In Food & Agriculture, We Set the Standard

The Stakeholder Panel on Agent Detection Assays

Standards for Next Generation Sequencing Applications

AOAC INTERNATIONAL's Stakeholder Panel on Agent Detection Assays (SPADA) was formed in 2007 to provide leadership within the biothreat community for the development of consensus standards, analytical methods and method validation criteria for the detection of biothreat agents. Its unique mission brings together experts from across the biothreat and regulatory communities to create guidance for a comprehensive and uniform approach to scientific analysis and detection of biothreat agents. SPADA members include many of the world's foremost biothreat detection experts from the U.S. Department of Defense, U.S. Department of Homeland Security, U.S. Environmental Protection Agency, U.S. Health and Human Services, and stakeholders within academia and industries. Since its inception, SPADA has produced more than 23 standards and guidance documents in support of its mission.

Two of the most recent and significant accomplishments of SPADA have been the June 2020 publications on guidance for *in silico* analysis and bacterial strain verification[1,2]. The *in silico* analysis guidance was successfully implemented immediately upon publication in the AOAC Research Institute's Emergency Response Validation (ERV) of methods for detection of SARS-CoV-2 (the causative agent of Covid-19) on stainless steel surfaces, exemplifying the rapid pace of innovation and response to emerging threats. As recommended by the guidance document, not only were molecular methods assessed for their ability to distinguish inclusivity genomes from exclusivity and background genomes using curated sequences from high quality databases, the primers, probes and their target sequences were also evaluated for unimolecular folding and bimolecular hybridization. Without revealing proprietary sequences, these evaluations included estimation of the energy required for binding to occur, i.e. energy needed to first unfold secondary structures in order to allow binding of primers and probes to their target sequences, as well as the strength of the bound state under assay conditions as an assessment of the efficiency of the molecular assay. Special consideration was given to isothermal amplification methods that rely on loop structures for amplification.

Expanding on the success of the initial ERV project, a related project evaluating methods for detection of SARS-CoV-2 in wastewater is planned. Validated methods for environmental surveillance for such emerging threats provide confidence in the ability to monitor group quarters noninvasively for potential disease hot spots requiring close attention. As evidenced by the impact of COVID-19 on the readiness of

the USS Theodore Roosevelt[3], once symptoms of disease first appear, asymptomatic or pre-symptomatic individuals have likely already spread the virus, which happens more quickly in tight quarters. Reliable and rapid environmental monitoring methods could potentially detect the presence of virus being shed by personnel before symptoms are reported, leading to a more rapid response and diminished impact on the security of the nation.

AOAC INTERNATIONAL is now proposing to build upon these last two bodies of work through the development of consensus standards for Next Generation Sequencing (NGS) applications.

BACKGROUND

Next-generation sequencing (NGS) technologies have revolutionized the world of forensics, epidemiology, environmental surveillance, clinical diagnostics and microbial detection. The last ten years have brought witness to a broad array of high-efficiency, high-throughput platforms that can rapidly sequence partial and complete genomes with greater depth of coverage and accuracy. These advances have been accompanied by an evolution of bioinformatic applications, *i.e.* data "pipelines" and software to handle and analyze the vast amounts of data generated by such high-throughput sequencing. Routine use of metagenomic sequencing - parallel sequencing of DNA from all organisms within a community, with high coverage for species-level detection - is now within reach.

For pathogen identification, traditional microbiological techniques and even PCR applications have now been largely supplanted by genomic sequencing. The ability to identify, serotype, and evaluate phenotypic traits, e.g. pathogenicity and antimicrobial resistance (AMR) patterns, through genomic sequencing makes the "one test" concept a reality. In addition, as confidence and practicality in metagenomic applications increase, a near limitless capacity to assess complex microbial communities free from *a priori* knowledge of an etiologic agent will now be possible. This contrasts with current conventional and molecular methodologies and workstreams that typically rely on a targeted approach. For biothreat and regulatory communities, there are many advantages to the implementation of NGS *e.g.* preparedness, surveillance, source identification, and clinical diagnostics, to ward against potential threats (unculturable and emerging, and AMR typing) and to investigate outbreaks. These include rapid time-to-results, and the increased breadth of information gained from this "one-test" approach.

