Research England GCRF QR Funding 2019-20
University of Cambridge

Scheme *Pump-priming*

Project title*: Local production of a Cas12-based typhoid diagnostic in Cameroon through a novel academic-community partnership*

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DAC-list country	Cameroon	Cameroon
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Project description

Typhoid fever is a systemic infection caused by the Gram-negative bacteria *Salmonella enterica serovar* Typhi (*S.* Typhi*)* and Paratyphi (*S.* Paratyphi*)* which causes approximately 17.8 million cases and 75,000–208,000 deaths worldwide each year (Antillón *et al.,* 2017), predominantly in those under five years of age (Arora *et al.,* 2019). Typhoid is widely recognised as a national public health concern in Cameroon where Global Health Data Exchange data (GHDx, 2017) estimates 45,000 cases and 580 deaths in 2016.

Typhoid fever is treatable with antibiotics but its clinical management faces two challenges:

I. the clinical signs and symptoms of typhoid fever are non-specific, hence laborious tests are essential for accurate diagnosis and take up to 7 days;
II. The emergence of multidrug-resistance (MDR) in African *S.* Typhi strains is common (Park et

al. 2018) and no data is currently available for MDR in Cameroon.

There is therefore a need for:

- Rapid and accurate diagnosis of typhoid fever;
- Identification of possible MDR genes within *S.* Typhi pathogens.

We propose developing a low-cost DNA-based diagnostic tool for *S.* Typhi using Cas12, which to our knowledge has never previously been used by researchers in Cameroon due to i) a need for capacity building and knowledge transfer and ii) the costs of reagents for molecular biology which can be prohibitively expensive. Cas12 is set to be a revolutionary and versatile platform for sensitive molecular diagnostics. It is an enzyme guided by RNA to detect very specific DNA sequences. Upon recognition, Cas12 cleaves a reporter DNA, releasing a fluorescent read-out (Chen *et al.,* 2018). This technology has lowered detection limits to attomolar quantities of DNA and offers the ability to design multiplex assays (Gootenberg *et al.,* 2018) in order to detect more than one specific DNA sequence e.g. MDR mutations.

Project Aim

We aim to develop a proof-of-principle DNA-based typhoid diagnostic using Cas12. The diagnostic tool will be capable of distinguishing *S.* Typhi from *S. Paratyphi*, will be produced in Cameroon and will have a reagent cost of < $1 per reaction.

Activities

Our work plan uses the respective strengths and resources of the partners to efficiently deliver the research via a two-way exchange (Fig 1). Dr Molloy and Dr Ajioka have expertise in protein purification, molecular diagnostics, cell-free protein expression; Dr Apinjoh and Mr Mboa have expertise in infectious disease epidemiology and immunology, drug-resistance surveillance, molecular biology, knowledge of local stakeholders and healthcare system. In addition to experimental research, MboaLab & Buea University will lead an engagement exercise interviewing local stakeholders[1] during M1-M3 to understand the requirements for diagnosing typhoid and pathways to impact within the local health system (e.g. adoption and use of molecular diagnostics within local hospitals, local policies to build capacity in public and private diagnostics laboratories).

The pace of the proposed work plan is achievable due to 12 months effort in establishing partnerships and molecular biology lab facilities in Cameroon and intensive capacity building for recombinant protein expression in a collaboration between the applicants supported by the Shuttleworth Foundation. Thanks to this progress, we are now in an ideal position to deliver short pump-priming projects providing evidence for our model of pairing universities and innovation hubs to tackle global challenges and to prepare the ground for major new funding and support.

[1] Stakeholders include medical doctors and biologists from public and private hospitals (e.g. Mbankomo District Hospital, Dimako District Medical Center), diagnostic lab technicians (e.g. from Prima Laboratory), academic researchers (e.g. from the University of Yaounde I and Catholic University of Central Africa: School of Health Science); government officials from the Ministry of Public Health (Directorate for the Fight against Disease, Epidemics and Pandemics) and NGOs (e.g. Cameroon Baptist Convention Health Services (CBCHS), Helen Keller International Cameroon).

