**A portable, quantitative microfluidic diagnostics for point-of-care malaria testing**

## Executive summary

Malaria is a major life-threatening disease in tropical Africa, especially Nigeria that records 26% global malaria deaths, whilst over 200 million people are exposed to malaria infection every day. Due to the laboratory cost (>#5000) and equipment required to perform a diagnostic, testing among populations in the rural areas or low-income settings is challenging, even though it would enable to curb fatalities, as affordable treatment is available. A low-cost (#50) diagnostic test that can accurately, sensitively and specifically detect the presence of malaria parasites at an early stage of infection can increase the chance of patients' survival in Nigeria. Moreover, diagnostic tests that can provide a quantitative result can give a rapid indication of treatment efficacy and patients response to treatment, currently not available. This project aims to commercialize diagnostic solution for malaria in Nigeria. We will overcome the clinical limitation of current tests based on *Plasmodium falciparum* histidine rich protein (PfHRP2), which has been affected by mutation and now is technically unreliable and should not be used widely clinical diagnosis. Here, we will make use of the high robustness of aptamers as molecular tools to capture antigens specifically (stable in a wide range of conditions, suitable for deployment in rural areas where cold chain is not available) and microfluidic technology, to develop a rapid (5min) diagnostic test (RDT) targeting *Plasmodium falciparum* lactate dehydrogenase (PfLDH), a WHO-accepted solution, in blood, reaching high specificity (99%) and sensitivity (95%) to malaria infection in real-life conditions in communities. All in all, this grant will enable the scale-up of the initial product to meet required standard for market demand and provide medical diagnostic services to Nigerian (primarily) at a lower cost than currently obtainable in the market.

## 1.0 Introduction

Malaria is a major burden to the public health in sub-Saharan Africa and many low-middle income countries (LMIC). *Plasmodium falciparum* malaria is particularly a life-threatening infectious disease, which disproportionately affects Nigeria with an estimated 26% of the global malaria deaths in 2023 (World Health Organization, WHO, 2024). Besides the prevalent high mortality rate, in 2018, the WHO began to report major complications (WHO, 2019) with approximately 11 million (29%) pregnant women were infected with malaria in Africa (38 countries), which resulted into preterm birth or low birth weight (a major factor for neonatal and infant mortality) of an estimated 872 000 children (WHO, 2022). Whilst progress has been made in the fight against malaria, especially with the provision of therapies bed nets, insecticides and recently vaccines (with support from Gavi Alliance), the WHO (WHO 2024) (and Nigerian government) have highlighted the importance of accurate and widespread diagnostic tools to sustain past successes.

Accurate malaria diagnosis in a quick response to suspected cases mitigates fatalities, but is often complicated by other febrile illnesses e.g. schistosomiasis or most recently – Covid19, that share similar symptoms in the early days of infection (when treatment is most efficient). This means that diagnosis (guiding to the right treatment) is often decided with prevalence: for example, in places where malaria is endemic, schistosomiasis could co-infect a patient (the patients are treated for malaria without specific diagnostic test), and vice versa, whilst in regions where they are co-endemic, one obscures the other and again is often missed, leading to inappropriate treatment and longer impacts on the patients.

Early detection of malaria could facilitate effective diagnosis, monitoring of treatment efficacy and surveillance. The most common among malaria markers that can determine the level of parasitaemia, and severity are molecules such as *P. falciparum* glutamate dehydrogenase (pGDHs), Histidine-rich

proteins (HRPs), aldolase and Lactate dehydrogenase (PfLDH) to overcome associated challenges with the conventional approaches e.g. microscopy of blood drawn from patients or, commercial antibody-based rapid diagnostic tests for only histidine-rich protein 2 – HRP2, before drug administration (WHO, 2019).

Whilst microscopy is adjudged a gold standard for malaria test, it requires specialists, difficult to access at the point-of-care, in communities, and the performance of HRP2 immunodiagnostics is compounded by factors such as mutation and its persistence in blood after treatments (Ding *et al.* 2017), as well as instability at high temperature and humidity (Albertini *et al*. 2012). Additionally, conventional devices for malaria testing lack sensitivity to asymptomatic cases and co-infection of malaria (where another disease is most abundant in the patient and malaria has a lower impact), which is needed to stop transmission. In Nigeria, presumptive treatment and self-medication is well-documented: anyone with fever is often first treated for malaria without testing because testing is difficult to access and expensive.

Therefore, development of stable, reliable and affordable biosensors that address the limitations of existing diagnostic tests will play a key role in the fight against malaria. In the place of antibodies, aptamers, sensitive and specific single stranded oligonucleotides (DNA) have demonstrated promising features such as lower cost of production and greater stability to high temperature to the benefits of low-cost diagnostic tests. A few DNA aptamers have been selected against malaria biomarkers namely, pLDH (Frith *et al*. 2018; Cheung *et al*. 2013), PfHRP 2 (Chakma *et al*. 2018) and PfGDH (Singh *et al*. 2018), which have not yet been integrated into affordable and practical sensing platforms.

**Product description**

Microfluidic technology is a robust and versatile platform that can process complex samples such as blood, in portable formats, where other techniques require multiple steps and devices for clinical samples. We functionalized such platforms with aptamer recognition agents to create a convenient and affordable biosensor with reliable analytical performance. Paper is an excellent platform for such devices, as used in lateral flow strips for example, as it contains hydrophilic cellulose fibers that allow fluid movement from one point to another by capillary action without requiring external forces e.g. pumps. Viewed from mass production perspective, paper is light weight and flexible, amenable to roll-to-roll manufacturing, made from plant making it a renewable material. Therefore, paper-based devices are low-cost, produce a fast response, biocompatible and biodegradable.

