PI: **Garrett, Patricia**	Title: Rapid Test for Recent HIV Infection	
Received: 09/09/2013	FOA: PA10-123	Council: 01/2014
Competition ID: ADOBE-FORMS-B1	FOA Title: NIAID ADVANCED TECHNOLOGY SBIR (NIAID-AT-SBIR [R43/R44])	
2 R44 AI098567-03	Dual:	Accession Number: 3620271
IPF: 2755701	Organization: IMMUNETICS, INC.	
Former Number:	Department:	
IRG/SRG: ZRG1 AARR-E (81)B	AIDS: Y	Expedited: Y
Subtotal Direct Costs (excludes consortium F&A) Year 3: Year 4: Year 5:	Animals: N Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Patricia Garrett	Immunetics, Inc.	PD/PI
Andrew Levin	Immunetics, Inc.	Other (Specify)-Scientific Director
Victor Kovalenko	Immeuntics, Inc	Other (Specify)-Principal Scientist
[redacted]	[redacted]	[redacted]

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: Immunetics, Inc.
Department: | Division:
* Street1: [redacted]
Street2:
* City: [redacted] | County / Parish:
* State: MA: Massachusetts | Province:
* Country: USA: UNITED STATES | * ZIP / Postal Code: [redacted]

Person to be contacted on matters involving this application
Prefix: Dr. | * First Name: Andrew | Middle Name: E.
* Last Name: Levin | 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:
Rapid Test for Recent HIV Infection

12. PROPOSED PROJECT:
* Start Date: 07/01/2014 | * Ending Date: 06/30/2017

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT:** MA-008

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION
Prefix: Dr. | * First Name: Patricia | Middle Name:
* Last Name: Garrett | Suffix:
Position/Title: Principle Scientist
* Organization Name: Immunetics, Inc.
Department: | Division:
* Street1: [redacted]
Street2:
* City: [redacted] | County / Parish:
* State: MA: Massachusetts | Province:
* Country: USA: UNITED STATES | * ZIP / Postal Code: [redacted]
* 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	0.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE E ECUTIVE 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: Dr. * First Name: Andrew Middle Name: E.

* Last Name: Levin Suffix:

* Position/Title: President and Chief Executive Officer

* Organization: Immunetics, Inc.

Department: Division:

* Street1: [redacted]

Street2:

* City: [redacted] County / Parish:

* State: MA: Massachusetts Province:

* Country: USA: UNITED STATES * ZIP / Postal Code: [redacted]

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

* Email: [redacted]

Signature of Authorized Representative: Andrew Levin

Date Signed: 09/09/2013

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

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

RESEARCH & RELATED Other Project Information

OMB Number: 4040-0001
Expiration Date: 6/30/2016

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:

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 - positive or negative - 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 [redacted] Add Attachment | Delete Attachment | View Attachment

8. Project Narrative [redacted] Add Attachment | Delete Attachment | View Attachment

9. Bibliography & References Cited [redacted] Add Attachment | Delete Attachment | View Attachment

10. Facilities & Other Resources [redacted] Add Attachment | Delete Attachment | View Attachment

11. Equipment Add Attachment | Delete Attachment | View Attachment

12. Other Attachments Add Attachments | Delete Attachments | View Attachments ☒

Monitoring the HIV epidemic to understand rates and patterns of growth, as well as targeting intervention efforts to populations exhibiting high rates of HIV transmission, are wholly dependent on determining the frequency of new infections using an assay that discriminates recent from long-term HIV infection. However, very few HIV assays have been developed specifically to distinguish incidence from prevalence. An added barrier has been the lack of an incidence assay for field work and resource-poor settings. Most HIV serologic assays are aimed at diagnostic use, while RNA assays have been used largely to determine viral load for clinical management purposes. Furthermore, HIV incidence testing currently requires access to well-equipped centralized laboratories capable of running the few sophisticated assays available for this purpose; these have been ELISAs requiring microplate handling and reading instrumentation, including the BED ELISA and the Vironostika detuned ELISA. Dependence on a central laboratory also implies the requirement for a system to transport serum specimens from where they have been collected to the laboratory, a separate and acute logistical challenge.

In this project, we propose to transform HIV serological incidence testing from ELISA methodology based on avidity and titer and requiring a laboratory with sophisticated infrastructure, to a field procedure using a simple, stable, and reliable rapid test. In Phase I, we have developed prototypes of a new, highly sensitive rapid test

To accurately monitor the HIV epidemic with respect to the rate of new infections, and to determine optimal interventions to prevent further transmission, a test that can identify recent infections is essential. Currently, such HIV incidence tests are few, mostly suboptimal in performance, and require a sophisticated laboratory to perform. We propose to develop the first rapid HIV incidence test than can be used in field conditions, without a laboratory, and will deliver results as or more accurate than the best laboratory tests now available. This test will put a powerful tool for identification of new HIV infections at the disposal of individuals and organizations responsible for monitoring and managing HIV prevention in the field.

FACILITIES AND RESOURCES

Immunetics, Inc.

Scientific environment:
Immunetics has developed effective capabilities and expertise for in vitro diagnostic assay development, manufacture and commercialization over a more than 20 year period devoted to this field. The company has pioneered a number of diagnostic assays for infectious diseases including the C6 peptide Lyme ELISA which is the first FDA-approved Lyme test based on a synthetic peptide, the Anthrax PA QuickELISA™ which is the first serologic test for anthrax approved by the FDA, and a variety of tests for esoteric pathogens including Babesia, Anaplasma, T. cruzi, T. solium and others. Immunetics was the first company to develop a neonatal dried blood spot-based HIV Western Blot for public health surveillance purposes, which became a CDC protocol in use for over a decade. Immunetics is one of a small number of companies which has the know-how and experience to manufacture and market kits for clinical diagnostics in a variety of resource-limited settings in Africa, Asia and Latin America. [redacted]

[redacted]

Clinical:
Immunetics does not have a clinical facility.

Animal:
Immunetics does not maintain animal facilities.

Computer:
Immunetics has a networked, state-of-the-art PC-based computer system supported by a secure server with password protection and including all peripherals, printers, scanners, data storage devices, etc.

Office:
Immunetics has an office area of 2,500 sf including all typical office services, computer stations and meeting facilities. Office support services including purchasing and accounting are available from staff, as included under indirect expense.

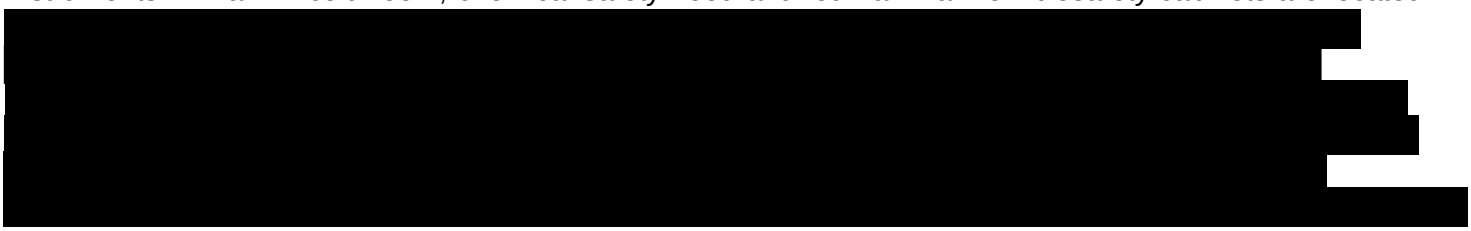
FACILITIES AND RESOURCES

Other:

Immunetics staff, including office and laboratory (R&D, manufacturing and regulatory personnel) are available for additional support where needed.

Major Equipment:

Immunetics' laboratory contains multiple workstations set up for assay development with electrophoresis and blotting equipment, ELISA readers, a FluoStar fluorescence Microplate reader, Biotek automated ELISA washer, Microplate agitator, flow-through Microplate manifold, protein concentration devices, Misonix probe sonicator, Labconco Lyph- Lock 6 shelf lyophilizer with automatic capping, argon hood for vial filling, liquid nitrogen tank, Waters HPLC system, low-pressure liquid chromatography system, floor and benchtop centrifuges, vacuum pumps, pH meters, balances, incubators, shakers, a deionized water supply system, a spectrophotometer, refrigerators and -20°C freezers, -80°C freezer, a variety of pipetters and small instruments. A walk-in cold room, chemical safety hood and four laminar flow biosafety cabinets are located



FACILITIES AND RESOURCES

[redacted] The company was started as an invention-based organization focused on development of sensitive colorimetric detection systems for rapid diagnostic tests and bioanalytical applications which use peroxidative enzymes as labels. In the six years since its inception, the company has developed many unique reagents for application in various membrane-based rapid tests and developed the concept for incorporation of a sensitive peroxidase-based detection system into a lateral flow format. The company staff is well experienced in the area of bioconjugation techniques, assay development and transfer of developed products to manufacturing. The company has performed several feasibility studies for application of the new technology to detection of antigens, disease markers, antibodies, and bacterial cells, which demonstrated the large potential of the new technology for development of highly sensitive rapid tests in various formats. The laboratory is well-equipped for development of membrane-based diagnostic tests and general immunochemistry work.

Laboratory:
[redacted] laboratory and office space (3,250 SF) in a business park with a separate entrance and security system. The laboratory is equipped for basic biochemistry and immunochemistry workoptimized for synthesis and analysis of conjugates, antigen and antibody characterization, and assay development. The laboratory is designed to have the nessesary utilities including power, water, pressurized airand additional air purification and humidity control equipment. The laboratory space can accommodate 8-10 scientists.

Clinical:
[redacted] does not have a clinical facility.

Animal:
[redacted] does not maintain animal facilities.

Computer:
[redacted] has 11 networked PC-based computers supported by a secure server with password protection and including all peripherals, printers, scanners, data storage devices, etc.

Office:
[redacted] has an office area for eight scientists and management including typical office services, computer stations and meeting facilities. Additional resources include storage and receiving areas and a kitchen facility.

Other:
[redacted] staff are available for additional support where needed.

Major Equipment:
The Company has a spectrophotometer, spectrophotometer/densitometer for membranes, microplate spectrophotometer, strip readers, HPLC and low pressure chromatography systems, water purification system, freeze dryer, plate washer, pH meters, conductimeter, balances, pumps, benchtop centrifuges, shakers, electrophoresis systems, dispensers for membranes (BioDot XYZ Dispensing system, CAMAG Linomat 4), automatic cutters for membranes (Kinematic Automation) , laminators, vacuum and convectional dryers, vacuum sealers, refrigerators/freezers, hoods, dehumidifiers, air purification systems, various accessories for enzyme immunoassays, drilling and cutting tools, air compressors, and photodocumentation systems.

FACILITIES AND RESOURCES

[redacted]

Scientific environment:

[redacted] is a full service developer and contract manufacturer of lateral flow assays and associated devices and electronics. We are ISO 9001:2008 and EN 13485 registered for development and production of assays, devices and electronics, and have a full range of manufacturing equipment for batch manufacture of lateral flow assays. DCN assists clients in rapidly developing, manufacturing and commercializing point of care lateral flow, flow through, microfluidic and ELISA assays for any market segment. We develop using fluorescent, visual (colloidal gold / colored latex) and paramagnetic labels. High sensitivity, quantitative and reader-based tests are our specialty.

[redacted] is the only OEM company in the rapid diagnostics market with the ability to contract develop entire point of care assay systems. We can design, develop, integrate, validate and transfer to manufacturing all aspects of a point of care assay system including the assay chemistry and biology, reagents, conjugates, strip design, cassettes, sample handling devices, and reader. We back up this capability with a broad network of partner companies in our Network of Affiliates and an on-staff group of highly experienced industry professionals that are available for consulting on any aspect of the development and commercialization of rapid diagnostics. We have developed products for markets as broad as clinical diagnostics, veterinary diagnostics, biowarfare, food science, microbiology, environmental health and safety, water quality, forensics, agriculture and consumer testing.

As of March 2011 [redacted] is both ISO 9001:2008 and ISO 13485 certified, and in March 2012 [redacted] was also ISO 13485 certified for Manufacturing.

Primary Services:

- Contract assay development
- Design and integration of cassettes, sample handling devices and strip readers
- Optimization and production of latex, colloidal gold and protein conjugates
- Education and training in lateral flow and flow through assay technology
- Consulting services
- Contract manufacturing
- Sales of supporting products, including digital readers, assay materials, and critical reagents

Laboratory:

[redacted] occupies an 8,000 sf suite in a business park. Of the 8,000 total sf, 3,000 sf area dedicated laboratory space housed in a class 10,000 capable clean-room. The laboratory is subdivided into three sections that can be closed off with doors. Laboratories are designed to have all the necessary utilities including power, water, pressurized air, etc. Inside the laboratory space, we have laboratory benches and islands to accommodate 16-20 scientists. Recently [redacted] added a 250 sf dry-room (15% relative humidity) to the facility. This temperature and humidity controlled room is used to assemble assay components that are sensitive to moisture, and to store materials that need a dry, desiccated environment.

Clinical:

[redacted] does not have a clinical facility.

Animal:

[redacted] does not maintain animal facilities.

Office:

The office and laboratory suite has a reception area, several offices for the scientists and management, a conference room, a server room, a storage room, rest rooms and a dedicated lunch room.

FACILITIES AND RESOURCES

Major Equipment:

[redacted] has a well-equipped 3,000 sf laboratory with sufficient bench space to accommodate 16-20 scientists. We have typical small laboratory equipment including timers, pipets, rockers,rotators, shakers, mixers and vortexers. We also have continuously monitored refrigerators and freezers(including ultralow temperatures) to store reagents, temperature sensitive raw materials, and samples. In addition, we have several incubators and ovens that are also continuously monitored. Larger equipment pieces that are housed in our laboratory include centrifuges, spectrophotometers, a microwell plate reader, a biological safety cabinet, a fume hood, a water purification system, and a lyophilizer. Equipment that dedicated to the production of lateral flow tests includes fluid-dispense systems, cutters, laminators and heat sealers. Recently we have added a 250 sf temperature-controlled dry-room (15% relative humidity) to assemble and store moisture-sensitive materials. All of our equipment has been validated, is calibrated if needed, and is under a preventive maintenance program.

FACILITIES AND RESOURCES

[redacted]

Laboratory:

[redacted] has state of the art equipment to support research related to blood and blood product safety in the areas of molecular biology, immunology, virology, tissue culture, cell processing and epidemiology. [redacted] staff has 8044 sq. ft. of labs (including a biosafety level 3 lab), and a 2896 square foot freezer room with capacity to store more than one million specimens. All [redacted] equipment is shared among investigators.

[redacted] has several core laboratories that are available to all investigators on a shared access basis. Relevant to this project is our Core Immunology Laboratory (CIL). The CIL is a successful resource for accomplishing high throughput immunological analysis of specimens that are part of larger research objectives. The CIL provides expertise in cellular, antibody or antigen detection assays such as high throughput T cell response screening (ELISPOT), single or multiplex analyte analysis (ELISA or Luminex), and cell sorting and general flow cytometry facilities (FACS Aria and LSR II).

Clinical:

Not applicable.

Animal:

Not applicable.

Computer:

PC's, laptops, and printers are connected to a Dell PowerEdge 2500 Dual Processor 1GHz Pentium III Server with antivirus, firewall, and user authentication on a Dell PowerEdge 1800 Quad 3 GHz Pentium 4 domain controller. Full nightly backups are performed. All users have workstations, laptops, or access to shared workstations, T-1 access to the internet, and secure remote access. Specialized and equipment-specific software programs available include Endnote (bibliography), Oracle 11i (financial), SAS 8.2 (statistical), ArcGIS (mapping and spatial analysis), Cardiff's Teleform 8.1 (image interpretation) and Freezerworks (repository management) software.

Office:

[redacted]

FACILITIES AND RESOURCES

[redacted]

Other:
Onan 350KW generator with a 900 gallon diesel tank available to provide emergency power to all temperature-critical equipment, computer server and other key equipment. Substantial plant operations support available, including carpentry and machine shop for minor repair/fabrication. Building security card key access. Secondary temperature monitoring system (Plexxium) for all temperature-critical equipment.

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: Patricia Middle Name:
* Last Name: Garrett Suffix:
Position/Title: Principle Scientist Department:
Organization Name: Immunetics, Inc. Division:
* Street1: [redacted]
Street2:
* City: [redacted] County/ Parish:
* State: [redacted] Province:
* Country: [redacted] * Zip / Postal Code: [redacted]
* 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: Ph.D.
Degree Year: [redacted]

Attach Biographical Sketch [redacted] 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: Andrew Middle Name: E
* Last Name: Levin Suffix:
Position/Title: Scientific Director and President Department:
Organization Name: Immunetics, Inc. Division:
* Street1: [redacted]
Street2:
* City: [redacted] County/ Parish:
* State: [redacted] Province:
* Country: [redacted] * Zip / Postal Code: [redacted]
* Phone Number: [redacted] Fax Number: [redacted]
* E-Mail: [redacted]

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

Project Role: Other (Specify) **Other Project Role Category:** Scientific Director

Degree Type: Ph.D.
Degree Year: [redacted]

Attach Biographical Sketch [redacted] 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: Victor Middle Name:

* Last Name: Kovalenko Suffix:

Position/Title: Principal Scientist Department:

Organization Name: Immeuntics, Inc Division:

* Street1: [redacted]

Street2:

* City: [redacted] County/ Parish:

* State: [redacted] Province:

* Country: [redacted] * Zip / Postal Code: [redacted]

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

* E-Mail: [redacted]

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

Project Role: Other (Specify) **Other Project Role Category:** Principal Scientist

Degree Type: Ph.D.

Degree Year: [redacted]

Attach Biographical Sketch [redacted] Add Attachment | Delete Attachment | View Attachment

Attach Current & Pending Support Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 3

Prefix: [redacted] * First Name: [redacted] Middle Name:

* Last Name: [redacted] Suffix:

Position/Title: [redacted] Department:

Organization Name: [redacted] Division:

* Street1: [redacted]

Street2:

* City: [redacted] County/ Parish:

* State: [redacted] Province:

* Country: [redacted] * Zip / Postal Code: [redacted]

* Phone Number: [redacted] Fax Number:

* E-Mail: [redacted]

Credential, e.g., agency login:

Project Role: Consultant **Other Project Role Category:**

Degree Type: [redacted]

Degree Year: [redacted]

Attach Biographical Sketch [redacted] 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 E CEED FOUR PAGES.**

NAME	POSITION TITLE
Garrett, Patricia E.	Principal, Pat Garrett Consulting
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
St. Francis College, Loretto, PA	B.S.	06/65	Chemistry
Georgetown University, Washington, D.C.	M.S.	01/68	Chemistry
University of Colorado, Boulder, CO	Ph. D.	08/70	Chemistry

A. Personal Statement

When I joined Boston Biomedica, Inc. in June, 1988, as the eighth employee and first Ph.D. scientist, BBIôs first HIV seroconversion panels had been in the marketplace for a year and were already gaining the tiny company visibility and influence far beyond its size. The utility of these panels in research and in diagnostics development and evaluation, and their commercial success, led us to assemble and manufacture other panels of samples carefully characterized for the marker(s) of interest, particularly in the fast-moving fields of blood- screening assays and HIV research. For this purpose, and for the line of quality control materials that we developed for HIV marker test methods, we collected HIV positive plasma units from plasma companies. That collection led to my first collaboration on incidence tests with Mike Busch, then of Blood Centers of the Pacific, and resulted in a poster presented at the Clinical Virology Symposium (CVS) in 1999. I have been working with leaders in the field on aspects of incidence testing ever since. Partially through my work, SeraCare has become a leader in the field of well-characterized HIV serum panels, including for assessment of test accuracy in detecting early infections.

In 2003, I organized an unfunded study with Tom Folks at CDC of randomly collected HIV positive units, each tested with 12 candidate incidence test methods performed in nine laboratories, to determine consensus or lack thereof for recent or long-standing infection. That study resulted in a poster presentation at CVS in 2004, and a commercially available panel of 15 plasma samples with unknown dates of infection that were consensus incident or consensus prevalent by at least 11 tests. Much of my work in HIV incidence since then has been the collection of those ógold standardô samples: whole blood or plasma from individuals with very closely estimated HIV infection dates (using the Fiebig algorithm, of which I played a part in the development). This work involved the same organizational and management skills that I have used for managing the design, development and evaluation of numerous other products.

I originated the idea of combining the sample collection with development of an innovative new HIV incidence assay, the concept underlying the present application. While at SeraCare I entered into a collaboration with Immunetics and Diagnostic Innovations which had developed a novel rapid test technology, that led to the Phase I SBIR grant 1R43 AI098567 supporting our joint efforts to develop both plasma panels and the rapid incidence test. I served as the Principal Investigator under the Phase I grant. During this time, I have expanded the project by engaging CDC, WRAIR and BSRI as collaborators, which will provide unparalleled access to expertise, field trial capabilities and well-characterized sample panels for test evaluation.

At the end of 2012, I retired from SeraCare and began a consulting practice, in which I continued to manage and represent SeraCareôs scientific interests with respect to the Phase I grant. Upon award of the Phase II grant, I will join Immunetics as an employee at Ó51% time, and will serve as the Principal Investigator for the grant. I believe that my extensive experience in the field of HIV testing and incidence measurement, literally from its beginnings through the present, is appropriate qualification supporting my role as Principal Investigator on the grant.

B. Positions and Honors

Positions and Employment

1981 ï 1988 Technical Director, Automated Chemistry, Immunoassay and STAT Laboratories, Lahey Clinic Medical Center, Burlington, MA

1988 ï 2004 Positions of increasing responsibility, culminating in Senior Vice President for Science and Technology, Boston Biomedica, Inc., West Bridgewater, MA

2004 ï 2012 BBI was purchased by SeraCare and I transitioned to Senior Director for Science and Technology, SeraCare Life Sciences, Inc., Milford, MA

2013 ï present Principal, Pat Garrett Consulting

Other Experience and Professional Memberships

Diplomate, American Board of Clinical Chemistry
Diplomate, National Registry of Clinical Chemists Member,
Association for Molecular Pathology
Former Member (6 year term), Board of Directors, American Board of Clinical Chemistry
Fellow, National Academy of Clinical Biochemistry
Volunteer writer and reviewer, Clinical Laboratory Standards Institute
Member, American Association for the Advancement of Science
Member, American Association for Clinical Chemistry
Member, Clinical Ligand Assay Society

[redacted]

C. Publications

1. Garrett, P.E. et al. ñSerial Plasma/PBMC Collections from Recently HIV-infected Individualsò 2012 HIV Diagnostics Conference, December 12-13, 2012, Atlanta, GA
2. Hallett, T. and the Incidence Assay Critical Path Working Group (2011). "More and Better Information to Tackle Epidemics: Towards Improved HIV Incidence Assays." PLoS Med. 8(6) e1001045. doi:10.1371/journal.pmed.1001045.
3. Hess, R. D., B. C. Gartner, et al. (2005). "Meeting report: Part I. Notes from the Molecular Virology Workshop, 23-24 April 2004, Clearwater Beach, Florida, USA." J Clin Virol **32**(3): 259-262, PMID 16180266.
4. Hess, R. D., B. C. Gartner, et al. (2005). "Meeting report: Part II. Notes from the Twentieth Annual Clinical Virology Symposium." J Clin Virol **32**(4): 342-346, PMID 15849874.
5. Hess, R. D., Gartner, BC, Garrett, PE. (2005). "Meeting Report Part III. Notes from the 20th Annual Clinical Virology Symposium, April 25-28, 2004 Clearwater Beach, FL, USA." J Clin Virol **33**(1): 83-87, PMID.
6. Garrett, P. E., S. Crush-Stanton, et al. (2004). ñMethod comparison of HIV tests for recent infection.ò 20th Annual Clinical Virology Symposium. Clearwater Beach, Florida. (Not peer-reviewed, but relevant to this project.)
7. Fiebig, E. W., PE Garrett, et al. (2003). "Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection." AIDS **17**(13): 1871-1879, PMID 12960819.
8. Garrett, P. E. (2001). "Quality control for nucleic acid tests: common ground and special issues." J Clin Virol **20**(1-2): 15-21, PMID 11163578.
9. Bondarenko, I. G., P. E. Garrett, et al. (1999). "Lack of correlation between sensitivity characteristics of the tests for hepatitis C virus antibodies estimated with serially diluted and natural low-reactive control specimens." Scand J Clin Lab Invest **59**(2): 153-158, PMID 10353330.
10. Garrett, P. E., V. L. MacKeen, et al. (1999). ñSeroconversion Profiles and Detuned Testing in the Determination of HIV Incidence.ò Clinical Virology Symposium. (Not peer-reviewed, but relevant to this project.)

