PI: **MacLeod, Iain James**	Title: PANDAA for universal, pan-lineage molecular detection of Lassa fever infection	
Received: 09/05/2018	FOA: PA18-574 Clinical Trial:Not Allowed	Council: 01/2019
Competition ID: FORMS-E	FOA Title: PHS 2018-02 Omnibus Solicitation of the NIH, CDC, and FDA for Small Business Innovation Research Grant Applications (Parent SBIR [R43/R44] Clinical Trial Not Allowed)	
1 R43 AI145704-01	Dual: TR	Accession Number: 4211012
IPF: 10036337	Organization: ALDATU BIOSCIENCES, INC.	
Former Number:	Department:	
IRG/SRG: ZRG1 IDM-V (12)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted]	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Early Stage Investigator:
Senior/Key Personnel:	*Organization:*	*Role Category:*
Iain MacLeod	ALDATU BIOSCIENCES, INC.	PD/PI
Nicholas Renzette	Aldatu Biosciences	Other (Specify)-Lead Scientist, Assay Development

OMB Number: 4040-0001
Expiration Date: 10/31/2019

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

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED | **Application Identifier**

5. APPLICANT INFORMATION

Organizational DUNS*: [redacted]

Legal Name*: ALDATU BIOSCIENCES, INC.
Department:
Division:
Street1*: [redacted]
Street2: Harvard Life Lab
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02134-1008

Person to be contacted on matters involving this application
Prefix: Dr. First Name*: Iain Middle Name: James Last Name*: MacLeod Suffix:
Position/Title: Chief Science Officer, Aldatu Biosciences
Street1*: [redacted]
Street2: Harvard Life Lab
City*: BOSTON
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02134-1008
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 46-4028776

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*
PANDAA for universal, pan-lineage molecular detection of Lassa fever infection

12. PROPOSED PROJECT
Start Date* Ending Date*
04/01/2019 03/31/2020

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MA-007

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Iain Middle Name: James Last Name*: MacLeod Suffix:
Position/Title: Chief Science Officer
Organization Name*: ALDATU BIOSCIENCES, INC.
Department:
Division:
Street1*: [redacted]
Street2: Harvard Life Lab
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 021340000
Phone Number*: [redacted] Fax Number: 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 EXECUTIVE ORDER 12372 PROCESS?*

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

DATE:

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

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

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

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: David Middle Name: Michael Last Name*: Raiser Suffix:
Position/Title*: Chief Executive Officer
Organization Name*: Aldatu Biosciences
Department:
Division:
Street1*: [redacted]
Street2: Harvard Life Lab
City*: BOSTON
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 02134-1008
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative*

[redacted]

Date Signed*

09/05/2018

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter_LASV_SBIR.pdf

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

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	ALDATU BIOSCIENCES, INC.
Duns Number:	[redacted]
Street1*:	[redacted]
Street2:	Harvard Life Lab
City*:	Boston
County:	
State*:	MA: Massachusetts
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	02134-1008
Project/Performance Site Congressional District*:	MA-007

Additional Location(s) File Name:

Funding Opportunity Number: PA-18-574. Received Date: 2018-09-05T16:54:59.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: 1 __ 2 __ 3 __ 4 __ 5 __ 6 __ 7 __ 8If

NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

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 the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract_LASV_9-2018.pdf
8. Project Narrative*	Project_Narrative_LASV.pdf
9. Bibliography & References Cited	Bibliography_LASV.pdf
10.Facilities & Other Resources	Facilities_LASV_final.pdf
11.Equipment	Equipment_Sept_2018.pdf

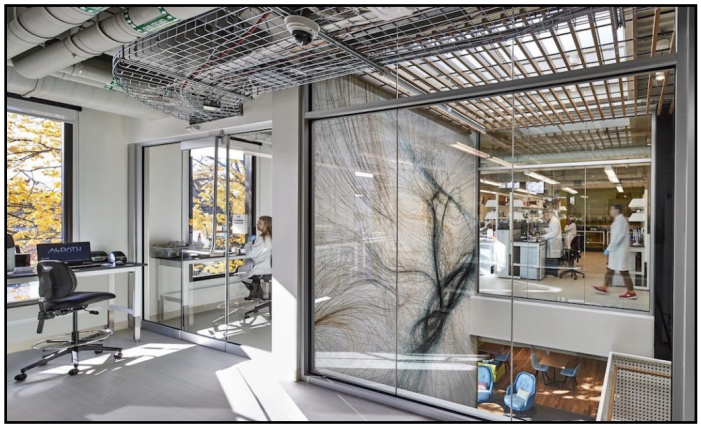
Funding Opportunity Number: PA-18-574. Received Date: 2018-09-05T16:54:59.000-04:00

Lassa virus (LASV), the causative agent of Lassa hemorrhagic fever (LHF), causes 2 million infections and 10,000 deaths each year, and further threatens global health security as a potential cause of epidemics and pandemics. Rapid and accurate diagnosis is critical to global health efforts, with a clear effect on LASV treatment, vaccine development, and outbreak containment. As observed in the 2018 Nigeria outbreak, burdensome and time-consuming diagnostic protocols delay results reporting (e.g. 4 days from sample collection), unnecessarily expose healthcare workers to infection, and, by delaying diagnosis in LASV-negative cases, push the healthcare infrastructure beyond its capacity. qPCR-based molecular assays offer the greatest potential for creating rapid and sensitive LASV diagnostic tools, but high genetic diversity has precluded a pan-lineage, universal diagnostic that sensitively and specifically detects all clades of LASV with equal performance. Multiple assays targeting different genomic regions are used in the clinic in an attempt to mitigate viral genetic variability, necessitating time-consuming, sequential diagnostic protocols. Aldatu's PANDAA technology is a novel platform which enables probe-based qPCR for target detection in highly variable genomic regions by simultaneously adapting and amplifying diverse templates. PANDAA uniquely mitigates the presence of target-proximal polymorphisms to allow otherwise divergent templates to be detected with consensus fluorescent probes with similar sensitivities. Building off of our team's success in development of PANDAA-based assays for SNPs in HIV, another highly polymorphic pathogen, we propose here to leverage the unique capabilities of PANDAA to mitigate lineage-associated genomic variability and develop a rapid, pan-lineage molecular assay for LASV detection. Preliminary feasibility studies have shown that even our as-yet unoptimized PANDAA reagents detect at least five divergent LASV lineages with near equal sensitivity. In this Phase I proposal, we plan to develop and validate an optimized PANDAA-LASV assay through the following aims: (1) initial design of PANDAA-LASV reagents (primers/probes and buffer) using optimized in-house design workflows; (2) refinement of PANDAA-LASV reagents on divergent genotypes representing all circulating lineages; and (3) analytical and clinical validation of a PANDAA-LASV diagnostic assay prototype with panels to rigorously assess sensitivity and specificity. Successful development and validation of the first pan-lineage PANDAA-LASV assay will precede a clinical diagnostic product that could significantly improve LHF diagnosis, management, and outbreak response, effectively reducing the testing algorithm from two tests to one. This novel, universal detection assay could ultimately be deployed in any endemic region on pre-existing qPCR equipment in central labs, and/or integrated into a closed, point-of-care system with sample processing to radically improve the LHF diagnostic workflow.

Rapid and accurate diagnosis of Lassa virus (LASV) infection is critical to global health efforts to prevent and manage outbreaks of Lassa hemorrhagic fever (LHF), with clear impacts on LHF treatment efficacy and LASV vaccine development, but high genetic diversity of circulating LASV lineages has precluded the development of a sensitive molecular diagnostic assay that can be used in all endemic areas and detect all LASV clades with equivalent sensitivity. We propose here to leverage the unique capabilities of Aldatu's qPCR-enabling PANDAA technology to mitigate lineage-associated genomic variability and develop a rapid, pan-lineage molecular assay for LASV detection. Successful development and validation of the first pan-lineage PANDAA-LASV assay will precede a clinical diagnostic product that could significantly improve LHF diagnosis, management, and outbreak response, effectively reducing the molecular diagnostic testing algorithm from two tests to one.

Facilities and Other Resources

Company Research Facilities



Pagliuca Harvard Life Lab: Aldatu Biosciences is based at Pagliuca Harvard Life Lab, an innovative, shared laboratory space for life sciences startups in Boston, MA. A state-of-the art 15,000 sq. ft. facility with fully equipped laboratory, office space, and administrative support that currently houses 16 startups, with room to accommodate approximately 20 ventures. The unique environment allows early-stage life science startups to take advantage of a vibrant, interactive community. Also provided are critical support services that startups need to transition from a science/technology focus to successful commercial-stage enterprises improving patient health. Harvard Life Lab, along with the Harvard Innovation Lab (i-Lab) and Harvard Launch Lab form an early stage life science and technology startup ecosystem designed to foster cross-disciplinary collaboration, entrepreneurship and innovation. Members of the ecosystem gain access to an extensive network of mentors, advisors and investors, both within the Harvard community and throughout the Boston area.

Research Facilities: The shared lab at Harvard Life Lab houses up to 36 scientists, and during the Phase I research project, Aldatu Biosciences will be provided with space in the open lab. There is a private laboratory suite designed to house 6 scientists that can accommodate small business growth, if required in Phase II. The open lab is fully equipped with centrifuges, vortex, shakers, glassware, and basic consumables.

Central Infrastructure: Harvard Life Lab provides autoclaves, bottle wash, ice machines, deionized water, chemical hoods, vacuum and gas supply. Each bench is provided with space in 4°C refrigerators, -20°C and -80°C freezers and liquid nitrogen storage freezers. The main open lab space is bio-safety lab level 1 with dedicated bio-safety level 2 rooms.

Reagent Supply Programs: Life Technologies and NEB, two of the largest global suppliers to customers in scientific research and applied sciences, have reagent programs established at Harvard Life Lab that provides convenience, value and a streamlined service for the supply of core reagents. Inventory is determined by usage patterns, allowing scientists to have instant access to the reagents they use most often, and simplifies the management of our most frequently used reagents. These programs also vastly reduce shipping costs by ensuring that the reagent supply orders are pooled.

Basic Services: Harvard Life Lab ensures the maintenance of permits for all lab operations, mandatory EHS training and compliance for all lab users, maintenance of equipment and technical support, contract research organization support available on-site, and cleaning and waste removal. Office Facilities: Business support services within an open-plan office environment at Harvard Life Lab are designed to foster collegial interactions among biotechnology startup scientists, investors, and academia.

BSL2 Tissue Culture Facilities: Multiple biosafety level 2 (BSL-2) facilities are available. Using BSL-3 practices (frequently denoted as BSL-2+), Aldatu has the resources to safely culture and isolate certain pathogens. Dedicated incubator and centrifugation equipment ensures proper security and biosafety isolation. These facilities are also suitable for handling additional pathogens that are also prevalent in LASV-infected patients.

Network and Computational Resources: State-of-the-art IT services are fully provided by Harvard Life Lab, and

each employee at Aldatu Biosciences is supplied with a laptop computer. In addition, Aldatu hosts a private intranet providing full connectivity between employee laptops, QuantStudio instruments, and a backup storage server. All Aldatu laptops are backed up daily to a Synology DS216+II network-attached storage (NAS) DiskStation, housed behind a firewall administered by Harvard IT. Aldatu utilizes the LabGuru electronic lab notebook service (BioData) for secure record-keeping and intra-company data sharing. LabGuru provides secure, SOC-compliant notebook hosting and daily data backups. Experiment signing and witnessing allow for full compliance with FDA CFR 21 part 11 regulations.

Additional Research Facilities

Harvard Medical School Biopolymers Facility: As members of the Harvard Life Lab, Aldatu has full access to the research core facilities of Harvard University at discounted rates. The Harvard Medical School Biopolymers Facility is a biotechnology service center in the Department of Genetics at Harvard Medical School, with the aim to provide investigators with access to technology services that would be time- and cost-prohibitive to establish and implement within their own research space. Access to the core is through a comprehensive web-based Laboratory Information Management System (LIMS) that provides Aldatu with access to order services and supplies as well as retrieve sequencing data and review and pay invoices all online.

Next Generation Sequencing: The core offers sequencing on Illumina MiSeq, which will be carried out only by facility personnel. Biopolymer facility staff are in constant contact with Illumina to ensure the use of up-to-date MiSeq protocols and reagents, as well as upgrading instrument parts as necessary. Facility staff will run initial quality control before next generation sequencing is initiated. Data will be processed on the Harvard Medical School Orchestra cluster and stored on the Harvard Medical School mass disk storage array, which Aldatu will be able to access directly.

Sanger Sequencing: The core also acts as a facilitator with a local commercial contract research organization to provide Sanger DNA Sequencing services at an extremely competitive price. This will allow Aldatu to perform Sanger sequencing for reagent authentication.as needed for PANDAA assay development. Results are quality checked by Biopolymer facility staff and returned online within one day.

Harvard Innovation Lab: Aldatu Biosciences is part of the Venture Incubation Program at the Harvard Innovation Lab (i-lab). This program provides Aldatu with mentoring, private workshops, foundational learning courses, community building events, and access to expert resources such as one-on-one appointments with legal and entrepreneurial experts-in-residence. With 30,000 square feet of space, with more than 250 workstations, 24 conference rooms, a workshop/prototyping room, a next generation classroom, and video conference suite, the i-lab provides Aldatu team members with opportunities for cross-disciplinary collaboration.

Other Research and Development Resources

Scientific and Business Advisory Board:

Marta Fern ndez Su rez, PhD: Dr. Fernández Suárez has over 10 years of scientific and industry experience commercializing scientific technologies for developing markets. Most recently, Marta served as VP of Assay R&D at Daktari Diagnostics, a HIV diagnostic company, where she developed a complex immunoassay platform designed for point-of-care medical testing. Prior to her role at Daktari, Dr. Fernández Suárez developed a point-of-care diagnostic for tuberculosis as a Postdoctoral Research fellow as Massachusetts General Hospital.

