| | | |
|---|---|---|
| PI: **LOCHHEAD, MICHAEL J** | Title: Point-of-Care HIV Antigen/Antibody Diagnostic Device | |
| Received: 05/07/2013 | FOA: PA13-088 | Council: 10/2013 |
| Competition ID: ADOBE-FORMS-B2 | FOA Title: PHS 2013-02 Omnibus Solicitation of the NIH, CDC, FDA and ACF for Small Business Innovation Research Grant Applications (Parent SBIR [R43/R44]) | |
| **2 R44 AI093289-02A1** | Dual: | Accession Number: 3586675 |
| IPF: 10026307 | Organization: MBIO DIAGNOSTICS, INC. | |
| Former Number: | Department: | |
| IRG/SRG: ZRG1 AARR-E (81)B | AIDS: N | Expedited: N |
| Subtotal Direct Costs (excludes consortium F&A)<br>Year 2: [redacted]<br>Year 3: [redacted] | Animals: N<br>Humans: Y<br>Clinical Trial: N<br>Current HS Code: 20<br>HESC: N | New Investigator: N<br>Early Stage Investigator: N |
| | | |
| *Senior/Key Personnel:* | *Organization:* | *Role Category:* |
| Michael Lochhead | MBio Diagnostics, Inc. | PD/PI |
| Daniel Nieuwlandt | MBio Diagnostics, Inc. | Other (Specify)-Senior Scientistg |
| Constance Benson | University of California, San Diego | Co-Investigator |
| Susan Little | University of California, San Diego | Co-Investigator |

OMB Number: 4040-0001
Expiration Date: 06/30/2011

**APPLICATION FOR FEDERAL ASSISTANCE**
# SF 424 (R&R)

| 3. DATE RECEIVED BY STATE | State Application Identifier |
|---|---|
| | |

**1. * TYPE OF SUBMISSION**
☐ Pre-application ☒ Application ☐ Changed/Corrected Application

**2. DATE SUBMITTED** | **Applicant Identifier**

**4. a. Federal Identifier** [redacted]

**b. Agency Routing Identifier**

**5. APPLICANT INFORMATION** *** Organizational DUNS:** [redacted]

* Legal Name: MBio Diagnostics, Inc.
Department: | Division:
* Street1: 5603 Arapahoe Avenue,Suite 1
Street2:
* City: Boulder | County / Parish: `
* State: CO: Colorado | Province:
* Country: USA: UNITED STATES | * ZIP / Postal Code: 80303-1377

Person to be contacted on matters involving this application
Prefix: | * First Name: Cynthia | Middle Name:
* Last Name: Moore | Suffix:
* Phone Number: [redacted] | Fax Number: [redacted]
Email: [redacted]

**6. * EMPLOYER IDENTIFICATION** *(EIN) or (TIN):* [redacted]

**7. * TYPE OF APPLICANT:** R: Small Business
Other (Specify):
**Small Business Organization Type** ☐ Women Owned ☐ Socially and Economically Disadvantaged

**8. * TYPE OF APPLICATION:**
☐ New ☒ Resubmission
☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).
☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration
☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

**9. * NAME OF FEDERAL AGENCY:** National Institutes of Health

**10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:**
TITLE:

**11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:**
Point-of-Care HIV Antigen/Antibody Diagnostic Device

**12. PROPOSED PROJECT:**
* Start Date: 10/01/2013 | * Ending Date: 09/30/2015

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT:** CO-002

**14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION**
Prefix: Dr. | * First Name: Michael | Middle Name: J
* Last Name: Lochhead | Suffix:
Position/Title: Vice President/CTO
* Organization Name: MBio Diagnostics, Inc.
Department: | Division:
* Street1: 5603 Arapahoe Avenue,Suite 1
Street2:
* City: Boulder | County / Parish: `
* State: CO: Colorado | Province:
* Country: USA: UNITED STATES | * ZIP / Postal Code: 80303-1377
* Phone Number: [redacted] | Fax Number: [redacted]
* Email: [redacted]

**15. ESTIMATED PROJECT FUNDING**

| | |
|---|---|
| a. Total Federal Funds Requested | [redacted] |
| b. Total Non-Federal Funds | 0.00 |
| c. Total Federal & Non-Federal Funds | [redacted] |
| d. Estimated Program Income | [redacted] |

**16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?**

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

**17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001)**

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

**18. SFLLL or other Explanatory Documentation**

Add Attachment | Delete Attachment | View Attachment

**19. Authorized Representative**

Prefix: Ms. * First Name: Cynthia Middle Name:

* Last Name: Moore Suffix:

* Position/Title: Director of Finance and Administration

* Organization: MBio Diagnostics, Inc.

Department: Division:

* Street1: 5603 Arapahoe Avenue,Suite 1

Street2:

* City: Boulder County / Parish: `

* State: CO: Colorado Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: 80303-1377

* Phone Number: [redacted] Fax Number: [redacted]

* Email: [redacted]

*** Signature of Authorized Representative**: Michael Lochhead

*** Date Signed**: 05/07/2013

**20. Pre-application** Add Attachment | Delete Attachment | View Attachment

# 424 R&R and PHS-398 Specific
# Table Of Contents

OMB Number: 4040-0010
Expiration Date: 08/31/2011

## Project/Performance Site Location(s)

**Project/Performance Site Primary Location** ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: MBio Diagnostics, Inc.

DUNS Number: [redacted]

* Street1: 5603 Arapahoe Avenue, Suite 100

Street2:

* City: Boulder County:

* State: CO: Colorado

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 80303-1377 * Project/ Performance Site Congressional District: CO-002

**Project/Performance Site Location** 1 ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of California, San Diego

DUNS Number: [redacted]

* Street1: 220 Dickson Street, Suite A

Street2:

* City: San Diego County:

* State: CA: California

Province:

* Country: USA: UNITED STATES

* ZIP / Postal Code: 92103-8950 * Project/ Performance Site Congressional District: CA-053

**Additional Location(s)** Add Attachment Delete Attachment View Attachment

# RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☒ Yes ☐ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☒ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☒ No

IRB Approval Date: 10/06/2011

Human Subject Assurance Number: [redacted]

2. * Are Vertebrate Animals Used? ☐ Yes ☒ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☐ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. * Is proprietary/privileged information included in the application? ☒ Yes ☐ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

**7. * Project Summary/Abstract** 1239-Project Summary.pdf Add Attachment Delete Attachment View Attachment

**8. * Project Narrative** 1240-Project Narrative.pdf Add Attachment Delete Attachment View Attachment

**9. Bibliography & References Cited** 1241-Bibliography.pdf Add Attachment Delete Attachment View Attachment

**10. Facilities & Other Resources** 1242-MBio Facilities Other Resources Add Attachment Delete Attachment View Attachment

**11. Equipment** 1243-MBio Equipment 20120823.pdf Add Attachment Delete Attachment View Attachment

**12. Other Attachments** Add Attachments Delete Attachments View Attachments ☐

MBio Diagnostics, Inc. proposes to develop a low-cost, point-of-care (POC), HIV-1/2 antigen/antibody combination diagnostic device. The goal is for the Ag/Ab combo system to deliver the performance of laboratory based, 4th generation antigen/antibody clinical analyzers, in a POC platform with cost and workflow of the widely used HIV rapid tests. Phase I research and development met or exceeded stated technical milestones. In particular, the MBio HIV antigen and antibody assays provided a combined performance that significantly exceeds performance FDA approved rapid tests, and provides near equivalence to much more complicated laboratory based tests. HIV infection remains a major public health crisis both in the United States and worldwide. There is increasing awareness that acutely infected individuals disproportionately contribute to disease spread. Yet these individuals remain the most difficult to identify, as infectivity is highest prior to the appearance of the HIV antibodies that serve as the basis for serological diagnostics. There are currently no FDA-approved point-of-care (POC) tests that directly target HIV viral antigens. An HIV-1 antigen/antibody (Ag/Ab) combination assay – the so-called "4th generation" immunoassay – in an inexpensive, simple to use, POC format would fundamentally improve HIV-1 screening efforts in the United States and worldwide. The specific aims of this proposal are to: (1) Combine the Phase I p24 antigen detection assay with the MBio multiplexed serology assay cartridge, addressing issues of final monoclonal antibody (mAb) pair selection, HIV-1 Ag and HIV-2 Ag selection, reagent conjugations, cross-reactivity, and minimization of assay steps and complexity. (2) Modify the MBio Cartridge, Rack, Reader, and Software to deliver an automated HIV-1/2 Ag/Ab combo result, and incorporate heat stable assay reagents into the MQ cartridge. The Aim 2 milestone is a portable, integrated system delivered to clinical collaborators that meets FDA CLIA waiver guidance requirements. (3) Validate system using well characterized early HIV infection specimens including a panel of 200 HIV positive specimens comprised of 20 acute infection samples, early seroconversion, and seropositive samples. 200 HIV-negative samples will be used for specificity testing. (4) Place systems in an intended use setting and capture operational and usability feedback in advance of design lock, and to use this site to generate a preliminary dataset on capillary whole blood samples. The outcome of this program will be a system design and dataset for an FDA investigational device exemption (IDE) meeting in advance of clinical trials.

The proposed point-of-care (POC) HIV-1 Ag/Ab combination diagnostic device will fill a critical unmet need for HIV screening technologies that detect recent and acutely infected individuals. The technology will be used in multi-test algorithms in public health laboratories, STD clinics, urban emergency departments, targeted outreach programs, etc., both in the United States and worldwide.

**MBIO DIAGNOSTICS, INC.**

## FACILITIES AND OTHER RESOURCES

### Biosafety and Biocontainment

MBio operates a dedicated Biosafety Level 2 Laboratory that has been designed per guidance in the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. MBio contracts certified industrial hygiene experts (Hellman and Associates, Wheat Ridge, CO) to perform annual training, safety audits, and written plan reviews. Per OSHA guidelines, MBio meets Bloodborne Pathogen training and vaccination requirements. Biological waste management is through a certified medical waste hauler (Colorado Medical Waste, Inc.).

All MBio staff with exposure to human samples are also up-to-date on required Protection of Human Subjects training.

### General Facility Description

MBio Diagnostics occupies a total of approximately 17,000 sq. ft. on Arapahoe in Boulder, CO with approximately 9,000 sq ft dedicated to laboratory and manufacturing. In addition to MBio's technical staff members, the company has a 3 person G&A team that provides accounting, purchasing services, and human resources support, a 4 person clinical & regulatory team providing clinical, regulatory and quality support, and a 2 person marketing department. The MBio facility includes employee offices, cubicles, computer workstations and network support, conference rooms, projectors, etc.

MBio's laboratories are divided into 5 controlled access work areas, described here.

*Assay Wet Lab*. The assay lab serves as the wet lab for standard assay development procedures. It is equipped with a sink, lab refrigerator, -20C freezer, dishwasher, sonicator and various mixers and shakers. Active glassware and lab disposables are stored here. The work space has lab benches for four scientists (~350 sq. ft).

*Biosafety Level 2 Laboratory.* MBio operates a dedicated Biosafety Level 2 Laboratory that has been designed per guidance in the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. The dedicated BL2 laboratory is a controlled access room housing a sink, three certified Class II biosafety cabinets, a state-of-the-art Beckman Allegra biosafety centrifuge. Refrigerators, a -20C freezer, and a -80C freezer are in the BL2 laboratory.

*PCR laboratories*. The facility has a three-room PCR laboratory set up. The first of these laboratories is the pre-PCR reagent clean room. This is a 110 sq. ft. area dedicated to PCR reagent preparation and is equipped with a HEPA-filtered UV-equipped forced-air hood to prevent PCR contamination. The second PCR station resides in the BL2 laboratory with a dedicated biosafety cabinet to allow for safe nucleic acid extraction from biohazardous material and PCR target addition in a controlled environment. The final PCR station resides within the Assay wet lab. This lab houses the thermocycler, gel electrophoresis equipment and imaging systems and has sufficient space and resources for many downstream analyses. The three PCR stations are spatially separated, have been designed for unidirectional traffic flow, and each contain dedicated 4C refrigerators, -20C freezers, pipettes, and other common, necessary lab equipment.

*Instrument Laboratory*. This ~190 sq. ft dedicated area houses the benches and optical tables used in instrument fluorescence waveguide instrument development. Test equipment, laboratory computers, and the fluorescence microscope are housed here.

*Array Fabrication Cleanroom*. The ~200 sq. ft. cleanroom facility houses MBio's Bio-Dot microarraying robot, enabling prototype waveguide substrate array printing in a cleanroom environment analogous to a full-scale production facility.

A second fabrication room (~120 sq. ft.) focuses on final waveguide preparation and cartridge assembly. This space includes a spin coater, pneumatic press, sonication baths used for cleaning, Sorvall centrifuge, a deionized water supply, and various scales and pumps..

*Cartridge Development Area*. A dedicated workspace has been created for disposable cartridge development. This area provides material storage, cutting tools, as well has peristaltic and syringe pump for controlled flowrate fluidic system development. Heat sealing equipment is available for cartridge packaging. Two Tenney environmental chambers, one with humidity control, are used for cartridge stability studies. The room also contains an AirClean Systems ductless chemical fume hood, benchtop vacuum ovens, and plasma treatment oven, used for surface chemistry preparations. The custom contact angle measurement tool in this room is used for surface characterization and quality control.

## Equipment

MBio Diagnostics operates a fully equipped R&D laboratory. Equipment is organized by location or affiliation to that location. The dedicated facilities listed here are described in detail in the Facilities document submitted with this grant.

Assay Wet Lab

- Gel electrophoresis equipment (PAGE and agarose; prep and analytical)
- Bio-Rad Versadoc gel imager
- Bio-Rad GelDoc Imaging System
- -20°C Laboratory freezer (Kenmore)
- Shaker (Barnstead/Labline)
- Microfuge (VWR Galaxy Mini)
- Various pipetters (Rainin LTS)
- Pipet-Aid automatic pipetters
- Hotplate-stirrers (VWR)
- Vortex mixers
- pH meters (Mettler)
- Analytical balance (A&D Limited)
- Laboratory refrigerators (Kenmore, Frigidaire)
- Microcentrifuges (Fisher accuSpin)
- Spectrophotometer (Thermo NanoDrop)
- Fisher Scientific IsoTemp temp block
- De-ionized and MilliQ water purification
- SPT Ice Maker
- PCR Thermal Cycler (Perkin Elmer 2400)
- BiioRad CFX96 Real-Time Thermal Cycler System
- Luminex MagPix
- BioCad 700E Perfusion Chromatography workstation

Cell Culture / Biosafety Level 2 Lab

- 3 Class II Biosafety Cabinets (4' SterilGard, 6' NuAire, 6' Labconco)
- $CO_2$ Incubator (VWR 2350)
- Centrifuge (Beckman Allegra X-12R)
- Microplate Thermoshaker (Kisker Biotech PSF-100HL)
- Microplate reader (BioTek ELX800)
- Autoclave (Napco)
- Laboratory refrigerator (VWR)
- -20°C Laboratory freezer (Frigidaire)
- Cell Culture Plate Incubator
- Microcentrifuge (Fisher accuSpin)
- Water Bath (VWR)
- Pipetters
- Rotators and mixers

Pre-PCR

- PCR Workstation (Airclean 600)
- -20C freezer
- Refrigerator
- Pipetters

Common Area

- -80°C upright freezer (Forma Scientific)
- -20C freezer (ScienTemp)
- Liquid Nitrogen Freezer (dewar equipment with cryobox rack system)
- 2 Tenney Environmental chambers (T2RC and TUJR)
- Heat sealers for packaging
- 4 x Refrigerator

Instrument Lab

- Digital oscilloscope & multimeters
- Function generator
- CMOS cameras
- Lock-in amplifier
- Spectrum analyzers
- DC current/voltage supplies
- Laser diode drivers
- Laser power meter
- Lasers
- Various optics + mounts + hardware
- Various electronics
- Olympus IX-71 inverted fluorescence microscope with phase optics and Retiga cooled CCD camera

Cleanroom/Chemistry Lab

- Ductless chemical fume hood, AirClean Systems XL10
- Vacuum ovens (VWR 1400E and VWR 1430M )
- Labconco Filtermate auxiliary air filter
- Nitrogen purged drybox
- 2 x Plasma Oven (PlasmaEtch PE-200; Harrick Plasma PDC-001)
- Vacuum Pumps (Edwards E2M8 and Edwards XDS-5 Dry Scroll)

- Vacuum desiccator for process development
- Labconco Centrivap cold trap (# = 2)
- Custom Contact Angle Goniometer
- Bio-Dot AD3200 robotic arrayer equipped with Bio-Jet print head, humidity control system, and dedicated workstation with AxSys software package

Cartridge Assembly Area

- Pneumatic press (Sonitek TS500)
- 2 x Spin Coater (CPK Industries; Laurell WS-650-23)
- Swinging Bucket Centrifuge (Sorvall T6000D)
- Bath sonicators (Crest, # = 2)
- Stereomicroscope with video capture
- Syringe pump
- Peristalitic pump (Ecoline)
- Sink
- USFilter de-ionizer
- Denver Instruments P-402 Balance
- Symphony SB70P pH Meter

OMB Number: 4040-0001
Expiration Date: 06/30/2011

# RESEARCH & RELATED Senior/Key Person Profile (Expanded)

**PROFILE - Project Director/Principal Investigator**

Prefix: Dr. * First Name: Michael Middle Name: J
* Last Name: Lochhead Suffix:
Position/Title: Vice President/CTO Department:
Organization Name: MBio Diagnostics, Inc. Division:
* Street1: 5603 Arapahoe Avenue,Suite 1
Street2:
* City: Boulder County/ Parish: `
* State: CO: Colorado Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 80303-1377
* Phone Number: [redacted] Fax Number: [redacted]
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** PD/PI **Other Project Role Category:**

**Degree Type:**
**Degree Year:**

***Attach Biographical Sketch** 1235-Lochhead_Biosketch_HIV-A Add Attachment Delete Attachment View Attachment
**Attach Current & Pending Support** Add Attachment Delete Attachment View Attachment

**PROFILE - Senior/Key Person 1**

Prefix: Dr. * First Name: Daniel Middle Name:
* Last Name: Nieuwlandt Suffix:
Position/Title: Senior Scientist Department:
Organization Name: MBio Diagnostics, Inc. Division:
* Street1: 5603 Arapahoe Avenue, Suite 1
Street2:
* City: Boulder County/ Parish:
* State: CO: Colorado Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 80303-1377
* Phone Number: [redacted] Fax Number: [redacted]
* E-Mail: [redacted]

Credential, e.g., agency login:

*** Project Role:** Other (Specify) **Other Project Role Category:** Senior Scientistg

**Degree Type:**
**Degree Year:**

***Attach Biographical Sketch** 1236-Nieuwlandt_Biosketch_P22 Add Attachment Delete Attachment View Attachment
**Attach Current & Pending Support** Add Attachment Delete Attachment View Attachment

# RESEARCH & RELATED Senior/Key Person Profile (Expanded)

**PROFILE - Senior/Key Person 2**

Prefix: Dr. * First Name: Constance Middle Name:
* Last Name: Benson Suffix:
Position/Title: Professor of Medicine Department:
Organization Name: University of California, San Diego Division:
* Street1: 200 W. Arbor Drive, MC#8208
Street2:
* City: San Diego County/ Parish:
* State: CA: California Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 82103-8960
* Phone Number: [redacted] Fax Number: [redacted]
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** Co-Investigator **Other Project Role Category:**

**Degree Type:**
**Degree Year:**

***Attach Biographical Sketch** 1237-Benson BioSketch.pdf Add Attachment Delete Attachment View Attachment
**Attach Current & Pending Support** Add Attachment Delete Attachment View Attachment

**PROFILE - Senior/Key Person 3**

Prefix: Dr. * First Name: Susan Middle Name: J
* Last Name: Little Suffix:
Position/Title: Professor of Medicine Department:
Organization Name: University of California, San Diego Division:
* Street1: 200 W. Arbor Drive, MC8208
Street2:
* City: San Diego County/ Parish:
* State: CA: California Province:
* Country: USA: UNITED STATES * Zip / Postal Code: 92103-8950
* Phone Number: [redacted] Fax Number: [redacted]
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** Co-Investigator **Other Project Role Category:**

**Degree Type:**
**Degree Year:**

***Attach Biographical Sketch** 1238-Little Biosketch.pdf Add Attachment Delete Attachment View Attachment
**Attach Current & Pending Support** Add Attachment Delete Attachment View Attachment

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| Michael Joseph Lochhead | Vice President, MBio Diagnostics, Inc. |
| eRA COMMONS USER NAME (credential, e.g., agency login) [redacted] | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | MM/YY | FIELD OF STUDY |
|---|---|---|---|
| University of Notre Dame, Notre Dame, IN | B.S. | 1988 | Chemical Engineering |
| University of Notre Dame, Notre Dame, IN | B.A. | 1988 | Government |
| University of Wisconsin, Madison, WI | Ph.D. | 1995 | Chemical Engineering |
| University of Washington, Seattle, WA | Post-Doc | 1996-1998 | Bioengineering |

### A. Personal Statement

The purpose of this project is to integrate the quantitative p24 direct antigen detection assay into the MBio POC diagnostics product development path. I have the expertise and leadership required to carry out the proposed work in a cost effective manner. Over the last 5 years, I have managed growth of the MBio Diagnostics from a 2 person R&D group within Precision Photonics Corporation (Boulder, CO) to a medical diagnostics company incorporated in early 2010. As Vice President since the Company's inception, I lead research, development, and commercialization efforts for MBio's product pipeline. I am Principal Investigator on multiple major contracts with the National Institutes of Health, and I manage clinical collaborations in the United States, Brazil, Kenya, and Mozambique. While at Accelr8 Technology Corp. (Denver, CO), I successfully moved two surface chemistry products targeted specifically for microarray applications from conception through R&D and commercialization. While at Accelr8 I was also project manager for the company's prototype microscopy-based microbiology instrument, which combined integrated plastic microfluidic disposables and a fluidic control system. My academic career included an Assistant Professor position in the Department of Chemical Engineering at the University of New Hampshire where my group focused on advanced materials for biosensor and biomaterial applications. This followed my post-doctoral training in the Bioengineering program at the University of Washington where my research focuses included biomaterials engineering and basic science and microfluidic device development. I am co-inventor on multiple issued and pending patents. In summary, I have a demonstrated record of successful project management in the areas of assay devices, surface chemistry, and instrumentation, all of which are relevant to the current project.

### B. Positions and Honors

**Positions and Employment**

1988-1990 **Staff Engineer**, ICF Incorporated, Fairfax, VA
1990-1995 **Graduate Studies**, Department of Chemical Engineering, University of Wisconsin, Madison, WI,
1996-1997 **Post-Doctoral Research Associate**, Department of Bioengineering, University of Washington, Seattle, WA
1998-2001 **Assistant Professor**, Department of Chemical Engineering, University of New Hampshire, Durham, NH
2001-2007 **Senior Scientist**, Accelr8 Technology Corporation, Denver, CO
2007-2008 **Diagnostics Team Leader**, Precision Photonics Corporation, Boulder, CO
2008-2010 **Vice President**, MBio Diagnostics division of Precision Photonics Corporation, Boulder, CO
2010-present **Vice President**, MBio Diagnostics, Inc., Boulder, CO

**Other Experience and Professional Memberships:**

| | |
|---|---|
| 1999-2001 | Participating Faculty, Center to Advance Molecular Interaction Sciences (CAMIS), University of New Hampshire (1999-2001) |
| 1995-2007 | Member, American Chemical Society |
| 2008- | Member, American Association for Clinical Chemistry |
| 2008- | Journal reviewer for *Analytical Chemistry* (American Chemical Society) |
| 2009- | Journal reviewer for *Lab on Chip* (Royal Society of Chemistry) |
| 2009- | Journal reviewer for *Physical Chemistry Chemical Physics* (Royal Society of Chemistry) |
| 2009- | Journal reviewer for *Analyst* (Royal Society of Chemistry) |
| 2010- | NIH National Cancer Institute, SBIR Program Peer Review Committee |

**Honors:**

| | |
|---|---|
| 1993 | Ragatz Outstanding Teaching Award, University of Wisconsin, Madison, WI |
| 1993-1994 | Amoco Research Fellowship, Chemical Engineering, University of Wisconsin, Madison, WI |
| 1993-1995 | Teaching Assistant Fellow, College of Engineering, University of Wisconsin, Madison, WI |

**C. Selected Peer-reviewed Publications**


1. **Lochhead, M. J.**; Letellier, S. R.; Vogel, V., Assessing interfacial electrostatics in oriented mineral nucleation at ordered organic monolayers. *J Phys Chem B* **1997,** 101, 10821.
2. Letellier, S. R.; **Lochhead, M. J.**; Campbell, A. A.; Vogel, V., Oriented growth of calcium oxalate monohydrate crystals beneath phospholipid monolayers. *Biochim Biophys Acta* **1998,** 1380, (1), 31-45.
3. **Lochhead, M. J.**; Touryan, L. A.; Vogel, V., In situ analysis of europium-calcium-oxalate crystallization using luminescence microspectroscopy. *J Phys Chem B* **1999,** 103, 3411.
4. Clarner, M. A.; **Lochhead, M. J.**, Hybrid Micro-Optical Sensors via Sol-Gel Soft Lithography. *Mat. Res. Soc. Symp. Ser.* **2000,** 628, CC9.3.1-CC9.3.6.
5. Metzger, S.; **Lochhead, M. J.**; Grainger, D. W., Surface technologies to improve performance in protein microarray based molecular diagnostics. *IVD Technology* **2002,** 8, (5), 39-45.
6. Touryan, L. A.; **Lochhead, M. J.**; Marquardt, B. J.; Vogel, V., Sequential switch of biomineral crystal morphology using trivalent ions. *Nat Mater* **2004,** 3, (4), 239-43.
7. Grainger, D. W.; Greef C. H.; Gong, P.; **Lochhead, M. J.**, Current Microarray Surface Chemistries, in Microarrays, Second Edition, Volume I: Synthesis Methods, J. Rampal, editor; Humana Press, Methods in Molecular Biology series, **2007**.
8. Saldarriaga Fernandez, I.C; van der Mei, H. C.; **Lochhead, M. J.**; Grainger, D. W.; Busscher, H. J., Bacterial adhesion onto surfaces of a multi-component cross-linked poly(ethylene glycol)-based polymer coating. *Biomaterials* **2007**, 28, 4105-4112.
9. Harbers, G. M.; Emoto, K.; Greef, C.; Metzger, S. W.; Woodward, H. W.; Mascali, J. J.; Grainger, D. W.; **Lochhead, M. J.**, Functionalized Poly(ethylene glycol)-based Bioassay Surface Chemistry that Facilitates Bio-Immobilization and Inhibits Nonspecific Protein, Bacterial, and Mammalian Cell Adhesion. *Chem. Mat.* **2007**, 19(18), 4405-4414.
10. Myatt, C.J.; Delaney, M.J.; Todorof, K.; Heil, J.R.; Givens, M.; Schooley, R.T.; **Lochhead, M.J**, Low-cost, multiplexed biosensor for disease diagnosis, Proc. SPIE 7167 Frontiers in Pathogen Detection: From Nanosensors to Systems, 716703 (2009)
11. **Lochhead, M.J.**, Insights from the 2010 HIV Diagnostics Conference. *Expert Rev Mol Diagn* **2010**, 10(5) 565-567.
12. **Lochhead M.J.**, Todorof K., Delaney M., Ives J.T., Greef C., Moll K., et al. Rapid Multiplexed Immunoassay for Simultaneous Serodiagnosis of HIV-1 and Co-Infections. J Clin Microbiol. 2011, 49(10) 3584-3590.
13. Devlin S, Meneely JP, Greer B, Greef C, **Lochhead MJ**, Elliott CT. Next generation planar waveguide detection of microcystins in freshwater and cyanobacterial extracts, utilising a novel lysis method for portable sample preparation and analysis. *Anal Chim Acta*. 2013; 769:108-13.
14. Meneely JP, Campbell K, Greef C, **Lochhead MJ**, Elliott CT. Development and validation of an ultrasensitive fluorescence planar waveguide biosensor for the detection of paralytic shellfish toxins in marine algae. *Biosens Bioelectron.* 2013; 41:691-7.