D. Research Support

CDC Contract [redacted] Garrett (PI) 09/10/10-09/09/11

Acquisition of Longitudinal Samples from HIV Infected Individuals

The goal of this project is to collect additional bleeds from 19 individuals with closely estimated infection dates who were recruited and previously bled under CDC Contract #200-2004-10206. As PI, my responsibility is to continue the relationships with the collection centers (San Francisco City Clinic and SeraCare's Donor Recruitment Group), work with the group to amend the protocol (completed) and to transition the shipment and processing of samples to CDC (also completed). This small contract is on track to be completed on time and within budget in September 2011.

CDC Contract [redacted] Garrett (PI) 09/01/04-08/31/10

Seroconverter Specimen Panels

The goal of this project was to collect serial bleeds over 15 months from documented dates of HIV infection from 70 individuals. As PI, I coordinated all aspects of the project, from protocol development through subcontractor agreements, protocol familiarization, process development, donor recruitment, scheduling and collection, sample shipment, processing, storage, and data collection, transfer, and organization for the prospective collections, and identification, negotiation, material transfer agreements, IRB approvals, and shipments to CDC for the retrospective collections. In six years, I worked with and/or managed a group that included three successive CDC Project Officers, four potential clinical sites, one actual clinical site, three retrospective collection sites outside of SeraCare, and two groups within SeraCare that recruited additional donors and processed, stored, and shipped all the samples and data. None of the people involved reported to me, yet cooperation was good and we brought the project to a successful conclusion.

CRS-2007-T 620 Manak (PI), Garrett (PI) 12/15/07-7/30/10

Subcontract to PPD NIH HHSN26620050022C

Characterization of HIV Subtypes in a GLP Environment Logistics and laboratory support for collection of HIV isolates from around the world, virus isolation, characterization, panel manufacture, and distribution. As Co-PI, I was responsible for all documentation and data handling, and was the contact person for donor organizations that were providing plasma or viral isolates for analysis and consideration for panel members.

BIOGRAPHICAL SKETCH	
NAME Andrew E. Levin	POSITION TITLE Scientific Director and President
eRA COMMONS USER NAME: [redacted]	

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
Princeton University, Princeton, NJ	A.B.	1972-1976	Biochemistry
University of Wisconsin, Madison, WI	Ph.D.	1977-1984	Molecular Biology
Harvard University, Cambridge, MA	Post-doc	1984-1987	Cell & Dev. Biology

A. Personal Statement.

As President and Scientific Director of Immunetics which I founded in 1987, I have spent over 20 years on the development and commercialization of in vitro diagnostic test products, mostly for esoteric infectious diseases. During this time, I have developed and/or launched new assay kits based on ELISA and Western Blot technologies, some of my own invention, for a wide range of diseases including HIV, HTLV, Lyme disease, Cysticercosis, Hydatid Disease, Babesiosis, Ehrlichiosis, SARS, Anthrax and others. In recent years I have led the development of a molecular diagnostics technology with broad application to clinical and public health needs for pathogen detection, and I have brought from concept to commercialization the novel BacTx® test for bacterial contamination in platelets. I have gained experience in clinical trial and regulatory pathways to FDA approvals for a range of in vitro diagnostic products. As President and Scientific Director of a profitable and growing in vitro diagnostic business, I have been successful in identifying opportunities where medical needs, scientific technology and business potential intersect. I believe that these attributes make me well-suited for the role of Principal Investigator on the proposed project.

B. Positions and Honors.

1976–1977 Massachusetts General Hospital, Boston, MA
Research Technician, Genetics Unit
1977–1984 University of Wisconsin, Dept. of Biochemistry, Madison, WI
Graduate Student, Recipient of NIH Predoctoral Fellowship Award
1981 Centre de Génétique Moléculaire, CNRS, Gif-sur-Yvette, France
NATO Exchange Researcher
1984–1987 Harvard University, Cambridge, MA, Dept. of Cellular and Developmental Biology
NIH Postdoctoral Fellow
1987-Present Immunetics, Inc., Boston, MA
Scientific Director, Founder and President

Professional Associations

American Association of Blood Banks
American Association of Clinical Chemistry
American Society for Microbiology
American Society for Tropical Medicine and Hygiene

C. Selected peer-reviewed publications (in chronological order):

1. "Single-tier testing with the C6 peptide ELISA kit compared with two-tier testing for Lyme disease", Wormser GP, Schriefer, Aguero-Rosenfeld ME, Levin A, Steere AC, Nadelman RB, Nowakowski J, Marques A, Johnson BJ, Dumler JS, *Diagn Microbiol Infect Dis* 2012 Oct 10 Epub (10.1016/j.diagmicrobio.2012.09.003)
2. "Identification of bacteria in scuba divers' rinse tanks", Washburn BK, Levin AE, Hennessy K and Miller, MR, *Undersea Hyperb Med* 37(4):233-40, 2010. PMID: 20737930.
3. "Serological diagnosis of *Taenia solium* cysticercosis using recombinant and synthetic antigens in QuickELISA™", Lee Y-M, Handali S, Hancock K, Pattabhi S, Kovalenko V, Levin AE, Rodriguez S, Castillo Berrios Y, Noh J, Silva-Ibanez M, Lin S, Scheel CM, Gonzalez AE, Gilman RH, Garcia HH, and Tsang VCW, Am. J. Trop. Med. Hyg. 84(4), 2010. PMID: 21460015.

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4. "Impact of clinical variables on *Borrelia burgdorferi*-specific antibody seropositivity in acute-phase sera from patients in North America with culture-confirmed early Lyme disease", Wormser GP, Nowakowski J, Nadelman RB, Visintainer P, Levin A, and Aguero-Rosenfeld ME.Clin. Vacc. Immunol. (2008) 15:1519-22. PMID: 18716009.
5. "Effect of *Borrelia burgdorferi* genotype on the sensitivity of C6 and two-tier testing in North American patients with culture-confirmed Lyme disease", Wormser GP, Liveris D, Hanincova K, Brisson D, Ludin S, Stracuzzi VJ, Embers ME, Philipp MT, Levin A, Aguero-Rosenfeld M and Schwartz I, Clin. Infect. Dis. (2008) 47:910-914. PMID: 18724824
6. "Detection of anti-Borrelia antibodies by immunoblotting in Lyme Borreliosis", Anan'eva LP, Studentsov EE, Levin A., *Klin Lab Diagn* (2002) Jun;(6):45-7.
7. "Diagnosis of babesiosis using an immunoblot serologic test", Ryan R, Krause PJ, Radolf J, Freeman K, Spielman A, Lenz R, Levin A., Clin Diagn Lab Immunol (2001) 8(6):1177-80.
8. "Human granulocytic ehrlichiosis (HGE) and babesiosis: tick-borne diseases" in Für die Praxis: Lyme-Borreliose, ed. T. Talaska, Frankfurt (Oder), 1998.
9. "Monoclonal anti-dipeptide antibodies cross-react with detyrosinated and glutamylated forms of tubulins", Kuriyama R, Levin A, Nelson D, Madl J, Frankfurter A, Kimble M., *Cell Motil Cytoskeleton* (1995) 30(3):171-82
10. "Checkerboard DNA-DNA Hybridization", Socransky, S.S., Smith, C., Martin, L., Paster, B.J., Dewhirst, F.E. and Levin, A.E., *Biotechniques* (1994);17:788-792.
11. "Purification and characterization of a calcium-dependent ATPase from Paramecium tetraurelia", A.E. Levin, S. Travis, L.D. DeVito, K.A. Park and D.L. Nelson, *J Biological Chemistry* (1989) 264: 4544-4551.
12. "Abolition of actin-bundling by phosphorylation of human erythrocyte protein 4.9", A. Husain-Chishti, A. Levin and D. Branton, *Nature* (1988) 334: 718-721.
13. "Assembled clathrin in erythrocytes", D. Bar-Zvi, A. Levin and D. Branton, *Journal of Biological Chemistry* (1987) 262: 17719-17723.
14. "Copper staining: A five-minute protein stain for sodium dodecyl sulfate-polyacrylamide gels", C. Lee, A. Levin and D. Branton, Analytical Biochemistry (1987) 166: 308-312.

Selected Abstracts

1. Erwin J.L., Ni X., Wang H., Krueger N.X., Telford S.R., Krause P.J., Busch M.P. and A.E. Levin (2012) "Sensitive and Specific Peptide-Based ELISA for Detection of Antibodies to Babesia microti", Poster SP412, American Association of Blood Banks Annual Meeting, Oct. 2012.
1. Furniss, C.S., Shekar, S., Hennessy, K., Perry, L., Doron, S., Snydman, D., Krueger, N.X. and A.E. Levin (2010) "Rapid Detection of Antibiotic Resistance Genes in Bacterial Pathogens Associated with Hospital-Acquired Pneumonia by Reverse Line Blot Assay", American Soc. of Microbiology Annual Meeting, May 2010.
2. Washburn, B.K., Hennessy, K.T., Levin, A.E., Libby, B.J., Castelblanco, A.S., Cubilos, G.F., Kett, D.H. and Snydman, D.R. 2008. Semi-automated reverse line blot assay for detection and identification of bacteria and fungi in clinical samples. American Society of Microbiology Annual Meeting, June 2008, Abstract #C-054.
3. Kirby, C., L. Beausang, V. Kovalenko and A. Levin (2005) „BacTx – A Rapid Assay for the Detection of Bacteria in Platelet Units", American Association of Blood Banks, Nov. 2005.
3. Levin, A., Beausang, L., Kirby, C., Syrkina, M. and V. Kovalenko (2005) "C6 ELISA Specificity vs. Two-Tier Testing Protocol", X International Conference on Lyme Borreliosis, Vienna, Sept. 2005.
4. Septak, M., P. Wilkins, L. Chalcraft, K. Stamey, M. Syrkina, V. Kovalenko, C. Quinn and A. Levin (2004) "Clinical Validation of a Novel Anthrax ELISA for the Detection of Antibodies to Protective Antigen", American Society of Microbiology, May 2004.
5. Andrew E. Levin, Peter Condon and Victor Kovalenko, (2002) "Lyme Borreliosis Serodiagnosis by ELISA Based on the C6 Peptide of VlsE", International Conference on Lyme Borreliosis, Belgium, 2001.
6. Levin, A., Condon, P. and Kovalenko, V. (2002) "C6 peptide ELISA for Lyme serodiagnosis: Equivalent to two-tier testing ?", IX International Conference on Lyme Borreliosis, New York, 2002. Oral presentation O-172.
7. Andrew Levin, Thomas Talaska and Victor Kovalenko (2002) "C6 Peptide ELISA: Universal assay for detection of European Lyme borreliosis ?", IX Int'l Conf. on Lyme Borreliosis, New York, 2002. Abstract P-149.
8. Levin, A.E. , Condon, P.C., Syrkina, M., Shmuter, G. and Oddo, S. (1999) "Fifteen-minute Lyme Western Blot with automated neural network interpretation",
VIII International Congress on Lyme Borreliosis and Related Tick-Borne Diseases, Munich.
9. Levin, A.E. and Talaska, T. (1998) "Serologic Evidence of Coinfection with HGE and *Borrelia burgdorferi* in Germany", First Congress of European Society for Emerging Infections, Budapest.

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10. Levin, A.E., Shmuter, G.M. and MacDonnell, P. (1998) “An accelerated membrane immunoassay for *Bartonella henselae* antibodies”, American Soc. for Trop. Medicine and Hygiene Annual Meeting, Abstract 174.
11. Levin, A.E., Shmuter, G.M. and Studentsov, Y. (1997) “Membrane-based assay distinguishes Chagas’ and Leishmania antibodies”, American Soc. of Trop. Med. and Hygiene Annual Meeting, 1997. Abstract 222.
12. Levin, A., Nicoll, C., Rowell, S. and Studentsov, Zh. (1997) “Canine Lyme disease kit distinguishes vaccinated from infected dogs”, American Veterinary Medical Association Annual Meeting, Abstract.
13. Levin, A. and Shmuter, G. (1996) “Evaluation of Western Blot assays for detection of human antibodies to *Taenia solium* and *Echinococcus granulosus*”, American Soc. for Microbiology Annual Meeting, Abstract V-51.

Patents:
Application #13/657,394 Sensitive and Specific Assay for Babesia spp. (pending), filed 10/22/2012.
“Rapid peptidoglycan-based assay for detection of bacterial contamination of platelets”, U.S. Pat. No. 7,598,054, issued 10/06/09
"Rapid Flow-Through Binding Assay Apparatus and Method", U.S. Pat. No. 6,303,389, issued 10/16/01
"Systems and Methods for Rapid Blot Screening", U.S. Pat. No. 6,194,160 issued 2/27/01
"Modified Western Blot Membrane and Method for Detecting Lyme Disease and Other Tick-Borne Diseases", U.S. Pat. No. 6,013,460, issued 1/11/00
"Binding Assay Device with Removable Cassette and Manifold", U.S. Patent No. 5,100,626 issued 3/31/92
"Apparatus for Blot Screening Numerous, Small Volume, Antibody Solutions", U.S. Patent No. 4,834,946 issued 5/30/89
"Fluid Flow Manifold for Blot Type Screening Apparatus", U.S. Patent No. 4,978,507 issued 12/18/90
"Templet for Simultaneous Screening of Several Antibodies and Method of Using the Same", U.S. Patent No. 4,713,349 issued 12/15/87
"Protein Detection by Negative Staining", U.S. Patent No. 4,782,027 issued 11/1/88

D. Research Support:
Current (selected):
SBIR Phase II Contract No. HHSN268201200067C “Screening and Confirmatory Tests for Human Babesia”
Agency: National Heart, Lung and Blood Institute/NIH
Performance Period: 08/31/2012 – 08/30/2014 Role: PI
Summary: Clinical evaluation under IND of an immunoassay for detection of antibodies to Babesia microti for use in blood screening. Based on an ELISA developed with specific *B. microti* synthetic peptide antigens.

SBIR Phase I Grant No. 1R43AI100471 “Immunoassay for Diagnosis of Babesia Infection”
Agency: National Institute of Allergy and Infectious Diseases/NIH
Performance Period: 12/15/2012 – 11/30/2014 Role: PI
Summary: Identification of peptide antigens for development of an immunoassay for detection of antibodies to *Babesia microti* and other human pathogenic Babesia species for in vitro diagnostic use.
Completed (selected):
Grant No. AI078567 “Detection of Antibiotic Resistance Genes in Bacterial Agents of Hospital-Acquired Pneumonia and Sepsis”
Agency: National Institute of Allergy and Infectious Diseases/NIH
Performance Period: 05/15/2008 – 04/30/2010
Summary: Further development and application of the reverse line blot assay developed under AI66564 to detect antibiotic resistance genes in bacteria derived from whole blood or respiratory samples, from patients with sepsis or hospital-acquired pneumonia.

Grant No. U01 AI66564 “Reverse Line Blot Diagnostic Test for Agents of Sepsis”
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health
Performance Period: 07/01/05 – 06/30/08 Role: PI
Summary: Development of a Reverse Line Blot assay to detect and identify bacterial agents of sepsis directly from whole blood samples, based on amplification with universal primers and hybridization with species-specific probes.

Grant No. 1 R43 AI064988-01 (PI: Kovalenko) “Peptide-Based Serodiagnostic Test for Cysticercosis”
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health

Andrew E. Levin, Ph.D.

Performance Period: 4/01/05 – 7/31/08 Role: Coinvestigator
Summary: Development of a serologic test for cysticercosis based on new peptide antigens in an antibody capture ELISA format.

Grant No. AI59329-01 "Identification of SARS Antigens for Serodiagnosis"
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health
Performance Period: 4/01/04-3/31/07 Role: PI
Summary: Identification of peptide epitopes suitable for use in immunodiagnostic assays for SARS infection. Synthetic and recombinant sequences of viral proteins will be produced to define antigenic epitopes.

Grant No. HL65877 (Phase II) "Rapid Assay for Bacterial Contamination of Platelets"
Agency: National Heart, Lung and Blood Institute/National Institutes of Health
Performance Period: 10/01/02 – 8/31/10 Role: PI
Summary: Continuation of the Phase I project. Development of an assay based on peptidoglycan-binding proteins of invertebrate hemolymph for the detection of bacteria in platelet units. The peptidoglycan-binding assay offers high sensitivity and specificity in a simple, inexpensive format for blood bank or point-of-care use.

Contract No. [redacted]00713 "Anthrax Serology Test"
Agency: Centers for Disease Control, National Center for Infectious Diseases
Performance Period: 9/26/02 – 9/26/07 Role: PI
Summary: This contract covered the development and production of an ELISA assay for antibodies to anthrax Protective Antigen (PA). The ELISA assay was based on Immunetics' QuickELISA™ format. It was developed in kit form, validated, and received FDA 510(k) approval. The assay kits will be provided to U.S. government public health laboratories for use in the event of an anthrax attack.

Grant No. AI51926 (Phase I) "Single Step Peptide ELISA for Lyme Serodiagnosis"
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health
Performance Period: 3/15/02 – 3/14/04 Role: PI
Summary: This project was aimed at a clinical trial comparison of Immunetics' C6 peptide Lyme ELISA with the current CDC two-tier protocol for Lyme serodiagnosis. The peptide ELISA would replace the two-tier protocol with a simpler, more accurate and more cost-efficient alternative for Lyme disease testing.

Grant No. AI44541 (Phase I) "Neural Network for Lyme Western Blot Interpretation"
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health
Performance Period: 3/1/99-8/31/99 Role: PI
Summary: Immunetics developed the first neural network software program for automated interpretation of Lyme Western Blot band patterns. The neural network yielded results equal to or exceeding those of human interpreters in sensitivity and specificity. With this software program, Western Blots could be scanned and interpreted instantly by computer with objectivity, reproducibility and high accuracy.

Grant No. AI43123-03 (Phase I and Phase II) "Tick-Borne Disease Panel Test"
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health
Performance Period: 3/1/98 – 2/28/02 Role: PI
Summary: Development of a panel of tests for tick-borne pathogens including Lyme, Human Granulocytic Ehrlichiosis, and Human Babesiosis. Lyme, HGE and Babesia native and recombinant antigens were tested in ELISA, dot blot and Western Blot formats. Development resulted in the first ELISA assays for HGE and Babesia based on semi-purified native antigen lysates. The Babesia Western Blot was validated in a study published in Clinical and Diagnostic Laboratory Immunology (2001) 8(6):1177-1180.

Grant No. AI40849 (Phase II) "Rapid Confirmatory Lyme Disease Test"
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health
Performance Period: 9/15/97 – 2/29/00 Role: PI
Summary: Continuation of CDC contract 200-93-0666, development of a rapid immunoassay for Lyme serodiagnosis. A wide range of recombinant Lyme antigens were evaluated for sensitivity and specificity. The C6 peptide sequence within VlsE was chosen for the final version. The C6 peptide Lyme ELISA was patented, validated, received FDA 510(k) approval, and has been launched commercially by Immunetics.

Program Director/Principal Investigator (Last, First, Middle): Kovalenko, Victor A.

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Kovalenko, Victor A.	Principal Scientist, Immunetics, Inc. President, Diagnostic Innovations, LLC
eRA COMMONS USER NAME (credential, e.g., agency login)	

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Moscow State University	M.S.	1970	Biochemistry
Institute of Bioorganic Chemistry, Russian Academy of Sciences	Ph.D.	1983	Bioorganic Chemistry

A. Personal Statement

I have spent all my scientific career developing sensitive bioanalytical and diagnostic detection methods utilizing a broad spectrum of detection labels – radioactive, enzymes, colloidal gold and carbon, pigment colloids, latex particles, and fluorescent labels. As a group leader at the Laboratory of Biotechnology, Institute of Bioorganic Chemistry, Moscow, I have supervised development of analytical methods and diagnostic test systems in various formats: ELISA, dot-blot, dipstick, passive diffusion, latex agglutination, and immunochromatography. These methods were applied to the development of various research diagnostic tests - human immunodeficiency virus, leptospirosis, tick-borne encephalitis, Streptococcus group A rapid antigen and antibody tests, detection of various recombinant proteins, neuropeptides, free and bound biotin, detection of *E.coli* contamination in recombinant proteins. As a Principal Investigator of the grant from the Russian Medical Academy I have develop HIV diagnostic test utilizing peptide antigens

In 1986 I have founded the Department of Bioanalytical Reagents and Diagnostic Methods, at the Peptide Engineering Center for Innovative Research "PEPTOS" (Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow). The department conducted research and development in the area of bioanalytical methods, focusing on application of the biotin-streptavidin system for development of amplified detection systems, diagnostic test systems and broad spectrum of reagents such as streptavidin, biotin derivatives, detector enzymes, enzyme conjugates, including polymerized HRP, colloidal carbon and pigment conjugates, affinity sorbents, blocking reagents and enzyme substrates. Several innovative approaches were related to application of polymerized forms of enzymes for signal amplification and using carbon and pigment colloidal particles for development of sensitive and simple rapid tests.

In 1994 I was invited to work in the USA as a Senior Scientist at BINAX, Inc. (Portland, ME), the developer and manufacturer of the ELISA and rapid lateral flow tests. There I was responsible for development of diagnostic reagents and amplified assays. I have developed new amplified tests in several ELISA formats for detection of bacterial antigens, chlamydial lipopolyssaccharide, gonorrhea, Legionella, Streptococcus group A, Streptococcus group B, Salmonella, and *E.coli*. I have developed lateral flow tests for detection of *Chlamydia trachomatis* and whole blood lateral-flow tests for detection of panel of human cardiac markers. My work also was directed on development of a new approaches for reduction of non-specific reactions in immunological tests using chemical modification of antibodies and developed methods for stable adsorption of analytes on colloidal gold based on analysis of affinity of various chemical groups to colloidal particles.

In 1999 I have moved to work at Immunetics, Inc (Boston, MA), where I became Principal Scientist in Diagnostic Methods and Reagent Development, and later in Rapid Tests Development. Here I made critical contributions to creation of several new products and intellectual property. Three tests, either developed by me or utilizing my inventions, were commercialized and received FDA approval, including the first C6 peptide test for Lyme disease (2002), the serological Anthrax PA test (2004), and a test for bacterial contamination in platelet units utilizing colorimetric method for detection of bacterial peptidoglycans (2012). New assay format utilizing peptide

conjugates (QuickELISA) was used successfully in numerous grants and contracts for development of sensitive and specific ELISA tests, including antibodies against C6 and C10 Lyme diagnostic peptides, antibodies against SARS virus nucleocapside and spike proteins, H5 avian influenza, serological test for *Taenia solium* tapeworm cysticercosis and taeniasis antigens, as well as for application in rapid test formats for antibody detection (Lyme, Cysticercosis, Influenza, and Chagas disease, Human Immunodeficiency Virus).
In 2007 I have founded Diagnostic Innovations, LLC (Scarborough, ME), as President and Research Director. This company is focused on invention-based development of the sensitive colorimetric detection system for rapid diagnostic tests and bioanalytical applications which use peroxidative enzymes as a label. In the five years since its inception, the company has developed many unique reagents of the substrate system for application in a various membrane-based rapid tests. The company performed several feasibility studies for application of the new technology for detection of antigens, disease markers, antibodies, and bacterial cells, which demonstrated the big potential of the new technology for development of highly sensitive rapid tests in various formats
In summary, I have demonstrated a combination of experience covering all major aspect of assay development and of productive research and development work leading to commercial products and new technologies.