William Rodriguez, MD: Dr. Rodriguez has been a leader in global health for more than two decades. He is the former founder and CEO of Daktari Diagnostics, an HIV diagnostic company, and is now a Managing Director at the Draper Richards Kaplan Foundation (DRK), a global venture philanthropy firm supporting early stage, high impact social enterprises. Dr. Rodriguez was the former Chief Medical Officer at the Foundation for Innovative New Diagnostics (FIND), a global NGO that aids in bringing new diagnostic tools to market in resource-limited settings, and from 2003-2007 he served as Chief Medical Officer of the Clinton Foundation, responsible for strategy and market development for global health products and clinical policies and programs.

Michael W. Henry, MBA: Mike Henry is Senior Vice President and General Manager Consumer Genomics at Centrillion Technologies, a genomics company in Palo Alto, California. He has over 20 years of senior leadership experience in biotechnology business development. Previously, Mike served as Vice President Business

Development at Ancestry, Quest Diagnostics, Athena Diagnostics (he sold Athena to Quest for $740 million in 2011) and Avant Immunotherapeutics. Prior to Avant, he worked in academic medical centers, serving as Senior Director Licensing and Ventures at University of Massachusetts Medical School, and Director Technology Transfer at Children's Hospital of Philadelphia. Prior to CHOP, he served as Manager New Business Development at Allelix, Mississauga, Ontario.

Mickey Urdea, PhD: Dr. Urdea is a Managing Partner for Halteres Associates, a biotechnology consulting firm. He serves as an expert consultant to the life sciences industry and is on the scientific advisory boards and boards of directors for a number of biotechnology, diagnostics, venture capital and philanthropic organizations. Dr. Urdea frequently consults for the Bill and Melinda Gates Foundation (BMGF) and other global NGOs to provide in-depth analyses of diagnostic challenges for a broad range of global health concerns. Formerly, he was the Chief Science Officer at Bayer Diagnostics and the founder of the Catalysis Foundation for Health.

Key Partners

Foundation for Innovative New Diagnostics (FIND): FIND is a non-profit organization based in Geneva, Switzerland in official relations with the World Health Organization (WHO) that functions as a global health product development partnership. Its mission is to turn complex diagnostic challenges into simple solutions to overcome diseases of poverty and transform lives. FIND catalyzes product development, evaluates opportunities, formulates positions, manages specimen banks, and accelerates access to tools and technologies that serve its mission. To achieve this goal, FIND has a network of over 150 partners, including health ministries, research and academic institutes, commercial partners, private-public partnerships, NGOs and over 80 clinical trial sites. For its partners in the private sector, FIND provides analysis of diagnostic needs, technical guidance during development, matching of diagnostic prototypes with researchers and clinical sites, assistance during the regulatory approval process, and support of diagnostic rollout and in country access. As part of Aldatu's pre-Phase I activities, FIND has confirmed the clinical need for improved Lassa virus diagnostics, and its Technology Scouting Team has recently favorably reviewed the core PANDAA technology as well as PANDAA-LASV preliminary data (see **Letter of Support**). Pending acceptable analytical performance, FIND will support Aldatu by providing technical guidance as PANDAA-LASV development progresses, and by supporting future clinical trials with its network of research partners. FIND will also work with its partners (including WHO and Bernard-Nocht-Institute (BNI)) to provide Aldatu with access to clinical LASV isolates representative of the breadth of circulating LASV lineages for the initial clinical sensitivity work proposed in Aim 3.

Bernard-Nocht Institute for Tropical Medicine: The Bernard-Nocht Institute for Tropical Medicine (BNI) (Hamburg, Germany) is Germany's largest institution for research, services and training in the field of tropical diseases and emerging infections. Research topics include clinical studies, epidemiology and disease control as well as the biology of the respective pathogens, their reservoirs and vectors. The current scientific focus is on malaria, haemorrhagic fever viruses, tissue nematodes and diagnostics development. To study highly pathogenic viruses and infected insects, the institute is equipped with laboratories of the highest biosafety level (BSL4) and a BSL3 insectary. Outstanding scientific achievements in the recent past include scientific work concerning the Ebola outbreak in West Africa. BNI also houses the **WHO Collaboration Centre for Arboviruses and Haemorrhagic Fever Reference and Research**. Stephan Günther, Director of the Centre and the Department of Virology at BNI, has committed to supporting the proposed analytical validation with access to a collection of 100+ LASV isolates, as well as supporting clinical evaluations together with FIND, WHO, and other local partners in Nigeria for post Phase 1 work (see **Letter of Support**).

Equipment

Real-Time PCR Capabilities: Aldatu has access to a range of different qPCR instrumentation: the company has a QuantStudio 3 (ThermoFisher), two QuantStudio 5 machines (ThermoFisher), and a Rotor-Gene Q (Qiagen), as well as access to two QuantStudio 3 instruments and one QuantStudio 5 with 96- and 384-well blocks as part of the shared resources within the Harvard Life Lab. Aldatu Biosciences also has a Quantstudio 3D Digital PCR System (ThermoFisher) for absolute nucleic acid quantitation and a ProFlex PCR Thermal Cycler (ThermoFisher)

Through the Harvard Life Lab, Aldatu Biosciences has direct access to critical molecular biology equipment, including: flow cytometers, PCR thermal cyclers, real-time PCR equipment, plate readers, imaging stations, luminometry, high-performance liquid chromatography, Illumina MiSeq 100, and gel electrophoresis boxes. (See also Facilities and Other Resources.)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Iain Middle Name James Last Name*: MacLeod Suffix:

Position/Title*: Chief Science Officer
Organization Name*: ALDATU BIOSCIENCES, INC.
Department:
Division:
Street1*: [redacted]
Street2: Harvard Life Lab
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 021340000

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: PhD Degree Year: 2007

Attach Biographical Sketch*: File Name: Biosketch_MacLeod_090518.pdf

Attach Current & Pending Support: File Name:

Tracking Number: GRANT12705780 Funding Opportunity Number: PA-18-574 . Received Date: 2018-09-05T16:54:59.000-04:00

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Nicholas Middle Name Last Name*: Renzette Suffix:

Position/Title*: Lead Scientist, Assay Development
Organization Name*: Aldatu Biosciences
Department:
Division:
Street1*: [redacted]
Street2:
City*: Boston
County:
State*: MA: Massachusetts
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 021340000

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

Project Role*: Other (Specify) Other Project Role Category: Lead Scientist, Assay Development

Degree Type: PHD Degree Year: 2009

Attach Biographical Sketch*: File Name: NR_Biosketch_Updated_09-04-18.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. **DO NOT EXCEED FIVE PAGES**.

NAME: **MacLeod, Iain J**

eRA COMMONS USER NAME: [redacted]

POSITION TITLE: **Chief Science Officer, Aldatu Biosciences**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	COMPLETION	FIELD OF STUDY
University of Glasgow, UK	B.Sc.	Jun 2003	Virology
University of Glasgow, UK	LL.M.	Sep 2004	International Law
University of Cambridge, UK	Ph.D.	Dec 2007	Pathology (Molecular Virology)
Harvard School of Public Health, MA	Post–Doctoral	Dec 2011	Retrovirology

A. Personal Statement

I have maintained a strong interest in virology throughout my academic career, developing experience with multiple viruses. My undergraduate degree at the University of Glasgow was the only one of its kind in the UK to focus in virology. In the lab of Prof. David Blackbourn, I was involved in the initial characterization of viral interferon regulatory factors (vIRFs) encoded by Kaposi's sarcoma-associated herpes virus. This interest led to a Ph.D. at the University of Cambridge focusing on the induction of intracellular signaling by herpes simplex type-1, with my doctoral training involving multiple technical and analytical facets of molecular virology.

A post-doctoral fellowship in the lab of Prof. Max Essex at the Harvard School of Public Health allowed me to develop my keen interest in the clinical applicability of molecular virology, where I had the opportunity to learn numerous quantitative analytical methods. A year at the Botswana-Harvard AIDS Institute in Gaborone, Botswana advanced my awareness of the role of co-infections in HIV progression and transmission. I have a strong interest in diagnostic molecular virology that have I applied to the development of a cost-effective real-time PCR approach for typing of human papillomaviruses (HPV) infection for resource-limited settings. In collaboration with colleagues at HSPH, I have pioneered the development of Pan Degenerate Amplification and Adaptation (PANDAA), a qPCR-based method for HIV drug resistance detection that is simple, low-cost, and highly sensitive, with point-of-care translatability and multi-parameter superiority to currently available commercial genotyping options.

B. Positions and Honors

Employment

2002 – 2003	Research Assistant, Centre for Drug Misuse Research, University of Glasgow
2004	Research Assistant, Weatherhall Institute for Molecular Medicine, Nairobi, Kenya
2005 – 2006	Research Assistant in International Law, University of Cambridge
2005 – 2007	Supervisor in Pathology, Robinson College, University of Cambridge
2008 – 2011	Post-doctoral Research Fellow, Department of Immunology and Infectious Diseases, Harvard School of Public Health
2010 – 2011	Post-doctoral Research Fellow, Botswana-Harvard AIDS Institute, Botswana
2008 – 2014	Teaching Fellow, Harvard University
2012 – Present	Research Associate, Department of Immunology and Infectious Diseases, Harvard School of Public Health
2014 – Present	Co-Founder and Chief Science Officer, Aldatu Biosciences

Awards

2004 – 2007	Wellcome Trust prize Studentship
2011	Young Investigator Award – Conference on Retroviruses and Opportunistic Infections
2012	Young Investigator Award – Conference on Retroviruses and Opportunistic Infections

C. Contribution to Science

PANDAA: My most recent contributions have been in the development of accessible diagnostics for resource-limited countries, primarily HIV drug resistance genotyping. I have pioneered the development of Pan Degenerate Amplification and Adaptation (PANDAA), a qPCR-based method for HIV drug resistance detection that is simple, low-cost, and highly sensitive, with point-of-care translatability and multi-parameter superiority to currently available genotyping options. PANDAA has resulted in the submission of two provisional patent applications, and an international patent application by Harvard University. Two associated manuscripts have recently been submitted for publication: the first is a point mutation assay design "guide" based on our analyses of the nucleotide variability across RT, protease, and integrase in all HIV-1 subtypes. The second is the core PANDAA methods paper in which we use the K65 and K103 of HIV, and the cleavage site of the Newcastle Disease Virus fusion protein, as proof-of-principle. Additionally, we also intend to submit a manuscript comparing PANDAA with Sanger and next generation sequencing with patients failing first-line ART in Botswana, and using a similar approach for ART-naïve women in Tanzania with transmitted drug resistance. These data have already been presented at several national and international conferences.

Although peer-reviewed publications are still forthcoming, PANDAA led to the formation of a Harvard-based startup company, Aldatu Biosciences, which has been successful in securing Direct to Phase II SBIR funding from the NIH. Furthermore, several collaborations are ongoing with other academic institutes to develop and implement PANDAA for other aspects of HIV drug resistance surveillance, such as in the prevention of mother-to-child transmission.

a. Duarte HA, Panpradist N, Beck IA, Lutz B, Lai J, Kanthula RM, Kantor R, Tripathi A, Saravanan S, **MacLeod IJ**, Chung MH, Zhang G, Yang C, Frenkel LM. *Current Status of Point-of-Care Testing for Human Immunodeficiency Virus Drug Resistance*. J Infect Dis. 2017 Dec 1;216(suppl_9):S824–S828. PMID: 29040621
b. Rowley CF, **MacLeod IJ**, Maruapula D, Lekoko B, Gaseitsiwe S, Mine M, Essex M. Sharp increase in rates of HIV transmitted drug resistance at antenatal clinics in Botswana demonstrates the need for routine surveillance. J Antimicrob Chemother. 2016 May;71(5):1361–1366. PMCID: PMC4830419
c. **Iain J. MacLeod**, Christopher F. Rowley, and Myron E. Essex. "*Methods of Determining Polymorphisms*." Application number: 61884352. 30 September 2013. Patent Pending.
d. **MacLeod IJ**, Rowley CF, Thior I, Wester C, Makhema J, Essex M, Lockman S. *Minor resistant variants in nevirapine-exposed infants may predict virologic failure on nevirapine-containing ART*. J Clin Virol. Elsevier B.V.; 2010;48(3):162–167. PMCID: PMC2909836
e. Rowley CF, Boutwell CL, Lee EJ, **MacLeod IJ**, Ribaudo HJ, Essex M, Lockman S. *Ultrasensitive Detection of Minor Drug-Resistant Variants for HIV After Nevirapine Exposure Using Allele-Specific PCR: Clinical Significance*. AIDS Res Hum Retroviruses. 2010;26(3):293–300. PMCID: PMC2864062

HPV: During my time in Botswana, I conducted a human papillomavirus (HPV) prevalence study in HIV-infected women, which was the first to identify the circulating HPV types in the country. These findings became central in the discussion of HPV vaccine policy in the region, and the Ministry of Health in Botswana incorporated my results into their decision-making process when evaluating which HPV vaccine to implement.

