challenges with respect to applying RMTMs and potential solutions that the Consortium could provide. Over 250 attendees from industry, government, academia, and other organizations participated in the workshop. Invited speakers and panelists provided stimulus for discussion, and feedback from the stakeholders was obtained via question submission and polling. This report summarizes the presentations, discussions, and poll results from the workshop. Overall, the stakeholders supported the three proposed topic areas for the Consortium: reference materials, testing methods, and interlaboratory studies. Based on input from the workshop and poll, the proposed scope of the reference materials topic was expanded to consider DNA and other reference materials, in addition to whole cell materials. Likewise, the testing methods scope was increased to encompass validation schema as well as the test methodologies. As a next step, the Consortium began monthly meetings in November 2020, for members to discuss and finalize the scope and proposed working groups for the Consortium. It is expected that the Consortium will lead to measurement assurance solutions and improved approaches for the community to develop, validate, and implement RMTMs.

Key words

Advanced therapy medicinal products; cell and gene therapy; consortium; microbial contamination; microbial detection; rapid methods; standards; workshop report.

Workshop Organizing Committee

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Workshop Team

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Workshop Overview

Advanced therapies such as cell and gene therapies and tissue engineered products show great promise as clinical therapeutics. As such, they need to be free of microbial contamination for safe administration to patients. However, the current compendial methods to detect bacterial contamination take 14 days to detect contaminants. Many biological products, particularly cell therapy products, have a limited shelf-life. Moreover, the recipient patients are often critically ill and may not be able to wait an additional two weeks for the potentially life-saving treatment. Alternative rapid microbial testing methods (RMTMs) that can assess microbial contamination in a shortened time period are needed to reduce the time to results and improve product quality assurance and patient safety. While this need is evident, the path to routine, validated, high confidence RMTMs for risk-based assessment of advanced therapy products is less clear.

NIST established the RMTM Consortium per the timeline in Fig. 1 to help facilitate validation and adoption of RMTMs for regenerative medicine and advanced therapy products. The Consortium is designed to convene stakeholders in the pre-competitive space to develop measurement solutions and standards that increase confidence in RMTM results and strengthen the framework needed to support the validation and use of RMTMs.

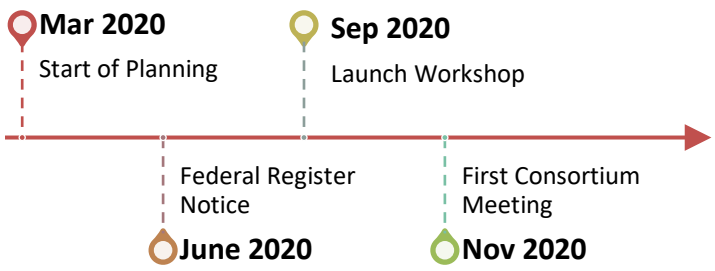


Fig. 1. Timeline of the NIST RMTM Consortium.

The purpose of the September 2020 public workshop was to publicize the launch of the RMTM Consortium, recruit members, disseminate Consortium goals and the proposed path forward, and inform potential Consortium directions via input from a broad range of stakeholders. As such, the workshop was free and open to all interested individuals.

Over 480 people registered, and over 250 attended the virtual workshop. The breakdown of registrants is provided in Fig. 2. While the majority of registrants were from industry, other sectors including academia, government, and non-profits were also represented. Registrants included individuals from Australia, Belgium, Canada, Colombia, France, Germany, India, Japan, Netherlands, Nigeria, Philippines, Switzerland, United Arab Emirates, United Kingdom, and the United States.

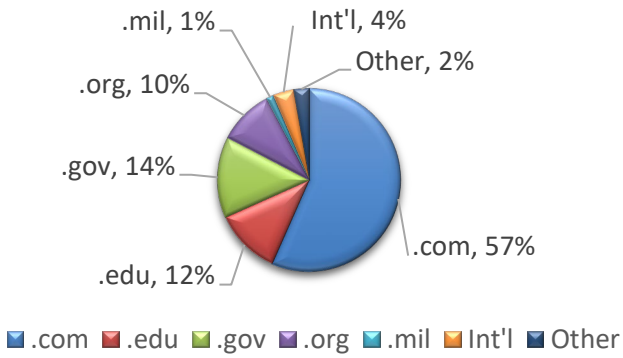


Fig. 2. Categories of workshop registrants.
Data is based on email addresses. Int'l = International (non-US).

As described in the agenda (Appendix A), the workshop consisted of a set of short introductory presentations made by NIST staff, two technical sessions with invited presentations and a panel discussion, and a final summary statement from NIST. For the technical sessions, Session 1 focused on the problem space: Challenges in Adopting RMTMs in the Cell and Gene Therapy Industry. Session 2 focused on the solution space: Ongoing Community Efforts and Potential Consortium Directions to Help Facilitate the Adoption of RMTMs. Speaker abstracts and bios are found in Appendix B.

The virtual format of the workshop allowed all audience questions to be easily archived. All questions submitted online during the workshop are listed in Appendix C. To obtain additional input, a pre-workshop poll was provided to all workshop registrants. Poll responses were discussed throughout the workshop and are provided in full in Appendix D.

The workshop contents are summarized in this report, and the full workshop recording is available online.[1]

Introductory Presentations

The workshop began with three presentations from NIST to introduce the Consortium, the NIST Advanced Therapy Program, and the NIST Microbial Metrology Program. The RMTM Consortium brings together expertise and experience from both programs to address the need for rapid microbial detection in advanced therapy products.

Workshop Introduction and Consortium Overview

Dr. Nancy J. Lin, Leader of the Biomaterials Group in the Biosystems and Biomaterials Division (BBD) of the Material Measurement Laboratory (MML) of NIST, opened the workshop with an introduction to the Consortium. She highlighted the benefits of a Consortium led by NIST, as NIST is a non-regulatory agency of the U.S. Department of

[1] https://www.nist.gov/news-events/events/2020/09/nist-workshop-launch-rapid-microbial-testing-methods-Consortium

Commerce, and a neutral convener with cross-disciplinary expertise in engineering and the physical, information, chemical, and biological sciences. Addressing the challenges faced when validating and implementing RMTMs requires a coordinated response with significant input from stakeholders. A NIST-led consortium lessens the risks being placed on any single entity, helps develop consensus, and leverages subject matter expertise from the community. Dr. Lin also described the goal and anticipated impact of the Consortium and outlined its three proposed focus areas:

- establish a repository of relevant microorganisms to use for interlaboratory studies and reference material development,
- develop an inventory of potential RMTM methods and protocols, and
- design and run interlaboratory studies to support the development of best practices and standard methods.

She then outlined the goals for the workshop, as described earlier. She also indicated that the work of the RMTM Consortium will likely have impact beyond advanced therapy products, as much of it is expected to be translatable to other fields in need of RMTMs, including microbial cell therapy (live biotherapeutic products), biothreat detection and biosurveillance, food/water safety, and environmental monitoring.

Overview of NIST Advanced Therapy Program

Dr. Sheng Lin-Gibson, Chief of the BBD in MML/NIST, described the NIST Regenerative Medicine and Advanced Therapy Program. This growing industry is represented by a diverse set of products (e.g., cell therapy, gene therapy, and tissue engineered products) that have shown promising clinical efficacy and are changing the paradigm for treating diseases and injuries. Clinical translation and patient access to this broad class of therapeutics requires better defined and characterized products and more robust, reliable, and cost-effective manufacturing processes. The NIST laboratory program supports the industry by addressing manufacturing, characterization, and testing challenges. Specially, NIST is developing advanced measurement capabilities, measurement assurance strategies, and supporting tools such as a suite of advanced biological and “living” reference materials to provide needed confidence for critical decision making regarding these complex biological products. To achieve these goals, NIST works extensively with key stakeholders, regulatory agencies, and international partners to identify industry-wide challenges and pre-competitive solutions.

Overview of NIST Microbial Metrology Program

Dr. Scott Jackson, Leader of the Microbial Metrology Group, BBD/MML/NIST, introduced the NIST Microbial Metrology Program. This program focuses on the development of measurement assurance strategies related to microbiomes, biofilms, and pathogen detection. Stakeholders include the burgeoning microbiome therapeutics and diagnostics industries, the biothreat detection community, the environmental biosurveillance community, and the infectious disease diagnostics community. NIST employs and advances a suite of technologies that include next generation sequencing (NGS)-based genomics and metagenomics, flow cytometry, nuclear magnetic resonance (NMR) and mass spectrometry-based metabolomics, digital polymerase chain reaction (PCR), particle enumeration methods,

and microfluidic device manufacturing. Major efforts currently include development of standards and measurement assurance strategies for cell enumeration and cell viability, nucleic acid-based detection and identification, and microbial metabolomics. These efforts facilitate the ability to characterize single species and multispecies microbial whole cell reference materials. In addition, NIST is developing a human gut microbiome (human fecal) reference material characterized for multi-omics measurements. This same suite of human fecal material is being evaluated as a potential tool to support detection of SARS-CoV-2 in wastewater and fecal material.

Session 1: The Problem Space

Overview

Session 1 focused on challenges in adopting RMTMs in the advanced therapy industry via two invited presentations and a panel discussion. The session highlighted the unique needs of industrial stakeholders for rapid sterility testing, the regulatory landscape associated with validation and adoption of new RMTMs, and fit-for-purpose RMTMs in terms of attributes related to analytical performance, cost, time, throughput, ease-of-use, etc.

Presentations

Key Characteristics of Rapid Microbial Test Methods for Cell and Gene Therapies
Spencer Hoover, Ph.D, Independent Consultant, former Director at the Centre for the Commercialization of Regenerative Medicine (CCRM)
Dr. Hoover discussed the unique challenges associated with assessing microbial contamination in cell and gene therapy products using compendial methods, including short product shelf-life, the need for timely product infusion into the patient, product reproducibility, the lack of terminal sterile filtration for cell products, detection of microbes within a mammalian cell background, limited batch size, cost, and variable manufacturing processes. Focusing on the manufacturing processes, he then discussed potential sources of microbial contamination and approaches to decrease the associated risk. Main sources to consider include people, process, materials, equipment, facility, and utilities. While not all risk can be mitigated by a single test, he explained how fit-for-purpose testing can be implemented at multiple steps to reduce overall risk and walked through an example Chimeric Antigen Receptor T-Cell (CAR-T) Therapy manufacturing process. Dr. Hoover also emphasized the importance of pre-planning the response to a positive test during manufacturing and the need for RMTMs with a high Technology Readiness Level (TRL) or technology maturity. Lastly, he suggested that other industries (radionuclides, biologics manufacturing, food and water safety, diagnostics, and platelets) face similar challenges and may be able to offer potential solutions. Questions were raised regarding the need for high throughput molecular testing and potential solutions from the pharmaceutical industry.

FDA Requirements and Recommendations for Sterility Testing of Regenerative Medicine Therapies

Judith Arcidiacono, M.S., International Regulatory Expert and Standards Liaison, at the U.S. Food and Drug Administration (FDA)

Ms. Arcidiacono discussed the FDA's strong interest in the development of more standards for the field of sterility testing to enable better product labeling, faster review, and clinical data exchange. She named additional potential sources of contamination not mentioned by the first speaker including incomplete skin disinfection, errors, cell lines, and cross contamination in multi-product manufacturing facilities. Currently 21 CFR 610.12 describes FDA biologics sterility testing requirements and emphasizes the importance of evaluating the viability of microbial contaminants. Several points from the first talk were reaffirmed, including the dependence of the verification approach on the testing method used, and the need for manufacturers to have a plan to follow up on positive test results. It is possible that an applicable standard might not be specific to regenerative medicine and could be borrowed from another field. Additional documents that may be useful include (1) the 2008 guidance for Chemistry, Manufacturing, and Control (CMC) information for human somatic cell therapy[2] and (2) International Conference on Harmonization (ICH) and FDA documents related to adventitious agent testing[3]. Questions in this section related to mycoplasma PCR testing, sterility testing in the context of product stability, the 2020 guidance on CMC Information for Human Gene Therapy Investigational New Drug Applications[4], and NGS-based adventitious agent testing.