B. Positions and Employment

1970-1973 Research Scientist, Institute of Biological Physics, Biological Center of the Russian Academy of Sciences, Pushchino, Russia
1973-1976 Research Scientist, Joint Laboratory of Protein Chemistry, Institute of Protein Research and Schemyakin Institute of Bioorganic Chemistry, Moscow, Russia
1976-1994. Senior Research Scientist**,** Shemyakin Institute of Bioorganic Chemistry, Moscow, Russia (Laboratory of Protein Chemisrty and Laboratory of Biotechnology)
1986-1993 Founder and Director of the Department of Bioanalytical Reagents and Diagnostic Methods**,** Institute-based Peptide Engineering Center for Innovative Research "PEPTOS", Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
1993 Visiting Scientist, Weizmann Institute of Science, Laboratory of Prof. Meir Wilchek, Israel
1994-1999 Senior Research Scientist and Project Manager, Binax, Inc., Portland, ME.
1999-Present Principal Scientist, Diagnostic Methods and Reagent Development, Immunetics, Inc., Boston, MA.
2007-Present Scientific Director, Founder and President/Managing Partner, Diagnostic Innovations, LLC, Scarborough, ME

Author of more than 35 publications and 5 patents

Patents :

Victor A. Kovalenko. U.S. Patent No. US7,125,517B2 “System and methods for detection of analytes in biological fluids” Issued 10/24/06
Victor Kovalenko. U.S. Patent No. 7,262,019 B2 “System and methods for detection of *Bacillus anthracis* related analytes in biological fluids”, Issued 9/28/07
Victor Kovalenko, Andrew E. Levin, Lee Anne Beusang. U.S. Patent 7,589,054 B2 “Rapid peptidoglycan-based assay for detection of bacterial contamination of platelets” Issued 10/6/2009
Victor Kovalenko. U.S. Application 61/494/232 Color-Producing Diagnostic Systems, Reagents and Methods. Filed June 7, 2012

Selected peer-reviewed publications

Yeuk-Mui Lee, Sukwan Handali, Kathy Hancock, Sowmya Pattabhi, Victor A .Kovalenko, Andrew E. Levin, Silvia Rodriguez, Yesenia Castillo Berrios, John Noh, Maria Silva-Ibanez, Sehching Lin, Christina M.Scheel, Armando E. Gonzalez, Robert H. Gilman, Hector H. Garcia, and Victor C.W. Tsang Serological diagnosis of *Taenia solium* cysticercosis using recombinant and synthetic antigens in QuickELISA$^{TM.}$ Am. J. Trop. Med. Hyg. 84(4), 587-593, 2011
Sukwan Handali, Sowmya Pattabi, Yeuk-Mui Lee, Maria Silva-Ibanez, Victor A. Kovalenko, Andrew E.Levin, Armando E.Gonzalez, Jacquelin M. Roberts, Hector H.Garcia, Robert H.Gilman,Kathy Hancock, and Victor C.W.Tsang.(2010) Development and evaluation of porcine cysticercosis QUICKELISA^{TM} in Triturus EIA analyzer. J Immunoassay Immunochem, 31:60-70
Pavlova I.S., Lukin Yu.V., Kovalenko V.A., Avdeev D.N., Kulshin V.A., Zubov V.P. (1994) Non-instrumental immunoassay based on colored polyacrolein latex: application to group-specific polysaccharide of *Steptococcrus pyogenes*.. Bioorg Khim (Russ.), 20, 731-739.

Stratieva-Taneeva P.A., Khaidukov V.A., Kovalenko V.A., Nazimov I.V., Samokhvalova L.V., Nesmeyanov V.A. (1993) Bispecific monoclonal antibodies to human interleukin 2 and horseradish peroxidase. Hybridoma ,12, 271-284.
Kiseleva V.I., Kolesnik T.B., Turchinsky M.F., Wagner L.L., Kovalenko V.A., Plaksin D.Yu., Koukalova B., Kuhrova V., Brabec V., Poverenny A.M.(1993) Trans-Diaminedichlorplatinum(II)-modified probes for detection of picogram quantities of DNA. Anal. Biochem., 206, 43-49.
Pomelova V.G., Zavodchicova E.N., Lavrova N.A., Kovalenko V.A., Gaidamovich S.Ia. (1992) The effectiveness of lanthanide immunofluorescence and immunoenzyme analyses in differentiating viruses of the tick-borne encephalitis complex. Vopr. Virusol.(Russ.), 37, 244-247.
Golovnina T.A.,Kasheverov I.E.,Plaksin D.Yu.,Yakunina N.B., Kovalenko V.A., Utkin Yu.N., Tsetlin V.I. (1992) New enzyme immunoassay for detection of Supstance P receptors based on biotin-streptavidin system. Bioorg Khim (Russ.), 18, 932-941.
Zavodchikova E.N., Lavrova N.A., Kovalenko V.A. (1991) Using of biotin-streptavidin complex in immunoenzyme analysis of tick-borne encephalitis. Advances in Science and Technology. Suppl.Virology, Moscow, pp.89-99.
Petrenko A.G., Kovalenko V.A., Shamotienko O.G., Surkova I.V., Tarasyuk T.A., Uskaryov Yu.A., Grishin E.V. (1990) Isolation and properties of the alpha-latrotoxin receptor. EMBO J, 9, 2023-2027.
Petrenko A.G., Shamotienko O.G., Surkova I.N., Kovalenko V.A., Grishin E.V. S. (1990) Studies on the receptor for neurotoxin from black-widow spider venom. 1. Characterization of membrane-bound and solubilazed receptor from bovine brain. Bioorg Khim, 16, 149-157.
Petrenko A.G., Surkova I.V, Shamotienko O.G., Kovalenko V.A., Krasnoperov V.N., Tretiakov L.A., Ushkaryov Y.A., Grishin E.V. (1990). Studies on the receptor for neurotoxin from black-widow spider venom. 2. Isolation and properties of the receptor from bovine brain membrane., Bioorg Khim , 16, 158-165.Petrenko A.G., Kovalenko V.A.,Shamotienko O.G.,Surkova I.V, Tarasyuk T.A., Uskaryov Yu.A.,Ovchinnikov Yu.A. (1988).Studies on the receptor for neurotoxin from black-widow spider venom, Neurochemistry Int.,138, 159.Grishin. E.V., Kovalenko V.A., Pashkov V.N., Shamotiyenko O.G. (1984) Isolation and characteristics of sodium channel components. "Biologhicheskiye membrany" (Russ.), 1, 858-867.
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Kovalenko V.A., Pashkov V.N., Grishin E.V., Ovchinnikov Yu.A., Shevchenko V.P., Myasoedov N.F. (1982) Synthesis of biologically active tritium labeled tetrodotoxin derivative. Bioorg Khym (Russ.),8, 710-712.
Kovalenko V.A., Vulphius E.A. (1978) Molecular organization, properties and isolation of acetylcholine receptor. Advances in Science and Biotechnology. Suppl. Biophysics, Moscow, pp.8-96

Selected Abstracts

Oksana Penezina, David Clapham, Jillian Collins, Victor Kovalenko, Neil Krueger, Sunitha Matthew, Isaac R.Rodriguez-Chavez, Andrew Levin (2012). HIV Selectest: a new peptide-based enzyme immunoassay successfully distinguishes HIV vaccine-induced seropositivity from HIV infections in RV144, VAX003 and VAX004 vaccine trial participants. Poster, XIX International AIDS Conference, Washington, DC. 22-27-July 2012
Lee YM, Patthabhi S, Kovalenko VA, Handali S, Hancock K, Garcia HH, Gonzalez AE, Gilman RH, Tsang VCW. (2006) Use of rT24H QuickELISA assay in diagnosis of cysticercosis. (poster) ASTMH Atlanta,
Levin A., Beausang L., Kirby C, M. Syrkina, V. Kovalenko (2005) C6 ELISA Specificity vs. Two-Tier Testing Protocol, X International Conference on Lyme Borreliosis , Sept. 2005
Kirby C., L. Beausang, V. Kovalenko and A. Levin (2005) “BacTx-A Rapid Assay for Detection of Bacteria in Platelet Units” , American Association of Blood Bank, Nov. 2005
M. Septak, P. Wilkins, L. Chalcraft, K. Stamey, M. Syrkina, V. Kovalenko, C. Quinn and A. Levin, (2004). Clinical Validation of a Novel Anthrax ELISA for the Detection of Antibodies to Protective Antigen Abstract of American Society of Microbiology annual meeting, May 2004, Los Angeles
Levin, A., Condon, P. and Kovalenko, V.(2002) “C6 peptide ELISA for Lyme serodiagnosis: Equivalent to two-tier testing?”, IX International Conference on Lyme Borreliosis, New York, 2002. O-172.
Andrew Levin, Thomas Talaska and Victor Kovalenko (2002) “C6 Peptide ELISA: Universal assay for detection of European Lyme borreliosis ?”, IX International Conference on Lyme Borreliosis, New York, 2002. Abstract P-149.
Andrew E. Levin, Peter Condon and Victor Kovalenko, (2002) “Lyme Borreliosis Serodiagnosis by ELISA Based on the C6 Peptide of VlsE”, International Conference on Lyme Borreliosis, Belgium, 2001.
Fedyuk N.V., Konovalov E.E., Pokrovsky A.G.,Loktev V.B., Kovalenko V.A.(1992) Detection of the core HIV-1 antigen p24 by enzyme immunoassay (EIA) and immunofluorescence assay. Abstracts VII International conference on AIDS/II STD World Congress, Amsterdam, The Netherlands, 19-24 July, p.20.
Fedyuk N.V., Konovalov E.E., Pokrovsky A.G.,Loktev V.B., Kovalenko V.A., Plaksin D.Yu. (1992) Detection and quantitation of p24 antigen of human immunodeficiency virus. Abstracts: Symposium "Structure and function of regulatory polypeptides", Moscow, p.18.

Kovalenko V.A. et al. (1988) Sensitive test-system for determination of free biotin, molecules modified by biotin and biotin-binding proteins. Abstract. All-Union Conference "Modern trends in creating of medical diagnosticum", 13-14 December, Moscow, p. 51

D. Research Support

Current:

Grant No. 1R43AI091291-01. “Rapid point-of-care test for Lyme serodiagnosis based on novel highly-sensitive detection technology”.
Agency: National Institute of Allergy and Infectious Diseases/NIH
Performance period: 07/01/2010 – 07/01/2013 (extended)
Summary: Development of a rapid test for Lyme disease for point-of-care application utilizing peptide antigens and new highly sensitive detection technology with peroxidase label and multicolor colorimetric substrate system
Grant Number: 1 R43 AI098567-01A1
Project Title: Rapid Test for Recent HIV Infection and Incidence Panels for Assay Evaluation
Period 07/12/2012-07/12/2014
Role: Co-investigator
Institution: SERACARE LIFE SCIENCES, INC./Immunetics, Inc. Principal Investigator: Garrett, Patricia Ellen
Summary: Development of rapid lateral flow test based on new colorimetric detection technology for analysis of antibody titer and avidity as a test for discrimination resent HIV infection from past and new incidence sera panel for assay validation.

Completed:

Grant N0 1R43AI064988-01and 2R44AI064988-02 “ Peptide-Based Serological Test for Cysticercosis”
Performance period: 4/01/2005-7/31/08
Role: PI
Summary: Development of a serological test for Cysticercosis in a ELISA format and rapid non-instrumental version with analytical sensitivity of the microplate ELISA
Grant SG348
Title: Chromogenic system for rapid diagnostic tests
Agency: Maine Technology Institute
Performance period: 03/18/2008- 09/08/2008
Role: PI
Previous:
Grant No. AI59329-01 (PI Andrew Levin)
Title: Identification of SARS Antigens for Serodiagnosis
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health
Performance Period: 4/01/04-3/31/06
Role: Principal Scientist
Grant No. HL65877 (Phase II, PI Andrew Levin)
Title: Rapid Assay for Bacterial Contamination of Platelets
Agency: National Heart, Lung and Blood Institute/National Institutes of Health
Performance Period: 10/01/02 – 8/31/06
Role: Principal Scientist
Contract No. [redacted]-00713
Title: Anthrax Serology Test
Agency: Centers for Disease Control, National Center for Infectious Diseases
Performance Period: 9/26/02 – 9/26/04
Role: Principal Scientist
Grant No. AI51926 (Phase I, PI Andrew Levin)
Title: Single Step Peptide ELISA for Lyme Serodiagnosis
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health
Performance Period: 3/15/02 – 3/14/04
Role: Principal Scientist

1990-1996 Palladin Institute of Biochemistry , Molecular Immunology Department , Academy of Sciences of Ukraine, Kiev, Ukraine
Research Scientist

- Developed matrices to control separation and adhesion of antibodies and conducted a solid phase synthesis of immunovector molecules (immunotoxins, radiolabeled antibodies), evaluated conformational stability, immunological activity and in vitro toxicity of the conjugates. Conducted experiments on chemical modification of different polymer substrates and antibodies.
- Researched chemistry of blood/cultured cell surface molecules by flow cytometry to establish targets/improve diagnostic methods.

SELECTED PATENTS

1. US Patent N 7628917, 2009. Porous Composite Membrane and Method for Making the Same". Penezina OP, Pacheco M, Tsou D.
2. USSR Patent N 1588137. New method for the synthesis of labeled antibodies. S.V. Komissarenko, G.N. Fomovskaya, O.P. Levchuk (**O.P. Penezina**), et.al. – 1988.

SELECTED PUBLICATIONS

1. **Penezina O**., Clapham D., Collins J., et al. "New HIV peptide-based immunoassay resolves vaccine-induced seropositivity in HIV vaccine (phase III) trial participants". Retrovirology 2012, 9(Suppl 2):P120
2. **Penezina O**, Komissarenko S, Tishenco L, et al. Revealing some oncofetal antigens in peripheral blood mononuclear cells of donors and patients with B-chronic lymphocytic leukemia. LEUKEMIA RES 22: (11) 1009-1013 NOV 1998
3. Fomovskaya GN, **Penezina OP**, Goroshnikova TV, et al. Expression of some oncofetal antigens in peripheral mononuclear-cells of human and monkey exposed to low-doses of ionizing-radiation. EKSP ONKOL 16: (2-3) [redacted]
4. Davenport R, Haddock T, **Penezina O**, et al. L-selectin expression on peripheral blood leukocytes over 48 hr. in stored blood. TRANSFUSION 38: (10) 17S, OCT 1998.
5. **Penezina OP**, Fomovskaya GN, Haddock TF, et al. Changes in the dextran purified human blood leukocyte cell subsets content after filtration via polyester filters coated with immobilized Chondroitin Sulfate A. TRANSFUSION 39: (10) 41S-41S Suppl. S OCT 1999
6. **Penezina OP**, Fomovskaia GN, Haddock TF, et al. Specific adhesion of human leukocytes to magnetic polystyrene beads, modified with Ca2+ independent ligands to L-selectin. FASEB J 11: (9) A1093 Suppl. S JUL 31 1997
7. Davenport RD, **Penezina OP**. Cleavage of high molecular weight kininogen induced by filtration of platelet concentrates. TRANSFUSION 37: (9) S416-S416 Suppl. S SEP 1997
8. **Penezina OP**. Fomovskaia GN. Haddock TF. Davenport RD. Partial dependence of human peripheral blood leukocyte binding to HMW fucoidan on divalent cations. UBZ,71(6):56-61, 1999
9. **Penezina OP**, Fomovskaya GN, Haddock TF, et al. Specific adhesion to immobilized chondroitin sulfate A varies for different cell subsets of dextran purified human blood leukocytes. MOL BIOL CELL 9: 56A NOV 1998
10. **Oksana Penezina**, Howard Neal, Navin Pathirana. "Proving the Suitability of Whatman Puradisc Syringe Filters for HPLC Sample Preparation Applications". LCGC Europe, The Applications Book, July 2006, page 82.

SELECTED POSTER PRESENTATIONS.

1. Penezina O., Clapham D., Collins J., et al. "New HIV peptide-based immunoassay resolves vaccine-induced seropositivity in HIV vaccine (phase III) trial participants". AIDS Vaccine 2012, September 2012, Boston, MA
2. Penezina O., Clapham D., Collins J., et al. "HIV Selectest: a new peptide-based enzyme immunoassay successfully distinguishes HIV vaccine-induced seropositivity from HIV infection in RV144, VAX003 and VAX004 vaccine trial participants". XIX International AIDS Conference (AIDS 2012), July, 2012, Washington, D.C.
3. Penezina O., Jose M. "Whatman nitrocellulose filterplates for chemiluminescent ELISA offer time, sensitivity and reagent-saving advantages over commonly used plastic 96-well microtiter plates", 13th SBS, April, 2007, Montreal, Canada.
4. Jose M, Penezina O. "Optimization of the Flow-through chemiluminescence ELISA protocol for Whatman Protran chemiluminescence ELISA filterplate", 13th SBS, April, 2007, Montreal, Canada.
5. Penezina O., Neal H. "Whatman FTA Cards, designed for collection, transport, archiving and isolation of nucleic acids at room temperature, inactivate human immunodeficiency virus type 1(HIV-1) and BVDV(HCV model) virus." 14th ISHEID, June, 2006, Toulon, France.
6. Penezina O., Neal H. "Whatman FTA Cards, designed for collection, transport, archiving and isolation of nucleic acids at room temperature, inactivate human immunodeficiency virus type 1(HIV-1)", 3rd IAS Conference on HIV Pathogenesis and Treatment, July, 2005, Rio de Janeiro, Brazil.
7. Penezina O., Harvey M. "Evaluation of Human Immunodeficiency Virus Type I(HIV-1) Inactivation by Whatman FTA Elute Microcards", XVII International AIDS Conference, August, 2008, Mexico City, Mexico.
8. Penezina O., Jose M., Harvey M. "FTA Elute Cards as Collection Devices for Blood, Urine and Saliva." PITTCON**,** March, 2008, New Orleans, USA.
9. Penezina O., et. al. "Whatman FTA cards designed for collection, transport, archiving and isolation of nucleic acids at room temperature, preserve poliovirus type-2 viral RNA for up to 30 days at room temperature". LabAutomation2007, Palm Springs, CA, January, 2007.

SELECTED ORAL PRESENTATIONS.

1. Oksana Penezina, David Clapham, Kristen Hennessy, David Crane, Victor Kovalenko, Neil Krueger, Isaac R. Rodriguez-Chavez, Michael P. Busch and Andrew E. Levin "New HIV peptide-based immunoassay resolves vaccine induced seropositivity in HIV vaccine RV144 and HVTN204 trials", 2012 CDC HIV Diagnostics Conference December 12-14, 2012, Atlanta, GA.
2. "New hydrophilization method of hydrophobic porous media", ICOM2005 (International Congress on Membranes and Membrane Processes), Seoul, Korea, August, 2005.
3. "New hydrophilization method for PVDF membrane", NAMS 2004, Honolulu, HI, June, 2004.
4. "The development of 95-well filterplate for DNA Sequencing reaction clean up", NAMS 2001, Lexington, KY, May, 2001.
5. "The development of 96-well ultrafiltration filterplates for genomics and proteomics applications." The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, New Orleans, LA, March, 2002: (PITTCON'2002)
6. "New Integrated Sample Preparation System for HPLC", NAMS 2005 (North American Membrane Society Annual Meeting), Providence RI, June, 2005.

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: [redacted]

Delete Entry **Start Date:** 07/01/2014 **End Date:** 06/30/2015 **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.	[redacted]		[redacted]		[redacted]	[redacted]	7.20			[redacted]	[redacted]	[redacted]
2.	Dr.	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]	0.60			[redacted]	[redacted]	[redacted]
3.	Dr.	[redacted]	[redacted]	[redacted]		[redacted]	[redacted]	7.20			[redacted]	[redacted]	[redacted]
4.	Dr.	[redacted]		[redacted]		[redacted]	[redacted]	1.20			[redacted]	[redacted]	[redacted]
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	Research Assoc	6.00			[redacted]	[redacted]	[redacted]
1	Research Assoc	6.60			[redacted]	[redacted]	[redacted]
1	QA Associate	1.20			[redacted]	[redacted]	[redacted]
1	MFG Associate	1.80			[redacted]	[redacted]	[redacted]
1	Clinicial Trial Director	1.20			[redacted]	[redacted]	[redacted]
5	**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: [redacted]

Delete Entry Start Date: 07/01/2014 End Date: 06/30/2015 Budget Period 1

C. Equipment Description

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

	Equipment item	Funds Requested ($)
1.		
2.		
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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: [redacted]

Delete Entry Start Date: 07/01/2014 End Date: 06/30/2015 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	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. [redacted]	[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.	Modified Total Direct Cost [redacted]	[redacted]	[redacted]	[redacted]
2.	Modified Total Direct Cost- [redacted]	[redacted]	[redacted]	[redacted]
3.	Modified Total Direct Cost- [redacted]	[redacted]	[redacted]	[redacted]
4.	Modified Total Direct Cost [redacted]	[redacted]	[redacted]	[redacted]
	Total Indirect Costs			[redacted]

Cognizant Federal Agency

(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 [redacted] 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: [redacted]

Delete Entry **Start Date:** 07/01/2015 **End Date:** 06/30/2016 **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.	[redacted]		[redacted]		PD/PI	[redacted]	7.20			[redacted]	[redacted]	[redacted]
2.	Dr.	[redacted]	[redacted]	[redacted]		Scientific Director	[redacted]	0.60			[redacted]	[redacted]	[redacted]
3.	Dr.	[redacted]		[redacted]		Senior Scientist	[redacted]	7.20			[redacted]	[redacted]	[redacted]
4.	Dr.	[redacted]		[redacted]		Project Leader	[redacted]	1.20			[redacted]	[redacted]	[redacted]
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	Research Associate	6.00			[redacted]	[redacted]	[redacted]
1	Research Associate	6.60			[redacted]	[redacted]	[redacted]
1	QA Associate	1.20			[redacted]	[redacted]	[redacted]
1	MFG Associate	1.80			[redacted]	[redacted]	[redacted]
1	Clincial Trial Director	0.60			[redacted]	[redacted]	[redacted]
5	**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: [redacted]

Delete Entry | Start Date: 07/01/2015 | End Date: 06/30/2016 | Budget Period 2

C. Equipment Description

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

	Equipment item	Funds Requested ($)
1.		
2.		
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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: [redacted]

Delete Entry Start Date: 07/01/2015 End Date: 06/30/2016 Budget Period 2

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. [redacted]	[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.	Modified Total Direct Costs- [redacted]	[redacted]	[redacted]	[redacted]
2.	Modified Total Direct Costs- [redacted]	[redacted]	[redacted]	[redacted]
3.	Modified Total Direct Costs- [redacted]	[redacted]	[redacted]	[redacted]
4.	Modified Total Direct Cost- [redacted]	[redacted]	[redacted]	[redacted]
	Total Indirect Costs			[redacted]

Cognizant Federal Agency

(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 [redacted] 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 3

ORGANIZATIONAL DUNS: [redacted]

Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry **Start Date:** 07/01/2016 **End Date:** 06/30/2017 **Budget Period** 3

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.	[redacted]	[redacted]		[redacted]		PD/PI	[redacted]	7.20			[redacted]	[redacted]	[redacted]
2.	[redacted]	[redacted]	[redacted]	[redacted]		Scientific Director	[redacted]	0.60			[redacted]	[redacted]	[redacted]
3.	[redacted]	[redacted]		[redacted]		Sr. Scientist	[redacted]	7.20			[redacted]	[redacted]	[redacted]
4.	[redacted]	[redacted]		[redacted]		Project Leader	[redacted]	1.20			[redacted]	[redacted]	[redacted]
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	Research Associate	6.00			[redacted]	[redacted]	[redacted]
1	Research Associate	6.60			[redacted]	[redacted]	[redacted]
1	QA Associate	1.20			[redacted]	[redacted]	[redacted]
1	MFG Associate	1.80			[redacted]	[redacted]	[redacted]
4	**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 3

ORGANIZATIONAL DUNS: [redacted]

Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry Start Date: 07/01/2016 End Date: 06/30/2017 Budget Period 3

C. Equipment Description

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

	Equipment item	Funds Requested ($)
1.		
2.		
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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 3

Next Period

ORGANIZATIONAL DUNS: [redacted]

Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: [redacted]

Delete Entry Start Date: 07/01/2016 End Date: 06/30/2017 Budget Period 3

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. [redacted]	[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.	Modified Total Direct Costs-[redacted]	[redacted]	[redacted]	[redacted]
2.	Modified Total Direct Costs-[redacted]	[redacted]	[redacted]	[redacted]
3.	Modified Total Direct Cost-[redacted]	[redacted]	[redacted]	[redacted]
4.	Modified Total Direct Cost-[redacted]	[redacted]	[redacted]	[redacted]
	Total Indirect Costs			[redacted]

Cognizant Federal Agency

(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 [redacted] Add Attachment Delete Attachment View Attachment

(Only attach one file.)