The high prevalence of genital HPV infection in HIV-infected women in Botswana highlighted the need for more robust surveillance in the country, particularly given the forthcoming introduction of an HPV vaccination program. I developed a triplex qPCR assay to screen for high-risk HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68), which was validated against the data obtained from our initial HPV prevalence study. In collaboration with Dr. Scott Peterson, we have implemented this assay in Botswana, training research staff at the Botswana Harvard AIDS Institute. This HPV assay is current being used in-country to estimate the proportion of head and neck squamous cell carcinomas that are linked to HPV infection, particularly those associated with vaccine types 16 and 18, and evaluate the change in the proportion of HPV-associated tumors over time comparing pre- and post-HAART periods. Furthermore, in collaboration with investigators from the Mount Sinai School of Medicine, my HPV diagnostic assay was used to screen biopsies from over 100 HIV-infected patients with anal high-grade squamous intraepithelial lesions. We found that wide range of high-risk HPV types, and that the presence of multiple types per lesion was common. Furthermore, infection with more than one high-risk HPV type was associated with more severe dysplasia.

a. Rantshabeng PS, Moyo S, Moraka NO, Ndlovu A, **MacLeod IJ**, Gaseitsiwe S, Kasvosve I. *Prevalence of oncogenic human papillomavirus genotypes in patients diagnosed with anogenital malignancies in Botswana*. BMC Infect Dis. 2017;17(1). PMCID: PMC5702116

b. **Macleod IJ**, O'Donnell B, Moyo S, Lockman S, Shapiro RL, Kayembe M, van Widenfelt E, Makhema J, Essex M, Wester C. *Prevalence of human papillomavirus genotypes and associated cervical squamous intraepithelial lesions in HIV-infected women in Botswana.* J Med Virol. 2011 Oct;83(10):1689–1695. PMCID: PMC3156037

c. Gaisa M, **MacLeod IJ**, Sigel K, Silverstein M, Kalir T and Goldstone S. *HPV Type Distribution in HIV-infected Patients with Anal HSIL and Impact on Recurrence.* (In Preparation)

HIV evolution and early infection: Both in Botswana and at the Harvard School of Public Health, I mentored a PhD student on a daily basis. We evaluated almost 700 single genome viral sequences generated from 20 patients infected with HIV-1 subtype C (HIV-1C) over 500 days' post-seroconversion, and established that specific amino acid signature patterns were apparent in primary HIV-1C infection compared with chronic infection. I undertook the in vitro characterization of tat mutations on long terminal repeat (LTR) activity and found significant linear relationships between Tat transactivation and patients' plasma viral loads and CD4 counts, highlighting the complex interplay between Tat mutations in early HIV-1C infection.

We performed a similar analysis on the accessory proteins Vif, Vpr and Vpu. Using phylogenetics, we reconstructed the transmitted / founder virus in single genome viral sequences from the same cohort, and identified amino acid signature patterns in Vif and Vpu that were more prevalent in the transmitted virus compared to those in chronic infection. This suggests that there may be a selection bias favoring viral genetic characteristics associated with increased fitness at the transmission bottleneck. Furthermore, I used a bioinformatics approach to evaluate changes in binding affinities of putative MHC class I epitope over 500 days' post-seroconversion. We found that Vif and Vpr epitope binding affinity decreased over time because of polymorphisms under possible immune selection pressure, and that these changes occurred more frequently in epitope anchor residues, resulting in significantly decreased epitope binding.

a. Rossenkhan R, **MacLeod IJ**, Sebunya TK, Castro-Nallar E, McLane MF, Musonda R, Gashe BA, Novitsky V, Essex M. *tat Exon 1 Exhibits Functional Diversity during HIV-1 Subtype C Primary Infection*. J Virol. 2013;87(10):5732–5745. PMCID: PMC3648179

b. Rossenkhan R, **Macleod IJ**, Brumme ZL, Magaret CA, Sebunya TK, Musonda R, Gashe BA, Edlefsen PT, Novitsky V, Essex M. *Transmitted/Founder HIV-1 Subtype C Viruses Show Distinctive Signature Patterns in Vif, Vpr, and Vpu That Are under Subsequent Immune Pressure during Early Infection*. AIDS Res Hum Retroviruses. 2016;32(10–11). PMCID: PMC5067802

Inflammation: Birth outcomes of infants born to HIV-infected mothers has remained a strong interest for a number of years, which began with my work with Prof. Sarah Rowland-Jones in Kenya in 2004 where we looked at inflammatory cytokines in breast milk to determine a possible association between cytokine-mediated transcription of proviral DNA and subsequent mother-to-child transmission. During my time in Botswana, I evaluated inflammatory cytokine transcription in women randomized to receive zidovudine/lamivudine/ABC (Trizivir) or lopinavir/ritonavir and zidovudine/lamividine (Kaletra/Combivir) from the third trimester through six-months postpartum for PMTCT. I found that abacavir may upregulate proinflammatory cytokines at the transcriptional level in this population, which added to the growing body of data demonstrating a small, increased risk of myocardial infarction in HIV-infected adults treated with abacavir, and possibly related to cytokine-mediated inflammation.

Following on from this, as part of a collaboration, we evaluated differences in inflammatory markers between gestation and postpartum periods in women randomized to either breastfeeding or formula-feeding. This was extremely important as limited data existed on inflammation during pregnancy or postpartum in HIV-infected women, despite certain inflammatory markers are associated with adverse health outcomes among HIV-infected persons. Our findings demonstrated that feeding method did not significantly alter inflammatory marker concentrations in young HIV-infected women in Botswana, nevertheless, postpartum TNF-α level was predictive of subsequent adverse clinical event in all women.

a. Russell ES, Mohammed T, Smeaton L, Jorowe B, **MacLeod IJ**, Hoffman RM, Currier JS, Moyo S, Essex M, Lockman S. *Immune activation markers in peripartum women in Botswana: Association with feeding strategy and maternal morbidity*. PLoS One. 2014;9(3). PMCID: PMC3962339

b. **MacLeod IJ**, Rowley CFCF, Lockman S, Ogwu A, Moyo S, Van Widenfelt E, Mmalane M, Makhema J, Essex M, Shapiro RLRL. *Abacavir alters the transcription of inflammatory cytokines in virologically suppressed, HIV-infected women*. J Int AIDS Soc. 2012;15(2):1–8. PMCID: PMC3499794

A complete list of my published work is available at:
http://www.ncbi.nlm.nih.gov/sites/myncbi/iain.macleod.1/bibliograpahy/40312537/public

D. Research Support

Ongoing Support

HHSN272201800050C MacLeod (PI) 09/01/18 – 8/31/21
NIH / NIAID [redacted] Role: **Principal Investigator**
ExPAND for rapid, sensitive, cost-effective detection of drug-resistant HIV minority variants

The primary technical objective of this contract is to develop an assay to detect minor populations of resistant variants in blood specimens from HIV-infected individuals with HIV RNA viral loads above 1000 copies/ml. The test must detect resistant variants that comprise 1% or more of the virus population or quasispecies, and must detect mutations causing resistance to NNRTIs, NRTIs, PIs and INIs in all subtypes of HIV. Sensitivity for qualitative detection of the minor variant at 1% or more must be at least 95% and specificity at least 98%, but the method must also yield quantitative results, showing the percentage of each resistant variant in the overall quasispecies. In this Phase II work, the goal of the proposed project is to advance the assay development work performed in Phase I and develop a product, ExPAND qDR-HIV, which will be a simple qPCR-based HIV genotyping kit capable of detecting drug resistant HIV minority variants.

R44AI128974 MacLeod (PI) 01/07/17 – 12/31/19
NIH / NIAID [redacted] Role: **Principal Investigator**
PANDAA for Rapid Genotyping of HIV-1 Infected Patients Failing Protease Inhibitor ART in Resource-Limited Settings

In this Phase II project, Aldatu will apply the PANDAA technology to the development of PANDAA PIDR+, a rapid, low-cost, thermostable test for detection of drug resistance in patients failing a PI-based ART regimen, which can radically improve clinical decision-making in low- and middle-income countries. Through the aims proposed here, we will 1) experimentally validate the design of PANDAA reagents to quantify mutations associated with protease inhibitor resistance comprising 10% of the viral quasispecies; 2) establish an extensive, collaborative proficiency panel of drug resistant and drug sensitive HIV-1 isolates for PANDAA PIDR+ validation; 3) assess PANDAA PIDR+ using established performance criteria for HIV drug resistance genotyping and produce PANDAA PIDR+ under GMP conditions; and 4) verify that end-user, multi-site implementation of PANDAA PIDR+ is highly reproducible. The first of its kind, a validated, GMP-produced PANDAA PIDR+ test kit from Aldatu will be poised to capture a significant share of this rapidly growing diagnostic market

Completed Research Support

272201600022C MacLeod (PI) 08/15/16 – 12/14/17
NIH / NIAID [redacted] Role: **Principal Investigator**
PANDAA for Rapid, Sensitive, Cost-Effective Detection of Drug-Resistant HIV Minority Variants

Antiretroviral therapy (ART) reduces mortality and morbidity in HIV-infected individuals. With successful therapy HIV RNA becomes undetectable, but drug resistance may occur. Specific HIV mutations are associated with resistance and these mutations can be detected through standard genotypic resistance tests, which have the ability to detect mutations only when they are present in approximately 20% of the virus population within an individual. Importantly, it is now known that the presence of certain resistance mutations, even at very low concentrations within a patient's virus population (1% or more), can contribute to virological failure. These drug resistant minor variants can reflect the early emergence of acquired resistance during therapy and can also be transmitted to newly infected individuals. These minor variants are not detected by standard drug resistance

assays and methods to detect minor variants that contribute to HIV virological failure are needed. These assays would need to detect mutations causing resistance to each of the antiretroviral drug classes (NRTI, NNRTI, PI and INI) in all HIV subtypes, and must be inexpensive, since large numbers of patients would need to be screened. The goal of this project is to develop an assay to detect minor populations of resistant variants in blood specimens from HIV-infected individuals with HIV RNA viral loads above 1000 copies/ml.

R44AI118441 MacLeod (PI) 04/01/15 – 05/31/18

NIH / NIAID [redacted] Role: **Principal Investigator**

A Rapid, Cost-Effective HIV Drug Resistance Diagnostic for Resource-Limited Settings

Existing drug resistance genotyping approaches are costly and complex, and their use in low- and middle-countries is precluded on account of high costs and limited resources. Aldatu Biosciences has pioneered the development of Pan-Degenerate Amplification and Adaption (PANDAA), a simple, low-cost, highly sensitive method for HIV drug resistance detection with multi-parameter superiority to currently available commercial genotyping options. In this Direct to Phase II SBIR, Aldatu will translate the validated lab-based assay into a clinical diagnostic product – PANDAA HIV6 - a thermostable, sample-in/answer-out kit that identifies six clinically actionable HIV mutations found in >95% of patients failing a WHO-recommended first-line regimen. In this Phase II SBIR, we will 1) confirm that an optimized thermostable PANDAA HIV6 prototype can sensitively and specifically quantify drug resistance from HIV-1 infected whole blood, plasma, and dried blood spots using existing RNA extraction methods; 2) demonstrate that PANDAA HIV6 performance metrics fulfill established criteria for both *in vitro* HIV drug resistance genotyping and quantitative assays; and 3) verify that PANDAA HIV6 intended end-user, multi-site implementation is highly reproducible. Successful commercialization of PANDAA HIV6 will result in the availability of an HIV genotyping option that is cost-effective to implement at first-line ART failure in low- and middle-income countries.

[redacted] MacLeod (PI) 08/01/13 - 03/31/15

[redacted] Role: **Principal Investigator**

Immune activation in response to HPV as a risk factor for HIV acquisition

HPV is one of the most common sexually transmitted infections and is considered the etiological agent for invasive cervical cancer (ICC). Epidemiological studies in sub-Saharan Africa and the USA have identified a possible correlation between HPV infection and an increased risk for HIV acquisition. Establishment of HIV infection at mucosal sites is an inefficient process, with a single founder virus responsible for productive infection in ~80% of successful transmissions. Few CD4+ cells are present in intact, non-inflamed mucosa so HIV has to exploit situations in which the innate immune system has promoted the influx of CD4-expressing cells. In this synergistic relationship between HIV and ongoing HPV infection, it has been suggested that individuals clearing HPV may be at increased risk of acquiring HIV as a result of CD4+ cells relocating to the genital mucosa. The clearance of HPV infection is typified by a sustained local increase in pro-inflammatory cytokines that correlate with enhanced lymphocyte trafficking. The interactions between HPV and HIV must be elucidated so appropriate interventions can be developed and implemented to reduce the burden of HIV. These studies will integrate our understanding of two distinct viruses that share a common transmission route.

[redacted] Walker (PI) 08/01/13 - 03/31/15

[redacted] Role: **Virologist**

HPV and HIV-Associated Head and Neck Squamous Cell Carcinoma in Botswana.

I developed an real-time qPCR genotyping assay for high-risk HPV types, used to ascertain HPV-association via immunohistochemistry and multiplex PCR of 300 HNSCC cases in Botswana. In addition, we develop a methodology to assess performance of proviral HIV DNA detection in achieved pathology tissue. These results were used to 1) estimate the proportion of HNSCC that are linked to HPV infection by HIV status, 2) estimate the proportion of HPV- associated HNSCC related to vaccine-contained HPV types (HPV 16/18), and 3) evaluate changes in the proportion of HPV-associated tumors over time comparing pre- and post-HAART periods.

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

BIOGRAPHICAL SKETCH

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

NAME: **Renzette, Nicholas J**

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: **Lead Scientist, Assay Development, Aldatu Biosciences**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	COMPLETION	FIELD OF STUDY
University of Delaware	B.S.	05/03	Biochemistry/Biology
University of Massachusetts, Amherst	Ph.D.	09/08	Molecular Genetics
University of Massachusetts Medical School, Worcester	Post-doctoral	09/15	Viral Molecular and Population Genetics

A. Personal Statement

A common theme of my scientific work for the past 15 years has been the novel application of molecular biology to the study of microbial populations – a theme which continues with the development of PANDAA diagnostic assays. During my doctoral work, I leveraged the vast molecular biological toolbox available for *E. coli* research to disentangle the genetic pathways regulating DNA repair and the SOS response within bacterial populations. As a postdoctoral researcher, I developed a novel molecular biological pipeline that rapidly amplified and deep sequenced human cytomegalovirus (HCMV) populations from a range of clinical samples and led to a rapid expansion of HCMV intra-host population data (~50-fold increase in four years). During this time and while transitioning to a role of Instructor, I built upon the molecular biological methods developed with HCMV to generate the analogous pipelines for the study of Epstein-Barr virus, human herpesvirus 6, influenza A virus and zika virus, leading to novel insights on viral population dynamics. During this time, I was helping to lead a large multi-disciplinary, multi-institution group of researchers focused on the evolution of antiviral resistance – an experience providing invaluable managerial and project management insight. This background has been highly impactful while continuing my career at Aldatu developing PANDAA assays. At Aldatu, I worked closely with Ian MacLeod, our CSO, to co-develop the ExPAND HIV diagnostics platform, a suite of assays with increased specificity, selectivity and sensitivity as compared to first-generation PANDAA assays. By drawing from past experiences with molecular biology and genetically diverse viral populations, I aided in our design, optimization, and validation of ExPAND reagents. This project was recently awarded a Phase II contract from the NIAID to continue our product development and field validation efforts. This work has allowed me to appreciate the power of the core PANDAA technology, while also contributing technical and design elements integral to ExPAND assays. Our proposed strategy to adapt PANDAA technology to pan-lineage Lassa virus diagnostics is a natural evolution of the platform and I am fully confident that my experience and expertise will be of much value as we develop sensitive and robust Lassa virus diagnostics.