Panel Discussion

This panel consisted of Dr. Spencer Hoover (Independent Consultant), Dr. Tom Leach (Associate Director, Drug Product Process Engineering and Packaging, AstraZeneca), and Dr. Claudia Zylberberg (CEO, Akron Biotech). See Appendix B for panelist bios. As part of the panel discussion, results from the workshop attendee poll were shared. The full poll results are provided in Appendix D. The discussion, which focused on requirements for RMTMs and challenges in adopting them, is summarized here, along with related poll results.

Of the poll respondents (up to 106, depending on the question), 60 % were from industry, with the remainder from US federal government, academia, and non-profits (Question 1). The respondents indicated that they test a variety of products for microbial contamination, including gene and/or cell therapy products (59 %), medical devices (30 %), biopharmaceuticals (29 %), raw materials (24 %), pharmaceuticals-small molecules (23 %), microbial cell therapies (18 %), foods (14 %), dietary supplements (13 %), cosmetics (12 %), and a wide variety of other products such as tobacco, consumer goods, additives, and water (Question 2).

[2] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/content-and-review-chemistry-manufacturing-and-control-cmc-information-human-somatic-cell-therapy
[3] https://www.ema.europa.eu/en/ich-q5a-r1-quality-biotechnological-products-viral-safety-evaluation-biotechnology-products-derived; https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q5a-viral-safety-evaluation-biotechnology-products-derived-cell-lines-human-or-animal-origin
[4] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/chemistry-manufacturing-and-control-cmc-information-human-gene-therapy-investigational-new-drug

Panelists were asked about changes that have occurred since an April 2018 workshop on Rapid Microbial Testing Methods organized by the Standards Coordinating Body (SCB) with NIST, BioFabUSA and NIIMBL.[5] The panelists indicated that they have seen progress, such as improved microbial laboratory capabilities and widely accepted molecular tests for mycoplasma. They noted that some needs have continued to grow, such as needs for reference materials and in process and in-line testing due to the transition from batch to continuous production. Currently, there are increased numbers of internal discussions on RMTMs, but the lack of scientific publications makes it difficult to de-risk the transition.

On the topic of validation and adoption of RMTMs, panelists brought up points including:

- sometimes there is an unwillingness to burden a project with the need for additional regulatory approval for new unproven technologies since current methods work;
- smaller companies are typically unable to move forward with RMTMs until larger companies take the risk, but even larger companies like AstraZeneca do not yet have a commercial product with a rapid microbial release test;
- rapid methods should demonstrate technical superiority over compendial methods; and
- dialogue with the FDA is critical.

Half of the poll respondents use rapid (undefined) methods (Question 3), but only 22 % of respondents currently have validated RMTM methods (Question 5). For those not currently using rapid microbial testing, the identified challenges fall into three general categories: regulations, technology, and cost (Question 4, results highlighted in Fig. 3). The participants noted that the US Pharmacopeia (USP) has guidance[6] for demonstrating equivalence between rapid and compendial methods, and the rapid method needs to correlate with the compendial method.

Criteria for a fit-for-purpose RMTM were also discussed. In terms of specific detection of bacteria, Dr. Hoover said there is power in being able to identify the contaminating microorganism. Dr. Zylberberg indicated that customers drive the needs, and Dr. Leach added that target organisms typically include compendial strains along with site isolates and any challenging microbes. When asked if a molecular signal is equivalent to a viable organism, the panelists indicated that a decision tree is needed, such as only testing for viable organisms if DNA is detected. From the poll results, 44 % of respondents indicated that microbial detection methods need to indicate microorganism viability, 35 % of respondents require taxonomic identification of the contaminant, and greater than 75 % define a rapid test as providing results in less than 24 h (Questions 6, 7, and 10). The ideal test throughput varied and ranged from greater than 1000 samples per week down to (1 to 5) samples per week (Question 9). Additionally, the most important attributes for a RMTM were identified as sensitivity, accuracy, speed, and specificity, followed by GMP compliance (Question 11).

[5] https://www.nist.gov/news-events/events/2018/04/rapid-microbial-testing-methods-workshop
[6] USP <1223> Validation of Alternative Microbiological Methods.

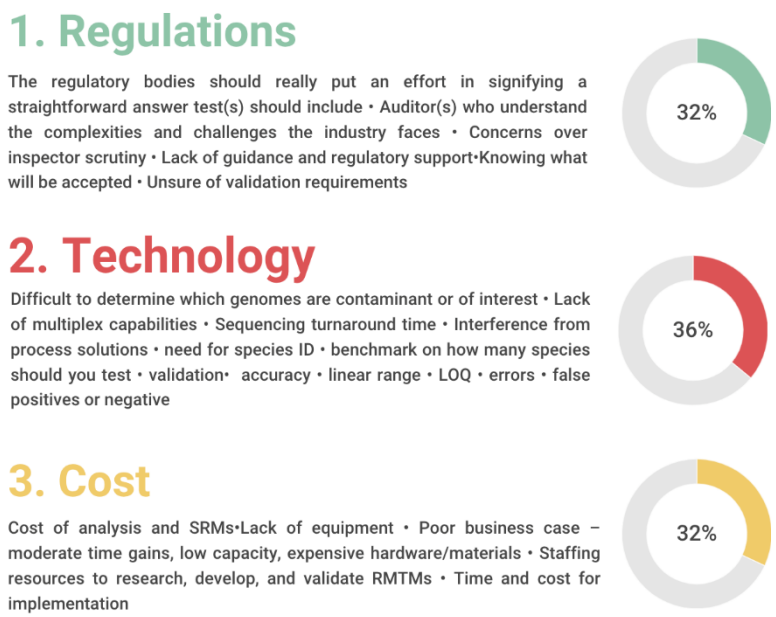


Fig. 3. Poll results indicating why respondents do not use RMTMs.

New RMTM technologies continue to emerge. When asked what their favorite RMTM technology is, panelists answered in-process in-line testing, recognizing it is still far off (Dr. Zylberberg); bioluminescence, molecular methods, and flow cytometry (Dr. Leach); and sensors within the production process (e.g., for oxygen) (Dr. Hoover). In the poll, respondents were asked which measurement technology they most hoped for; the most popular response was a non-sequencing-based molecular method such as PCR (57 %), followed by a sequencing-based method (43 %), and in-line sensor technology (34 %) (Question 12).

Overall, the discussion highlighted the variability in the application of RMTMs, with the test requirements depending on when and where the RMTMs will be applied, emphasizing the need for more than one validated RMTM.

Session 2: The Solution Space

Overview

Session 2 transitioned from challenges to potential solutions by discussing ongoing community efforts and potential Consortium directions to support validation and adoption of RMTMs. The format was the same as Session 1: two invited presentations followed by a panel discussion. This session included discussion of progress towards documentary standards to support the implementation of RMTMs, a use case from industry for validating a new RMTM, existing efforts in the stakeholder community, and potential directions for the new NIST RMTM Consortium.

Presentations

Progress Toward Documentary Standards to Support Rapid Microbial Testing
Dawn Henke, Ph.D., Senior Technical Program Manager at the Standards Coordinating Body (SCB)
Dr. Henke presented an overview of SCB, a US-based, non-profit standards coordination organization that assists in the development and dissemination of standards for regenerative medicine by engaging the stakeholder community as a neutral partner. SCB's Regenerative Medicine Standards Landscape Report[7] compiles over 250 standards for cell therapy, gene therapy and tissue engineering across many standards development organizations. The report discusses the many RMTM documentary standards already published by national and international standards organizations. The scope of these standards varies based on their focus, for example, cell preparations versus release testing. Ongoing standards development efforts in International Organization for Standardization (ISO) and ASTM International were highlighted. The working draft (WD) document, ISO/WD 24190 Biotechnology — Analytical Methods — Risk based approach for design and validation of methods for rapid microbial detection in bioprocesses, seeks to standardize a risk-based approach to RMTM implementation without mandating specific assays. Another document, still in an early draft stage, is ASTM WK70143 New Guide for Sampling Methods of Tissue Engineered Medical Products (TEMPs) for Sterility Assurance, whose goal is to provide guidance on addressing the challenges associated with sampling TEMPS to develop sterility assurance test methods. Both of these draft standards need additional input from the stakeholder community. Information about other educational and outreach resources, including the SCB annual meeting and standards implementation courses, is available on the SCB website.

Rapid Detection of Bacteria and Fungi in ATMPs Prior to Treatment – Validation of a Real-time PCR-based Test
Kai Nesemann Ph.D., Product Manager, Microbiology, Sartorius Lab Instruments GmbH & Co. KG
This presentation provided an example validation scheme for a RMTM for advanced therapy medicinal products (ATMPs). Although there are some efforts to standardize RMTM for bacteria and fungi, these methods do not provide enough guidance for validating sterility of products with a short shelf-life, i.e., under five days. Sartorius Instruments took on the challenge of developing a RMTM for total bacteria and total fungi using a qPCR kit that will return results in several hours. Dr. Nesemann described the process used to create the validation method, which was designed around principals of specificity, sensitivity, ruggedness, robustness, and equivalency to compendial methods. Getting a high specificity was difficult for fungi since they are also eukaryotes, but putative fungal contaminants of cell cultures are detected. For sensitivity, the concentration at which each species passed was reported. For robustness and ruggedness, Sartorius performed a device comparability study between 4 qPCR instruments. Another important assay performance metric is the ability to detect microbial free-DNA that could be present. The presence of eukaryotic cells provides an addition source of background DNA with which to challenge the assay. For equivalency, the new qPCR assay was found to perform as well as or better than the growth-based assays. The mycoplasma, total bacteria and total fungi kits are identically designed in terms of qPCR

[7] https://www.standardscoordinatingbody.org/landscape

temperature profile, so only one DNA extraction is needed, and all three assays can be performed in the same run.

Panel Discussion

This panel discussion focused on potential solutions and paths forward for the Consortium.

Panel members were Ms. Judith Arcidiacono (International Regulatory Expert, Standards Liaison, FDA), Mr. Richard Hammond (Technology Director, Cambridge Consultants LTD), Dr. Richard McFarland (Chief Regulatory Officer, ARMI|BioFabUSA), Dr. Stacy Springs (Senior Director of Programs; Executive Director Biomanufacturing Initiatives, Massachusetts Institute of Technology's Center for Biomedical Innovation), and Dr. Radhakrishna Tirumalai (Principal Scientific Liaison, US Pharmacopeial Convention). Panelist bios are provided in Appendix B. Poll results related to reference materials and the proposed Consortium directions were also discussed. Full poll results are provided in Appendix D. The discussion is summarized here.

During their introductions, the panelists were asked to describe their organization's efforts related to RMTM. Dr. Tirumalai indicated the USP has developed a risk-based approach for short shelf-life products and is making individual methods for adenosine triphosphate (ATP) luminescence and respiration. Mr. Hammond said Cambridge Consultants is working with automated, streamlined, closed systems and is developing methods for in-line testing with real-time information. Dr. Springs leads two consortia on biomanufacturing and adventitious agents, with a new research group developing early stage rapid sterility testing. Dr. McFarland stated that BioFabUSA is charged with supporting tissue engineered products where they have challenges in developing strategies to collect samples for microbial testing. Ms. Arcidiacono is working with NIST, SCB, ISO, and ASTM to contribute to consensus standards related to RMTMs, and the FDA has a lab program to help support the work. Besides the efforts from the panelists' organizations, the panelists and workshop participants indicated they were not aware of many other community efforts focused on RMTMs, listing only the Microbiology Modernisation Cross-industry Consortium (MMCC) established in 2018,[8] and the revision of the 2013 version of Parental Drug Association (PDA) Technical Report No. 33, Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods.[9]

Regarding a question on technology roadmapping, Dr. McFarland highlighted that it would be better for the Consortium to focus on a short-term roadmap and then take some action, such as developing materials and running interlaboratory studies, as long-term roadmapping can be a lengthy process. Mr. Hammond noted that planning should focus on translating RMTMs into practice, ensuring consistency in validation, and allowing for alignment across groups.