BUDGET JUSTIFICATION – IMMUNETICS

Patricia Garrett, Ph.D. (Principal Investigator, Immunetics), 60% effort (7.2 cm)
Dr. Garrett has 25 years of experience in HIV diagnostics and has worked extensively in HIV incidence, in particular. She is the originator of the concept for the Phase I grant in progress, and was the PI until a management change at SeraCare resulted in her transition to a consultant role there earlier this year. She is skilled in project design and management, has been principal investigator or co-principal investigator on four other grants and contracts, and has published and presented widely on topics related to infectious disease diagnostics, method evaluation, and quality control. She has worked with this project team for more than a year, fostered good working relationships among team members and excellent progress on the grant work, and will be responsible for all aspects of the project.

Andrew E. Levin, Ph.D. (Scientific Director, Immunetics), 5% effort (0.6 cm)
Dr. Levin will provide overall coordination and guidance for the work to be carried out at Immunetics. He has extensive experience in diagnostic assay development and has served as PI on a number of previous infectious disease assay projects. Dr. Levin will coordinate work with Diagnostic Innovations, Diagnostic Consulting Network and with clinical collaborators at CDC, MHRP, and BSRI as needed with respect to acquisition and serological testing of human serum samples. He will analyze assay data for performance of the rapid test with respect to sensitivity, specificity, and other parameters, which is a prerequisite to further development by Dr. Kovalenko and partner company Diagnostic Innovations. Dr. Levin will be responsible for the Immunetics component in reports to NIH, in scientific publications, and in presentations at other venues.

Victor A. Kovalenko, PhD (Senior Scientist), 60% effort (7.2 cm)
Dr. Kovalenko as the inventor of the new rapid test technology which is the subject of this application will provide overall scientific leadership for the project. With his extensive experience in numerous areas of analytical biochemistry, bioorganic chemistry, diagnostic reagent and infectious disease assay development, he has served as principal scientist on a number of grants and as PI on a recent immunodiagnostics grant. He will assist in generating scientific reports for publications or presentation.

[redacted], Ph.D. (Project Leader), 10% effort (1.2 cm)
Dr. [redacted] will be the primary scientist responsible for carrying out laboratory evaluations of the HIV rapid test at Immunetics on serum samples acquired from collaborators and internally, determining quantitative performance characteristics, investigating and analyzing deviations from performance targets, running ELISA and Western Blot assays to characterize samples and for comparison with the rapid test, analyzing comparative assay performance qualitatively and quantitatively, and overseeing documentation of laboratory work and compliance with Quality Systems Regulations (GMP). Dr. [redacted] has over 15 years of experience in development of in vitro diagnostic products in both public and private sector organizations, including 5 years focused on membrane assay development and validation, directly relevant to the present project.

Quality Assurance Associate, 10% effort (1.2 cm)
A QA Associate at Immunetics will provide about 4 hours of effort per week to ensure that the project is documented in accordance with Immunetics Quality System and Design Control requirements, and to audit laboratory and clinical records as needed.

[redacted] Research Associate, 50% effort (6.0 cm)
The Research Associate will be responsible for carrying out reagent syntheses for assay development, evaluations of the HIV rapid test at Immunetics on serum samples acquired from CDC, MHRP and BSRI or internally, determining qualitative and quantitative performance characteristics, investigating and analyzing deviations from performance targets, running ELISA and Western Blot assays to characterize samples and for comparison with the rapid test, documentation of laboratory work and compliance with Quality Systems Regulations (GMP).

[redacted] Research Associate, 55% effort (6.6cm)
A Research Associate at Immunetics will support the Senior Scientist (Dr. [redacted] with about 20 hours of effort per week in year 1, during which most of the assay development work will be carried out requiring intensive testing of serum samples under various experimental conditions, both on rapid test prototypes and on other incidence ELISAs, conventional ELISAs and Western Blot assays. The Research Associate will perform assays, document and interpret results, and provide general laboratory assistance.

[redacted] MT (ASCP), MPH (Director, Clinical Affairs), 1.2 cm
[redacted] is Immunetics' Director of Clinical Affairs and is responsible for all aspects of design, monitoring, and compliance for clinical studies. Prior to joining Immunetics, [redacted] was Director of Clinical Research at [redacted] Corporation overseeing all activities related to clinical research studies. She has over 20 years of experience in clinical affairs management, including regulatory affairs and quality assurance. Her experience includes medical device, pharmaceutical, and academic arenas in the therapeutic areas of diabetes, anesthesiology, oncology and dermatology.

Consultants

[redacted] - Statistical Analyst
There are no funds requested for the first year.
Total Year 1 Direct Cost: [redacted]

Consortium

Diagnostic Innovations (DI). [redacted] will maintain a 20% effort at Diagnostic Innovations, coordinating project development activities performed at this facility. Diagnostic Innovations will concentrate on developing conjugates and the enzyme label detection system, substrate and test strip components which are the key elements of the HIV rapid test. Tasks will specifically include synthesis of colorimetric detection system reagents and peptide conjugates, development of stabilization chemistry for all diagnostic strip components, assay format optimization and diagnostic cassette design. Following completion of an optimized prototype rapid test, DI will provide formulations, protocols and documentation for manufacture of bulk reagents and test strip manufacture.
Total Year 1 Direct Cost: $[redacted]

[redacted] will oversee design and engineering of the proposed assay. [redacted] will perform manufacturing procedures (impregnation, dispensing, drying, lamination, cutting, assembly) of the diagnostic cassette components, including the test strip, dried reagent pad, and flow cell housing. Quality control of HIV rapid test kits will be performed at the component level. Further details are described in the Consortium Arrangements section.
Total Year 1 Direct Cost: $[redacted]

Blood Systems Research Institute.
BSRI as the research division of Blood Systems, Inc. has been a leader in investigations of new blood screening technologies and the development of new regulatory and industry policies for blood screening and donor management. The work at BSRI will be overseen by [redacted] is an expert on HIV incidence testing and has an extensive history of involvement in major studies of every pathogen affecting blood safety over the past several decades. The study of HIV incidence involving comparison of whole blood vs plasma proposed in this project will be managed [redacted]
Total Year 1 Direct Cost: $[redacted]

Other:

[redacted]

[redacted]

Controls from SeraCare.
SeraCare will provide HIV serum or plasma controls for use with the HIV rapid incidence test. [redacted]

Shipping
Reimbursement is requested for shipping costs for shipping of samples and rapid test kits ([redacted]

Total Year 1 Cost: $[redacted]

Equipment
None.
Total Year 1 Cost: $[redacted]

Travel
Travel for Principal Investigator and one scientist to one national meeting (e.g., American Society of Microbiology, CDC Emerging Infectious Diseases) per year is requested [redacted]
Total Year 1 Cost: $[redacted]

Fringe
Fringe benefits are calculated at the rate of [redacted] as approved by NIH DFAS.
Total Year 1 Cost: $[redacted]

Indirect Cost (F&A)
Indirect costs are charged at Immunetics' DFAS-approved F&A rate of [redacted].
Total Year 1 Cost: $[redacted]

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		
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		

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **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.	[redacted]	[redacted]	[redacted]	MD	PD/PI	[redacted]	0.60			[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** [redacted]

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	Staff Scientist II		0.60			[redacted]	[redacted]	[redacted]
1	Research Associate II		1.50			[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: GRANT11483849

OMB Number: 4040-0001
Expiration Date: 04/30/2008

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **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: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **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	
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	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency DHHS, DCA Western Field Office, Arif Karim, 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-BSRI_Budget_Justification.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:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2015 *** End Date:** 06-30-2016 **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.		[redacted]	[redacted]	[redacted]	MD	PD/PI	[redacted]	0.60			[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** [redacted]

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	Staff Scientist II	0.60			[redacted]	[redacted]	[redacted]
1	Research Associate II	1.50			[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: GRANT11483849

OMB Number: 4040-0001
Expiration Date: 04/30/2008

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2015 *** End Date:** 06-30-2016 **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: [redacted]

*** Start Date:** 07-01-2015 *** End Date:** 06-30-2016 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)**
Total Other Direct Costs	

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	**27,894.00**

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. MTDC	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency DHHS, DCA Western Field Office, Arif Karim, [redacted]

(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: [redacted] Mime Type: application/pdf

(Only attach one file.)

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2016 *** End Date:** 06-30-2017 **Budget Period: 3**

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.	[redacted]	[redacted]	[redacted]	MD	PD/PI	[redacted]	0.60			[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** [redacted]

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

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

Tracking Number: GRANT11483849

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2016 *** End Date:** 06-30-2017 **Budget Period: 3**

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 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2016 *** End Date:** 06-30-2017 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)
Total Other Direct Costs	

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	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency DHHS, DCA Western Field Office, Arif Karim, [redacted]

(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-BSRI_Budget_Justification.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		
[redacted]		
[redacted]		[redacted]
[redacted]	[redacted]	
[redacted]		
[redacted]		[redacted]
[redacted]		[redacted]
[redacted]		[redacted]
[redacted]		

OMB Number: 4040-0001
Expiration Date: 04/30/2008

Budget Justification
[redacted]

Personnel
[redacted] Consortium Principal Investigator for the project will oversee the work performed at [redacted] will provide intellectual input to the project both in the technical performance of the work and the interpretation of the results. He will attend the management and advisory meetings, as necessary. [redacted] will contribute 0.6 calendar months to the project each year.

[redacted] Staff Scientist II and Manager of the Core Immunology Laboratory (CIL) at [redacted] will provide scientific support and guidance to the research associate performing the work in the CIL, will assist in the preparation of standard SOPs for the work, and analyze results. Dr. [redacted] will provide 0.6 calendar months effort during Years 1 and 2.

[redacted], Research Associate II, has 13 years' experience performing various incidence assays. She will be responsible for receiving, subaliquoting, cataloging (using a Freezerworks specimen management program/system) all samples that will be tested and will perform incidence assays at [redacted] as directed by the study group. [redacted] will devote 0.15 calendar months to the project during Years 1 and 2.

Fringe benefits are calculated at our federally-negotiated rate of [redacted]

Materials and Supplies
During Year 1, $[redacted] has been budgeted for the purchase of disposables, including gloves and tips.

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **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.	[redacted]		[redacted]	PhD	Other Investigator	[redacted]	0.60			[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** [redacted]

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	Scientist	1.20			[redacted]	[redacted]	[redacted]
1	Associate Scientist	1.20			[redacted]	[redacted]	[redacted]
1	Laboratory Assistant	1.20			[redacted]	[redacted]	[redacted]
1	Design Engineer	1.20			[redacted]	[redacted]	[redacted]
4	**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: GRANT11483849

OMB Number: 4040-0001
Expiration Date: 04/30/2008

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **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)	[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 - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **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	
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. Facilities, Overhead	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency None

(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: [redacted] 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:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2015 *** End Date:** 06-30-2016 **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.	[redacted]		[redacted]	PhD	Other Investigator	[redacted]	0.60			[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** [redacted]

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	Scientist	1.20			[redacted]	[redacted]	[redacted]
1	Research Associate	1.20			[redacted]	[redacted]	[redacted]
1	Research Assistant	1.20			[redacted]	[redacted]	[redacted]
1	Design Engineer	1.20			[redacted]	[redacted]	[redacted]
4	**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 2

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2015 *** End Date:** 06-30-2016 **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)	[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 - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2015 *** End Date:** 06-30-2016 **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	
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. Facilities, Overhead	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency None

(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: [redacted] Mime Type: application/pdf

(Only attach one file.)

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2016 *** End Date:** 06-30-2017 **Budget Period: 3**

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.	[redacted]		[redacted]	PhD	Other Investigator	[redacted]	0.60			[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** [redacted]

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	Scientist	1.20			[redacted]	[redacted]	[redacted]
1	Research Associate	1.20			[redacted]	[redacted]	[redacted]
1	Research Assistant	1.20			[redacted]	[redacted]	[redacted]
1	Design Engineer	1.20			[redacted]	[redacted]	[redacted]
4	**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: GRANT11483849

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2016 *** End Date:** 06-30-2017 **Budget Period: 3**

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)	[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 - SECTIONS F-K, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2016 *** End Date:** 06-30-2017 **Budget Period: 3**

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	
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. Facilities, Overhead	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency None

(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: [redacted] 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	12
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	
Section D, Travel	[redacted]
1. Domestic	[redacted]
2. Foreign	
[redacted]	
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

BUDGET JUSTIFICATION-[redacted]

Personnel

[redacted] will serve as the Principal Investigator at [redacted] (3 calendar years, 5% effort). His responsibilities include study design and supervision of research activities, review and interpretation of data, communications with Immunetics.

Scientist TBD, will serve as (3 calendar years, 10% effort).
Oversight, planning and management of the program at [redacted] liaison with Immunetics.

Assoc. Scientist TBD, will serve as (3 calendar years, 10% effort).
Translation of documents provided to [redacted] into [redacted] spefications and SOPs. Validation of processes and equipment, technical troubleshooting, production of parts for evaluation at Immunetics.

Lab Technician TBD, will serve (3 calendar years, 10% effort) to carry out the laboratory work of the research project as per protocol under the guidance of the Principal Investigator.

Design Engineer TBD will serve (3 calendar years, 10% effort)
Design and development of the cartridge for the Immunetics device, production of rapid prototype parts, design and implementation of the prototype mold.

Supplies
Supplies to be purchased in year 1 will include: assay specific material, stereo-lithographic plastic parts, plastic parts for prototype mold, miscellaneous lab and computer supplies and services and shipping

Total Year 1 Cost: $[redacted]

Travel
Support for a trip [redacted] to allow the two collaborating companies to discuss progress in face-to-face meeting per year is requested (travel plus 1 night lodging plus per diem)
Total Year 1 Cost: $[redacted]

Fringe
[redacted] fringe rate approved by DFAS is [redacted]
Indirect Cost (F&A)
[redacted] F&A rate approved by DFAS is [redacted]

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **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.	[redacted]	[redacted]	[redacted]		PD/PI	[redacted]	4.20			[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** [redacted]

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	[redacted]	7.20			[redacted]	[redacted]	[redacted]
1	[redacted]	7.20			[redacted]	[redacted]	[redacted]
1	[redacted]	9.60			[redacted]	[redacted]	[redacted]
3	**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: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **Budget Period: 1**

C. Equipment Description

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

Equipment Item	* Funds Requested ($)
1. Virtis Advantage Plus Freeze Dryer XL,	[redacted]
2. Bio-Dot	[redacted]

Total funds requested for all equipment listed in the attached file

Total Equipment [redacted]

Additional Equipment: File Name: Mime Type:

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 - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2014 *** End Date:** 06-30-2015 **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	
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. Total Direct Cost	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency

(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: [redacted] 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:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2015 *** End Date:** 06-30-2016 **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.	[redacted]	[redacted]	[redacted]		PD/PI	[redacted]	4.20			[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** [redacted]

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	[redacted]	7.20			[redacted]	[redacted]	[redacted]
1	[redacted]	7.20			[redacted]	[redacted]	[redacted]
1	[redacted]	9.60			[redacted]	[redacted]	[redacted]
3	**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: GRANT11483849

OMB Number: 4040-0001
Expiration Date: 04/30/2008

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

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2015 *** End Date:** 06-30-2016 **Budget Period: 2**

C. Equipment Description

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

Equipment Item	* Funds Requested ($)
1. spectrophotometer (Shimadzu UV1800)	[redacted]

Total funds requested for all equipment listed in the attached file

Total Equipment [redacted]

Additional Equipment: File Name: Mime Type:

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 - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2015 *** End Date:** 06-30-2016 **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	
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. Total Direct Cost	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency

(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: [redacted] Mime Type: application/pdf

(Only attach one file.)

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

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2016 *** End Date:** 06-30-2017 **Budget Period: 3**

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.	[redacted]	[redacted]	[redacted]		PD/PI	[redacted]	4.20			[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** [redacted]

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	[redacted]		7.20			[redacted]	[redacted]	[redacted]
1	[redacted]		7.20			[redacted]	[redacted]	[redacted]
1	[redacted]		9.60			[redacted]	[redacted]	[redacted]
3	**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: GRANT11483849

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2016 *** End Date:** 06-30-2017 **Budget Period: 3**

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)	[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 - SECTIONS F-K, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

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

Enter name of Organization: [redacted]

*** Start Date:** 07-01-2016 *** End Date:** 06-30-2017 **Budget Period: 3**

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	
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. Total Direct Cost	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency

(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: [redacted] 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	9	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		[redacted]
Section D, Travel		[redacted]
1. Domestic	[redacted]	
2. Foreign		
[redacted]		
[redacted]		
[redacted]		
[redacted]		
[redacted]		
[redacted]		
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[redacted]		[redacted]
[redacted]		

HIV Incidence rapid test

BUDGET JUSTIFICATION – [redacted]

[redacted] **(Scientific Director, P.I.), 35% effort**
Dr. [redacted] as a inventor of a new technology and assay formats will provide overall scientific leadership (supervision) of project, coordination and guidance for the project He will write scientific reports for publications or presentations

[redacted] **(Senior Scientist) , 60% effort**
Dr. [redacted] as a specialist in protein biochemistry will be responsible for synthesis of particles and polymers with hydrogen donors and peptides, development of strategy of stabilization of conjugates, components of substrate system, supervise stability study of the reagents, develop QC methods for reagents

[redacted] **(Scientist), 60% effort**
will be responsible for the development and optimization of diagnostic strips, integration plasma separation elements for whole blood testing, reagents stabilities studies, writing documentation

[redacted] **(Associate Scientist) 80% effort**
Will assist [redacted] [redacted] in development of reagents, stability study, optimization of components of diagnostic strips, analysis of various biological matrixes and materials for lateral flow tests

Supplies

Supplies to be purchased will include : various chemical reagents, cross-linking reagents, blocking reagents, stabilization products, biochemicals, materials for rapid test development (membranes, adsorbents, lamination films, cards, vacuum sealer bags, miscellaneous lab supplies (plastic ware, tubes, filters, desiccants, etc.)
Total three years cost: $[redacted]

Equipment

Equipment to be purchased will include benchtop freeze dryer with shelf system and stoppering (Virtis Advantage Plus Freeze Dryer XL, Cost ~$18,000), upgrade for spectrophotometer (Schimadzu UV1800 as a replacement of current UV1240, additional cost ~ $8,000) and rotary cutter for membranes (Bio-Dot, ~ $16,000) New freeze dryer need for manufacture of key reagents that require more precise control of drying conditions and stoppering under vacuum. Currently use freeze drier model do have this functions. Spectrophotometer is important equipment in reagent synthesis and analysis. New model should significantly reduce time for performing spectrophotometric data analysis in comparison with current UVmini1240, which do not have good data managing software. Rotary cutter will be used instead current

guillotine-type cutter to attain more precise strip cutting with minimal contamination of the membrane edges with adhesive

Total cost: $ [redacted]

Travel

[redacted]

Total 3 Years Cost: $ [redacted]

Indirect Cost (F&A)
F&A rate is 30%.
Total for three years : $ [redacted]

SBIR/STTR Information

OMB Number: 4040-0001
Expiration Date: 6/30/2016

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. 35
☐ 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: [redacted] 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 ☐ 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. * Attach File: [redacted] Add Attachment Delete Attachment View Attachment
☒ Yes ☐ 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 ☐ No	* 10. Please indicate whether the answer to BOTH of the following questions is TRUE: (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 (2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?
☐ Yes ☐ 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?

RAPID HIV INCIDENCE TEST COMMERCIALIZATION PLAN

Value of the SBIR Project, Expected Outcomes, and Impact

Overview.
Assessment of the state of the HIV epidemic for epidemiologic purposes and targeting intervention efforts to populations exhibiting high rates of HIV transmission are wholly dependent on determining the frequency of new infections. However, very few HIV assays have been developed specifically to distinguish incidence from prevalence. Most HIV serologic assays are aimed at diagnostic use, while RNA assays have been used largely to determine viral load for clinical management purposes. The general lack of tools for this purpose reflects the greater focus on diagnostic applications by assay developers. We aim to serve a major public health need through the development of a new test capable of rapidly identifying new HIV infections.
Secondly, determining the frequency of new HIV infections has required access to well-equipped centralized laboratories capable of running the few sophisticated assays available for this purpose; these have been ELISAs requiring microplate handling and reading instrumentation, including the BED ELISA and the Vironostika detuned ELISA. Dependence on a central laboratory also implies the requirement for a system to

[redacted]

. Immune complexes comprising antibodies and peroxidase conjugates migrate through membrane pores in lateral flow tests without formation of aggregation/agglutination migratory artifacts which are known to be a problem in lateral flow tests using large detector conjugates.

Antibody detection format. The proprietary QuickELISA™ format is based on using peptide conjugates for capture and labeling of specific antibodies without participation of secondary antibody conjugate binding reagents. This format allows for testing of antibodies in undiluted samples and detects all classes of antigen-specific antibodies. The higher sensitivity and specificity of this technology have been demonstrated by Immunetics in several past products including the FDA-approved Anthrax PA QuickELISA™ test and a Lyme ELISA test marketed in Europe.

Novel peptide antigens for improved sensitivity. The IDR-M antigen recently developed by Dr. Parekh at CDC overcomes the shortcomings of previous antigens (e.g. the BED antigen) in detection of multiple HIV subtypes, an essential requirement for a worldwide assay. We have transformed IDR-M into its constituent peptides for enhanced performance in the HIV rapid incidence test, allowing us to take full advantage of the QuickELISA methodology (above) in a rapid assay format.

Expected outcomes.
At the conclusion of the proposed Phase II effort, we will have completed optimization of an HIV rapid test capable of discriminating incidence from prevalence in serum or plasma within a 15 minute turnaround time. Working together with [redacted], a highly experienced manufacturing partner for rapid tests, we will have configured, optimized and produced the HIV incidence rapid test in cassette format. Based on initial test performance data and on input from our collaborators at [redacted], we will design and perform multi-site field studies designed to determine the test sensitivity, specificity and false recency rate with both well-characterized incidence/prevalence panels and prospective samples, and will evaluate the reproducibility, stability and impact of potentially interfering substances on test performance. These data will be submitted to FDA to support a waiver for non-diagnostic surveillance use of the assay, to our European notified body (Obelis SA) to support commercial introduction in Europe, and to regional regulatory agencies in other countries (e.g., in Africa, Asia and elsewhere ex-U.S.) as needed. With appropriate regulatory approvals or waivers, we expect to launch the rapid incidence test commercially in the U.S. and worldwide by the end of Phase II.