B. Positions and Honors

Positions and Employment

2002 - 2003	Research Assistant, DuPont Corporation
2003 - 2008	Research Associate, University of Massachusetts, Amherst
2008 - 2015	Postdoctoral Fellow, University of Massachusetts Medical School
2015 - 2016	Instructor, University of Massachusetts Medical School
2016 - 2018	Technology Specialist, Aldatu Biosciences
2018 - Present	Lead Scientist, Aldatu Biosciences

Other Experience and Professional Memberships

2006 - Member, American Society of Microbiology
2011 - Member, Society for Molecular Biology and Evolution

Honors

2008 Most Outstanding Thesis Research, Curtis B. Thorne Award, University of Massachusetts, Amherst
2009 Ruth L. Kirschstein Individual Postdoctoral Fellowship, NIH
2010 Invited Speaker and Travel Grant Recipient, International Herpesvirus Workshop
2012 Invited Speaker and Travel Grant Recipient, International Herpesvirus Workshop
2014 Young Investigator Award, American Society for Microbiology General Meeting

C. Contributions to Science

1. My thesis research focused on disentangling the genetic pathways of DNA repair and recombination in *Escherichia coli*. Decades of research had shown that RecA-mediated recombination, and specifically the RecA:DNA complex, induces the SOS genetic pathway, a bacterial stress response activated after the detection of DNA damage. SOS induction is a key checkpoint that can drive a cell towards DNA repair or DNA replication and cellular division. However, our in vivo studies showed that RecA:DNA complexes are common, even in cells without high levels of DNA damage and without an activated SOS response. These results led to further genetic studies to dissect the mechanisms regulating SOS induction in the presence of RecA filament formation. We showed that the SOS-inducing activity of the RecA:DNA complexes is variable in populations of *E. coli* and highly dependent on the source of the DNA damage, RecA ATPase activity as well as RecA accessory proteins, such as DinI and RecX. In total, my thesis refined our understanding of a critical checkpoint in bacterial stress response and DNA repair, with implications in studies of gene regulation, antibiotic resistance and cell cycle control.

   a. Renzette N, Gumlaw N, Nordman JT, Krieger M, Yeh SP, Long E, Centore R, Boonsombat R, Sandler SJ. Localization of RecA in Escherichia coli K-12 using RecA-GFP. Mol Microbiol. 2005 Aug;57(4):1074-85. PubMed PMID: 16091045.
   b. Renzette N, Gumlaw N, Sandler SJ. DinI and RecX modulate RecA-DNA structures in Escherichia coli K-12. Mol Microbiol. 2007 Jan;63(1):103-15. PubMed PMID: 17163974.
   c. Renzette N, Sandler SJ. Requirements for ATP binding and hydrolysis in RecA function in Escherichia coli. Mol Microbiol. 2008 Mar;67(6):1347-59. PubMed PMID: 18298444.
   d. Gruenig MC, Renzette N, Long E, Chitteni-Pattu S, Inman RB, Cox MM, Sandler SJ. RecA-mediated SOS induction requires an extended filament conformation but no ATP hydrolysis. Mol Microbiol. 2008 Sep;69(5):1165-79. PubMed PMID: 18627467; PubMed Central PMCID: PMC2538424.

2. As a postdoctoral researcher, I changed model systems from *E. coli* to human cytomegalovirus (HCMV) to increase the relevance of my research to public health. During this time, I focused on the molecular and population genetics of HCMV in vivo infections. Others had shown that genes or even genomes of HCMV, a large dsDNA virus, were variable between patients. These results led to the model that HCMV *inter*-host diversity is a consequence of HCMV *intra*-host diversity. We tested this model with the use of high throughput sequencing and population genetic analysis, showing that HCMV intrahost populations are indeed highly genetically diverse. These findings were the result of novel molecular biological pipeline generated that could amplify diverse viral populations from difficult (i.e. low viral load) samples without biasing the underlying population structure. Using this pipeline, additional studies described a large-scale analysis of HCMV genetic diversity, identifying mutational hot- and cold-spots. Additional analyses elucidated the roles of natural selection and stochastic genetic drift in shaping HCMV diversity, both within individual patients and throughout the HCMV species. Our work has revealed that the majority of HCMV genetic polymorphisms are governed by stochastic mechanisms, which is an important consideration when attempting to develop predictive models of HCMV intrahost and interhost evolution. Collectively, these studies provide the most highly detailed views of HCMV in

vivo evolutionary dynamics and have served as a basis for studies of other DNA viruses, such as HHV-6 and EBV.

a. Renzette N, Bhattacharjee B, Jensen JD, Gibson L, Kowalik TF. Extensive genome-wide variability of human cytomegalovirus in congenitally infected infants. PLoS Pathog. 2011 May;7(5):e1001344. PubMed PMID: 21625576; PubMed Central PMCID: PMC3098220.

b. Renzette N, Gibson L, Bhattacharjee B, Fisher D, Schleiss MR, Jensen JD, Kowalik TF. Rapid intrahost evolution of human cytomegalovirus is shaped by demography and positive selection. PLoS Genet. 2013;9(9):e1003735. PubMed PMID: 24086142; PubMed Central PMCID: PMC3784496.

c. Renzette N, Kowalik TF, Jensen JD. On the relative roles of background selection and genetic hitchhiking in shaping human cytomegalovirus genetic diversity. Mol Ecol. 2015 Jul 24;PubMed PMID: 26211679.

d. Renzette N, Pokalyuk C, Gibson L, Bhattacharjee B, Schleiss MR, Hamprecht K, Yamamoto AY, Mussi-Pinhata MM, Britt WJ, Jensen JD, Kowalik TF. Limits and patterns of cytomegalovirus genomic diversity in humans. Proc Natl Acad Sci U S A. 2015 Jul 28;112(30):E4120-8. PubMed PMID: 26150505; PubMed Central PMCID: PMC4522815.

3. I have studied the evolution of influenza A virus (IAV), and specifically, viral adaptation to novel environments. Antiviral resistance is an area of study with clear links to public health as well as viral evolution. The most commonly used antiviral to treat IAV infections is oseltamivir (Tamiflu) and a resistance mutation (H275Y) in the NA gene had been identified through epidemiological and genetic studies. However, a quantification of the fitness effects of the mutation in the presence and absence of antiviral drug was lacking. Through a large multi-institution collaboration, we used in vitro evolution and population genetics to study the adaptation of IAV to oseltamivir. As for HCMV, I developed a molecular biological pipeline for the unbiased amplification and sequencing of diverse IAV populations. Using a novel statistical method that we developed, we quantified the strong beneficial fitness effect of the resistance mutation in the presence of antiviral and the neutral (i.e., same as wild type) fitness effect in the absence of antiviral drug. This work suggests the persistence of the resistance mutation in natural IAV populations is a result of the fitness effects in the two environments. We have also studied how the virus adapts to changing genetic backgrounds, through the mechanism of segment reassortment, and showed that reassortment follows a largely deterministic, and highly predictable, pathway. These studies have revealed that in contrast to antiviral resistance, IAV adaptation to new hosts involves many loci that are targeted by both positive and negative selection and recurrent “waves” of selection.

a. Renzette N, Caffrey DR, Zeldovich KB, Liu P, Gallagher GR, Aiello D, Porter AJ, Kurt-Jones EA, Bolon DN, Poh YP, Jensen JD, Schiffer CA, Kowalik TF, Finberg RW, Wang JP. Evolution of the influenza A virus genome during development of oseltamivir resistance in vitro. J Virol. 2014 Jan;88(1):272-81. PubMed PMID: 24155392; PubMed Central PMCID: PMC3911755.

b. Foll M, Poh YP, Renzette N, Ferrer-Admetlla A, Bank C, Shim H, Malaspinas AS, Ewing G, Liu P, Wegmann D, Caffrey DR, Zeldovich KB, Bolon DN, Wang JP, Kowalik TF, Schiffer CA, Finberg RW, Jensen JD. Influenza virus drug resistance: a time-sampled population genetics perspective. PLoS Genet. 2014 Feb;10(2):e1004185. PubMed PMID: 24586206; PubMed Central PMCID: PMC3937227.

c. Zeldovich KB, Liu P, Renzette N, Foll M, Pham ST, Venev SV, Gallagher GR, Bolon DN, Kurt-Jones EA, Jensen JD, Caffrey DR, Schiffer CA, Kowalik TF, Wang JP, Finberg RW. Positive Selection Drives Preferred Segment Combinations during Influenza Virus Reassortment. Mol Biol Evol. 2016 Jun;32(6):1519-32. PubMed PMID: 25713211; PubMed Central PMCID: PMC4462674.

d. Jiang, L., Liu, P., Bank, C., Renzette, N., Prachanronarong, K., Yilmaz, L.S., Caffrey, D.R., Zeldovich, K.B., Schiffer, C.A., Kowalik, T.F., Jensen, J.D., Finberg, R.W., Wang, J.P., Bolon, D.N.A. A balance between inhibitor binding and substrate processing confers influenza drug resistance. Journal of Mol. Biol. 2016. 428(3):538-53. PubMed PMID: 26656922. PubMed Central PMCID: In Process.

Complete List of Published Work in My Bibliography – 24 Publications in Total:
http://www.ncbi.nlm.nih.gov/sites/myncbi/nicholas.renzette.1/bibliography/9281431/public/?sort=date&direction=descending

D. Additional Information: Research Support

Ongoing Research Support

HHSN272201800050C MacLeod (PI) 09/01/18 – 08/31/21
NIH / NIAID [redacted] Role: **Lead Scientist**

PANDAA for Rapid, Sensitive, Cost-Effective Detection of Drug-Resistant HIV Minority Variants

The goal of this Phase II work is the further validation, field testing and commercial development of PANDAA assays for the detection of HIV minority drug resistance variants. The assays are based on the ExPAND technology, a second generation PANDAA derivative technology that increases sensitivity and selectivity of the diagnostic platform. Phase I validation work showed the assays detect drug resistance variants at very low frequency (< 1%) from samples with low copy number (< 1000 copies/mL). ExPAND assays were developed to cover resistance mutations associated with all HIV drug classes (NRTI, NNRTI, PI and INI). Phase II activities include further streamlining the assays to reduce costs and end user labor, large scale clinical sensitivity and specificity testing, and site-to-site variability studies.

Completed Research Support

[redacted] Finberg (PI) 09/15 - 09/20
Role: Co-Investigator
Anticipating Influenza Resistance Evolution (AIRe): Pathways and Strategies
Investigating the evolution of influenza A virus in avian, swine and human host environments and the adaptive barriers to influenza host switching.

[redacted] Finberg (PI) 09/11 - 04/14
Role: Co-Investigator
[redacted]
Algorithms to Limit Viral Epidemics (ALiVE)
Developed novel approaches to sequence influenza virus genomes that seed nascent algorithms with datasets for the development of predictive modeling.

F32 AI084437 Renzette (PI) 09/09 – 08/11
Role: Principal Investigator
Genomic Variability of Cytomegalovirus in a Clinical Setting
The goal of this fellowship was to use population genetics with high throughput sequencing technology and other assays to better understand the population dynamics of HCMV within human hosts.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: ALDATU BIOSCIENCES, INC.

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	Iain	James	MacLeod		PD/PI	[redacted]	1.2			[redacted]	[redacted]	[redacted]
2 .	Dr.	Nicholas		Renzette		Lead Scientist, Assay Development	[redacted]	3.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Associate	10.8			[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-18-574 . Received Date: 2018-09-05T16:54:59.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: ALDATU BIOSCIENCES, INC.

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **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	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

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**	**0.00**

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

Tracking Number: GRANT12705780 Funding Opportunity Number: PA-18-574 . Received Date: 2018-09-05T16:54:59.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: ALDATU BIOSCIENCES, INC.

Start Date*: 04-01-2019 **End Date*:** 03-31-2020 **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 . G&A	[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 ($)*
	[redacted]

K. Total Costs and Fee	Funds Requested ($)*
	[redacted]

L. Budget Justification*	File Name: Budget_Justification_LASV_final.pdf (Only attach one file.)

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

Tracking Number: GRANT12705780 Funding Opportunity Number: PA-18-574 . Received Date: 2018-09-05T16:54:59.000-04:00

Budget Justification

Please note that this grant proposal falls under NIAID Division of Microbiology and Infectious Diseases waiver topic B: "*Development of rapid, highly sensitive and specific clinical diagnostics that are easy to use, cost-effective and can diagnose individuals infected with pathogens or individuals that have been exposed to toxins*".

The additional budget over the typical cap is requested for the necessary primer synthesis in Aim 1, as well as for a full-time research associate, who will be closely managed by our Lead Scientist for Assay Development (Dr. Renzette) and supervised by the project PI (Dr. MacLeod), in order to complete the proposed assay development work (all Aims).