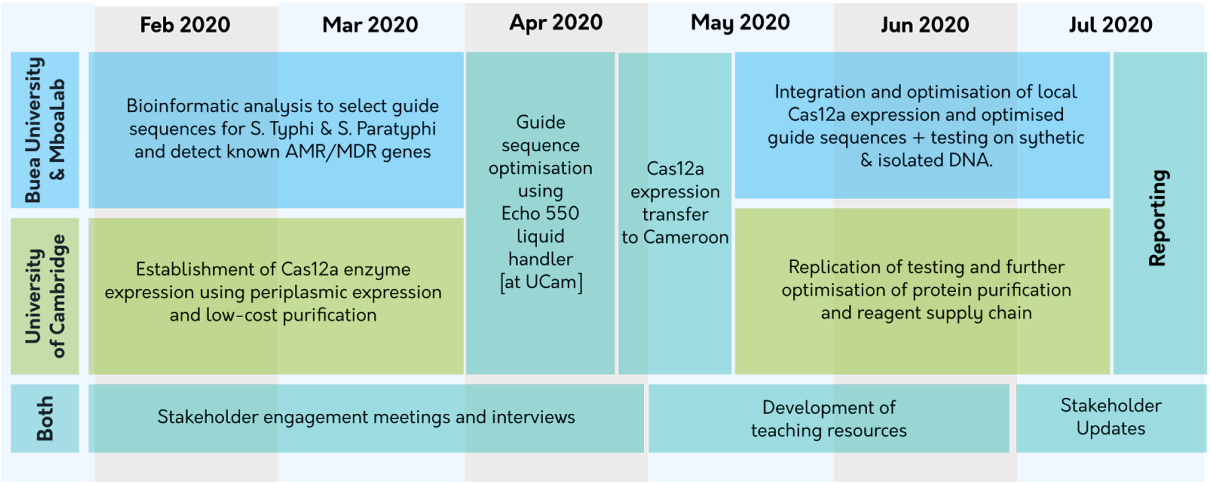

Fig 1: *Work plan showing division of effort between partners over the six months of the project*

Outputs

1. Proof-of-concept for a locally produced diagnostic tool based on the state-of-the-art molecular biology tool Cas12, detecting S. Typhi and S. Paratyphi in purified DNA samples.
2. Accessible protocols for expressing, purifying and handling Cas12 as a diagnostic tool using local resources (both in English and French).
3. Report on stakeholder engagement, detailing pathways to impact for typhoid diagnosis and treatment in Cameroon and identifying other priority diseases where Cas12 diagnostics might be impactful.

Impact

In the immediate term, this pump-priming project will build capacity in Cameroon for use of a cutting-edge research and diagnostic platform technology based on Cas12, providing valuable research skills. In the longer term, a locally produced, effective, low-cost detection method for typhoid has potential to improve clinical treatment of typhoid fever and avoid some of the >500 deaths per year in Cameroon. We see at least three paths to expanding impact: i) transferring the resulting diagnostic to other parts of Sub-Saharan Africa and even further afield lowering the 75,000–208,000 deaths worldwide each year (Antillón *et al.,* 2017), this should be feasible with adaptation where health systems operate similarly to Cameroon; ii) application of Cas12 to other communicable and non-communicable diseases that are a priority for Cameroon, based on findings of stakeholder engagement exercise; iii) expansion to multiplex detection of MDR genotypes based on the findings of our feasibility study. This pump-priming project will provide proof of principle for planned larger proposals to Wellcome Trust (Collaborative Awards in Science Scheme) and UKRI GCRF (future calls and submitted Challenge Cluster on local production of health technologies) to support further work establishing local production of enzymes for molecular diagnostics.

Type of activity

Capacity and capability building. Specifically:

i) **Establishing or enhancing equitable and sustainable partnerships with researchers and other organisations in developing countries:** this project will prime the transfer of cutting edge synthetic biology techniques to local production with an innovative model

of academic-community interaction between MboaLab and Buea University. It transfers and extends the work of the GCRF-funded Low Cost Viral Diagnostics Project (UK and South Africa, led by Dr Ajioka) in a very different context and leverages the establishment of a biotechnology laboratory at the MboaLab innovation hub in Yaounde by the Shuttleworth Foundation-funded Open Bioeconomy Lab project led by Dr Molloy.

ii) **Growing people-based capacity and capability to undertake ODA-eligible research across career stages in the UK and developing countries:** through the research internship programme with Buea University, MboaLab and the University of Cambridge we are developing researchers who can apply state-of-the-art biotechnology to achieve sustainable development goals within their own context and build enterprises that will generate employment. The planned two-way capacity-building exchange visit will also make best use of the resources and existing skills of both partners and give both the experience of undertaking ODA-eligible research in both the UK and Cameroon.

b) **Interdisciplinary and collaborative research activity:** our collaborative research activity promotes the adoption of synthetic biology tools, bioinformatics and engineering approaches e.g. lab automation, design of experiment analysis, to develop a response to a local health challenge that makes equitable use of the strengths and resources of both research partners and leaves a sustainable partnership and increased in-country capacity. This will strengthen future funding applications to build on the preliminary data generated.

