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. Environmental Protection Agency, U.S. Health and Human Services, U.S. Centers for Disease Control, U.S. Food and Drug Administration, 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 2020 publication on guidance for *in silico* analysis and bacterial strain verification[1,2].

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 these devices.

AOAC 2020 Initiative

AOAC INTERNATIONAL is proposing the formation of a funded, multi-year standards development program to be initiated during the 2020 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 "NGS Guidance") for use to evaluate NGS biothreat agent detectors[3]. 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[4].

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, acceptance criteria, and validation design. Such consensus standards will form the foundation for future method development, provide guidance to detection assay developers, 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 program, the *Performance Tested Methods* (PTM) program within AOAC INTERNATIONAL's Research Institute (RI). SMPRs® document acceptance criteria in precise detail for method development and evaluation to include parameters such as inclusivity; exclusivity; interference/inhibition; limit of detection; bias; precision; repeatability (RSDr, intra-laboratory relative standard deviation); and,

reproducibility, (RSDR, inter-laboratory relative standard deviation). The mission of the AOAC Research Institute is to promote and carry out activities related to the development, improvement and validation of proprietary methods. Within the RI, the *Performance Tested Methods*[SM] (PTM) program provides an independent third-party review of proprietary test method performance. Test methods demonstrated to meet acceptable performance criteria are granted PTM status. Method developers of approved PTM test methods are licensed to use the PTM certification mark. The PTM certification mark affirms and certifies that an independent assessment has determined that the performance of a test method meets the established consensus performance standards (SMPRs) for the claimed intended use. *(See the AOAC RI Policy page for more information on the PTM Program and its expert review process)*.

Proposal

This multi-year program will be divided into 4 phases that will encompass AOAC-driven working group process and the *AOAC Research Institute/ Performance Tested Methods* (PTM) *Program*. The projected cost for the overall program is **$418,000 USD** *(See Attachment 1 for details)*

Phase 1 *(Year 1)*

Working Group1:

- Adapt the FDA's *Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers Draft Guidance* (hereafter "NGS Guidance") for use to evaluate NGS biothreat agent detectors,

Working Group 2

- Create a *Standard Methods Performance Requirement* (SMPR) for 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,
    - Development of 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

- 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)

- Select NGS biothreat detection assay for evaluation using developed SMPR,

- Conduct *Performance Tested Methods* (PTM) review of selected NGS biothreat detection assay.
- Assess and refine SMPR and NGS Guidance documents,
- Establishment of a self-sustaining PTM program for all future NGS biothreat detection assays.

Phase 4 *(Year4)*

- Permanent PTM program for NGS biothreat detection assays available for evaluation of all future NGS biothreat detection assays.

Timeline

Months 1-3	Assemble working group to review Guidance document.
	Assemble working group to develop SMPR.
Months 3-6	Review and adapt Guidance for environmental testing.
	Develop SMPR for NGS assays to detect biothreat agents in the environment.
	Create validation criteria for the use of *in silico* processes: • Primer design. • Development of Confidence Parameters for biothreat agent identify 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.
Month 11	Select NGS assay to be evaluated.
Months 12 – 36	
	• **Validation of genotype-to-phenotype determinations** • Survey NGS biothreat detect assays. • Select NGS biothreat detect assay for evaluation using developed SMPR. • Conduct *Performance Tested Methods* (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 biothreat and ensure the well-being and safety of the warfighter.

For DHS communities

- Provide the performance metrics needed for the development 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 standards consensus development process,
- Provide peer-reviewed publication of the outcomes for wider distribution and adoption by communities outside of AOAC/SPADA,
- Encourage the development of *Official Methods of Analysis* and *Performance Tested Methods* programs which provide the highest level of analytical confidence,
- AOAC *Official Methods of Analysis* are recognized as the benchmark for regulatory applications and biosecurity to ensure public safety and national confidence.

Method developers and laboratories

- Influence the development of consensus standards, which will be used by AOAC Expert Review Panels to evaluate your candidate methods for possible adoption as AOAC *Official Methods of Analysis* and *Performance Tested Methods* in support of national biosafety surveillance efforts.

Contact Information

Palmer A. Orlandi, Jr., Ph. D.
Deputy Executive Director & Chief Science Officer
AOAC INTERNATIONAL
2275 Research Blvd., Suite 300
Rockville, MD 20850
Tel: 301-924-7077; ext. 163
porlandi@aoac.org www.aoac.org

[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] *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*

[4] *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):* $418,000 USD

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

The base fee per working Group is $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(s).*
- ***AOAC Stakeholder Panel Meeting****. Working Group Chairs will present draft SMPRs for approval by the stakeholders. Stakeholders will deliberate and reach consensus on and thereby approve a final version of the SMPR(s).*
- ***Publication Costs.*** *SMPRs adopted by each working group (3) and approved by the stakeholder community will be published in The Journal of AOAC INTERNATIONAL.*

Year 2: $150, 000 USD *(2 working groups)*

Year 3: $43, 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: **$33,000 USD**,
    - Application fee**, $15,000 USD**
    - Laboratory fee, **$15,000 USD**
    - Consultant fee, **$ 3,000 USD**
- Assess and refine SMPR and NGS Guidance documents (WG 2 & 3, no-cost extension),
- Establishment of a self-sustaining PTM program for all future NGS biothreat detect assays: **$10,000 USD**,

Year 4: $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 an Official Method of Analysis - $10,000 per method
   - 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 during the 2-year tracking period, if required
   - Includes Publications of methods and method manuscripts

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 and coordination of laboratory work if needed. New application fees for resubmission, if ERP does not approve initial method submission.