A Personnel

Iain MacLeod, LLM PhD, Principal Investigator (Effort: Year 1 – 1.2 calendar months). Dr. MacLeod is co-founder and Chief Science Officer at Aldatu Biosciences. Dr. MacLeod also holds a part-time academic appointment in the Department of Immunology and Infectious Diseases, Harvard School of Public Health. Dr. MacLeod developed the novel PANDAA technology that is the focus of this SBIR application. He will be responsible for general oversight of the work proposed in this SBIR submission. Dr. MacLeod will play an integral role in the design of proprietary primer and probe reagents for use in PANDAA-based detection of LASV. He will be responsible for the overall direction of the project and interpretation and dissemination of the study results, including the associated statistical analyses, and will coordinate data storage and security for all aspects of the work. He will be responsible for ensuring that any clinical samples obtained during these studies fulfil any IRB requirements, and that all Aldatu employees undergo the necessary human subject research training. Dr. MacLeod will also be responsible for compiling and submitting progress reports as required by the NIAID.

Nicholas Renzette, PhD, Lead Scientist, Assay Development (Effort: Year 1 – 3 calendar months). Dr. Renzette has extensive experience in virology and infectious diseases, with a focus on developing tools to study of diverse viral populations. He has published extensively in the development of novel next generation sequencing approaches for polymorphic DNA and RNA viral pathogens. At Aldatu Biosciences, Dr. Renzette has spent more than two years designing, optimizing, and validating “second-generation” PANDAA assays for drug-resistant HIV detection, and during such time has become an expert in the unique design and assay development considerations for PANDAA-based assays. During these proposed studies, he will be primarily responsible for designing and validating PANDAA reagents for LASV detection, in close collaboration with and under the supervision of Dr. MacLeod. Given his expertise, he is extremely well-suited to drive the work proposed in this SBIR submission.

Stephan G nther, MD, will be an **Other Significant Contributor** on this project with no measurable effort. Dr. G nther will provide ad hoc advisory support for the proposed work as an unpaid consultant. Dr G nther (MD) is the Head of Department of Virology, the Biosafety level 4 laboratory, and WHO Collaborating Centre for Arboviruses and Hemorrhagic Fever Reference and Research at the Bernhard-Nocht-Institute for Tropical Medicine in Hamburg, Germany, and adjunct professor at the University of Hamburg. He studied medicine and specialised in virology, microbiology, and infection epidemiology. Dr. G nther’s research is dedicated to viral hemorrhagic fevers (VHF), including Ebola virus disease, Lassa fever, and Crimean-Congo hemorrhagic fever. The clinical research programs he manages are focused on pathophysiology of VHF and the development of diagnostic tools. He and his research team developed the RT-PCR-based LASV assay on which the most widely used commercial LASV molecular diagnostic test (RealStar® Lassa Virus RT-PCR Kit CE) is based. Dr. G nther is ideally suited to advise the Aldatu R&D team in this LASV diagnostic assay development work, and beyond. (See Letter of Support).

TBD, Research Associate (Effort: Year 1 – 10.8 calendar months). The TBD research associate (Ph.D. or M.S. + 2yrs industry experience) will be responsible for the execution of research and development experiments related to PANDAA-LASV. Primary duties will include testing of primers to identify optimal primer binding sites, comparison of PANDAA probe sensitivity, evaluation of PANDAA primer design, optimization of PANDAA-LASV buffer formulation and primer / probe concentrations, and performance of analytical sensitivity and specificity efforts. The research associate will also be responsible for thorough record-keeping and effective communication of results to the other members of the PANDAA-LASV R&D team. Dr. MacLeod and Dr. Renzette will work closely with the research associate to ensure completion of the Phase I milestones in a manner consistent with the proposed timeline.

Fringe rate: Aldatu’s fringe rate for the duration of this Phase I SBIR i[redacted], which includes: paid time off (PTO), all payroll-associated taxes, workers’ compensation, unemployment compensation, limited life insurance, and employer contributions to medical and dental insurance.

B SBIR Fee

We request an SBIR fee of [redacted] in Year 1.

C Supplies

General lab reagents and supplies: [redacted] is budgeted for general consumables. These supplies include plasticware (Eppendorf tubes, Falcon tubes, PCR tubes, micropipettes tips,) and general lab supplies (gloves, face masks, protective eyewear, parafilm, syringes, needles, filters, pipettes etc.).

D Breakdown of Materials and Supplies Requested for Aims 1 to 3

Oligonucleotides	
Synthetic DNA templates	[redacted]
Primer synthesis	[redacted]
Probe synthesis	[redacted]
PANDAA qPCR Reagents	
PANDAA custom mastermix	[redacted]

We have provided a breakdown of our justification for the materials and supplies required.

D1 Aim 1 [redacted]

Aim 1 Design and Optimization of PANDAA-LASV primers, probes and buffer.

Aim 1 costs cover the synthesis of primers and probes to empirically determine the optimal design LASV RNA detection. Costs also cover the synthesis of synthetic DNA and in vitro transcription of RNA used in the evaluations, as well as the custom qPCR mastermix that has been developed for multiplexed PANDAA.

Oligonucleotides	
Primer synthesis	[redacted]
Synthetic DNA templates	[redacted]
PANDAA qPCR Reagents	
PANDAA custom mastermix	[redacted]

D2 Aim 2 [redacted]

Aim 2 Refinement of PANDAA reagents on divergent genotypes.

Aim 2 costs cover the synthesis of modified variants of primers developed in Aim 1 to increase pan-lineage sensitivity, as well as the large volume manufacturing of the optimal custom mastermix, determined in Aim 1.

Nucleic Acids	
Arenaviruses/Other Pathogens	[redacted]
Human Serum	[redacted]
PANDAA qPCR Reagents	
PANDAA Custom Mastermix	[redacted]

D3 Aim 3 [redacted]

Aim 3 Analytical and clinical validation of PANDAA diagnostic prototype.

Costs for Aim 3 are associated with obtaining and purifying nucleic acid from closely related arenaviruses, pathogens causing febrile illness, and human sera from commercial sources. In additions costs are associated with the large scale studies for analytical and clinical validation of the PANDAA-LASV prototype.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		[redacted]
Section K, Total Costs and Fee (I + J)		[redacted]

OMB Number: 4040-0001
Expiration date: 10/31/2019

SBIR/STTR Information

Agency to which you are applying (select only one)*

❍ DOE ● HHS ❍ USDA ❍ Other:

SBC Control ID:* [redacted]

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)

Application Type (select only one)*

● Phase I ❍ Phase II ❍ Fast-Track ❍ Direct Phase II ❍ Phase IIA ❍ Phase IIB

❍ Commercialization Readiness Program (See agency-specific instructions to determine application type participation.)

Phase I Letter of Intent Number:

* Agency Topic/Subtopic:

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

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?* ● Yes ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 8

1c. Is your small business majority owned by venture capital operating companies, hedge funds, or private equity firms?* ❍ Yes ● No

1d. Is your small business a Faculty or Student-Owned entity?* ❍ Yes ● No

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

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?* ● Yes ❍ No

If no, provide an explanation in an attached file. Explanation:*

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?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

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 email address of the official signing for the applicant organization to state-level economic development organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ❍ No

7.Commercialization Plan: The following applications require a Commercialization Plan: Phase I (DOE only), Phase II (all agencies), Phase I/II Fast-Track (all agencies). Include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

OMB Number: 4040-0001
Expiration date: 10/31/2019

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.

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.* ● Yes ❍ No

Attach File:* Commercialization_History_Sept_2018.pdf

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ● Yes ❍ No

STTR-Specific Questions:

Questions 10 - 12 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 - 12 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ❍ Yes ❍ No

(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?

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?* ❍ Yes ❍ No

12. Provide DUNS Number of non-profit research partner for STTR.*

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Commercialization History/Prior Phase II SBIR Awards

Aldatu Biosciences has not received more than 15 SBIR Phase II awards from the Federal Government during the preceding five fiscal years. The Phase II SBIR awards that Aldatu has received are listed below:

R44AI118441 MacLeod (PI) 06/01/15 – 5/31/18
NIH / NIAID
A Rapid, Cost-Effective HIV Drug Resistance Diagnostic for Resource-Limited Settings

R44AI128974 MacLeod (PI) 01/17/17 – 12/31/19
NIH / NIAID
PANDAA for rapid genotyping of HIV-1 infected patients failing protease inhibitor ART in resource-limited settings

HHSN272201800050C MacLeod (PI) 09/01/18 – 8/31/21
NIH / NIAID
ExPAND for rapid, sensitive, cost-effective detection of drug-resistant HIV minority variants

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ❍ Yes ❍ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

❍ Yes ❍ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*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)

Tracking Number: GRANT12705780 Funding Opportunity Number: PA-18-574 . Received Date: 2018-09-05T16:54:59.000-04:00

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3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

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://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ❍ Yes ● No

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

*Previously Reported: ❍ Yes ❍ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12705780 Funding Opportunity Number: PA-18-574 . Received Date: 2018-09-05T16:54:59.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
2. Specific Aims	Specific_Aims_LASV_final.pdf
3. Research Strategy*	Research_Strategy_LASV.pdf
4. Progress Report Publication List	
Other Research Plan Section	
5. Vertebrate Animals	
6. Select Agent Research	Select_Agents_LASV_final.pdf
7. Multiple PD/PI Leadership Plan	
8. Consortium/Contractual Arrangements	
9. Letters of Support	LoS_all_fixed_margins_LASV_final.pdf
10. Resource Sharing Plan(s)	
11. Authentication of Key Biological and/or Chemical Resources	Authentication_of_Key_Reagents_LASV.pdf
Appendix	
12. Appendix	

Tracking Number: GRANT12705780
Funding Opportunity Number: PA-18-574. Received Date: 2018-09-05T16:54:59.000-04:00

Background and Significance: Lassa virus (LASV), the causative agent of Lassa hemorrhagic fever (LHF), causes 2 million infections and 10,000 deaths each year, and further threatens global health security as a potential cause of epidemics and pandemics. Rapid and accurate diagnosis is critical to global health efforts, with a clear effect on LASV treatment, vaccine development and outbreak containment. The efficacy of current antiviral treatment strategies is limited to early stage infection and thus requires diagnostics capable of delivering results during this time. While the WHO has prioritized the development of a vaccine against Lassa, they have also recognized that the first step towards this goal is an improvement of Lassa diagnostics, as the current diagnostics do not provide reliable incidence or distribution data and are insufficient for any future vaccine efficacy study. Lastly, the failings of diagnostics for outbreak containment became clear during the 2018 Nigeria LASV outbreak, the largest of its kind on record. Burdensome and time-consuming diagnostic protocols delay results reporting (e.g. 4 days from sample collection), unnecessarily expose healthcare workers to infection, and, by delaying diagnosis in LASV-negative cases, push the healthcare infrastructure beyond its capacity.

Of the molecular assays available for LASV, qPCR offers the greatest potential for creating a rapid and sensitive clinical diagnostic tool. However, the genetic diversity of the virus has precluded a pan-lineage, universal diagnostic that sensitively and specifically detects all clades of LASV with equal performance. This shortcoming is well-documented in the literature and is addressed in the clinic by employing multiple assays targeting different genomic regions, in an attempt to mitigate viral genetic variability. Even with this approach, dubious results occur and thus multiple, independent, time-consuming diagnostic protocols need to be employed.

Innovation: Aldatu Biosciences has pioneered the use of PANDAA technology, which enables probe-based qPCR for target detection in highly variable genomic regions by simultaneously adapting and amplifying diverse templates. PANDAA uniquely mitigates the presence of target-proximal polymorphisms to allow otherwise divergent templates to be detected with consensus fluorescent probes with similar sensitivities. Our experience in reaction buffer optimization further enables PANDAA to maximize assay sensitivity and specificity.

Preliminary Feasibility: Aldatu Biosciences is uniquely positioned to deliver a rapid pan-lineage qPCR-based LASV diagnostic. Our technology has been successfully applied to development of subtype-independent drug resistance mutation (DRM) detection in HIV. We have designed assays for more than fifteen DRM targets covering all major HIV drug classes. Analytical and clinical validation studies have shown quantification of DRMs at very low frequency (<1%) and low copy number (>5 copies) across HIV subtypes. Excitingly, we have performed preliminary studies with LASV templates from multiple lineages, showing that even our as-yet unoptimized PANDAA reagents detect at least five lineages with near equal sensitivity and outperform the current gold standard assay by greater than an order of magnitude in terms of cross-lineage sensitivity.

Approach: We propose to leverage the unique capabilities of PANDAA to develop a rapid, sensitive molecular diagnostic assay for LASV detection, and the first with pan-lineage coverage, through the following specific aims:

Aim 1 Design of PANDAA-LASV primers and probes and reaction optimization **Months 0 - 6**

We will draw on our experience and optimized workflows to develop PANDAA-LASV primers and probes against a novel target in highly conserved regions of the LASV genome, as well as a custom reaction buffer. Reagent sensitivity will be analyzed on LASV reference sequences and empirically optimized. Milestone: Optimized PANDAA primers/probes and buffer with limit of detection (LoD) ≤10 RNA cps/reaction for LASV strain Josiah.

Aim 2 Refinement of PANDAA-LASV reagents on divergent genotypes **Months 6 - 9**

Reagents from our preliminary studies and Aim 1 will be evaluated on divergent LASV templates encompassing all circulating lineages. Iterative designs incorporating pre-established molecular techniques, such as PANDAA ProAmp and/or universal bases, will be evaluated to normalize sensitivity across lineages. Milestone: Refined PANDAA-LASV assay for which LoD ≤10 RNA cps/reaction and sensitivity deviation <25% between lineages.

