S&T Bioaerosol Threat Detection Capability Improvements

February 6, 2023

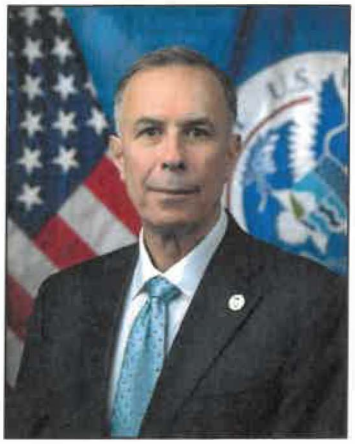
Fiscal Year 2022 Report to Congress

Homeland Security

Science and Technology Directorate

Message from the Office of the Under Secretary for Science and Technology

February 6, 2023



I am pleased to submit the following report, "S&T Bioaerosol Threat Detection Capability Improvements," which was prepared by the Science and Technology Directorate (S&T).

This document was compiled pursuant to direction in the Joint Explanatory Statement that accompanies the Fiscal Year (FY) 2022 Department of Homeland Security (DHS) Appropriations Act (P.L. 117-103). Included is an overview.

Pursuant to congressional requirements, this report is being provided to the following Members of Congress:

The Honorable Dave Joyce
Chairman, House Appropriations Subcommittee on Homeland Security

The Honorable Henry Cuellar
Ranking Member, House Appropriations Subcommittee on Homeland Security

Chair, Senate Appropriations Subcommittee on Homeland Security

Ranking Member, Senate Appropriations Subcommittee on Homeland Security

Inquiries about this report may be directed to the Office of Legislative Affairs at (202) 447-5890.

Sincerely,

Dimitri Kusnezov, Ph.D.
Under Secretary
Science and Technology

Executive Summary

This report provides information on the development of technologies for improving detection capabilities for aerosolized biological threats and describes the progress that S&T made to develop and test a prototype digital Matrix-Assisted Laser Desorption and Ionization (digitalMALDI) Triggered Mass Spectrometer used to test air samples retrieved from multiple operational demonstration venues. This new tool will help to enable near-real time detection of aerosolized biological threat agents, including bacteria, viruses, toxins, and biologically active chemicals.

The purpose of this report is to provide members of Congress with a comprehensive update on S&T's activities to improve the detection of aerosolized biological threats in FY 2022. Specifically, this report details the effort, Triggered Mass Spectrometry for Environmental Surveillance, a Small Business Innovation Research Phase III effort, that S&T's Office of Mission and Capability Support is sponsoring within the Defense Technologies activity. DHS S&T currently is executing the fourth and final option period of performance of the awarded contract, under which it is developing a prototype device that combines a trigger and detector function, based upon the Matrix-Assisted Laser Desorption/Ionization – Time-of-Flight Mass Spectrometry (MALDI-TOF MS) analysis technique. The technique adapts the classical laboratory method of MALDI-TOF MS by enabling the technique to be applied to individual aerosol particles "on the fly," which allows for individual mass spectra to be generated particle by particle, rather than from a collected aggregate sample. This adaptation of the classical MALDI-TOF MS technique is dubbed "digitalMALDI" by the performer.

The digitalMALDI approach allows for much less-intensive data processing to extract spectral features of interest (i.e., those of an aerosolized biological threat agent) from those of the background (i.e., innocuous environmental particulates). When combined with advances in computing power, and implementation of innovative data processing algorithms, the digitalMALDI technique shows promise in enabling the presumptive identification of an aerosolized biological threat agent within minutes of an aerosol being sampled by the device. If realized, this approach may represent an evolutionary leap that will enable the rapid, sensitive, and specific sensing of biological threat agent aerosols. Additionally, the MALDI-TOF MS technique is capable of detecting multiple forms of biological threats, to include vegetative bacteria, bacterial endospores, viruses, and toxins, and the digitalMALDI technology may allow for detection of multiple biological threat agent types in a single platform, which cannot be accomplished, or can be accomplished in only a limited fashion because of technical constraints, using current biological detection systems. The digitalMALDI technology may be a potential candidate for technology transition to the Countering Weapons of Mass Destruction Office for advanced development and injection into the Biological Detection for the 21st Century acquisition.