15. Logan C, Givens M, Ives JT, Delaney M, **Lochhead MJ**, Schooley RT, et al. Performance evaluation of the MBio Diagnostics point-of-care CD4 counter. *J Immunol Methods*. 2013; 387(1-2):107-13. PMCID: 3529779.

**D. Research Support**

**Ongoing Research Support:**

NIH NIAID 5R44AI070052-05 Lochhead (PI) 03/01/2006 – 02/28/2014
Low Cost Laser Diagnostic for CD4+ T Cell Counting
The goal of this project is to build on the cell enumeration instrument developed under NIST-ATP FY2007 70NANB7H7053, improving count statistics, adding additional CD markers, and performing larger volume clinical sample testing.
Role: Principal Investigator

NIH NIAID 5R01AI096189-02 Lochhead (PI) 07/05/2011 – 06/30/2016
Integrated System for Rapid Detection of Respiratory Pathogens
The goal of this project is development of a low-cost, no amplification, nucleic acid diagnostic technology for respiratory pathogens.
Role: Principal Investigator

NIH NIAID 2R44AI68543-06 Myatt (PI) 09/01/2009 – 08/31/2013
Development of Low Cost Multi-Pathogen Laser Diagnostics for HIV and AIDS Co-Infections
The goal of this project is development of an inexpensive, multiplexed serotyping and antigen detection point-of-care diagnostics platform, targeted at HIV and opportunistic infection screening.
Role: Project Manager, Scientist

**Completed Research Support** (last 3 years)

NIH U54 AI065359 Barbour (PI) 05/01/2009 – 04/30/2011
Subaward No. 2009-2167 Myatt (Subaward, PI)
Pacific Southwest Regional Center of Excellence for Biodefense and Emerging Infectious Diseases. Project 2.5: Benchtop Devices for Detection of BoNT in Clinical Samples
The goal of this project is development of a clinical assay system based on the botulinum toxin Assay with Large Immono-Sorbent Surface Area (ALISSA) format. The aim of the subaward project is to create, test, and validate waveguide-based detection system that will be integrated with the automated ALISSA instrument.
Role: Project Manager, Scientist

NIH NIAID 1R43AI093289-01A1 Lochhead (PI) 08/10/2011 – 07/31/2012
Point-of-Care HIV Antigen/Antibody Diagnostic Device
The goal of this project is to develop a low-cost, point-of-care (POC), HIV-1 antigen/antibody combination diagnostic device using MBio's novel multiple particle assay approach. The system goal is to deliver performance of laboratory based, clinical analyzers, in a POC platform with cost and workflow of widely used HIV rapid tests.

NIH NIAID 7R43AI091097-02 Lochhead (PI) 07/03/2010 – 01/31/2011
Multiple Antigen Point-of-Care Device for Improved Syphilis Diagnosis
The goal of this project is development of a quantitative non-treponemal assay to complement mBio's treponemal-specific rapid diagnostic. The combined treponemal / non-treponemal test will significantly advance point-of-care syphilis testing.
Role: Principal Investigator

NIH NIAID 1U01AI074521-04 Schooley (PI) 07/01/2007 – 06/30/2012
Multiplex Nucleic Acid Detection Device for the Diagnosis of Respiratory Viruses

The goal of this project is development of the low-cost, field deployable instrumentation and analysis platform for multiplexed nucleic acid assays, targeting influenza virus subtyping specifically and respiratory pathogen identification in general.
Role: Project Manager, Scientist

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| Daniel Terry Nieuwlandt | Senior Scientist, MBio Diagnostics, Inc. |
| eRA COMMONS USER NAME (credential, e.g., agency login) | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | MM/YY | FIELD OF STUDY |
|---|---|---|---|
| California State University, Fullerton, CA | B.A. | 1985 | Biological Science |
| Iowa State University, Ames, IA | M.S. | 1988 | Microbiology |
| Ohio State University, Columbus, OH | Ph.D. | 1992 | Microbiology |
| University of Colorado, Boulder, CO | Post. Doc. | 1992-1994 | Molecular Biology |

### A. Personal Statement

With over 25 years of research experience, including 18 years with a primary focus on assay development at multiple early-phase biotechnology companies, I bring a breadth of research and project management experience to this project. Of particular relevance is my extensive experience with IVD development and bioconjugation techniques. As a Senior Scientist immersed in HIV and influenza IVD R&D at MBio Diagnostics, I have extensive experience with all aspects of the company's planar waveguide technology. As Director of Assay Development at Lyzer Diagnostics, Inc., I brought an "Enhanced Velocity Electroimmunoassay" platform (high sensitivity solution phase immunoassay) from concept to a functioning prototype. At Great Basin Scientific, I led a team of scientists that developed a rapid automated molecular assay for Staphylococcal species. As an assay development Group Leader at SomaLogic, Inc., I led the successful development of sensitive and highly specific nucleic acid-based microarray diagnostic assays based on the company's photoaptamer microarray technology. I additionally held the positions of Director of Biology and Vice President, Biology, at Invenux, Inc., and Associate Research Director at NeXstar Pharmaceuticals, Inc. At Invenux, I directed a project aimed at discovering antibiotics active against MRSA, a research effort that required the cloning, expression, and purification of PBP2a and amino acid adding enzymes (peptidoglycan biosynthesis pathway) and the development of assays for enzyme activity and methods for catalytic RNA partitioning. My role at NeXstar included the development of aptamer reagents specific for Group A Streptococci and *Chlamydia* elementary bodies and the management of a collaborative diagnostics research effort with Becton Dickinson. Each of the research and development efforts at MBio Diagnostics, Lyzer Diagnositcs, Somalogic, Invenux, and NeXstar required the conjugation of proteins or nucleic acids to dyes and microspheres and the use of these constructs as IVD reagents. I am a co-inventor on six issued patents. In brief, I am a microbiologist with extensive molecular biology experience and a track record of successful IVD development.

### B. Positions and Honors

**Positions and Employment**

1992-1994 **Postdoctoral Research Fellow**; University of Colorado, Boulder, CO
1994-1996 **Senior Scientist**; NeXagen, Inc. / NeXstar Pharmaceuticals, Inc., Boulder, CO
1996-1999 **Associate Director**; NeXstar Pharmaceuticals, Inc., Boulder, CO
2000-2001 **Director of Biology**; Invenux, Inc., Denver, CO
2001-2003 **Vice President of Biology**; Invenux, Inc., Denver, CO
2003-2008 **Scientific Advisory Board Member**; Cropsolution, Inc.
2004-2007 **Senior Scientist & Assay Development Group Leader**; Somalogic, Inc., Boulder, CO
2007-2008 **Director of Assay Development**; Lyzer Diagnostics, Inc. Boulder, CO
2008-2010 **Director of Research**; Great Basin Scientific, Longmont, CO
2010-2010 **Director of Assay Development** (Jan-Aug); Lyzer Diagnostics, Inc. Boulder, CO
2010-present **Senior Scientist**; MBio Diagnostics, Inc., Boulder, CO

**Honors**

1987 Gamma Sigma Delta Honor Society of Agriculture membership.
1991 Phi Kappa Phi Honor Society membership.
1991 Proctor and Gamble Research Fellowship for “Transfer RNA Processing in the Archaebacterium *Haloferax volcanii*."
1991 Graduate Student Alumni Research Award from Ohio State University for “Characterization of Ribonuclease P from the Archaebacterium *Haloferax volcanii*."

**C. Peer-Reviewed Publications**


1. **Nieuwlandt, D. T**., and P. A. Pattee. **1989**. Transformation of a conditional peptidoglycan-deficient mutant of *Staphylococcus aureus* with plasmid DNA. J. Bacteriol. 171: 4906-4913.
2. Thompson, L. D., L. D. Brandon, **D. T. Nieuwlandt**, and C. J. Daniels. **1989**. Transfer RNA intron processing in the halophilic archaebacteria. Can. J. Microbiol. 35: 36-42.
3. **Nieuwlandt, D. T**., and C. J. Daniels. **1990**. An expression vector for the archaebacterium *Haloferax volcanii*. J. Bacteriol. 172: 7104-7110.
4. **Nieuwlandt, D. T**., E. S. Haas, and C. J. Daniels. **1991**. The RNA component of RNase P from the archaebacterium *Haloferax volcanii*. J. Biol. Chem. 266: 5689-5695.
5. **Nieuwlandt, D.T**., M. B. Carr, and C. J. Daniels. **1993**. In vivo processing of an intron-containing archael tRNA. Molec. Microbiol. 8: 93-99.
6. Palmer, J. R., **D. T. Nieuwlandt**, and C. J. Daniels. **1994**. Expression of a yeast intron-containing tRNA in the archaeon Haloferax volcanii. J. Bacteriol. 176: 3820-3823.
7. **Nieuwlandt, D. T**., M. Wecker, and L. Gold. **1995.** In vitro selection of RNA ligands to substance P. Biochemistry 34: 5651-5659.
8. Lin, Y., **D. Nieuwlandt**, A. Megallanez, B. Feistner, and S. D. Jayasena. **1996**. High-affinity and specific recognition of human thyroid stimulating hormone (hTSH) by in vitro-selected 2’-amino-modified RNA. Nucleic Acids Res. 24: 3407-3414.
9. **Nieuwlandt, D**. **1998.** In vitro selection of functional nucleic acid sequences. Pp. 117-132 In: Genetic Engineering with PCR. R. M. Horton and R. C. Tait, eds. Horizon Scientific Press. Portland, Oregon.
10. **Nieuwlandt, D**. **2000.** In vitro selection of functional nucleic acid sequences. Curr. Issues Mol. Biol. 2: 9-16.
11. Dewey, T. M., **D. Nieuwlandt**, and T. Tarasow. **2002**. Integrated drug discovery technology in a test tube. Current Drug Discovery. July 2002: 21-25.
12. **Nieuwlandt, D**., M. West, X. Cheng, G. Kirshenheuter, and B. E. Eaton. **2003**. The first example of an RNA urea synthase: selection through the enzyme active site of human neutrophile elastase. Chembiochem. 4: 651-654.
13. Tarasow, T.M., E. Kellogg, B.L. Holley, **D. Nieuwlandt**, S.L. Tarasow, and B.E. Eaton. **2004**. The effect of mutation on RNA Diels-Alderases. J. Am. Chem. Soc. 126(38): 11843-51.
14. Gold, L., D. Ayers, J. Bertino, C. Bock, A. Bock, E.N. Brody, J. Carter, et al. **2010**. Aptamer-based multiplexed proteomic technology for biomarker discovery. PLoS One 5(12): e15004.
15. Pasko, C., B. hicke, J. Dunn, H. Jaeckel, **D. Nieuwlandt**, D. Weed, E. Woodruff, X. Zheng, and R. Jenison. **2012**. Staph ID/R: a rapid method for determining staphylococcus species identity and detecting the mecA gene directly from positive blood culture. J. Clin. Microbiol. 50(3): 810-7.


**D. Research Support**

**Ongoing Research Support:**

NIH NIAID 5R01AI096189-02 Lochhead (PI) 07/05/2011 – 06/30/2016
Integrated System for Rapid Detection of Respiratory Pathogens
The goal of this project is development of a low-cost, no amplification, nucleic acid diagnostic technology for respiratory pathogens.
Role: Senior Scientist

## BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| **Constance A. Benson, M.D.** | **Professor of Medicine** |
| eRA COMMONS USER NAME [redacted] | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training and residency training if applicable..)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| The Ohio State University, Columbus, OH | B.A. | 1975 | Zoology |
| The Ohio State U. College of Med., Columbus, OH | M.D. | 1978 | Medicine |
| Rush University Medical Center (Chicago, IL) | Residency | 1979-81 | Internal Medicine |
| Rush University Medical Center (Chicago, IL) | Fellowship | 1986 | Infectious Diseases |

### A. Personal Statement

My role on the project will be to participate as the UCSD collaborative partner PI, and to participate in and oversee the design, development, implementation and conduct of the research at UCSD; to facilitate and oversee the recruitment, monitoring and followup of research participants; to oversee site and laboratory acquisition of samples and conduct of study procedures by research staff; to oversee sample and data collection and submission; and to participate in the analysis and interpretation of data and publication of results as appropriate. I am highly qualified for this role based on my experience as an internationally recognized translational and clinical researcher in the field of HIV/AIDS, serving in the investigative and scientific leadership of the NIH/NIAID AIDS Clinical Trials Group (ACTG) continuously from 1996 to 2010 (as PI/Chair from 2003-2010), and in these roles managing this multinational clinical trials network comprising 72 clinical research sites and laboratories in 14 different countries including the U.S., Haiti, Brazil, Peru, South Africa, Uganda, Zambia, Botswana, Malawi, Zimbabwe, Tanzania, Kenya, India, and Thailand. My responsibilities in the leadership of the ACTG included establishing and mentoring the development of investigators and research staff at 22 international clinical research sites in resource-limited settings and training more than 200 international investigators, affiliated research and laboratory personnel at these sites to conduct scientifically rigorous randomized clinical trials and laboratory research. In the context of these activities, I have overseen the establishment, training and development of four major reference laboratories to conduct sophisticated and quality controlled viral genotypic resistance testing and sophisticated diagnostic testing for tuberculosis (TB) in support of the ACTG Network trials. My own research experience has included development of new drugs and strategies for treatment of HIV-1 infection, HIV-related opportunistic infections, and for the treatment and prevention of TB and other mycobacterial infections. I have chaired numerous multicenter clinical trials including serving as protocol chair, vice chair or co-investigator for 12 current ACTG clinical trials related to treatment or prevention of HIV and mycobacterial infections. In addition to these research roles, I am a member of the NIH/NIAID/DAIDS TB Laboratory Working Group; a member of the WHO Stop TB/HIV Working Group; a member of the ACTG's TB Transformative Science Group; a co-investigator in another SBIR grant to develop a point-of-care diagnostic instrument and assays for rapid quantitation of CD4 cell count and serodiagnosis of other infectious diseases; and a member of the External Scientific Advisory Group for the NIAID/DMID's TB Clinical Diagnostics Research Consortium.

### B. Positions and Honors

**Professional and Academic Appointments**

| | |
|---|---|
| 1981-1984 | U.S. Navy Staff Physician, Great Lakes Naval Regional Medical Center; North Chicago, IL |
| 1986-1987 | Instructor in Medicine, Rush Medical College/Rush-Presbyterian-St. Luke's Medical Center |
| 1987-1992 | Assistant Professor of Medicine, Rush Medical College; Chicago, IL |
| 1993-1997 | Associate Professor of Medicine, Rush Medical College; Chicago, IL |
| 1997-2004 | Professor of Medicine, University of Colorado Health Sciences Center; Denver, CO |
| 2004-Present | Professor of Medicine, University of California, San Diego; San Diego, CA |
| 2006-Present | Infectious Diseases Training Program Director, UCSD, San Diego, CA |

**Administrative and Committee Positions**

| | |
|---|---|
| 1994-Present | Co-Chair, USPHS/IDSA Treatment and Prevention of Opportunistic Infections Working Group |
| 1994-Present | Member, Scientific Program Committee, Conference on Retroviruses and Opportunistic Infections (Vice Chair 1997-1999; Chair 2000-2002) |
| 1995-2002 | Vice Chair, Adult AIDS Clinical Trials Group, NIAID/NIH |
| 1995-2003 | Chair, Scientific Agenda Steering Committee, Adult AIDS Clinical Trials Group, NIAID/NIH |
| 1996-Present | Member, Board of Directors, International AIDS Society – USA |
| 2000-2003 | Member, NIH Office of AIDS Research Advisory Council (OARAC) (Chair 2001-2003) |
| 2003-2010 | Chair and Principal Investigator, AIDS Clinical Trials Group network, NIAID/NIH |
| 2006-Present | Director, UCSD Antiviral Research Center, San Diego, CA |

**C. Selected peer-reviewed publications relevant to this project (from over 140, in chronological order).**


1. **Benson CA**, Williams PL, Currier JS, et al. A prospective, randomized trial examining the efficacy and safety of clarithromycin in combination with ethambutol, rifabutin, or both for the treatment of disseminated *Mycobacterium avium* complex disease in persons with acquired immunodeficiency syndrome. *Clin Infect Dis* 2003; 37(9):1234-1243.
2. **Benson CA**, Kaplan JE, Masur H, Pau A, Holmes KK. Treating opportunistic infections among HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association/Infectious Diseases Society of America. *MMWR* 2004; 53(RR-15):1-112. (also published in *Clin Infect Dis* 2005)
3. **Benson CA**, Vaida F, Havlir DV, et al. A randomized trial of treatment interruption before optimized antiretroviral therapy for persons with drug-resistant HIV: 48-week virologic results of ACTG A5086. *J Infect Dis* 2006; 194(9):1309-1318.
4. Bosch RJ, Bennett K, Collier AC, Zackin R, **Benson CA**. Pre-treatment factors associated with 3-year (144-week) virologic and immunologic responses to potent antiretroviral therapy. *J Acquir Immune Defic Syndr* 2007; 44(3):268-277.
5. Smurzynski M, Collier AC, Koletar SL, Bosch RJ, Wu K, Bastow B, **Benson CA**. AIDS Clinical Trials Group Longitudinal Linked Randomized Trials (ALLRT): rationale, design, and baseline characteristics. *HIV Clin Trials* 2008; 9(4):269-282.
6. Kitahata MM, Gange SJ, Abraham AG, Merriman B, . . . **Benson CA**, et al. Effect of early versus deferred antiretroviral therapy for HIV on survival. *N Engl J Med* 2009; 360(18):1815-26.
7. Kumarasamy N, Madhavan V, Venkatesh KK, Saravanan S, Kantor R, Balakrishnan P, Devaleenal B, Poongulali S, Yepthomi T, Solomon S, Mayer KH, **Benson C**, Schooley R. High frequency of clinically significant mutations after first-line generic highly active antiretroviral therapy failure: implications for second-line options in resource-limited settings. *Clin Infect Dis* 2009; 49(2):306-9.
8. Smith DM, May SJ, Perez-Santiago J, Strain MC, Ignacio CC, Haubrich RH, Richman DD, **Benson CA**, Little SJ. The use of pooled viral load testing to identify antiretroviral treatment failure. *AIDS* 2009; 23:2151-8. Park D, Qin H, Jain S, Preziosi M, Minuto JJ, Mathews WC, Moser KS, **Benson CA.** Tuberculosis due to *Mycobacterium bovis* in patients co-infected with HIV. *Clin Infect Dis* 2010; 51:1343-6.
9. Ribaudo HJ**, Benson CA**, Zheng Y, Koletar SL, Collier AC, Lok JJ, Smurzynski M, Bosch RJ, Bastow B, Schouten JT, for the ACTG A5001/ALLRT Protocol Team. No immediate and long-term risk of mycocardial infarction associated with initial antiretroviral treatment containing abacavir: results from ACTG A5001/ALLRT over 1 and 6 years. *Clin Infect Dis* 2011; 52:929-40.
10. Krishnan S, Schouten JT, Jacobson DL, **Benson CA**, Collier AC, Koletar SL, Mitsuyasu R, for the ACTG-ALLRT Protocol Team. Incidence of non-AIDS defining cancer (NADC) in antiretroviral treatment (ART) naïve subjects after ART initiation: an ALLRT (ACTG Longitudinal Linked Randomized Trials) analysis. *Oncology* 2011; 80:42-49.
11. Daar ES, Tierney C, Fischl MA, Sax PE, Mollan D, Budhathoki C, Godfrey C, Jahed NC, Myers L, Katzenstein D, Farajallah A, Rooney JF, Pappa KA, Woodward WC, Patterson K, Bolivar H, **Benson CA**, Collier AC, for the AIDS Clinical Trials Group Study A5202 Team. Atazanavir plus ritonavir or efavirenz as part of a three drug regimen for initial treatment of HIV-1: a randomized trial. *Ann Intern Med* 2011; 154:445-56.
12. Krishnan S Wu K, Smurzynski M, Bosch RJ, **Benson CA**, Collier AC, Klebert MK, Feinberg J, Koletar SL, for the ALLRT/A5001 Team. Incidence rate of and factors associated with loss-to-follow-up in a longitudinal cohort of anti-retroviral treated HIV-infected persons: an AIDS Clinical Trials Group (ACTG) Longitudinal Linked Randomized Trials (ALLRT) analysis. *HIV Clin Trials* 2012; (in press).

13. Lochhead MJ, Todorof K, Delaney M, Ives JT, Greef C, Moll K, Rowley K, Vogel K, Myatt C, Zhang X-Q, **Benson C**, Reed S, Schooley RT. Rapid multiplexed immunoassay for simultaneous serodiagnosis of HIV-1 and co-infections. *J Clin Microbiol* 2011; (Epub ahead of print] (in press).
14. Eastburn A, Scherzer R, Zolopa AR, **Benson C,** Russell T, Do T, Bacchetti P, Shlipak M, Grunfeld C, Tien PC. Association of low-level viremia with inflammation and mortality in HIV-infected adults. *PLoS ONE* 2011; 6(11):e26320.
15. Thompson MA, Aberg JA, Hoy JF, Telenti A, **Benson C,** Cahn P, et al. Antiretroviral treatment of adult HIV infection: 2012 recommendations of the International Antiviral Society-USA panel. *JAMA* 2012; 308:387-402.

**D. Research Support**
**Active:**

5 UM1 AI069432 (**Benson, Constance**) 02/01/2007-11/30/2013
National Institutes of Health
UCSD Department of Medicine HIV/AIDS Clinical Trials Unit
Major goals: The UCSD Department of Medicine HIV/AIDS Clinical Trials Unit conducts investigator-initiated clinical trials of the NIH/NIAID Adult AIDS Clinical Trials Group (AACTG), intended to develop new or improve existing antiviral therapies or strategies for the treatment and prevention of HIV infection and its complications in adults and older adolescents infected with HIV. (Role: PI)

7 UM1 AI68636 (Kuritzkes) 06/29/2006-5/31/2013
NIH/Social and Scientific Systems, Inc.
AIDS Clinical Trials Group Network Leadership Group
Major goals: This is the Central Group Core for the AIDS Clinical Trials Group (AACTG), a large international multicenter clinical trials network that conducts investigator initiatied therapeutic clinical trials to develop new or improve existing antiviral drugs and strategies for the treatment and prevention of HIV infection and its complications. Dr. Benson serves as the immediate past Principal Investigator of the U01 and a current member of the Executive Committee of this Group. (Role: Co-Investigator)

5 P01 AI074621 (Little, Susan) 03/15/2008-02/28/2013
NIH/NIAID
Determinants of HIV Transmission
Major goals: To focus on the identification and systematic evaluation of individuals who have been recently infected with HIV and the sexual partners who transmitted HIV to them (Transmission Pairs) to elucidate and quantify epidemiologic, behavioral, biologic, virologic, and host factors that contribute to transmission. (Role: Co-Investigator)

Social & Scientific Systems, Inc (Gilbert, Tari) 07/02/2007-06/30/2013
NIH/NINR
Durability of Adherence in Self-Management of HIV (DASH)
Major goals: To evaluate the impact of individualized self-management interventions on adherence to highly-active antiretroviral therapy (HAART) through assessment and modification of an existing intervention intended to improve long-term durability of HAART adherence. (Role: Co-Investigator)

2 R44 AI068543 (Myatt, Chris) 09/01/2010-08/31/2012
NIH/NIAID
Low Cost Multi-Pathogen Laser Diagnostic for HIV and AIDS Co-Infection
Major goals: Develop a low cost point of care platform for the diagnosis of HIV-1 associated infections in resource limited settings. (Role: Co-Investigator)

1 R24 TW008908-01 (Noormahomed, Emilia) 09/30/2010-09/29/2015
NIH
The Medical Education Partnership Initiative
Major Goals: Assist PI in her efforts to develop clinical and translational research capacity at UEM. She will

assist PI in the further development of the translational research laboratory both in terms of equipping the laboratory and in terms of providing training for technical staff, trainees and junior faculty in laboratory techniques; serve as a member of the UCSD Core Program Faculty and be available to mentor faculty in translational research related to HIV transmission, drug resistance and molecular diagnosis. (Role: UCSD PI of Research Component; Co-Investigator)

R21 AI080353 (Smith, David M.) 04/01/2011-03/31/2013
NIH
Pooled Nucleic Acid Testing to Identify HIV Treatment Failure in Mozambique
To determine the effectiveness of a pooled nucleic acid testing strategy for detecting antiretroviral therapy failure and the development of HIV drug resistance in the resource limited setting of Mozambique. (Role: Co-Investigator)

NIH 2R44 AI070052-04A1 (Schooley) 03/01/2012 – 02/28/2013
Low Cost Laser Diagnostic for CD4 T Cell Counting
To develop and test in clinical settings a novel, 2-color fluorescence detection method in a low cost point-of-care platform to identify and quantify CD4+T cells.