Aim 3 Analytical and clinical validation of PANDAA-LASV diagnostic prototype **Months 9 - 12**

A pan-lineage analytical validation panel and probit analysis will be used to determine 95% detection limit. Serial dilutions of spiked serum will quantify LoD for purified samples. Specificity evaluation will be carried out with LASV-negative human serum, related arenaviruses, and other pathogens that cause febrile illness. Clinical sensitivity will be quantified with diverse-lineage LASV clinical isolates obtained via partnerships with FIND/BNI. Milestone: Prototype PANDAA-LASV assay with the following specifications: pan-lineage 95% detection limit of ≤10 RNA cps/rxn, negative signal from non-LASV templates (Cq >37 cycles), and clinical sensitivity >95%.

Long-Term Goal: Successful development and validation of the PANDAA-LASV assay will precede a clinical diagnostic product that could significantly improve LHF diagnosis, management, and outbreak response, effectively reducing the testing algorithm from two tests to one. This novel, pan-lineage detection assay could ultimately be deployed in any endemic region on pre-existing qPCR equipment in central labs, and/or integrated into a closed, point-of-care system with sample processing to radically improve the LHF diagnostic workflow.

Research Strategy

A Significance

A1 Lassa Virus is a global health threat. Lassa virus (LASV), the causative agent of Lassa fever, is endemic to West Africa, where 100-300,000 infections, and 5,000 LASV-associated deaths, occur each year.[1] Moreover, serology studies in endemic areas suggest that many infections are unreported and the true incidence is likely much higher.[2–5] Globally, up to 2 million infections and 10,000 deaths occur per year due to LASV.[6] Due to long incubation periods, LASV is one of the most commonly exported causes of viral hemorrhagic fever, raising concerns about pandemic preparedness and global health security.[7,8] Although the natural LASV reservoir is the multimammate rat, human-to-human transmission in community or hospital settings is a public health concern.[9] Indeed, due to the high probability of LASV epidemics, a single suspected LASV infection in endemic countries is cause for mandatory reporting to the WHO's Integrated Disease Surveillance and Response (IDSR) system. With continued economic and population growth in West Africa, along with environmental disruptions associated with climate change, the impact of LASV on global health will likely increase.[6] By 2070 it is predicted that the geographical distribution of LASV endemic areas will double and the frequency of LASV outbreaks will increase over two-fold.[10]

A2 Timely diagnosis of LASV infection is critical to effective treatment. The sole approved therapeutic for LASV infections is the broad-spectrum antiviral ribavirin. Ribavirin treatment is significantly associated with decreased mortality rates if administered within the first six days of symptom onset. After this period, the benefits of treatment decrease and mortality rates are similar to that of a no treatment control group.[11] Thus, timely diagnosis of LASV infections is critical to effective treatment. Insufficient diagnostic infrastructure is the primary barrier to timely LASV treatment without which clinicians must rely on differential diagnosis. However, Lassa fever typically presents with symptoms also displayed by other infectious agents endemic to West Africa e.g. malaria, yellow fever, dengue fever and influenza.[12] Consequently, antimalarials or antibiotics are initially prescribed, and if symptoms do not resolve, LASV infection is suspected. This delay reduces the likelihood of effective ribavirin treatment while increasing LASV transmission to patient visitors and healthcare workers.[12]

A3 Current diagnostic strategies lack speed or sensitivity. Traditional laboratory diagnosis relies on either viral culture or indirect fluorescent-antibody (IFA) assays, though neither is suitable for rapid diagnostics.[12,13] ELISA-based assays to detect LASV antigen, and host IgM and IgG antibodies, are faster and demonstrate improved sensitivity.[13,14] A critical limitation of immunoassays, though, is that the detection window after symptom onset for antigen (~7.9 days) and host IgM (~13.1 days) is outside the window for successful ribavirin treatment (<7 days).[13]

RT-PCR provides timelier diagnosis of LASV, detecting viral RNA 3-5 days after symptom onset.[15,16] Several RT-PCR protocols have been developed with the most commonly used assays based on Demby et al's protocol from 1994 (GPC RT-PCR/1994).[15] This was refined by Ölschläger et al. in 2010 (GPC RT-PCR/2007),[17] and subsequently commercialized (altona Diagnostics, Hamburg, Germany). GPC RT-PCR/2007 modified primer design to improve sensitivity after clinical samples in West Africa demonstrated that viral genomic heterogeneity led to false negatives with the original primer.[17] Despite the improved design, the limit of detection varied ~8-fold among strains due to strain-specific polymorphisms.[17] Although a RT-PCR could improve linkage between diagnosis and effective treatment, a universal RT-PCR assay with equal performance for all LASV infections is not currently available.

A4 2018 Nigerian Outbreak – A case study of current diagnostic needs. In 2018, Nigeria experienced the largest reported Lassa fever outbreak with at least 2,395 suspected cases.[18] Although a concerted effort was put forth to improve the healthcare system following the 2014-15 Ebola virus epidemic, the recent LASV outbreak revealed ongoing diagnostic gaps.[19] The current diagnostic standard is two RT-PCR assays, targeting separate genomic regions to mitigate false negatives associated with viral variability: the altona test combined with a secondary RT-PCR e.g. Nikisins et al.[20] When conflicting results are presented, the RT-PCR products are sequenced or resolved by agarose gel to confirm amplicon size. Employing multiple tests is burdensome particularly in an epidemic of this scale where it took up to 4 days to receive test results.[21] In some states, up to 60% of LASV cases are not diagnosed until after death, partially due to the slow diagnostic turnaround times.[19] The diagnostic delays unnecessarily exposed healthcare workers to LASV, and at least 39 healthcare workers were infected.[18] Delays in confirming those patients *without* LASV infections - almost 80% of all suspected cases in this outbreak[18] - compromised disease containment due to limited isolation ward capacity.[14,22] The outbreak exposed significant weaknesses in the current diagnostic protocols and emphasized the need for a simple and sensitive assay that reduces the diagnostic bottleneck.

A5 LASV genetic diversity is a hurdle for a universal diagnostic assay. LASV clusters into at least four evolutionary lineages that correlate with geographical distribution.[23–25] Lineages vary 19-27% at the nucleotide level.[23] As noted, viral sequence heterogeneity is a significant obstacle in generating a pan-lineage PCR diagnostic assay, due to polymorphisms in primer- and/or probe-binding sites.[26] Given the high level of genetic diversity and geographical clustering of LASV, it has been proposed that country- or region-specific diagnostic assays be developed.[12] This approach is not ideal as it requires the development, validation and deployment of multiple kits.[19]

A6 PANDAA overcomes the hurdles preventing rapid, pan-lineage LASV diagnostics. Despite its assumed limitations, particularly when targeting heterogenous viral populations, quantitative real-time PCR (qPCR) maintains the greatest potential for LASV diagnostic development, rather than more resource-intensive approaches such as population sequencing, due to its high detection sensitivity and short turnaround time. Aldatu's Pan-Degenerate Amplification and Adaptation (PANDAA) technology uniquely enables probe-based qPCR for target detection in highly variable genomic regions by mitigating variability in both the primer and probe-binding sites without sacrificing assay specificity. PANDAA will be used to mitigate the cross-clade nucleotide diversity of LASV, where other PCR approaches cannot, to develop a first-ever universal LASV clinical diagnostic (PANDAA-LASV). The unique capabilities of PANDAA will allow Aldatu to develop a significant solution for dramatically reducing false negatives and provide an impactful tool in the global effort to respond to Lassa epidemics.

B Innovation

B1 A novel diagnostic concept: Pan-Degenerate Amplification and Adaptation (PANDAA). Focused genotyping is a diagnostic approach that identifies substitutions at key positions in clinically-relevant genomic targets rather than more resource-intensive approaches such as population sequencing. Point mutation assays (PMAs; e.g. allele-specific PCR, oligonucleotide ligation assay, and probe-based quantitative real-time PCR [qPCR]) are the best candidates for focused genotyping. PMAs have high detection sensitivities for single nucleotide changes. However, they return a false negative result in the presence of a secondary mutation (e.g. a proximal nucleotide change that is clinically irrelevant). This genomic variability is the primary bottleneck restricting clinical implementation of PMAs for focused genotyping of LASV. Although the negative impact of sequence variability of probe-based qPCR performance has been known for almost 20 years, numerous attempts to overcome it have failed.

PANDAA is a novel qPCR design concept that is superior to current genotyping approaches in its simplicity and economy (Figure B.2). Although population nucleotide diversity reduces, or even eliminates, primer and probe-binding in traditional qPCR, PANDAA overcomes this limitation through two complementary design characteristics (Figure B.1). PANDAA mitigates high intra- and inter-strain genomic variability by removing secondary polymorphisms, minimizing their impact on qPCR sensitivity and specificity. Simultaneous mutagenesis during qPCR amplification by PANDAA removes secondary polymorphisms that would otherwise inhibit probe-binding. Aldatu's PANDAA technology can be augmented to provide a universal assay for LASV diagnosis despite genomic diversity.[27]

B2 Aldatu has made significant advancements in PANDAA reagent design. Recognition of PANDAA's potential for widespread clinical implementation uniquely positions us to develop a focused genotyping diagnostic for the clinical implementation. Aldatu has pioneered the commercial development of PANDAA for HIV drug resistance genotyping (Table C.1), establishing a sample-in, answer-out HIV genotypic resistance test within ~2 hours of sample purification. While the LASV diagnostics market presents some significant barriers to entry due to competition and resource constraints in many high-burden markets, it is also ripe for innovation.[28]

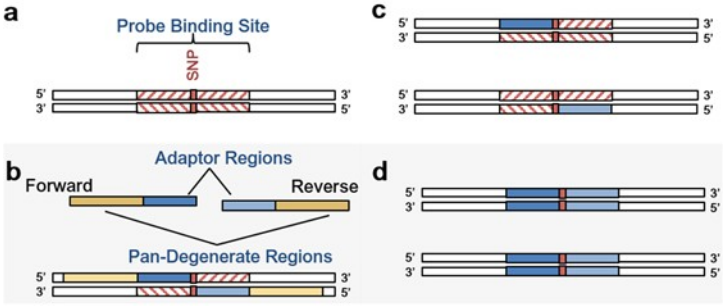


Figure B.2 Removal of secondary mutations by PANDAA. (a) Highly polymorphic genomes prevent the use of probe-based qPCR. (b) By using primers to adapt a probe-binding site flanking a SNP of interest, PANDAA compensates for secondary polymorphisms that would otherwise abrogate probe hybridization. (c) and (d) As qPCR proceeds, the template-derived amplicon will contain a perfectly matched probe-binding site and the SNP of interest will be unchanged.

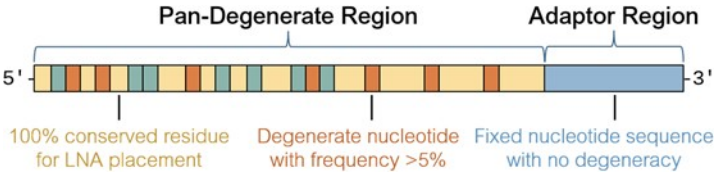


Figure B.1 PANDAA primers contain two key features: a 3' adaptor region that is matched to the probe-binding site; and a pan-degenerate (PDR) region that incorporates nucleotide degeneracy seen in the primer-binding site of the target. PDR design accounts for the high degree of variability in primer-binding sites. LNA bases are incorporated into the 5' region at 100% conserved positions to address concerns regarding primer-dimer formation that could reduce qPCR sensitivity and / or efficiency. This results in little to no dimer formation as LNA are significantly less tolerant of mismatch hybridization, and binding with a matched template is more thermodynamically stable.

C Approach

Phase I Overview: This proposal will allow Aldatu Biosciences to perform a feasibility study of a pan-lineage LASV detection assay based on PANDAA. We will leverage our experience and expertise gained from developing HIV diagnostics to create a LASV diagnostic prototype that detects diverse strains with high sensitivity and specificity.

Next generation sequencing data have increased our knowledge of LASV genetic diversity.[23,29–31] From this we developed more complete maps of genome-wide heterogeneity to better predict and mitigate the influence of genetic diversity on diagnostic assay performance (Figure C.1).

Assay	Overview	Funding
PANDAA qDx HIVDR RTI	Commercial development of PANDAA to detect six drug resistance mutations (DRMs) in >99% of HIV-infected patients failing first-line antiretroviral treatment.	NIAID Direct-to-Phase II SBIR MacLeod R44AI118441 (Completed) [redacted] 06/2015 to 03/2018.
	Trials with clinical samples due to be undertaken by partners in Johannesburg and Durban, South Africa in Q3 2018. Research-use only reagents to be launched in USA Q3 2018.	
PANDAA qDx HIVDR PIDR+	Focused genotyping assay to detect multiple DRM associated with treatment failure on protease inhibitor-based antiretroviral therapy in resource-limited settings.	NIAID Direct-to-Phase II SBIR MacLeod R44AI128974 (Ongoing) [redacted] 01/2017 to 12/2019
ExPAND qDx	Derivative PANDAA technology with increased the specificity, selectivity and sensitivity to detect low-level variants present at ≤1%. Successfully designed and optimized for all HIV-1 antiretroviral drug classes. Phase I (completed) and Phase II (ongoing) SBIR contract proposals funded by NIAID.	NIAID Phase II SBIR Contract MacLeod 272201800050C (Ongoing) [redacted] 09/2018 to 08/2021

Table C.1 Clinical diagnostics and research-use reagents in production and under development by Aldatu.

Design: We performed a preliminary study to determine if PANDAA could improve detection of divergent LASV strains. As a comparison, we tested the reagents and reaction conditions for the GPC RT-PCR/2007 assay.[17] Five LASV strains, with inter-strain nucleotide identify of 79.5%, were selected to represent three genetic lineages from five different countries (Figure C.1). We generated synthetic templates encoding the S segment 5' region. PANDAA primers were designed using patented PANDAA design criteria, including strategically placed LNA bases. Base degeneracy at variable sites covered 99% of strains using full length LASV S Segment sequence data (n=377). PANDAA reagents were compared to GPC RT-PCR/2007 in a qPCR reaction using a universal reporter and 10^5 total DNA copies / reaction.