In terms of helping companies overcome resistance to being the first to adopt new RMTMs because of the risk, Dr. Tirumalai said that the Consortium can help lower the bar by making validated methods available. Mr. Hammond indicated that a Consortium could help the

[8] Gonzalez, M. "Microbiology Modernisation Cross-industry Consortium takes shape." Cleanroom Technology. 2018. https://www.cleanroomtechnology.com/news/article_page/Microbiology_Modernisation_Crossindustry_Consortium_takes_shape/149849
[9] PDA Technical Report No. 33, Revised 2013 (TR 33) Evaluation, Validation and Implementation of Alternative and Rapid Microbiological Methods. https://www.pda.org/bookstore/product-detail/4381-tr-33-revised-2013-rapid-microbiological-methods

community recognize that there will be support if they try to implement RMTMs, and the community effort may help give management confidence that RMTMs can be successfully adopted.

A significant portion of the panel discussion was centered around reference materials (RMs). Panelists indicated that RMs are needed for areas including molecular methods, NGS, and viability measurements, and RMs should be well characterized, easily acceptable materials to enable comparison among organizations. Scalability for RMTM is critically important (Dr. Hammond), with real-time, integrated systems more promising than manual, end-point tests. Dr. Tirumalai indicated an RM should include organisms commonly seen in product failure and contamination. Dr. Springs brought up the NIST monoclonal antibody Standard Reference Material (SRM), which is useful because it is so well characterized. Having a RM with substantial amounts of data, including molecular methods and long-read sequencing would be valuable. Likewise, nucleic acid standards would also be useful, in addition to cells. Dr. McFarland said the community needs viable cell standards. Mr. Hammond said robust standards that people trust are needed as the foundation for methods, and those standards should have widespread acceptance and adoption. It was noted that RMs for viable (or non-viable) cells do not exist. Ms. Arcidiacono indicated that RMs consisting of viable organisms should be a priority since methods must be able to detect viable organisms (per 21 CFR 610.12 - Sterility). She also indicated that testing for nucleic acid first and then going back to find viable microbes may not be the optimal approach because it may result in a lengthier process that in the end does not provide a strong advantage. Moreover, the point was also raised that false positives for nucleic acid tests are expensive.

The word cloud shown in Fig. 4 highlights where respondents currently acquire reference materials for sterility testing (Question 13).

The poll also asked about the number of species needed in a microbial whole cell reference material to validate a new RMTM workflow (Question 14). The most popular answer was 11 species to 20 species (43 %); while 28 % of respondents indicated less than 10 species is sufficient and 16 % of respondents indicated the need for more than 30 species. In addition to whole cell microbial reference material, nucleic acid standards (e.g., RNA, DNA, XenoDNA (synthetic DNA analogs with a different sugar backbone) were identified as another class of reference materials that would be useful to accelerate assay validation (Question 15).

Panelists and the workshop participants were asked about what the RMTM Consortium could and should do. Mr. Hammond indicated it would be helpful to provide a method validation framework with data-rich measurements to reduce the burden of getting technology to market. Dr. Springs said that anything the Consortium can do to help de-risk the process would be valuable, especially considering existing methods are cheap. Dr. McFarland shared that PCR assay development is challenging for smaller companies, and a consensus list of RMs that can be made available at a reasonable cost and validated with interlaboratory studies would lower the risk for assay companies. He also said there is a need for a standardized approach to perform a validated RMTM. Dr. Tirumalai indicated there is a need to foster new technologies. Ms. Arcidiacono suggested the Consortium provide a pre-competitive environment where manufacturers, kit makers, reagent developers, and other stakeholders can come together to share data and protocols to create broadly useful approaches, adding that the FDA can be flexible if there is a data-driven approach. Dr.

McFarland added that the FDA should increase efforts to achieve global harmonization in this space.

Fig. 4. Word cloud of reference material sources.
This word cloud represents the open text responses from 60 respondents (some with multiple answers) to the question: "Where do you currently acquire your reference materials for sterility testing?" Larger font size indicates more individuals identified that source (graphic: Wordcloud).

Attendees were asked to identify the top two priorities that the RMTM Consortium should seek to address first. The top response was to lead development of best practices for method validation schema (47 %), followed closely by development of fit-for-purpose reference materials (41 %), development and validation of a new fit-for-purpose RMTM technology, and identification of an existing RMTM technology that is fit-for-purpose (35 %) (Question 16). A more complete list of actions items recommended for the Consortium can be found in the full responses to Questions 16a and 17 (Appendix D).

Overall, the panelists supported the Consortium as a mechanism to bring the community together to address the challenges facing RMTM validation and adoption. Further, the panelists indicated that publications on behalf of the Consortium would be a very impactful way to spread best practices to the community.

Summary and Next Steps

Overall, this workshop convened stakeholders to discuss hurdles associated with implementing RMTMs for advanced therapy products. Input from the presenters, panelists, and attendees emphasized the need for the community to work together to efficiently overcome challenges that limit the use of RMTMs. There are tradeoffs between speed and sensitivity, and thus a single validated RMTM to serve all purposes is not likely. Rather, a suite of fit-for-purpose validated methods will need to be combined with a risk-based testing strategy for successful implementation. Some of the initial work of the Consortium will be focused on gathering information to enable the forward movement toward rapid, risk-based testing of advanced therapy products.

At the beginning of the workshop, NIST proposing three focus areas for the Consortium: reference material development, testing methods, and interlaboratory studies. The workshop discussions and the poll results indicated general support for these three topics while also suggesting slight expansion on two of the topics. On the topic of reference materials, the need for other types of RMs, in addition to the proposed whole cell RMs, was indicated. For testing methods, the need for validation schema was expressed. Based on this feedback, NIST adjusted the three proposed focus areas, with expanded scopes for the topics of reference materials and methods to better address stakeholder needs.

Organizations interested in joining the Consortium were invited to submit a Letter of Interest[10] to indicate their desire to join the Consortium and to initiate the required paperwork, in most cases a Cooperative Research and Development Agreement (CRADA). Prior to the workshop, NIST had received 14 Letters of Interest from organizations or individuals expressing interest in joining the Consortium. Within one month of the workshop, 16 additional Letters of Interest were received, with 7 of them being received the first week after the workshop.

The next steps for NIST included completing the necessary paperwork with interested organizations; initiating regular meetings for Consortium members; and drafting the scope, proposed working groups, and a potential timeline for the Consortium. The first monthly Consortium meeting was held on Tuesday, November 17, 2020.

The discussions from this workshop will be used to help inform the direction of the Consortium, but ultimately it will be up to the Consortium members to draft, via consensus, a path forward for the Consortium to have a lasting impact on the use of RMTMs for microbial detection in advanced therapy products.

[10] https://docs.google.com/forms/d/e/1FAIpQLSc9vlSdSIxUMu-GJv8iPZm7AXi-sdIeEo7_OLEnLXhu-kdU0w/viewform

Appendix A: Workshop Agenda

Thursday, September 17, 2020, 1 PM to 5 PM

INTRODUCTORY REMARKS	
1:00 PM – 1:10 PM	**Welcome and Overview of the RMTM Consortium and Workshop Goals** *NANCY LIN, Leader of the Biomaterials Group, Biosystems and Biomaterials Division (BBD), Material Measurement Laboratory (MML), NIST*
1:10 PM – 1:20 PM	**Overview of NIST Advanced Therapy Program** *SHENG LIN-GIBSON, Chief of the BBD, MML, NIST*
1:20 PM – 1:30 PM	**Overview of NIST Microbial Metrology Program** *SCOTT JACKSON, Leader of the Complex Microbial Systems Group, BBD, MML, NIST*

SESSION 1: CHALLENGES IN ADOPTING RMTMS IN THE CELL AND GENE THERAPY INDUSTRY *Moderator: Jason Kralj, NIST*	
1:30 PM – 2:00 PM	**Key Characteristics of Rapid Microbial Test Methods for Cell and Gene Therapies** *SPENCER HOOVER, Independent Consultant*
2:00 PM – 2:20 PM	**FDA Requirements and Recommendations for Sterility Testing of Regenerative Medicine Therapies** *JUDITH ARCIDIACONO, International Regulatory Expert, Standards Liaison, FDA*
2:20 PM – 3:05 PM	**PANEL DISCUSSION: Measurement Challenges and Needs** Moderator: Scott Jackson, NIST ▪ *SPENCER HOOVER, Independent Consultant* ▪ *TOM LEACH, Associate Director, Drug Product Process Engineering and Packaging, AstraZeneca* ▪ *CLAUDIA ZYLBERBERG, CEO, Akron Biotech*
3:05 PM – 3:15 PM	**BREAK**

SESSION 2: ONGOING COMMUNITY EFFORTS AND POTENTIAL CONSORTIUM DIRECTIONS TO HELP FACILITATE THE ADOPTION OF RMTMS *Moderator: Sandra Da Silva, NIST*	
3:15 PM – 3:20 PM	**Introduction to Session 2** *NANCY LIN, NIST*
3:20 PM – 3:35 PM	**Progress Toward Documentary Standards to Support Rapid Microbial Testing** *DAWN HENKE, Senior Scientific Program Manager, Standards Coordinating Body*
3:35 PM – 3:55 PM	**Rapid Detection of Bacteria and Fungi in ATMPs Prior Treatment – Validation of a Real-time PCR-based Test** *KAI NESEMANN, Product Manager, Microbiology, Sartorius Lab Instruments GmbH & Co. KG*
3:55 PM – 4:45 PM	**PANEL DISCUSSION: Potential Solutions and Paths for the Consortium** Moderator: Nancy Lin, NIST • *JUDITH ARCIDIACONO, International Regulatory Expert, Standards Liaison, FDA* • *RICHARD HAMMOND, Technology Director, Cambridge Consultants LTD* • *RICHARD MCFARLAND, Chief Regulatory Officer, ARMI|BioFabUSA* • *STACY SPRINGS, Senior Director of Programs; Executive Director Biomanufacturing Initiatives, Massachusetts Institute of Technology's Center for Biomedical Innovation* • *RADHAKRISHNA TIRUMALAI, Principal Scientific Liaison, US Pharmacopeial Convention (USP)*

CONCLUDING REMARKS	
4:45 PM – 5:00 PM	**Summary and Next Steps** *NANCY LIN and SCOTT JACKSON, NIST*

Appendix B: Abstracts and Bios

SPEAKER AND PANELIST LIST – Abstracts and Bios

SPEAKERS

SHENG LIN-GIBSON, Ph.D., Chief of the Biosystems and Biomaterials Division, NIST

Presentation Title: Overview of NIST Advanced Therapy Program

Abstract: Advanced therapies, including cell therapy, gene therapy, and tissue engineered products, have shown promising clinical efficacy and are changing the paradigm for treating diseases and injuries. Clinical translation and patient access to this broad class of therapeutics requires better defined and characterized products and more robust, reliable, and cost-effective manufacturing processes. Our Program supports the growing industry by addressing manufacturing, characterization, and testing challenges. Our efforts include 1) developing measurement assurance strategies and innovative measurement solutions, 2) convening and working stakeholders to identify industry-wide challenges and pre-competitive solutions, and 3) leading and contributing to the development of global documentary standards and reference materials.

