Development of Assay Panel for Vector-Borne Disease Research Cooperative Agreement D17AC00026

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FINAL REPORT

Submitted by:

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DEVELOPMENT OF ASSAY PANEL FOR VECTOR-BORNE DISEASE

RESEARCH COOPERATIVE AGREEMENT D17AC00026

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Research Cooperative Agreement D17AC00026

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KEYWORDS

Real-time, polymerase chain reaction, Biomeme two3™ and three9™, handheld, lightweight, portable

"The views expressed are those of the authors' and do not reflect the official views or policy of the Department of Defense or its Components"

1.0 ABSTRACT

This report is submitted in fulfillment of the Milestone Task "**Engineering Technical Report: Technical Reports of findings, results and engineering changes resulting from DT&E activities for each product/device**" for the Vector-Borne Disease Panel, to be submitted to the government technical point of contact (TPOC) 22 months from Award.

The Air Force Medical Support Agency has the responsibility for identifying, developing and fielding advanced technologies to address known capability gaps in military medicine. The disease detection and diagnostic product line is an especially important focus area for military medicine due to the prevalence and nature of illnesses that affect DoD personnel in performance of their mission responsibilities.

Biomeme technology provides for rapid, portable, field-forward sample preparation and surveillance detection in insect vectors. The device is simple, user friendly, and weighs less than 1lb. The current lack of far-forward environmental surveillance tests for identifying and differentiating between pathogens causing vector-borne disease in austere environments hinders disease prevention and control measures, causes lost duty days and decreased productivity in military and civilian sector. The primary goal of the Vector-Borne Disease Panel project was to transition the following Biomeme disease detection and diagnostic products from TRL 3/4 to a program of record for incorporation at TRL 6/7 into a complete system:

a. two3™: Two-Color/3-Well (6 targets) Biomeme PCR Device

b. three9™: Three-Color/9-Well (27 targets) Biomeme PCR Device

c. QPrep-9™: Automated Sample Prep Prototype

d. Mosquito Vector Pathogen Panel: Vector-borne PCR Assays to include Malaria, Chikungunya, Dengue, Zika, and Yellow Fever

Biomeme is a Philadelphia-based technology company that produces commercially available handheld, portable real-time Polymerase Chain Reaction (RT-PCR) platforms that can detect pathogenic diseases including federal select agent pathogens and regional endemic infectious diseases in syndromic assay panels that enhance point-of-care (POC) diagnosis of diseases of operational concern to the U.S. military. The Biomeme platform includes: 1-minute field sample prep, lyophilized shelf-stable reagents and test kits, a mobile thermocycler, and user-friendly software that together enables assay analysis in the field without the use of other specialized equipment or trained technicians. The remainder of this Report provides findings, conclusions and recommendations for each product/device.

2.0 INTRODUCTION

This acquisition provides for the development, design, refinement, evaluation, and transition of innovative dual-use technologies that will help revolutionize the detection and diagnostic ability of human disease targets for patients possibly exposed to such illness-causing pathogens.

The inability to reliably identify microbial pathogens in the environment at the point of exposure has led to vector-borne infection illness with lost duty days and decreased productivity in military and civilian sector. The Biomeme pathogen surveillance system provides a powerful method to distinguish pathogen infection etiologies and guide appropriate epidemiological remedies. The Biomeme technology addressed some of the existing DOD challenges with molecular diagnosis and integration with operational assessment, at the point of acute illness in austere environments. Currently, rapid epidemiological tests are used to provide quick point-of-exposure results, but have been shown to have moderate sensitivity (50-70%) compared to viral culture or real-time polymerase chain reaction (PCR), resulting in many cases going undetected. Real-time PCR is considered the "gold standard" for testing of the proposed targets of human exposure; however, this currently requires specimens to be collected, frozen and sent to an approved laboratory for testing, while maintaining a cold-chain for the specimens. This lengthy process, which can take up to 1-2 weeks in the US, can be especially difficult in remote or resource-poor settings and does not typically provide results quick enough for timely initiation of treatment of acute illness and implementation of infection control practices.