The content of this report covers all initiatives (i.e., contract periods of performance) from the base award to the option period currently under execution to demonstrate the progress that the performer has made since the FY 2018 base contract award. The background section includes a high-level breakdown of funding by contract period and fiscal year in Table 1. Section III

provides a breakdown of funding by contract period, associated deliverables, and accomplishments in Table 2. The expenditure data presented in this report has a reporting cutoff date of June 10, 2022.

S&T Bioaerosol Threat Detection Capability Improvements

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I. Legislative Language

The report was compiled pursuant to direction in the Joint Explanatory Statement that accompanies the Fiscal Year (FY) 2022 Department of Homeland Security (DHS) Appropriations Act (P.L. 117-103), and which states:

> *Improving Detection Capabilities for Aerosolized Biological Threats*.—Within 90 days of the date of enactment of this Act, S&T shall provide a report on DHS future plans to complete advanced development, transition, fielding and sustainment of these critical detection capabilities for aerosolized biological threats.

II. Background

The DHS Science and Technology Directorate (S&T) is the primary research and development (R&D) agency of DHS; providing federal, state, local, tribal, and territorial (FSLTT) officials with the technology and capabilities to protect the homeland. The Nation's national infrastructure is vulnerable to biological attacks on major facilities such as large office buildings, subways, stadiums, and airports. A biological threat agent dispersed as an aerosol through a facility's heating ventilation and air conditioning (HVAC) system or in an enclosed area could spread quickly before the attack is recognized and an appropriate response is activated. S&T is developing and testing biological aerosol sensors that can alert to the release of aerosolized biological threat agents within minutes. S&T intends to mature and transition this technology to the Countering Weapons of Mass Destruction Office (CWMD) for acquisition, operational deployment, and sustainment to improve facility biological threat detection capabilities and to support DHS's mission to secure America.

Biological attacks remain a significant threat because nation states and terrorist groups alike are pursuing biological weapons. CWMD's Biological Detection for the 21st Century (BD21) major acquisition program is addressing the goals of the 2018 National Biodefense Strategy and is attempting to alleviate critical biodetection capability gaps in the Nation's ability to detect intentional releases of biological threat agents. BD21 is critical to our ability to counter such attacks effectively through swift detection and response.

Expertise within S&T and CWMD is being utilized to understand the maturity of the BD21 concept. S&T leverages not only previous R&D discoveries, but also executes programs, projects, and activities aligned with the CWMD Strategic Plan's Objectives 1.3 and 2.3 to fill knowledge gaps in the biothreat space.[1]

The technology being developed under the Triggered Mass Spectrometry for Environmental Surveillance effort described in this report addresses two crucial areas: trigger sensors and presumptive identification sensors. Trigger sensors are able to determine rapidly if an aerosol is biological in origin. Trigger sensors can initiate a "low regret" alarm and activate a nearby presumptive identification sensor or can initiate changes to air flows to minimize the spread of a potential contaminant. Presumptive identification sensors autonomously collect and process air samples when initiated by a trigger alert and, within 15 minutes, can determine if the collected aerosol may contain a biological threat. Presumptive identification sensors provide first responders with a basis for "high regret" alarms to initiate building evacuation and public health response.

The technology described below combines trigger and presumptive identification sensors into an integrated platform. Although the technology shows promise, S&T must demonstrate several technical metrics—including rapid sensing in cluttered backgrounds; coverage for up to 20 biological threat agents; and reliability, availability, and maintainability data—before

[1] DHS, *Countering Weapons of Mass Destruction Strategy 2020 – 2024*, pp. 5, 7, and 9.

transitioning technologies to DHS Components. According to a recent independent assessment, no significant risks to maturing the system to meet these metrics are anticipated.[2]

S&T conducts research to assess, prevent, detect, prepare for, respond to, and recover from incidents involving chemical and biological (CB) threats and hazards. This program:

- Supports DHS, its Components, and the Homeland Security Enterprise with risk awareness, knowledge products, and technical solutions needed to protect the Nation from incidents involving CB hazards, and
- Executes and enhances a portfolio of capabilities and supporting activities to counter CB threats.