SSS, Inc Subct (Schooley) 11/01/2011 – 03/31/2013
CRB-DCR01-S-09-00299 IRC004
IRC 004: A Randomized Double-Blind Study Comparing Oseltamivir versus Placebo for the Treatment of Influenza in Low Risk Adults

**Completed Research Support**

2 R44AI070052 (Myatt, Chris – PI) 09/01/2009 - 08/31/2011
NIH/NIAID
Low Cost Laser Diagnostic for CD4+ T Cell Counting
Major Goals: To develop a novel, 2-color fluorescence detection method to identify and count CD4+ T-cells. (Role: Co-Investigator)

3 U01 AI27670-18S2 (Benson, Constance - PI) 01/01/2000 – 11/30/2006
NIH/NIAID
San Diego AIDS Clinical Trials Unit
Major Goals: The UCSD AIDS Clinical Trials Unit conducts investigator-initiated clinical trials of the NIH/NIAID Adult AIDS Clinical Trials Group (AACTG), intended to develop new or improve existing antiviral therapies or strategies for the treatment and prevention of HIV infection and its complications in adults and older adolescents infected with HIV. (Role: PI)

1 R01 AI081321 (Garfein, Richard – PI) 12/01/2009 – 11/30/2014
NIH/NIDA
Cross Border Dynamics of MDR/XDR TB Epidemiology by HIV Status in Tijuana, Mexico
Major Goals: This project aims to describe tuberculosis drug resistance patterns and correlates of drug-resistant TB in the cross-border region of northern Mexico and southern California, and to determine the critical pathogen transmission dynamics of TB transmission in a high prevalence endemic area in Tijuana and the cross-border region of southern California
Role: Co-Investigator

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| **Susan J. Little, M.D.** | |
| eRA COMMONS USER NAME [redacted] | **Professor of Medicine** |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| Carleton College; Northfield, MN | B.A. | 1983 | Biology |
| Washington Univ. School of Med.; St. Louis, MO | M.D. | 1987 | Medicine |
| Barnes Hospital; St. Louis, MO | Residency | 1987-1990 | Internal Medicine |
| University of California, San Diego; San Diego, CA | Fellowship | 1990-1993 | Infectious Diseases |

### A. Personal Statement

The overarching goal of this application entitled, "Point of Care HIV Antigen/Antibody Diagnostic Device", is to a develop a point-of-care HIV-1/2 antibody and p24 antigen detection assay on a single point-of-care platform with performance equivalent to FDA-approved laboratory approved 4$^{th}$ generation AHIV-1 Ag/Ab combination assays. Specifically, Dr. Little will work with MBio Diagnostics, Inc. to develop and execute the plans to provide clinical samples from patients with acute and chronic HIV infection that will include de-identified archived samples, as well as newly acquired samples of serum/plasma and potentially other biological fluids from patients participating in clinical research at the UCSD Antiviral Research Center (AVRC). Dr. Little will maintain her highly successful recruitment program for persons with acute HIV infection (HIV RNA+/Ab-) paired with recruitment of appropriate control populations (both HIV+ and HIV-) and provide well-characterized clinical and behavioral data linked to regularly collected and banked biological specimens that will be made available to MBio and UCSD collaborating investigators. Dr. Little works with a large international collaborative team to assess viral replication dynamics and host immune dynamics during acute HIV infection. She directs an NIH-funded Program Project at UCSD to evaluate determinants of HIV transmission. She oversees the integration of pathogenesis, translational, and clinical research in the UCSD Acute and Early HIV Infection (AEH) Program and mentors post-doctoral research fellows and faculty. Dr. Little is an active investigator in the AIDS Clinical Trials Group (ACTG) and has developed numerous ACTG protocols to evaluate new strategies for treatment of acute infection. She has been heavily involved in the design of novel approaches to screen and identify acutely infected individuals, as well as the evaluation of important questions related to HIV prevention, transmission, pathogenesis and treatment. Below, fifteen publications are listed pertinent to the proposed topic, especially #s 1, 3, 7, 9, 12, 14, 15 as selected from over 80 peer-reviewed manuscripts.

### B. Positions and Honors

**Positions**

| | |
|---|---|
| 10/93-09/97 | Associate Physician, University of California, San Diego; San Diego, California |
| 10/93-present | Attending Physician, Veteran's Administration Medical Center; La Jolla, CA |
| 10/97-06/03 | Assistant Adjunct Professor of Medicine, University of California, San Diego; San Diego, CA |
| 07/03-06/05 | Associate Adjunct Professor of Medicine, University of California, San Diego; San Diego, CA |
| 07/05-06/09 | Associate Professor of Medicine in Residence, University of California, San Diego; San Diego, CA |
| 07/09-present | Professor of Medicine in Residence, University of California, San Diego; San Diego, CA |

**Current National/Scientific Committees and Service**

| | |
|---|---|
| 07/01-present | World Health Organization HIV Resistance Network (HIVResNet) Scientific Committee |
| 07/03-present | International HIV Drug Resistance Workshop Scientific Committee |
| 01/04-present | *Current HIV/AIDS Reports* Editorial Board |
| 10/04-present | The HIV Prevention Science Committee: A Joint HPTN-AACTG Committee |
| 12/04-present | *Antiviral Therapy* Editorial Board |

02/06-present International Workshop on HIV Transmission Scientific Committee
07/08–present Member of the AIDS Clinical Trials Group (ACTG) Acute Infection Task Force
01/09-present HIV Acute Infection Meeting Session Chair and Scientific Organizing Committee

**Honors**

1996 LIFE Achievement Award: For dedication to improve the lives of those with HIV infection. Awarded by the Los Angeles LIFE Lobby HIV/AIDS organization.
1997 Award of Excellence: For service and support to HIV and AIDS education. Awarded by Being Alive San Diego and Christie's Place.
2000 Health Hero Award: In recognition of contributions in HIV research and community leadership. Awarded by Combined Health Agencies, a federation of twenty-six chapters of local and national health agencies.
2000 Spirit of Being Alive: In recognition of an individual who reflects the spirit of dedication and service to the ever-evolving HIV-positive communities. Awarded by Being Alive San Diego.
2006 HIV Emerging Leader in Research Award: In recognition of outstanding scholarly achievement in the field of HIV research. Awarded by the HIV Medicine Association (HIVMA) of the Infectious Diseases Society of America (IDSA).
2010 Nominee for the San Diego Brad Truax Award and recipient of the HIV Care, Treatment and/or Support Services Award for persons living with HIV/AIDS.
2011 Nominee for San Diego Magazine "Woman of the Year" award.

**C. Selected peer-reviewed publications (from over 80, in chronological order).**


1. **Little SJ**, McLean AR, Spina CA, Richman DD, Havlir DV. Viral dynamics in acute HIV-1 infection. *Journal of Experimental Medicine* 190(6): 841-850, 1999. [PMCID PMC2195636]
2. **Little SJ**, Holte S, Routy JP, Daar ES, Markowitz M, Collier AC, et al. Antiretroviral drug resistance among subjects recently infected with HIV. *New England Journal of Medicine* 347(6): 385-394, 2002.
3. Strain MC, **Little SJ**, Daar ES, Havlir DV, Gunthard HF, Lam RY, Daly OA, Nguyen J, Ignacio CC, Spina CA, Richman DD, Wong JK. Effect of treatment, during primary infection, on establishment and clearance of cellular reservoirs of HIV-1. *J Infect Dis* 2005;191(9):1410-1418.
4. **Little SJ**, Frost SD, Wong JK, Smith DM, Pond SL, Ignacio CC, et al. Persistence of transmitted drug resistance among subjects with primary human immunodeficiency virus infection. *Journal of Virology* 82(11): 5510-5518, 2008. [PMCID PMC2395184].
5. Gorbach PM, Drumright LN, Javanbakht M, Pond SL, Woelk CH, Daar ES, **Little SJ**. Antiretroviral drug resistance and risk behavior among recently HIV-infected men who have sex with men. *J Acquir Immune Defic Syndr* 2008; 47(5):639-643.
6. Smith DM, May SJ, Tweeten S, Drumright L, Pacold ME, . . . **Little SJ**. A public health model for the molecular surveillance of HIV transmission in San Diego, California. *AIDS* 23(2): 225-232, 2009. [PMCID PMC2644048].
7. Markowitz M, Vaida F, Hare CB, Boden D, Mohri H... **Little S**. The virologic and immunologic effects of cyclosporine as an adjunct to antiretroviral therapy in patients treated during acute and early HIV-1 infection. J Infect Dis. 2010 May 1;201(9):1298-302. [PMCID:PMC2851487]
8. DeGruttola ScD V, Smith DM, **Little SJ**, Miller V. Developing and evaluating comprehensive HIV control strategies: issues and challenges. *Clin Infect Dis* 50 (Suppl 3): S102-07, 2010.
9. Morris SR, **Little SJ**, Cunningham T, Garfein RS, Richman DD, Smith DM. Evaluation of an HIV nucleic Acid testing program with automated internet and voicemail systems to deliver results. *Ann Int Med* 152:778-785, 2010. (NIHMSID: 223933)
10. DeGruttola V, Smith DM, Little SJ, Miller V. Developing and evaluating comprehensive HIV infection control strategies: issues and challenges. *Clin Infec Dis* 50(Suppl 3):S102-S107, 2010. (PMC2913596)
11. Gianella S, Delport W, Pacold ME, Young JA, Choi JY, Little SJ, *et al.* Detection of minority resistance during early HIV-1 infection: natural variation and spurious detection rather than transmission and evolution of multiple viral variants. *Journal of Virology* 2011,85:8359-8367. (PMCID: PMC3147985).
12. Hecht FM, Wellman R, Busch MP, Pilcher CD, Norris PJ, Margolick JB, Collier AC, **Little SJ**, Markowitz M, Routy JP, Holte S; Acute Infection Early Disease Research Program. Identifying the early post-HIV antibody seroconversion period. *J Infect Dis*. 2011 Aug 15;204(4):526-33. PubMed PMID: 21791654; PubMed Central PMCID: PMC3144168.

13. Wertheim JO, Kosakovsky Pond SL, **Little SJ**, De Gruttola V. Using HIV Transmission Networks to Investigate Community Effects in HIV Prevention Trials. *PLoS ONE* 2011,6:e27775. (PMCID: PMC3218056).
14. Hogan CM, Degruttola V, Sun X, Fiscus SA, Del Rio C, Hare CB, Markowitz M, Connick E, Macatangay B, Tashima KT, Kallungal B, Camp R, Morton T, Daar ES, **Little S**; A5217 Study Team. The setpoint study (ACTG A5217): effect of immediate versus deferred antiretroviral therapy on virologic set point in recently HIV-1-infected individuals. J Infect Dis. 2012 Jan;205(1):87-96. Epub 2011 Dec15. PubMed PMID: 22180621; PubMed Central PMCID: PMC3242744.
15. Karris MY, Anderson CM, Morris SR, Smith DM, **Little SJ**. The Cost of Missing Acute HIV Infection: Testing Antibodies, Antigens, and Nucleic Acids. *J Clin Microbiol*. 2012. Epub 2012/03/24. doi: 10.1128/JCM.00106-12. PubMed PMID: 22442319.

## D. Research Support

**Ongoing Research Support**

5 P01 AI074621-05 (**Little, Susan J.**) 03/15/2008 – 02/28/2013
National Institutes of Health
Determinants of HIV Transmission
Major goals: To focus on the identification and systematic evaluation of individuals who have been recently infected with HIV and the sexual partners who transmitted HIV to them (Transmission Pairs) to elucidate and quantify epidemiologic, behavioral, biologic, virologic, and host factors that contribute to transmission.

5 R01 AI087164-02 (Woelk, Christopher) 12/1/2009 – 11/30/2014
National Institutes of Health
Gene Expression Biomarkers of Immune Recovery in HIV Infected Patients
Major goals: This research will identify patient genes that contribute to immune recovery and represent new targets for drugs that can be used to increase immune recovery. This proposal will also identify which drugs an HIV-infected patient should be treated with in order to maximize the extent of their immune recovery.

3 P01 AI074621-03S1 (UCSD #2010-3954) (**Little, Susan J.**) 05/03/2010 – 09/26/2012
National Institutes of Health/NIAID NOT-OD-09-056 (1st Suppl in 03 yr)
Recovery Act Administrative Supplement
Determinants of HIV Transmission
Major goals: To develop and implement the infrastructure to combine data across three highly successful acute/early HIV infection cohorts (UCSD, UCSF, and MGH) and utilize the unified dataset to address key scientific questions.

3 P01 AI074621-03S1 (UCSD #2010-3029) (**Little, Susan J.**) 09/27/2010 – 09/26/2012
National Institutes of Health/NIAID NOT-OD-056 (2nd Suppl in 03 yr)
Recovery Act Administrative Supplement
Determinants of HIV Transmission
Major goals: To estimate and evaluate predictors of universal HIV testing acceptance and use statistical techniques applied to HIV sequences to infer a molecular epidemiological transmission network within the study population.

1 DP1 DA034978-01 (Smith, David) 08/01/2012 – 07/31/2017
National Institutes of Health/NIDA
Molecular Epidemiology for HIV Prevention for Drug Users and Other Risk Groups
Major goals: To integrate subject data from local research, clinical and public health entities that are screening for and treating HIV infected individuals.

**Completed Research Support**

RN07-SD-702 (**Little, Susan J.**) 10/01/2007 – 12/31/2008
University of California, California HIV/AIDS Research Program

Acute Detection and Early Prevention Trial
Major goals: To evaluate a program of nucleic acid testing (NAT) to screen for acute HIV infection at Rapid HIV testing and counseling sites in the County of San Diego with a plan to coordinate cross-cohort analyses of HIV incidence rates, transmission of HIV during primary infection, cost effectiveness of NAT screening, and characterization of the demographics of acutely HIV infected clients among participating funded study sites.

5 U01 AI043638-09 (Richman, Douglas D.) 08/01/1998 – 06/30/2008
National Institutes of Health
Southern California Primary Infection Program
Major goals: A collaborative program between UCSD and Harbor/UCLA Medical Center to combine resources and expertise in patient recruitment, clinical trials, and pathogenetic research. This program will identify, recruit, and retain subjects with acute or early HIV infection, complete two innovative trials, and answer a series of important pathogenesis questions.

County of San Diego (Smith, David) 11/01/2006 – 02/29/2008
Early Intervention Services
Major goals: To develop and implement nucleic acid testing in San Diego County HIV Testing and Counseling sites to increase the ability to identify individuals with acute and early HIV infection.

County of San Diego (Smith, David) 07/01/2007 – 06/30/2008
Early Intervention, Bridge, and Positive
Changes Program
Major goals: These programs serve newly diagnosed HIV infected individuals in San Diego County through a multidisciplinary approach, including case management, primary medical and psychological care, health education and risk reduction.

5 R01 NS051132-04 (**Little, Susan J.**) 07/15/2005 – 06/30/2010
National Institutes of Health/University of California, San Francisco
HIV Adaptation in the CNS
Major goals: A collaborative program between UCSF and UCSD investigators to evaluate of the persistence of HIV within functional reservoirs among patients receiving potent antiretroviral therapy.

3 P01 DA012065-10S1 (**Little, Susan J.**) 05/01/2009 – 04/30/2011
National Institutes of Health/NIDA
NeuroAIDS: Effects of Methamphetamine and HCV
Major goals: To determine whether methamphetamine use potentiates depletion of gut-associated lymphocytes, triggering a cascade of bacterial translocation, and peripheral and central immunopathogenesis that leads to early, and potentially persistent, brain injury.

3 P01 AI074621-02S1 (**Little, Susan J.**) 09/12/2009 – 08/31/2011
National Institutes of Health
Recovery Act Diversity Supplement
Determinants of HIV Transmission
Major goals: To characterize the risk behaviors and attitudes related to HIV prevention efforts among San Diego Latinos and the prevalence of HIV infection within this community, particularly among the non-gay identified men who are not historically well represented in HIV surveillance sampling.

OMB Number: 4040-0001
Expiration Date: 06/30/2011

## RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

**Enter name of Organization:** MBio Diagnostics, Inc.

Delete Entry *** Start Date:** 10/01/2013 *** End Date:** 09/30/2014 **Budget Period** 1

### A. Senior/Key Person

| | Prefix | * First Name | Middle Name | * Last Name | Suffix | * Project Role | Base Salary ($) | Cal. Months | Acad. Months | Sum. Months | * Requested Salary ($) | * Fringe Benefits ($) | * Funds Requested ($) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Dr. | Michael | J | Lochhead | | PD/PI | [redacted] | [redacted] | | | [redacted] | [redacted] | [redacted] |
| 2. | Dr. | Daniel | | Nieuwlandt | | Senior Scientist | [redacted] | [redacted] | | | [redacted] | [redacted] | [redacted] |
| 3. | | | | | | | | | | | | | |
| 4. | | | | | | | | | | | | | |
| 5. | | | | | | | | | | | | | |
| 6. | | | | | | | | | | | | | |
| 7. | | | | | | | | | | | | | |
| 8. | | | | | | | | | | | | | |
| 9. | **Total Funds requested for all Senior Key Persons in the attached file** | | | | | | | | | | | | |
| | | | | | | | | | | | **Total Senior/Key Person** | | [redacted] |

**Additional Senior Key Persons:** Add Attachment Delete Attachment View Attachment

### B. Other Personnel

| * Number of Personnel | * Project Role | Cal. Months | Acad. Months | Sum. Months | * Requested Salary ($) | * Fringe Benefits ($) | * Funds Requested ($) |
|---|---|---|---|---|---|---|---|
| | Post Doctoral Associates | | | | | | |
| | Graduate Students | | | | | | |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
| 1 | Kurt Vogel, Engineering Manager | 0.60 | | | [redacted] | [redacted] | [redacted] |
| 1 | Associate Scientist | 12.00 | | | [redacted] | [redacted] | [redacted] |
| 1 | Clinical Site Manager | 0.60 | | | [redacted] | [redacted] | [redacted] |
| 1 | Research Associate | 6.00 | | | [redacted] | [redacted] | [redacted] |
| 3 | Engineering (Mechanical, Process and Software) | 2.40 | | | [redacted] | [redacted] | [redacted] |
| | | | | | | | |
| 7 | **Total Number Other Personnel** | | | | | **Total Other Personnel** | [redacted] |

**Total Salary, Wages and Fringe Benefits (A+B)** [redacted]

## RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

**Enter name of Organization:** MBio Diagnostics, Inc.

Delete Entry *** Start Date:** 10/01/2013 *** End Date:** 09/30/2014 **Budget Period 1**

**C. Equipment Description**

**List items and dollar amount for each item exceeding $5,000**

| | Equipment item | * Funds Requested ($) |
|---|---|---|
| 1. | | |
| 2. | | |
| 3. | | |
| 4. | | |
| 5. | | |
| 6. | | |
| 7. | | |
| 8. | | |
| 9. | | |
| 10. | | |
| 11. | **Total funds requested for all equipment listed in the attached file** | |
| | **Total Equipment** | |

**Additional Equipment:** Add Attachment Delete Attachment View Attachment

**D. Travel** **Funds Requested ($)**

| | | |
|---|---|---|
| 1. | Domestic Travel Costs ( Incl. Canada, Mexico and U.S. Possessions) | [redacted] |
| 2. | Foreign Travel Costs | |
| | **Total Travel Cost** | [redacted] |

**E. Participant/Trainee Support Costs** **Funds Requested ($)**

| | | |
|---|---|---|
| 1. | Tuition/Fees/Health Insurance | |
| 2. | Stipends | |
| 3. | Travel | |
| 4. | Subsistence | |
| 5. | Other | |
| | **Number of Participants/Trainees** **Total Participant/Trainee Support Costs** | |

RESEARCH & RELATED Budget {C-E} (Funds Requested)

## RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: MBio Diagnostics, Inc.

Delete Entry * Start Date: 10/01/2013 * End Date: 09/30/2014 Budget Period 1

| F. Other Direct Costs | Funds Requested ($) |
|---|---|
| 1. Materials and Supplies | [redacted] |
| 2. Publication Costs | |
| 3. Consultant Services | [redacted] |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | [redacted] |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8. Other: Tooling and Instrument Components | [redacted] |
| 9. | |
| 10. | |
| **Total Other Direct Costs** | [redacted] |

| G. Direct Costs | Funds Requested ($) |
|---|---|
| **Total Direct Costs (A thru F)** | [redacted] |

H. Indirect Costs

| | Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | * Funds Requested ($) |
|---|---|---|---|---|
| 1. | F&A | 45.00 | [redacted] | [redacted] |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |
| | **Total Indirect Costs** | | | [redacted] |

**Cognizant Federal Agency** NIH, Division of Financial Services, Frank Mihalovic, 301-443-
(Agency Name, POC Name, and POC Phone Number)

| I. Total Direct and Indirect Costs | Funds Requested ($) |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | [redacted] |

| J. Fee | Funds Requested ($) |
|---|---|
| | [redacted] |

**K. * Budget Justification** 1254-Budget Justification.pdf Add Attachment Delete Attachment View Attachment
(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

## RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

**Enter name of Organization:** MBio Diagnostics, Inc.

Delete Entry *** Start Date:** 10/01/2014 *** End Date:** 09/30/2015 **Budget Period** 2

### A. Senior/Key Person

| | Prefix | * First Name | Middle Name | * Last Name | Suffix | * Project Role | Base Salary ($) | Cal. Months | Acad. Months | Sum. Months | * Requested Salary ($) | * Fringe Benefits ($) | * Funds Requested ($) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Dr. | Michael | J | Lochhead | | PD/PI | [redacted] | 0.60 | | | [redacted] | [redacted] | [redacted] |
| 2. | Dr. | Daniel | | Nieuwlandt | | Senior Scientist | [redacted] | 4.80 | | | [redacted] | [redacted] | [redacted] |
| 3. | | | | | | | | | | | | | |
| 4. | | | | | | | | | | | | | |
| 5. | | | | | | | | | | | | | |
| 6. | | | | | | | | | | | | | |
| 7. | | | | | | | | | | | | | |
| 8. | | | | | | | | | | | | | |
| 9. | **Total Funds requested for all Senior Key Persons in the attached file** | | | | | | | | | | | | |
| | | | | | | | | | | | | **Total Senior/Key Person** | [redacted] |

**Additional Senior Key Persons:** Add Attachment Delete Attachment View Attachment

### B. Other Personnel

| * Number of Personnel | * Project Role | Cal. Months | Acad. Months | Sum. Months | * Requested Salary ($) | * Fringe Benefits ($) | * Funds Requested ($) |
|---|---|---|---|---|---|---|---|
| | Post Doctoral Associates | | | | | | |
| | Graduate Students | | | | | | |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
| 1 | Kurt Vogel, Engineering Manager | 0.60 | | | [redacted] | [redacted] | [redacted] |
| 1 | Associate Scientist | 12.00 | | | [redacted] | [redacted] | [redacted] |
| 1 | Clinical Site Manager | 3.60 | | | [redacted] | [redacted] | [redacted] |
| 1 | Research Associate | 6.00 | | | [redacted] | [redacted] | [redacted] |
| 3 | Engineering (Mechanical, Process and Software) | 2.40 | | | [redacted] | [redacted] | [redacted] |
| | | | | | | | |
| 7 | **Total Number Other Personnel** | | | | | **Total Other Personnel** | [redacted] |

**Total Salary, Wages and Fringe Benefits (A+B)** [redacted]

## RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

**Enter name of Organization:** MBio Diagnostics, Inc.

Delete Entry *** Start Date:** 10/01/2014 *** End Date:** 09/30/2015 **Budget Period 2**

**C. Equipment Description**

**List items and dollar amount for each item exceeding $5,000**

| | Equipment item | * Funds Requested ($) |
|---|---|---|
| 1. | | |
| 2. | | |
| 3. | | |
| 4. | | |
| 5. | | |
| 6. | | |
| 7. | | |
| 8. | | |
| 9. | | |
| 10. | | |
| 11. | **Total funds requested for all equipment listed in the attached file** | |
| | **Total Equipment** | |

**Additional Equipment:** Add Attachment Delete Attachment View Attachment

**D. Travel** **Funds Requested ($)**

| | | |
|---|---|---|
| 1. | Domestic Travel Costs ( Incl. Canada, Mexico and U.S. Possessions) | [redacted] |
| 2. | Foreign Travel Costs | |
| | **Total Travel Cost** | [redacted] |

**E. Participant/Trainee Support Costs** **Funds Requested ($)**

| | | |
|---|---|---|
| 1. | Tuition/Fees/Health Insurance | |
| 2. | Stipends | |
| 3. | Travel | |
| 4. | Subsistence | |
| 5. | Other | |
| | **Number of Participants/Trainees** **Total Participant/Trainee Support Costs** | |

RESEARCH & RELATED Budget {C-E} (Funds Requested)

## RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 2

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: MBio Diagnostics, Inc.

