

**Department of Homeland Security (DHS) Science and Technology
Directorate (S&T) Chemical and Biological Defense Division (CBD) OBAA 14-
003/Call 0016**

1. **Announcement Number:** Open Broad Agency Announcement Number (OBAA) 14-003/Call 0016
2. **FBO Solicitation Number:** HSHQDC-14-R-B0009
3. **Call 16 Event Dates/Time (Local Eastern Time):**
 - Notification to Submit Full Proposals–**February 16, 2016**
 - Full Proposal Due Date–**March 16, 2016 (COB)**
 - Notification of Selection/Non Selection of Full Proposals–**April 16, 2016**

There will be no exceptions to the time and date on which responses are due, unless determined otherwise by the Government. Full Proposals received after the designated closing date/time will not be considered.

Note: This Call will be conducted in accordance with the Single-Phased Evaluation Process as described under Section 1.6 of the OBAA. The OBAA 14-003/Solicitation HSHQDC-14-R-B0009 was posted on Federal Business Opportunities on June 16, 2014. See Link.

<https://www.fbo.gov/index?s=opportunity&mode=form&id=3935288433485a6ee877134ac7d2a8a9&tab=core>

This Call will consist of the solicitation, receipt, and evaluation of a Full Proposal, limited to 30 pages, excluding the Formal Transmittal Letter, Cover Page, Summary of Costs and Related Information, Table of Contents and resumes/biographical information for proposed performers. Once the Full Proposal review process has been completed, offerors will be notified via email, or in writing, that its proposal has been selected, selected but not funded, or not selected for award.

4. **OBAA Call Technical Topic Area (TTA) of Interest:**

CBD.01 – Diagnostics and Agent Characterization: Research to develop rapid, robust, and affordable diagnostic tools to support detection, response, recovery, and real-time bio-surveillance and situational awareness. CBD's interest in diagnostics includes efforts in the areas of biological assays, sample preparation, advanced diagnostics (e.g. multiplex, high throughput, low-cost, field-deployable, complex sample matrices, multiple target types), and agent characterization of chemical or biological materials.

CBD.02 – Surveillance and Detection: Advance the capability to provide early warning and detection of a chemical or biological incident in a cost-sustainable way. Effective surveillance provides essential information to decision authorities on a timescale that allows them to take actions towards mitigating or responding to the threat. Efforts in this area include bioinformatics, open area and facility surveillance through sensing and data integration, and development or improvement of chemical and biological sensors.

4.1. Research Opportunity Description

4.1.1. DHS S&T: Directed-Specific PCR of Virulence Genes w/ Detection by Next Generation Sequencing.

Background

The U.S. Department of Homeland Security (DHS) is committed to using cutting-edge technologies and scientific talent in its mission to make America safer. The DHS Directorate of Science and Technology (S&T) is tasked with researching and organizing the scientific, engineering, and technological resources of the United States and leveraging these existing resources into technological tools to help protect the homeland. The Chemical and Biological Defense Division of S&T supports this mission by identifying and developing technologies for the DHS operational components that are needed to reduce the probability and potential consequences of a biological pathogen or a chemical attack on the nation's civilian population, its infrastructure, or its agricultural system.

DHS's mission space includes preventing, detecting, responding to, and recovering from intentional or accidental introduction of biological and chemical agents which present a threat against the Nation's human population and critical infrastructure. To support this mission, DHS and its state and local partners have a need to quickly collect reliable information to enable a swift and confident response to a biological and/or chemical threat. The Chemical and Biological Defense (CBD) Division within DHS S&T is working toward developing and transitioning technologies that ***demonstrate significant improvements*** to current analytical approaches in sensing and identifying chemical or biological contaminants in all types of environmental samples (solid, vapor, liquid, serum, blood, growth media) with high confidence.

The goal of the Federal Interagency Biosurveillance projects has been the early detection of biothreat agents to prevent or decrease mass civilian or military casualties. These systems have relied upon real time PCR to give a binary answer regarding the presence/absence of the biothreat agent target nucleic acids. The major challenge in this endeavor has been the complexity of the environmental samples, where tens-of-thousands of microorganisms exists, many of which are highly similar to the target pathogens. BioWatch is an example where numerous false positive results have been generated due to poorly known near-neighbor species confusing individual assays.