Results: GPC RT-PCR/2007 performed well for the reference strain, Josiah, but sensitivity was reduced ~16-fold for other strains, consistent with previous reports (Figure C.2).[17] In contrast, PANDAA reagents detected all strains with near equal sensitivity, matching that of GPC RT-PCR/2007 for the reference strain. Thus, our preliminary results suggest that PANDAA can enable a sensitive universal, pan-lineage diagnostic.

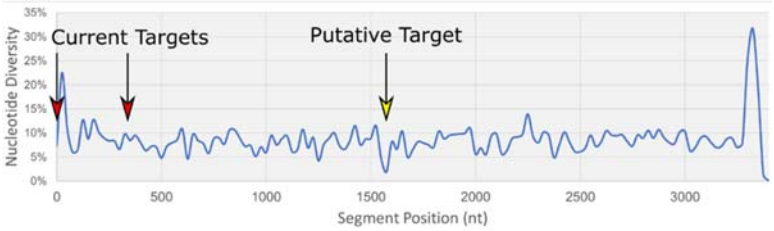
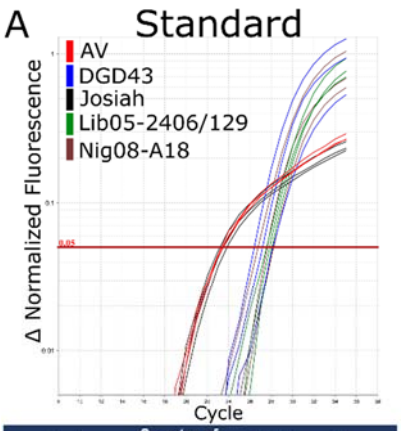


Figure C.1: Segment wide map of LASV nucleotide diversity. Full length S segment sequences retrieved from GenBank in August 2018 (n = 377) were used to calculate nucleotide diversity. The primer binding sites of the current LASV RT-PCR diagnostics are highlighted with red arrows, and a more conserved putative binding site is highlighted with a yellow arrow.

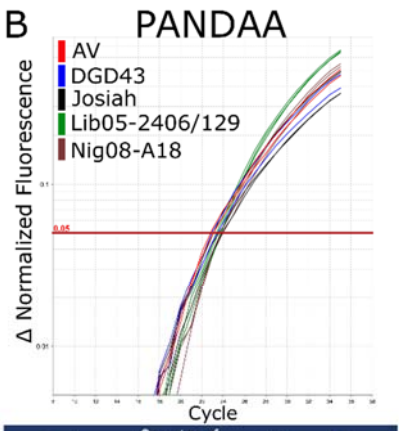
General Experimental Approach

Outlined below are the standard procedures to be used throughout this Phase I proposal.

LASV diversity panel: Synthetic DNA templates will be designed for the entire genome of LASV strain Josiah. DNA of the homologous regions will be synthesized for nine LASV strains that represent all genetic lineages and were isolated from all countries where LASV is endemic (Table C.2). A terminal T7 promoter will allow the DNA templates to be in vitro transcribed into RNA templates, which then will be diluted from 10^5 – 0 copies/µL (cp/µL). Template normalization will be performed with pre-established Aldatu protocols that target non-LASV sequence at the 3' end of the synthetic template. All LASV viral RNA samples (BEI Resources, Manassas, Virginia) are prepared from cultured virus and are eluted in a background of cellular nucleic acid, thereby testing assay sensitivity in the presence



Strain	Country of Origin	C_t (SD)	C_t Range
AV	Ivory Coast	23.4 (0.07)	[23.4 - 27.8] 4.4 cycles
DGD43	Guinea	27.2 (0.88)	
Josiah	Sierra Leone	23.4 (0.34)	
Lib05-2406/129	Liberia	27.8 (0.24)	
Nig08-A18	Nigeria	27.5 (0.69)	



Strain	Country of Origin	C_t (SD)	C_t Range
AV	Ivory Coast	22.9 (0.14)	[22.9 - 23.8] 0.9 cycles
DGD43	Guinea	23.0 (0.53)	
Josiah	Sierra Leone	23.6 (0.44)	
Lib05-2406/129	Liberia	23.5 (0.19)	
Nig08-A18	Nigeria	23.8 (0.12)	

Figure C.2: PANDAA offers superior sensitivity for divergent LASV strains. Sensitivity for divergent templates was tested with synthetic templates encoding a kilobase region of five LASV strains isolated from five countries. The GPC RT-PCR/2007 assay (**Panel A**) was compared to reagents generated with PANDAA design elements (**Panel B**).

The standard reagents had a 4.4 cycle (~21-fold) difference in detection sensitivity. In contrast, PANDAA reagents generated near equal signal among all strains. Further, the sensitivity was comparable to or better than the sensitivity with standard reagents. The results suggest that PANDAA reagents allow for sensitive and broadly specific LASV detection.

of extraneous template. Throughout, RNA will be isolated with the QIAamp Viral RNA kit (Qiagen).[12]

Controls and replicates: Negative controls will contain 0.05ng / reaction human genomic DNA only. All reactions containing >10^4 copies / reaction (cp / rxn) will be performed in triplicate and those <10^4 cp/rxn in 15 replicates.

Data analyses for PANDAA-LASV will be performed on the QuantStudio 3 / 5 qPCR systems (Thermo Fisher, CA, USA). Through this proposal, qPCR Cq data will be log-transformed and plotted relative to input copy number. A positive signal is defined as Cq < 37 cycles and a negative signal Cq > 37 cycles.

Strain	Genetic Lineage	Country of Origin
LP	I	Nigeria
Nig08-04	II	Nigeria
Nig08-A18	III	Nigeria
Josiah	IV	Sierra Leone
BA366	IV	Guinea
Lib05-2406/129	IV	Liberia
AV	IV	Ivory Coast
Soromba-R	V	Mali
Togo	VI	Togo

Table C.2: Strains for PANDAA-LASV Evaluation

Aim 1 Design of PANDAA-LASV primers and probes and reaction condition optimization

Milestone: 1) *In silico* design and validation of optimal primer-binding sites within conserved regions; 2) Selection of PANDAA-LASV primers to adapt putative probe-binding sites within conserved regions; 3) Validation of PANDAA-LASV probe design; 4) PANDAA-LASV reaction buffer optimization to provide a lower limit of quantification (LLOQ) ≦10 RNA copies / reaction (cp/rxn) for the prototypic LASV strain, Josiah.

Rationale: Inadequate performance of traditional PCR-based diagnostics, due to Lassa virus genomic diversity, can be mitigated by PANDAA. Recent whole genome LASV sequence data provide rich diversity maps that will inform primer- and probe-binding site selection and facilitate PANDAA-LASV development. By developing a custom reaction buffer, we can improve sensitivity and specificity beyond what is achievable with existing commercial formulations.

Experimental Design

Aim 1.1 Determination of optimal PANDAA primer and probe design. From genome wide diversity data, we identified the most conserved regions (top 5% by rank, n = 6). The nucleotide diversity for these regions is 5.4% as compared to 8.9% for the targets of the current LASV diagnostics.

Primer-binding site selection: Within these more conserved regions, 25-40 bp forward and reverse non-PANDAA primers will be designed. The design criteria are that a) the forward and reverse primers 3' termini are proximal to the same, strictly conserved base and b) the 5' terminus of each primer is at a conserved base. Due to the unique PANDAA design characteristics, annealing temperature (T_m) is rarely a consideration for primer-binding site selection. Using these criteria ~6 forward and reverse primers will be evaluated per region, which allows ~36 primers pairs to be evaluated simultaneously on a single 96-well plate. Amplification will be detected by incorporating a 5' CMV-sequence tag in the reverse primers, which we have shown does not interfere with amplification. For each region, the primer pair with the greatest sensitivity will determine PANDAA primer-binding sites.

PANDAA primer design: The PANDAA primers PDRs include degenerate and LNA bases to allow for efficient binding to a diverse template pool. We have established a rigorous in-house process to evaluate PANDAA primer performance *in silico*. A core PANDAA feature is the use of highly degenerate primers that would, with traditional qPCR, raise concerns about non-specific PCR products.

Despite a high degree of degeneracy, non-specific products do not impede PANDAA performance (Figure C.3). For example, in our PANDAA qDR HIV diagnostics, we have successfully used a forward primer that is 41,472-fold degenerate and two reverse primers with a combined degeneracy of 34,560-fold. As such, the cumulative primer pool in a single PANDAA reaction contains 80-110,000 distinct primer sequences without detrimental specificity issues. The PANDAA primers ADRs (i.e. the 3' regions) perfectly match the probe-binding sequence. Thus, this design element is selected during PANDAA probe evaluation and will not be altered.

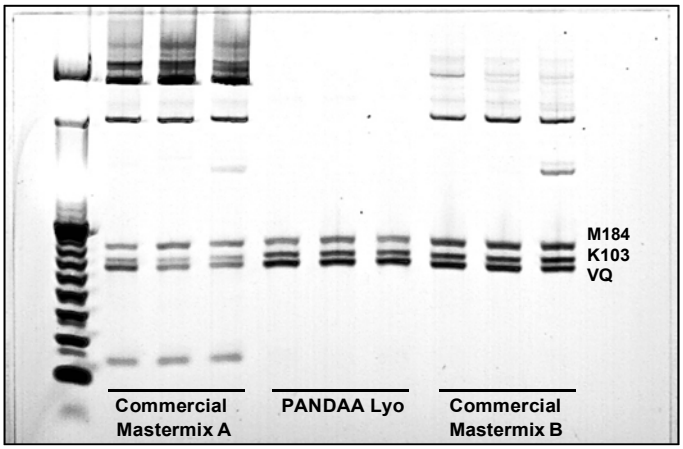


Figure C.3: Comparison of commercial vs optimized PANDAA qPCR mastermix. Three codons in HIV were amplified in a triplex PANDAA reaction and resolved on a 4% agarose gel. The PANDAA custom mastermix had no non-specific bands and higher intensity for all targets as compared to commercial mastermixes.

Using a similar computational approach as we have used for PANDAA-HIV design, we will design site-specific PANDAA primers. Degeneracy will range from 75-95% (nucleotides with a frequency of 5-25% will be incorporated) in the primer Pan-Degenerate Region with the optimal degeneracy determined empirically. 100% conserved nucleotides within the binding sites will be identified for LNA nucleotide placement. The substantial mismatch intolerance of LNA nucleotides also enhances PANDAA specificity and are refractory for the formation of non-specific

products, as we have demonstrated in previous PANDAA assays.

Selection of PANDAA primers: We will select the primer set for each region using the synthetic RNA template across a dynamic range of 10^4 to 10^1 cp/rxn. We will evaluate primer degeneracy and the placement of LNA nucleotides on assay performance. We will perform this preliminary evaluation using 200nM primers in our customized PANDAA real-time PCR buffer that was developed in collaboration with Promega (Figure C.3). Negative controls and replicates are as described in General Experimental Approach. Selection of the optimal primer set will allow us to proceed with the refinement of a custom buffer for PANDAA-LASV (Aim 1.2). Performance will also be evaluated on full length LASV RNA of strain Josiah.

PANDAA probes design and selection: During primer-binding site selection, the assay readout used 5' CMV-sequence tag that was targeted by a FAM-labelled probe. This ensures that any performance differences between primer set is exclusively due to amplification efficiency and not related to probe-binding kinetics. For increased specificity, LASV-specific probes will be used with PANDAA. Probe sequence will match that of the most common LASV haplotype at the selected primer-binding site as PANDAA primers overlap with and adapt the probe-binding sites, the PANDAA probe-binding site position is wholly dependent on primer-binding sites. Probes of various lengths (typically 11-15 nts) and orientations (forward or reverse) will be designed *in silico*. Based on our experience with HIV PANDAA design, probes with a predicted T_m between 56-60°C will be synthesized and evaluated for sensitivity. Probes with the highest sensitivity and shortest length from each region will be selected.

Data analyses: Primer sets will be ranked separately based on sensitivity for the LASV diversity panel samples, determined by the lowest copy number detectable at ≤37 cycles. Negative controls will be analyzed by agarose gel electrophoresis to ensure that no products have formed in the absence of template. The primer pair within each region (n=6) that returns the lowest Cq at 10^1 cp/rxn will be used for downstream validation. Although our expected results may demonstrate sub-optimal specificity, assay optimization (e.g. enzyme, dNTP, and $MgCl_2$ concentrations) can help to overcome reduced specificity of PANDAA and is addressed in Aim 1.2.

Aim 1.2 Optimize amplification through reaction buffer development and refinement. Aldatu's previous development work of PANDAA-based diagnostics generated a fully optimized qPCR mastermix (PRxB) for PANDAA, and distinctly outperforms commercial mastermixes (Figure C.3). With industry partners, we have developed custom buffers for other PANDAA technologies. These kits contain optimal concentrations of Mg^{2+} and dNTPs, as well as a DNA polymerase that is tolerant of primer:template mismatches within PANDAA reactions.

Our previous work has shown that target GC-content is an important determinant of optimal buffer formulation. The average GC content of the HIV and LASV genomes is 42.2% and 44.2%, respectively.[32] Thus, our current PRxB buffer serves as an ideal precursory formulation for PANDAA-LASV, and indeed, our preliminary data (Figure C.2) was generated with PRxB without optimization. Each of the region-specific primers identified in Aim 1.1 will be evaluated at the preliminary LOD. Primer concentrations will be held constant at 200nM and $MgCl_2$ will be titrated from 3.5-7.0 mM. Optimal $MgCl_2$ concentration will be determined as the concentration with the lowest LOD that does not results in non-specific signal with the negative control. As RT inhibits Taq polymerase activity,[33] there is a balance between generating maximal cDNA without inhibiting PCR amplification, thus absolute concentrations and ratios of Taq polymerase and reverse transcriptase will be optimized.