Delete Entry * Start Date: 10/01/2014 * End Date: 09/30/2015 Budget Period 2

| F. Other Direct Costs | Funds Requested ($) |
|---|---|
| 1. Materials and Supplies | [redacted] |
| 2. Publication Costs | [redacted] |
| 3. Consultant Services | [redacted] |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | [redacted] |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8. Other: Tooling and Instrument Components | [redacted] |
| 9. | |
| 10. | |
| **Total Other Direct Costs** | [redacted] |

| G. Direct Costs | Funds Requested ($) |
|---|---|
| **Total Direct Costs (A thru F)** | [redacted] |

**H. Indirect Costs**

| | Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | * Funds Requested ($) |
|---|---|---|---|---|
| 1. | F&A | 45.00 | [redacted] | [redacted] |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |
| | **Total Indirect Costs** | | | [redacted] |

**Cognizant Federal Agency** NIH, Division of Financial Services, Frank Mihalovic, 301-443-
(Agency Name, POC Name, and POC Phone Number)

| I. Total Direct and Indirect Costs | Funds Requested ($) |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | [redacted] |

| J. Fee | Funds Requested ($) |
|---|---|
| | [redacted] |

**K. * Budget Justification** 1254-Budget Justification.pdf Add Attachment Delete Attachment View Attachment
(Only attach one file.)

**BUDGET JUSTIFICATION**
**MBio p24 SBIR Phase II Application**

The budget outlined here covers personnel, supplies, and travel, as well as instrument and cartridge engineering and testing at MBio Diagnostics.

**Key Personnel**

**Michael Lochhead**. Dr. Lochhead is Vice President at MBio Diagnostics serves as PI on the project. Dr. Lochhead has been managing the immunoassay product development at the company since July 2007. He provides overall program management and strategy and he will commit 5% effort to this project.

**Daniel Nieuwlandt.** Dr. Nieuwlandt is a Senior Scientist at MBio and he will lead assay development efforts under Aims 1 – 3. Dr. Nieuwlandt is an expert in bioconjugation techniques and assay development, and he was the senior scientist on Phase I of this program. He commits 40% effort to the project (4.8 calendar months).

**Other Personnel**

**Associate Scientist.** MBio will assign one of its Associate Scientists to work full time on the project through the program duration. Candidates include **Katie Todorof**, **Phil Papst** and **Greg Husar**, all of whom are experience immunoassay development scientists.

**Research Associate**. MBio will assign a Research Associate at 50% effort throughout the project. The role of the Research Associate will provide basic laboratory support, including running assays, preparing reagents, etc.

**Kurt Vogel**. Dr. Vogel is Director of Engineering at MBio Diagnostics. His group is responsible for the instrument hardware and software development as well as disposable cartridge manufacturing. Dr. Vogel will coordinate system modifications associated with the p24 assay. He will commit 5% effort to this project.

**Kevin Moll**. Dr. Moll is Senior Scientist at MBio. In addition to providing optical design expertise, he serves as MBio's primary in-house software developer. Dr. Moll develops the company's proprietary image analysis algorithms and coordinates system software development. He will commit 33% effort to this project.

**Keagan Rowley**. Mr. Rowley is the lead mechanical design engineer on the project. He is responsible for modifying MBio cartridge designs in support of the p24 project. He will also be responsible for part design, vendor management, and builds for Aim 2. Mr. Rowley has a 20% percent commitment to the project.

**Process Engineer**. The Process Engineer is a new hire at MBio, scheduled for September 2012. The process engineer will perform process development research related to cartridge integration tasks under Aim 2. The process engineer will dedicate 30% effort throughout the project.

**Clinical Site Manager**. During year 2 when clnical evaluation is taking place at UCSD, MBio will support a clinical site manager who works on data management, logistics, sample transfers, etc. with the UCSD clinical team. This position will be at a 5% level during year 1, increasing to 30% during year 2.

**Equipment**
No capital equipment is requested under this award.

## Supplies

The Supplies budget worksheets are provided in the tables below.

| Supplies | | Item Subtotal | Item Total | |
|---|---|---|---|---|
| Antigens and Antibodies | $ | [redacted] | | See calculation table |
| Labware (gloves, tubes pipets, etc.) | $ | [redacted] | | $[redacted]/month based on past experience |
| Cartridges (materials & labor) | $ | [redacted] | | [redacted] $ [redacted] Cost includes materials and labor |
| Reference Test Kits | $ | [redacted] | | |
| | | **Supplies:** | **$** [redacted] | |

Antibody and Reagent Cost Calculation:

| ANTIGEN/ANTIBODY CALCULATOR: | | |
|---|---|---|
| Analytes/ Reagents: | 6 | antibodies |
| Labeling kits: | 2 | e.g., Invitrogen |
| Repeat orders: | 3 | |
| Price per order: | $[redacted] | average |
| TOTAL: | $[redacted] | |

Reference test kits are a substantial part of the budget. For example, 4th generation HIV Ag/Ab Combo assays (e.g., Bio-Rad) cost > $[redacted] per microplate. Reference test costing is based on experience from Phase I.

## Other: Tooling and Instrument Components

In addition to the standard laboratory and cartridge supplies listed above, we designate tooling and supplies for instrument development in their own "other" category.

| Other: Tooling and Instrument Components | | | |
|---|---|---|---|
| Cartridge Molds | $ | [redacted] | |
| Blister pack vendor support | $ | [redacted] | |
| SnapEsi System (dedicated to p24) | $ | [redacted] | |
| | | **Other:** | **$** [redacted] |

The Cartridge Mold line item assumes that during each year we will do one revision of the molded cartridge. MBio works with multiple molding vendors. The current SnaEsi cartridge body mold was $[redacted] (steel tool to hold polish and throughput), and we use that number as an estimate for revisions. The cartridge also has a lid which will be based on less expensive aluminum tooling, such as through Protomold. We budget $[redacted] for the lid mold and two iterations at Protomold. Total mold budget with run time costs is estimated at $[redacted] during year 1.

We will work with custom blister pack vendors to incorporate on board liquid reagents for the final wash step outlined under Aim 2. MBio has experience with several of these vendors.

Instrument builds for the program will be at $[redacted] per unit, with one built in year 1 and two in year 2.

## Travel

The travel budget anticipates two trips per year. One will be for two people to visit the clinical collaborators at UCSD and coordinate the field evaluation. For each person, we budget $[redacted] transportation (air and ground), $[redacted] lodging, and $[redacted] meals ($[redacted] per person). The total for two people is $[redacted]

## Consultants

We request funding for specific regulatory support associated with the 4th Gen HIV product. Because such a test will require a full pre-market approval (PMA) through CBER at FDA, we will be looking for specific guidance from our regulatory consultants. MBio currently engages Myraqa for strategic regulatory consulting. We budget $[redacted] per year for these consulting services.

## Fee

We include the allowed 7% fee in our budget. The 7% is calculated on the MBio Direct and Indirect costs, excluding the UCSD subaward.

## RESEARCH & RELATED BUDGET - Cumulative Budget

| | | Totals ($) |
|---|---|---|
| **Section A, Senior/Key Person** | | [redacted] |
| **Section B, Other Personnel** | | [redacted] |
| Total Number Other Personnel | 14 | |
| **Total Salary, Wages and Fringe Benefits (A+B)** | | [redacted] |
| **Section C, Equipment** | | |
| **Section D, Travel** | | [redacted] |
| **1.** Domestic | [redacted] | |
| **2.** Foreign | | |
| **Section E, Participant/Trainee Support Costs** | | |
| **1.** Tuition/Fees/Health Insurance | | |
| **2.** Stipends | | |
| **3.** Travel | | |
| **4.** Subsistence | | |
| **5.** Other | | |
| **6.** Number of Participants/Trainees | | |
| **Section F, Other Direct Costs** | | [redacted] |
| **1.** Materials and Supplies | [redacted] | |
| **2.** Publication Costs | [redacted] | |
| **3.** Consultant Services | [redacted] | |
| **4.** ADP/Computer Services | | |
| **5.** Subawards/Consortium/Contractual Costs | [redacted] | |
| **6.** Equipment or Facility Rental/User Fees | | |
| **7.** Alterations and Renovations | | |
| **8.** Other 1 | [redacted] | |
| **9.** Other 2 | | |
| **10.** Other 3 | | |
| **Section G, Direct Costs (A thru F)** | | [redacted] |
| **Section H, Indirect Costs** | | [redacted] |
| **Section I, Total Direct and Indirect Costs (G + H)** | | [redacted] |
| **Section J, Fee** | | [redacted] |

## RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

**Enter name of Organization:** The Regents of the Univ. of Calif., San Diego

*** Start Date:** 10-01-2013 *** End Date:** 09-30-2014 **Budget Period: 1**

**A. Senior/Key Person**

| Prefix | * First Name | Middle Name | * Last Name | Suffix | * Project Role | Base Salary ($) | Cal. Months | Acad. Months | Sum. Months | * Requested Salary ($) | * Fringe Benefits ($) | * Funds Requested ($) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Constance | | Benson | MD | Consortium PI | [redacted] | 0.60 | | | [redacted] | [redacted] | [redacted] |
| 2. | Susan | | Little | MD | Co-Investigator | [redacted] | 0.30 | | | [redacted] | [redacted] | [redacted] |

**Total Funds Requested for all Senior Key Persons in the attached file**

**Additional Senior Key Persons:** File Name: Mime Type: **Total Senior/Key Person** **18,409.00**

**B. Other Personnel**

| * Number of Personnel | * Project Role | Cal. Months | Acad. Months | Sum. Months | * Requested Salary ($) | * Fringe Benefits | * Funds Requested ($) |
|---|---|---|---|---|---|---|---|
| | Post Doctoral Associates | | | | | | |
| | Graduate Students | | | | | | |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
| 1 | Jill Kunkel, Research Nurse | 1.20 | | | [redacted] | [redacted] | [redacted] |
| 1 | Denise Lovec-Jenkins, Regulatory Specialist | 1.20 | | | [redacted] | [redacted] | [redacted] |
| **2** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | [redacted] |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | [redacted] |

RESEARCH & RELATED Budget {A-B} (Funds Requested)

## RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

**Enter name of Organization:** The Regents of the Univ. of Calif., San Diego

*** Start Date:** 10-01-2013 *** End Date:** 09-30-2014 **Budget Period: 1**

**C. Equipment Description**

**List items and dollar amount for each item exceeding $5,000**

| Equipment Item | * Funds Requested ($) |
|---|---|

**Total funds requested for all equipment listed in the attached file**

**Total Equipment**

**Additional Equipment:** File Name: Mime Type:

**D. Travel** **Funds Requested ($)**

1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

**Total Travel Cost**

**E. Participant/Trainee Support Costs** **Funds Requested ($)**

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

**Number of Participants/Trainees** **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

## RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

**Enter name of Organization:** The Regents of the Univ. of Calif., San Diego

*** Start Date:** 10-01-2013 *** End Date:** 09-30-2014 **Budget Period: 1**

| F. Other Direct Costs | Funds Requested ($) |
|---|---|
| 1. Materials and Supplies | [redacted] |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8. Communications and Computing | [redacted] |
| 9. Outpatient Care Costs | [redacted] |
| **Total Other Direct Costs** | [redacted] |

| G. Direct Costs | Funds Requested ($) |
|---|---|
| **Total Direct Costs (A thru F)** | [redacted] |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | * Funds Requested ($) |
|---|---|---|---|
| 1. MTDC, On Campus Rate | 55.00 | [redacted] | [redacted] |
| | | **Total Indirect Costs** | [redacted] |

**Cognizant Federal Agency** DHHS Region IX 415-437-7820

(Agency Name, POC Name, and POC Phone Number)

| I. Total Direct and Indirect Costs | Funds Requested ($) |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | [redacted] |

| J. Fee | Funds Requested ($) |
|---|---|
| | |

**K. * Budget Justification** File Name: 1234-UCSDBudgetJustification.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

## RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** 8043557900000

*** Budget Type:** ❍ Project ● Subaward/Consortium

**Enter name of Organization:** The Regents of the Univ. of Calif., San Diego

*** Start Date:** 10-01-2014 *** End Date:** 09-30-2015 **Budget Period: 2**

**A. Senior/Key Person**

| | Prefix | * First Name | Middle Name | * Last Name | Suffix | * Project Role | Base Salary ($) | Cal. Months | Acad. Months | Sum. Months | * Requested Salary ($) | * Fringe Benefits ($) | * Funds Requested ($) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | | Constance | | Benson | MD | Consortium PI | [redacted] | 0.60 | | | [redacted] | [redacted] | [redacted] |
| 2. | | Susan | | Little | MD | Co-Investigator | [redacted] | 0.30 | | | [redacted] | [redacted] | [redacted] |

**Total Funds Requested for all Senior Key Persons in the attached file**

**Additional Senior Key Persons:** File Name: Mime Type: **Total Senior/Key Person** **18,638.00**

**B. Other Personnel**

| * Number of Personnel | * Project Role | Cal. Months | Acad. Months | Sum. Months | * Requested Salary ($) | * Fringe Benefits | * Funds Requested ($) |
|---|---|---|---|---|---|---|---|
| | Post Doctoral Associates | | | | | | |
| | Graduate Students | | | | | | |
| | Undergraduate Students | | | | | | |
| | Secretarial/Clerical | | | | | | |
| 1 | Jill Kunkel, Research Nurse | 1.20 | | | [redacted] | [redacted] | [redacted] |
| 1 | Denise Lovec-Jenkins, Regulatory Specialist | 1.20 | | | [redacted] | [redacted] | [redacted] |
| **2** | **Total Number Other Personnel** | | | | | **Total Other Personnel** | [redacted] |
| | | | | | | **Total Salary, Wages and Fringe Benefits (A+B)** | [redacted] |

RESEARCH & RELATED Budget {A-B} (Funds Requested)



**Tracking Number: GRANT11392590**

**OMB Number: 4040-0001**
**Expiration Date: 06/30/2011**

## RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

**Enter name of Organization:** The Regents of the Univ. of Calif., San Diego

*** Start Date:** 10-01-2014 *** End Date:** 09-30-2015 **Budget Period: 2**

**C. Equipment Description**

**List items and dollar amount for each item exceeding $5,000**

| Equipment Item | * Funds Requested ($) |
|---|---|

**Total funds requested for all equipment listed in the attached file**

**Total Equipment**

**Additional Equipment:** File Name: Mime Type:

**D. Travel** **Funds Requested ($)**

1. Domestic Travel Costs ( Incl. Canada, Mexico, and U.S. Possessions)
2. Foreign Travel Costs

**Total Travel Cost**

**E. Participant/Trainee Support Costs** **Funds Requested ($)**

1. Tuition/Fees/Health Insurance
2. Stipends
3. Travel
4. Subsistence
5. Other:

**Number of Participants/Trainees** **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

## RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

**Enter name of Organization:** The Regents of the Univ. of Calif., San Diego

*** Start Date:** 10-01-2014 *** End Date:** 09-30-2015 **Budget Period: 2**

| F. Other Direct Costs | Funds Requested ($) |
|---|---|
| 1. Materials and Supplies | [redacted] |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8. Communications and Computing | [redacted] |
| 9. Outpatient Care Costs | [redacted] |
| **Total Other Direct Costs** | [redacted] |

| G. Direct Costs | Funds Requested ($) |
|---|---|
| **Total Direct Costs (A thru F)** | [redacted] |

**H. Indirect Costs**

| Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | * Funds Requested ($) |
|---|---|---|---|
| 1. MTDC, On Campus Rate | 55.00 | [redacted] | [redacted] |
| | | **Total Indirect Costs** | [redacted] |

**Cognizant Federal Agency** DHHS Region IX 415-437-7820

(Agency Name, POC Name, and POC Phone Number)

| I. Total Direct and Indirect Costs | Funds Requested ($) |
|---|---|
| **Total Direct and Indirect Institutional Costs (G + H)** | [redacted] |

| J. Fee | Funds Requested ($) |
|---|---|
| | |

**K. * Budget Justification** File Name: 1234-UCSDBudgetJustification.pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

## RESEARCH & RELATED BUDGET - Cumulative Budget

| | Totals ($) |
|---|---|
| **Section A, Senior/Key Person** | [redacted] |
| **Section B, Other Personnel** | [redacted] |
| Total Number Other Personnel | 4 |
| **Total Salary, Wages and Fringe Benefits (A+B)** | [redacted] |
| **Section C, Equipment** | |
| **Section D, Travel** | |
| 1. Domestic | |
| 2. Foreign | |
| **Section E, Participant/Trainee Support Costs** | |
| 1. Tuition/Fees/Health Insurance | |
| 2. Stipends | |
| 3. Travel | |
| 4. Subsistence | |
| 5. Other | |
| 6. Number of Participants/Trainees | |
| **Section F, Other Direct Costs** | [redacted] |
| 1. Materials and Supplies | [redacted] |
| 2. Publication Costs | |
| 3. Consultant Services | |
| 4. ADP/Computer Services | |
| 5. Subawards/Consortium/Contractual Costs | |
| 6. Equipment or Facility Rental/User Fees | |
| 7. Alterations and Renovations | |
| 8. Other 1 | [redacted] |
| 9. Other 2 | [redacted] |
| 10. Other 3 | |
| **Section G, Direct Costs (A thru F)** | [redacted] |
| **Section H, Indirect Costs** | [redacted] |
| **Section I, Total Direct and Indirect Costs (G + H)** | [redacted] |
| **Section J, Fee** | |

**UCSD Budget Justification**

**PERSONNEL:**

**Constance Benson, M.D. (Consortium PI – 0.60 Calendar Months).** Dr. Benson is a Professor of Medicine at UCSD and Director of the UCSD Antiviral Research Center. She will coordinate and supervise all research activities for the project, and will be responsible for recruitment and enrollment of research participants. She will recruit individuals from participants in clinical and translational research studies at the AVRC, obtain informed consent and abstract correlative clinical information for the SBIR project.

**Susan Little, M.D. (Consortium Co-Investigator – 0.3 Calendar Months).** Dr. Little is a Professor of Medicine at UCSD and a co-investigator of the UCSD Antiviral Research Center. She directs the Acute and Early HIV Infection (AEH) Program at the AVRC, and coordinates the clinical and specimen repository for the AEH Program. She will coordinate access to stored samples for assay development, and will assist Dr. Benson in participant recruitment, obtaining informed consent, and abstracting correlative clinical information for the SBIR project.

**Jill Kunkel, R.N. (Study Coordinator and Chief Research Nurse of the Antiviral Research Center – 1.20 Calendar Months).** Ms. Kunkel will assist Dr. Benson in participant recruitment as well as in logistics of blood acquisition and data extraction.

**Denise Lovec-Jenkins (Regulatory Specialist – 1.20 Calendar Months).** Ms. Lovec-Jenkins will coordinate regulatory submissions and renewals.

**OTHER DIRECT COSTS:**

**Supplies ($[redacted]** are budgeted to cover costs of laboratory disposables such as gloves, blood collection materials, etc.

**Patient care costs ($[redacted]** are budgeted to cover costs of reference testing using HIV EIA and NAT testing for 200 samples prospectively collected for the project (HIV EIA x 200 samples x $[redacted] per sample = $[redacted] HIV NAT x 200 samples x $[redacted] per sample = $[redacted]

**NGN/Communications/Computing ($[redacted]** costs have been included for telephone and associated voice and data communication charges which are directly related to the individuals working on the project.

# SBIR/STTR Information

OMB Number: 4040-0001
Expiration date: 06/30/2011

*** Program Type (select only one)**

☒ SBIR ☐ STTR

☐ Both *(See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)*

*** SBIR/STTR Type (select only one)**

☐ Phase I ☒ Phase II

☐ Fast-Track *(See agency-specific instructions to determine whether a particular agency participates in Fast-Track)*

## *Questions 1-7 must be completed by all SBIR and STTR Applicants:*

| | |
|---|---|
| ☒ Yes ☐ No | * 1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement? |
| | * 1b. Anticipated Number of personnel to be employed at your organization at the time of award. 30 |
| ☐ Yes ☒ No | * 2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies? * If yes, insert the names of the Federal laboratories/agencies: |
| ☐ Yes ☒ No | * 3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov |
| ☒ Yes ☐ No | * 4. Will all research and development on the project be performed in its entirety in the United States? If no, provide an explanation in an attached file. * Explanation: Add Attachment / Delete Attachment / View Attachment |
| ☐ Yes ☒ No | * 5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work? * If yes, insert the names of the other Federal agencies: |
| ☒ Yes ☐ No | * 6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)? |
| | * 7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions. * Attach File: 1252-Commercialization Plan p24 Add Attachment / Delete Attachment / View Attachment |

# SBIR/STTR Information

## SBIR-Specific Questions:

***Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.***

| | |
|---|---|
| ☒ Yes<br>☐ No | * 8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.<br>* Attach File: 1253-Commercial History - Less [Add Attachment] [Delete Attachment] [View Attachment] |
| ☒ Yes<br>☐ No | * 9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award? |

## STTR-Specific Questions:

***Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.***

| | |
|---|---|
| ☐ Yes<br>☐ No | * 10. Please indicate whether the answer to BOTH of the following questions is TRUE:<br>(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND<br>(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project? |
| ☐ Yes<br>☐ No | * 11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work? |

## COMMERCIALIZATION PLAN

### Executive Summary

The mission of MBio Diagnostics, Inc. is to improve health outcomes by providing the right information, in the right place, at the right time. Our initial focus is on low cost point-of-care technology emphasizing simplicity while providing laboratory quality results.

The company is advancing two product applications based on its proprietary fluorescence assay platform. Both have initial products in the in the field of HIV/AIDS. The first is a multiplexed serodiagnostic tool that either individually or simultaneously provides diagnostic results for HIV and common AIDS opportunistic infections (hepatitis B & C, syphilis, HHV-8, etc.), all from a single drop of blood. The technology for p24 viral antigen detection in this proposal will enable a high sensitivity HIV test that can detect the presence of virus soon after the onset of viremia. The technology will be included in a antenatal screening panel in development at MBio, a panel that will screen for HIV, HBV, syphilis, and anemia all on a single device with a single 10ul sample. The second product is a simplified cytometer for routine enumeration of CD4 cells for management of HIV-infected individuals. HIV-1 mediated CD4 cell destruction is the central immunologic feature of HIV-1 infection. Thus, the CD4 count is a critical measurement in initial disease staging, in monitoring antiretroviral therapy and in managing primary and secondary prophylaxis for opportunistic infections.

This Phase 2 application seeks funding for an innovative approach to serological testing for HIV that shows promise in detecting HIV at the earliest stages, yet with the high specificity that makes it a valued test for screening. The company is seeking SBIR Phase 2 funding to develop the technique and demonstrate its utility in the clinic. At the same time, MBio is pursuing strategic partnerships and venture capital investment that will be used to drive development through manufacturing, regulatory filings, sales, and distribution.

MBio scientists and engineers are driven to make an impact on health, and a simple rapid tool that allows detection of HIV at the earliest, most infectious stages can greatly slow the spread of the disease. In general, the sensitive and specific detection of circulating proteins has value in many diagnostic applications, in both infectious disease (e.g. HBV surface antigen, cryptococcal antigen) and non-infectious disease (e.g. cardiac troponin).

### A. Value of the SBIR/STTR Project, Expected Outcomes, and Impact

**Value**

The research that will be conducted under this grant will lead to a low-cost, ultra-sensitive POC HIV test, enabling detection of disease at its earliest stages in settings where HIV prevalence is highest. In general, the core technology serves as a platform for the development of other critical diagnostics tools, such as diagnostic panels for hepatitis, other infectious diseases, and non-communicable diseases. The value of all the applications in aggregate is substantial; here we will focus on the value of a highly sensitive POC HIV test that can directly detect acute infections in the clinic, and describe the overall market for products that could be enabled by the technology.

In the United States, it is estimated that there are 1.3 million individuals infected with HIV, and another 50,000 per year are newly infected. Approximately 250,000 individuals do not know their status, and those that do not know drive about half of the new infections, according to CDC estimates. The financial cost of treating HIV over the course of a lifetime is estimated at

$[redacted] representing $[redacted] billion dollars for the current caseload, and increasing by $[redacted] billion each year. This is just in the US, and similarly staggering sums can be calculated for the caseload worldwide of nearly 33 million individuals. In short, the value of a targeted screening program within the most impacted communities in the US, and leading to a decrease of 10,000 infections per year represents a cost savings of $6 billion. The CDC has advocated both wide screening approaches, such as for any person seeking care at an emergency department, and focused screening for populations at highest risk. The goal is to directly impact the new infection rate by increasing awareness of HIV status. A technology that is highly sensitive to acute infections (~30x increase in infectivity during the acute phase of the disease), can enable immediate contact tracing (as opposed to a weeks-long delay for getting lab-based nucleic acid testing results), and is available in a format that is simple to run and that allows many individuals to be screened in parallel with a low-cost device (as opposed to any of the POC nucleic acid devices under development), is a clear enabling technology for such a screening program.