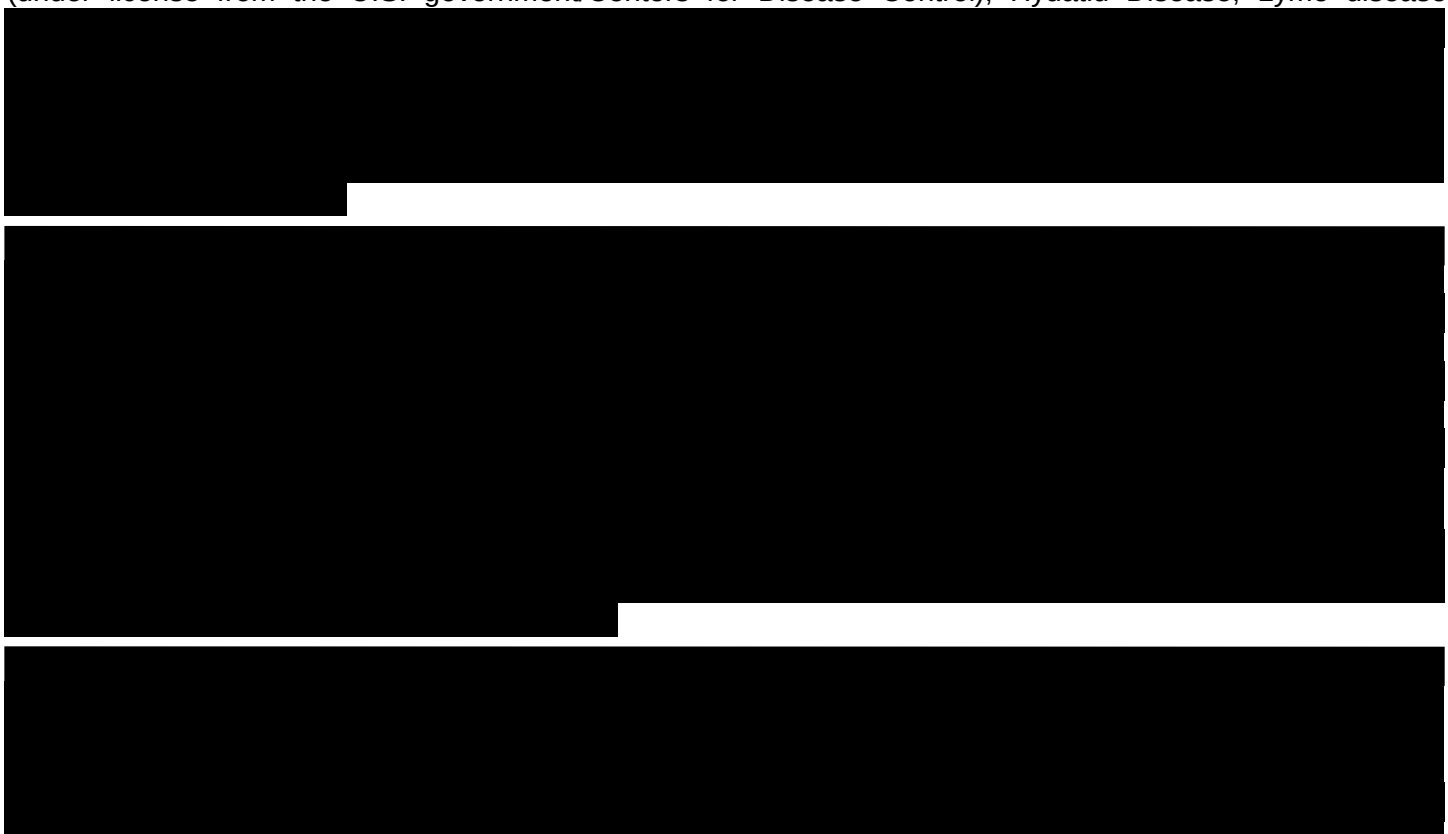
Impact.
The availability of the first rapid test for HIV incidence will transform the field by putting a powerful tool for monitoring the HIV epidemic at the disposal of organizations and individuals operating primarily in the field, without the need for support services from a highly sophisticated central laboratory as currently required. Pending the results of field studies and the scope of the claims, this rapid test could ultimately lead to a change in the testing algorithm for HIV incidence by eliminating the need for RITA's (Recent Infection Testing Algorithms) dependent on multiple tests to overcome the shortcomings of each individual test. Finally, commercialization of the HIV incidence rapid test will enable Immunetics to enter the growing rapid test market and demonstrate the utility of a novel detection technology with the potential to improve the standard of performance for HIV and rapid tests in general.

Company.
Immunetics is a small business, currently with a staff of approximately [redacted] full-time employees, focused on infectious disease diagnosis and pathogen detection. The company, started by Andrew Levin in 1987, has developed and commercialized a range of diagnostic assay products. Since inception, the Company has been funded by revenues from product sales, private (angel) investments, NIH grants and NIH contracts. About [redacted] million in equity investment has been raised over the past 10 years. Total revenues exceeded [redacted] million in 2012, about 20% growth over 2011. The Company has been profitable since 2005 and is not dependent on equity investments to maintain and grow the business. Current staff include [redacted] administrative/finance people, [redacted] in marketing/sales, [redacted] in Manufacturing and Quality Assurance, and [redacted] in Product Development. In 2004, the Company moved from Cambridge, MA to its present location in Boston, where it occupies about 18,000 square feet of newly built-out office and laboratory space. It is currently expanding its laboratory with another 9,000 sf to accommodate growth needs. The facility has passed four successive FDA inspections with no findings and operates with GMP infrastructure ensured by a Quality Assurance director supported by an external regulatory consultant. In 2009, the Company achieved ISO13485 certification, a mark of excellence requiring adherence to the highest quality standards throughout all company operations. Pertinent to this application, Immunetics has recently recruited as CEO John Yonkin, the former President of Alere, Inc., a global firm specializing in development and marketing of point-of-care tests for physician and consumer use. Mr. Yonkin brings extensive and directly relevant industry experience to the launch of this novel point-of-care test. Immunetics' Vice President, Richard Pinkowitz, was likewise a marketing and sales executive at Alere's predecessor Inverness Medical, where he managed the sales of point-of-care tests for diabetes and brings significant marketing and sales experience to the company. Dr. Andrew Levin, Immunetics' President and Scientific Director, has over 20 years of experience in the development of HIV assays, most recently as Principal Investigator of a $10 million contract from the National Heart, Lung and Blood Institute for development of the HIV Selectest, an ELISA for discrimination of HIV infection from vaccine-induced antibody responses.

Development of the rapid test will be guided by [redacted], Dr. Victor Kovalenko. He is the inventor of the antibody detection technology (QuickELISA) and colorimetric detection system for rapid diagnostic tests and has extensive experience in diagnostic reagents and assay development in various formats, gained at Immunetics and prior academic and industrial positions. Both Immunetics' FDA-approved C6 Lyme IgG/IgM ELISA and Anthrax Protective Antigen ELISA kits were developed under his supervision. He is also a principal at Diagnostic Innovations, Immunetics' consortium partner in several joint projects for rapid diagnostic test development.

Blot assay devices. The Miniblotter® instrument, a device developed and patented by Dr. Levin for incubation and processing of multiple samples on western blot and other membranes, was the Company's first product. The Miniblotter® product line continues to be a source of revenue for the Company, with over 5,000 users worldwide. These instruments have been used by Immunetics and its customers to develop many specialized assays for both research and clinical purposes, including miniaturized assays for HIV antibodies in neonatal dried blood spots, the checkerboard assay format for detection of multiple antibody-antigen pairs, and for multiple nucleic acid hybridizations, which has led to the development of tests now in widespread use for clinical evaluation of periodontal pathogens, the spoligotyping method, an epidemiological and diagnostic tool for analysis of Mycobacteria strains, and numerous others. Immunetics has also developed the CodaXcel™ instrument, a device which accelerates the processing time for western blots and nucleic acid hybridization blots, providing a start-to-finish time of approximately 15 minutes in place of hours.

Diagnostic assay products. Immunetics' main diagnostic product line comprises a series of immunoassay kits for detection of antibodies as markers of infection. Diagnostic assays which were brought from inception to market include Western Blot assay kits for HIV-1/2 (under license from the U.S. government), Cysticercosis (under license from the U.S. government/Centers for Disease Control), Hydatid Disease, Lyme disease



for HGE based on a recombinant antigen. These are the first and only products of their type brought to market. Grant AI45809 supported the development of a recombinant antigen-based rapid test for Lyme disease. Under this grant, Immunetics began work with VlsE, a newly discovered protein antigen of Borrelia burgdorferi. This work has led to the development of a new, highly sensitive and specific ELISA for Lyme disease based on the C6 peptide of VlsE, a patented technology now licensed exclusively to Immunetics. This represents the first peptide-based serodiagnostic test for Lyme disease, which has been shown by laboratories in the U.S. and European countries to offer the highest combination of sensitivity and specificity currently possible in a single assay. A series of published studies have documented the advantages of the C6 ELISA over other serologic tests. The Company has conducted clinical trials of the new Lyme ELISA, filed a submission to the FDA, and received 510(k) clearance in May, 2001. The C6 Lyme ELISA is now in use in major clinical laboratories in the U.S. and Europe, has arguably become the standard of performance in the field, and is the Company's top source of sales revenue. Under grant AI51926, Immunetics evaluated the clinical performance of the C6 ELISA with and without an additional peptide component (C10 from the OspC protein) proposed to enhance sensitivity. This grant supported initial clinical studies comparing the C6 ELISA to the widely accepted ELISA+Western Blot protocol recommended by CDC in 1994. The Company extended these studies in a multicenter clinical trial, carried out in 2005-2006, which was the largest and most stringent ever conducted for

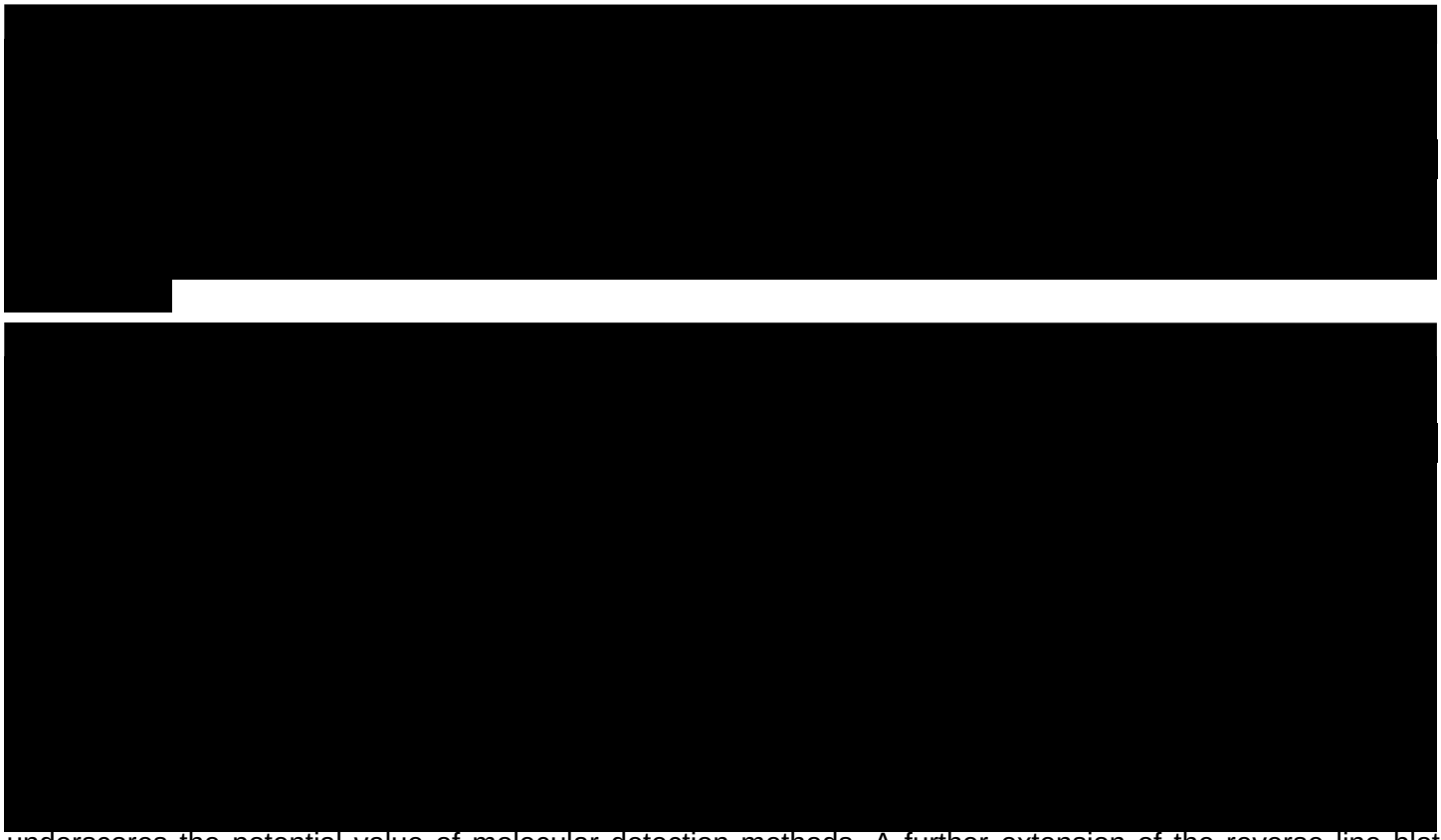
a Lyme disease diagnostic test – involving approximately 500 well-characterized retrospective clinical samples, 1,200 prospective patient samples and over 2,000 controls (published recently in Diagnostic Microbiology and Infectious Diseases (2013) 75(1):9-15). Immunetics has submitted a 510(k) application to the FDA based on the trial data, supporting the claim that the C6 ELISA can be used as a single-step test for Lyme disease, with no confirmatory Western Blot needed – a precedent of significance for the diagnosis of Lyme disease, with ramifications for the Company's market position with this product. Through this clinical trial, Immunetics has acquired experience in the design and management of a multicenter clinical trial, data management, regulatory aspects, FDA interaction, HIPAA compliance, Quality Assurance requirements and infrastructure, business and administrative organization, and financial requirements.

With support from NIAID Phase I SBIR grant R43 AI091291, Immunetics has developed a rapid test for Lyme disease. The prototype rapid test employs the proven C6 antigen used in the FDA-approved Lyme C6 ELISA test together with a second peptide, C10, that increase sensitivity for antibody detection at earliest stages of disease. The rapid test utilizes Immunetics' proprietary QuickELISA assay technology, which exhibits high sensitivity and specificity. Detection of reactive samples will employ a proprietary detection system, which uses enzyme catalysis and a novel stable multicolor substrate system (Patent application 61/494,232, filed

has completed clinical trials of the BacTx™ kit, and received FDA (CBER) 510(k) approval in June 2012 for testing of whole blood-derived platelets and in August 2013 for testing of apheresis platelets. This experience with a regulatory approval for a blood test through CBER will be relevant to other blood screening tests under development for Chagas disease, babesiosis and HIV. We consider this a major strength supporting our capability to carry out the proposed project successfully from start to finish.

Chagas disease test. With support from NIAID grant R44 AI080021, Immunetics is developing an immunoblot-based serological confirmatory test for Chagas' disease, a chronic infection with the parasite *Trypanosoma cruzi*, which has emerged recently as a significant new threat to transfusion safety in the U.S. and elsewhere. As a result of the large number of immigrants from Latin America into the United States and other countries, up to several hundred thousand individuals in the U.S. are currently believed to be infected with *T. cruzi*. *T. cruzi* serology is limited in accuracy, though, by a high level of cross reactivity with Leishmania, as well as other microbial pathogens and disease conditions. To address this concern, the confirmatory test under development will include both native Chagas protein fractions and Leishmania antigen peptides. The goal of the project is to complete development of the test and to generate validation data in preparation for clinical trials. We will seek to receive clearance of the test from CBER for use in confirmatory blood screening as well as from CDRH for use in patient diagnosis.

HIV Selectest - discriminatory test for infection vs. vaccine-induced seropositivity. The vaccine trial market is the target for a project at Immunetics supported by a recently awarded major contract from the NHLBI under its REDS-II (Retrovirus Epidemiology Donor Study) project. Under this contract, Immunetics is developing an ELISA aimed at distinguishing between the immune response to HIV infection and to experimental HIV vaccine antigens. Currently, individuals participating in clinical trials of HIV vaccines test positive on standard HIV screening assays based on cross-reaction between vaccine and native virus antigens. The new test under development is based on recently identified peptide antigens which react with sera from infected individuals but not from HIV vaccine recipients. When development is completed, this ELISA will be evaluated in a large-scale clinical trial and an application will be submitted to the FDA for approval for vaccine trial use. The test kit will then be made available for use in government and industry-sponsored HIV vaccine trials. Through this project, Immunetics is gaining experience in the regulatory and QA compliance requirements for development, manufacture and release of a blood screening test, and likewise in the regulatory approval pathway defined by the Center for Biologics Evaluation and Research (CBER) at the FDA. Experience gained with the CBER requirements and regulatory pathway will be directly translatable to other blood screening tests under development, including tests under development for Chagas disease and babesiosis.



underscores the potential value of molecular detection methods. A further extension of the reverse line blot system in another direction is underway supported by NIAID grant AI080004, in which primers and probes are being developed to permit detection and identification of trypanosomal diseases, in particular African trypanosomiasis caused by *T. brucei* subspecies, and to differentiate between these and other pathogenic and non-pathogenic kinetoplastids.

Immunetics staffing and financial base.

As a small but growing business, Immunetics faces the same challenges that many other small technology-based companies face. Recruiting talented people for creative and managerial positions is a key requirement for success. Based in Boston, Immunetics presents an attractive opportunity in a location well-known for its large biomedical community. We consider that our success in recruitment is due in large part to the pipeline of products, most of which have been developed with support from NIH grants. Since 2005, the company has been operating at a profit, based on increasing sales of its core products (above). With management capable of delivering both profitable operations and growth in revenues at about 15%-20% per year based on current products (last 2 years), the Company is well-positioned to expand its business. Equity financing will be sought based on market conditions, but the Company is not dependent on it for operations and growth. Over the next 5 years, Immunetics envisions accelerating growth in all aspects, including sales revenue from the launch of

the BacTx® kit and other products, the new product development pipeline, manufacturing capability, and sales and marketing impact. The Company's long term objective is to return value to its shareholders, which may be achieved by an eventual merger or acquisition, public offering, or management buyout.

Market, Customer and Competition

Market size and share. An estimated 60,000-100,000 HIV incidence tests for population studies are currently performed annually worldwide (Scenario 1, below). This figure is based on a 2009 study funded by the Bill and Melinda Gates Foundation [7]. HIV incidence testing is thus an "orphan" market, yet one which plays an essential role in public health efforts to contain the epidemic and interdict HIV transmission. The study cited three scenarios with different market size estimates. Because the scenarios are relevant to our plans for intended use of the HIVI rapid test, we have quoted them directly here:

(1) Scenario 1: The current situation, in which HIV incidence assays are used only on HIV-seropositive specimens and demand is influenced by suboptimal performance.

(2) Scenario 2: An improved HIV incidence assay is widely available at a low cost and performs consistently well across HIV-1 subtypes. This new assay is used only on HIV-seropositive specimens.

(3) Scenario 3: A novel HIV incidence assay is developed that can be used both to determine HIV-seropositivity and to identify how recently a given sample was infected. This assay would be used for public health and research purposes, rather than individual patient diagnosis.

of approaches to incidence testing in past years have relied on modifications of existing diagnostic tests – typically by detuning assay sensitivity, practiced on the Vironostika EIA, Vitros EIA, Architect and Axsym EIA, Inno-LIA and several others [7]. However, none of these assays have adequately satisfied desired performance criteria including low false recency (FRR) rates [5,10]. The CDC LAg ELISA based on rIDR-M, now offered commercially by Sedia and Calypte, represents the only assay which appears to come close to meeting desired FRR and other criteria. Currently there are no direct competitors with an HIV incidence *rapid* test on the market, although some efforts are being made to modify conventional rapid HIV screening tests for incidence use, such as the Uni-Gold Recombigen test [7]. These modified assays will almost by definition have fairly complex test protocols, and in some cases a RITA (recent infection testing algorithm) is anticipated. In a RITA scenario, multiple assays or a combination of test results and clinical information are used to establish and/or confirm recency [5], adding to the overall complexity and cost of determining HIV incidence by

population surveillance. Based on our data to this point, we believe we can produce, validate and commercialize a single, simple, accurate, HIVI rapid test with a false recency rate equivalent to the LAg ELISA, eliminating the need for a complex and costly RITA. Thus we expect to gain market share rapidly, and project capturing [redacted] TAM share (as above) within 3 years of product launch.

[redacted]

Intellectual property protection

The HIV incidence rapid test employs a proprietary antibody capture and detection method, covered by U.S. patents 7,262,019, 7,125,517, and 7,105,311 issued to Immunetics. This method, termed QuickELISA™, allows capture of labeled antibodies in one step, and has been used by Immunetics in commercially available and FDA-approved serological tests for Lyme disease and Anthrax.

The IDR-M peptide used in the HIV rapid test is in the public domain and not covered by an issued patent based on the publication of its sequence and properties in 2010 [13]. CDC has acknowledged that no patent has been filed on IDR-M. Our patent attorneys are available to resolve any freedom-to-operate issues which may arise, whether merited or not.

The rapid test also employs a new highly-sensitive, multi-color detection system (U.S. Patent application 61/494,232 filed by Immunetics June 7, 2011). Immunetics has also reviewed the intellectual property landscape for lateral flow rapid tests in general, and has concluded that we will have freedom to operate based on the intended test design and on the impending expiration of several patents covering lateral flow technology.

Finance plan

[redacted]

Final rapid test kit manufacturing.

Final production steps for the HIV incidence rapid test kits will be performed at Immunetics, including manufacturing and dispensing of wash buffer and kit controls, product labeling, and assembly of components into kits comprising 10 tests. Immunetics has the facilities and capacity to perform such kit production and packaging tasks within our FDA registered manufacturing facility located in Boston, as we currently do for the FDA-cleared BacTx®, Anthrax and C6 Lyme ELISA kits. Following manufacture and QC, HIV incidence rapid test kits will be sequestered in a cold room as inventory ready to ship. Space will be set aside to accommodate sufficient kits to maintain a rolling inventory adequate to cover anticipated shipments within the next 90 days. For this purpose, a 400 sf cold room and separate 200 sf cold room are available. Immunetics has adequate unused space to install additional cold storage if needed to maintain larger inventory levels. Should production requirements rise beyond Immunetics' and DCN's capacity, we will contract production to a higher volume manufacturer of rapid tests, of which a variety of companies are known in the U.S. and Asia which are FDA-certified and meet high quality assurance standards.

Marketing plan and sales strategy.

Product positioning and regulatory position.

Previous HIV incidence ELISA assays have been positioned for regulatory purposes as population surveillance

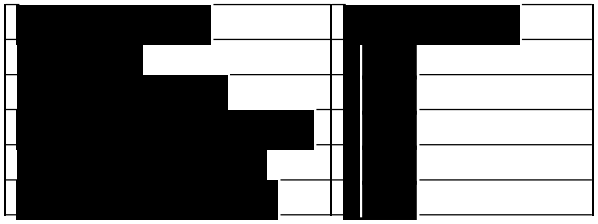
[redacted]

Sales strategy.

[redacted]

Revenue stream

[redacted]



COMMERCIALIZATION HISTORY

Current:

Contract No. [redacted] (Phase II)
Title: Screening and Confirmatory Tests for Human Babesia
Agency: National Heart, Lung and Blood Institute/National Institutes of Health
Performance Period: 08/31/2-12 ï 08/30/2014
Total award: $3,667,660

Commercialization status:
The Babesia ELISA test is currently in IND clinical trials leading to an FDA submission for BLA licensure.

Summary:
The aim is development and regulatory approval of an immunoassay for Babesia seropositivity, to be used as a high throughput blood screening test to prevent transfusion-transmitted babesiosis. The test employs unique immunodominant antigens specific for *B. microti*.

Phase III and other funding:
Follow-on funding from the Companyôs current product revenue sources will be used to support initial manufacture and release of product following FDA licensure. Funding has also been received from BSRI, our partner in clinical trials and in commercialization of the product.

Grant No. [redacted] (Phase II)
Title: Confirmatory Immunoblot Test for ChagasôDisease
Agency: National Institute of Allergy and Infectious Diseases/National Institutes of Health
Performance Period: 06/01/08 ï 02/28/14
Total award: $2,410,129

Commercialization Status:
Product in development, planned for clinical trials in 2013-2014 followed by FDA submission and commercial launch upon FDA clearance.

Summary: Development of an immunoblot assay for antibodies to *T. cruzi*, the agent of Chagasôdisease, for use as a confirmatory test in clinical diagnosis and blood screening. The immunoblot assay will be significantly easier to use than radioimmunoprecipitation (RIPA), the current standard, which is only practiced in a handful of laboratories nationwide. Chagasôhas been identified as a threat to the blood supply and a confirmatory test is required for blood screening. The disease affects over 25 million people worldwide, principally in Latin America, and confirmatory testing for clinical diagnosis is likewise a critical need.

Previous:

Grant No. [redacted] (Phase II)
Title: Rapid Assay for Bacterial Contamination of Platelets
Agency: National Heart, Lung and Blood Institute/National Institutes of Health
Performance Period: 10/01/02 ï 8/31/10
Total award: $[redacted] (through 8/31/07)

Phase III Funding:
Follow-on funding from the Companyôs current product revenue sources plus an investment round in 2006 yielding about $[redacted] Additional product revenues projected from sale of product following FDA approval.

Commercialization status:
FDA 510(k) clearance for the BacTx® rapid test for bacterial detection in whole blood-derived platelets obtained in June 2012. FDA 510(k) clearance for use on apheresis platelets received in August 2013. Product launch planned for Q4 2013. This is only the second product cleared by the FDA for this application.

Summary: Development of an assay based on peptidoglycan-binding proteins for the detection of bacteria in platelet units. This assay addresses an acute need in the blood bank and transfusion unit, where bacterial contamination of platelets causes both morbidity and mortality. The peptidoglycan-binding assay offers high sensitivity and specificity in a rapid, simple and inexpensive format for blood bank or point-of-care use.

Grant No. [redacted] (Phase II)
Title: Rapid Confirmatory Lyme Disease Test
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health
Performance Period: 9/15/97 ï 2/29/00
Total award: $[redacted]

Phase III Funding:
Investment rounds led by Stonegate Partners during this period raised about $5 million, which in part supported commercialization of the C6 Lyme ELISA developed under this grant.