Biomeme's surveillance capabilities allow for real-time results with high sensitivity and specificity in patients suffering from life-threatening illnesses, revolutionizing the delivery and efficacy of appropriate care at the point of acute trauma and illness. The Biomeme product line is an especially important focus area for military medicine due to the prevalence and nature of injury and illnesses that affect Department of Defense personnel in performance of their mission responsibilities in field-forward, austere environments. At the clinical care level, test results are easily integrated with existing telemedicine structures, allowing clinicians to receive the results of tests performed remotely and better understand exposure risk prior to an in-person visit. At the infection control level, de-identified data is easily transmittable/accessible by clinicians and public health officials for real-time disease surveillance. Additionally, the current device allows for characterization of up to 6 pathogens/subtypes at a time, with different assays easily switched in or out depending on the circumstance and currently circulating disease strains.

The Biomeme detects pathogenic diseases including federal select agent pathogens and regional endemic infectious diseases in syndromic assay panels that enhance point-of-care (POC) diagnosis of diseases of operational concern to the U.S. military. Successful assay development on the Biomeme includes a vector-borne panel consisting of assays for detection of Chikungunya Virus, Zika Virus, Yellow Fever Virus, Dengue Virus and Malaria.

3.0 MATERIALS AND METHODS

3.1 two3™ Two-Color/3-Well (6 targets) Device

3.2 Findings and Results

The technology development engineering for this device has been completed, it has been successfully field- and lab-tested, and the units are now being manufactured in quantity and deployed in the field for use by select DoD operators, as well as numerous commercial applications including food and water testing.

Biomeme's two3 is an "open platform" which offers appreciable time advantages vs. closed architecture / cartridge-based microfluidic systems when responding to a disease outbreak or other time critical scenarios. It is designed to allow for efficient and rapid porting of existing Real-Time PCR assays onto the system's optional Open version of the sample-to-strip cards. The time to port an existing wet assay is literally the seconds required to spike it into the card's reaction wells. For situations requiring more validation or development, the process may require as little as a few weeks.

The purpose of the two3 development effort was to build on Biomeme's earlier TRL 6 prototype one3™ systems, by increasing the number of targets that could be tested in a single run, and increasing the overall stability of the platform. The two3 met all of its design objectives and performance specifications. It is an easy-to-use, hand portable device well-suited for use in the field and in low resource and/or austere environments, capable of running a variety of assays and sample types. The two3 is manufactured to ISO standards, and it complies with FCC Part 18, and MIL-STD-882D and 60950-1 requirements.

The following table presents the as-built production specification (e.g., operating temperature, storage temperature, run time, operating tilt range, operating decibel level, dimensions, weight, power specifications, test data, etc.):

Characteristic	Specification
Sample capacity	3 wells
Sample volume per well	20-50 uL
Sampling channels	2
Sampling Ch. 1 excitation wavelength	466 ± 20 nm
Sampling Ch. 1 emission wavelength	522 ± 20 nm
Sampling Ch. 2 excitation wavelength	581 ± 15 nm
Sampling Ch. 2 emission wavelength	647 ± 33 nm
Operating temperature range	32° - 95° F

Storage temperature range	-4° to 113° F (limits of iPhone SE)
Operating tilt range, front/back axis	±15°
Operating tilt range, left/right axis	±15°
Dust ingress rating of storage case	code 6: dust tight
Water ingress rating of storage case	code 7: immersion in water up to 1 m

Fig. 1. Biomeme two3 as-built Product Specifications

Figures 2 and 3 below provide an illustration of the complete two3 system, including the two3 thermocycler hardware, the iPhone SE, the wired data transfer port adapter, and the optional external power supply.