S&T's Chem-Bio Threat Surveillance & Defense Project primarily focuses on developing effective surveillance, prevention, and operational capabilities for detecting and countering biological threats. S&T takes a system-level approach to integrating information into surveillance architectures, to developing and testing advanced detection systems, and to implementing a cross-domain focus on biological, chemical, and agricultural threats. Activities within the project focus on the following:

- Further development, demonstration, and delivery of a proof-of-concept surveillance system for identifying endemic, trans-boundary, and emerging disease outbreaks in livestock;
- Development of affordable, effective, and rapid detection systems and architectures to provide advance warning of a biological attack at indoor, outdoor, and national security events; and
- Development of a Common Operating Picture Platform prototype that integrates multiple data streams to support decision-making during a biological event as well as to inform training tools for state responders.

The Defense Technologies: Triggered Mass Spectrometry for Environmental Surveillance (i.e., digital Matrix-Assisted Laser Desorption and Ionization (digitalMALDI) activity effort) is developing and testing a single-particle, time-of-flight mass spectrometer as a combined trigger sensor and presumptive identifier to detect aerosolized biological threat agents. S&T's Office of Mission and Capability Support and CWMD's BD21 Program are working together to develop, test, and deploy new technologies that will decrease the time required to detect a biological incident. The currently deployed BioWatch system:

- Requires 12 to 36 hours to detect an aerosolized biological threat agent;
- Is limited to detection of a relatively low number of legacy priority biological threat agents; and
- Lacks the capacity to be expanded to address new biological threat agents as emerging pathogens are identified and added to the Biological Select Agents and Toxins list.

[2] DHS Systems Engineering and Standards Division, *Technology Readiness Assessment Report*, September 2022.

S&T has a need to develop and transition technologies to BD21 that can decrease this timeline significantly, thereby enabling a more rapid response resulting in decreased casualties and that readily can be expanded to identify new biological threat agents as they are listed. In addition, S&T has a need to develop and transition technologies to FSLTT partners that can detect the release of a biological threat agent rapidly to enable a rapid response. More specifically, the technology being developed under the effort described here will improve current capabilities for BD21 and FSLTT partners in at least three critical ways:

- Reduce the time that it takes to detect the presence of an aerosolized biological threat agent following its release from the current 12 to 24 hours down to minutes;
- Leverage the increasing wealth of biological threat agent genomic and proteomic information available through modern bioinformatics techniques to increase the number of biological threat agents that can be detected from the current 5 to 20 or more, and provide the capability to support whole-of-government response actions to develop and deploy rapidly technologies that detect emerging pathogens that achieve breakthrough into the global population and that present a pandemic threat to public health, safety, and the economy (e.g., Coronavirus Disease 2019 [COVID-19]); and
- Lower operations and maintenance cost on a per-sensor basis.

This type of sensor approach is made feasible by new developments in trigger technologies and Matrix-Assisted Laser Desorption/Ionization – Time-of-Flight Mass Spectrometry (MALDI-TOF MS). The objective is to develop a sensor technology that provides presumptive identification analysis in the field within seconds to minutes. The scope of this work is to develop and test a novel prototype sensor technology that enables near-real time detection of aerosolized biological threat agents including vegetative and sporulated bacteria, viruses, and toxins. The prototype sensor will be a unity of two component technologies that together have sample collection and preparation functions and trigger and detector functions.

The technology will ingest and sort individual airborne biological threat agents (herein referred to as particles), will coat these particles with MALDI matrix, and will send them into the detector one particle at a time. The advantage of sending one particle at a time into the detector is that it can be operated in environments with potentially heavy particle backgrounds, such as subways, convention centers, and airports. The MALDI-TOF MS produces a single spectral "molecular fingerprint" for each particle, independent of potential confounding spectral signals from environmental background "clutter," which greatly simplifies the analysis of generated spectra.