Metagenomic sequencing attempts to sequence "all" of the DNA in a sample and then to deconvolute its content computationally. Sequencing all of the DNA in a complex sample is difficult, slow and very expensive. The computational approaches are improving but still contain many flaws that lead to false conclusions, rendering the method unsuitable for reliable biodetection. While our knowledge of near-neighbors and of the target biothreat agents is rapidly increasing, it is unrealistic to ever expect complete knowledge of either. Furthermore, the presence of bacteriophages for hundreds of soil and environmental bacteria, plant viruses, plant and animal DNA, fungal DNA, and other unknown organisms, create an extremely diverse and complex matrix of interfering nucleic acids, that most likely will be found in vastly higher

amounts than the select agents being sought. This huge excess of non-select agent DNA makes it all the more difficult to find the *needle-in-the-haystack*. A recent sensationalized report describing the detection of DNA from anthrax and plague bacteria in the NYC subways illustrates the pitfalls of such endeavors (Mason *et al. Cell Systems*), which is fraught with false alarms and inability to detect the targets of interest. Unfortunately, although significant amounts of data are produced with metagenomics, it is frequently insufficient for the detection of reliable, informative signatures to differentiate pathogens from near neighbors. Moreover, deep sequencing of single specimens may cost nearly \$1000 and take several weeks to generate data of sufficient quality to make a call of positive or negative, plus the sequencing platforms and necessary computational servers are very expensive. Metagenomic sequencing is clearly not ready at this time for implementation for biosurveillance. An alternative method with greater discrimination, but not yet realized, is needed and a more tightly focused method of DNA sequencing may offer a potential solution.

One such alternative may be a variant of *amplicon sequencing*, which is now a mature and simple method that forms the technological basis for the explosion in microbiome research. Thousands of papers have been published using this method that involves the deep (>5,000X) sequencing of the 16S gene PCR amplicon product to identify individual components of mixed bacterial communities. It is powerful precisely because the PCR primers are not specific but the intervening sequences are highly informative and can be used to discriminate among bacterial taxa. Unfortunately for biothreat detection, the 16S gene has insufficient discrimination power to differentiate biothreat pathogens from their near neighbors. Discrimination power is a function of gene diversity and the 16S gene in many bacterial biothreat agents has **low** or **no** diversity among closely related bacteria. Therefore, we cannot effectively identify a biothreat agent or distinguish from near-neighbor species using this method by itself either.

However, it may be possible to increase the discrimination power of amplicon sequencing through comparative genomic analysis to identify diverse genomic regions. This method would be most effective when large genome databases are available and could be highly specific in both clinical diagnostics and environmental detection of bacterial biothreat agents DNA. Properly validated, this approach can be used to identify multiple pathogens and to predict their virulence and resistance to antibiotics. PCR primers specific to a pathogen genera or species are sufficient, if there is additional DNA sequence information that can be leveraged for precise agent identification. Multiplex systems of several hundred amplicons are becoming common and could provide coverage for dozens of pathogens. Sample preparation that works for real time PCR should also work for targeted amplicon sequencing methods, so current sampling schemes would adapt well to this type of assay. A multiple amplicon sequencing system could be easily adapted to changing targets with addition of new amplicons. Because of the multiplex nature of the assay, redundant amplicons can easily be included to verify the identification of a biothreat agent and even provide a differential identification of a near-neighbor species. Variation within the amplicons could also be analyzed to identify drug resistance, virulence factors and subtype (strain).

4.1.2. Description Technical Topic Areas

**Department of Homeland Security (DHS) Science and Technology
Directorate (S&T) Chemical and Biological Defense Division (CBD) OBAA 14-
003/Call 0016**

The performer will *develop* and *validate* a novel, multiple amplicon sequencing system for identifying major bacterial biothreat agents (e.g., *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Burkholderia mallei* and *Burkholderia pseudomallei*). The system should distinguish between the biothreat agent and its near-neighbor species using both amplification positive/negative criteria and qualitative analysis of sequence within the amplicons. This latter analysis will result in strain identification, drug susceptibility and usable strain identification data. The analytical system will be supported by automated, interpretive software that generates actionable reports. Such a system will include quality assurance data to identify sample and/or process issues rapidly, to limit the effect of QC issues on final results.