**Expected Outcomes**

The direct outcome of this program is a highly sensitive, specific, and affordable technology for detecting and quantifying proteins in a biological sample in a clinic or other point-of-care setting. This technology enables multiple products, including a 4$^{th}$ generation HIV test, a p24 antigen quantification test for ART therapy monitoring, a cardiac troponin test, an improved hepatitis B screening test, and a combination test for antenatal screening including HIV, hepatitis B, and syphilis, all on a single device. Each of these tests can make a significant impact on health, both in the US and in a global context.

**Impact**

To understand the impact of this proposal, it is necessary to put the resulting products in the context of the overall platform under development at MBio. While each test we develop has intrinsic value, there is an even greater value for an overall platform that delivers a menu of tests at the point of care. In the case of HIV, the MBio platform can simultaneously screen for multiple diseases including HIV, then provide a confirmation test that separately reports each of several markers for HIV, then perform a CD4 test, screen for multiple co-infections, and measure viral load for a confirmed case of HIV. Each of these indications is under development at MBio, and shows how a single platform can provide a comprehensive set of diagnostic information. A successful completion of this development, followed by commercialization and market acceptance, would lead to critical enabling of HIV ART therapy throughout the world. It would permit wider distribution of such therapy, helping to limit the tragedy wrought by HIV. In the US, an improved HIV screening technology that is sensitive during the viremic stage of the disease can have a real impact on the ongoing epidemic and its human and financial costs.

In the broader context of society, there is a critical need to drive efficiency in the healthcare system—and if this is not achieved, it will bankrupt the United States. Increased efficiency will demand earlier and more accurate interventions—the cornerstones of personalized medicine.

## B. Company

MBio Diagnostics, Inc. was established in 2007 as a division of Precision Photonics Corporation (PPC), a leading manufacturer of laser technology, located in Boulder, Colorado. MBio was spun off as an independent company in 2010. Founded by optical measurement scientists from Nobel-prize winning laboratories at the University of Colorado, MBio's mission is to improve health outcomes by providing the right information, in the right place, at the right time. To complement the instrumentation expertise of the company's founders, MBio has assembled a team of diagnostic industry veterans and has established strong relationships with a world-class

group of clinical collaborators. The company also has an active Silicon Valley based investor group that has provided seed financing and ongoing business guidance. MBio Diagnostics, Inc. is now a 24 person, privately held company based in Boulder.

**Leadership**
The company's leadership team is comprised of:

*Dr. Christopher Myatt, PhD – CEO* – Chris has over 20 years of experience in technology development and building companies that capitalize on advanced measurement technology and scalable manufacturing. He founded Precision Photonics Corporation, an optical device design and manufacturing business that was successfully sold in 2012. The MBio technology was initially developed at Precision Photonics, and then spun out into a separate company prior to the sale of the parent company. Chris earned his PhD at University of Colorado/JILA, where his research work under Prof. Carl Wieman culminated in the first creation of Bose-Einstein Condensation (BEC) in Prof. Wieman's laboratory. Prof. Wieman was awarded the 2001 Nobel Prize in Physics for BEC.

*Dr. Michael Lochhead – Vice President* – Mike has over 10 years experience leading development teams in the life sciences and diagnostics field, with successful products on the market. He has led the growth of MBio from a two-person research project within Precision Photonics to a 24-person independent diagnostics company. He brings critical expertise in both the science and project management of a multi-disciplinary team. Dr. Lochhead completed his post-doctoral training in the Bioengineering Department at the University of Washington, and has a PhD in Chemical Engineering from the University of the Wisconsin, Madison.

*Michael Seely – Chief Commercial Officer* – Michael is an expert at building international commercial organizations, having founded and run medical technology businesses in South America, Southeast Asia, and Europe. Michael will lead our efforts to commercialize MBio products world-wide.

*Cindi Moore – Controller* – Cindi has over 20 years experience in accounting and finance, including biotech firms and manufacturing companies.

In addition to internal company resources, MBio has partnered with Dr. Robert Schooley and Dr. Constance Benson from University of California, San Diego (UCSD) Medical School. Drs. Schooley and Benson provide clinical guidance to the company and will be working with MBio on validation of the SnapCount™ technology. Dr. Schooley is Head of the Division of Infectious Diseases at UCSD and currently serves on the Scientific Advisory Board of the U.S. President's Emergency Plan for AIDS Relief (PEPFAR). Through his affiliation with the NIH AIDS Clinical Trials Group (ACTG), his international network includes clinical research sites in Latin America (Peru and Brazil), the Caribbean (Haiti), Africa (Mozambique, South Africa, Botswana, Zimbabwe, Malawi, Zambia, Kenya and Uganda), and Asia (India and Thailand). Working with investigators from these sites he has developed an initial scientific agenda for the ACTG International Program. One of the major goals of this agenda is the development of accurate and inexpensive point-of-care diagnostics that can be deployed in resource limited settings where disease management is often syndromic because of the lack of local diagnostic capabilities. In addition, he collaborates with investigators in Kenya (Dr. Fred Sawe, Director, Kericho Walter Reed Program), Zimbabwe (Dr. James Hakim, Chairman, Department of Medicine, University of Zimbabwe) and Brazil (Dr. Roberto Badaro, Chief, Division of Infectious Diseases, Federal University of Bahia).

**Funding History**
MBio's funding to date is from four sources: research grants and contracts, funding by former parent company Precision Photonics Corporation (PPC), private investment, and product sales.

To date, the company has received research grants and development contracts totaling over [redacted] million, with [redacted] million remaining on various contracts and grants. This research funding not only provides key development resources, but winning competitive proposals is an indirect validation of MBio's technology and approach.

From the time the group was founded through the end of 2010, parent company PPC provided significant direct and in-kind support to the MBio effort, covering major costs not addressed by research contracts and grants, including intellectual property investment as well as facility expansion, business development, and overhead. In addition, MBio has a core group of private investors, led by Milton Chang of Incubic, who have recently invested [redacted] into MBio to achieve initial regulatory approvals and product launch. This financing was a re-investment of a portion of the proceeds from the recent sale of PPC, representing a significant vote of confidence in the MBio team. MBio will continue to seek investment financing to expand its product offerings and bring those products to market.

Finally, MBio has begun shipping products, currently for research use only, including a panel for detecting shellfish toxins in a food safety application. First regulatory approvals for diagnostic tests are expected in [redacted] with growing product sales beginning in the [redacted] timeframe. This growing business will continue to fund product improvements and corporate development.

**Business Strategy**
MBio has a platform diagnostic technology that offers multiplexing, quantitation, high sensitivity, and data connectivity in a simple, affordable package that enables point-of-care testing. As a platform technology, there are many applications that can be developed. As a small company, we have limited resources for addressing this multitude of applications. Thus our business strategy is to complete the technology development, demonstrate its utility in certain applications that the company will develop and bring to market, and look to partners to develop other applications, either as licensees or acquirers of the technology for specific applications.

MBio has chosen to focus our internal development on unmet market needs within infectious disease testing. MBio is developing initial applications in testing for HIV, hepatitis, influenza, and syphilis with external R&D funding and has established strong and active working relationships with key opinion leaders in the global health field. The technology proposed here will significantly advance our product offering by enabling market leading performance in HIV, and open up other high sensitivity applications in fields such as cardiology and rheumatology.

MBio is in active collaboration or discussion for development of a variety of other applications of our technology. Examples include an ongoing development of a food safety application, a joint project to develop a TB serology diagnostic, and several discussions about development of specific panels as companion diagnostics for therapeutic. This last application is particularly exciting, as it would enable personalized medicine directly in the clinic, and speed up the clinical decision process.

**Regulatory Strategy**
In anticipation of United States FDA and European (CE Mark) regulatory filings, MBio is implementing Quality Systems under 21 CFR 820 and ISO 13485. MBio's initial products are being developed under Design Control, and we anticipate that our new pilot production facility

will be cGMP compliant in [redacted] MBio's regulatory strategy is to gain approval in Europe and other markets outside of the US first, and then bring the technology for approval in the US. Our first indication, a CD4 enumeration assay, is a relatively low risk device that can be self-certified in Europe (expected [redacted] and faces a 510(k) process in the US (expected [redacted] The HIV 4th generation test anticipated by the present proposal would require more elaborate regulatory approvals, since an HIV diagnostic test is included in Annex 2a under the European system, and requires a PMA for approval in the US. These regulatory hurdles will be addressed starting in [redacted]

**Reimbursement Strategy**
MBio has chosen to develop first products for indications with established CPT codes and purchasing channels. This focus on known reimbursement and purchasing reduces risk in the business plan. The products anticipated from the proposed effort, such as a 4th generation rapid HIV test, or adjacent products such as a HBV diagnostic or a cardiac troponin test, all have well defined reimbursement codes. For the HIV test, there is a chance for code stacking, since the test reports both the antibody and antigen status.

## C. Market, Customer, and Competition

**Market**
MBio estimates that the global market for in-vitro diagnostics exceeds $75B annually. MBio intends to establish itself as a leader in the professional point-of-care testing (POCT) segment of this market, which is estimated to be, over all applications, between $4B and $5B. The initial markets for the MBio technology are defined by the need for low-cost rapid tests for HIV, both diagnosing the disease itself and its common co-infections, and tests to manage therapy. The HIV/AIDS testing market, both POC and lab-based, is estimated to be greater than US$1B annually and growing at a rate of 15% per year. This growth is due to the increased prevalence of the disease and the increased funding being applied to testing patients worldwide, while being moderated by price decreases as more competitors enter the space.

Analysts at the Clinton Health Access Initiative (CHAI) are developing an analysis of the global demand for point-of-care (POC) testing. CHAI has shared preliminary results of this analysis with MBio. We have supplemented this analysis with several market reports, and with internal analysis. We are projecting the demands to 2017, given the long lead times for regulatory approval and market acceptance. Factored in are the initiatives that are underway to increase HIV screening (e.g. US CDC mandate to screen all emergency department visitors) and HIV therapy (e.g. the "15x15 Initiative", to put 15 million persons on HIV ART by 2015). The standard of care for HIV ART monitoring in the US is a viral load and CD4 measurement 4 times per year, while currently in the developing world 1-2 CD4 tests per year are standard. However, the desire is to increase this monitoring, to 2x per year for viral monitoring (either a viral load test or a semi-quantitative "viral monitor" test that indicates viral replication when a signal is above a set threshold), and 1x per year for CD4 testing. This is in addition to viral load and CD4 tests that are used for disease staging and therapy initiation. Additional tests include antenatal screening of the 125 million births per year worldwide, testing for a panel that at least includes HIV and syphilis. Further, there are confirmation tests and co-infection panels that are either current standard of care or increasingly part of the standard in the emerging markets of the world. The market for this family of HIV tests is shown in the following table, indicating a nearly $1 billion market for POC HIV testing in the near future.

| | US and Europe | | | Rest of World | | | 2017 |
|---|---|---|---|---|---|---|---|
| | Total Units* | POC Share | POC ASP | Total Units* | POC Share | POC ASP | total Market* |
| Clinical HIV screening | 60.0 | 20% | $[redacted] | 120.0 | 70% | [redacted] | $[redacted] |
| Antenatal screening | 10.0 | 5% | $[redacted] | 120.0 | 70% | $[redacted] | $[redacted] |
| Confirmation: HIV | 2.0 | 50% | $[redacted] | 5.0 | 50% | $[redacted] | $[redacted] |
| Co-Infection Panel | 0.5 | 15% | $[redacted] | 2.0 | 100% | $[redacted] | $[redacted] |
| CD4 | 8.0 | 40% | $[redacted] | 20.0 | 50% | $[redacted] | $[redacted] |
| Viral Monitor | 8.0 | 15% | $[redacted] | 60.0 | 50% | $[redacted] | $[redacted] |
| | | | | | | | **$[redacted]** |

*Figures in millions

In addition to infectious disease diagnostics, personalized medicine is also an attractive application of this technology. As pharmaceutical companies produce more specialized drugs that serve more narrow markets, the MBio technology will help identify potential customers at a reasonable cost. In 2005 the pharmaceutical industry spent over $25B in promoting new drugs. Prescription drug spending by consumers is projected to remain the fastest growing sector of health care costs. Spending on drugs is expected to account for 14.5 percent of $3.1 trillion health care expenditures by 2012 (for a total of $465B), compared to approximately 10 percent in 2001. MBio's POCT technology could be a key tool for pharmaceutical companies to lower cost of patient acquisition and expand markets for their drugs.

Other key applications for our serology and cytometry POC technologies include:

1. *Influenza Rapid Testing* – The portability, low power and multi-pathogen detection provide an ideal tool for detection of flu strains. MBio is part of a multi-year funded program aimed at improved POC testing for respiratory viruses.
2. *Cardiac Trauma Testing* – Cardiac panels for chest pain are an established segment in the POC market. Products analyze various markers in a panel, including troponin I, troponin T, CK-MB, BNP, and myoglobin. This market segment, for both lab and POC testing, has been estimated at $800 million. With the high sensitivity and multiplexing of the proposed technology, this is an attractive segment.
3. *Systemic Inflammatory Response Syndrome (SIRS)*. This predecessor to sepsis kills over 300,000 people in the US alone. Septic shock is the most common cause of mortality in the intensive care unit. It is the 10th leading cause of death overall (2003) and is the most common cause of shock encountered by internists in the U.S. There is a continuum of clinical manifestations from SIRS to septic shock. Our technology can provide a rapid quantitative diagnostic that could diagnose the indicators of septic shock.
4. *Allergy Testing* – Traditional tests are done partially at the point of care and require both quantitative and multi-analyte tests. It is estimated that in the allergy market alone, with only 1% penetration revenues could be greater than $25MM in the US and $50MM globally.
5. *Autoimmune Disease* – This very large market currently has no good diagnostic solutions. Evidence suggests the disease diagnosis will likely be based on simultaneous measurement of multiple parallel biomarkers. MBio's multiplexed technology is uniquely suited for this application.

In short, there are significant opportunities across the spectrum of medical diagnostics.

**Customers**

We initially focus on international customers for our CD4 cytometry technology, our first product. Our customers are the clinics, hospitals, and healthcare systems within emerging economies; and international organizations and non-governmental organizations (NGO's) that purchase and distribute diagnostics for the developing world. For NGO's and system-wide purchases, the World Health Organization (WHO) has defined a set of criteria for the inclusion of a product into the bulk procurement scheme. The WHO states that for limited resource environments the diagnostics must operate on battery power, require no refrigeration and be simple to operate. Our product will meet these specifications and perform high quality tests for [redacted] per test kit.

MBio will work with its' partners and affiliates to be included in bulk procurement schemes, both for specific countries as well as through umbrella organizations, such as the WHO and SCMS. These qualifications will add credibility and provide an objective review of our product for end-use customers. The UN procurement agencies, non-governmental organizations, the Global Fund to fight AIDS, TB, and Malaria, the Clinton Foundation, the PEPFAR program, and developing nations increasingly rely on the guidance provided by WHO on diagnostics and equipment that have been evaluated and intend to purchase products at reduced prices.

The strategy also includes the global AIDS Medicines and Diagnostics Service (AMDS), which will ensure that poor countries have access to quality medicines and diagnostic tools at the best prices. The service, which will be operated by WHO, UNICEF and other partners, will help countries to forecast and manage supply and delivery of necessary products for the treatment and monitoring of AIDS. AMDS will also include a medicines and diagnostics evaluation component, which will ensure that manufacturers, products, procurement agencies and laboratories meet international quality, safety and efficacy standards.

The customers for our CD4 product will directly overlap with customers for a 4$^{th}$ generation HIV test. Thus the channel we are currently building, through distributors and partners, will be ideally suited to the product envisioned in the current proposal.

**Competition**

The high growth rates for POC technology have attracted a number of competitors. There are a number of established competitors in specific segments, such as Abbott Point of Care (glucose and blood chemistry), HemoCue (hematology), and Siemens (blood gas). Traditional lateral flow tests are marketed by Alere, Quidel, and Becton Dickinson (BD). For the segments we are focused on, the biggest established competitor are Alere, Trinity Biotech, and OraSure, with multiple brands that compete in the HIV space. The rapid tests for diagnosis of HIV marketed by these three competitors are all traditional lateral flow devices, and are visually read. The electronic readout and control features, such as procedure controls and operator lockout, offered by the MBio technology are valued in a professional setting, where the license of the lab director is on the line when point-of-care tests are not run properly. Alere is introducing a rapid 4$^{th}$ generation test that is based on lateral flow technology, but it requires a large sample (50 microliters!) and has a complicated visual readout—virtually ensuring errors.

In the CD4 application, Alere has introduced the PIMA CD4 counting technology for point-of-care use. The PIMA system uses a complex pumping scheme along with immunostaining and fluorescence imaging to generate a CD4 cell count. The MBio system has significant throughput and cost advantages relative to the PIMA. We also believe that a "fast follower" market entry is the best business model for this application. Because POC CD4 testing is a new concept, Alere is undertaking a number of clinical studies just to establish the operational characteristics of POC CD4 testing. Our market introduction will benefit from the market

development that Alere has had to do to gain acceptance for POC CD4 testing. We see an outstanding business opportunity with a superior new product offering that can achieve rapid uptake and acceptance.

Our competitive status against select competitors is summarized below:

| Competitor | Product | MBio advantage |
|---|---|---|
| Alere | Determine HIV test | More sensitive and specific, lower sample volume, electronic readout |
| Trinity | UniGold HIV test | Higher sensitivity, electronic readout |
| OraSure | OraQuick | Higher sensitivity, electronic readout |
| Alere | PIMA CD4 | Higher throughput, simpler technology (more robust) |
| Zyomyx | CD4 test | Simpler procedure, electronic readout |
| Daktari | CD4 test | Lower cost, higher throughput |

Competition in the point of care diagnostic market includes higher speed realization of traditional diagnostic approaches, ranging from rapid delivery of samples to hospital labs and third party diagnostic service companies such as Quest Diagnostics and LabCorp. The importance of POC diagnostics is pointed out by Quest Diagnostics in the 2006 annual report: "The diagnostic testing industry is faced with changing technology and new product introductions. Advances in technology may lead to the development of more cost-effective tests that can be performed outside of a commercial clinical laboratory such as (1) point-of-care tests that can be performed by physicians in their offices and (2) home testing that can be performed by patients or by physicians in their offices. Development of such technology and its use by our customers would reduce the demand for our laboratory testing services and negatively impact our net revenues." The clear validation of this concern is the purchase by Quest of HemoCue in 2007.

**D. Intellectual Property (IP) Protection**

There are three major pillars of our intellectual property strategy. First, we must protect our ideas and methods; we combine an exclusively licensed portfolio with new IP filings to provide protection. Second, we must establish freedom to operate for our products; we have the presumption of freedom to operate through our issued patents, and will supplement this with further analysis and licensing of any necessary IP. Finally, we will investigate the blocking ability of our IP, whereby we could extract royalties from infringing products on the market.

We have exclusively licensed an extensive patent portfolio from [redacted] for the use of waveguides for making biosensors and diagnostic tests. This portfolio provides the base of our IP protection: (1) the portfolio provides basic coverage of the waveguide illumination technique, (2) the technology has been commercialized previously, showing that it can pass the FDA and withstand scrutiny of freedom to operate, and (3) the patents range in date from [redacted] to the present, giving both assurance of precedence and of ongoing protection. In addition, the company has filed multiple applications with improvements to the waveguide technique; recently we have received two Notices of Allowance on filed utility patents. We will continue to prosecute new innovations around the platform, including a filing that covers innovation in this proposal. New innovation continues in sample preparation, signal enhancement, electronic connectivity, new assays, and new biomarkers. We have dedicated one staff member to IP prosecution, and have a significant budget for IP development.

Protecting IP also includes managing disclosure of our ideas. We require our employees, consultants, outside collaborators and other advisors to execute confidentiality agreements upon the commencement of employment or consulting relationships with us. These agreements provide that all confidential information developed by or made known to the individual during the course of the individual's relationship with with the company, is to be kept confidential and not disclosed to third parties except in specific circumstances. In the case of employees, the agreements provide that all inventions conceived by the individual during his or her tenure are the exclusive property of the company. Documents are reviewed for confidential information prior to public disclosure.

Establishing absolute freedom to operate is a challenging task, as it requires complete knowledge of all IP in existence. In practice, the existence of patents covering one's technology brings a presumption of validity of those patents, and consequently the right to manufacture and sell those products. This was one of the key reasons we were pleased to secure an exclusive license to the [redacted] portfolio of patents covering the work of [redacted], a leader in the field of biosensors. We have the freedom to operate on these patents. Nonetheless, we have had discussions with potential partners about formally assessing the freedom to operate in the field of waveguide sensors. The relevant fields of concern are waveguide sensors, fluorescence immunoassays, and diagnostic microarrays. We are continuing to evaluate potential conflicts, and are budgeting [redacted] over the next year for analysis and up to two freedom-to-operate opinions to clarify our position for a partnership.

In addition to IP on the platform, we must secure licenses to and/or supply agreements for the reagents used in our tests. We anticipate being able to achieve this on commercially acceptable terms, and it will be a major driver for securing partnerships.

## E. Finance Plan

MBio funding history was discussed above, and the company has already secured the funding for gaining initial regulatory approvals and launching first products. Moving forward the company will use a combination of federal funding, equity investment, and partnerships. The equity investment requirements are modest. While we have the cash on hand to gain approvals for the first products, we plan to raise an additional [redacted] to complete commercialization of the MBio technology over the next 18 months. Funding will be used to complete the development of the product, secure further intellectual property protection, scale up manufacturing, and develop marketing relationships to promote and to distribute the product.

The revenue generated by our technology can come from:
1) Sales of the instrument and test kits by MBio
2) Royalties from the sales through strategic partners
3) Licensing or sale of the Intellectual Property to a strategic partner

Under the first scenario MBio would retain all of the rights to the technology and develop manufacturing and distribution relationships to get the technology to market. This strategy is feasible with equity fundraising, from investors and/or partners. Distribution would initially be through third party agents.

In the second scenario, MBio would outsource the sales and marketing to a larger distribution partner while managing and overseeing the design and manufacturing processes. Under this scenario MBio would need to increase resources in its' manufacturing areas and engineering

capabilities and capacity. Development funding and working capital would be provided by the distribution partner. These resources would ensure that MBio had the ability to mass-produce (with contract manufacturing partners) the instrument and cartridges, with QA/QC done in-house.

The last scenario would be to grant an exclusive license for manufacturing and distribution of the products or other variation of the products. MBio has entered discussions with large diagnostics companies. MBio would work closely with the acquirer of the Intellectual Property to ensure the highest probability of commercial success.

A combination of the above sources of revenue could develop. For example MBio could grant an exclusive license for the manufacturing and distribution of the product in South America while producing the product for distribution to the rest of the world. Further, we are considering licenses for certain product areas where we don't intend to develop products.

## F. Production and Marketing Plan

### Operations Plan

MBio is preparing to launch a CD4 test, and the requirements for a 4$^{th}$ generation HIV test would be similar. The company is undertaking the following significant development actions.

- Regulatory compliance: Engineering development of a medical device must occur under an established quality system that meets ISO 13485 and/or FDA QSR requirements, and must be documented in a Design History File (DHF) that is submitted to the FDA for regulatory approval. A Device Master Record (DMR) is also required that fully documents the bill of materials, approved suppliers, all hardware and software design specifications, and all manufacturing procedures. Additionally, to assess and mitigate potential harm to the user and patient, risk management efforts incorporating human factors engineering must be fully documented throughout the product lifecycle. The quality system is currently being implemented, and the design history file for the CD4 product is actively being assembled. Any product resulting from the proposed development would benefit from the quality system currently being implemented.
- Design for manufacture/value engineering: For viable product, material and labor costs must be substantially reduced from those of the prototype. Detailed engineering studies need to be performed to determine less expensive options for materials, components, and assembly methods. For the instrument, this primarily involves transitioning from expensive machined components to injection-molded parts, and re-designing the electronics boards and optical components to best balance performance with cost. For the disposable cartridges, there are two areas for value engineering: (1) tooling for more efficient injection molding, and (2) reducing labor time. The company is investing in mold tooling to lower cost, and investigating automation to reduce labor time and increase capacity. Validating laser welding of the cartridge and validating multi-cavity mold tooling are the primary objectives for moving the cartridge to high capacity production.
- Supply chain management: Ramping to projected volumes will require close partnerships with a variety of outside contract manufacturers (CM's) that have been approved under MBio's Supplier Quality Assurance procedures. With regards to instrument production, the modest volumes (1000 to 10,000 per year) can easily be produced by one of many CM's that have in place the required quality system and assembly infrastructure. During development, MBio will evaluate and qualify several CM's, choosing one for instrument production. On the other hand, the much higher anticipated volumes for the CD4 disposable cartridge, up to 20 million units per year, present significant challenges to production capacity. Initial production, up to 2 million units per year, is being implemented

in a new facility that MBio has leased. As the product scales up, we will investigate CM's for the cartridge. MBio is currently vetting possible CM's to do this specialized high capacity production, such as [redacted]

**Marketing Plan**

We will roll out our products through a combination of direct sales, strategic partnerships and independent distributors. Our marketing strategy is to raise awareness through a full array of marketing activities, which include trade shows, print advertising, special programs and distributor promotions, to support sales in each target market.

We will market diagnostic products directly to customers in the public health market for HIV testing and therapy management. This market consists of a broad range of clinics and hospitals and includes countries, ministries of health, states, and specific agencies such as the Centers for Disease Control and Prevention ("CDC") in the United States. We anticipate that there will be increasingly centralized purchasing for devices and tests for world health; as an example, the Global Fund is looking to centralize purchasing for the various programs it funds. There are also a number of organizations in the public health market, such as AIDS service organizations and various community-based organizations set up primarily for the purpose of encouraging and enabling HIV testing. We plan to register our diagnostics in a number of countries, progressing country by country in order of market size. We will also enlist the help of NGOs to raise awareness of these tests and facilitate acceptance.