Bio: Dr. Sheng Lin-Gibson is the Chief of the NIST Biosystems and Biomaterials Division. She oversees a multidisciplinary research portfolio that includes advanced therapies, precision medicine, synthetic biology, and complex microbial systems. She leads or contributes to the development of several international standards particularly relevant to emerging biotechnology.

SPENCER HOOVER, Ph.D., Independent Consultant

Presentation Title: Key Characteristics of Rapid Microbial Test Methods for Cell and Gene Therapies

Abstract: This talk will identify some of the reasons why RMTM are needed for Cell and Gene Therapies and why developing those methods are challenging. Ideas on where to look for relevant technologies and how to speed their uptake using a risk-based approach will also be discussed.

Bio: Building on his PhD in Microbiology, Spencer led the development of high multiplex in vitro diagnostics for pathogen detection for several years at Luminex. He has spent the past five years in the cell and gene therapy industry, most recently as the Director of Process and Analytical Development at CCRM in Toronto as part of the collaboration with Cytiva. In 2020, he has been working as an independent consultant to help various cell and gene therapy companies with facility design, automation, and analytical method development. He co-chaired the RMTM workshop organized by the Standard Coordinating body in 2017

and is looking to neighboring industries for approaches and technologies to address the needs for RMTM in CGT.

JUDITH ARCIDIACONO, M.S., International Regulatory Expert, Standards Liaison, FDA

Presentation Title: FDA Requirements and Recommendations for Sterility Testing of Regenerative Medicine Therapies

Abstract: When developing rapid microbial testing methods, it is important to understand the local or regional regulatory requirements for testing Regenerative Medicine Advanced Therapy (RMT) products. This presentation will cover the U.S. regulations for sterility testing described in the US Code of Federal Regulations (CFR), 21 CFR 610.12, and expectations for RMT products outlined in FDA Guidance Documents. Working towards the development of rapid microbial testing methods will require a community effort where developers of testing methods, end users and regulatory agencies collaborate on the development of tests appropriate for RMT products.

Bio: Judith has been with the FDA for 30 years, the first 17 years as a researcher/reviewer and currently is leading international regulatory activities and policy on standards for Regenerative Medicine Therapies. She represents FDA in ISO Technical Committee 276, Biotechnology, the American Society for Testing Materials (ASTM) F04 Committee on Medical and Surgical Materials and Devices, Tissue Engineered Medical Products, and the Parenteral Drug Association Standards Development Organization. She serves as the secretariat for the International Pharmaceutical Regulators Programme (IPRP), Cell Therapy Working Group and Gene Therapy Working Group. Judith represents FDA as a Subject Matter Expert in regenerative medicine policy for the Asia Pacific Economic Cooperation Regulatory Harmonization Steering Committee Priority Work Area for Advanced Therapies. She is also a faculty member for the Northeastern University Center of Excellence for Advanced Therapies and Duke Medical School at the National University of Singapore Center of Regulatory Excellence.

DAWN HENKE, Ph.D., Senior Scientific Program Manager, Standards Coordinating Body

Presentation Title: Progress Toward Documentary Standards to Support Rapid Microbial Testing

Abstract: This talk will be focused on standards related to rapid microbial testing methods for regenerative medicine manufacturing. Current published standards, standards under development, and needed standards will be discussed. A focus of this talk will be engagement and education about standards.

Bio: Dawn Henke is the senior scientific program manager at the standards coordinating body. She has a Ph.D. in genetics and genomic sciences from University of Alabama at Birmingham and was a postdoctoral fellow at the National Institutes of Health.

KAI NESEMANN, Ph.D., Product Manager, Microbiology, Sartorius Lab Instruments GmbH & Co. KG

Presentation Title: Rapid detection of bacteria and fungi in ATMPs prior treatment – Validation of a real-time PCR-based test

Abstract:

- First rapid sterility test for short-shelf life ATMPs compliant to international guidance including the new USP<1071> as well as EP 5.1.6., EP 2.6.27 and USP<1223>.
- Obtain the QC result PRIOR to treatment and safeguard the patient´s health by avoiding exposure to additional risks.
- Full validation results of a qPCR method for sensitivity, specificity, robustness and equivalency of total bacteria and fungi detection.
- KEYWORDS: rapid final release of ATMPs; 16S/18S ribosomal DNA detection; Paul-Ehrlich-Institute; quantitative Polymerase Chain Reaction (qPCR); TaqMan® probe.

Bio: Kai Nesemann joined Sartorius Lab Instruments GmbH in July 2016. He is global product manager microbiology and responsible for the DNA-based rapid detection portfolio as well as for continuous air monitoring in microbiological quality control. Special focus is given on the biopharmaceutical production for in-process and final release testing of Mycoplasma as well as total bacterial and fungal contamination. Validation of robust rapid tests with results prior treatment is of particular importance for patient safety in regenerative medicine. Nesemann graduated at the Georg-August-University in Goettingen, Germany. He has many years of experience in academic research, and he worked as a Ph.D. student in the department for molecular microbiology and genetics of Prof. Dr. Gerhard.

PANELISTS

RICHARD HAMMOND, M.S., Technology Director, Cambridge Consultants Ltd.

Bio: Richard is Head of Bioinnovation at Cambridge Consultants, leading a multi-disciplinary team in the design and development of novel equipment and processes for ATMP research and manufacture. Prior to joining Cambridge Consultants, Richard worked for Alere (now part of Abbott) leading teams developing in-vitro diagnostics for infectious diseases using molecular techniques such as isothermal DNA amplification. Richard trained as an engineer and holds BA and MEng degrees from the University of Cambridge. See https://www.linkedin.com/in/richard-hammond-15774a8/.

TOM LEACH, Ph.D., Associate Director, Drug Product Process Engineering and Packaging, AstraZeneca

Bio: I am a biochemist and chemical engineer by training and have worked in biopharmaceutical drug product development for 17 years. Most of my career has involved formulation and process development for monoclonal antibody drug products. Currently, I am working in cell and gene therapy, and serve on a global AstraZeneca microbiology forum which aims to modernize and advance microbiological controls in manufacturing and in drug products.

RICHARD MCFARLAND, M.D., Ph.D., Chief Regulatory Officer, ARMI|BioFabUSA

Bio: Richard McFarland is an immunopathologist and the Chief Regulatory Officer at the Advanced Regenerative Manufacturing Institute (ARMI) where he oversees regulatory affairs for ARMI and its BioFabUSA program. Currently he is also serving as President of the Standards Coordinating Body. Prior to joining ARMI in 2017, Dr. McFarland was Associate Director for Policy of the Office of Tissues and Advanced Therapies (and its predecessor office) at the Food and Drug Administration's Center for Biologics Evaluation and Research (FDA/CBER) for eleven years after six years as a reviewer in FDA/CBER. In addition, he, served on the federal government's interagency committee for tissue engineering and regenerative medicine, the Multi-agency Tissue Engineering Sciences group (MATES) for fifteen years, including five years as its Chair.

STACY SPRINGS, Ph.D., Senior Director of Programs; Executive Director Biomanufacturing Initiatives, Massachusetts Institute of Technology's Center for Biomedical Innovation

Bio: Dr. Stacy Springs serves as the Senior Director of Programs at MIT's Center for Biomedical Innovation and as the Executive Director of Biomanufacturing Initiatives including MIT's Biomanufacturing Program, (BioMAN), it's Consortia on Adventitious Agent Contamination in Biomanufacturing, (CAACB), and the BioACCESS initiative. The objective of BioMAN is to develop knowledge, science, technologies, and strategies that advance the global manufacture and delivery of high-quality biopharmaceuticals. The CAACB pools biopharmaceutical manufacturing expertise in the area of adventitious agent contamination to better enable a safe and dependable delivery of life-saving biologics. BioACCESS seeks to better understand the growing need for and barriers to safe, effective, and affordable health services in low- and middle-income countries, especially biologic therapies for chronic and non-communicable diseases. In addition, Dr. Springs serves as the co-captain for the Flagship Project 2 team of the Singapore-MIT Critical Analytics for Manufacturing Personalized-Medicines (SMART CAMP) project where she addresses rapid critical quality attributes (CQA) for safety of cell sources and cell therapy products, informing process analytic technologies and speeding product release.

She holds a PhD in Chemistry from the University of Texas at Austin and gained postdoctoral training in protein and biophysical chemistry at Princeton University.

RADHAKRISHNA TIRUMALAI, Ph.D., Principal Scientific Liaison, US Pharmacopeial Convention (USP)

Bio: Dr. Tirumalai has been at the USP since 2003 and is currently a Principal Scientific Liaison-General Chapters in the Science Division. He is the Liaison to the USP Expert Committee on Microbiology. He works with the industry, regulatory agencies and other external science-based organizations in the development and revision of General Chapters. Dr. Tirumalai represents USP on PDA expert task forces and committees related to Microbiology and Sterility Assurance 2005-till date, the organizing committee of PDA Global Microbiology Conference 2006-2018, on AAMI expert working groups related to Microbiology, Sterilization, Sterility Assurance and Biocompatibility 2004-till date, and on the editorial board of FDA's Pharmaceutical Microbiology Manual.

Dr. Tirumalai's prior industry experience encompasses process and product research and development, transfer, and product manufacturing. He has a Ph.D. degree in Biochemistry. He has authored numerous publications, review articles and several book chapters. He has organized numerous workshops and conferences on Pharmaceutical Microbiology topics and is a frequent speaker at conferences and has taught Pharmacopeial Microbiology courses at numerous locations globally.

CLAUDIA ZYLBERBERG, Ph.D., CEO, Akron Biotech

Bio: Claudia Zylberberg, Ph.D. is a leader in regenerative medicine. She is the founder and CEO of Akron Biotechnology, a manufacturer of cGMP-grade ancillary materials for the tissue, cell, and gene therapy industry. She also co-founded AssureImmune, an adult stem cell bank. Dr. Zylberberg holds numerous patents and has developed several patent-pending platform technologies in cryopreservation, novel formulations, and others. She has authored and co-authored several peer-reviewed publications and has received grants from the NIH and Department of Defense, among others. In her early years, Dr. Zylberberg worked at Nabi Biopharmaceuticals, specializing in human plasma-derived products. Her experience in product development and protein manufacturing has been instrumental for the development of key materials to accelerate the regen med industry. In addition, she co-founded the Standards Coordinating Body (SCB) and is a board member of ISCT, ARM, AABB's NBF, and the NAS (Regenerative Medicine Forum). Other advisory positions include ISO US TAG, BioFlorida, ISSCR, CBA, and Biomedical Engineering, University of Miami.

Dr. Zylberberg is also the author of a children's book, *You're Full Genes* and recently launched an updated version. Originally introduced in 2001, *You're Full of Genes* renews Dr. Zylberberg's call for an educated public. Sales proceeds will support three foundations working on scientific advancements and education in the field: the CCRM Foundation, the ARM Foundation for Cell & Gene Medicine and Duke University's Center for Autism and Brain Development.

Appendix C: Audience Questions and Comments

These questions, responses, and comments were entered into the online platform during the workshop. Note that some comments and questions were directed at specific speakers, who may have responded verbally in the workshop or via the chat.