As the particle enters the detector, it trips a laser-trigger that initiates activation of an ionization laser. The ionization laser pulse interacts with the MALDI matrix, causing the particle to vaporize and generate ionized fragments. The ionized fragments produced are unique to individual species of bacteria and viruses, as well as to individual protein toxins, and affect a detector within the MALDI-TOF MS mass analyzer, which generates a unique and repeatable spectral signature for a given biological threat agent. Comparison of individual particle MALDI-TOF spectra against a library of known biological threat agent MALDI-TOF spectra allows for extremely rapid presumptive identification of a biological threat agent with high confidence, potentially after a very small number of particles (on the order of 10s) have been sampled, and with the potential for extremely low false alarm rates. The adaptation of MALDI-TOF MS

technology to generate molecular fingerprints from an individual particle is dubbed "digitalMALDI" by its creators. This novel technological approach offers potential for continuous air monitoring and the ability to identify biological particles indicative of a bioterrorism event rapidly and confidently with limited human intervention required.

digitalMALDI Accomplishments

- The digitalMALDI effort successfully completed its Base Option and Options 1, 2, and 3 periods of performance (POP). The Option 4 POP is being executed currently.
- Capture of ambient aerosol background samples and particle concentration data from two mass transit facilities leveraged CWMD's BD21 Technology Demonstration Program and S&T's Chem-Biodefense Testbed (CBT) sites. The collected samples and data were used to create synthetic aerosols in a laboratory setting to demonstrate prototype mass spectrometer performance and synthetic data processing and analysis techniques in lieu of planned deployment for operational test and evaluation (OT&E), which was disrupted by impacts of the COVID-19 pandemic.
- digitalMALDI prototype instruments were designed, fabricated, integrated, and refined, and the first-generation (Gen 1) Triggered Mass Spectrometer (TMS) prototype instrument was deployed to a mass transit facility and a large event venue for OT&E:
    - Deployment to a mass transit facility leveraged a BD21 Technology Demonstration Program site for extended OT&E.
    - A rigorous and independent technology readiness assessment (TRA) of the Gen 1 prototype conducted by the DHS S&T Office of Science and Engineering (OSE) Systems Engineering and Standards (SES) Division resulted in a "high" Technology Readiness Level (TRL) 5 being assigned to the prototype in which the prototype also met many DHS TRL 6 criteria.[3] This is an example of another successful matrix partnership established within S&T.
    - More than 5 million single particle spectra were captured to date during these deployments.
- Resulting OT&E data sets have been analyzed by the S&T Chemical Security Analysis Center and have been shared with BD21 stakeholders.
- Engagement with BD21 Program personnel is driving the current Option 4 POP scope to reduce the size of the second-generation (Gen 2) TMS prototype.

[3] See the *DHS TRA/Manufacturing Readiness Assessment Guide* of May 2022. The guidance and methods in the *DHS TRA* conform with Government Accountability Office TRA best practices.

History of Funding to Date

Table 1. Triggered Mass Spectrometry for Environmental Surveillance Budget and Expenditures by Fiscal Year and Period of Performance

Fiscal Year	Period of Performance	Funding Obligated	Funding Expended
FY 2018	Base Period	$1,500,000	$1,500,000
FY 2019	Option Period 1	$1,509,711	$1,509,711
FY 2020	Option Period 2	$2,002,512	$2,002,512
FY 2021	Option Period 3	$915,422	$915,422
FY 2022	Option Period 4	$1,374,061	$418,020*
Total		**$7,301,706**	**$6,345,665**
*Expenditures as of June 10, 2022			

Past Challenges and Progress Made to Address Challenges

- CWMD requirements:
  - Continued engagement with BD21 stakeholders via the S&T OSE Office of Requirements Analysis and the CWMD Portfolio Manager to obtain validated requirements for current and future scope.
- COVID-19 impacts to supply chain and ability to conduct OT&E:
  - Use of no-cost extensions (NCE) to extend POPs to allow for delayed delivery of materials and components from vendors.
  - Use of alternate methods to satisfy OT&E by obtaining aerosol samples from OT&E environments to use in laboratory settings with simulant spiking, and fusion of background particle spectral data with laboratory-acquired simulant spectral data to produce synthetic datasets.
- Lack of ability to conduct OT&E with simulants:
  - Use of synthetic datasets described above to test data processing and analysis algorithms.
- Lack of ability to conduct testing with biological threat agents:
  - Planning for follow-on R&D to conduct live or inactivated biological threat agent testing under appropriate biological safety level (BSL) containment laboratory settings.
- Front-end aerosol sampling technologies to enhance overall system sensitivity:
  - Commission independent survey for available commercial off-the-shelf (COTS)/government off-the-shelf (GOTS) technologies that may meet Option 4 scope objectives.
  - Accept risk that no such technologies exist and develop alternative aerosol sampling solutions.

