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LLNL-TR-748959

Highly Multiplexed Assays for Detection of Biothreat and Food Safety Agents Final Report CRADA No. TC02156.0

P. Naraghi-Arani, M. Beal

April 4, 2018

Disclaimer

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Highly Multiplexed Assays for Detection of Biothreat and Food Safety Agents

Final Report
CRADA No. TC02156.0
Date Technical Work Ended: April 12, 2014

Date: 5/19/2014 Revision: 1

A. Parties

This project was a relationship between Lawrence Livermore National Laboratory (LLNL) and BioSearch Technologies, Inc.

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B. Project Scope

This was a collaborative effort between Lawrence Livermore National Security, LLC as manager and operator of Lawrence Livermore National Laboratory (LLNL) and BioSearch Technologies Inc. (BTI) of Novato, California, to develop Multiplexed Molecular Assays for the Rapid and Sensitive Detection of Microbial Agents of Concern for Bioterrorism and Food Safety for the Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN). There are no outstanding leans for this project.

C. Technical Accomplishments

The specific technical accomplishments were the development of 3 separate multiplex molecular assay panels of value to testing of various foods for presence of multiple organisms.

Assay Panel 1 was developed during the first year of funding and fulfilled Task1 on the CRADA. A 13-plex molecular assay able to simultaneously detect *Bacillus anthracis*, *Yersinia pestis*, and *Francisella tularensis* was developed and extensively tested both at LLNL and by the customer at FDA. LLNL report issued to BioSearch: LLNL-TR-487352

Assay Panel 2 was to incorporate signatures for detection of Norovirus into Assay Panel 1 during the second year of the project. However, extensive attempts to accomplish this task met with failure due to lack of specific sequences and samples available for norovirus to enable development of tests for this agent. BioSearch and the FDA sponsor were informed of these issues.

Assay Panel 3 was to incorporate signatures for the detection and differentiation of *Brucella abortus* and *Brucella melitensis* into the assay panel already developed during the third year of the project. A 16-plex assay was developed which added three signatures for the detection of *Brucella abortus*, and *Brucella melitensis* to the existing assay able to detect *Bacillus anthracis*, *Yersinia pestis*, and *Francisella tularensis.* Additionally, as the initial goal of incorporating an RNA virus into the mix of the targets to be detected failed, the enzyme used was replaced from one that utilized RT-PCR to one that utilized PCR at greatly reduced cost. LLNL report issued to BioSearch: LLNL-TR-622832

Assay Panel 4 was to incorporate signatures for the detection and differentiation of *Salmonella enterica* into panel 3 and was developed during the fourth year of the project. An 18-plex assay was developed which added two signatures for the detection of *Salmonella enterica* to the existing assay able to detect *Bacillus anthracis, Yersinia pestis*, *Francisella tularensis*, *Brucella abortus, and Brucella melitensis.* LLNL report issued to BioSearch: LLNL-TR-639673

An additional task order for modification of the assay panel to run on the newest Luminex instrument, the MagPix system was issued by FDA and responded to. However, due to sequester cuts, that task order was never funded by FDA and thus no work took place.

D. Expected Economic Impact

The assays developed are expected to be used by the FDA CFSAN (and shared by them with others in the public health arena) to improve rapid detection of intentionally adulterated food and for diagnosing food poisoning cases. All information has been transferred to FDA in order to enable them to directly purchase needed assay reagents from Luminex, BioSearch, and Thermo Corps. Additionally, Luminex has expressed interest in producing test kits for use by the public health laboratory infrastructure to purchase and use.

D.1 Specific Benefits

Benefits to DOE

Demonstrated the rapid development and testing of novel multiplex molecular assays that enhance the security of the country.

Benefits to Industry

As the range and depth of multiplex molecular assays expands, there will be a concomitant increase in the safety of the food produced and the premium that industry can get for food demonstrated to be safe for consumption.

E. Participant Contribution

BioSearch Technologies generated all oligonucleotide primers and probes for this project. Additionally, they generated all artificial template materials used to test specificity and sensitivity of the assay panels developed and provided other reagent material for assay development.

