SUMMARY STATEMENT

PROGRAM CONTACT: **(Privileged Communication)** ***Release Date:*** **12/17/2018**
Patricia Repik ***Revised Date:***
[redacted]

Application Number: **1 R43 AI145704-01**

Principal Investigator

MACLEOD, IAIN JAMES

Applicant Organization: ALDATU BIOSCIENCES, INC.

Review Group: **ZRG1 IDM-V (12)**
Center for Scientific Review Special Emphasis Panel
Small Business: Non-HIV Diagnostics, Food Safety, Sterilization/ Disinfection, and Bioremediation

Meeting Date:	**11/29/2018**	***RFA/PA:***	**PA18-574**
Council:	**JAN 2019**	***PCC:***	**M32B B**
Requested Start:	**04/01/2019**	***Dual PCC:***	**OSA13**
		Dual IC(s):	**TR**

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

SRG Action: **Impact Score:10**
Next Steps: **Visit https://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
1	[redacted]	[redacted]
TOTAL	[redacted]	[redacted]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

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1R43AI145704-01 MacLeod, Iain

RESUME AND SUMMARY OF DISCUSSION: An outstanding investigator with expertise in basic and diagnostic virology proposes to develop and validate a rapid, sensitive, and molecular diagnostic assay to detect Lassa virus (LASV), the causative agent of Lassa hemorrhagic fever (LHF). Reviewers agreed that development of a novel, rapid, accurate, and improved pan-lineage Lassa virus detection assay that can be deployed, at either inpatient or outpatient settings, in endemic regions is highly significant because of its potential to reliably monitor and measure Lassa infections leading to effective treatment, advances in vaccine development, improvements in global health, and significantly reduce outbreaks and healthcare costs. A significant global health concern, critical clinical need, and incredibly innovative approach are strengths of this application. Reviewers agreed on strong feasibility of the project based on proven and promising technology and convincing premise supported by successful application in HIV diagnostics. Some minor concerns were raised about the potential challenges in deploying technology at the point-of-care due to somewhat complex chemistry and high rates of false positives and negatives. However, these minor concerns were significantly reduced based on successful application of the technology in HIV detection and thoughtful assay optimization strategy in their approach. There is exceptionally high overall enthusiasm for this outstanding application based on an excellent investigative team, feasibility, and high biomedical, clinical, and commercial potential.

DESCRIPTION (provided by applicant): 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.

PUBLIC HEALTH RELEVANCE: 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.

CRITIQUE 1

Significance: 1
Investigator(s): 2
Innovation: 1
Approach: 1
Environment: 2

Overall Impact: PANDAA for universal, pan-lineage molecular detection of Lassa fever infection. This application seeks to solve the problems with diagnosing a very important virus, Lassa fever virus (LASV). LASV is endemic in east Africa, causing 2 million infections each year and 10,000 deaths. The only treatment, ribavirin, must be started soon after presentation to have any effect, but current diagnostic methods are not rapid enough to meet this deadline. The delay in identifying LASV also puts health care workers at risk because LASV is spread by direct contact. Part of the problem is the divergence of LASV strains, necessitating multiple qRT-PCR tests, and the low sensitivity of these tests. Aldatu has developed a modified PCR approach to this type of broad diversity problem that has been used effectively to identify drug resistant mutations in minor HIV populations. The goal of this application is to develop a rapid, accurate and sensitive qRT-PCR test to identify LASV in clinical specimens. The approach uses degenerate primers in a very clever manner to isolate short signature sequences that identify the different strains of LASV.

1. Significance:

Strengths

- Lassa virus (LASV), the causative agent of Lassa hemorrhagic fever (LHF), causes 2 million infections and 10,000 deaths each year, and is a potential cause of epidemics and pandemics. Rapid and accurate diagnosis is critical for treatment, vaccine development, and outbreak containment.
- Delays in diagnosis unnecessarily expose healthcare workers to infection, and in LASV-negative cases, push the healthcare infrastructure beyond its capacity.
- qPCR assays have the greatest potential for creating rapid and sensitive LASV diagnostic tools, but high genetic diversity has prevented a diagnostic that sensitively and specifically detects all clades of LASV.
- The Aldatu PANDAA platform enables probe-based qPCR for target detection in highly variable genomic regions by simultaneously adapting and amplifying diverse templates.
- Clinical diagnostic product that could significantly improve LHF diagnosis, management, and outbreak response.

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- Iain MacLeod, trained in virology at the Universities of Glasgow, Cambridge and Harvard, is a co-founder and the CSO of Aldatu Biosciences, a Harvard-based company.
- Nicholas Renzette is the Lead Scientist for assay development at Aldatu. He received his PhD in Molecular Genetics from UMass, Amherst, and did postdoctoral training at UMass Medical Center in Viral Molecular and Population Genetics. He is a Lead Scientist at Aldatu and co-developed PANDAA with MacLeod.

Weaknesses

- None noted.