As with any significant advances in technology, adoption of routine NGS applications are hindered by questions of confidence in specificity and reliability of results.

For greater international acceptance of such an innovative technology, it is essential to evaluate standard sets of pre-analytical and analytical protocols for optimization and operation, particularly for those designed specifically for biothreat detection. This is important because there are many variables that markedly influence the performance characteristics of devices *i.e.* instrument platforms and diagnostic kits that incorporate NGS work streams.

AOAC 2021 Initiative

AOAC INTERNATIONAL is proposing the formation of a funded, multi-year standards development program to be initiated during the 2021 calendar year under the umbrella of SPADA to develop consensus standards and methods for the use and further development of NGS and bioinformatic applications.

In 2016, the FDA drafted the *Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers Draft Guidance* (hereafter "FDA NGS Guidance") for use to evaluate NGS biothreat agent detectors[4]. The Agency has also taken steps to create a database for infectious disease reference genomes that incorporates quality control metrics to ensure that expansion of this reference genome database meets the highest standards for accuracy[5].

While this guidance provides a good starting point for biothreat agent assays and its utility for biosurveillance, it is focused solely on clinical diagnostics. Through the present proposal, AOAC seeks to adapt and expand this concept to environmental surveillance of high priority unculturable and emerging biothreat pathogens. Though similar in scope, use of NGS applications for environmental surveillance will necessarily require the development of a unique set of performance requirements *e.g.* limits of detection, inclusivity/exclusivity panels, validation design, and acceptance criteria. Such consensus standards will form the foundation for future method development, provide guidance to detection assay manufacturers, and establish confidence in data obtained through a "one-test" NGS work stream.

To accomplish these objectives, AOAC INTERNATIONAL will integrate its working group process for adopting *Standard Method Performance Requirements*, SMPRs® with its premier conformity assessment programs, *i.e.,* the Official Methods Program and the *Performance Tested Methods* (PTM) Program. SMPRs® will document acceptance criteria - in precise detail - for method development and evaluation to include parameters such as inclusivity; exclusivity; interference/inhibition; probability of detect (POD) with confidence intervals ; and reproducibility (RSDR, inter-laboratory relative standard deviation). Whereas the Official Methods Program will be used to ensure and demonstrate method reproducibility, the PTM Program, specifically geared for kit-based methods, will be used to affirm, and certify that the method meets the established consensus performance standards (SMPRs) as well as manufacturing quality and labeling standards. Additionally, the Emergency Response Validation (ERV) process will be implemented to expedite the conformity assessment at one independent laboratory *(See the AOAC RI Policy page for more information on the PTM Program and its expert review process).* In concert, these two programs will provide well characterized methods - whether propriety or non-proprietary – that perform with a high level of confidence and reproducibility.

Proposal

This multi-year program will be divided into 4 phases that will encompass the AOAC-driven working group process for standards and methods development and the *AOAC Research Institute Performance Tested Methods* (PTM) *Program*. The projected cost for the overall program is **$417,000 USD.** *(See Attachment 1 for details.)*

Phase 1 *(Year 1)*

Working Group 1:

- Adapt the FDA NGS Guidance for use to evaluate NGS biothreat agent detectors.

Working Group 2

- Create a *Standard Methods Performance Requirement* (SMPR) for targeted and non-targeted NGS biothreat agent detectors.

Working Group 3

- Create validation criteria – confidence in reference genome database accuracy and expansion.
- Create validation criteria for the use of *in silico* processes:
    - All aspects of assay design.
    - Develop Confidence Parameters for the identification and phenotypic attribution of biothreat and genetically modified agents by NGS- bioinformatic applications.

Phase 2 *(Year 2)*

Working Group 4

- Develop validation criteria for genotype-to-phenotype determinations.

Working Group 5

- Conduct a gap analysis for the availability and performance suitability of all NGS biothreat-related detection assays to include targeted and metagenomic (non-targeted) approaches.