LLNL performed bioinformatics analysis to identify signatures to include in the assays developed as well as performing all analytical testing of the assays developed on whole genome target organisms as well as background, near-neighbor and artificial template testing to determine sensitivity and selectivity of the assays developed.

LLNL scientists also traveled to FDA CFSAN in Laurel, MD to train personnel there on the use of the assays developed.

FDA CFSAN scientists conducted tests on food samples spiked with various pathogens to determine the sensitivity and specificity of the assays developed relative to existing molecular assays. By every measure, the assays developed were superior to existing assays.

F. Documents/Reference List

Reports

The list of reports issued to BioSearch Technologies and to the FDA sponsor are listed below:

Year 1 Report: Multiplex Assay Development for Detection of Agents with Bioterrorism Potential in Food. LLNL-TR-487352

No year 2 report was issued due to lack of progress on Norovirus assay development and the need to move on to addition of other signatures into the multiplex assay.

Year 3 Report: Multiplex Assay Development for Detection of Agents with Bioterrorism Potential in Food. LLNL-TR-622832

Year 4 Report: Multiplex Assay Development for Detection of Agents with Bioterrorism Potential in Food. LLNL-TR-639673

Copyright Activity

None

Subject Inventions

None

Background Intellectual Property

LLNS disclosed the following Background Intellectual Property for this project:

U.S. Patent No. 7,494,772 - Nucleotide Sequences Specific to Yersinia pestis and methods for detection of Yersinia pestis; Inventors: Paula M. Mccready, Thomas R. Slezak, Elizabeth A. Vitalis, Lyndsay Radnedge, Linda L. Ott, Thomas A. Kuczmarski, Gary L. Andersen; Issued 2/24/09. (IL11030)

U.S. Patent No. 7,494,778 - Nucleotide Sequences Specific to Francisella tularensis and methods for detection of Francisella tularensis; Inventors: Lyndsay Radnedge, Linda L. Ott, Thomas A. Kuczmarski, Vladimir L. Motin, Gary L. Andersen, Paula M. Mccready, Thomas R. Slezak; Issued 2/24/09 (IL11031)

LLNL Docket No. IL12252
[Patent Application not pursued]

BioSearch Technologies, Inc. disclosed the following background intellectual property for this project:

'Black Hole Quencher' (BHQ) fluorescent quenching technology

G. Acknowledgement

Industrial Participant's signature of the final report indicates the following:

1) The Participant has reviewed the final report and concurs with the statements made therein.
2) The Participant agrees that any modifications or changes from the initial proposal were discussed and agreed to during the term of the project.
3) The Participant certifies that all reports either completed or in process are listed and all subject inventions and the associated intellectual property protection measures generated by his/her respective company and attributable to the project have been disclosed and included in Section E or are included on a list attached to this report.
4) The Participant certifies that if tangible personal property was exchanged during the agreement, all has either been returned to the initial custodian or transferred permanently.
5) The Participant certifies that proprietary information has been returned or destroyed by LLNL.

5/27/2014

Marc Beal, Director of Corporate Development — Date
BioSearch Technologies, Inc.

Pejman Naraghi-Arani, PhD. LLNL Principal Investigator — Date 05/19/14
Lawrence Livermore National Laboratory

9-15-14

Richard A. Rankin, Director, Industrial Partnerships — Date
Lawrence Livermore National Laboratory

Attachment I – Final Abstract

Highly Multiplexed Assays for Detection of Biothreat and Food Safety Agents

Final Abstract (Attachment I)
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B. Purpose and Description

This was a collaborative effort between Lawrence Livermore National Security, LLC as manager and operator of Lawrence Livermore National Laboratory (LLNL) and BioSearch Technologies Inc. (BTI) of Novato, California, to develop Multiplexed Molecular Assays for the Rapid and Sensitive Detection of Microbial Agents of Concern for Bioterrorism and Food Safety for the Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN). There are no outstanding leans for this project.

C. Benefit to Industry

As the range and depth of multiplex molecular assays expands, there will be a concomitant increase in the safety of the food produced and the premium that industry can get for food demonstrated to be safe for consumption.

D. Benefit to DOE/LLNL

Demonstrated the rapid development and testing of novel multiplex molecular assays that enhance the security of the country.

E. Project Dates

April 18, 2010 thru April 12, 2014