Commercialization status:
In Phase III, the C6 peptide Lyme ELISA was validated, received FDA 510(k) approval, was patented, and has been launched commercially by Immunetics. It is now the Companyôs lead product by revenue, showing about 20% growth in sales per year. It is in routine use in major clinical laboratories in the U.S. and Europe.

Summary: The aim was development of a rapid immunoassay for Lyme serodiagnosis. Immunetics evaluated a wide range of recombinant Lyme antigens, focused on antigens with high sensitivity and specificity. Antigens were evaluated on serum panels including Lyme-positive, normal and potentially cross-reactive sera. The VlsE antigen was selected, and the C6 peptide sequence within VlsE ultimately chosen for use in the final ELISA version.

Grant No. [redacted] (Phase II)
Title: Tick-Borne Disease Panel Test
Agency: National Institute of Allergy and Infectious Diseases/ National Institutes of Health
Performance Period: 8/31/99 ï 2/28/02
Total award: $[redacted]

Phase III Funding:
Two investment rounds led by the Companyôs Board of Directors during this period raised about $1 million, which in part supported commercialization of the Babesia and Ehrlichia tests developed under this grant.

Commercialization status:
Immunetics was awarded U.S. patent #6,013,460 for a modified Western Blot for detection of multiple tick-borne diseases. The Babesia Western Blot was validated in a study published in Clinical and Diagnostic Laboratory Immunology (2001) 8(6):1177-1180. The Babesia Western Blot kit and HGE ELISA and Western Blot kit were produced and marketed as the first commercial kits of their type for serodiagnosis of these diseases.

Summary: This project was aimed at development of a panel of tests for tick-borne pathogens including Lyme, Human Granulocytic Ehrlichiosis, and Human Babesiosis. Immunetics evaluated Lyme, HGE and Babesia native and recombinant antigens in ELISA, dot blot and Western Blot formats. Development resulted in the first ELISA and Western Blot assays for HGE and Babesia based on semi-purified native antigen lysates.

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

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

Prefix: Dr.
* First Name: Patricia
Middle Name:
* Last Name: Garrett
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:
Middle Name:
* Last Name:
Suffix:

* Phone Number:
Fax Number:
Email:

* Title:

* Street1:
Street2:
* City:
County/Parish:
* State:
Province:
* Country:
* Zip / Postal Code:

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:GRANT11483849 Funding Opportunity Number:PA-10-123 Received Date:2013-09-09T18:13:49-04:00

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)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	[redacted]	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	[redacted]	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	[redacted]	Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities	[redacted]	Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table	[redacted]	Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children	[redacted]	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	[redacted]	Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	[redacted]	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	[redacted]	Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments | Remove Attachments | View Attachments

Specific Aims

Accurate estimates of HIV incidence (the rate of new HIV infections) are critical for policy decisions, for monitoring the epidemic, and for planning and evaluating interventions. Despite more than a decade of effort, significant barriers to progress remain [5,10]. Two such barriers have been the lack of an incidence assay for field work and resource-poor settings, and the unavailability of serially collected plasma panels for assay evaluation from individuals with closely estimated infection dates. In this project, we propose to transform HIV serological incidence testing from ELISA methodology based on avidity and titer and requiring a laboratory with sophisticated infrastructure, to a field procedure using a simple, stable, and reliable rapid test. In Phase I, we have developed prototypes of a new, highly sensitive rapid test based on novel colorimetric detection technology, which allows the analysis of antibody titer and avidity in less than 15 minutes within a single cassette. The rapid assay is based on synthetic peptides derived from a recently developed multisubtype gp41 HIV peptide antigen (IDR-M) which is the basis for the currently state-of-the-art limiting antigen (LAg) ELISA for incidence testing [13,15,16]. Evaluated with a CDC panel of 188 samples (57 recent, 131 long-term), the new rapid assay with one synthetic peptide yielded > 98% correlation with the LAg ELISA (Table 2, Research Plan). In Phase II, we will evaluate the accuracy of the new rapid test for classification of recent vs. long-term HIV infections using well-characterized serum/plasma panels developed specifically for this purpose. These will include a panel representing recently HIV-infected individuals (HIV RNA positive/anti-HIV negative at recruitment) collected over a two year period as a specific aim in Phase I. Field evaluations of the rapid test will be carried out in collaboration with public health agencies. In Phase II, we plan to optimize, validate and commercialize the HIV incidence rapid assay with the following specific aims:

Aim 1. Complete development of an optimized HIV incidence rapid test based on the rIDR-M antigen or synthetic peptides derived from it.

a. Optimization of antibody capture format, colorimetric detection system, dissociation conditions, interpretation criteria, test strip format and housing to yield <2% false recency compared to LAg ELISA;

b. Preparation and validation of plasma controls (incident, prevalent, negative) using dried tube specimens [2] or lyophilization. Validation will involve comparison with standard controlsô ability to detect introduced error. Correlation of ≥95% will signal success.

c. Simultaneous preparation of instructions for use, interpretation spreadsheet and training package, including photographs or video of test method process and interpretation;

d. Design of cassette, transfer to manufacturing and manufacture of pilot lots of the rapid test for laboratory and field evaluations.

e. Field testing of device and peripherals for accuracy, ease of use, reproducibility, and clarity of interpretation; data review and analysis; modifications as required. Four resource-limited sites from among CDC sites in Malawi, Botswana and Kenya and MHRP sites in Nigeria, Tanzania, and Thailand will test 200 sequential unlinked samples each in parallel with LAg ELISA or their current population surveillance method. The goal will be ≥98% correlation. Discrepants will be investigated to the extent possible, and appropriate modifications made to the assay.

Aim 2. Validate the final-format HIV incidence rapid test performance using plasma panels with closely estimated HIV infection dates and other relevant sample sets from DOD, CDC, CEPHIA, Blood Systems Research Institute and SeraCare.

a. Analysis of sensitivity and specificity of antibody detection and discrimination of recent vs. long-term infections across multiple subtypes with seroconversion series and other repository samples from known long-term infections, elite controllers, and ART-treated and non-treated individuals with a goal of Ò 2% false recency;

b. Evaluation of a rapid plasma separation device [17] to allow population surveillance with whole blood samples when necessary: comparison of fresh plasma samples (incident and prevalent) from spun whole blood and membrane-separated whole blood will be performed at Blood Systems Research Institute with a goal of ≥98% correlation between specimen types.

Aim 3. Prepare data from all studies for publication and for submission to FDA for waiver of approval to allow intended use for population surveillance, for submission for CE mark, and for other regulatory approvals as appropriate.

a. To make the HIVI rapid test commercially available, we will pursue regulatory waiver from the FDA and approvals in target countries/regions, including Africa and Asia.

SIGNIFICANCE

Over three decades into the global HIV epidemic, identifying new HIV infections is critical to the evaluation of prevention and intervention strategies, because these must be specifically directed at interdicting new infections. The cost of HIV prevention programs exceeds $10 billion/year worldwide, while UNAIDS estimates about 2.5 million new infections annually, such that each new infection is worth about $4,000 in prevention efforts [15]. As such, improvements in targeting prevention efforts more specifically to populations with high rates of new infection should lead to greater cost-effectiveness and greater success in reducing HIV transmission. A significant challenge has been to identify new infections, however.

Very few HIV assays have been developed specifically to distinguish new from established HIV infections, i.e. to distinguish incidence from prevalence. Most HIV serologic assays are aimed at diagnostic use, while RNA assays have been used largely to determine viral load for clinical management purposes. Acute HIV infection can be detected by tests such as p24 antigen capture, but the time frame for such acute infection is limited to about 3 weeks post-infection, while a ñrecentò infection for purposes of determining incidence is typically defined as within one year. Assessment of the state of the HIV epidemic for epidemiologic purposes is wholly dependent on determining the frequency of new infections. The general lack of tools for this purpose reflects the greater focus on diagnostic applications by assay developers due to the perceived larger market for traditional clinical diagnostics. A first generation of incidence assays have been developed, such as the well-known BED ELISA and Vironostika detuned ELISA, along with assays targeting specific immunodominant epitopes, p24 antigen detection and others, but their limitations have become increasingly obvious [5,10,15,16]. The World Health Organization (WHO) formally advised to discontinue use of the BED assay in 2005 due to its low level of accuracy, and other assays have shown similar problems. Given the limitations in accuracy of single first generation incidence assays, dependence on RITAôs or Recent Infection Testing Algorithms combining results of more than one type of test is increasing. Recognizing this need, the WHO with support from the Bill & Melinda Gates Foundation appointed a Technical Working Group on HIV Incidence Assays in 2008 (http://www.who.int/diagnostics_laboratory/links/hiv_incidence_assay/en/index.html) to develop recommendations for assay development, validation and commercialization [5]. At a recent (2010) meeting on the state of HIV incidence assays, participants concluded that a ñstandard, accurate, inexpensive and easy to use commercial kitòis urgently needed to estimate HIV incidence [10]. We aim to serve a major public health need through the development of a new test capable of rapidly identifying new HIV infections.

Secondly, determining the frequency of new HIV infections has required access to well-equipped centralized laboratories capable of running the few sophisticated assays available for this purpose; these have been ELISAs requiring microplate handling and reading instrumentation, including the BED ELISA and the Vironostika detuned ELISA [7]. Dependence on a central laboratory also implies the requirement for a system to transport serum specimens from where they have been collected to the laboratory, a separate and acute logistical challenge in low-resource settings where refrigeration may not be available or dependable. A rapid test capable of identifying new infections that could be administered in real time in the field, where the subject is located rather than in a laboratory, would offer major benefits.

In Phase I, we have developed a prototype serological assay in a rapid (< 15 minute), point-of-care format for distinguishing recent from long-term HIV infection using antibody avidity, and we have generated an incidence/prevalence serum panel useful in characterizing such assays through serial collections from six recently HIV-infected individuals over almost two years. The simplicity of this rapid assay, in contrast with conventional laboratory-based assays, offers to make incidence testing available to all potential users, especially in low-resource settings. As has been demonstrated in other fields where the advent of point-of-care tests has resulted in more timely and effective clinical management, the HIV incidence rapid test will provide a new and powerful tool for public health which may similarly lead to significant advances in the field.

We propose in Phase II to complete development, validate and pursue appropriate regulatory approvals or waivers for this transformative HIV incidence (HIVI) rapid test, intended for population surveillance, that combines three innovative components: a capability to distinguish recent from long-standing infection that uses a unique one-step approach to determining antibody avidity; a novel multicolor colorimetric enzymatic detection chemistry with ELISA-equivalent sensitivity, and a synthetic peptide antigen combination with significantly improved detection equivalence for multiple group M HIV subtypes. The new rapid HIVI test will fill the acute need for an accurate incidence test that meets the needs of all stakeholders involved in public health prevention and monitoring efforts and clinical research, from central facilities and laboratories to the field.

INNOVATION

This project will introduce a significant innovation in the field of HIV incidence measurement - the first rapid test for identification of recent HIV infections designed for field use rather than performance in a central, well-equipped laboratory. This development will reduce the dependence on well-established laboratory infrastructures for epidemiologic surveillance in limited-resource areas where the HIV epidemic is most prevalent, including regions in Africa and Asia. The ability to carry out testing of HIV incidence in the field will eliminate the need to transport serum specimens in a stable way to a frequently distant central laboratory, thereby simplifying logistics and reducing cost. The real-time turnaround of results will likewise enable faster response time for public health efforts aimed at identifying populations with high HIV incidence. These features in synergy will transform the paradigm for HIV incidence testing from the current centralized and slow laboratory-based system to a rapid, field-based, decentralized and locally empowered system.

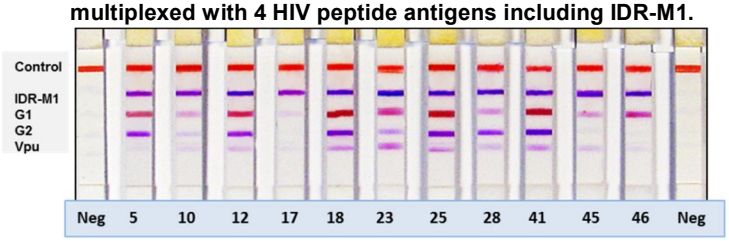
The Immunetics HIVI rapid test will be the first to offer a combination of sensitivity and specificity for determining HIV recency in a rapid (< 15 minute) format. The test will use a novel, highly sensitive and stable colorimetric detection technology based on peroxidase-catalyzed dye formation with oxidative coupling to membrane immobilized dye-formation reagents, which was developed by Principal Scientist Dr. Kovalenko. The new detection chemistry will open the door to development of other highly sensitive rapid tests with unique ability for multicolor targeting in multiplexed assays, ideally suited for visual reading or simple densitometry.

The IDR-M antigen on which the test is based is a recent innovation developed by Dr. Parekh et al. at CDC, and has shown significant advantages in serological detection of the complete spectrum of HIV clades [13]. Recombinant IDR-M is a tandem sequence of three gp41-derived peptides with varying sequences to optimize immunoreactivity with all HIV clades. While IDR-M has been employed for incidence ï prevalence discrimination in a limiting antigen (LAg) ELISA format which has arguably become the current standard for incidence testing [15,16], application to rapid tests is a new and relatively unexplored area. A recent study by Granade et al. [1] demonstrates the potential for IDR-M to be used in a lateral flow format, but no commercial rapid test has yet been developed. Hence the use of the new IDR-M antigen in our unique, proprietary rapid test format is a second significant innovation.

With respect to the underlying assay technology, the new HIV incidence (HIVI) rapid test embodies three further, distinct innovations:

1) HIVI will combine antibody *titer* and *avidity* analysis in a simple lateral flow format with the sensitivity and specificity of an ELISA and the speed and simplicity of a rapid, point-of-care test.
2) The HIVI rapid test will use a new, simplified approach to discriminate between recent and long-term infection: a one-step avidity assay specially developed for rapid tests, based on preventing low affinity antibody binding by addition of a chemical reagent upon sample addition rather than subsequent dissociation of a preformed complex. This new approach to prevent binding of low affinity antibodies, combined with a proprietary antibody capture format using peptide conjugates without secondary detection reagents (ñQuickELISAÊ ò), makes it possible to run a rapid test with minimal sample dilution or even undiluted, compared to typical sample dilutions of 1:20-1:200 required for most ELISAs.
3) The high sensitivity of the HIVI rapid test is based on an innovative, stable, multi-color colorimetric detection system using peroxidase that is applicable to a broad spectrum of diagnostic tests (U.S. Patent application 61/494,232 filed by Immunetics June 7, 2011). For the HIVI test, this detection system is integrated into the most popular rapid test format, a lateral flow strip test, providing sensitivity that significantly exceeds conventional colloidal gold or colored latex labels. An example of multiplexed detection of antibodies to several different envelope-derived HIV peptides using different colors for each is shown in Figure 1. The small sizes of the horseradish peroxidase (HRP)-labeled conjugate and the antibody capture conjugate eliminate many problems with agglutination and migration of labeled immune complexes through typical lateral flow membranes.

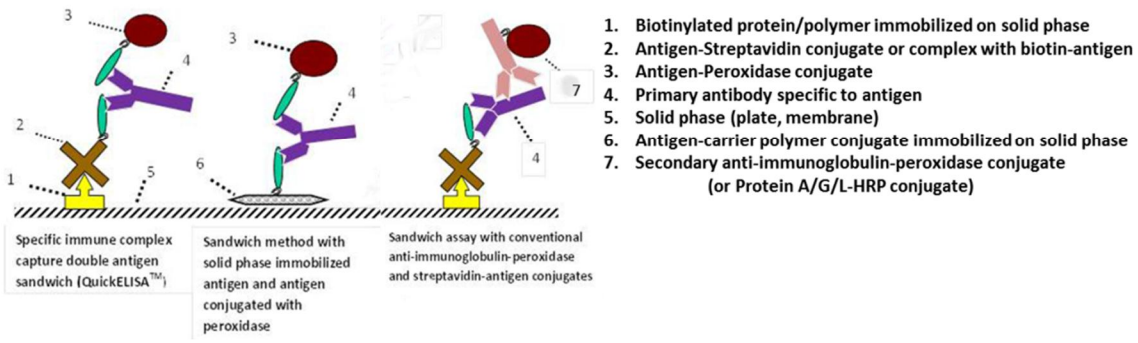
Figure 1. HIV positive sera. Sample dilution 1:40. 15 min test, multiplexed with 4 HIV peptide antigens including IDR-M1.



Antibody detection with peptide antigen conjugates (QuickELISA™). The assay format based on antibody capture as a complex with two independent peptide conjugates (Figure 2) eliminates the use of labeled secondary antibody conjugates and provides a significant increase in specificity, capture of all antibody classes and ability for antibody analysis without sample dilution, which is critical to the simplicity of a rapid test protocol

and increases analytical sensitivity. This method, termed ñQuickELISAÊ ò was developed by Principal Scientist Dr. Kovalenko and is covered under U.S. patents 7,105,311 and 7,125,517. In a QuickELISA assay, one molecule of peptide antigen is conjugated with detector label, while the second is conjugated to a component of a high-affinity binding pair (streptavidin). The second component of this high affinity binding pair (e.g. biotinylated protein) is immobilized on the solid phase at high density. Unlike conventional tests with secondary conjugates, where samples must be diluted typically 20-200 fold before transfer to wells, the QuickELISAÊ test with peptide conjugates can be run with undiluted sera. Only the label involved in specific complex formation is captured and detected, making the test insensitive to most factors related to non-specific site reactions. The absence of a species- and isotype-specific secondary antibody enables detection of both IgG and IgM along with other multivalent antibody classes in a single assay. This approach has been used successfully by Immunetics in a commercial, FDA-cleared serological test for Anthrax Protective antigen and a Lyme C6 QuickELISA test marketed in Europe.

Figure 2. Antibody capture schemes for ELISA and membrane-based rapid tests.

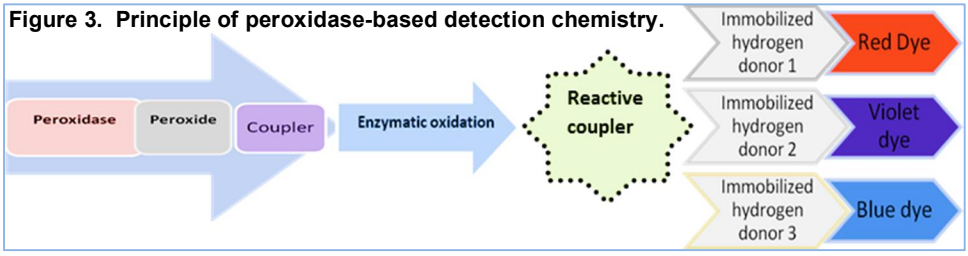


APPROACH

Phase I Report.

Rapid test detection technology. Our rapid test technology integrates a catalytic peroxidase detection system (Figure 3) with a new type of two-component stable substrate system, where one component (hydrogen donor) can be passively immobilized on membranes through use of a soluble polymeri coupled hydrogen donor conjugate or incorporated into membrane pores as particle-loaded reagents. The second component (coupler) is present in a free form, and is oxidized by peroxidase in the presence of a peroxide as primary peroxidase substrate. The oxidized coupler reacts with the membrane-immobilized hydrogen donor with formation of stable, light-insensitive contrast dyes. The availability of a plurality of hydrogen donor compounds opens unique possibilities for lateral flow tests where the internal control zone and several test zones produce different colors, simplifying visual analysis. The sensitivity of this detection system equals or exceeds the sensitivity of ELISAs with common HRP labeled reagents and the best colorimetric tetramethylbenzidine (TMB) HRP substrate. The new rapid test can detect antibodies at nanogram/ml concentrations and at dilutions up to 10 million-fold for some strongly positive samples. This substrate system also produces signals free from the diffusion frequently seen with precipitating peroxidase substrates. All major components of the new detection technology can be incorporated into lateral flow cassettes in a stable dry form (substrate reagents, capture/detector reagents), allowing long term storage at ambient temperature. Lateral flow tests with HRP as a label can be fabricated in a configuration requiring only one liquid component, an assay wash buffer, as in conventional lateral flow tests. The availability of efficient commercial immunoassay stabilizers and blockers, and experience with peroxidase stabilization, suggested to us that HRP or other thermostable peroxidases can be used as a label in rapid diagnostic tests with stability similar to conventional particle-based colorimetric labels. We have developed reagents which function efficiently as membrane blockers/stabilizers, conjugate drying buffers, and dry substrate reagents containing a coupler and a stabilized peroxide compound.

Figure 3. Principle of peroxidase-based detection chemistry.



Approach for simultaneous analysis of antibody titer and avidity. Analysis of antibody avidity is the best currently available approach for serological analysis of HIV incidence. Several ELISA-based methods have been developed and commercialized for avidity analysis, designed for discriminating recent from past or

chronic infection (e.g., for CMV, herpes, toxoplasmosis, and hepatitis, as well as HIV), all based on comparison of antibody binding to a solid phase immobilized antigen before and after incubation with reagents that dissociate low affinity antibodies (i.e., low pH, or chaotropic reagents such as urea, ammonium thiocyanate, guanidine). The dissociation reagent must be removed before treatment with secondary detection conjugate. The difference between signal before and after dissociation correlates with the proportion of low avidity antibodies.

For the HIVI rapid test, we plan a simpler version of avidity detection, more suitable for a lateral flow device. To eliminate the additional dissociation and washing steps, we have changed the function of the dissociation reagent from dissociation to prevention. In our new HIVI method, the dissociation reagent is present when sample is incubated with antigen, and thus binding of low affinity antibodies is prevented. Combining this method with the use of two antigen conjugates in an antibody capture format (ñQuickELISAò format described above) results in a one-step assay where prevention of low avidity antibody binding and labeling of bound antibodies proceed together in one incubation step. Following washing to remove non-bound label, the test is ready for detection of bound label (i.e., color development/substrate reaction).

sensitivity of the signal to the dissociation reagent between recent and long term infection samples (i.e., incident vs. prevalent, as defined by LAg ELISA results) is shown on the score card in Figure 6. The score card illustrates expected band patterns obtained with a range of incidence and prevalence serum samples as a working reference standard. An internal positive control line (red) is included to qualify an assay as valid. Test results for an unknown sample can accordingly be interpreted by comparison to a similar score card which will be included in the final kit. The presence of three antibody bands at increasing intensity provides a greater dynamic range over which to interpret assay results; however, further evaluations during Phase II will indicate whether a single band can effectively substitute for the three separate bands.

When the test is optimized and validated, a similar scoring chart will be prepared with high quality color images of results from pedigreed samples representing different HIV subtypes and a range of known infection dates from early Recent to very Long Term. The requirement for a single chart with accurate results from several widely circulating HIV subtypes or clades is the basis for Specific Aims 1d and 2a, which require that all widely circulating HIV subtypes show equivalent results.

Figure 4. Comparison of two antibody capture methods for rapid incidence tests with two-strip format +/- dissociation reagent, on SeraCare Incidence/Prevalence Panel PRB601.