Data analyses will be performed as in Aim 1.1 using the same performance criteria. Our expected results should identify reaction component concentrations that meet our quantitative milestones. As an alternative approach, we will evaluate 12 PCR buffers from a second industry collaborator that we previously evaluated for existing PANDAA-based diagnostics. The final buffer formulation will be used throughout Aims 2 and 3.

Aim 2 Refinement of PANDAA reagents on divergent genotypes

Milestone: 1) Validation of Aim 1 PANDAA-LASV reagents using diverse LASV template; 2) Refinement of PANDAA-LASV design to account for performance differences across divergent LASV genomes to ensure 95% LOD ≦10 RNA copies/reaction (±2.5 copies). Rationale: Aim 1 will generate a set of PANDAA-LASV reagents capable of sensitively detecting LASV strain Josiah. PANDAA-LASV performance reagents will be evaluated on divergent templates sampled from circulating strains of LASV covering all known genetic lineages (General Experimental Approach). Reagent design will be iteratively updated to increase and normalize detection across divergent LASV strains.

Experimental Design

Validation of PANDAA-LASV with divergent genotypes: Using the optimal PANDAA-LASV reagents from Aim 1, sensitivity will be evaluated on all samples in the LASV diversity panel at 10^2 cp/rxn. Given our preliminary results, we expect that sensitivity across strains will be comparable. If so then we will determine the LOD for each of the nine strains using 2-fold serial dilutions of diversity panel samples. The PANDAA-LASV reagents with the lowest LOD will be selected for further optimization.

Iterative PANDAA-LASV design update: Selected primers and probe will be analyzed to identify design features that may limit sensitivity across all lineages. We can incorporate design elements from prior PANDAA development

work, including paired degenerate PANDAA primers and probes, and inclusion of the universal base 5-nitroindole, which does not discriminate between the four natural nucleotide bases.[34] To offset any primer T_m reduction by 5-nitroindole, 100% conserved nucleotides within the cluster will be identified for LNA nucleotide placement.

Reaction condition optimization: After iterative PANDAA design optimization, PANDAA-LASV will be optimized. In the past, asymmetrical PCR has yielded superior sensitivity as compared to symmetrical reactions. The primer that generates the probe target template (e.g. the reverse primer when the probe is in the forward orientation) is typically increased to achieve this end. Our assay optimization workflow has been fine-tuned during PANDAA-HIV development and is expected to be similar for optimization of PANDAA-LASV. Data analyses will be performed as previously described. If our expected LOD criteria are not met then an alternative approach would be to incorporate PANDAA ProAmp, which offsets the low amplification efficiency in early qPCR cycles due to primer:template mismatches and creates a pool of partially adapted templates that are more effectively amplified.

Aim 3 Analytical and clinical validation of PANDAA diagnostic prototype

Milestone: Prototype PANDAA-LASV assay with the following specifications: Pan-lineage 95% LOD ≦10 RNA copies/reaction, specificity >99% using non-LASV templates, and preliminary clinical sensitivity >95%.

Key Feature	Ebola - Desired	Ebola – Acceptable	PANDAA-LASV
Analytical Sensitivity	> 98%	> 95%	>95%
Analytical Specificity	> 99%	> 99%	>99%
Number of Steps	< 3	< 10	3-5
Time to Result	< 30 minutes	< 3hours	90 minutes

Table C.3 Ebola Target Product Profile (TPP).

Rationale: Using a WHO TPP for Ebola (another viral hemorrhagic fever [VHF] outbreak disease) as a model, we have set preliminary performance specifications for PANDAA-LASV (Table C.3).[35] Analytical validation of the PANDAA-LASV prototype will be performed with serial dilutions of a LASV RNA panel covering all circulating lineages and with spiked sera samples. Specificity will be confirmed with LASV negative human sera, and nucleic acid from a closely related arenavirus as well as other pathogens that cause febrile illness in West Africa. Additional sensitivity and specificity metrics will be obtained using a panel of 100 LASV isolates from Stephen Günther at Bernhard-Nocht Institute for Tropical Medicine (see Letter of Support). Clinical validation will be supported through a partnership with FIND (see Letter of Support).

Experimental Design

Analytical Sensitivity: 1) Synthetic Templates: The 95% LOD of PANDAA-LASV on RNA templates representing the nine strains (Table C.2) will be determined by Probit analysis. The GPC RT-PCR/2007 assay (see Preliminary Data) will be run in parallel for comparison. Using a 384-well plate, 64 reactions will be run for each strain at 10^4 – 10^1 cp/rxn, as well as 64 reactions containing only negative controls. In total, nine 384-well plates will be required.

2) LASV Spiked Sera: LASV (Josiah) RNA will be spiked into normal human serum (Lampire Biological Laboratories, Pipersville, PA) at 10^4-10^0 copies/mL to determine PANDAA-LASV LOD from sera.

Analytical Specificity: 1) Nucleic acid from a related arenavirus, other VHFs, and pathogens that may circulate in the blood of patients in West Africa will be evaluated (see Select Agents Research). 10-fold serial dilutions of RNA or DNA, over three orders of magnitude, will be used in the PANDAA-LASV assay and positive signal will. Reactions will be analyzed on agarose gel to identify potential amplification products that were not detected by qPCR. 2) Human Sera: Purified total RNA from pooled normal human sera, without spiked LASV RNA, will be diluted across six 10-fold dilutions. 64 PANDAA-LASV reactions will be performed for each dilution to verify negative signal in each.

Clinical Sensitivity: Through our partnership with FIND, a non-profit dedicated to the development, evaluation and implementation of novel diagnostics in resource limited countries, FIND has agreed to grant Aldatu access to its extensive network of research partners as well as collection of rare LASV samples. Clinical sensitivity will be evaluated in parallel with the GPC RT-PCR/2007 assay, results will be compared between assays to access clinical sensitivity of the PANDAA-LASV prototype.

Data will be analyzed with the generalized linear model and confidence interval functions of the R statistical package[36]. The median and 95% LOD will be quantified for each strain. The LOD is expected to deviate by <25% for all lineages with PANDAA-LASV, based on our preliminary work in which signal deviation from a non-optimized assay is < 2-fold. It is expected that no non-specific signal will be generated in the absence of LASV RNA. Our previous experience with PANDAA optimization provides mitigation strategies if specificity is sub-optimal.

The specificity of our pool of candidate PANDAA-LASV reagents developed in Aim 1 can be evaluated, while also being mindful of the pan-lineage sensitivity of the assays. Second, if a homologous sequence in a non-LASV template generates non-specific signal (which can be confirmed by sequencing the non-specific product), then incorporation of mismatch intolerant LNA bases at sites conserved within the LASV species but divergent between species can increase specificity. Lastly, our previous HIV development work has shown that adjustments of $MgCl_2$ concentration over a small range tend to alter specificity to a greater degree than sensitivity. As such, our ability to generate custom buffer allows for assay performance to be finely tuned.

PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002

Expiration Date: 03/31/2020

Are Human Subjects Involved	❍ Yes ● No
Is the Project Exempt from Federal regulations?	❍ Yes ❍ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	● Yes ❍ No
If Yes, provide an explanation of why the application does not involve human subjects research	Human_Subjects_Exemption_LASV.pdf
Other Requested information	

Human Subjects Sections

5. Protection of human subjects

In **Aim 3** of the proposed research, there is a requirement for human sera in order to test PANDAA-LASV sensitivity and specificity. Human sera will be obtained from a commercial source (e.g. Lampire Biological Laboratories, Pipersville, PA) that is committed to ensuring the privacy of their donors, and as such no identifying information will be provided to Aldatu Biosciences.

In **Aim 3** of the proposed research plan, there is a requirement for determining clinical sensitivity with RNA isolates derived from clinical samples previously obtained from Lassa-infected individuals. These samples will be obtained from research partners of FIND, who have previously collected and characterized these samples, and no patient identifying information will be provided to Aldatu Biosciences by FIND or its partners. This research falls under exemption 4.

Exemption 4: Human Subjects and Characteristics:

RNA extracted from sera samples from unidentified human adults will be obtained. No identifying information will be provided. Prior to publication of any associated data, Aldatu Biosciences will consult with FIND and its research partners solely to confirm that there is no risk to subjects, and that adequate patient protection remains safeguarded.

Exemption 4: Sources of material: Lassa clinical isolates will be obtained from research partners of FIND. These samples are publicly available to a Principal Investigator, Laboratory Director or equivalent at public or academic institution, or the Director of Research or equivalent at a private or for-profit institution. No subjects will be recruited by Aldatu Biosciences.

Exemption 4 Justification: We are claiming exemption 4 for this research because (a) the specimens/data were not collected specifically for the research through an interaction or intervention with a living person, and (b) the investigators cannot “readily ascertain” the identity of the individual who provided the specimen/data. The tissue we obtain for this research will be completely devoid of identifiers linked to the subjects. There therefore will be no way for the investigators to identify the subjects.

Select Agent Research

Lassa virus is a select agent under the Federal Select Agent Program.

However, according to the Centers for Disease control and Prevention (CDC) and the Animal APHIS Select Agent Program, and in accord with 42 CFR §§ 73.3, 73.4; 9 CFR §§ 121.3, 121.4; 7 CFR § 331.3:

"select agents or toxins that meet any of the following criteria are excluded from the select agent regulations:

… *Non-viable select agents or nontoxic select toxins are excluded*."

As our work will be exclusively performed with inactivated, purified Lassa virus RNA, and inactivation will be certified by commercial vendors or partners (e.g. FIND, BNI), the reagents will be excluded from regulation under the select agent program.

Similarly, we will be performing specificity studies with purified nucleic acid isolated from other pathogens (see Table below), including the select agents Marburg virus, Rift Valley fever virus and Crimean-Congo Hemorrhagic Fever Virus. The purified nucleic acid will be obtained from government or commercial vendors and inactivation will be certified by the suppliers.

All purified, inactivated DNA and RNA reagents used in these studies can be worked with under BSL-1 conditions.

Species	Material	Source	Biosafety Level
Mopeia Virus	Genomic RNA	PHE	1
Dengue Viral Types 1-4 Panel	Genomic RNA	BEI	1
Yellow Fever Virus	Genomic RNA	BEI	1
Malaria Plasmodium falciparum	Genomic DNA	ATCC	1
Crimean-Congo Hemorrhagic Fever Virus	Genomic RNA	BEI	1
Marburg Virus	Genomic RNA	BEI	1
Rift Valley Fever Virus	Genomic RNA	BEI	1
HIV-1, 7 subtypes	Synthetic RNA	Aldatu	1

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Authentication of Key Biological and/or Chemical Resources

Our institution fully recognizes the importance of ensuring the identity and validity of key biological and chemical resources used in our studies, including the proposed study. We fully understand that unreliable or misidentified resources can misdirect research, negate reproducibility and derail further expansion of our research by either us or others. It is of the utmost importance to us to ensure the identity and validity of key biological and chemical resources in order to ensure the reproducibility of results and consistency of data.

In light of the above we have general practices in our labs ensuring the authentication of our key biological and chemical resources, both for the purpose of the proposed project and otherwise.

In many cases, purchased or established resources have already been authenticated prior to receipt, and the vendor has included a specification sheet with the product. Even If the authentication data provided by the vendor meets our needs in terms of how the product will be used, we also plan to independently verify the identity and activity of the product before use, for example by performing an assay with the new lot compared to a previous lot. Products that will be used long-term, are assessed regularly at appropriate time intervals for identity and validity.

Resources that will be used in this research that require confirmation of identity and validity that are in the Class of Biologicals:

1. Linear oligonucleotides for probe-based quantitative real-time PCR (qPCR), i.e. the primers and fluorescently labeled probes.
2. Synthetic DNA fragments
3. Real-time PCR reaction buffer (including DNA polymerase and additives)
4. Purified genomic RNA of Lassa (strain Josiah), and purified RNA or DNA of other pathogens.
5. Ribonucleotides and T7 RNA Polymerase

Methods that we use or plan to use for authentication depend on the key resource type. In any case, Authentication plans for key biological and/or chemical resources will be based on accepted practices:

Biologics: Key specialty biologics will be validated by accepted practice for the type of biologic in use:

- Linear oligonucleotides for probe-based quantitative real-time PCR (qPCR), i.e. the primers and fluorescently labeled probes. These are commercially manufactured under GMP by Thermo Fisher Scientific/Life Technologies, Integrated DNA Technologies (IDT), and/or Eurogentec. Product Specification Sheets include analytical Mass Spectrophotometry reports.
- Synthetic DNA fragments are commercially manufactured GeneArt™ Strings™ DNA fragments (Thermo Scientific). The fragments are bulk sequence-controlled before shipment.
- Real-time PCR reaction buffers (including DNA polymerase and additives) are produced by Promega and undergo extensive quality control prior to lot release. As buffer optimization progresses as part of the proposed SBIR work, Aldatu will develop in-house quality control protocols for lot testing to ensure consistent performance of buffer components.
- Purified genomic RNA of LASV and other pathogens will be obtained from ATCC, BEI Resources and Public Health England National Collection of Pathogenic Viruses, widely-used and reliable sources of genomic reagents. All RNA or DNA will be subject to extensive quality control at ATCC, and Aldatu will perform targeted Sanger sequencing of all genomic RNA or DNA to validate the commercially-purchased reagents; for LASV, Aldatu will use sequencing to verify the integrity of the PANDAA-LASV assay target.
- Ribonucleotides and T7 RNA Polymerase will be obtained from New England Biolabs and are provided with product specification sheets verifying purity and activity. Activity of the reagents will be independently assessed by Aldatu via an in-house in vitro transcription protocol. Product quality will be evaluated with agarose gels and targeted qPCR assays to confirm the reagents generate pure, full length product.

Chemical: We also purchase the following chemical reagents from commercial sources:

- Ethanol, obtained from MilliporeSigma, is supplied with product specification sheets verifying purity.

Fluorophores: VIC®, NED® and FAM®. These will be validated by readings in the respective wavelength.

We also will be in periodic communication with the scientific community to establish updated guidelines for the authentication of various types of key resources.