- I strongly recommend for NIST to reach out to some of the participants logged in today, who have 20+ years practical experience in validating rapid methods, developing guidance standards for Pharma, and gaining worldwide regulatory acceptance. The expertise will enhance NIST's goals.
- Why is the USP not included when the Food, Drug and Cosmetic Act defines the USP as the recognized standard-setting organisms for drug products?
- NIST has had a long-standing collaboration with USP. One example is a recent joint effort on CD34 reference material. It should be included in the documentary standards slide.
- Does the adoption of a molecular test for mycoplasma mean we're not so concerned about non-viable bugs being picked up?
- Only a few organisms you tested used an LOD < 10 CFU; the majority was at 50 CFU or higher. Since many regulators expect rapid sterility test validations to include LOD testing at the single cell level, how will end-users demonstrate this with your system?
- @JUDITH: Can you comment on the acceptability of rapid sterility tests in FDA's 2020 guidance document: CMC Information for Human Gene Therapy IND Applications?
- Are there any plans for providing standards on Next-generation sequencing based Adventitious agent testing?
- Can you comment on the utility of the rapid PCR methods for release of CAR-T products?
- Regarding viral safety and animal derived materials where a batch is a single piece (lot of n=1), can rapid methods apply?
- Will NIST consider the need for determining the viable but non-cultural microbial cells in the development of rapid methods?
- We do talk about this, as this is a critical shortcoming of the culture-based methods, and a potential benefit of some rapid methods.
- That is definitely a challenge and something we're taking into consideration. We would welcome input on this, esp. specific organisms that should be studied or serve as a standard.
- Would the use RNA as a target using RT-PCR be a superior approach as RNA is better measure of viability?
- Another industry-based effort will be the revision process for PDA Technical Report #33, which is planned to start later this year.
- For Scott: Have you been able to show similar counts between different methods - e.g., CFU vs microscopy vs flow cytometry?
- Methods can have different measurands, meaning they measure different aspects of the microbes. For example, measuring culturable cells (CFU) vs cells with intact membranes (via exclusion of fluorescent dyes). In those situations, you probably should not expect similar counts from different methods. That being said, when we

use multiple methods that measure the same attribute (e.g., total object count via Coulter counter, flow cytometer, or microscope), we do get similar counts.

- Thanks for the response. For the reference standards, are we thinking of including a CoA with different measurements? e.g., 1e9 cfu/g - total plate count, 1e10 cells/g - flow cytometry
- Potentially yes. Any reference value we provide with a reference material would be linked to a specific measurand, and the measurement method used will be described. One would not necessarily expect to get the same number when measuring a different measurand on that same reference material.
- How do you show equivalence of RMTMs to compendial methods?
- Have any PCR based methods been successfully registered with regulatory agencies for ATMP final product release?
- Verax testing is FDA approved for platelets. Could this be used for other biological products? https://www.veraxbiomedical.com/products/pgd-technology/
- clearance appears limited to platelets. So likely not generally applicable without further validation and verification
- Yes, also Pall eBDS system is another that maybe could be modified but as you say would need further validation
- Do the molecular methods provide an advantage for viable but non-culturable organisms?
- Yes. Universal (e.g., 16s) and untargeted (e.g., metagenomics) methods with detect uncultivable organisms. Microbial "dark matter"
- Follow up to the question about how you show equivalence to compendial methods: please refer to the strategies outlined in PDA Technical Report #33, USP 1223, Ph. Eur. 5.1.6 and the guidance at rapidmicromethods.com.
- there are some traditional universal primers like 27F or 336R has been used in PCR for 16s rRNA. Would you be able to share if you evaluated the traditional universal primers or developed novel universal primers/probes sequences used in your qPCR for bacterial?
- Kai - for high throughput testing, do you have any data using automated nucleic acid extraction systems for samples with and without cells?
- Are these [Sartorius] tests commercially available?
- Yes, all three of them
- Is the consortium still looking for members to join?
- YES! Please see https://www.nist.gov/programs-projects/nist-rapid-microbial-testing-methods-consortium
- This is just the beginning of the recruiting phase
- Why did NIST eliminate the particulate standard for visual inspection of sterile drug products?
- Can independent consultants join the consortium?
- yes!
- NIST has had a long-standing collaboration with USP. One example is a recent joint effort on CD34 reference material. It should be included in the documentary standards slide.
- A general Question: Are the NIST microbe standards, are being sent to labs as a part of HAMQAP?

- Thanks! We're going to explore this.
- Spencer, do you see the requirement for high sample through put using molecular testing?
- You can look to conventional Pharma manufacturing as well, as we have already validated and gained regulatory acceptance for rapid sterility tests based on a variety of scientific principles.
- You talked about the [time to results] and LOD but what about the range of organisms that need to be detected? Would assume a broad range including molds and slow growers like C. acnes should be detected?
- How can you differentiate between microbial testing and microbial toxin testing especially in the GCT
- How can you differentiate between microbial testing and microbial toxin testing especially in the CAR-T cells?
- For Mycoplasma- DNA PCR is valid or need RNA RT-PCR?
- Is RNA as reliable?
- Sorry I don't understand the answer "Is RNA as reliable? I think PCR to detect RNA will be better than DNA. So my question still: What are CMC requirements?
- for Judith - what is the context / rationale behind sterility testing on stability? Is this container-closure assurance only or are there other aspects of sterility assurance this is in support of?
- Is NIST going to provide standards for the RAPID testing?
- Difference on Fit for Purpose and Risk Base
- Some blood derived products are exempt for the 21 610.12 sterility requirements. Can you see ATPMs processed in closed systems also as candidates for exemption?
- We recognize there is a fear to be the first to try new RMTM technologies, as the risk is high. What do you think can be done by industry leaders/NIST/regulatory authorities to encourage cell therapies companies to adopt new RMTM?
- We hope the Consortium will help with this!! Rather than one organization taking on all the risk to be first, we can build the infrastructure together to reduce the risk for everyone.
- Can I help with the bugs question? You choose compendia organisms and organisms that may pose an issue, including EM isolates, sterility and media fill failures, slow growers for growth-based methods, etc.
- The molecular biology methods require nucleic acid extraction and there has been different extraction kits preferentially extracting certain types of bacteria (e.g., cell wall composition), how do we circumvent this?
- This is a big issue in the microbiome scientific community. Microbiome samples can have >1000 different types of bacteria and there are no DNA extraction methods that are 100% efficient across all cell types. But you're absolutely right, DNA extraction is a source of bias in DNA-based detection methods
- Will there be funding or collaboration opportunities for process development and validation work for small companies that don’t have an internal cross-disciplinary microbiology group? What does the consortium plan to do to support these smaller entities beyond the development of reference materials?
- We'd like to learn more about how we can address the needs of smaller companies.

- To Dawn: Can you say more about the effort to coordinate communication among standards development organizations?
- This is an effort to bring together representatives of the organizations together to increase cooperation and harmonization among ongoing efforts. The first meeting will be in mid-November. There will be regular meetings after this.
- Q. for Dawn Henke - some of my colleagues are just starting out in tissue engineering - which are the best upcoming events and resources from them? Really impressed by the breadth of resources and events the SCB has to offer!
- I would suggest getting involved with BiofabUSA. They are a great resource for tissue engineering. SCB will also have our January workshop to increase education and provide an opportunity to learn more. We will be discussing tissue engineering in this event. TERMIS is also a great event for learning more about tissue engineering field. If they are interested in standards for TEMPs I would suggest ASTM F04.
- In addition to the events offered by SCB and TERMIS, you might want to checkout the Meeting in the Millyard on Tuesdays in October the BioFabUSA is running virtually. Also Columbia University has recently started a great series of academic, open access seminars in the area.
- If anyone needs the link to the landscape report, here you go - https://www.standardscoordinatingbody.org/landscape
- And here is this one https://www.standardscoordinatingbody.org/needed
- Is Burkholderia under consideration from an expansion of coverage perspective?
- Burkholderia, as a prokaryote, should be detected by the universal 16s assay that Kai described.
- Would you be able to share some information on the standard curved DNA used for bacterial? It is a mixture of DNAs or from one single strain?
- @Kai: When you spike in 10 copies of genomic DNA, how do you know that you have added 10 copies? How do you confirm your dilution?
- Kai, could you customize the bacteria?
- We offer pre-quantified CFU validation standards that are ready to use and inactivated - so non-infectious (for Bacteria, Fungi and Mycoplasma)
- The USP is working on the development of RMTMs? Should we concentrate on mycoplasma detection, sterility testing and specified microorganisms screening? What test area should we advance first and what technologies would we use?
- It possible to use headspace analysis cultures to detect microbial contamination. Does the panel support this non-invasive approach?
- How would NIST link microbial standards to specific test methods? How is this type of decision made?
- Question for the 'Potential Solutions' Panel - What requirements must a 'Potential Solution' meet in order to be a 'Real Solution' for RMTM in CGT manufacturing? To what extent must a potential solution be scalable to have longevity in the field?
- When is USP getting back to validating a USP 71.1 chapter?
- Are there in-line sensors specific for microbial detection currently in development/under consideration?
- Tony--we can investigate behavior of our standards on these methods and publish, as would fit the needs of the community. We can undertake that work from community-driven suggestions.

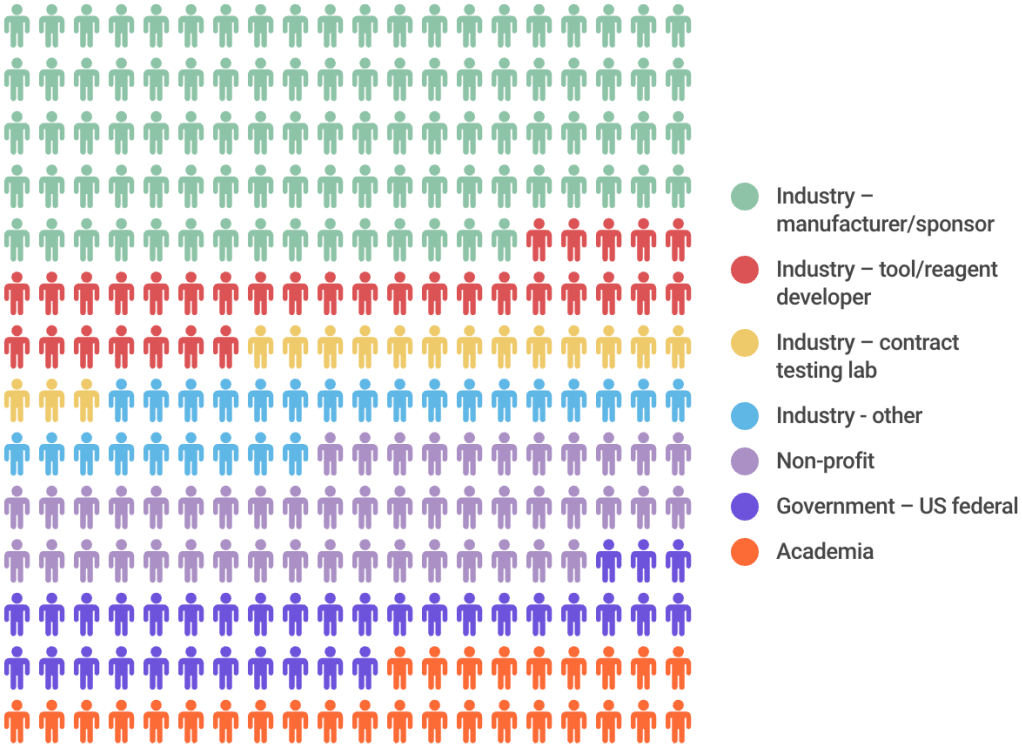
- Note: Another industry-based effort is the Kilmer RMM Consortium.
- In addition to validation, is comparability to compendial methods in the sponsor's drug substance/product required by regulatory agencies for RMMs
- Qn to Radhakrishna: There is only one anaerobe C. sporogenes in the USP62 using the RCM plate. For most LBPs, the products usually comprise of anaerobes and maybe in the Clostridia group and these products can grow on RCM plates. The molbio methods (eg. PCR) would be too specific to C. sporogenes.
- Is there a need for technology highway/roadmap here...where there's an ultimate goal - in line/real time, yet rapid off line methods are an intermediary solution..at each step validation, acceptance and trust have to be secured .....
- Detection of viable contaminant/organism is important, and the method has to be Rapid and with high sensitivity. DNA/RNA PCR based methods detect organism but will not ascertain viability. What kind of methods one should integrate to confirm presence of viable organism?
- Continuation to my question below: What would you recommend as a workaround for detecting extraneous anaerobic organisms in LBPs?
- I have to drop off soon. What are the next steps for the consortium?
- Please let us know if you are interested in joining the consortium and get to work, please contact us with details. and thanks!
- Scott Jackson answered - Find the letter of interest here: https://www.nist.gov/programs-projects/nist-rapid-microbial-testing-methods-consortium
- Michael Lehmicke replied - This isn't my area of expertise, but it is important for the Regen med field (and therefore important for ARM members). Is there a "listen only mode" where I could get copies of minutes going forward, etc.?
- Michael Lehmicke replied - Dropping off now. Feel free to reach out to me @ mlehmicke@alliancerm.org. I will pass the LOI on to our member companies.
- Can Ref materials be non-viable materials e.g. DNA/RNA/Lipid combinations of a library of microbes? That way it is easier for many labs to do the work. This would be first step, to develop/test method. And then go into viable microbe library/reference standards.
- Scott Jackson answered - All options are on the table. We're already developing microbial DNA mixtures and whole cell (viable) mixtures
- Q for Stacy: How do you circumvent the sequencing errors in current sequencing technologies and is that forgiving at the strain level?
- Intensification of microbial reads over host reads and novel bioinformatics development. It is a known issue with ONT, but our experience so far (still early days) is that it looks feasible
- thanks, is the contamination of phylogenomic database with metagenomic-constructured genomes a concern for eventual strain identity?
- Depends on how well curated your database is.
- Can case by case scenarios be shared?
- Estimated time commitment for consortium members?
- Mass Spectrometry based method is an emerging technology for identification of microorganisms. Where do you see its applications for RMM application?
- MALDI already being used: microbial ID