## G. Revenue Stream

MBio has developed an extensive financial model for our products, both in the research use only markets, and the clinical diagnostics market. In addition, we have revenue from R&D contracts, such as the current proposal. We group the R&D contracts and our "research use only" product revenues into a Life Sciences business line. We group our clinical diagnostics products, such as the CD4 product and a 4th generation HIV test, into a Clinical Diagnostics line of business. We anticipate introducing our CD4 product in [redacted] with country roll-outs through [redacted] We anticipate introducing our HIV/syphilis serology product into world markets starting in [redacted] with country-level introductions extending over several years. The proposed HIV 4th generation test technology would likely be introduced into world markets in [redacted] and in the US in [redacted] Additional indications, not discussed here, are included in the model. We assume 3rd party distribution for all these products, and manufacturing at MBio's new facility in Boulder, CO. Not included in this are licensing fees and royalties, which are possible (and even likely), but we are establishing a baseline through this model. Based on these expectations, we predict the following revenue for MBio, expressed in millions of $:

**SBIR/STTR Information**

Question 8.

MBio Diagnostics, Inc. *has not received* more than 15 SBIR Phase II awards from the Federal Government during the preceding five fiscal years.

# PHS 398 Cover Page Supplement

OMB Number: 0925-0001

## 1. Project Director / Principal Investigator (PD/PI)

Prefix: Dr.
* First Name: Michael
Middle Name: J
* Last Name: Lochhead
Suffix:

## 2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

## 3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:
* First Name: Cynthia
Middle Name:
* Last Name: Moore
Suffix:

* Phone Number: [redacted]
Fax Number: [redacted]
Email: [redacted]

* Title: Director of Finance and Administration

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County/Parish:
* State: CO: Colorado
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 80303-1377

# PHS 398 Cover Page Supplement

## 4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

**Cell Line(s):** ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.



Tracking Number:GRANT11392590 Funding Opportunity Number:PA-13-088 Received Date:2013-05-07T02:15:52-04:00

OMB Number: 0925-0001

# PHS 398 Research Plan

## 1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☐ New ☒ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

## 2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

| | | | | |
|---|---|---|---|---|
| 1. Introduction to Application (for RESUBMISSION or REVISION only) | 1245-Introduction.pdf | Add Attachment | Delete Attachment | View Attachment |
| 2. Specific Aims | 1246-Specific Aims.pdf | Add Attachment | Delete Attachment | View Attachment |
| 3. *Research Strategy | 1247-Research Strategy.pdf | Add Attachment | Delete Attachment | View Attachment |
| 4. Inclusion Enrollment Report | | Add Attachment | Delete Attachment | View Attachment |
| 5. Progress Report Publication List | | Add Attachment | Delete Attachment | View Attachment |

Human Subjects Sections

| | | | | |
|---|---|---|---|---|
| 6. Protection of Human Subjects | 1248-p24 Ag SBIR Protection | Add Attachment | Delete Attachment | View Attachment |
| 7. Inclusion of Women and Minorities | 1249-p24 Ag SBIR Inclusion | Add Attachment | Delete Attachment | View Attachment |
| 8. Targeted/Planned Enrollment Table | 1250-Targeted Enrollment.pd | Add Attachment | Delete Attachment | View Attachment |
| 9. Inclusion of Children | 1251-p24 Ag SBIR Inclusion | Add Attachment | Delete Attachment | View Attachment |

Other Research Plan Sections

| | | | | |
|---|---|---|---|---|
| 10. Vertebrate Animals | | Add Attachment | Delete Attachment | View Attachment |
| 11. Select Agent Research | | Add Attachment | Delete Attachment | View Attachment |
| 12. Multiple PD/PI Leadership Plan | | Add Attachment | Delete Attachment | View Attachment |
| 13. Consortium/Contractual Arrangements | | Add Attachment | Delete Attachment | View Attachment |
| 14. Letters of Support | | Add Attachment | Delete Attachment | View Attachment |
| 15. Resource Sharing Plan(s) | | Add Attachment | Delete Attachment | View Attachment |

16. Appendix Add Attachments | Remove Attachments | View Attachments

**INTRODUCTION**

Here we provide an overview of revisions included in this grant resubmission. According the Summary Statement for the original submission, "there was significant enthusiasm for this Phase II application." Cited strengths included the success of Phase I studies, promising preliminary data, and the significance of point-of-care (POC) technology. Cited weaknesses were minor. Here we attempt to address all weaknesses in order of appearance in the Summary Statement. Reviewer comments are in bold, followed by the MBio response. Italic items highlight where edits have been made to the Research Strategy.

**Not as sensitive as PCR**. PCR is definitely more sensitive than antigen-based approaches, including the approach outlined here. We believe, however, that the cost and ease-of-use advantages of immunoassays will continue to result in high impact diagnostic tests, particularly at the point-of-care in limited resource settings. Very low cost POC PCR is still years away (in our opinion). *Cost and ease-of-use are addressed more clearly in the Significance section.*

**Uses serum or whole blood not saliva which may be more difficult**. Finger stick whole blood is widely accepted worldwide and will be necessary for the antigen detection part of the proposed assay. Oral fluid is very convenient, but antigen detection in saliva is not likely to meet analytical sensitivity requirements.

**Clinical assays are available to detect HIV-1 antibody/antigens**. Clinical lab assays indeed exist (e.g., Abbott and Bio-Rad). But high quality POC antigen/antibody combination assays have still not been well established. Published data for the antigen detection component of the Alere Determine® HIV-1 Ag/Ab Combo assay (e.g., recent posters from CROI 2013, Atlanta) show significant opportunity for improvement, particularly in the context of acute infection detection.

**POC kits based on nucleic acid based testing for early HIV infection may be a more appropriate technology**. Nucleic acid tests perhaps pose the greatest commercial threat to the proposed antigen-based system. As discussed above however, we believe the cost advantages and ease-of-use of immunoassay approaches will keep antigen detection very competitive in cost-driven applications such HIV screening in global markets. *Cost and ease-of-use are addressed more clearly in the Significance section.*

**No details are given for VDSA (trade secret)**. A detailed description of the viral disruption formulation is not critical for the scientific review of this proposal. As the reviewer correctly notes, viral disruption and immune complex disruption have "standard" elements, and the details of the formulation are not at the core of our innovation claim. Instead, our innovation is around integration of the disruption approaches into the POC system.

**It is not clear what was innovative in these efforts**. We believe the combination of low cost, quantitation, and sensitivity is unique in this proposal. *We try to more clearly articulate this in the Innovation section.*

**Of few quantitative results presented, judging from figure 3 it is not convincing whether the results support the claimed analytic sensitivity and detection limit.** We articulate our method of defining reactivity in Fig. 3, where we run the no target control samples in triplicate and define cutoff as mean plus 5 standard deviations. We stand by this definition. Regardless, the performance of the particle assay is not where we wanted it to be for making the transition to product, and we clearly stated that the "One-Step protocol does not meet the target analytical sensitivity milestone." It was this assessment that led us to develop the direct waveguide sandwich assay, which yielded the Table 1 performance. The direct waveguide assay is at the center of the proposed Phase II research.

**Why are there huge reading variations or errors at higher concentrations and almost no variation or error at lower concentration? Is it because the signal is too low to produce any reading variation?** Data are shown on a linear plot which compresses the appearance of relative variation. The variation, however, is high across the entire dynamic range and is concentration independent. The high variability makes the particle approach less attractive in general. This was part of the justification for our proposed transition to the direct waveguide sandwich immunoassay approach. *This point is augmented in the resubmission*.

## SPECIFIC AIMS

HIV infection remains a major public health crisis both in the United States and worldwide. There is increasing awareness that acutely infected individuals disproportionately contribute to disease spread (1). Yet these individuals remain the most difficult to identify, as infectivity is highest prior to the appearance of the HIV antibodies that serve as the basis for serological diagnostics (2). There are currently no FDA-approved point-of-care (POC) tests that are sensitive to acutely infected individuals. An HIV-1/2 antigen/antibody (Ag/Ab) combination assay – the so-called "4$^{th}$ generation" immunoassay – in an inexpensive, simple to use, POC format would fundamentally improve HIV-1/2 screening efforts in the United States and worldwide (3, 4).

MBio Diagnostics, Inc. is developing a point-of-care infectious disease testing platform for multiplexed HIV and coinfection serodiagnostic screening. Prototype devices have been placed in field sites in San Diego, Mozambique, Kenya, and Brazil. Due to cost and labor constraints, current acute infection diagnosis is typically based on pooled sample nucleic acid amplification testing algorithms, with 7 to 14 day turnaround times. 4$^{th}$ gen Ag/Ab assays in the clinical laboratory have been approved recently (Abbott ARCHITECT HIV Ag/Ab combo, Bio-Rad GS HIV 1/2 Ag/Ab Combo), but the 4$^{th}$ gen clinical analyzers do not offer the improved linkage to care associated with rapid, POC HIV testing. Here we propose an inexpensive device that delivers the performance of lab-based 4$^{th}$ gen Ag/Ab combo assays in a simple, POC package. We build on successes of our Phase I SBIR program and propose continuation of our translational research on a novel system with high commercial potential, offering:

- Parallel HIV-1/2 antibody and p24 antigen detection on a single point-of-care platform.
- Workflow and ease-of-use comparable to conventional HIV rapid tests.
- Robust, low cost, minimally instrumented system for use in emergency departments, public health labs, STD clinics, and targeted outreach programs.

***Aim 1*: *Assay Development***. Combine the Phase I p24 antigen detection assay with the MBio multiplexed serology assay cartridge, addressing issues of final monoclonal antibody (mAb) pair selection, HIV-1 Ag and HIV-2 Ag selection, reagent conjugations, cross-reactivity, and minimization of assay steps and complexity. The Aim 1 milestone is an HIV-1/2 antigen/antibody detection assay with performance equivalent to FDA-approved laboratory 4$^{th}$ gen Ag/Ab combo assays for the MBio early/acute sample collection [a set of 5 commercially available HIV-1 seroconversion/performance panels, two anti-HIV-1/2 combo performance panels, an anti-HIV-2 performance panel, and a unique collection of acute samples from San Diego.]

***Aim 2*: *Cartridge Integration***. Modify the MBio Cartridge, Rack, Reader, and Software to deliver an automated HIV-1/2 Ag/Ab combo result, and incorporate heat stable assay reagents into the MQ cartridge. The Aim 2 milestone is a portable, integrated system delivered to clinical collaborators that meets FDA CLIA waiver guidance requirements.

***Aim 3*: *Assay Validation***. Validate system using well characterized early HIV infection specimens including a panel of 200 HIV positive specimens comprised of 20 acute infection samples (RNA+ / Ab -), early seroconversion (Western Blot indeterminate) and seropositive (HIV-1 and HIV-2) samples. 200 HIV-negative samples will be used for specificity testing. The Aim 3 milestone is a dataset demonstrating performance equivalent to FDA-approved laboratory 4$^{th}$ gen HIV-1/2 Ag/Ab assays.

***Aim 4***: ***Pre-Market Field Evaluation***. Place systems in intended use setting and capture operational and usability feedback in advance of design lock; and generate a preliminary dataset on capillary whole blood samples from 100 study participants in San Diego. The Aim 3 and 4 milestone is a system design and dataset for an FDA investigational device exemption (IDE) meeting in advance of clinical trials.

The assembled group of investigators is uniquely capable of executing this project in a timely and cost efficient manner. The PI, Michael Lochhead, Ph.D. has led successful life science product commercialization efforts and manages MBio R&D programs, including several NIH grants projects. The MBio team includes established diagnostics industry veterans, development engineers, and bioassay scientists. Complementing the MBio group is a world class clinical team at the University of California, San Diego, with a well-established early HIV infection research program.

**[Edits to the original submission are included in bold brackets in this resubmission]**

## SIGNIFICANCE

HIV/AIDS remains a critical public health crisis in the United States and worldwide:

- CDC estimates there are 1.2 million Americans with HIV, and 1 in 5 do not know their disease status (1).
- Because the early/acute phase of infection is marked by very high titers of active viral particles (>3E05 particles/mL), infectivity is significantly higher for individuals during the early/acute phase of infection compared to those with chronic HIV infection and a mature antibody response.
- New guidelines from CDC for laboratory testing of HIV infection recommend initial screening with a sensitive, "4$^{th}$ generation" antigen/antibody combination assay (2).
- The global impact of HIV/AIDS remains enormous, with approximately 2.7 million new infections per year, 2 million AIDS-related deaths, and ~33 million people living with HIV (3).

**Figure 1**. MBio multiplexed immunoassay system, including a reader and disposable cartridges.

Current point-of-care HIV testing in the United States is based on FDA-approved rapid tests. Although these antibody-based tests have excellent clinical sensitivity and specificity for seroconverted individuals, they do not identify pre-seroconversion, acutely infected individuals (4, 5). Recent studies have demonstrated that measurement of HIV p24 antigen in combination with antibody measurements can significantly improve identification of acute infection cases (5). 4$^{th}$ generation HIV-1/2 Ag/Ab combo assays on clinical analyzer instruments have been commercially available outside of the United States for several years. But these 4$^{th}$ gen systems, such as the Abbott ARCHITECT® (FDA approval issued June 2010), are large clinical instruments not designed for point-of-care use. Inverness Medical recently launched a point-of-care, lateral flow based p24/antibody combo assay (Determine® HIV-1 Ag/Ab Combo, not currently approved in the US). Recent results suggest that the antigen feature of the Determine® test provides an advantage over antibody-only HIV-1/2 rapid tests, but that the antigen sensitivity is inferior to the clinical analyzers (6-9), and the specificity of the antigen line is a concern (10). PCR-based methods deliver the sensitivity required for acute infection diagnosis, but current PCR-based molecular tests do not meet the cost, turnaround time, or ease-of-use requirements needed for the large-scale public health screening.

Effective ELISA tests for the HIV-1 p24 antigen have been available for some time, but are not approved for human diagnostics. Importantly, the ELISA protocol includes pre-treatment to break antigen-antibody (Ag-Ab) complex, long incubations, various wash protocols, and added cost that make this test format incompatible with sensitive POC testing. The system proposed here avoids the Ag-Ab complex issue by reporting parallel Ag and Ab results. Other sensitive p24 assays have appeared in the literature recently (11), including POC devices with clinically relevant sensitivities and workflow (12, 13). The POC systems are promising, but they require multiple steps and, because they are built on lateral flow technology, those systems will not have the multiplexing capabilities of the system described in this proposal.

**[While PCR and lab-based methods provide outstanding sensitivity, they will be limited in impact in high disease burden, resource-limited settings where cost and ease-of-use are major drivers. The system proposed here addresses a major unmet public health screening need.]**

## INNOVATION

A robust, POC device with HIV Ag/Ab with performance equivalent to laboratory testing would significantly advance HIV screening efforts in the United States and globally by identifying the most infectious individuals quickly and cost effectively. The research proposed here has the ultimate goal of delivering direct detection of HIV-1/2 viral antigen at picogram/ml sensitivity, and HIV-1/2 antibody reactivity, from fingerstick whole blood in a simple point-of-care device. In order to do this, a highly innovative cartridge-based immunofluorescence assay approach is proposed. The major accomplishment of Phase I is a substantial

dataset on the MBio system demonstrating equivalent performance to significantly more complex laboratory tests – **feasibility is clearly established**. The major innovation of Phase II will be integration of the simple, finger stick whole-blood assay into the cartridge and reader system capable of operation in non-CLIA laboratories. Performance is achieved through a highly innovative combination of fluidic cartridge design, optical instrumentation, viral lysis techniques, and (if needed) an integrated wash mechanism. The disposable cartridge is assembled from injection-molded plastics with feature sizes within well-established manufacturing tolerances. The cartridge incorporates a molded waveguide that enables sensitive fluorescence immunoassays without the fluid handling requirements of ELISAs or the alignment, pumping, or interface issues of microfluidic devices—and the innovation of the key features of this waveguide technology were recently recognized by the patent office, with a Notice of Allowance for a patent on the technology. **MBio's technical innovations are directed at simplicity of the user experience** (minimal steps, small sample, error tolerance, heat-stable on-board reagents, etc) while not compromising on performance. Cartridge assembly costs will be comparable to those of existing lateral flow rapid tests, which have well established < $1 per piece manufactured cost. The novel, waveguide-based Cartridge-Reader System solves the light coupling and reproducibility issues that have plagued waveguide based fluorescence sensors for decades.

To our knowledge, there is no POC assay system on the horizon in the United States that can deliver the performance and ease-of-use expected from the MBio system. NIAID funding will provide the critical resources required to complete development, positioning the company to justify investment in clinical trials and manufacturing scale-up. Phase II will position the company for FDA pre-IDE meetings, and will provide the groundwork for pre-market approval (PMA) clinical trials and subsequent CLIA waiver evaluations.

## APPROACH

### Preliminary Studies and Phase I Final Report

The overall goal of Phase I was the development of a highly sensitive HIV p24 antigen assay, with post-Phase I efforts leading to an HIV-1/2 Ag/Ab combination assay in an inexpensive, simple to use, point-of-care (POC) format. To achieve high p24 Ag detection sensitivity, a multi-particle immunoassay sandwich approach was proposed (Fig. 2). With capture antibody conjugated to a paramagnetic particle, and detect antibody conjugated to an intensely fluorescent particle, the feasibility of combining target antigen concentration and labeling was demonstrated in the preliminary studies. HIV-1/2 Ag/Ab combination assay development, via integration of the developed multi-particle p24 Ag assay with the MBio HIV-1 antibody assay plus incorporation of HIV-2 antibody detection, was slated for Phase II.

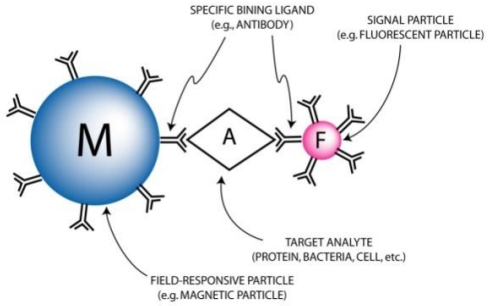


**Figure 2**. Particle complex approach to sandwich immunoassays. The magnetic particle provides concentration and specific signal enhancement. The fluorescent particle provides signal amplification.

*Phase I, Specific Aim 1. Establish a core collection of well-characterized clinical serum/plasma samples focused on acute and recent HIV infection. The Phase I collection will be based on samples from existing archives and sources, with additional testing under this award. The Phase I milestone will be a collection of 60 HIV-1 positive and 40 negative sera.*

The Aim 1 milestone was achieved. A collection of 40 HIV-negative serum samples was obtained from Valley Biomedical (Winchester, VA). The following HIV-1 seroconversion and performance panels, each including challenging acute/early HIV-1 infection samples and/or low anti-HIV-1 titer samples, were acquired: seroconversion panels HIV 9075, HIV 9079, and HIV 12008 from ZeptoMetrix (Buffalo, NY), and seroconversion panel PRB965 and anti-HIV-1 low titer performance panel PRB109 from SeraCare Life Sciences (Milford, MA). Added to this set were two HIV-1 positive control serum samples (SeraCare).

In addition to these commercially available samples, we assembled a panel of early/acute samples from our clinical collaborators in San Diego. For Phase I we obtained five acute (HIV RNA+/EIA-) plasma samples and 10 HIV RNA-positive plasma samples that yield indeterminate Western blot band patterns (Dr. Susan Little, UCSD Antiviral Research Center).

*Phase I, Specific Aim 2. Demonstrate MBio's magnetic multi-particle approach for rapid, ultra-sensitive, no-enzyme detection of HIV p24 antigen. The milestone for this aim is a model assay standard curve for p24 antigen detection with a lower limit of detection of 20 pg/ml.*

The Aim 2 multi-particle assay concept was demonstrated using the following key reagents: capture anti-p24 mAb conjugated to paramagnetic beads, biotinylated detect anti-p24 mAb, and NeutrAvidin (NA)-coated fluorescent beads. The start of Phase I used the following preliminary assay protocol: the antibody-conjugated magnetic beads were combined with the sample and biotinylated mAb then incubated for 25 min at ambient temperature. The magnetic beads where "pelleted" with a magnetic separator, the supernatant was discarded. The magnetic beads were then suspended in buffer containing the NA-coated fluorescent beads and incubated for 10 min. Following a wash step, this reaction mixture was transferred to a custom-designed disposable cartridge and magnetic particles were translocated with a magnet to separate magnetic bead:fluorescent bead complexes from free fluorescent beads. The cartridge was imaged on a fluorescence microscope and image analysis software was used to generate fluorescent bead counts.

Phase I goals were to optimize the assay reagents and to simplify this preliminary protocol. A range of magnetic particle types, differing in size and/or surface functionality (carboxylic acid, amine, N,N'-carbonyldiimidazole) where evaluated. Coupling to carboxylic acid MyOne™ particles via sulfo-NHS/EDC chemistry provided the highest yields of conjugated active mAb. Dark red fluorescent particles (Thermo Scientific) of varying sizes and surface functionalities were tested. The best assay performance achieved to-date has been with NeutrAvidin adhered to 0.39 μm sulfate-functionalized fluorescent particles.

A set of candidate anti-p24 antibodies, consisting of three polyclonal antibodies and 13 monoclonal antibodies, were screened for multi-particle assay performance (protocol as outlined above). Although the assay protocol as outlined above performed well with p24 Ag spiked into buffer, serum samples (50% v/v in assembled bead binding reactions) initially posed problems: significant aggregation of the magnetic beads and adherence of magnetic beads to the cartridge surface. Additionally, certain HIV-negative serum samples yielded false-positive results. These were subsequently identified as samples containing heterophilic antibodies (HAs). Both problems were alleviated through assay buffer optimization and the introduction of an HA competitor.

*"Two-Step" Multi-Particle Assay*. The following optimized "Two-Step" protocol was utilized for p24 Ag low limit of detection (LLOD) determination, for calculating the %CV at the limits of detection, and for evaluating assay performance with the Aim 1 sample set. Complete assay protocol details are not included here due to space constraints. Briefly, 25 µl of serum or plasma was added to reagent buffer containing both the the mAb-magnetic particles and biotinylated detection mAb. Following a 30 min room temp incubation, a magnetic separator was used to collect the particles, which were then resuspended in NeutrAvidin-coated fluorescent particles. Following a brief incubation, a magnetic wash-resuspension was used to remove free fluorescent particles. Particles were then magnetically focused and imaged on a fluorescence microscope and custom image analysis software provided the number of magnetic bead-fluorescent bead complexes.

We note here that we use the World Health Organization International Standard HIV-1 p24 reagent (NIBSC code: 90/636), expressed in international units (IU/mL). The NIBSC reference reagent is commonly used for cross-platform analytical comparisons (14), and it serves as the basis for analytical sensitivity performance standards from the European Commission; HIV-1 antigen assays used for diagnostics purposes must have an analytical sensitivity of **2 IU/mL** or better using the NIBSC standard.
The lower limit of p24 Ag detection with the Two-Step multi-particle protocol was determined via serial dilutions. As shown in Fig. 3(a), the limit of detection with this protocol is approximately **1.2 IU/mL p24 Ag**. This compares well with the limits of detection of this same p24 reference standard measured with four

commercial HIV Ag/Ab combination assays and one HIV p24 Ag only assay: ARCHITECT HIV Ag/Ab Combo (1.24 IU/ml), AxSYM HIV Ag/Ab Combo (1.94-2.25 IU/ml), VIDAS HIV DUO Quick (0.43 IU/ml), VIDAS HIV DUO Ultra (0.66 IU/ml), and VIDAS HIV p24 II (0.73-1.15 IU/ml) (14).

Having delivered a multi-particle assay with analytical sensitivity approaching the Abbott ARCHITECT (2 IU/mL versus 1.24 IU/mL) is a major accomplishment of Phase I Aim 2. As the Fig. 3 standard deviation bars suggest, however, assay variability is higher than desired. An additional Aim 2 goal was to simplify the protocol, reducing the assay time to ≤ 30 min and the number of steps to no more than two requiring user interactions. Efforts shifted to protocol minimization and improved reproducibility.

*"One-Step" Multi-Particle Assay*. The Two-Step protocol has the kinetic advantage of an extremely high affinity biotin-NA interaction bringing the magnetic beads, with bound p24 Ag immunocomplexes, and fluorescent beads together. The tradeoff, however, is a requirement for the separate addition of NA-coated fluorescent beads and a second binding reaction incubation. To achieve the minimal protocol as outlined in Fig. 2, the preparation of fluorescent beads pre-bound/conjugated to detect antibody was necessary. Extensive optimization of chemistry, bead ratios, incubation times, etc. resulted in the following simplified protocol: A 25-μl sample is added to a reagent buffer containing mAb-magnetic particles and mAb-fluorescent particles (see Fig. 2). Following a 30-min incubation at room temperature with rotational mixing, a magnetic wash is performed, particles are magnetically focused, and then analyzed as described above.

The lower limit of p24 Ag detection with the "One-Step" multi-particle protocol was determined by assaying 12.5, 25, 50, 100, and 200 IU/ml WHO Standard p24 Ag spiked into normal serum. Each concentration was assayed in triplicate. An intra-experiment cutoff value [mean + (5 X SD)] was calculated from results obtained with five replicates of a no-spike normal serum control. As shown in Fig. 3(b), the LLOD is approximately 30 to 40 IU/mL, a 20-fold higher concentration than achieved with the Two-Step protocol. Unlike with the Two-Step protocol, the number of detected p24-dependent bead pairs increased almost linearly with incubation periods beyond 30 min (1 hr and 2 hr tested; data not shown), demonstrating the slow binding kinetics between these two relatively large particles. The One-Step protocol does not meet the target analytical sensitivity milestone.