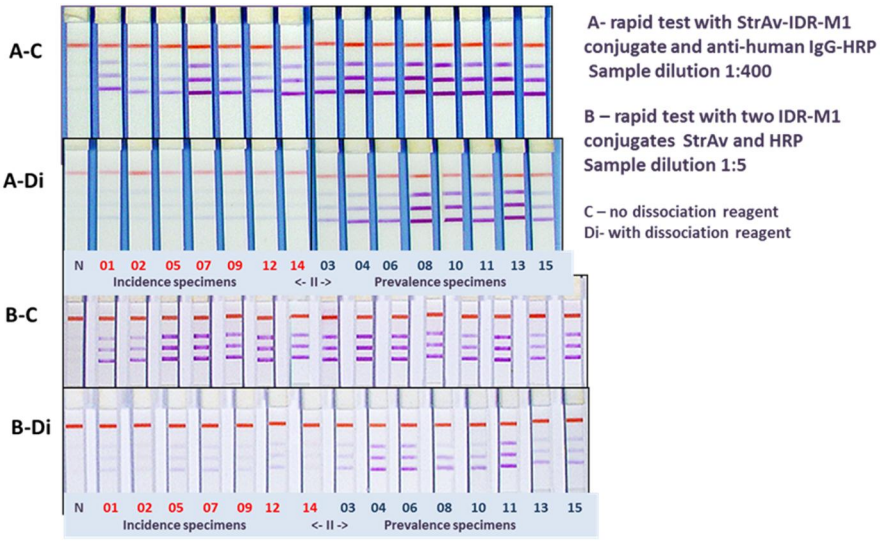


Figure 5. Proposed rapid HIV incidence test cassette design and scoring chart. Mock up of cassette with strips inserted after running.
X: Long-term infection with high LAg ODn; Y: Long-term infection with LAg ODn near LAg ELISA cut-off; Z: Recent infection.
Sample windows:
C- Control non-treated sample window
D - Window for sample with dissociation reagent

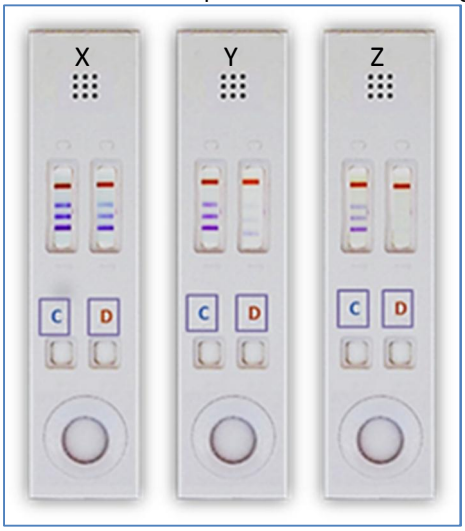


Figure 6. Scoring chart for HIVI rapid incidence test interpretation. R= Recent; LT = Long-term infection. **C**- Control non-treated sample window. **D** - Window for sample with dissociation reagent.

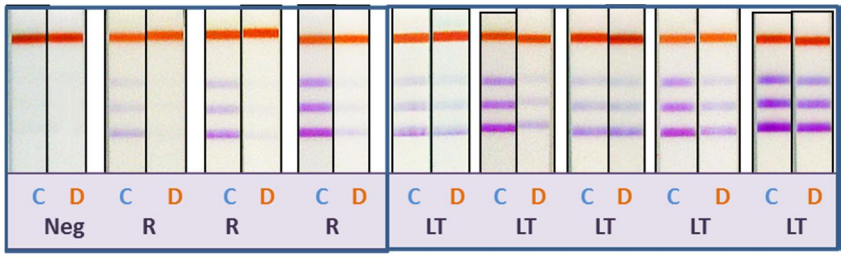
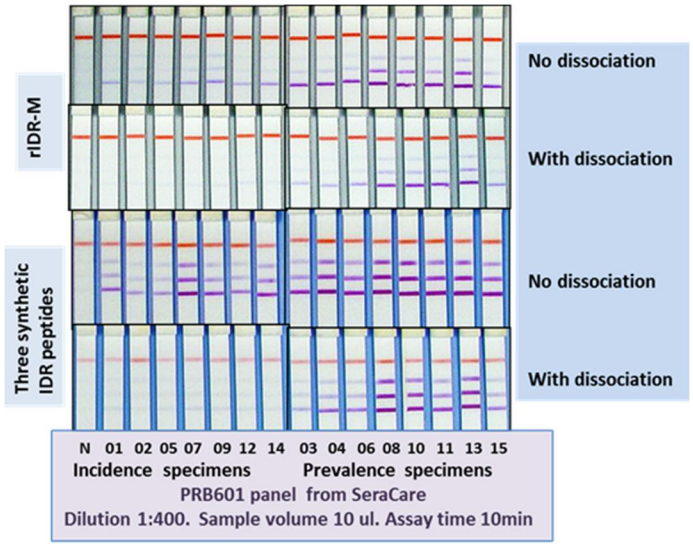


Figure 7. Comparison of sensitivity of synthetic peptides vs. recombinant IDR-M in HIVI rapid incidence test. rIDR-M and peptides M1, M2 and M3 at equimolar concentration as streptavidin conjugates. Detection with anti-human IgG-HRP.

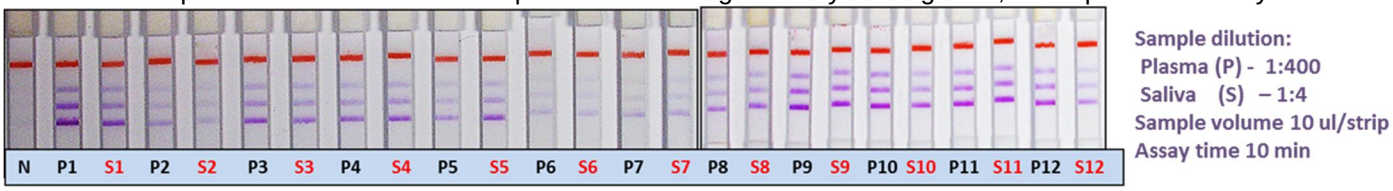


Peptides for rapid incidence test. While the original IDR-M protein was produced by CDC as a recombinant containing tandem gp41 sequences covering different sets of HIV clades, we determined that the high hydrophobicity of the large rIDR-M protein, even after incorporation of hydrophilic linkers, prevented solubility in buffer over a wide concentration range. The high hydrophobicity also limited the possibilities for synthesis of the soluble conjugates required for the antibody detection format described above. Additionally, StrAv and HRP conjugates synthesized from recombinant rIDR-M showed strong association tendencies, leading to non-specific signal from binding with immobilized biotin in the absence of antibodies. To circumvent these problems, we generated synthetic peptides recapitulating each of the tandem gp41 sequences in the rIDR-M protein. To optimize the synthesis method for conjugates from hydrophobic peptides, we selected one peptide (IDR-M1) covering HIV subtypes A,B,C,F,G,H,J,K and recombinant forms AG,AB,AC,BF and BG [13] and combined it with hydrophilic linkers used for conjugation. We also synthesized the three peptides contained within rIDR-M in a biotinylated form with additional hydrophilization by linkage with a polyethylene glycol spacer. All peptides were adequately immunoreactive as antigen complexes with additionally hydrophilized StrAv. The mixture of conjugated individual peptides provided much higher sensitivity than rIDR-M, possibly due to greater availability of the antigenic epitopes in the synthetic peptide configuration (Figure 7). Covalent conjugates of IDR-M1 with StrAv and HRP with additional hydrophilic linkers added during conjugation demonstrated good solubility and complete absence of non-specific association, making these

conjugates well suited for antibody detection in a format with two peptide conjugates. The same strategy for hydrophilization will be applied for the other two rIDR-M peptides, which cover clades D, AD and AE.

The HIVI rapid test with the hydrophilic IDR-M1 peptide has very high analytical sensitivity. We demonstrated that some high positive samples can produce positive signal at dilutions up to 13 million, 10-20 times more sensitive than in an ELISA version of the test. This high sensitivity provides the possibility to explore use of oral fluid/saliva as the sample in place of serum in future assay versions, which would enable replacement of a blood draw with a non-invasive sampling procedure. In a preliminary experiment, the HIVI rapid test with IDR-M1 detected HIV antibodies in saliva, which correlated well with detection in paired plasma samples (Figure 8).

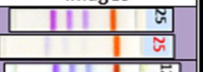
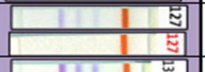
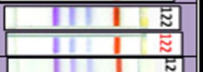
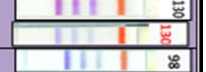
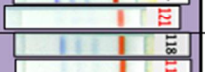
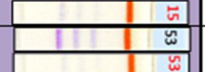
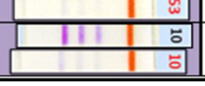
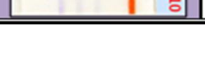
Figure 8. Paired plasma/saliva HIV panel tested with HIVI rapid incidence lateral flow test, detecting IgG, IgM and IgA antibodies. Separate detection lines correspond to increasing antibody binding level, not separate antibody classes.



The saliva/plasma HIV panel was provided by the University of California at San Francisco SCOPE study.

Testing HIV serum panels with known Recent/Long Term classification based on LAg ELISA (CDC). A serum panel originally from BocaBiolistics (Coconut Creek, FL) comprising 132 samples and a second panel from SeraCare comprising 89 samples were evaluated with the prototype HIVI rapid test using IDR-M1 peptide. Results for these panels obtained with the rIDR-M-based LAg ELISA were provided by CDC for comparison. Interpretation for the HIVI is based on visual analysis of the intensity of color in test lines. The LAg ELISA's ODn values correlated >98% for Recent and >99% for Long Term samples. Four samples were negative in both the LAg ELISA and the HIVI rapid test. Table 1 compares strip photographs with ELISA data for selected samples representing recent infection with ODn values in a medium range and close to the ELISA cut-off of 1.5 ODn, and for long term samples selected because their line color intensity in the absence of dissociation reagent is close to that of Recent samples. With one exception (sample 11-SC-0025), the Recent samples show a significant decrease in signal intensity in the presence of dissociation agent, while the Long Term samples show equivalent intensities. The two strip format thus provides unique visual discrimination between Recent and Long Term infection. The data as summarized in Table 2 clearly demonstrate excellent correlation between the two tests.

Table 1. Examples of HIVI rapid test with LAg-defined Recent and Long Term infection.

	HIV-1 Incidence		Incidence	Rapid test		HIV-1 Incidence		Incidence	Rapid test
Specimen ID	CDC LAg ODn	LAg Classif	rapid test	images	Specimen ID	CDC LAg ODn	LAg Classif	rapid test	images
BOCA-064	0.787	RECENT	R		11-SC-0025	1.928	Long Term	LT	
BOCA-127	0.532	RECENT	R		BOCA-122	1.995	Long Term	LT	
BOCA-132	1.000	RECENT	R		BOCA-123	1.799	Long Term	LT	
BOCA-102	0.554	RECENT	R		BOCA-130	1.971	Long Term	LT	
BOCA-115	0.910	RECENT	R		BOCA-098	2.672	Long Term	LT	
BOCA-104	0.677	RECENT	R		BOCA-114	1.903	Long Term	LT	
BOCA-064	0.787	RECENT	R		11-SC-0076	2.983	Long Term	LT	
BOCA-121	1.349	RECENT	R		11-SC-0032	3.881	Long Term	LT	
BOCA-118	1.437	RECENT	R		11-SC-0047	3.030	Long Term	LT	
11-SC-0015	1.342	RECENT	R		BOCA-030	3.587	Long Term	LT	
11-SC-0053	1.417	RECENT	R		BOCA-020	2.897	Long Term	LT	
11-SC-0010	1.800	Long Term	R		BOCA-098	2.672	Long Term	LT	

Phase I Progress Summary:

1. Hydrophobic peptides from rIDR-M were adapted for use in an assay format with two antigen conjugates (StrAv and HRP) that offers multiple advantages for antibody testing in a rapid test format, requiring one step for antibody labeling and capture in a test line.
2. Peptide conjugates from the major rIDR-M peptide that covers multiple subtypes (A,B,C,F,H,J,K and recombinants AC,AB,AC,BF,BG), and biotinylated forms of peptides for clades AE, D, and AD were synthesized and hydrophilized.
3. A new approach for one step avidity testing based on combining antigen antibody binding and dissociation reagent treatment was developed.
4. New dissociation reagents were developed that are compatible with HRP, do not inactivate HRP activity, and can be conveniently incorporated into the sample addition step to prevent complex formation between low-avidity antibodies and peptide conjugates.
5. Stable dry forms of dissociation reagent were developed for a separate sample application zone on lateral flow strips, as a potential alternative to the liquid form.
6. A stable dry form of color developer/substrate that can be integrated into a lateral flow cassette was developed.
7. The 2-strip design for the HIVI rapid test, with multiple test zones that improve its ability to discriminate samples based on antibody titer in addition to comparison of antibody avidity was further optimized.
8. A prototype two-strip lateral flow cassette design was developed for use with either liquid or dry forms of substrate, both enabling an assay time <12 min from sample application to result.
9. A scoring chart for simple and convenient visual interpretation of rapid test results was proposed.
10. Extensive testing of an HIVI rapid test prototype using the IDR-M1 peptide with an HIV panel characterized with LAg ELISA data demonstrated more than 98% agreement with ELISA interpretation.
11. The high sensitivity of the prototype HIVI lateral flow test using the IDR-M1 and several other HIV peptides for antibody detection was demonstrated. .
12. The capability of the HIV rapid test for detection of HIV antibodies of multiple clades was confirmed by testing an HIV serum panel including clades A, B and C (sera from multiple sources).

Table 2. Comparison of LAg ELISA with rIDR-M peptide vs. HIVI rapid test with IDR-M1 peptide conjugates.

LAg ELISA ODn	Number	LAg Recent/Neg	LAg LongTerm	Rapid Recent/Neg	Rapid LongTerm
Recent <0.4/Neg	17	13/4	0	13/4	0
Recent >0.4 <1.0	29	29	0	29	0
Recent >1.0<1.5	11	11	0	10	1
Long Term >1.5<2.0	12	0	12	1	12
Long Term >2.0<3.0	27	0	27	1	27
Long Term >3.0 <4.0	42	0	42	0	42
Long Term >4.0	50	0	50	0	50
Total	188	57	131	58	130
Correlation with LAg ELISA		100%	100%	98.3%	99.2%

RESEARCH PLAN

The overall objective of the proposed Phase II project is to optimize and configure the prototype HIVI rapid test for scalable manufacture, to carry out a field evaluation at several sites, to validate the optimized assay, to publish the data and submit for an FDA waiver for population surveillance use. Immunetics will be responsible for the overall project including work performed by collaborators, final assay design and development, bulk reagent manufacture, assembly and QC, retrospective and prospective field evaluation, and submission preparation and follow-through. Consortium partner Diagnostic Innovations (DI) will carry out reagent development and optimization, while Design Consulting Network (DCN) will perform optimization and scale-up of test strip manufacturing processes as well as diagnostic cassette mold design, manufacture and assembly. Retrospective and prospective field studies of HIVI rapid test performance aimed at supporting FDA waiver and CE mark submissions will be carried out in collaboration with CDC, MHRP, and BSRI. Immunetics will manage the project in accordance with our Quality System and Design Control guidelines.

Product Definition.

The target specifications for an HIV incidence test have been summarized in a recent paper by Hallett et al. which represents the consensus of the HIV Incidence Assay Working Group convened in a 2011 meeting sponsored by NIH and the Bill and Melinda Gates Foundation [10]. In Table 3, we compare the specifications of our HIV incidence rapid test with those proposed in this paper. Key attributes of an incidence test are the mean duration (ω) and the false recency rate (FRR). The mean duration indicates the time at which an individual converts from ñrecentò to ñestablishedò infection. The false recency rate reflects the inaccuracy of the

test in classifying longstanding infections as recent. The LAg ELISA as the current standard in the field demonstrates a mean duration in the range of 4 ï 5 months, and a false recency rate of slightly less than 2% [15,16]. Our preliminary data show correspondence between the HIVI rapid test and the LAg test of > 95%, suggesting that the HIVI rapid test will yield mean duration and FRR values similar to those observed for the LAg ELISA, which meet the target criteria below. With respect to other criteria, the HIVI rapid test is designed to meet the intended use on target populations comprising all HIV clades, and to meet requirements for sample type and volume, infrastructure and training, storage and shipping, and shelf life. As Immunetics is ISO13485 certified and manufactures products under GMP, that requirement will be met; the need for regulatory clearance by FDA will be determined by the proposed claims. Previous HIV incidence tests have been exempt from FDA approval based on their use for population studies but not individual diagnosis; should the HIVI rapid test be used for the same purpose, it is anticipated that it will be exempted from FDA review. However, given the potential to use the test for diagnostic purposes based on the high diagnostic potential of the IDR-M antigen, the possibility to pursue FDA approval in the future will be considered.

Table 3. Target specifications for an HIV incidence assay. From Incidence Assay Critical Path Working Group (2011). More and Better Information to Tackle HIV Epidemics: Towards Improved HIV Incidence Assays. PLoS Med 8(6): e1001045. Published 06/14/2011.

Specification	Acceptable Performance	Ideal Performance	IDR-M Rapid Test
Intended Use	Population-based incidence estimate	Population-based incidence estimate, prevention-trial planning, community-level prevention intervention studies	Population-based incidence estimate, prevention-trial planning, community-level prevention intervention studies
Target Population	Specific to clade	All clades	All clades
False Recent Rate (FRR)	≤ 2% in different populations (with different clades, epidemic phases, treatment coverage etc)	0% in all population (No evidence of false-recent classifications).	TBD
Mean Duration	4 months (95% CI, +/- 0.2)	1 year (95% CI, +/- 0.2)	TBD
Algorithm	Included in a RITA	None required	TBD, objective = none
Analyte	Any	Any	IDR-M antibodies
Sample Type	Frozen serum, frozen plasma	Frozen serum or plasma , dried blood spots (or other easily obtained and stored sample)	Serum or plasma. Potential for whole blood and dried blood spots.
Sample Volume	1 mL	10 uL or fingerstick	10 uL or fingerstick
Infrastructure requirements	Centralized laboratory facility (clean water and electricity available)	None (all reagents and necessary materials to run assay are in self-contained kit)	None (all reagents and necessary materials to run assay are in self-contained kit)
Storage/Shipping Conditions	4-25 °C	Ambient temperature	Ambient temperature
Incubation Temperature	4-25 °C	Ambient temperature	Ambient temperature
Shelf Life	9 months	>18 months	TBD but probably >18 months
Training	Laboratory technician can be proficient with one week's training based on proficiency testing	Minimal training would allow any health worker to conduct the assay	Minimal training allowing any health worker to conduct the assay
Regulatory Pathway	GMP or ISO 13485 or equivalent, and/or approval by national governing body	FDA and equivalents	To be determined. Minimally, GMP, ISO13485, and local regulatory bodies in countries of use.

Phase II Research and Development work will comprise:

Specific Aim 1: Complete development of an optimized HIV incidence rapid test based on the rIDR-M antigen or synthetic peptides derived from it.

Aim 1a. Optimization of antibody capture format, colorimetric detection system, dissociation conditions, interpretation criteria, test strip format and housing to yield <2% false recency compared to LAg ELISA. Activities will include:

- Synthesis of the StrtAv and HRP conjugates from two IDR-M peptides (M2, M3) specific to clades AE, D, and AD using hydrophilization approach validated for M1 peptide. Analyze possibility for synthesis of StrAv and HRP multi-peptide conjugates containing two or all three peptides in one conjugate without loss of solubility and without tendency to self-association.
- Optimization of the rapid test with all peptides covering the full spectrum of IDR-M specificity using panels of HIV positive samples representing all major subtypes.

- Evaluation of differences between two antibody detection methods: one for simultaneous detection of all classes of anti-peptide antibodies (method of choice with two peptide conjugates) and methods with secondary detection reagents binding IgG antibodies as in conventional LAg ELISA.
- Optimization of binding capacity of reagents in three tests lines to provide better discrimination of differences in antibody titer. Comparison vs. a single test line for greater simplicity.
- Finalization of development of stabilization chemistry to produce strips with dry peptide conjugates, dissociation reagent, substrate reagents and for liquid sample diluents .
- Introduction of two-color (color-coded) tracking dye system allowing simple visual monitoring to verify that test strips are being run with correctly diluted sample.
- Development of single dose packs with wash buffer.
- Comparison serum and plasma as sample matrix for rapid test.
- Evaluation of use of dry tube or filter paper-dried serum/plasma samples for rapid incidence testing.
- Evaluation of commercially available plasma separation devices for preparation of plasma for rapid incidence testing from whole blood
- Finalization of development of cassette design optimized for new detection system in two versions, with dry substrate reagent and for use with liquid substrate reagent.
- Development of storage materials and conditions for completely assembled tests (packaging materials, desiccants).
- Development of QC criteria for peptide conjugates, substrate reagents and dissociation reagents.
- Development of a simple approach for result interpretation using a scoring chart with representative images of test strips showing recent or long-term HIV band patterns obtained with samples of known infection status based on LAg ELISA criteria.

The final stages of rapid test development at Immunetics will include scale-up of bulk reagent manufacture, peptide conjugates, substrate system reagents developed at Diagnostic Innovations, and writing and validation of manufacturing and QC procedures. QA reagents and procedures will be developed for in-process QC to verify that each lot of the rapid test detects antibodies to all HIV peptides included on the test strip.

Aim 1b. Preparation and validation of plasma controls (incident, prevalent, negative) using dried tube specimens. Validation will involve comparison with standard controls' ability to detect introduced error. Correlation of ≥95% will signal success. Activities will include:

- Development of dry tube forms of Positive and Negative control samples for rapid test kits. Dry controls provide longer term stability than liquid controls and are less demanding in transport and storage. The method described by Ramos et al. for preparation of air-dried control sera will be compared with preparation by lyophilization. Air dried controls possess two disadvantages: the requirement for overnight drying and a similar requirement for overnight rehydration. Lyophilization can be carried out more rapidly and will result in a dry preparation that can be resolubilized within seconds to minutes. Immunetics has experience with lyophilization procedures and will prepare lyophilized controls in its pilot-scale lyophilizer in the Manufacturing Dept. Stability and reactivity of controls prepared by various protocols will be compared to select an optimal protocol for production of controls for HIV rapid incidence test kits.

Aim 1c. Simultaneous preparation of instructions for use, interpretation spreadsheet and training package, including photographs or video of test method process and interpretation.

- Instructions and training package will be prepared and tested on naïve operators to optimize them prior to external evaluations. Photos and video will be generated using available equipment and expertise at Immunetics. The interpretation spreadsheet will be prepared in Excel and made available for downloading from Immuneticsô website by test users. The spreadsheet will facilitate record keeping, accuracy and objectivity in interpretation by providing a standard form for documentation of results.

Aim 1d: Design of cassette, transfer to manufacturing and manufacture of pilot lots of the rapid test for laboratory and field evaluations.

- DCN In collaboration with Diagnostic Innovations will optimize and perform manufacturing operations for the rapid test cassette and test strips. An injection molded cassette will be designed to meet the specific characteristics of the HIV rapid test (fluid volume, diagnostic line placement, delivery of peroxide substrate) and to allow simple, low-cost assembly. Initial cassette prototypes will be produced and tested to enable design refinement. Three pilot lots of 1-2,000 cassettes each will be produced to meet the anticipated needs of test evaluations in-house and at field sites. The molded cassette will likely be a derivative design

based on existing cassettes which applicants have found suitable for the rapid test with some minor modifications.

- Test strip production involves sequential impregnation, dispensing, drying, cutting and laminating steps, each of which must be carefully optimized and controlled to ensure reproducible and sensitive test performance. Accurate and consistent dispensing of reagents will be preferentially performed using non-contact ink-jet technology (e.g. Bio-Dot). Strip manufacture will be done at DCN which has all equipment necessary for this process. A batch method will be applied for application of control and test line reagents on diagnostic membranes attached to a backing card with subsequent membrane blocking and drying. Cards with diagnostic membranes will then be loaded with conjugate pads and sample application pads, or additional plasma separation material. All work will be performed under humidity control. Completely assembled cards will be cut on rotary cutters. Immunetics will provide positive and negative control sera for Quality control. Prototype test kits will be evaluated on a small scale at one or more collaborating sites and at Immunetics to obtain input on usability and preliminary performance and enable improvements, if needed. *Stability studies.* Reagents and completely assembled devices will be subject to real time stability studies at 4°C, room temperature (20-25°C), and accelerated stability at 37°C. Performance of each lot of reagents and assembled devices will be verified using a QC panel comprising 10 - 12 samples containing a range of incidence and prevalence sera, and negative controls. The target shelf life for the rapid test will be 12 months at room temperature.

Aim 1e: Field testing of the rapid test for accuracy, ease of use, reproducibility, interpretation.

- We will design and carry out a study comparing the HIV incidence rapid test with the conventional LAg ELISA method. Immunetics has extensive experience in the design, management, execution and analysis of clinical trials in support of regulatory submissions (its last clinical trial resulted in a 510(k) approval by CBER in August 2013). IRB approval will be obtained for the protocol, potentially exempting it from human subjects regulations as individual subjects will not be recruited but testing carried out anonymously.
- The market for HIV incidence assays has been well understood since at least the mid-1990s to be relatively small and very price-sensitive, yet extremely important to stakeholders, including national and regional governments, so-called normative organizations that set standards and provide guidance, other NGOs, charities, and academic and research institutions [10]. Given this situation, many of these organizations, as well as some small or civic-minded (or both) for-profit companies, have tried different approaches to developing HIV incidence assays and evaluating what they have developed. These efforts, in turn, have led over time to some consensus as to steps required for validation, and we will be guided by this consensus. An example of the guidance for validation of an HIV incidence test, used at the CDC [Y. Duong], is shown in Table 4.