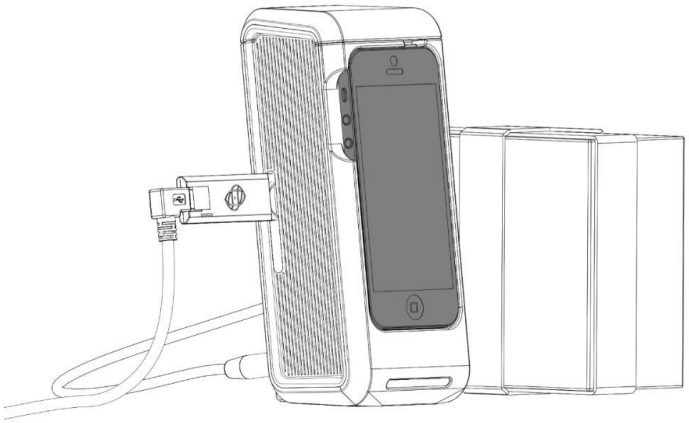

Fig. 2. The complete Biomeme two3 JHBI system, with iPhone SE, two3 thermocycler, and EIT data transfer cable.

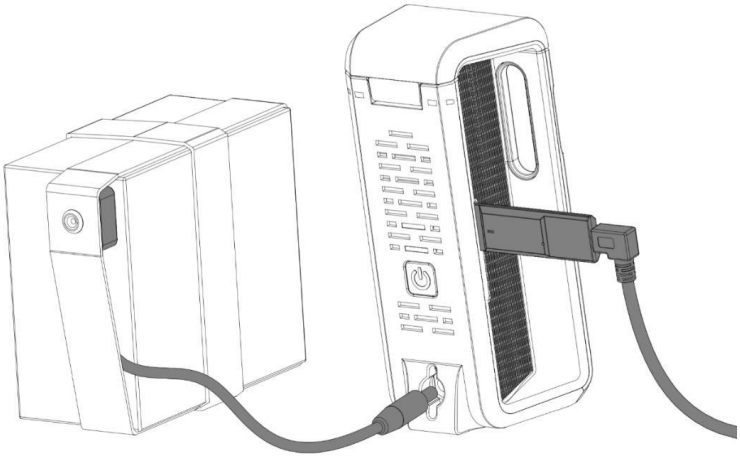

Fig. 3. Rear view of the Biomeme two3 thermocycler, shown with an external battery plugged into the DC jack.

Operation of the two3 is relatively straightforward and requires minimal user training. To operate the Biomeme two3 system, the user must prepare the biological sample, insert the sample into the two3 hardware, and operate the two3 User App to begin a test. The results are then displayed on the two3 User App. The most difficult aspect of the operation from a user point of view is the preparation of the biological sample, and the transfer of the prepared sample into the device.

4.0 Franklin three9™ Three-Color/9-Well (27 targets) Device

4.1 Findings and Results

The technology development engineering for this device has been completed, it has been successfully field- and lab-tested, and the units are now being manufactured in quantity and deployed in the field for use by select DoD operators, as well as numerous commercial applications including food and water testing.

Like the two3, the three9 is an "open platform" which offers appreciable time advantages vs. closed architecture / cartridge-based microfluidic systems when responding to a disease outbreak or other time critical scenarios. It is designed to allow for efficient and rapid porting of existing Real-Time PCR assays onto the system's optional Open version of the sample-to-strip cards. The time to port an existing wet assay is literally the seconds required to spike it into the card's reaction wells. For situations requiring more validation or development, the process may require as little as a few weeks.

The primary purpose of the three9 development effort was to further increase the number of targets that could be tested in a single run by adding an additional color, and 6 additional wells. The three9 met all of its design objectives and performance specifications. It is an easy-to-use, hand portable device well-suited for use in the field and in low resource and/or austere environments, capable of running a variety of assays and sample types.