The independent TRA of the Gen 1 digitalMALDI prototype found that TRL 5 criteria were met, or exceeded, and that many TRL 6 criteria also were met. The TRA team did not note any major risks to the maturation and manufacturing of the system. Findings captured in the TRA report

will inform subsequent scopes required to mature the technology in preparation for transition to the BD21 Program for advanced development and for potential acquisition, deployment, and sustainment. The TRA report will be shared with BD21 stakeholders.

III. Results and Expenditure Plan

The digitalMALDI effort currently is executing its fourth and final option period of contract performance. S&T issued a 3-month NCE to the Option 4 POP to commission an independent survey of available COTS/GOTS aerosol sampling devices; these devices will assist performers with achieving their Task 11 scope. The independent survey is being conducted by Battelle Memorial Institute. Per the Option 4 statement of work, the Task 11 scope follows:

> The contractor analyzes the existing Wetted Wall Cyclone (WWC) system and identifies opportunities to improve collection efficiency. The two primary objectives are to decrease the output fluid flow by at least 10x (i.e., reduce the flow rate from 100µl/min to 10µl/min) ±20 percent and to increase the consistency of the output flow. Considerations shall include design optimization of the WWC fluidics and potential addition of an aerosol concentrator. The contractor conducts an analysis of alternatives for commercially available collectors and produces a final prototype of the Gen 2 TMS sensor.
>
> **Milestone:** One Gen 2 TMS prototype with increased aerosol sampling efficiency. Delivery of a Task 11 test report and test data package 10 months following execution of Option 4.

The current contract option period is focused on identifying potential improvements to the efficiency and sensitivity of the aerosol sampling device utilized by the current Gen 1 digitalMALDI prototype to optimize this technology for development of a Gen 2 digitalMALDI prototype design.

The recently completed TRA final report provided additional analyses of the current WWC's performance. Importantly, the TRA determined that the Gen 1 digitalMALDI prototype met or exceeded TRL 5 criteria and met many TRL 6 criteria. The TRA captured recommendations for maturation activities needed to achieve TRL 6 if follow-on development activities are funded. The TRA also noted numerous benefits of using the MALDI-TOF MS technology for biodetection.

S&T also is working with CWMD to coordinate with the CWMD BD21 stakeholder community to identify opportunities and requirements for additional maturation of the digitalMALDI technology to serve as a potential technology injection into the BD21 acquisition. S&T's vision is that the digitalMALDI technology would serve as both a trigger and presumptive identification subsystem for indoor environmental biological aerosol monitoring of the overall BD21 system.

Please refer to Table 1 for a high-level expenditure plan covering all contract POPs. A more detailed description of contract activities, obligations, expenditures, objectives, deliverables, and accomplishments follows below in Table 2.

Table 2. Breakdown of Funding by Contract Period, Associated Deliverables, and Accomplishments

Title	**Triggered Mass Spectrometry for Environmental Surveillance Base Award**
Duration	15-month POP
Task Objectives and Deliverables	**Task 1:** Project management (monthly and quarterly reports; active across all awards/POPs) **Task 2:** Design of subsystem components; preliminary design review **Task 3:** Fabrication of subsystem components; integration of subsystem components into an initial prototype instrument; testing of initial prototype with simulants in a laboratory setting; and critical design review
Accomplishments	A prototype instrument was designed and fabricated, and demonstrated during laboratory testing with simulants the ability to satisfy goals of 90-percent probability of detection of biological particles of interest at a concentration of 2,000 particles per liter of air in fewer than 15 minutes necessary for a successful Go/No Go decision for Option 1 contract award.
Obligations	$1,500,000
Expenditures	$1,500,000