Table 4. Validation and characterization for HIV incidence assays – CDC guidance

Objectives	Specimen Type	Sample Size	HIV Subtype or Cohort	Output Result	Pass Criteria
Laboratory Validation					
Optimization	Serum/plasma	>100 runs on controls	Applicability to all subtypes	Assay precision - %CV for controls	<10% (in the dynamic range)
Commercial kit or accessory kit for modifying diagnostic kit	Serum/plasma and DBS	N/A	N/A	Manufactured product	Manufacturer can produce multiple lots consistently by passing stringent external QC criteria
Mean duration of recency (ω)	Seroconversion panels (ART-naïve)	20-30 panels per subtype	A, B, C, D, AE, and/or other CRFs	• Biomarker kinetics • ω for each subtype • ω overall	• Defined for single subtype • For worldwide application, statistically equivalent ω for all subtypes
FRR	• >1 year (ART-naïve) • AIDS	500 per study population	Minimum 2-3 subtypes	Level of misclassification	<2%
FRR	Elite Controllers	20-30	Minimum 1 set of specimens	Level of misclassification	<2%
Impact of ART	Individuals on ART with clinical information on ART use	50-100	Minimum 1 subtype	Level of misclassification	Inclusion/ Exclusion criteria for ART use

Field Validation					
Incidence Estimates	Cross-sectional survey populations	Varies	Minimum 2-3 survey populations, containing subtype(s) for which an assay will be used	Comparison with modeled or observed incidence, plausibility	Assay-derived incidence estimate should be similar to other methods
Sub-category risk analysis	Cross-sectional survey populations	Varies	Minimum 2-3 survey populations, containing subtype(s) for which an assay will be used.	Comparison with observed risks, plausibility	Odds ratios should be plausible and comparable to observed risks

We have modified this process somewhat to gain field experience with a prototype assay. The prototype assay will be built with the final antigen formulation, and will have demonstrated both satisfactory reproducibility and excellent correlation (>98%, taking into account possible differences in the cutoff) with the LAg lab-based assay across multiple widely occurring HIV Group M subtypes or clades, a major requirement for a test expected to have worldwide distribution. (Differing classification as recent or long term for different subtypes will lead to unacceptably inaccurate estimates of incidence in some populations.) When this milestone has been reached, an evaluation of operability, or the quality of results from field-trained individuals in two sites (one MHRP and one CDC site) will be done. Feedback from this evaluation will be used to make final adjustments in the test configuration itself, if required, but especially in the Instructions for Use (IFU) and the training package.

After the final test kit formulation has been transferred to Manufacturing and three lots have been satisfactorily made, validation largely according to the above table will begin. Here we are fortunate in that large repositories of plasma samples with incidence test results from LAg and various more complex RITAs (recent infection test algorithms), and with clinical data confirming recency or long term infection from several populations of interest, have already been assembled by CDC, CEPHIA (testing at BSRI) and MHRP, and these organizations will test the Immunetics HIVI rapid test against those samples. Populations included among the repository samples (see also the Table above) are

- Seroconverters (individuals with known or closely estimated HIV infection dates) with HIV infections from several different subtypes and circulating recombinant forms). These samples will be tested first, as they are used to evaluate the mean duration of recency, which in turn sets the cutoff for the assay. Statisticians from CDC who have experience with this aspect of the evaluation will work with Dr. Parekhôs group and the Immunetics team to determine the MDR, for which the symbol ór is used.
- False recency samples (from 1. individuals known to have been infected for more than a year but who test órecentô; 2. individuals with AIDS, whose immune systems have been degraded so that they also test recent; 3. elite controllers (individuals whose immune systems control their infections without anti-retroviral therapy, who also sometimes test órecentô) will be tested to determine or confirm a false recency rate (FRR) in different populations. If other aspects of the assay are satisfactory or better, but FRR is >2% in a given populations, exclusion criteria could be considered, or a RITA in which órecentsôare all confirmed (or not) by another incidence assay, by clinical information, or by reflexing to another test such as CD4 or HIV RNA.
- Samples from individuals taking anti-retroviral therapy (ART), which is known to slow or seemingly abort the anti-HIV immune response will be tested to determine whether ART use is an exclusion criterion for the Immunetics HIVI rapid test.

If the results of the laboratory validations confirm that the HIVI rapid test meets the goals established in the final column of the table above, field evaluations using prospective, sequential samples from populations under study, and comparing results with the incidence assay or RITA currently in use at the site, will be launched at two to four sites within and outside the U.S. by CDC and MHRP. In these studies, surveillance technicians working in-country in settings where a rapid test format is preferable to a high-volume system will be trained to run the Immunetics HIVI rapid test, and then will test up to 100 sequential samples as just described in cross-sectional survey populations for incidence estimates or sub-category risk analysis. Correlation of HIVI results with percent recency as determined by other means in this setting will be calculated. The CDC validation protocol above describes a goal for these studies of ósimilarôincidence estimates, because the locally used method may be less well validated. Results will be analyzed by surveillance experts and reported in a publication or presentation. We anticipate that field evaluations will be scheduled to take place starting in the first half of year 3 of the grant. The epidemicôs evolution and multiple other factors will influence where surveillance is being done at that time, but sites will be chosen where different HIV-1 Group M subtypes are circulating.

- Data will be collected in real time as studies proceed, and will be analyzed on an ongoing basis. Publications and presentations will be prepared as analysis becomes available. For statistical analyses of data and objective verification of study results, interpretation and conclusions, we will engage Dr. [redacted]

[redacted] biological statistician. [redacted] has assisted Immunetics in previous clinical studies with similar statistical analyses.

Aim 2a. Analysis of HIVI rapid incidence test sensitivity, specificity and discrimination of recent vs long-term infections.

- The capability of the HIVI rapid test to discriminate recent from long-term infection will be evaluated on well-characterized serum/plasma panels containing known incidence and prevalence specimens, as well as samples from elite controllers and ART-treated or non-treated individuals. The CDC LAg ELISA with false recency rate < 2 at cut-off of 1.5 will be used as the standard of comparison. Panels will be obtained from [redacted] (see letters of support). [redacted] will assist in requesting access to incidence-prevalence panels from CEPHIA (Consortium for the Evaluation and Performance of HIV Incidence Assays, http://www.incidence-estimation.com/cephiaqueries/cephiaDB/overview, supported by a grant from the Bill & Melinda Gates Foundation), including the 200-member Qualification Set and the 3,000 member Evaluation Set which includes a preponderance of global, non-clade B sera. The HIVI rapid incidence test must also exhibit a level of sensitivity of detection of HIV antibodies roughly comparable to that of other HIV screening tests in order to serve the indicated use. Sensitivity will be evaluated using panels of well-characterized HIV-positive sera, low titer sera, seroconversion panels, and global clade panels, all of which are available within Immuneticsôcurrent collection. Specificity will be tested on healthy blood donor sera, of which thousands are available in Immuneticsôfreezers.

Aim 2b. Evaluation of a rapid plasma separation device to enable whole blood testing.

- The HIVI incidence rapid test will be adapted for testing of whole blood samples by a simple, independent plasma separation device (Pall, GE Healthcare, or MDI Membrane Technologies) designed for sample application and transfer of plasma to a diagnostic strip. Our collaborators at [redacted] and [redacted] have extensive experience in development of whole blood rapid tests. Optimization of test performance in whole blood will be performed using a test panel prepared by spiking normal blood samples with a range of titers of HIV sera. The testing of whole blood samples will be evaluated at [redacted] (see letter of support), which will have access to whole blood samples drawn from local HIV patients participating in the Phase I-initiated collection of samples from recently infected, newly diagnosed individuals [redacted] (see letter of support). Assay performance on whole blood vs. serum/plasma will be compared with respect to accuracy of recency classification, and overall sensitivity and specificity.

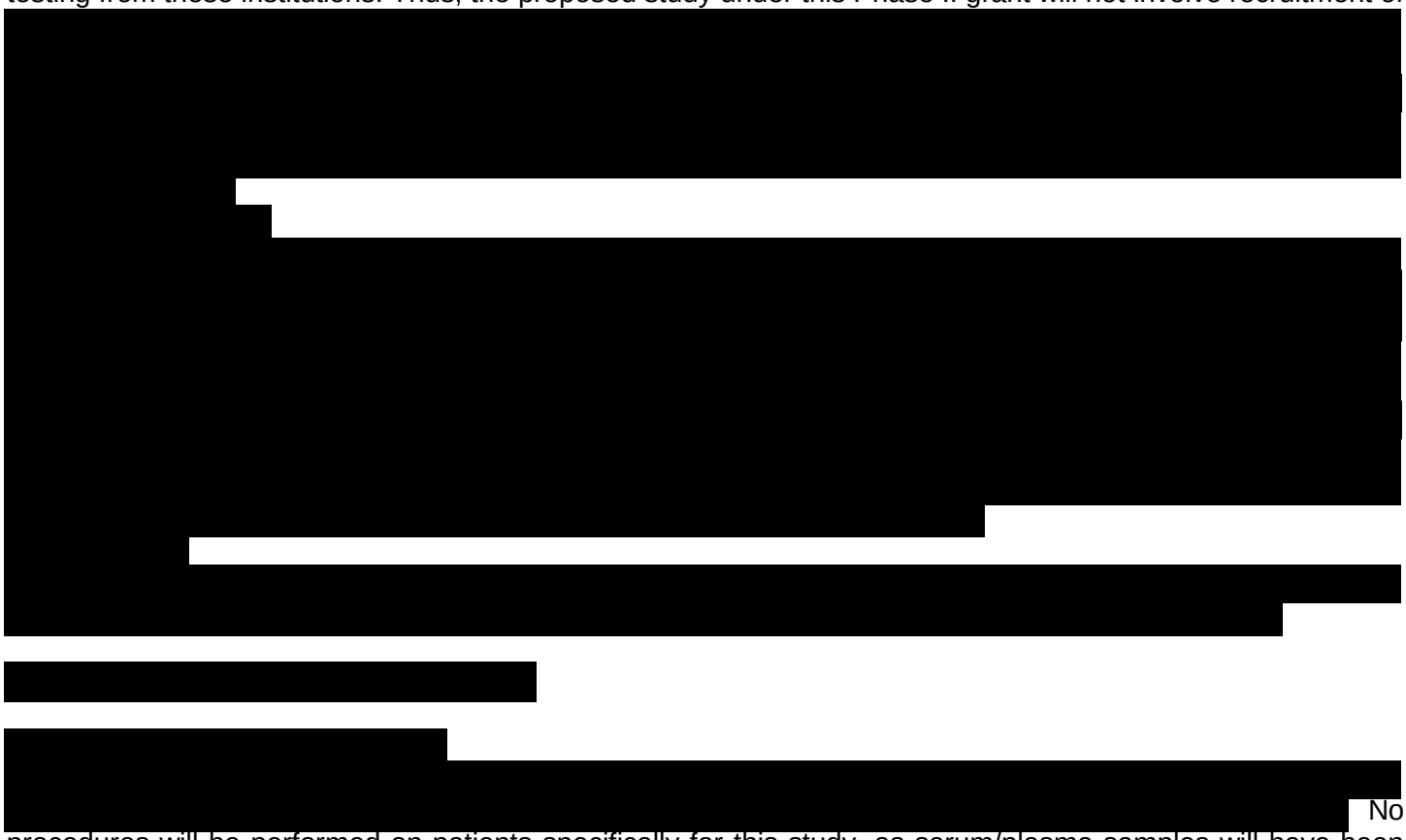
Aim 3. Prepare data from all studies for publication and for submission to FDA for waiver of approval to allow intended use for population surveillance, for submission for CE mark, and for other regulatory approvals as appropriate.

- We intend to submit, together with our collaborators, results of evaluations of the HIVI rapid incidence test for publication in order to promptly disseminate information on its performance and availability. The regulatory situation worldwide will be monitored, and the Immunetics team and our collaborators will stay in touch with regulators and prepare submissions as soon as data analysis allows. As a test to be applied to a population for surveillance rather than to an individual for diagnosis, the HIV incidence rapid test will not be subject to FDA regulation as a diagnostic device. Previous HIV incidence tests including the BED ELISA which is commercially available from Sedia (http://www.hivincidence.com/uploads/LN-6011-02 Product Insert BED EIA sm .pdf) have been waived by the FDA based on the intended use for surveillance only, and the specific exclusion of diagnostic use. However it will be necessary to obtain CE mark registration for commercialization in Europe, and other regulatory requirements may pertain to other regions of the world. We will obtain CE mark registration through [redacted] of the world will be pursued on a case by case basis. We expect the regulatory environment to evolve over the next few years, and we plan to monitor it, and apply for clearances, approval, or waiver as appropriate, for an intended use for population surveillance.

Human Subjects.
IRB approvals will be sought for the proposed studies, although we anticipate that they may be considered exempt. Immunetics possesses FWA #8606 covering work with Human Subjects.

1. Risks to the subjects.

Human subjects involvement and characteristics.
The project's goal is the development of a rapid assay for HIV incidence which will allow discrimination between recent and long-term HIV infection. In the course of the project, we will make use of panels of HIV-positive sera to be provided by CDC, MHRP (WRAIR/DOD), Blood Systems Research Institute (San Francisco), SeraCare and other sources. These sera were collected under IRB approvals by the source institutions which allow their use in our research project. CDC and MHRP will additionally carry out field studies of the rapid HIV incidence test. These studies will be carried out at existing study sites in various global locations (expected to include Africa and Asia) in conjunction with existing, IRB-approved protocols for HIV testing from these institutions. Thus, the proposed study under this Phase II grant will not involve recruitment of



No procedures will be performed on patients specifically for this study, as serum/plasma samples will have been collected as part of ongoing studies by the collaborating organizations.
Protection against risk.
There will be no risk to patients' health presented by this study, as no new procedures will be carried out for this study. The study will rely on serum/plasma samples collected as part of ongoing studies by collaborating organizations including CDC, MHRP, and BSRI. Clinical samples to be provided in the course of the study will be provided to Immunetics devoid of patient identifiers to protect individual privacy.

3. Potential benefits of the proposed research to the subjects and others.

As serum/plasma samples will be de-identified, there will be no human subjects and hence no direct benefit to them. The goal of the project is the development of a rapid test for discrimination of recent vs. long-term HIV infection. As no such rapid test is currently available, development of the proposed test will aid future public efforts which will ultimately affect HIV infected individuals. As such, the test resulting from this work will represent a significant asset to the global HIV prevention effort.

4. Importance of the knowledge to be gained.

While the principal result of the project will be the development of a rapid test for discrimination of recent from long-term HIV infection, it is anticipated that the process of assay development will generate useful knowledge regarding HIV peptide antigens, the immune response in HIV infection and immunoassay methods. The application of a novel rapid assay methodology will be demonstrated, and its discriminatory power, analytical sensitivity and specificity shown. A successful discriminatory test will set a significant precedent, providing an essential monitoring and diagnostic tool for future HIV incidence studies. The value of such information far outweighs the negligible risks posed by the study. Significant findings from the project will be published as scientific papers, while new intellectual property will be patented or protected.

Inclusion of Women and Minorities

Women and minorities will be included to the extent that they are represented in the population of individuals from whom samples have been or will be collected, as no subjects will be enrolled for this study and all samples will be de-identified. As the source regions from which samples will be obtained include the U.S., Africa and Asia, it is expected that a large percentage of samples will be obtained from individuals who correspond to minorities in the U.S. including individuals of black racial and African and Asian ethnic origins. The exact distribution of genders and racial/ethnic origins in the sample population is not under our control, however, but dependent on the study protocols and organizations of collaborating organizations including [redacted]

Program Director/Principal Investigator (Last, First, Middle): Garrett, Patricia.

Targeted/Planned Enrollment Table

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

Study Title: Rapid Test for Recent HIV Infection

Total Planned Enrollment: 0

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	0	0	0
Not Hispanic or Latino	0	0	0
Ethnic Category: Total of All Subjects	0	0	0
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	0	0	0
White	0	0	0
Racial Categories: Total of All Subjects	0	0	0

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

Inclusion of Children

Children will be included in this study to the extent that they are represented in the population of individuals from whom samples have been or will be collected, as no subjects will be enrolled for this study and all samples will be de-identified. The frequency of children in the population to be studied will not be under our control.

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Consortium Arrangements

Overview. This project will be carried out principally at three sites: [redacted] have overall responsibility for administering the grant and managing the work of all consortium partners, including responsibility for all grant finances. The investigator responsible for carrying out the work at each site will be responsible for that site's finances and work product. All consortium participants will abide by NIH policies for grantees and will maintain grant financial records so as to meet NIH financial audit requirements. Disagreements between consortium participants will be resolved by good faith discussions or if necessary, by decision of the Principal Investigator.

Dr. Patricia Garrett, the Principal Investigator, will organize and oversee the performance of all grant work, directly handling the relationships among the organizations developing the assay and those working with us to perform various evaluation tasks outside of the consortium. [redacted] employee at [redacted] Dr. Garrett originated the idea for the joint [redacted] project that led to the Phase I SBIR, and was Principal Investigator for the Phase I SBIR grant held by [redacted] until [redacted]. She worked for [redacted] and its predecessor company Boston Biomedica for more than 24 years. During that time, she was responsible for multiple new panel and control products, and managed many different areas of the growing company, including clinical trials, product development, quality and regulatory, CLIA licensed clinical laboratories, business development and intellectual property. She has worked in HIV incidence since before 1999, and has managed CDC-funded [redacted] to provide serial samples from individuals whose dates of HIV infection can be closely estimated.

[redacted], will direct the assay development work at Immunetics. He has been Principal Investigator on over a dozen grants and contracts from the National Institutes of Health and the Centers for Disease Control and Prevention. He has developed ELISA and Western Blot assays for a range of parasitic, bacterial and viral diseases including Lyme disease ELISA and Western Blot, Cysticercosis Western Blot, Echinococcosis Western Blot, *Babesia microti* ELISA and Western Blot, *T. cruzi* Western Blot, Human Granulocytic Ehrlichiosis (HGA) ELISA and Western Blot, Anthrax ELISA and Western Blot, *H. pylori* Western Blot, *Bartonella henselae* Western Blot, HIV Western Blot, *Ehrlichia risticii* Western Blot, SARS ELISA, Parvovirus B19 Western Blot, and others. Dr. Levin has brought a number of these assays from conception to final product, organized and managed clinical trials to support FDA submissions, developed means to scale up manufacturing, and launched the FDA-approved tests as in vitro diagnostic test kits.

The Principal Scientist, [redacted] as a spin-off in order to develop rapid test methods and technologies. He has specialized in the area of rapid test chemistry for over 30 years, and has developed numerous tests for a wide range of diagnostic applications. His expertise includes synthesis of immunochemical reagents, devising novel detection strategies based on various means of signal amplification, and design and construction of complete rapid test systems. Dr. Kovalenko's group at [redacted] will concentrate specifically on developing antigens, conjugates, substrates, and membrane assay compositions that are the key elements of the HIV incidence rapid test. The resources, equipment, and staff at [redacted] are appropriate for these R&D activities.

[redacted] contribution will be commercial product development and test kit production. [redacted] has a long history of development of ELISA and Western Blot assays for infectious diseases, and has achieved regulatory approval and commercialized many of these in the form of diagnostic kits for clinical laboratory use. Immunetics will provide the complementary capability to carry out assay evaluation studies using clinical specimens, to develop the assay in kit format and launch it commercially. Furthermore, Immunetics has filed a patent application covering the new rapid test technology for multiple other applications. To develop and launch a commercial HIV incidence rapid test, Diagnostic Innovations, Immunetics and [redacted] will cooperate to share the technology owned by each for purposes of this project. Dr. Kovalenko will coordinate work between chemistry development at Diagnostic Innovations, development of diagnostic cassette and integration of strip design at [redacted] and technology transfer to production at Immunetics.

[redacted]

[redacted]

In summary, the contributions [redacted] are necessary to carry out the proposed project. The combination of strengths and capabilities offered by this consortium and scientific partnership will be melded with a straightforward division of responsibilities, and the history of productive work by Drs. Garrett, Kovalenko and Levin will ensure a high probability of success in achieving the project's short and long term goals.

[redacted] specializes in rapid development, manufacture and commercialization of POC tests in various formats - lateral flow, flow through, microfluidic and ELISA for any market segment. The company is ISO 9001-2008 Registered and operates under design controls and GMP as required. [redacted] can design, develop, integrate, validate and transfer to manufacturing all aspects of a point of care assay system, including the assay chemistry and biology, reagents, conjugates, strip design, cassettes, and sample handling devices. [redacted] has performed over 40 successful product development programs for companies in the rapid testing field, in application areas as diverse as medical diagnostics, veterinary diagnostics, bio-warfare, forensics, food microbiology and agricultural product testing.

Additionally, [redacted] of providers includes experts in all aspects of rapid diagnostic device design and development, including reagent developers, reader designers, cassette design and development companies, molders, materials suppliers and manufacturing equipment suppliers, as well as consultants in areas including regulatory affairs, quality systems, market analysis and product distribution. [redacted] will be responsible for development of the cassette housing for the HIV incidence lateral flow test, strip integration and manufacture of pilot lots of rapid test strips and cassettes. [redacted] will be provided with all key reagents, peptide conjugates and substrate system reagents from Immunetics, and will provide manufacturing documentation for all procedures in a package that can be provided to high-volume manufacturing companies for eventual commercial production on a larger scale. [redacted] will communicate with [redacted] a regular basis, including an initial in-person meeting and periodic visits to assess progress and participate in critical decisions regarding product development.

CDC. The U.S. Centers for Disease Control and Prevention has led the effort to develop tests for HIV incidence worldwide, and has funded numerous projects in the U.S. and in resource-constrained regions to build repositories of samples to evaluate these tests. The current most widely used HIV incidence test worldwide (BED, for the HIV subtypes covered), and the current most advanced laboratory incidence test (LAg, for Limiting Antigen) were both developed at CDC by [redacted]. [redacted] has performed, documented, and published thorough validations of their and other candidate incidence test methods. This group has worked with commercial and non-profit organizations interested in assays, sample panels or evaluations of HIV incidence tests for many years. [redacted] are funded in part by PEPFAR (President's Emergency Plan for AIDS Relief), and they also work in the field in Africa and Asia to help agencies in these countries estimate HIV incidence in their general populations and populations at risk. [redacted] also developed a prototype rapid HIV incidence test, citing areas needed for improvement that will inform our efforts [1]. Since Immunetics first contacted CDC about our interest in using the breakthrough technology from [redacted] to develop an HIV incidence assay and offered preliminary data for review, CDC has shared samples of the antigen that is the basis for the LAg assay as well as plasma samples with data from both LAg and BED assays. CDC has communicated the serious need throughout the world for a simple, accurate rapid test for HIV incidence, and has strongly encouraged the Immunetics team to move forward with this assay. [redacted] will assist with the optimization of the assay, particularly advising on the decision for the cutoff to distinguish recent from long term HIV infection, and will conduct laboratory and field studies to evaluate the assay once it is optimized. As a U.S. government agency, CDC cannot receive funding under this grant, but will provide services at its own expense, and is accordingly not included in the grant budget. [redacted] is listed as a key person on the grant in the capacity of consultant.

[redacted]

[redacted]

[redacted] Ten Years of Progress and New Challenges in the Development and Application of Assays for HIV Incidence Estimation from Surveys. AIDS 24:2763-2771, 2010). The group is currently funded by the Gates Foundation in a 3-year initiative called CEPHIA with the UK Health Protection Agency to evaluate 10 lab-based incidence assays, and as part of that effort has assembled panels of more than 3000 pedigreed samples for these evaluations. [redacted] will test the optimized Immunetics HIVI rapid test with these samples, comparing the data generated with results already gathered from LAg and more complex HIV incidence determination algorithms. They will also undertake a small study comparing fresh plasma samples generated by conventional centrifugation to plasma from membrane-based whole blood separation systems to determine whether membrane-separated plasma delivers equivalent results. [redacted] himself is perhaps the leading world expert in HIV diagnostics, and will function as an advisor to Immunetics in addition to leading the studies described here. [redacted] is listed as a key person on the grant.

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: [redacted]
* First Name: [redacted]
Middle Name: [redacted]
* Last Name: [redacted]
Suffix: [redacted]

☐ 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