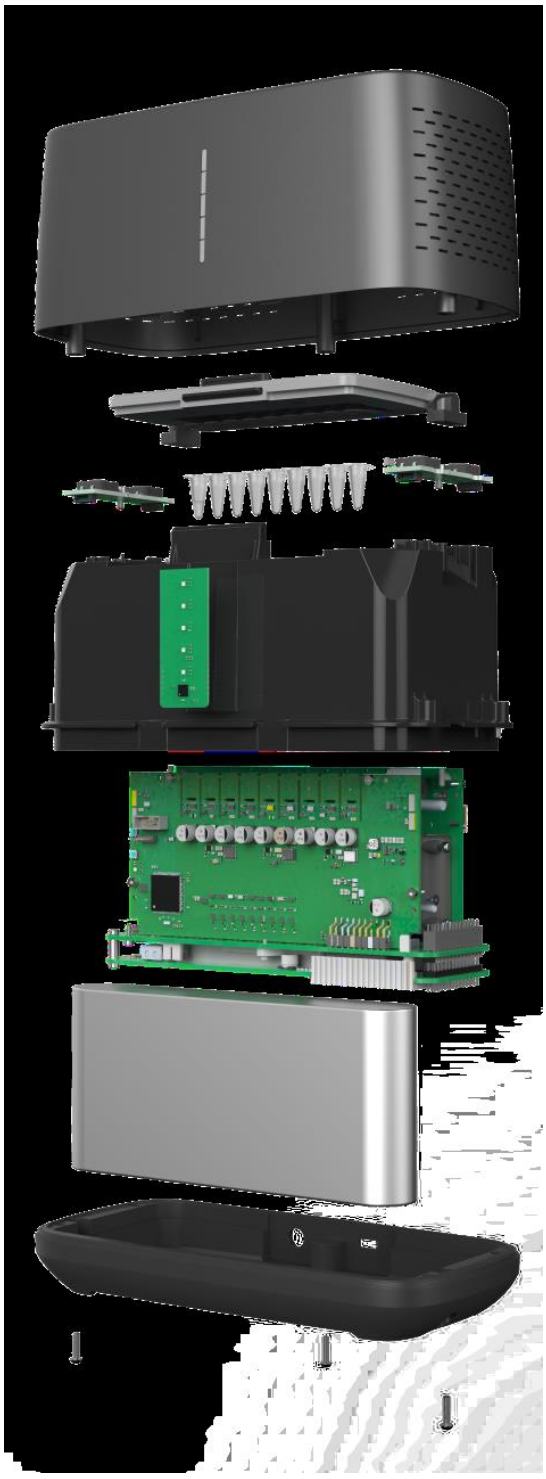

Fig. 4. The Biomeme three9 system Internals and Enclosure

Fig. 5. Biomeme Franklin™ (three9) Thermocycler

The following table highlights the performance parameters and design requirements for the Franklin three9:

Performance Requirements
Ability to transfer data via wired port to an external information technology
System must be able to effectively run within the temperature range 45-90F
Time to answer with genomic DNA in buffer (40-cycles at 23C) must be <50min
System must be compatible with Mil-standard battery (e.g., BA 2590)
Battery life of internal rechargeable battery must allow at least 4 complete runs
Splash resistant with or without case (objective: IPX2 rating = Protected from water spray less than 15 degrees from vertical)
Transportable to 30K ft MSL and still function when returned below 10,000 ft MSL (within case if necessary)
3-color system with 9 wells
Total system must weigh <3lbs

Fig. 6. Biomeme three9™ Requirements

SPECIFICATION	DIMENSION
Sample Capacity	9 Wells
Reaction Volume per Well	20 µL
Total Channels	3
Franklin one9 Fluorophore*	FAM / SYBR (Green)
Franklin two9 Fluorophores*	FAM / SYBR (Green), ATTO647N (Red)
Franklin three9 Fluorophores	FAM / SYBR (Green), TexasRedX (Amber), ATTO647N (Red)
System Control & Data Transfer	Wireless (BLE)
Integrated Barcode Scanner	Yes
Max Samples per Run	9
Max PCR Targets per Run	27
Weight	1.20 kg / 2.65 lb
Operating Ambient Temperature	4 - 40°C / 39 - 104°F
Operating Humidity Limit	0 - 99%
Operating Altitude Limit	3,048 m / 10,000 ft
Wall Power (VAC)	100 - 240V
Voltage	19V
Full Load Current	3.3A
Internal Battery	5 hrs
Quantitative	Yes
IP Rating	IP30
Indoor/Outdoor?	Indoor or Outdoor in a Covered Area
Pollution Degree	2
Degree of Ingress Protection	Keep 5 cm Clearance Around the Thermocycler for Proper Performance