Phase 3 *(Year 3)*

AOAC Research Institute/Performance Tested Method Program (RI/PTM) Project

- Select NGS biothreat detection assay for evaluation using developed SMPR.
- Develop specific validation protocols tailored to the intended use and developed SMPR.
- Conduct *Performance Tested Methods* (PTM) review of selected NGS biothreat detection assay using the ERV framework..
- Assess and refine SMPR and NGS Guidance documents.
- Establish a self-sustaining PTM program for all future NGS biothreat detection assays.

Phase 4 *(Year4)*

- Implement permanent PTM program for evaluation of all future NGS biothreat detection assays.

Timeline

Months 1-3	Assemble Working Group 1 to review FDA Guidance document.
	Assemble Working Group 2 to develop SMPR.
	Assemble Working Group 3 to develop validation criteria – databases and in silico processes
Months 3-6	WG1 - Review and adapt Guidance for environmental testing.
	WG2 - Develop SMPR for NGS assays to detect biothreat agents in the environment.
	WG3 - Create validation criteria for the use of *in silico* processes: • Primer/probe design for targeted NGS. • Development of Confidence Parameters for biothreat agent genetic and phenotypic identification by NGS bioinformatic applications.
Months 6-9	Public review and comment period for Guidance and SMPR documents.
Month 10	SPADA Meeting and review of Guidance and SMPR documents.
	Assemble Working Group 4
	Assemble Working Group 5
Months 11 - 14	WG4 - Develop validation criteria – genotype to phenotype

	WG5 - Survey and select NGS assay to be evaluated.
Months 15 – 36	• Establish ERV framework and develop validation protocols • Conduct PTM review of selected NGS biothreat detect assay. • Assess and refine SMPR and NGS Guidance documents. • Create enduring PTM program for all future NGS biothreat detect assays.
Months 37 – 48	Permanent PTM program for NGS biothreat detect assays available for evaluation of all future NGS biothreat detect assays.

Benefits

For the warfighter

- AOAC INTERNATIONAL is a globally recognized standards and methods development organization that has served DoD and the warfighter since 2007 and has produced a significant portfolio of analytical tools needed to protect its fighting force from biothreats,
- AOAC INTERNATIONAL unites DoD leaders and scientists, academicians, and detection kit manufacturers and renowned scientists with the tech sector to establish analytical performance metrics that are needed to guide the development of those tools (methods) necessary to ensure the accuracy and precision of in-the-foxhole diagnostic applications to protect the warfighter against biothreats and ensure the well-being and safety of the warfighter.
- Rapid validation through the use of the ERV framework allows validated methods to be implemented sooner, increasing the confidence in biothreat surveillance.

For DHS communities

- Provide the performance metrics needed for the development of high-performance biothreat detection platforms needed to monitor and protect against potential biothreats among the general population.

For members of the biothreat and regulatory communities

- Ensure that your needs are met through AOAC INTERNATIONAL's unique consensus standards development process,
- Provide peer-reviewed publication of the outcomes for wider distribution and adoption by communities outside of AOAC/SPADA,
- Encourage the development of the *Performance Tested Methods* program, which provides the highest level of analytical confidence for kit-based methods,
- AOAC *Standard Method Performance Requirements* (SMPRs) and AOAC *Official Methods of Analysis*^{SM} are recognized as the benchmark for regulatory applications and biosecurity to ensure methods are appropriate for public safety and national confidence.

Method developers and laboratories

- Influence the development of consensus standards, which will be used by AOAC Expert Reviewers to evaluate your candidate methods for possible adoption as AOAC *Performance Tested Methods* in support of national biosafety surveillance efforts.
- Make use of the ERV process to obtain expedited ERV PTM certification.