- interesting that COST is not in top 3
- Cost matters to me and my company!

Appendix D: Poll Results

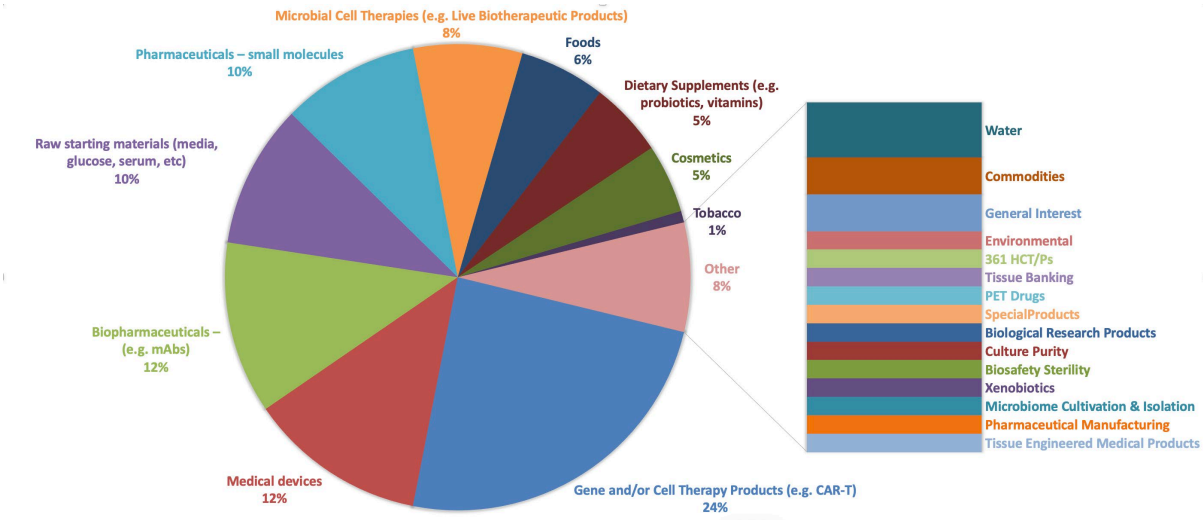
The poll was opened 1 day before the workshop and kept open for 2 days after the workshop to ensure that interested parties were able to complete the poll. All registrants were invited to complete the poll, including the speakers and panelists. Results were shared during the panel discussions at the workshop.

Question 1: **What type of organization do you represent?** (single answer question, 106 responses, graphic: Infogram)

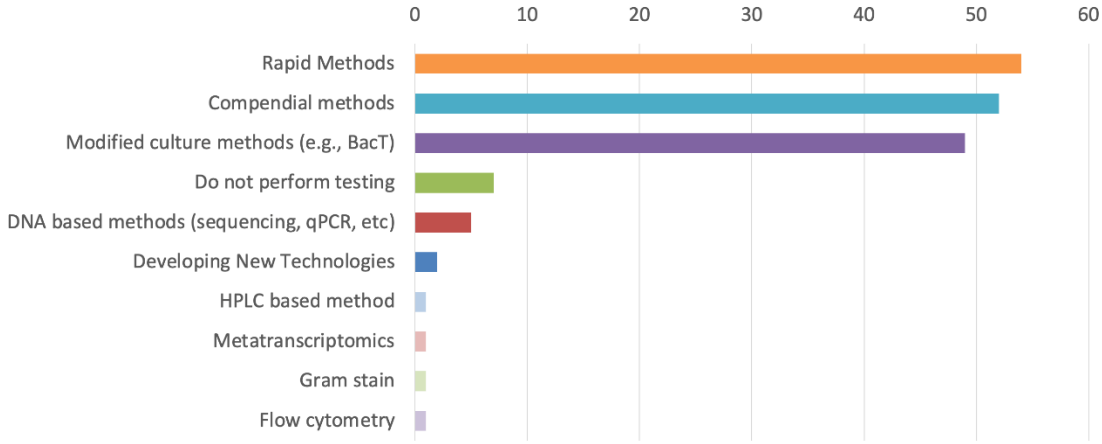


Individuals working in industry represent the largest fraction (60 %) of the poll respondents. The remaining 40 % of respondents self-reported as representing non-profits (17 %), the U.S federal government (12 %), and academia (10 %).

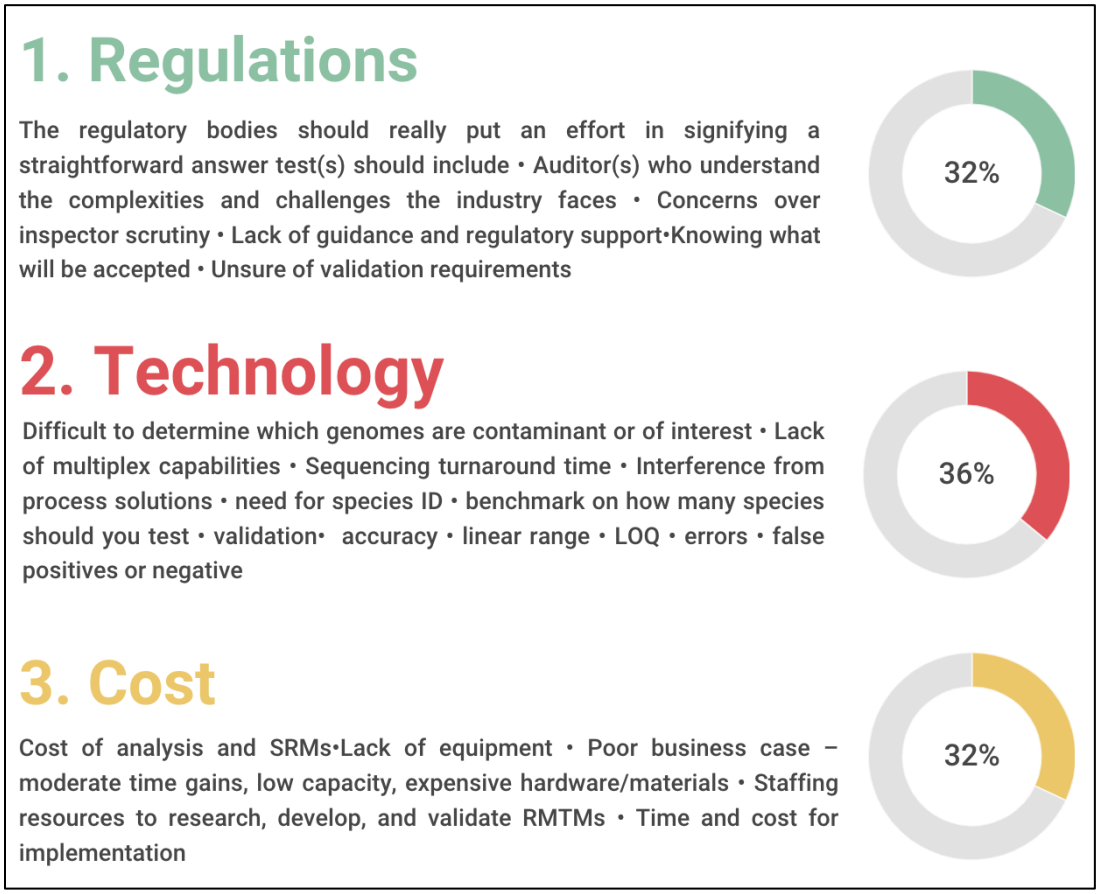
Question 2: **What types of products do you test for microbial contamination?** (open text poll, multiple answers permitted, 104 responses)



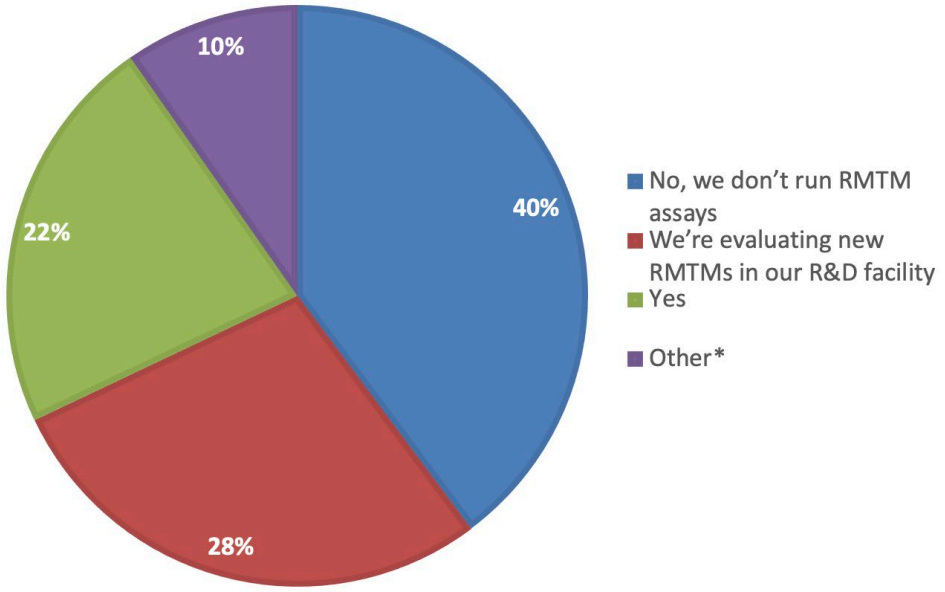
Question 3: What type of microbial testing do you currently perform? (multiple answers permitted, 102 responses)



Question 4: If you don't use rapid methods, why not? What are the bottlenecks in implementing rapid methods? (open answer, 33 responses, graphic: Infogram)