Fig. 7. Biomeme Franklin™ (three9) Specifications

4.2 Engineering Changes Resulting from DT&E Activities

Based on the DT&E activities undertaken as part of the current program, the key recommended engineering change involves replacing the current "moving carriage" design with a static carriage design, and adding a 4th color for passive reference dye that can then be used for normalization, as described more fully in the May 2019 "Recommendations Report". This change has not been implemented in the current device design, but development efforts are ongoing.

5.0 QPrep-9TM Automated Sample Prep

5.1 Findings and Results

The QPrep-9TM (also referred to as the Dx or ISP – integrated sample prep) is a man-portable, sample-to-answer system comprising the following components:

- Integrated sample preparation module
- A Franklin three9TM thermal cycler "core"
- An enclosure with lid assembly for auto-triggering the sample prep process
- Android smart device user interface for initiating runs and viewing results
- Lyophilized panel assays stable for at least 1 year at room temperature

The system specifications, and an overview of the system workflow are provided in the Figures 8 and 9 below:

Characteristic	Specification
Sample capacity	9 wells
Sample volume per well	33 uL
Sampling channels	3
Time to Result	<70 minutes including sample prep
Operating temperature range	32° - 95° F
Instrument size	~140 cubic inches
System (instrument and phone) weight in tactical carry configuration	~5 lbs.
PCR runs per battery charge	4
Storage temperature range	-4° to 113° F

Fig. 8. QPrep-9TM Characteristics

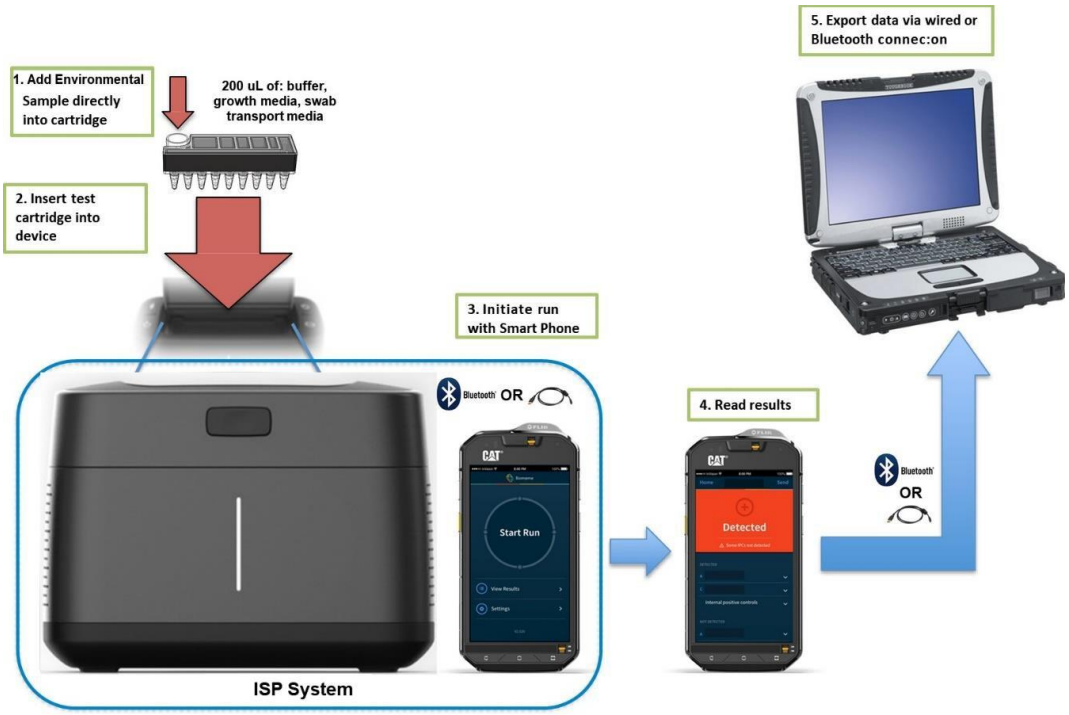

Fig. 9. Block diagram overview of the QPrep-9TM workflow.