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[1]*OMA Appendix Q: Recommendations for Developing Molecular Assays for Microbial Pathogen Detection Using Modern In Silico Approaches (http://www.eoma.aoac.org/app_q.pdf)*

[2]*OMA Appendix R: Guidelines for Verifying and Documenting the Relationships Between Microbial Cultures (http://www.eoma.aoac.org/app_r.pdf)*

[3]*An Outbreak of Covid-19 on an Aircraft Carrier.*
https://www.nejm.org/doi/full/10.1056/NEJMoa2019375

[4]*Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers Draft Guidance.*
https://www.fda.gov/regulatory-information/search-fda-guidance-documents/infectious-disease-next-generation-sequencing-based-diagnostic-devices-microbial-identification-and

[5]*FDA-ARGOS is a database with public quality-controlled reference genomes for diagnostic use and regulatory science. https://www.nature.com/articles/s41467-019-11306-6.*

APPENDIX 1

SPADA 2020 Program Cost Projections

Total Program Cost *(4 years):* $\underline{\$417,000\ USD}$

Year 1: $225,000 USD *(3 working groups)*

The base fee per working Group is $\underline{\$75,000}$ USD and includes:

- ***Advisory Panel Meeting****. AOAC will hold an Advisory Panel Meeting to identify renowned subject matter experts and to identify additional key authorities and experts to participate on AOAC working groups.*
- ***AOAC Stakeholder Panel Meeting****. Working Group Chairs will present the Working Group launch presentation and the stakeholders will refine fitness for purpose.*
- ***AOAC Working Group Meetings****. The Working Groups will hold a series of teleconferences, as needed, to complete the draft SMPR, adapt the FDA NGS guidance, and create validation criteria..*
- ***AOAC Stakeholder Panel Meeting****. Working Group Chairs will present draft SMPR and other working group documents for approval by the stakeholders. Stakeholders will deliberate and reach consensus on and thereby approve a final version of the SMPR, adapted NGS guidance, and validation criteria..*
- ***Publication Costs.*** *SMPR and guidance documents adopted by each working group (3) and approved by the stakeholder community will be published in The Journal of AOAC INTERNATIONAL.*

Year 2: $\underline{\$150,\ 000\ USD}$ *(2 working groups)*

Year 3: $\underline{\$42,\ 000\ USD}$ *(AOAC Research Institute/Performance Tested Method Program)*

- Select NGS biothreat detection assay for evaluation using developed SMPR (WG4, no-cost extension),
- Conduct *Performance Tested Methods* (PTM) review of selected NGS biothreat detect assay: **$\underline{\$32,000\ USD}$** (includes publication of manuscript and certification if successful),
- Assess and refine SMPR and NGS Guidance documents (WG 2 & 3, no-cost extension),
- Establish a self-sustaining PTM program for all future NGS biothreat detect assays: **$\underline{\$10,000\ USD}$**.

Year 4: $\underline{\$0.00}$ *(AOAC Research Institute/Performance Tested Method Program)*

- Permanent PTM program for NGS biothreat detect assays available for evaluation of all future NGS biothreat detection assays: Self-funded through application fees.

Additional fees (as applicable):

1. Application Fees for *Official Methods*^{SM} Review - $35,000 USD per method
   - Includes recruitment of Expert Review Panel (ERP) Members (Volunteer Experts)
   - Includes Preparation and Review of Methods for Review
   - Includes ERP Orientation and Facilitating ERP Meetings
     - Initial in-person meeting and, if methods are adopted, maintenance of ERP over the two (2) year method tracking period
   - Includes ERP review of Method Modifications during the 2-year tracking period
   - Includes Publications of methods and method manuscripts

2. Application Fees for Modifying or Extending a *Performance Tested Method* - $5000 or $10,000 per method, depending on the modification/matrix extension.
   - Includes publication of method modification manuscript

Optional Enhancements (per method):

- Consultation on validation test protocols: $3,000 USD
- Drafting Protocols & Review of Protocol: $3,000 USD
- Drafting of Method in AOAC Format: $2,000 USD
- Drafting of Method Manuscript in AOAC Format: $5,000 USD

Costs Not Covered:

- Travel of ERP members,
- Independent laboratory fees for method evaluation,
- New application fees for resubmission if ERP does not approve initial method submission.