*Direct Waveguide Sandwich Immunoassay*. Concurrent with multi-particle assay development, MBio also developed a direct p24 Ag sandwich assay on MBio's SnapEsi™ planar waveguide platform under Aim 2. The sandwich assay was initially a reference method for particle assay development, but improvements to the direct sandwich assay approach progressed to the point where it was out-performing the multi-particle assays both in terms of sensitivity and simplicity. Fig. 4 provides a schematic cross-section of the MBio planar waveguide cartridge (15). The sandwich assay protocol is discussed in more detail below.

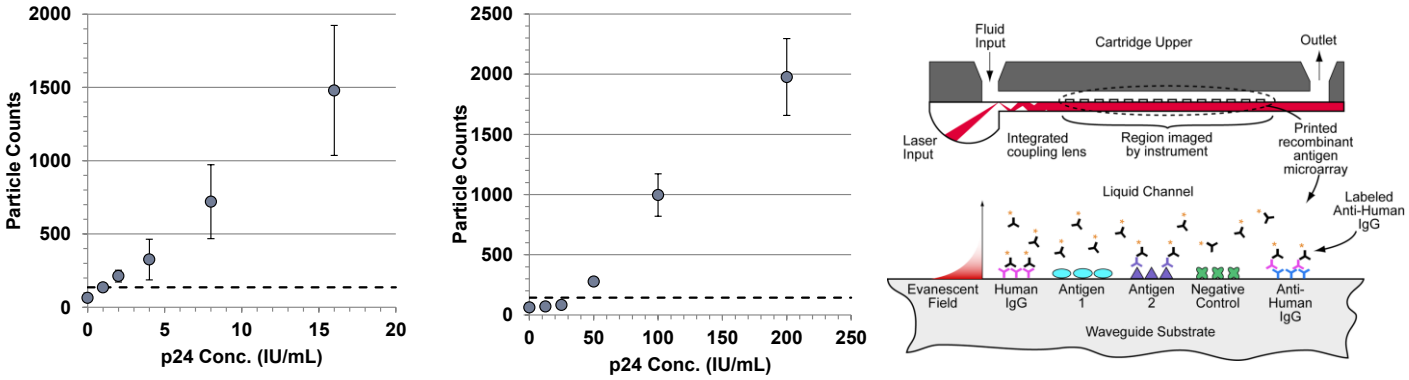


**Figure 3**. **(a)** Two-Step Multi-Particle Assay analytical sensitivity. **(b)** One-Step Multi-Particle Assay analytical sensitivity. NIBSC p24 antigen reference standard spiked into normal serum. Dashed line is cutoff, defined as mean signal + 5 standard deviations of 8 replicate measurements of the normal serum zero. Error bars are ± 1 stdev. **(c)** Schematic of MBio multiplexed immunoassay cartridge (image from reference (15)). For the direct p24 sandwich immunoassay, capture antibody is printed to the waveguide substrate, and detect antibody carries a label. See text.

**The main conclusion of the Aim 2 effort is that although the multi-particle assay approach came close to achieving the stated sensitivity milestone, an alternative approach that is both simpler and more sensitive was discovered during assay development.** The direct sandwich immunoassay using the MBio planar waveguide has become the focus of Phase II.

*Phase 1, Specific Aim 3. Quantitatively compare performance of the MBio antigen/antibody assay on the Aim 1 recent infection panel with gold-standard methods. The milestone for this aim is detection of 80% of HIV-1 positive samples not detected with the current market-leading FDA-approved HIV-1/2 rapid test.*

The performance of the Two-Step multi-particle p24 Ag assay, the SnapEsi™ planar waveguide p24 Ag sandwich immunoassay, and the SnapEsi™ planar waveguide 3rd Generation HIV-1 serology assay were compared with the "recent infection" panel (developed under Aim 1). With each of these assays, cutoff values were determined by assaying 40 HIV-negative serum samples; positive assay cutoffs were calculated as follows: cutoff = mean + (5 X Standard Deviation). For comparative purposes, the same recent infection panel was assayed at MBio with the following two rapid tests and two EIA's: OraQuick Advance® Rapid HIV-1/2 Antibody Test (OraSure Technologies, Inc., Bethlehem, PA), Uni-Gold™ Recombigen® HIV (Trinity Biotech, PLC, Wicklow, Ireland), Alliance HIV-1 P24 Antigen ELISA (PerkinElmer, Waltham, MA), and GS HIV-1/2 Combo Ag/Ab EIA (Bio-Rad, Hercules, CA). The product insert protocols were strictly followed for each of these commercial tests. The results are provided in Table 1.

Table 1 Notes:
All tests were run at MBio Diagnostics, with the exception of the Abbott ARCHITECT (package insert data provided)
(A) High Ab titer HIV-1 positive control serum samples (SeraCare)
(B) Anti-HIV-1 low titer performance panel PRB109 (SeraCare).
(C) Seroconversion panel PRB965 (SeraCare).
(D) Seroconversion panel HIV 9075 (ZeptoMetrix).
(E) Seroconversion panel HIV 9079 (ZeptoMetrix).
(F) Seroconversion panel HIV 12008 (Zeptometrix).
(G) Acute infection plasma samples (HIV RNA+/EIA-, Dr. Susan Little, AVRC, UCSD).
(H) HIV RNA+ samples that yielded indeterminate Western blot band patterns (Dr. Susan Little).
† PRB109-04 is a bleed from the same donor as PRB109-03, collected 2 days later.
‡ PRB109-11 is a bleed from the same donor as PRB109-10, collected 2 days later. †† PRB109-13,14, and 15 are same donor bleeds collected 5 days and 2 days apart, respectively.
‡‡ PRB109-18 is a bleed from the same donor as PRB109-17, collected 2 days later.

The anti-p24 antibody screen and heterophilic antibody (HA) background reduction investigations completed during multi-particle assay development aided development of the waveguide direct sandwich assay. The same detection mAb is used for both assays and a similar HA blocking strategy is employed. The following protocol was used to generate the "MBio Rapid HIV p24 Ag" assay results shown in Table 1: 21 μl of sample was mixed with 9 μl of detect reagent (including biotin-mAb) and added to the cartridge entrance port. Following a 20-min room temp incubation, SA-SureLight (SLP3) was added to the cartridge inlet port, displacing the sample volume. After a 15-min incubation at room temperature, wash buffer was added to the inlet port and the cartridge was analyzed on the MBio Reader (read time ~ 30 seconds).

*MBio HIV-1/2 Antibody Assay*. Although Phase I development focused primarily on p24 antigen detection, a useful POC 4th gen assay must combine HIV-1/2 antibody detection as well. The MBio rapid HIV-1 Ab assay currently under development is a 3rd generation assay that employs immobilized HIV-1 antigen gp41 and fluorescently-labeled detect gp41; sample anti-gp41 antibodies bridge the capture and detect antigens. In addition to being simpler than 2nd generation assays, the 3rd generation test does not rely on the specificity of an anti-human IgG detect Ab and therefore has the advantage of additionally detecting anti-gp41 IgM antibodies in early infection samples. The protocol used to generate the "MBio Rapid HIV-1 Ab (gp41)" assay data in Table 1 is as follows: 15 μl of sample was added to a detect reagent containing A647-labeled gp41 and then immediately transferred to the cartridge inlet port. Following a 20-min room temp incubation, wash buffer was added and the cartridge was analyzed on the MBio reader.

**Several important conclusions can be drawn from the Table 1 reference method comparison**

- **The MBio HIV-1 Ab assay significantly outperforms the FDA-approved rapid tests**

- **When MBio HIV-1 Ag and Ab data are considered together, the combination compares very well to FDA-approved laboratory 4th generation Ag/Ab Combo assays (Bio-Rad and Abbott)**
- **Assay performance feasibility is established**; Phase II will focus on system integration

**Table 1**. Summary of Results – Platform Comparison (see text for description)

| | | HIV Rapid Tests | | Enzyme Immunoassays (EIA) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Panel Member I.D. | OraQuick Advance | Trinity Uni-Gold | PerkinElmer HIV p24 Ag (S/CO) | Bio-Rad GS HIV Combo Ag/Ab (S/CO) | Abbott ARCHITECT Ag/Ab Combo* (S/CO) | MBio HIV-1 Ab (gp41) (S/CO) | MBio Direct p24 Ag (S/CO) | MBio Multi-Particle HIV p24 Ag (S/CO) |
| A | SC-9182257 | POS | POS | 0.4 | > 16.4 | | 36.8 | 0.2 | 0.5 |
| | SC-9148134 | POS | POS | 0.5 | 16.3 | | 35.9 | 0.1 | 0.4 |
| B | PRB109-01 | NEG | NEG | 22.9 | 14.8 | 40.9 | 2.7 | 14.3 | 3.2 |
| | PRB109-02 | NEG | NEG | 31.8 | > 16.4 | 281.3 | 2.1 | 115.3 | 8.1 |
| | PRB109-03† | NEG | NEG | 0.9 | 5.8 | 3.5 | 1.0 | 0.5 | 0.4 |
| | PRB109-04† | NEG | NEG | 2.0 | 13.0 | 10.7 | 2.7 | 0.4 | 0.2 |
| | PRB109-05 | NEG | POS | 0.4 | 14.6 | 10.2 | 14.3 | 0.4 | 0.2 |
| | PRB109-06 | NEG | POS | 3.6 | 7.3 | 4.1 | 2.2 | 5.1 | 1.3 |
| | PRB109-07 | NEG | NEG | 0.6 | 1.7 | 3.9 | 0.8 | 1.1 | 0.4 |
| | PRB109-08 | NEG | NEG | 0.3 | 0.2 | 0.3 | 0.5 | 0.2 | 0.3 |
| | PRB109-09 | NEG | NEG | 2.4 | 13.3 | 10.6 | 3.1 | 3.2 | 0.5 |
| | PRB109-10‡ | NEG | POS | 0.6 | 14.6 | 20.5 | 5.7 | 0.2 | 0.2 |
| | PRB109-11‡ | POS | POS | 1.1 | 15.0 | 36.5 | 5.0 | 1.0 | 0.4 |
| | PRB109-12 | NEG | POS | 1.8 | 15.1 | 34.1 | 14.4 | 2.0 | 0.3 |
| | PRB109-13†† | NEG | NEG | 2.7 | 10.6 | 23.6 | 0.3 | 0.4 | 0.3 |
| | PRB109-14†† | NEG | POS | 9.6 | > 16.4 | 127.5 | 5.4 | 0.7 | 0.6 |
| | PRB109-15†† | POS | POS | 7.6 | 16.2 | 127.9 | 11.8 | 0.6 | 0.1 |
| | PRB109-16 | NEG | POS | 21.2 | 15.7 | 116.7 | 2.5 | 40.8 | 6.8 |
| | PRB109-17‡‡ | NEG | NEG | 4.2 | 15.3 | 41.6 | 1.8 | 11.2 | 0.6 |
| | PRB109-18‡‡ | POS | POS | 3.8 | 14.9 | 58.6 | 7.2 | 1.9 | 0.3 |
| | PRB109-19 | POS | POS | 0.5 | 15.0 | 23.5 | 9.0 | 0.5 | 0.2 |
| | PRB109-20 | NEG | NEG | 0.8 | 10.7 | 13.7 | 3.4 | 0.1 | 0.2 |
| C | PRB965-01 | NEG | NEG | 0.2 | 0.3 | 0.2 | 0.1 | 0.3 | 0.2 |
| | PRB965-02 | NEG | NEG | 0.5 | 3.2 | 2.8 | 0.2 | 0.6 | 0.5 |
| | PRB965-03 | NEG | NEG | 0.6 | 3.4 | 3.5 | 0.2 | 1.1 | 0.5 |
| | PRB965-04 | NEG | POS | 0.7 | 13.3 | 15.6 | 4.8 | 1.2 | 0.6 |
| | PRB965-05 | POS | POS | 0.5 | 15.8 | 22.4 | 6.1 | 0.5 | 0.6 |
| | PRB965-06 | POS | POS | 0.3 | 15.2 | 18.8 | 9.9 | 0.3 | 0.4 |
| D | ZM 9075-02 | NEG | NEG | 0.2 | 0.2 | 0.1 | 0.4 | 0.3 | 0.1 |
| | ZM 9075-03 | NEG | NEG | 1.6 | 5.8 | 7.3 | 0.7 | 3.9 | 0.1 |
| | ZM 9075-04 | POS | POS | 2.0 | 14.7 | 31.9 | 11.5 | 4.0 | 0.2 |
| | ZM 9075-05 | POS | POS | 0.6 | 15.0 | 51.0 | 13.1 | 0.7 | 0.1 |
| E | ZM 9079-08 | NEG | NEG | 0.2 | 0.2 | 0.1 | 0.5 | 0.1 | 0.4 |
| | ZM 9079-09 | NEG | NEG | 0.9 | 5.0 | 3.6 | 0.5 | 1.7 | 0.8 |
| | ZM 9079-10 | NEG | NEG | 3.4 | 14.0 | 23.9 | 0.5 | 17.9 | 0.6 |
| | ZM 9079-11 | NEG | NEG | 6.4 | 15.5 | 43.1 | 1.1 | 23.4 | 2.6 |
| | ZM 9079-12 | NEG | NEG | 11.3 | 16.2 | 68.7 | 2.8 | 53.0 | 4.7 |
| | ZM 9079-13 | NEG | IND** | 0.8 | 12.3 | 34.8 | 2.1 | 0.7 | 0.2 |
| | ZM 9079-14 | NEG | NEG | 0.6 | 11.1 | 3.8 | 1.9 | 0.6 | 0.3 |
| | ZM 9079-15 | NEG | NEG | 0.3 | 9.9 | 4.1 | 3.6 | 0.2 | 0.3 |
| | ZM 9079-16 | NEG | IND** | 0.3 | 9.8 | 8.4 | 3.2 | 0.2 | 0.8 |
| F | ZM 12008-06 | NEG | NEG | 0.2 | 0.2 | 0.1 | 0.2 | ND | 0.1 |
| | ZM 12008-07 | NEG | NEG | 0.2 | 0.2 | 0.1 | 0.3 | 0.6 | 0.1 |
| | ZM 12008-08 | NEG | NEG | 0.3 | 0.6 | 0.5 | 0.2 | 0.5 | 0.1 |
| | ZM 12008-09 | NEG | NEG | 7.8 | 15.0 | 50.3 | 0.2 | 13.6 | 3.2 |
| | ZM 12008-10 | NEG | NEG | 28.4 | 16.3 | 167.7 | 0.8 | 57.6 | 8.1 |
| | ZM 12008-11 | NEG | NEG | 11.8 | 14.1 | 53.7 | 6.8 | 2.0 | 0.4 |
| | ZM 12008-12 | POS | NEG | 0.6 | 15.4 | 28.4 | 8.9 | 0.3 | 0.6 |
| | ZM 12008-13 | POS | NEG | 0.3 | 15.7 | 30.2 | 8.9 | 0.4 | 0.1 |
| | | | | 0.0 | | | | | |
| G | 177-SL (Acute) | NEG | NEG | 21.0 | 16.1 | | 0.3 | 0.1 | 0.4 |
| | 189-SL (Acute) | NEG | NEG | 0.8 | 2.6 | | 0.6 | 0.2 | 0.4 |
| | 190-SL (Acute) | NEG | NEG | 6.5 | 14.2 | | 0.4 | 2.8 | 0.6 |
| | 191-SL (Acute) | NEG | NEG | 10.4 | 13.7 | | 0.4 | 6.7 | 1.0 |
| | 198-SL (Acute) | NEG | NEG | 0.3 | 0.3 | | 0.9 | 0.0 | 0.2 |
| H | 178-SL (IND WB) | NEG | POS | 1.2 | 13.8 | | 5.2 | 0.3 | 0.2 |
| | 187-SL (IND WB) | POS | NEG | 0.7 | 14.4 | | 2.1 | 0.1 | 0.2 |
| | 192-SL (IND WB) | NEG | POS | 1.0 | 14.8 | | 2.1 | 0.3 | 1.0 |
| | 193-SL (IND WB) | NEG | POS | 4.5 | 14.2 | | 2.4 | 0.7 | 0.5 |
| | 194-SL (IND WB) | POS | NEG | 1.0 | 15.4 | | 1.8 | 0.9 | 0.4 |
| | 195-SL (IND WB) | NEG | POS | 0.5 | 6.4 | | 5.5 | 0.0 | 0.3 |
| | 196-SL (IND WB) | POS | POS | 0.9 | 15.7 | | 8.6 | 0.5 | 0.1 |
| | 197-SL (IND WB) | POS | NEG | 6.4 | 14.7 | | 2.7 | 1.1 | 0.6 |
| | 199-SL (IND WB) | POS | POS | 0.8 | 16.0 | | 7.9 | 0.1 | 0.3 |
| | 200-SL (IND WB) | POS | POS | 7.0 | 15.9 | | 2.8 | 0.8 | 0.3 |

* Data provided in product inserts

** Indeterminant (one observer called as negative; two observesr called as very weak positive.

Of the 64 samples in Table 1, the Bio-Rad GS HIV Combo Ag/Ab EIA and Abbott ARCHITECT Ag/Ab Combo results indicate that 56 are HIV-positive (sample 198-SL, with an HIV RNA concentration of approximately 2,000 copies/ml, was missed by both assays). The MBio p24 Ag and HIV Ab serology combination identified 52 of these 56 as HIV-positive, a 92.9% sensitivity. The OraQuick Advance HIV 1/2 Ab and Trinity Uni-Gold HIV-1 rapid tests identified 18 and 24 of the 56 as HIV-positive, respectively, for assay sensitivities of 31.1% and 42.9%. Of the HIV-positive samples not detected by the OraQuick Advance and Trinity Uni-Gold rapid tests, 84.2% and 83.1%, respectively, were identified as HIV-positive by a combination of the MBio Rapid p24 Ag and HIV-1 Ab Rapid Ab planar waveguide assays. **Thus, a combination of the Ag/Ab planar waveguide formats fully meets the goals of the Phase I program.**

Although the MBio p24 Ag assay performed very well, it is sensitive only to extraviral p24 antigen. In contrast, the PerkinElmer ELISA assay includes an immune complex dissociation procedure that both denatures sample antibodies, releasing bound p24, and disrupts virus particles, releasing additional detectable p24. An advantage of 4$^{th}$ generation Ag/Ab assays is that immune complex dissociation is not required—antigen and antibody detection overlap within the seroconversion window. However, we recognized that increasing the concentration of detectable p24 via virus disruption without significantly compromising the serology component of a 4$^{th}$ generation assay would improve assay sensitivity.

To this end, non-ionic detergents alone and in combination with ionic detergents were evaluated for effectiveness at improving p24 Ag detection without compromising the ability to detect sample antibodies. Although further optimization is pending (Phase II), a preliminary virus disruption sample additive (VDSA) has been developed. Details of the VDSA formulation are being held as trade secret by MBio. For the purposes of this grant review, we can say we are using a combination of ionic detergents, salts, and chelating agents. **[We are not claiming details of the viral disruption formulation as a core innovation of this grant proposal. Instead, it is incorporation of disruption into the simple worklflow that will be unique.]** The assay protocol was as described above for the MBio Rapid HIV p24 Ag assay with following exception; with the 'VDSA" protocol, assays were initiated by combining sample with the VDSA reagent, with mixing via aspiration. Detect reagent (includes biotin-mab and blockers) was then added to the sample-VDSA mixture and the entire mix was then transferred to the cartridge inlet port. A comparison of this "virus disruption" protocol to the MBio Rapid HIV p24 Ag assay protocol described above was performed with the five acute HIV infection samples listed in Table 1 (G). Results from this comparison are shown in Table 2. **By combining the MBio planar waveguide Ab and Ag detection, along with a novel viral disruption technique, the most sensitive HIV point-of-care screening test will be achieved, at a cost point that enables broad roll-out.** Note that differences in the "direct" method data between Table 1 and Table 2 are the result of an improved capture mAb print buffer instituted prior to the later Table 1 experiment. We also note that HIV Ab detection assay does not appear to be adversely affected by the detergent treatment (data not shown).

**Table 2**. MBio p24 Ag assay sensitivity comparison. Effect of virus disruption sample additive

| | MBio HIV p24 Ag (direct) | MBio HIV p24 Ag +VDSA |
|---|---|---|
| **Sample I.D.*** | **S/CO** | **S/CO** |
| 177-SL (acute) | **0.6** | **1.9** |
| 189-SL (acute) | **0.1** | **1.0** |
| 190-SL (acute) | **1.7** | **12.0** |
| 191-SL (acute) | **2.3** | **16.0** |
| 198-SL (acute) | **0.3** | **0.7** |

*Acute samples are RNA+ / EIA-

*HIV-1/2 Antibody Assay Improvements.* Since the goal of Phase II will be integration of an HIV 1/2 Ag/Ab combo assay, we include antibody detection results here as well. The 3$^{rd}$ generation MBio rapid HIV-1 Ab assay protocol has been simplified by removal of the post-incubation wash step. Performance of the one-step whole blood assay demonstrated using 59 whole blood samples. Samples from 34 HIV-positive donors were collected at the UCSD AVRC. Participants provided a venous whole blood tube under an IRB-approved protocol. Tubes were shipped overnight to MBio and assayed the same day as receipt. 25 HIV-negative samples were sourced commercially and were assayed in triplicate. Fig. 5(a) boxplots show Ab assay signal intensities for a gp41 antigen sandwich assay for the whole blood samples. Positive and negatives are well differentiated. Preliminary work on HIV-2 Ab detection has also been performed, focusing on detection of antibodies to the highly conserved env gp36 ectodomain. Although we have just recently begun the investigation of gp36 antigens in our 3$^{rd}$ generation serology assay format (and have not yet built a significant HIV-2 sample set), early results are promising, as shown in Fig. 5(b).

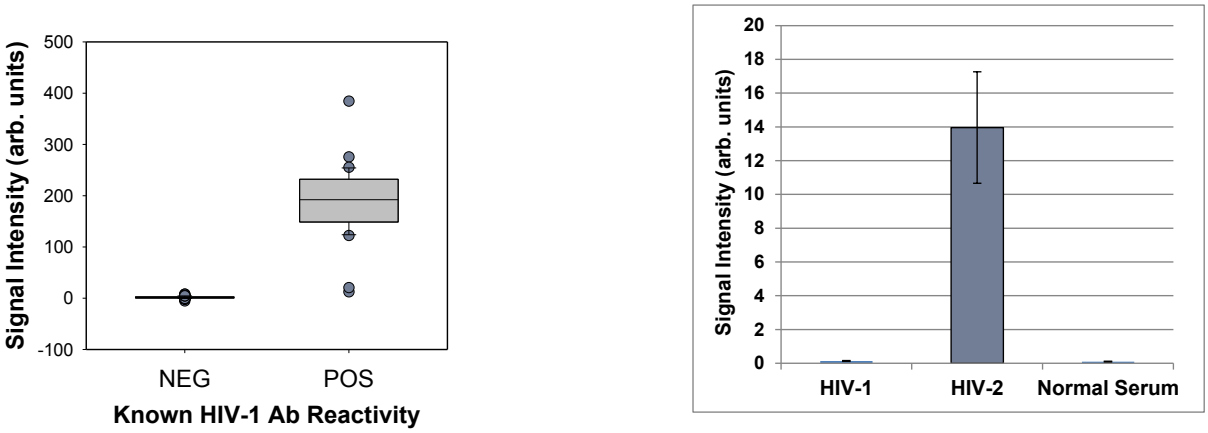


**Figure 5. (a)** Whole blood HIV-1 Ab reactivity results for known negative (NEG, N=25) and known positive (POS, N=34) samples, reported as signal intensity of gp41 antigen sandwich. **(b)** HIV-2 Ab reactivity results for known HIV-1 positive control, HIV-2 positive control, and normal serum, reported as signal of gp36 antigen sandwich. Control samples were run in triplicate; error bars are ± one stdev. Different normalization methods result in the absolute signal intensity differences between (a) and (b).

## PHASE II EXPERIMENTAL RESEARCH DESIGN AND METHODS

**Our goal is to develop and validate a 4$^{th}$ gen HIV-1/2 Ag/Ab combination assay that meets or exceeds the performance of clinical laboratory assays, in a low-cost, low-complexity, POC format.**

**Strategy.** The general strategy for achieving the Phase II project goals is to finalize the selection of antibody and antigen reagents, to reduce the complexity of the p24 Ag detection component to the level of the demonstrated HIV-1 Ab assay protocol, and to then combine the HIV-1/2 serology and p24 Ag immunoassay components on a single POC platform. Improvements in assay sensitivity will be sought through a re-design of the cartridge channel and the introduction of a virus disruption reagent. Further protocol simplification, to a level that meets FDA CLIA waiver guidelines, will be accomplished through cartridge design modifications and the development of a method for on-cartridge lyophilization of assay reagents. If necessary to reach performance goals, a mechanism for washing the cartridge waveguide array without user intervention will be integrated into the final cartridge and existing reader instrument. The integrated system will then be validated with a large carefully chosen set of HIV infection and control samples with pre-market evaluation in a clinical setting.