The performer will work with Government guidance to validate the final assays according to Public Health Actionable Assays requirements. DHS may also conduct specialized and specific analysis, test, evaluation, and validation of assays to help characterize and deploy the assays for use by DHS customers with areas of responsibility for biological defense, detection, and surveillance.

The Government has established metrics for assessing the capabilities and qualifications of the offeror to successfully meet the requirements of the task. The criteria shown in the Evaluation Criteria section below will be given **equal** weight in determining the final decisions of the source selection committee. An Offeror may submit a **full** proposal to this technical topic area. The proposal will be reviewed by a panel of Government subject matter experts for several criteria as described below in Evaluation Criteria section. Failure to address each criterion fully will result in rejection of the proposal as non-responsive.

Note also that the emphasis with respect to past performance for the topic area will be based on demonstrated and prior experience as judged by reviewers to yield the highest possible quality of performance to assist the DHS in its biological detection and surveillance portfolio. Offerors are encouraged to submit brief and concise plans to execute the tasks, and to include information that will allow the reviewers to judge against the criterion shown in the Evaluation Criterion section.

Specific tasks would include:

Task 1 - Bioinformatic identification of target signature sequences that can be amplified specifically for the biothreat agents and provide phenotypic or stain identification data.

- a) Use comparative genomics to identify **target signature sequences** that will differentiate target agents from their near neighbor relatives. This should be focused on regions that amplify across all known target strains, but could also include regions that amplify near-neighbor species and, hence, would provide a differential identification. This differentiation should focus upon sequences for assay primer design.
- b) Define the phylogenetic relatedness of the target agent and its near neighbors via a comparison of amplicon sequences. While regions identified in Task 1-a will be conserved within the target, the internal sequence regions will vary. This information gathered from sequencing of amplicons

should allow strain identification, drug susceptibility and virulence predictions. Typically, the exposed genomes from the more easily disrupted cell walls of the gram-negative bacteria and RNA viruses are much less stable. A majority of the select agents of interest are not stable on dry filter matrices, and therefore loss of the RNA and DNA signatures from gram-negative organisms are challenging. Target sequence information will also differentiate near neighbor from target species in the event of target presence/absence signal erosion.

Task 2 - Design and validate assay primers that will amplify all strains of the threat agent, but will not amplify the near neighbors: Target Specific Amplicons.

- a) Assay primer design should be consistent with standard PCR method but amenable to analysis using next generation sequencing methods. This approach should include the addition of “barcodes” to allow for indexing of amplicons that can be sequenced in single batch runs.
- b) Validate the exclusivity and inclusivity of these primers target signature primer sequences via *in silico* analysis of all available target threat agents (inclusive) genome sequences and near-neighbor (exclusive) genome sequences. Because a large number of genomic sequences now exist for target and non-target organisms, an *in silico* validation step should eliminate any primers that would be predicted to be non-exclusive to the biothreat target. In addition, *in silico* validation should include genomes from commonly found contaminants, to enable the exclusion of such interfering DNAs as from humans, animals, plants, *etc.* and soil organism DNAs.
- c) Test the assay primers against the target and near-neighbor DNA templates to validate them under actual assay conditions.

Note – In some cases, exclusive amplicons may not be feasible and the combination of amplification and internal sequence will be needed to distinguish target from near-neighbors. In the absence of exclusive-target amplification, the amplicon sequence could provide definitive identification of the target and non-target agents.

Task 3 - Design and validate assay primers that will amplify all strains of the near-neighbor, but will not amplify the target: Differential Target Amplicons.

- a) Differential identification assays should be included in the multiplex assay to help identify and exclude any false positive results. Although the addition of differential assay components is less critical than those components which can produce specific target amplicons, they offer interpretive value in evaluating complex target species.
- b) Assay primer design should be consistent with standard PCR methods but amenable to analysis using next generation sequencing methods. This approach should include the addition of “barcodes” to allow for indexing of amplicons that can be sequenced in single batch runs.

**Department of Homeland Security (DHS) Science and Technology
Directorate (S&T) Chemical and Biological Defense Division (CBD) OBAA 14-
003/Call 0016**

- c) Validate the exclusivity and inclusivity of these primers *in silico* across all available threat agents and near neighbor genomes. Because a large number of genomic sequences now exist for target and non-target organisms, an *in silico* validation step should eliminate any primers that would be predicted to be non-exclusive to the near-neighbor species. In addition, *in silico* validation should include genomes from commonly found contaminants, to enable the exclusion of such interfering DNAs as from humans, animals, plants, *etc.* and soil organism DNAs.
- d) Test the assay primers against the target and near-neighbor DNA templates.