3. Innovation:

Strengths

- Probe-based qPCR for target detection in highly variable genomic regions by simultaneously adapting and amplifying diverse templates
- Enhancing sensitivity by careful optimization of reaction conditions.
- Have successfully developed PANDAA-based assays for SNPs and drug resistance mutations in HIV, another highly polymorphic pathogen.
- Modifications introduced during PCR to enhance amplification of diverse variants with specificity and sensitivity.
- Capture a unique “SNP” that identifies a LASV subtype.

Weaknesses

- None noted.

4. Approach:

Strengths

- Nucleotide diversity in a highly diverse virus population greatly reduces primer and probe binding in traditional qPCR. PANDAA mitigates this problem by removing secondary polymorphisms, thereby minimizing their impact on qPCR sensitivity and specificity.
- PANDAA uses invariant bases where possible, degenerate bases where variable and the universal base, 5-nitroindole, when needed in the primers.
- Preliminary studies with LASV templates from multiple lineages show that PANDAA could detect at least five lineages with near equal sensitivity and outperform the current gold standard assay by greater than an order of magnitude. These results did not include any optimization which should greatly increase the sensitivity.
- Aim 1 will determine the optimal primers and probe sequences.
- Aim 2 will refine the reagents on divergent genomes.
- Aim 3 will validate the PANDAA diagnostic prototype, analytically and clinically. A collaborator, Stephen Günther, will supply 100 LASV strains for testing.

Weaknesses

- None noted.

5. Environment:

Strengths

- Aldatu is housed in the Harvard Life Lab, with an open lab layout and contact with other entrepreneurs and with support facilities.
- All necessary equipment is available for this study.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable

Vertebrate Animals:

Not Applicable

Biohazards:

Unacceptable

Select Agents:

Unacceptable

- Lassa fever virus is a Risk Group 4 agent. Stephan Günther, Director of the WHO Collaboration Centre for Arboviruses and Hemorrhagic Fever Reference and Research will provide "access to a collection of 100+ LASV isolates". The Harvard Life Lab where Aldatu is situated has access to BSL2 and 3 lab space, but not BSL4. It is not clear from the application or from Dr. Gunther's letter if Aldatu would receive virus or extracted RNA.

Resource Sharing Plans:

Not Applicable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommended budget modifications or possible overlap identified:

- Acceptable

CRITIQUE 2

Significance: 1

Investigator(s): 1
Innovation: 1
Approach: 1
Environment: 1

Overall Impact: Lassa fever virus is a rodent-borne RNA virus endemic to West Africa, the cause of annual outbreaks with thousands of deaths each year. Symptoms are presented in a wide clinical range of severity, yet the high morbidity of those with severe hemorrhagic fever necessitate early diagnosis such that isolation and antiviral therapies can be offered. Acute diagnosis of Lassa fever virus is hampered by the great genetic diversity seen in the two RNA genome segments, which have made RT-qPCR assays very difficult and impossible to cover the different lineages present in West Africa. There is a great need to design a sensitive assay to detect the earliest Lassa fever infections. This phase I application offers a solution with a novel approach of detecting highly divergent RNA genomes. Using their PANDAA strategy where chimeric primers with a region of degeneracy and a region that binds the virus with conserved specificity allow detection of highly diverse sequences, while also providing a target for a fluorescent probe to specifically bind and allow quantitation in a Real Time qPCR assay. Aldatu Biosciences has shown the PANDAA approach to detect variants in HIV, which is also known to have a high degree of quasi-species. The approach to detect a set of diverse Lassa fever lineages is logical and provides rigor with direct comparison with the published GPC RT-PCR assay. Although the genetic diversity between the Lassa lineages poses a significant challenge, Figure C.2 shows the high probability of having a sensitive molecular assay to detect early Lassa Fever infection. This approach, if shown to work for the detection of Lassa virus, would also be of great benefit to numerous, important RNA viruses which also have great genetic diversity.

1. Significance:

Strengths

- Like all arenaviruses, Lassa Fever virus persistently infects its rodent host while generating a diverse repertoire of virus genomes. People are infected from exposure to the rodent's urine and often outbreaks are independent introductions from rodent to human, making detection of the viral RNA genome difficult due to the high degree of nucleotide diversity. There is no approved diagnostic assay for Lassa Fever virus and current assays rely on nested RT-PCR or primer sets for a particular lineage, all not appropriate for most hospital settings.
- Lassa Fever infection is most frequently in resource poor settings. The PANDAA assay has already been proven to be useful to detect HIV in Africa and could be applied to clinics and hospitals in West Africa

Weaknesses

- None noted.

2. Investigator(s):

Strengths

- Dr. MacLeod is an expert with the design and application of the PANDAA assay
- The team at Aldatu have experience in development and commercialization of molecular diagnostic assays.
- Dr. Gunther is an expert in Lassa fever diagnosis in Africa and can provide excellent guidance and advice

Weaknesses

- None noted.

3. Innovation:

Strengths

- The PANDAA strategy of adapting diverse RNA sequences such that they may be detected using a sensitive probe in a Real Time assay is novel and would be applicable to numerous RNA virus assays.
- The ability to specifically detect Lassa given the huge amount of genetic diversity of West African lineages.