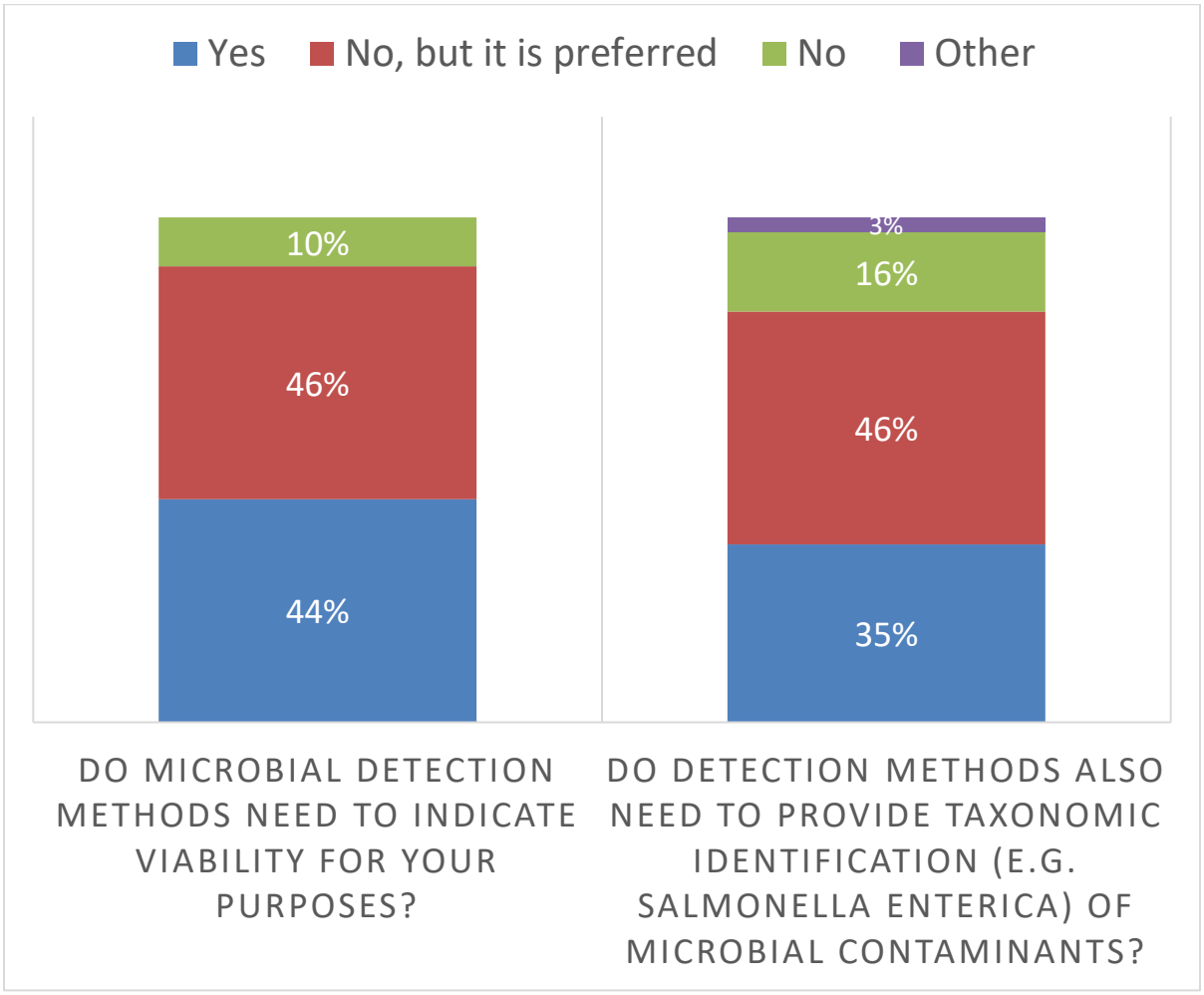


Question 5: Do you currently have a validated RMTM assay that you're using? (single answer response, 103 responses)



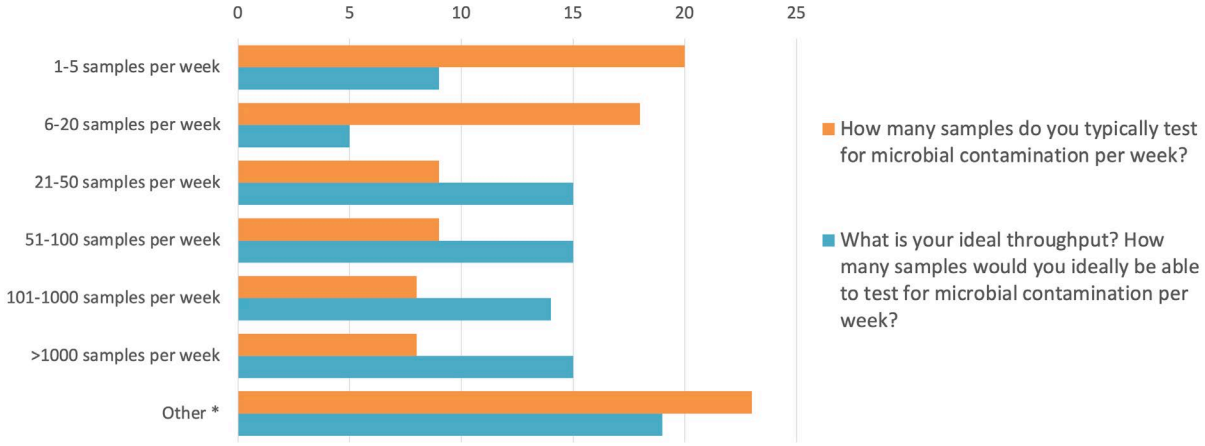
Other*: 3 responders are in Method development; 4 responders are not involved in testing but ancillary activities; 5 responders are not currently testing but have plans to in the future

Question 6 (single answer response, 104 responses)/ **Question 7** (single answer response, 103 responses):

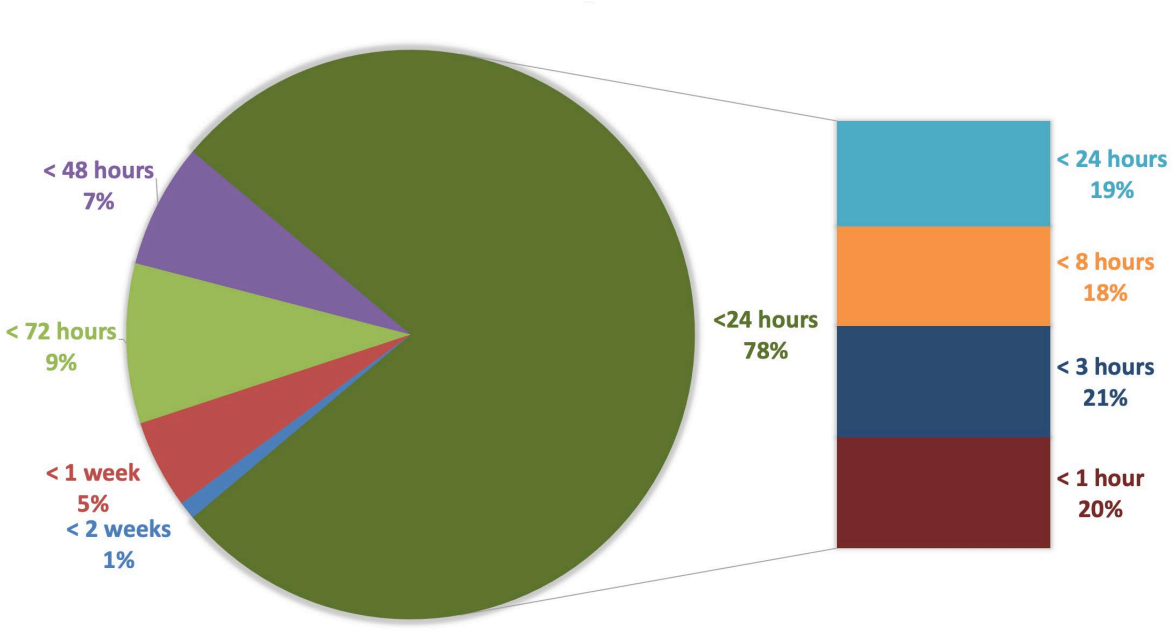


Note: Question 7 other responses included: sometimes, not sure, unapplicable, above a certain action level and/or depending on the area (A/B, C/D), and our customers are wanted strain level resolution to distinguish the microbes from the microbes they are using as a therapeutic and also to determine pathogen from non-pathogen.

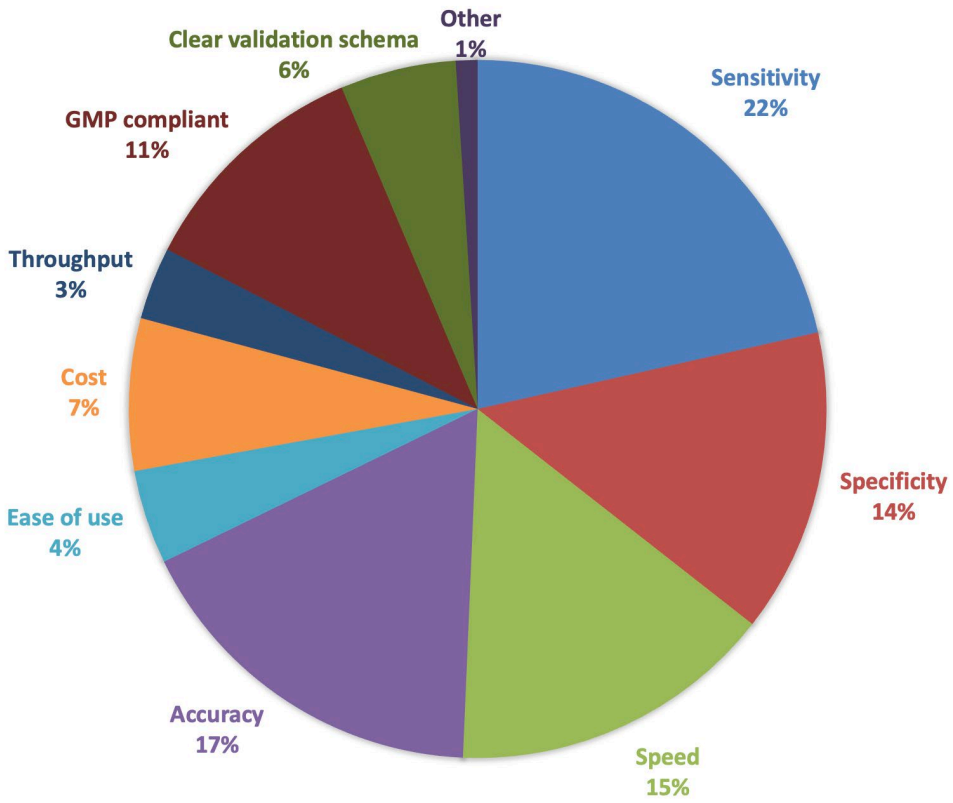
Question 8 (single answer, 95 responses)/ **Question 9** (single answer, 92 responses):



Question 10: What does rapid mean to your process? How rapid is necessary? (single answer, 99 responses)

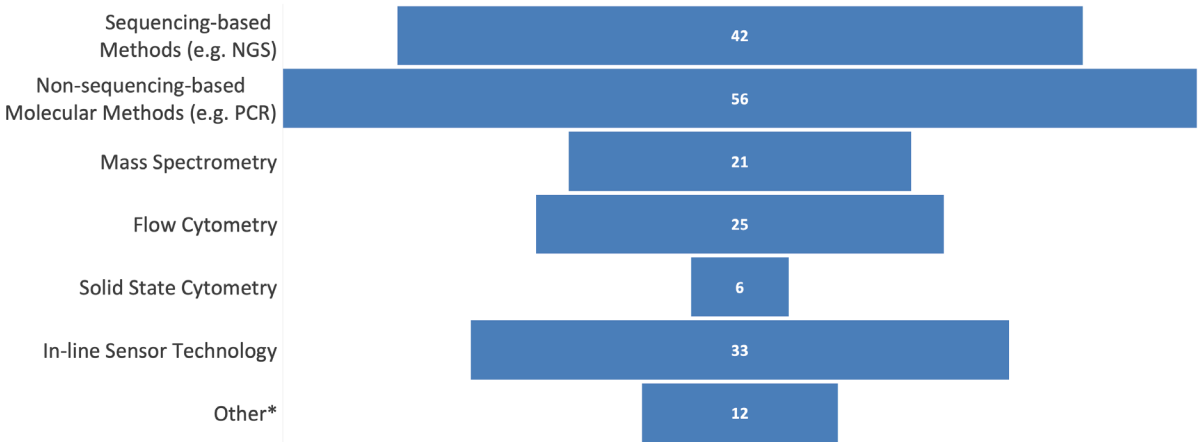


Question 11: Select the three most important attributes for a rapid microbial test method, based on your application(s). (Select up to 3 answers, 102 responses)