Task 4 - Design, validate and implement multiplex assays for each of biothreat target agent.

- a) Multiplexing of many amplicons will be optimized as a complete assay for a single biothreat agent – *B. anthracis*. This should include phenotypic predictors (e.g., virulence, drug susceptibility) and strain identification amplicons.
- b) Repeat this task individually for *F. tularensis*, *Y. pestis*, *B. mallei*, *B. pseudomallei*, **Brucella melitensis*, **B. abortus*. (*Optionally funded)
- c) For single biothreat agent assays, determine important test parameters such as linearity, LOD, sensitivity, specificity, quantitative performance (absolute and relative), contaminant interference, performance with environmental samples (spikes), *etc.*

Task 5 - Design and validate a multi-agent amplicon sequencing assay.

- a) Develop combined multi-agent amplicon sequencing assays for bacterial biothreat agents and validate under laboratory conditions.
- b) For the combined biothreat agent assays, determine important test parameters such as linearity, LOD, sensitivity, specificity, quantitative performance (absolute and relative), contaminant interference, performance with environmental samples (spikes), *etc.*

Task 6 - Design and develop Interpretive Analytical software for the multi-agent amplicon sequencing assay.

- a) Develop prototype software that will analyze *B. anthracis* amplicon sequence data and provide actionable information (*i.e.*, agent presence with confidence metrics, presence of virulence and antibiotic resistance factors, phylogenetic classification, *etc.*)
- b) Develop final software to include other agent targets (*F. tularensis*, *Y. pestis*, *B. mallei*, *B. pseudomallei*, *Brucella melitensis*, and *B. abortus*) and allow for on-site and remote reporting.

5. Number of Selections: DHS S&T expects to make one award using its FY 2016 funds

6. Anticipated Ceiling: Although subject to official fiscal appropriation and availability, it is anticipated that approximately \$3,000,000 of Fiscal Year (FY) 2016 funds will be available for any

**Department of Homeland Security (DHS) Science and Technology
Directorate (S&T) Chemical and Biological Defense Division (CBD) OBAA 14-
003/Call 0016**

resultant awards under this OBAA Call. **The Government will reserve the right to incrementally fund any resultant contracts awarded from this OBAA Call as provided by the FAR 52.232-22, “Limitation of Funds.”** Contracts or other agreements that obligate funds will not have an initial period of performance that exceeds 18 months from the date of contract award. However, Offerors will be able to propose a base effort with additional option years.

7. **Anticipated Award Type:** Award type is anticipated to be in the form of Cost Reimbursement type contracts. However, the Government reserves the right to award Fixed Price or Interagency Agreements (IAs) to appropriate parties should the situation warrant.

In the event an offeror or subcontractor is a Federally Funded Research and Development Center (FFRDC), Department of Energy National Laboratory, or other Federally funded entity, DHS/S&T will work with the appropriate sponsoring agency to issue an interagency agreement pursuant to the Economy Act (31 U.S.C. 1535) or other appropriate authority.

8. **Anticipated Award Dates:** The 3rd Quarter of Fiscal Year 2016 is when the government anticipates making any resultant contract awards under this Call for those Proposals are selected. However, the award date for any resultant contract award may vary based on the quality of the proposals received and the availability of funds.

9. **White Paper Instructions:** NA – No white papers are being requested in response to this Call.

10. **Full Proposal Instructions:** Offerors shall submit their Full Proposals in accordance with OBAA 14-003, Section 5.4 - Format and Content of Full Proposals. See FBO link above for access to OBAA 14-003/Solicitation Number HSHQDC-14-R-B0009.

11. **Evaluation Criteria:** The evaluation of Full Proposals will be accomplished through an independent technical review using the following criteria:

Criterion I: Scientific Merit: The Offeror must demonstrate understanding of the critical technology and scientific challenges required to achieve the desired performance metrics and strategy as described elsewhere within this announcement. The research approach should be scientifically sound, practical, and technically defensible. The technical approach is innovative and has advantages over other solutions, if successfully implemented. The research must contribute to scientific knowledge in the topic area and the proposal must enumerate the potential benefits of the proposed research. The proposal shall demonstrate an awareness of the state-of-the-art. The proposal should be well-prepared with supportive information that is self-explanatory.