Title	**Triggered Mass Spectrometry for Environmental Surveillance Option 1 Award**
Duration	9-month Option 1 POP
Task Objectives and Deliverables	**Task 1:** Project management (monthly and quarterly reports; active across all awards/POPs) **Task 4:** Refinement of prototype subsystem components and integration of refined subsystem components into a refined prototype instrument **Task 5:** Delivery of test and evaluation report
Accomplishments	Two prototype instruments were produced incorporating refinements based on the Base POP critical design review to upgrade subsystem components. The first prototype, TMS-1, was integrated into a mobile cart to test concepts for planned deployment of prototype instruments for OT&E at an independent testbed in subsequent option POPs and was used to conduct integrated system level testing in a laboratory setting. The second prototype, TMS-2, served as a laboratory testbed of improved subsystem components and testing of enhanced data acquisition and processing protocols; these improved components then were integrated into the TMS-1 prototype for integrated system level testing. Laboratory integrated system level testing demonstrated the

	ability of the integrated prototype to distinguish presence of simulants in a mixture of simulant and background materials.
Obligations	$1,509,711
Expenditures	$1,509,711

Title	**Triggered Mass Spectrometry for Environmental Surveillance Option 2 Award**
Duration	9-month Option 2 POP
Task Objectives and Deliverables	**Task 1:** Project management (monthly and quarterly reports; active across all awards/POPs). **Task 6:** Improvement of prototype subsystem components based on OT&E data from Option 1 POP; integration of improved subsystem components into improved prototype instrument. **Task 7:** Delivery of an improved prototype instrument to an independent testbed; delivery of a test and evaluation report.
Accomplishments	An improved prototype instrument, TMS-3, was fabricated. Because of disruptions caused by the COVID-19 pandemic, it was not possible to deploy the TMS-3 prototype instrument to an independent testbed for OT&E as planned under this option period. S&T worked with the performer to develop an alternative plan to deploy aerosol monitoring and collection devices to two separate mass transit facilities, leveraging the BD21 Technology Demonstration Program's site at Newark Liberty International Airport and the S&T CBT site at Grand Central Station in New York City, to capture ambient background aerosol samples and particle concentration data. These samples and data were utilized at the performer's laboratory to generate aerosols spiked with simulants, which demonstrated the ability of the prototype instrument to detect simulants in aerosol mixtures anticipated to be similar to those encountered in operational settings intended for OT&E. The ambient background aerosol concentration data collected was used to demonstrate the process of combining laboratory-acquired simulant spectra with the real-world ambient particle data to develop synthetic data sets. These synthetic data sets were used to test data processing and analysis protocols and to demonstrate improved signal-to-noise ratio that will allow for rapid and sensitive detection of anomalous biological events.
Obligations	$2,002,512
Expenditures	$2,002,512

Title	**Triggered Mass Spectrometry for Environmental Surveillance Option 3 Award**
Duration	6-month POP
Task Objectives and Deliverables	**Task 1:** Project management (monthly and quarterly reports; active across all awards/POPs). **Task 8:** Delivery of an improved prototype instrument to the operational demonstration testbed; delivery of an operational demonstration report.
Accomplishments	The TMS-3 prototype instrument was deployed to the BD21 Technology Demonstration Program's Newark Liberty International Airport site on August 16, 2021. The TMS-3 prototype instrument was operated via a mixture of in-person and remote control through December 3, 2021, and a total of 3,139,407 single particle spectra were captured with no anomalous events detected in the ambient background. This OT&E event demonstrated the ability of the TMS prototype to be operated remotely for extended periods of time in a setting representative for operational deployment of a production model instrument. The performer demonstrated the ability to create synthetic data sets utilizing the ambient background data and laboratory-generated simulant data to develop refined signal processing algorithms that demonstrate separation of a signal (simulant) from noise (ambient background) with as few as 5 to 10, on average, spectra, which indicates that the instrument is capable of achieving highly sensitive detection with low probabilities of false alarm.
Obligations	$915,422
Expenditures	$915,422

Title	**Triggered Mass Spectrometry for Environmental Surveillance Option 4 Award**
Duration	9-month POP (extended at no cost to 12 months)
Task Objectives and Deliverables	**Task 1:** Project management (monthly and quarterly reports; active across all awards/POPs). **Task 9:** Development of a manufacturing and commercialization plan; development of a comprehensive test plan; delivery of a comprehensive test plan; delivery of a manufacturing and commercialization plan. **Task 10:** Delivery of a prototype instrument to an operational demonstration testbed; delivery of an operational demonstration report. **Task 11:** Improvement of aerosol sampling subsystem efficiency: Reduction of fluid sample output by at least 10x from that of the previous subsystem design; improved consistency of sample output volume from that of the previous subsystem design; and delivery of an improved aerosol sampling test report and test data package.