5.2 Engineering Changes Resulting from DT&E Activities

The QPrep-9TM is still under development. A final prototype has been successfully tested and demonstrated. The final field-ready version is scheduled for completion in early 2020. Current efforts, which are continuing, focus on optimization of the sample prep and PCR performance.

Key engineering changes implemented to date as a result of DT&E activities include:

- Redesigning the lid and closing mechanism to increase and more evenly distribute the force applied to the cartridge to ensure full piercing of the seals.

- Merging the individual heater block wells of the thermalcycler core into a single "monolith" configuration in order to decrease well-to-well positional variation

- Over the course of several iterative development and test cycles involving a series of injection-moldings, various modifications to the design of the Sample Prep Cartridge and Cards were introduced to improve sample prep and PCR performance, including:

    - Changes to the nozzle geometry
    - Selection of different materials for top disk and other components
    - Adjustments to fluid channel widths

    - Addition of a sample well funnel
    - Modifications to gasket designs to improve sealing performance
    - Replacing manually cut valve and other parts with molded ones
    - Modifications to the shroud portion of the cartridge to allow for better equilibration during waste absorption.

- Addition of design elements to prevent release of biohazards, chemical/reagent spills, and carry-over contamination, including:

    - Sample well cap
    - Self-sealing plugs guarding all exit paths requiring two-way air flow
    - Transient seals (dry side to wet side, test card to device, nozzles to PCR wells)
    - Additional gaskets
    - Rib feature to prevent sponge sealing
    - Vent plugs
    - Polished nozzle to avoid use of gel sealants
    - Sample funnel and displacement "valves"

- Modifications to the formulation of the sample prep reagents and sample prep protocol to optimize performance and minimize sample-to-answer time

- Optimizing fluidics balancing through numerous (iterative) adjustments to the size and placement of pumps

- Switch from laser cutting of the PSA to using a converter instead to further minimize low Signal/PCR Inhibition

- Changes to assay packaging materials and packaging system for the consumables based on the results of accelerated stability testing

6.0 Vector-Borne Assay Development and Sample Preparation

6.1 Findings and Results

The current capability developed during this effort consists of a manual sample prep protocol and a lyophilized assay panel for the Biomeme Franklin three9 PCR device. The current effort involved development and evaluation of a lyophilized panel of real-time reverse transcriptase polymerase chain reaction (RT-PCR) assays for detection of the following vector-borne pathogens on the Biomeme Franklin three9 device: Yellow Fever virus, Chikungunya virus, Dengue virus, Zika virus, and *Plasmodium* species.

In addition to evaluating sensitivity and specificity of the assays with purified nucleic acids, a working limit of detection (LoD) was determined for each pathogen in blood, using Biomeme manual sample prep reagents and consumables.

CB.56 packages for the assays are included as Appendices to this Technical Report.

6.2 Engineering Changes Resulting from DT&E Activities

Attached to this Report as Appendices are updated versions of:

- Pilot Lot Report
- Sensitivity and Specificity Report
- Sample Prep SOP for the Vector-Borne Panel

The above updated reports, along with the CB.56 packages, summarize the final assays including changes resulting from DT&E activities.

List of Appendices (See attachments for Appendices A-F)

Appendix A. Pilot Lot Report Updated July 26, 2019
Appendix B. Sensitivity and Specificity Report Updated July 26, 2019
Appendix C. Sample Prep SOP Updated July 26, 2019
Appendix D. DENV/ZIKV CB.56 Rev.0
Appendix E. Plas/EndoIPC_RNaseP CB.56 Rev.0
Appendix F. YFV/CHIKV/ExoIPC_MS2 CB.56 Rev.0