Expected project start and end dates *(all funding must be spent by 31 July 2020):*

1 Feb 2020 to 31 July 2020 (six months)

Funding requested and X5 number:

Funder	Research England (short name: RESENG)	
X5 number	**X5:37196**	
Total Funding Requested (Cambridge staff and non-staff costs + External partner costs, if any)	**£79,923**	
Cambridge staff costs	£ 21,443	
Cambridge Non-Staff Costs	£ 36,161	
External partner costs (funds to be transferred to external partner(s)	Partner name (add columns if more than one external partner)	Name:
	Salaries	£ 2,730
	Consumables	£ 7,500
	Travel & Subsistence	£ 6,360
	Equipment	£ 1,000
	TOTAL DI COSTS	£ 16,590
	Investigators	£ 2,700
	Estates	£ 0
	Other Directly Allocated	£ 0
	TOTAL DA COSTS	£ 2,700
	Indirect Costs	£ 2,029
	Total external partner costs	£ 22,319

If any partner(s) needs an advance payment please specify who and the reasons why (please note that, generally, only DAC-list partners will be considered for advance payments):

Buea University, to ensure that reagents and consumables can be purchased in good time.

Sustainable Development Goal addressed (*Select the UN SDG that best describes the focus of this activity*):

Addressing affordability and availability of typhoid diagnostics brings Cameroon closer to meeting Sustainable Development Goal 3 (SDG3), "Good health and well-being". Furthermore, establishing a path to local production of health technologies could be a significant boost to the industrial sector (SDG9, "Industry, innovation and infrastructure"), and would create skilled jobs (SDG8, "Decent work and economic growth").

Expected benefits to and impacts on DAC-list nations.

The expected benefits come of this project come in the longer term from the potential for improved typhoid fever diagnostics and more immediately from the capacity building outcomes of the grant.

The ability to detect typhoid and its MDR genotypes will contribute to:

- improved patient management, especially where typhoid is a co-infection, resulting in faster treatment and lower morbidity and mortality. Reducing the >500 deaths annually in Cameroon and the morbidity and length of illness is an obvious goal and there are benefits not only for the patient but also caregivers, family members and community;
- detection of asymptomatic infections, resulting in lower transmission. This would reduce expense and stigma for residents of cities like Douala and Yaounde where outbreaks of typhoid have led to distrust of the water supply and people buying mineral water instead which is not always affordable;
- surveillance, resulting in more effective and timely health systems planning;
- detection of drug resistance, leading to more effective treatment and antimicrobial stewardship through judicious use of different antimicrobials and targeted use of the new typhoid conjugate vaccine (TCV) that was pre-qualified via the World Health Organization (WHO) in January 2018.

Culturally, our partners note that many Cameroonians label typhoid as a disease that can only be treated by native doctors or by using local herbs and partly this arises from a distrust of medical science due to repeated positive results from existing diagnostics such as the antibody-based Widal Test even after antibiotic treatment. This is an artefact of the antibody-based test that means it is positive in patients who have previously been infected with *S.* Typhi. A more accurate diagnostic tool has the potential to improve trust in medical facilities and slowly change cultural practices with regards to typhoid treatment and diagnosis. There are many steps between success in the laboratory and achieving this impact, which would require cooperation and communication by a range of stakeholders including community organisations**.**

Social and economic impact also emerges from capacity building. The Cameroon research team will be given the skills and technical know-how to use novel biological techniques and initiate further projects in their own context due to the lower cost, increased reliability of supply and reduction of

reliance on imports achieved through local manufacture of at least one key enzyme required for the research. Our pump-priming project specifically promotes local innovation and eventually production, which is believed by the World Health Organisation (2011) to build health security and promote diagnostics that are more suitable for local health needs, local entrepreneurship and skilled employment. Local production of a diagnostic product is a 10+ year goal but including that goal by design at this pump-priming stage is important for ensuring equitable starting conditions and setting a direction for our partnership that is focused on economic benefits to Cameroon.

In terms of **academic capacity building**, the introduction of CRISPR/Cas based techniques into Cameroon and the partnership with the University of Cambridge will enable more innovative and lower-cost academic research projects that are internationally excellent while focused on local social impact and economic development. Our Cameroon partners are committed to sharing their knowledge through the educational resources generated in on-going workshop and internship series with graduate students in which around 6-8 students are expected to pass through the MboaLab each year. We estimate that as a result of this project, 40-50 researchers and students will be trained in the next two years in methods that are revolutionising molecular diagnostics

Other impact not included in the above, if any (max 150. words)

The novel partnership between MboaLab and the University of Buea will promote new modes of institutional collaboration and partnership in Cameroon and demonstrate the efficacy of community-based innovation hubs and academic institutions combining strengths to address sustainable development goals in resource-limited environments. Moreover, the tripartite relationship with the University of Cambridge that this grant enables will move from the bilateral capacity building project which started in May 2019 to a much deeper partnership around development-focused biotechnology led by Cameroonian colleagues and forming a solid foundation for larger funded projects. It will serve as a model for future projects in Africa including in Ghana where Dr Molloy has similarly established a partnership between KNUST and the Kumasi Hive innovation hub.

References

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