Weaknesses

- None noted.

4. Approach:

Strengths

- The design of primers and probes is the most challenging part of the assay development, yet is well described with an appreciation of possible limitations and approaches to mitigate issues.
- The strategy to optimize the MgCl2 and the primer concentrations is logical and necessary given the degeneracy of the primers.
- Comparison of the PANDAA assay with a gold standard, published RT-PCR assay is valid and offers metrics when optimizing.

Weaknesses

- There is little mention (only once) of the reverse transcriptase step and no details about its source.
- The Lassa genome consists of two segments, S and L. The L segment also has regions of sequence conservation and should also be in the analysis when designing primers.
- Current Lassa RT-PCR assays are often affected by off target binding and amplification of host human RNA instead of viral. Off target amplifications could also be a major problem given the degeneracy of the assay, but they describe how they would sequence verify products.

5. Environment:

Strengths

- Aldatu Biosciences has adequate laboratory space and equipment in the Pagliuca Harvard Life Lab incubator and they have access to the Harvard core facility for sequencing support.

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 3

Significance: 1
Investigator(s): 1
Innovation: 1
Approach: 2
Environment: 1

Overall Impact: The applicants propose a universal, pan-lineage molecular technology (PANDAA) for the detection of Lassa virus (LASV) infection. The new assay could potentially overcome the major limitation of the current molecular tests caused by the genetic diversity of the virus. Lassa hemorrhagic fever (LHF), caused by LASV infection is a global threat as a potential cause of epidemics and pandemics. qPCR has been widely used in the diagnostics of LASV infection and the technique has the longer-term potential for incorporation into an integrated point-of-care device. Yet the genetic diversity of the virus created a diagnostic conundrum that would necessitate the use of multiple different test addressing different genetic regions, which is time-consuming, labor-intensive, and adds to the overall costs of testing. The proposed assay, especially if it could be used in the point-of-care setting, could significantly improve the accuracy of testing, significantly reduce the time to diagnosis, and help decrease the threat of further spreading the disease world-wide.

1. Significance:

Strengths

- The PANDAA technology, as proposed, may enable probe-based qPCR for target detection in highly variable genomic regions by simultaneously adapting and amplifying diverse templates, and thus might mitigate the presence of target-proximal polymorphisms to allow otherwise divergent templates to be detected with consensus fluorescent probes.
- It incorporates previously established molecular techniques that may reduce the time required for assay development.

Weaknesses

- While it has been noted that the assay could eventually integrated into a point-of-care device, the complexity of the chemistry may pose some difficulty for such transition. The test, as described, appears to be more suitable for use in well-established laboratories thus requiring significant laboratory infrastructure that may be problematic in resource-pour settings.

2. Investigator(s):

Strengths

- The PI has significant experience in academic virus research and diagnostic molecular virology, both in the laboratory, and in the field settings in Africa. His contributions to science include the development of the Pan Degenerate Amplification and Adaptation (PANDAA) assay, a qPCR-based method for HIV drug resistance detection that is simple, low-cost, and highly sensitive. He is highly qualified to lead the proposed project.
- The Lead Scientist worked in the field of viral molecular assay development and is also well qualified.

Weaknesses

- None noted.

3. Innovation:

Strengths

- Innovative and unique assay design overcoming some of the most significant limitations of the LAVS assays currently in use.
- The proposed chemistry for simultaneous and accurate detection of multiple genotypes of LASV draws heavily from the design of the company's proven, proprietary PANDAA technology-based Early HIV Drug Resistance Detection Assay.
- Simultaneous, high sensitivity detection of multiple LASV genotypes, decreasing assay turnaround time and laboratory technologists' time when compared to using multiple, complex central laboratory-based tests.

Weaknesses

- None noted by the reviewer.

4. Approach:

Strengths

- Pan-lineage LASV detection assay based on PANDAA. Preliminary data presented in the application support feasibility.
- *In silico* cost-effective design and validation of optimal primer-binding sites within conserved region, cost-effective and proven approach as seen in the company's HIV assay.

Weaknesses

- Complexity of the assay design may not enable POCT development, keeping the test initially in well-equipped central laboratory facilities.

5. Environment:

Strengths

- Aldatu Biosciences is based at Pagliuca Harvard Life Lab, an innovative, shared laboratory space for life sciences startups in Boston. Based on the detailed information provided the lab space of state-of-art and the investigators have access to shared and core facilities such as a BSL-2 tissue culture laboratory, NGS and Sanger sequencing core facilities. The facilities are adequate for the proposed research project.
- The collaborators have excellent general research environment along with the Harvard Innovation Lab (i-Lab) and furnished Letters of Support from the WHO and the University of Cambridge (UK).

Weaknesses

- None noted.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Select Agents:

Acceptable

Resource Sharing Plans:

Acceptable

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

Footnotes for 1 R43 AI145704-01; PI Name: MacLeod, Iain James

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.