***Specific Aim 1: Assay Development***. *Combine the Phase I p24 antigen detection assay with the MBio multiplexed serology assay cartridge, addressing issues of final monoclonal antibody (mAb) pair selection, HIV-1 Ag and HIV-2 Ag selection, reagent conjugations, cross-reactivity, and minimization of assay steps and complexity. The Aim 1 milestone is an HIV-1/2 antigen/antibody detection assay with performance equivalent to FDA-approved laboratory 4$^{th}$ gen Ag/Ab combo assays for the MBio early/acute sample collection.*

*Selection of p24 antibody pair*. Although significant progress has been made on the identification of a high affinity anti-p24 monoclonal antibody sandwich pair, the search has not been exhaustive. Additional candidate antibodies will be tested as both detection and capture agents. Availability of long term supply agreements will be part of the mAb evaluation. Antibody-dye conjugation will be optimized at MBio. A design of experiments (DOE) approach will be used to optimize Ab print buffers. Dye labeled recombinant p24 and anti-species Ab will be used as fluorescent probes, and spot signal/noise, morphology (intra-spot %CV), and lot-to-lot reproducibility as primary metrics. A protocol that mimics the steps of the current HIV-1 Ab assay will be followed. Briefly, the sample will be combined with detect antibody and added to the cartridge entrance port. Following a 20-min incubation period, a wash volume will be added and the cartridge will be imaged on the MBio Array Reader. In addition to the NIBSC p24 reference standard, additional control samples from the Phase 1 collection will be validated for assay development. p24 antigen

detection sensitivity and specificity will be analyzed with a three-point titration of the NIBSC p24 standard and an 8-member panel of HIV-negative serum comprised of four non-heterophilic antibody (HA) samples and four known HA-containing samples. Iterative screening will be performed.

Results from a titration of the A647-labeled detect Ab will inform a decision on whether substituting the significantly brighter SLP3 for A647 will be necessary to meet assay performance goals. If yes, Columbia Biosciences (the SLP3 source) has provided MBio with a quote for custom conjugation of SLP3 to a supplied antibody.

*Selection of HIV-1 and HIV-2 antigens.* The MBio 3rd gen HIV-1 Ab assay utilizes purified recombinant HIV-1 gp41 protein from Fitzgerald. While assay performance with this antigen preparation has been satisfactory, alternative antigens will be tested in order to further improve performance and to ensure multiple reliable sources for product reagents. MBio is currently in a confidential contract negotiation with a cGMP supplier of HIV-1 and -2 recombinant proteins. Antigenic peptides will also be considered, as improved HIV-1/HIV-2 specificity may be achieved (16, 17). The screening path will be similar to that followed for anti-p24 mAb selection, including incorporation of print buffer optimization. Detect antigens will be labeled with A647 (or an equivalent dye). The assay sensitivity and specificity provided by the candidate HIV-1 and HIV-2 antigens will be measured by assaying an anti-HIV-1/2 combo performance panel (Seracare # PRZ207). Non-specific binding of candidate capture antigens to the anti-p24 detect mAb, and of the anti-p24 capture mAb to candidate detect antigens, will be investigated in detail.

*Concentration of detect antigens*. The stoichiometry of the labeled detect Ag and sample target antibody heavily influences assay sensitivity. In low titer samples, a high detect Ag concentration can saturate Ag binding sites thus preventing the antibody from serving as a bridge between the immobilized capture Ag and the detect Ag. Conversely, with very high Ab titer samples, a relatively low detect Ag concentration can result in most captured antibodies being unbound to detect Ag ("hook effect"). The Phase I HIV-1 Ab assay results (Table 1) suggest that a lower detect Ag concentration would have improved assay sensitivity. High titer Ab samples yielded s/co values of approximately 36; a two-fold reduction in detect Ag concentration would have increased the signals on low titer samples without sacrificing detection of the high titer samples. The selected HIV-1 and HIV-2 detect antigens will be carefully titrated with a panel of very high titer and very low titer samples to arrive at their final assay concentrations.

*Addition of a virus disruption step.* To improve the sensitivity of p24 immunoassays, samples are often subjected to detergents and heat, or acid followed by neutralization, to release Ag from both anti-p24 antibodies and viral particles (18, 19). These antibody-denaturing methods are not, however, available to the proposed rapid 4th gen assay. In the preliminary studies section, a detergent-based virus disruption sample additive (VDSA) was described that improved p24 Ag detection with a small set of acute infection samples without adversely affecting the serology component of the assay. An Aim 1 activity will be development of the VDSA reagent for best performance, including development of lyophilized, heat stable formulations. Reagent lyophilization is discussed in more detail under Aim 2.

*Combining HIV-1/2 Ab detection and p24 Ag detection.* The current anti-gp41 Ab and p24 Ag detection assays share a common sample additive buffer (minus HA-blockers for the serology assay) and wash buffer. Post-print processing of waveguides and cartridge assembly is identical for the two assay formats. Challenges with combining the detection reagents within a single array and sample additive should therefore be limited to cross-reactivity issues. While cross-reactivity will be addressed in the final antibody and antigen selections, adjustments in the sample additive buffer and wash buffer formulations may be required. Incubation times are also similar between the existing assays and it is expected that a mutually effective incubation time of between 10 to 20 min will be targeted.

The envisioned minimal assay protocol will consist of: (1) addition of sample to cartridge entrance port; (2) following an incubation period (10-20 min), insert the cartridge into fluorescence reader and, 15-30 sec later, receive assay results. A distinguishing advantage of the planar waveguide technology is only fluorescent molecules on, or very near, the waveguide service is exposed to the input light; thus free fluor in

the solution above the array is not significantly excited. The whole blood assay results discussed in the preliminary studies section were obtained with a version of this simple protocol. However, an assay performance boost can be obtained with a single wash of the array surface. Whether this post-incubation wash step is necessary to meet assay performance goals will be determined under Specific Aim 1.

*Assay performance.* The FDA-approved Bio-Rad GS HIV Combo Ag/Ab EIA run at MBio will serve as the reference test for Aim 1 method development. Performance of the MBio HIV-1/2 Ag/Ab combination assay will be tested with a set of 5 commercially available HIV-1 seroconversion/performance panels (SeraCare PRB109 and PRB965; ZeptoMetrix 9075, 9079, and 12008), two anti-HIV-1/2 combo performance panels (SeraCare PRZ207 and PRZ208), and an anti-HIV-2 performance panel (SeraCare PRF203). We have also secured an expanded, 80 sample "challenge panel" from subaward collaborator Dr. Susan Little at UCSD. Samples were selected from a large set (2,755 samples) collected during a prospective HIV screening program (NIH P01-AI074621; The San Diego Early Test program (20)). The 80 samples are HIV rapid-test negative, and have been characterized by HIV NAT (PCR) and Abbott ARCHITECT HIV Ag/Ab Combo.

***Expected outcomes, risks, and alternatives*.** The expected outcome of Specific Aim 1 is the demonstrated performance (clinical sensitivity and specificity) of a rapid HIV 1/2 Ag/Ab combo assay that is equivalent to that of an FDA-approved laboratory-based 4$^{th}$ gen assays. By reducing the complexity of the p24 Ag detection component of the assay, we risk losing the assay sensitivity required for early Ag detection in acute infection samples. It is expected, however, that direct conjugation of SLP3 to the detect Ab, a re-design of the cartridge channel (Aim 2), and incorporation of an VDSA reagent will more than recover any lost assay sensitivity. If an increase in sensitivity is still required, the potential of utilizing biotinylated detect reagents paired with delivery of SA-SLP3 from an on-cartridge reagent reservoir will be investigated.

***Specific Aim 2: Cartridge Integration***. *Modify the MBio Cartridge, Rack, Reader, and Software to deliver an automated HIV-1/2 Ag/Ab combo result, and incorporate heat stable assay reagents into the MQ cartridge. The Aim 2 milestone is a portable, integrated system delivered to clinical collaborators that meets FDA CLIA waiver guidance requirements.*

Representative MBio cartridge components are shown here. Location of lyophilized reagents is not shown (confidential). A detailed description of each component is beyond scope this proposal. Modifications to the assay cartridge will address liquid flow, sample loading, introduction of test quality controls, on-cartridge lyophilized assay reagents, and potentially integration of a wash buffer reservoir.

*Fluidic channel geometry for improved assay sensitivity.* The current cartridge geometry places 0.6 mm diameter printed antibody or antigen features within a fluid channel with a width of 3 mm and height of 0.145 mm. The time between sample addition and channel filling via capillary flow is only 3-4 seconds. On-rate and diffusion limitations to analyte binding should be reduced by both reducing the volume of the channel and the flow rate. With existing cartridge components, channel geometries can be altered by simply changing the dimensions of a die-cut adhesive gasket. The assay performance effect of 2 mm width channels with heights of 0.145, 0.1, 0.075 and 0.50 mm will be tested. The reduced channel volumes will both slow flow rate and "funnel" a greater fraction of the sample over printed proteins.

*Sample Loading.* Robustness of sample loading is a critical design feature in a CLIA-waivable test. The cartridge will be design to directly accept finger stick whole blood, without the use of transfer pipets. The inlet port will be designed such that direct contact with the blood droplet will result in capillary fill into the cartridge. The same inlet port will be designed to be compatible with a range of transfer devices (e.g., pipets) when the sample is a blood tube. The approximate blood volume from a finger stick sample is 17 μl (21). With the current 3 mm wide X 0.145 mm high cartridge channel, an 18-μl sample volume is sufficient to cover the planar waveguide array. The channel re-design described under Aim 1 should reduce this

required volume to not more than 10 μl. The design goal is to have the assay perform with precision for a sample volume range of 10 to 30 μl.

*Internal Quality Controls.* On-cartridge controls will be integrated to provide confirmation of proper sample introduction and reagent formulation. The addition of anti-detect reagent antibodies to the printed array, downstream of the array capture reagents, will serve as reagent formulation controls (e.g., anti-mouse IgG Ab, anti-gp41Ab and anti-gp36 Ab).

*On-Board Reagents.* On-cartridge dried reagents will be necessary to meet FDA CLIA waiver guidance requirements and to achieve long-term storage and thermal stability. Formulations and lyophilization conditions are separately being developed at MBio to obtain stable products, and this will be adapted for the proposed device. A requirement is that the dried reagents rapidly hydrate and disperse evenly throughout the sample, properties that will be targeted with a careful study of excipients and caking agents. An additional requirement is that the sample volume brought in contact with lyophilized reagent is controlled. MBio cartridges use controlled hydrodynamic forces (channel geometry and tuned surface energy) and capillary gates to control flow. For the current whole blood cartridge, the channel fills and then flow stops once the end of the channel is reached. The head pressure of excess sample volume in the entrance port is not sufficient to push sample volume into the exit port, even when the cartridge is held vertically.

Dried reagents will be maintained by sealing cartridges in vapor barrier pouches with desiccant material. MBio has the necessary temperature- and humidity-controlled environmental chambers for testing of both real time and accelerated stability. If the assay protocol demands a wash step, a means to do so without a manual intervention step will be developed. One approach is to place a “blister pack” containing the wash buffer on the cartridge. The blister pack will sit above a reservoir and channel that directs the flow of wash buffer to the channel. A simple solenoid-driven plunger in the instrument would depress the blister pack releasing the buffer contents.

***Expected outcomes, risks, and alternatives.*** Aim 2 activities are expected to produce an assay system that delivers HIV-1/2 Ag/Ab combo assay results with only two user intervention steps- addition of sample to the assay cartridge and insertion of the cartridge into the reader instrument. The main risk is that reconstitution of the lyophilized reagents is not sufficiently rapid and/or does not yield a reproducible concentration of these reagents in the cartridge channel volume. Alternative cartridge designs that further restrict sample volume flow as well as alternative caking reagents and lyophilization protocols would be investigated as correcting measures. An additional risk is that a post-incubation wash becomes a requirement and that the delivery of wash buffer from a blister pack is unsuccessful. An alternative approach would be the addition of wash buffer to the entrance port with a dropper bottle. Although this would require a manual intervention step, the volume would not require metering.

***Specific Aim 3: Assay Validation****. Validate system using well characterized early HIV infection specimens including a panel of 200 HIV positive specimens comprised of 20 acute infection samples (RNA+ / Ab -), early seroconversion (Western Blot indeterminate) and seropositive (HIV-1 and HIV-2) samples. 200 HIV-negative samples will be used for specificity testing. The Aim 3 milestone is a dataset demonstrating performance equivalent to FDA-approved laboratory 4$^{th}$ gen HIV-1/2 Ag/Ab assays.*

The performance of the developed assay system will be validated by assaying a panel of ≥ 200 HIV-positive samples, including a minimum of 20 acute infection samples (HIV RNA+ / Ab -) sourced through collaborator at UCSD. The remainder of the set will include early infection samples, high Ab titer samples, commercially available HIV-1 subtype performance panels, a commercially available HIV-1 Incidence/Prevalence panel, and the HIV-1 and HIV-2 panels listed under Aim 1. 200 HIV-negative (RNA-) samples will be acquired from Valley BioMedical and Dr. Susan Little for specificity testing. Assume 95% sensitivity and specificity, these sample numbers give 95% confidence intervals of ± 3%. In addition, it will be determined whether a panel of the following potentially interfering substances have effects on assay performance: lipemic samples (≥ 1000 mg/dL triglycerides, icteric samples (≥ 20 mg/dL bilirubin), hemolyzed samples (≥ 500 mg/dL hemoglobin), and proteinemic samples (≥ 12 g/dL protein).

***Expected outcomes, risks, and alternative***. The objective of Aim 3 is to demonstrate that the performance (clinical sensitivity and specificity) of the rapid HIV 1/2 Ag/Ab combo assay system is that is equivalent to FDA-approved 4th gen assays (95% confidence intervals match the Bio-Rad GS HIV-1/2 Ag/Ab Combo. The main risk is that performance does not meet expectations and that additional system development is required. In the event that performance equivalent to 4th gen laboratory tests is not achieved, system advantages in the context of a 3rd gen HIV assay relative to FDA-approved rapid tests will be pursued; 3rd gen product launch is considered low technical risk given existing data (e.g. Table 1).

***Specific Aim 4 - Pre-Market Evaluation***. *Place systems in intended use setting and capture operational and usability feedback in advance of design lock; and generate a preliminary dataset on capillary whole blood samples from 100 study participants in San Diego. The Aim 3 and 4 milestone is a system design and dataset for an FDA investigational device exemption (IDE) meeting in advance of clinical trials.*

The Pre-Market Evaluation will take place at UCSD HIV care clinics (AVRC and Owen Clinic), under the direction of subaward co-investigators Benson and Little, who will direct AVRC staff. A study of approximately 100 finger stick donors will be designed. The study will accomplish two goals: (1) generate usability dataset, and (2) establish performance in intended use setting.

*Usability Testing*. Given that intended use is a CLIA-waived product that will be operated by non-professional staff, a first aspect of the pre-market evaluation will be an assessment of how operators interact with the system, including patient, cartridge, and reader user interface/software. Human factors analysis will follow FDA and ISO 13485 guidance, and will be based on surveys and direct observation. Usability testing will be performed in the context of MBio's Quality Management System, and will be part of the Design and Risk Management Processes.

*Intended Use Setting Performance*. Although not a powered clinical study, the analysis of 100 study participants will provide critical feedback on system performance. Donors will provide both finger stick and venous samples. (NOTE: Human Subjects topics are addressed in a separate section of this proposal). Sensitivity relative to the FDA approved rapid tests and the Bio-Rad GS HIV-1/2 Ag/Ab Combo will be established. Reproducibility of the system will be established by running venous samples in triplicate. Importantly, accuracy and precision of finger stick samples relative to venous will be established.

The outcome of Phase II should be a system design and dataset that the company will use as the basis for a product launch decision. Data generated under Phase II will form the basis of the pack MBio takes to a preliminary FDA investigational device exemption (pre-IDE) meeting in advance of clinical trials.

| PROGRAM TIMELINE | Yr1: Q1 | Yr1: Q2 | Yr1: Q3 | Yr1: Q4 | Yr2: Q1 | Yr2: Q2 | Yr2: Q3 | Yr2: Q4 |
|---|---|---|---|---|---|---|---|---|
| **Aim 1: Assay Development** | | | | | | | | |
| 1.1 p24 mAb selection / optimization | ■ | ■ | ■ | ■ | | | | |
| 1.2 HIV-1 and -2 antigen selection | ■ | ■ | ■ | ■ | | | | |
| 1.3 Viral disruption assay | | | ■ | ■ | ■ | | | |
| **Aim 2: Cartridge Integration** | | | | | | | | |
| 2.1 Fluidic channel geometry | ■ | ■ | ■ | | | | | |
| 2.2 Sample loading / inlet port design | | ■ | ■ | ■ | | | | |
| 2.3 Internal QC | | | ■ | ■ | ■ | ■ | ■ | |
| 2.4 On-board reagents | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| **Aim 3: Assay Validation** | | | | | | | | |
| 3.1 HIV positive panel | | | ■ | ■ | ■ | | | |
| 3.2 HIV negative panel | | | | ■ | ■ | | | |
| 3.3 Interfering substance | | | | | | ■ | | |
| **Aim 4: Pre-Market Field Evaluation** | | | | | | | | |
| 4.1 Study plan | | | ■ | ■ | | | | |
| 4.2 Usability testing | | | | ■ | ■ | ■ | ■ | |
| 4.3 Prospective study (100 donors) | | | | | ■ | ■ | ■ | ■ |

***Human Subjects***

All studies involving human subjects conducted under this project will comply with federally-mandated human subjects regulations as presented in 45 CFR 46 (Regulations for Research Involving Human Subjects). In addition, the project will also be in compliance with OHRP guidance regarding the use of repositories for human serum, tissue, urine and other body fluids. All clinical sites that recruit subjects for donation of specimens will hold a Federal Wide Assurance (FWA) and strictly enforce the protections outlined for human subjects in accordance with the FWA and local policy. Key personnel who participate in clinical research are required to complete education in protection of human research participants which is recognized by the NIH and acceptable to local human subjects review boards. Certification of human subjects training will be kept on file for all key personnel and will be provided upon request. No research will be implemented without prior approval of the local Institutional Review Board or Ethics Committee or other relevant regulatory.

**Risks to Subjects**

*Involvement and Characteristics of Human Subjects.* Patients recruited to donate specimens and for whom clinical data will be collected for assay studies will include male and female adults (> 18 years of age) who are at risk for, infected with, or being tested for human immunodeficiency virus (HIV) and willing and able to provide informed consent. The health status of study participants from whom specimens have been or will be obtained will include those with signs or symptoms of acute HIV infection and asymptomatic or otherwise healthy individuals. Specific protocols will note the eligibility, inclusion, and exclusion criteria for recruitment to the project. Vulnerable populations such as prisoners and children will not be included as they will derive no discernable benefit from study participation, and will not be able to provide independent informed consent. Pregnant women, if they are undergoing testing for HIV and are willing and able to provide informed consent, may be referred to the project and will be offered the opportunity to donate specimens for this project. Pregnancy is unlikely to alter performance characteristics of the assay development, and in this context pregnant women will be eligible to participate because there is reasonable expectation that the assay being evaluated will have no adverse consequences to the woman or her fetus.

*Sources of Research Material.* Likely sources of research material will include samples of blood, serum, or other sterile body fluids obtained from study volunteers or archived in the UCSD Acute and Early HIV infection (AEH) Program repository with patient consent for future testing on file. Patients' existing medical records will be reviewed to obtain correlative clinical data. Surveys or questionnaires may also be used as a source of research data. The clinical data collected will be used solely for the purpose of research as defined by the project. Most of this information, however, will be available to the clinicians providing care to the patients if needed. All data, including laboratory data, will be identified by a patient-specific patient identification number (PID). Data submitted to the project database will not bear patient identifiers. Only local study personnel will have access to any link between PID and patient identifiers. Aliquoted specimens may be retained at the study site for use in research studies that are necessary to support the primary findings of the studies, and any future research studies of stored specimens will be allowed only after specific proposals for their use are reviewed and approved by the project, its partners and the NIH project officer have determined that use is covered by existing informed consent.

*Potential Risks of Study Participation.* Possible physical risks to study participants are limited to those associated with venipuncture for blood samples, interviews, and questionnaires. The risk from these procedures is considered minimal. It is possible that psychological trauma may result from a diagnosis of HIV infection. Potential social risks include embarrassment and stigmatization if there is inadvertent disclosure of confidential information such as diagnosis of HIV. The informed consent for obtaining samples and data for this project will include the alternatives to study participation and these will be reviewed with potential study subjects, as will risks and benefits.

**Adequacy of Protection Against Risks**

*Recruitment and Informed Consent.* Recruitment strategies for the project will be determined by the clinical investigators and recruitment will occur only after full approval for the study is obtained from the local Institutional Review Board or Ethics Committee and other relevant regulatory entities. Written informed consent documents for participation provide complete descriptive information about the research. They will be translated in a language that the participant understands as needed, and will be back translated into English to assure the adequacy of the translation. If potential study participants are unable to read, the clinical study site

will comply with their local Institutional Review Board or Ethics Committee guidelines for obtaining informed consent. In addition to the written document, verbal discussions will take place between the study participant and investigators before the consent form is signed. Additional information to be provided in the informed consent document includes adverse effects, risks, painful procedures, and the need for any follow-up visits. Compensation will also be addressed in the consent form. After approval of the consent form, written informed consent will be obtained by a member of the medical research team from the study participant. The signed informed consent document will be maintained in the patient's medical record and the study site source document; the patient will receive a copy. The informed consent process will be documented in each participant's study chart, and where appropriate, additional IRB-approved educational or recruitment materials will be provided to the participants. All clinical care required for study participants identified with HIV who agree to donate samples and clinical data to this project will be at the discretion of their primary physicians. Any relevant information necessary to their clinical care will be provided to the primary physician in a timely manner.

*Protections Against Risk.* Efforts will be made to limit any psychological impact for the study participant and his or her family. The investigators will maintain all patient information in strict confidence. All information about study participants will be contained in the medical record that is maintained in a secure facility. All research records will identify the patient only by a coded number. The master linkage between patient name and coded number will be accessible only by study personnel. We will follow all guidelines for confidentiality in research.

**Potential Benefits of the Proposed Research to the Subjects and Others**

Benefits of participation may include the provision of information that could result in more rapid identification and diagnosis of HIV that might afford appropriate referral for treatment or other interventions to reduce morbidity. The proposed research may result in the development of new technology that may allow earlier detection of HIV, which might benefit public health by reducing risk of transmission of HIV from infected persons to others.

**Importance of Knowledge to be Gained**

The proposed research may result in the development of new technology that might allow earlier detection of HIV, which might benefit public health by reducing transmission of HIV from infected persons to others and personal health by allowing earlier initiation of appropriate treatment for HIV.

**Data and Safety Monitoring Plan**

Data and safety will be monitored on multiple levels. At the individual institution, we will monitor patients for the occurrence of any adverse events associated with specimen or data collection and report these events as directed by the institution's adverse event reporting policy. Any event is reported directly to the study team. Data collection and data integrity will be monitored by the team through conference calls and review of data at face to face meetings. NIH project officers will be included in the data monitoring plans.

**Inclusion of Women and Minorities in Research**

Women and minorities will be appropriately represented in patient populations recruited to provide specimens for project studies. The substantial portion of our research participants are likely to be racial/ethnic minorities, based on HIV demographics in our region. The populations will be typical of the HIV epidemic in San Diego County, and in each additional area from which specimens are recruited. There will be representation of both males and females in project studies, and the distribution by sex will reflect nearly the proportions affected or at risk for HIV based on the demographics of local statistics. There are no data currently to suggest that diagnostic test results will differ by sex. Any differences will be explored in secondary analyses of study results. In addition, we will also conduct exploratory analyses to determine any racial/ethnic differences where the enrolled population allows.

**Inclusion of Children in Research**

Children will not be included in the research, based on their general inability to provide independent informed consent and provision of high quality samples for use in the assays. Should a sensitive and specific assay be produced as a result of this research, children will be included in the next phases of diagnostic testing.

## Inclusion of Women and Minorities in Research

Women and minorities will be appropriately represented in patient populations recruited to provide specimens for project studies. The substantial portion of our research participants are likely to be racial/ethnic minorities, based on HIV demographics in our region. The populations will be typical of the HIV epidemic in San Diego County, and in each additional area from which specimens are recruited. There will be representation of both males and females in project studies, and the distribution by sex will reflect nearly the proportions affected or at risk for HIV based on the demographics of local statistics. There are no data currently to suggest that diagnostic test results will differ by sex. Any differences will be explored in secondary analyses of study results. In addition, we will also conduct exploratory analyses to determine any racial/ethnic differences where the enrolled population allows.

# Targeted/Planned Enrollment Table

**This report format should NOT be used for data collection from study participants.**

**Study Title:** HIV Antigen/Antibody Diagnostic

**Total Planned Enrollment:** 100

| TARGETED/PLANNED ENROLLMENT: Number of Subjects | | | |
|---|---|---|---|
| **Ethnic Category** | **Sex/Gender** | | |
| | **Females** | **Males** | **Total** |
| Hispanic or Latino | 4 | 12 | 16 |
| Not Hispanic or Latino | 6 | 78 | 84 |
| **Ethnic Category: Total of All Subjects *** | 10 | 90 | 100 |
| **Racial Categories** | | | |
| American Indian/Alaska Native | 0 | 2 | 2 |
| Asian | 0 | 4 | 4 |
| Native Hawaiian or Other Pacific Islander | 2 | 4 | 6 |
| Black or African American | 4 | 8 | 12 |
| White | 6 | 70 | 76 |
| **Racial Categories: Total of All Subjects *** | 12 | 88 | 100 |

* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.”

**Inclusion of Children in Research**

Children will not be included in the research, based on their general inability to provide independent informed consent and provision of high quality samples for use in the assays. Should a sensitive and specific assay be produced as a result of this research, children will be included in the next phases of diagnostic testing.

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# PHS 398 Checklist

OMB Number: 0925-0001

## 1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☐ New ☒ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier: [redacted]

## 2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

## 3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

**4. * Program Income**

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

| *Budget Period | *Anticipated Amount ($) | *Source(s) |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |

**5. * Disclosure Permission Statement**

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No