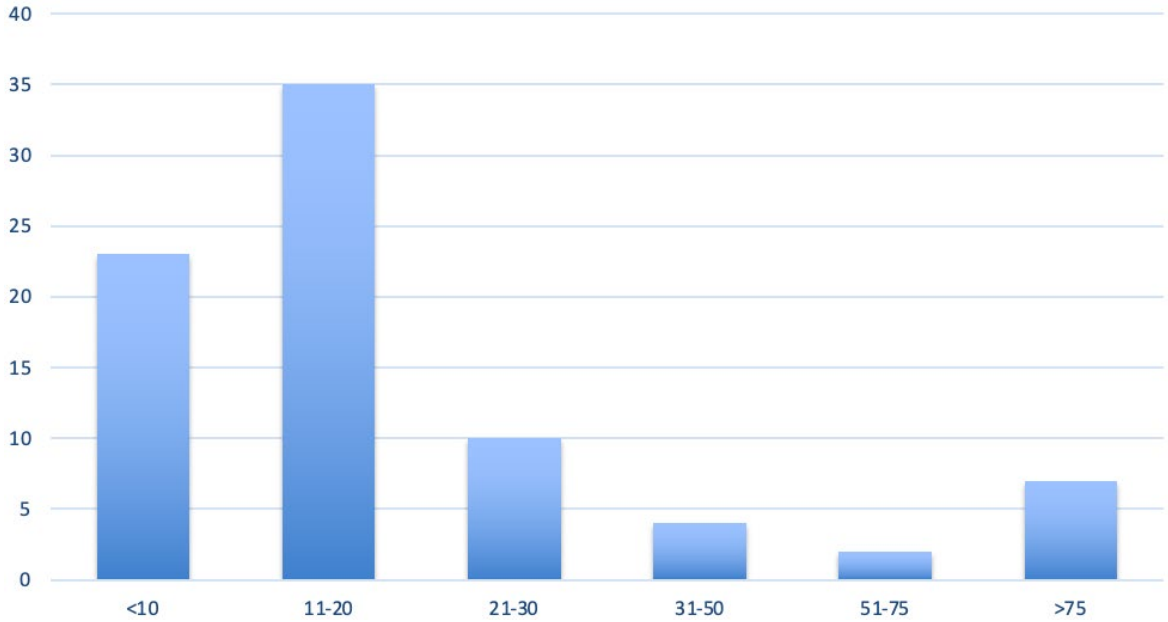
Other (1 response each): My actual answer is all of the above; Limit of detection; Automated early detection systems

Question 12: What rapid microbial measurement technologies are you most hopeful to be adopted in your industry? (multiple answers permitted, 98 responses)

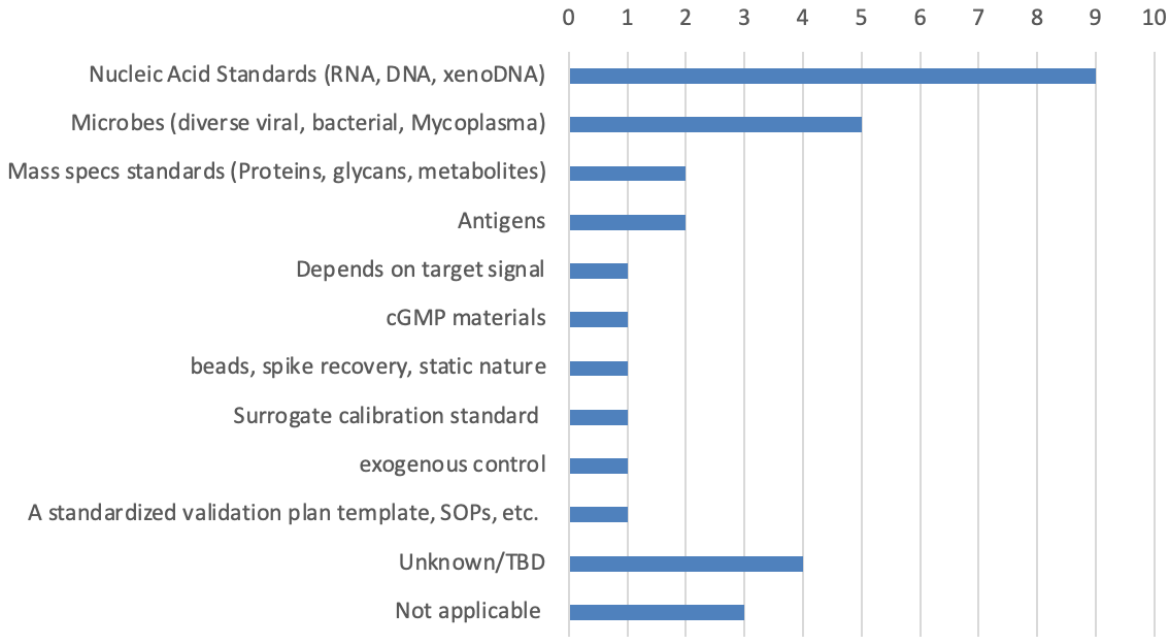


Question 13: Where do you currently acquire your reference materials for sterility testing? (open answer, multiple answers, 60 responses, graphic:Wordcloud)

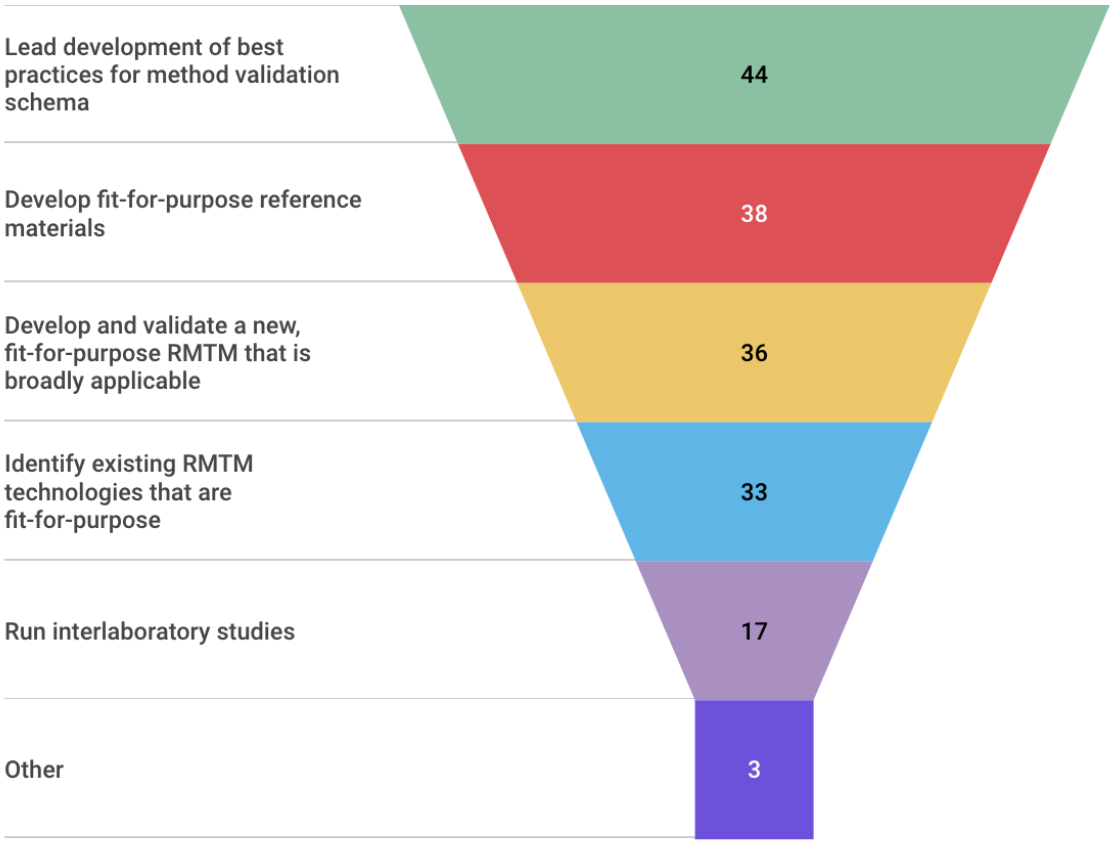
Question 14: How many species of microbes need to be represented in a microbial whole cell reference material designed to validate a new RMTM workflow? (single answer, 81 responses) *(Histogram of responses received as a function of number of species)*



Question 15: What reference materials (other than whole cell microbes) would be useful to accelerate assay validation? (open answer, 26 responses)



Question 16: What are the top two priorities that this Consortium should seek to address first? (select up to 2 responses, 93 responses)



Question 17: What are some specific actions that you think the Consortium should start with? (open answers, 29 responses) *(responses were compiled into 4 general categories)*

1. Mission statement and goals
    a. Define and prioritize remaining gaps
    b. Purpose and Scope
    c. Benchmark priorities
    d. Develop a scope, prioritize initiatives, assign a project manager, regular meetings to keep things moving forward so they don't languish
    e. Think different from sterility testing and move to rapid microbial screening
    f. Questioning the current concepts of rapid microbiological methods and rethink drug safety.

2. Surveys and stakeholder communication
    a. Collect information from product developers on what methods they used and how they validated those methods
    b. Recruit diverse and representative experts to join Consortium for significant impact
    c. Communicate with key stakeholders regarding specific method needs
    d. Initiate a national user survey to identify needs and gaps
    e. Collating samples from many manufacturers and sequencing them to better understand the microbes that affect these processes
    f. User Requirement Specification

3. Harmonization of protocols
    a. Develop a compendium of available methods and pros and cons
    b. Harmonization of RMTM technologies
    c. Standardizing rapid test protocols
    d. Validation
    e. Providing guidance for validation studies
    f. Sharing standards of validated assays
    g. Consideration of the application of these testing methods across different industries (for example, medical device versus LBT), and how the fit-for-purpose/compliance/validation schemes may differ.
    h. best practices for both fresh and frozen cell-based gene therapies (Investigational New Drugs – INDs / Investigational medicinal product - IMPs or products)
    i. Emphasize the need for product specific qualifications/validations and addressing uncertainty behind the recent revocation of 610.30
    j. make strong recommendations for preferred RMTM and fit-for-purpose RMs.
    k. What method and purpose of test
    l. setting standards for INDs and products that can be administered with less than perfect sterility test results (i.e., distinguishing no-go contaminants from ones that only pose a slight risk)
    m. Best reference in Mass Spectrometry.

4. Integration of guidance documents
    a. Confirm current guidance on validation strategies via online reference material (websites); PDA; USP; Ph. Eur.
    b. Guidance on testing methods that address 1271 guidelines.
    c. Achieve alignment of current guidelines for RMM validation i.e. in USP and EP.
    d. Submit and seek FDA acceptance/approval of the top candidates
    e. Best practices for decentralized, point-of-care, and centralized manufacturing models
    f. Finding ways to help industry streamline in process controls and lot release testing to ensure that the cells get to the patients who need them most

Question 18: What are your next steps regarding the Consortium? (single answer, 16 responses)