	Task 12: Improvement of matrix coating performance: Selection of a liquid matrix compound as an alternative to a solid matrix compound; 1-month deployment of an improved matrix coating process utilizing a liquid matrix compound to an operational testbed; delivery of an improved matrix coating test report and test data package. **Task 13:** Development of a reduced size prototype design: Threshold of 25-percent reduction in instrument volume; objective of 50-percent reduction in instrument volume; delivery of a reduced size concept design package, an analysis of alternatives report, a comparative performance test report, and a prototype instrument incorporating upgrades developed under Option 4.
Accomplishments	The TMS-3 prototype was deployed to a large venue operational demonstration testbed at Capital One Arena in Washington, D.C., on February 24, 2022. TMS-3 is being operated completely via remote control, and initial lessons learned resulted in the performer incorporating additional upgrades to the TMS-3 prototype instrument. To date, 4,095,924 single particle spectra have been captured under a variety of different testbed facility HVAC venue configurations (e.g., various types of sporting events, concerts, and non-event days). OSE/SES is completing a TRA of the TMS-3 prototype on the basis of data acquired under laboratory settings and from the operational demonstration deployments. The TRA determined that the TMS-3 prototype met, or exceeded, TRL 5 requirements, and met many TRL 6 requirements. S&T is capturing recommendations for activities to complete maturation to TRL 6 and supplemental information to support execution of Task 11 scope. CWMD R&D staff have requested a copy of the TRA report once the document is completed. The TRA was completed in September 2022, and the associated report is undergoing revisions by OSE/SES per request from the CWMD Portfolio Manager to make minor edits to style prior to being accepted for transition in the first quarter of FY 2023.
Obligations	$1,374,061
Expenditures	$418,020 (as of June 10, 2022; on track for completion in the first part of FY 2023)

IV. Discussion

Biological attacks remain a significant threat because nation states and terrorist groups alike are pursuing biological weapons. CWMD's BD21 major acquisition program is addressing the goals of the 2018 National Biodefense Strategy and is attempting to alleviate critical biodetection capability gaps.

The BD21 value proposition includes the following: (1) identify threats in a timely manner; (2) enable the initial response sooner; (3) inform decisions for the U.S. Department of Health and Human Services and other senior government officials to mobilize medical countermeasures sooner; (4) enable more effective post-attack Federal Bureau of Investigation investigations; and (5) provide a common operating picture to FSLTT stakeholders.

The S&T digitalMALDI effort described in this report represents development of a potential enabling technology for transition as a technology injection into the BD21 acquisition program. This technology could address the requirement for a bioaerosol surveillance device that would yield improvements to the timeliness of alerting to the presence of a potentially hazardous biological aerosol within minutes. However, the digitalMALDI technology still requires additional maturation to reduce the size of the instrument and to validate that existing biological threat agent spectral features derived in laboratory settings from the conventional MALDI-TOF MS technique are sufficiently well replicated by the digitalMALDI technique, to provide biological threat agent spectral signature matches. Initial results obtained by the performer with simulants indicate that this is likely to be the case, but validation is necessary and will require follow-on R&D to test the prototype with live biological threat agents under appropriate BSL containment laboratory conditions and/or work with inactivated biological threat agents under lower BSL containment laboratory conditions. The performer has established relationships with potential partners that have expertise in conducting inactivated and live biological threat agent aerosol challenge studies and test and evaluation of prototype and commercially produced biosensors under appropriate BSL containment laboratory conditions.

Obtaining validated CWMD requirements from BD21 will shape the scope of such maturation activities. As the digitalMALDI instrument is refined further via additional rounds of R&D, OT&E, and testing with live and/or inactivated biological threat agents, it is anticipated that the technology will achieve TRL 7 and will be transitioned to BD21 for advanced development, acquisition, deployment, and sustainment.