Criterion II: Sound Technical Approach: Of importance is how the proposed technology will meet or exceed the performance requirements for this program and be commercially applicable (how the proposed technology will be transitioned into a sustainable commercial or government market and what the intended use, or concept of operations, would be). All critical scientific and technical issues and risks are clearly identified, and the planned development approach and risk mitigation efforts are clearly defined and feasible. The merit of the technical approach over other competing approaches should be clearly delineated.

**Department of Homeland Security (DHS) Science and Technology
Directorate (S&T) Chemical and Biological Defense Division (CBD) OBAA 14-
003/Call 0016**

Criterion III: Sound Management Approach: Presentation of a sound managerial approach to the proposed work, including a demonstrated understanding of the issues and challenges associated with achieving the goals of the topic, and a strategy to address those issues and challenges. A successful team will possess multidisciplinary expertise to address the complexity of the effort.

Criterion IV: Capability to Perform and History of Performance: Demonstration of a capability to perform the proposed work, including history of previous successful performance in developing related solutions and technologies. Specific considerations will include:

- The Offeror must possess clear and convincing qualifications and must have a proven record of performance and experience, including successful production of and authorship on peer reviewed publications related to projects associated with the creation, and assessment of performance of select agent detection assays, *i.e.*, amplicon sequencing, next generation sequencing, *etc.*
- The Offeror must have access to a comprehensive repository of bacterial select agents and near-neighbors sufficient to produce the required nucleic acid samples for developing, testing, and validating the putative assays required for production in this BAA.
- The Principle Investigator must possess at a minimum, a doctoral level degree from an accredited university.
- The Offeror must have an expert understanding and knowledge of assay development and technical requirements associated with the specialized development and genetic analysis of bacterial select agents involved in this effort.
- Offeror's knowledge of current and in-use biological detection methods and systems.
- Offeror's team is sufficiently complete: key personnel are identified with the required range of competencies to execute this effort and the team includes appropriate experience and publication record.
- Possess the required sequencing platforms, bioinformatic resources and knowledge, and the necessary computational base and staff in place.

Criterion V: Cost Realism/Reasonableness: Presentation of accurate, well-founded and reasonable estimates of all costs related to performance of the proposed effort, including an appropriate allocation of labor resources. Members of the Review panel will be looking for overall *best* value to the government.

12. Foreign Concerns: Foreign persons are advised that their participation may be subject to Export Control restrictions in accordance with OBAA 14-003 Section 8.3. Any such restrictions shall be reviewed on an individual award basis.

13. iDURC Requirements: The performer and any proposed sub-performer(s) working under any award resulting from this BAA Call shall conduct all research involving agents and toxins identified in sections III.1 and 6.2.1 of the USG Policy for Oversight of Dual Use Research of Concern and USG Policy for the Institutional Oversight of Dual Use Research of Concern, respectively, in accordance with both policies referenced above and in accordance with any additional requirements set forth in related DHS policies and instructions. Each performer and any sub-performer(s) planning to perform research involving agents and toxins identified in sections

**Department of Homeland Security (DHS) Science and Technology
Directorate (S&T) Chemical and Biological Defense Division (CBD) OBAA 14-
003/Call 0016**

III.1 and 6.2.1 of the USG DURC policies under this award must attest at the time of seeking funding that they are in compliance with all aspects of the Policies.

- 14. Questions:** Any questions concerning this call must be submitted via email to the Contracting Officer at Michael.Jones@hq.dhs.gov no later than **March 11, 2016 3:00 PM EST** in the following format:

Question #	Reference	Contractors' Question
1	General (if there is no specific document reference)	
2	(Example) OBAA 14-003, page 15, Section 5.2, first paragraph, second sentence	
3	(Example) OBAA 14-003/Call 0016, page 2, Section 9, first sentence	

Please include "Questions for OBAA 14-003/ Call 0016" in the subject line. All questions and responses will be posted on the Federal Business Opportunities website <http://www.fbo.gov> and <https://baa2.st.dhs.gov> . Questions will only be accepted or answered electronically.