Planning conducted by S&T and the performer during the current Option 4 POP will yield an initial commercialization and manufacturing plan that will inform post-transition acquisition of the digitalMALDI technology. As part of this process, the performer is engaging in discussions with potential vendors that will be able to manufacture production design components for a commercialized digitalMALDI instrument in their own facilities. The performer will assemble the commercialized digitalMALDI instrument at low- to medium-rate production levels. Should acquisition of a commercialized digitalMALDI instrument eventually be undertaken by BD21, it is possible that full-rate production will be necessary, which may necessitate expansion of the vendor base and partnering with a manufacturer capable of producing sufficient quantities of

instruments, with the required quality to maintain instrument performance across production batches, to meet deployment demands.

The digitalMALDI technology represents a potential major advancement in the field of biodetection that may allow for realization of near-real time detection of aerosolized biological threat agents. The digitalMALDI technology also may provide an advancement toward the newly identified goal of threat agnostic detection of biothreats, because of MALDI-TOF MS being well-suited for the detection and identification of multiple types of biological threat agents, including known bacteria, viruses, and toxins, and emerging and unknown threats.

V. DHS Action Plan

S&T issued a 3-month NCE to the Option 4 POP while the S&T team coordinated tasking to have an independent organization with subject matter expertise conduct a survey of potentially available COTS/GOTS technologies as alternatives for the current TMS-3 prototype's aerosol-sampling front end. During this time, S&T intends to coordinate with the CWMD Portfolio Manager to continue to engage with BD21 stakeholders to identify methods to scope additional development activities and to mature digitalMALDI technology further once the Option 4 POP concludes, which is the end of the Triggered Mass Spectrometry for Environmental Surveillance contract. Should S&T successfully conduct additional maturation activities for the digitalMALDI technology, the anticipated outcome is transition of the technology to CWMD BD21. Obtaining BD21 stakeholder buy-in is critical for transitioning the digitalMALDI prototype technology to CWMD for advanced development, acquisition, deployment, and sustainment.

The TRA determined that the digitalMALDI prototype met, or exceeded, TRL 5 requirements, and satisfied multiple TRL 6 requirements. This assessment will inform potential development pathways for S&T to mature the technology for transition to CWMD for either advanced development or direct injection into the BD21 acquisition. S&T is coordinating with CWMD, via the CWMD Portfolio Manager, to determine a course of action; one potential outcome would be for CWMD to request that S&T sponsor additional R&D to mature the technology to a higher TRL that would support transition. S&T is preparing FY 2023 spend plan activities to program $2.27 million of Defense Technologies funding to conduct additional technology maturation scope and internal S&T activities necessary to support execution of this scope.

S&T appreciates the continued congressional interest in development of the digitalMALDI technology.

Appendix: Abbreviations

Abbreviation	Definition
BD21	Biological Detection for the 21st Century
BSL	Biological Safety Level
CB	Chemical and Biological
CBT	Chem-Biodefense Testbed
COTS	Commercial Off-the-Shelf
COVID-19	Coronavirus Disease 2019
CWMD	Countering Weapons of Mass Destruction Office
DHS	Department of Homeland Security
digitalMALDI	digital Matrix-Assisted Laser Desorption and Ionization
FSLTT	Federal, State, Local, Tribal, and Territorial
FY	Fiscal Year
Gen 1	Generation 1
Gen 2	Generation 2
GOTS	Government Off-the-Shelf
HVAC	Heating Ventilation and Air Conditioning
MALDI-TOF MS	Matrix-Assisted Laser Desorption/Ionization – Time-of-Flight Mass Spectrometry
NCE	No-Cost Extension
OSE	Office of Science and Engineering
OT&E	Operational Test & Evaluation
POP	Period of Performance
R&D	Research and Development
S&T	Science and Technology Directorate
SES	Systems Engineering and Standards Division
TMS	Triggered Mass Spectrometer
TRA	Technology Readiness Assessment
TRL	Technology Readiness Level
WWC	Wetted Wall Cyclone