The paper microfluidic diagnostic consists of channels, sample loading, assay buffer and detection zones (Figure 1). The sample loading zone allows blood to flow vertically through the paper and horizontally (lateral flow) on the paper surface to reduce non-specific interactions. The detection zones are functionalized with aptamers to capture the target marker molecule from the sample (PfLDH), whilst all other molecules (impurities) continue to waste. The assay buffer applied allows to develop the color signal through a sensitive and rapid biochemical reaction. Thus, these innovative geometries are used as practical valves that minimize interference from blood complex matrix to increase aptamer-PfLDH interaction in the malaria colorimetric diagnostic testing. In addition, the device is laminated to enable portability, whilst maintaining the ease-of-use and low-cost of lateral flow, leading to a step change in sensitivity and specificity of the assay, diagnostic analysis using smartphone.

Overall, this device is configured to: (i) minimize colour signal interference from blood, thus yielding high signal over noise performance and thus high sensitivity (detecting low levels of infection), (ii) ensure even distribution of target antigens (PfLDH) to the detection zone (consequently enhancing

sensitivity further) and (iii) the nanoparticle conjugates of the aptamer are immobilized at the test line to capture PfLDH in fractionated blood sample to generate amplified signal that enables better analytical sensitivity and specificity during test. Test results are captured using smartphone (containing image analysis software) and transmitted via mobile technology. This new product is designed with a novel methodology to produce excellent analytical performance in the field, on a par with target product profile (TPP) requirements by the WHO.

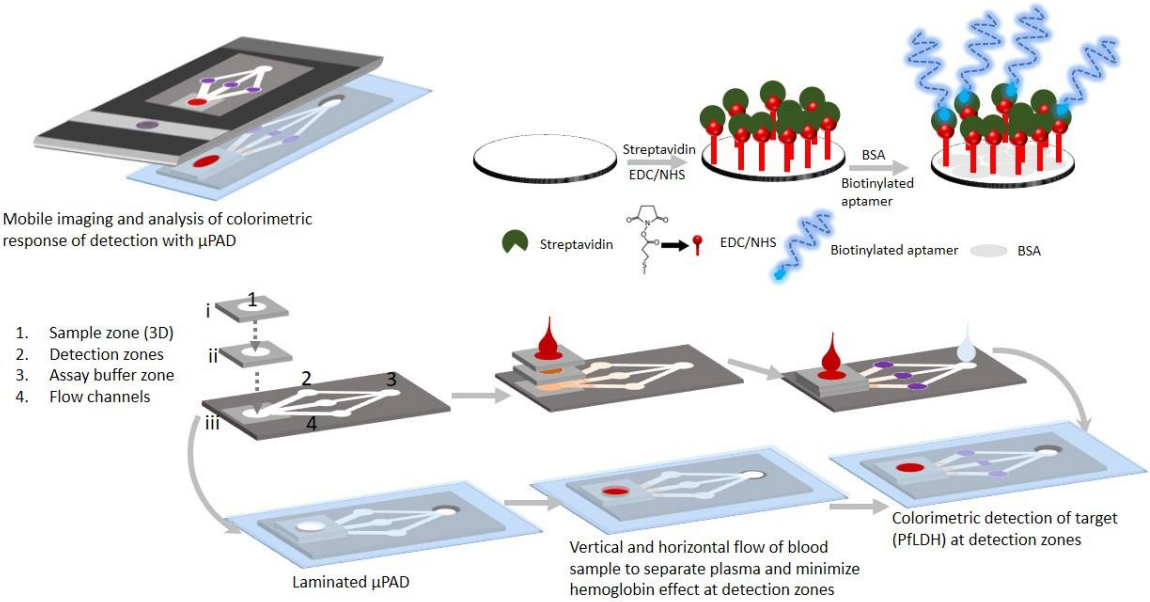


**Figure 1**: Construction of microfluidic paper analytical device (µPAD) enabling detection of malaria via aptamer tethered enzyme capture. The µPAD will be fabricated using screenprinting technology, whilst aptamer immobilization at the detection zones will be performed by the combination of carbodiimide chemistry (covalent) and streptavidin-biotin interactions (non-covalent). The sample zone is designed to 3D by stacking the paper discs (i & ii) layers together into iii for effective separation of blood and detection of target without steric hindrance.

**Preliminary results**

During my PhD, I developed microfluidic paper analytical devices (µPADs) for detection of pLDH in malaria (Ogunmolasuyi *et al*. 2022; Figure 2A), paper-based enzyme-linked oligonucleotide assay (pELONA; Figure 2B), and paper-based aptamer tethered enzyme capture (APTEC; Figure 2C-E).Following my PhD, I was awarded USAID-ARNTD funding ($29689) in 2021, with which I set up a biosensor research laboratory from scratch and developed the colorimetric detection of PfLDH using rLDH7 DNA aptamer conjugated to gold nanoparticles (Figure 3). **These positive results have been demonstrated up to TRL 5 outside of the lab, in an industrial setting** (but TRL 4 for the use in communities) **and** serve as basis for scaling up the biosensor to an aptamer-based microfluidics paper analytical device that can uniquely handle direct blood separation to get an enhanced visual response in a lateral flow configuration.

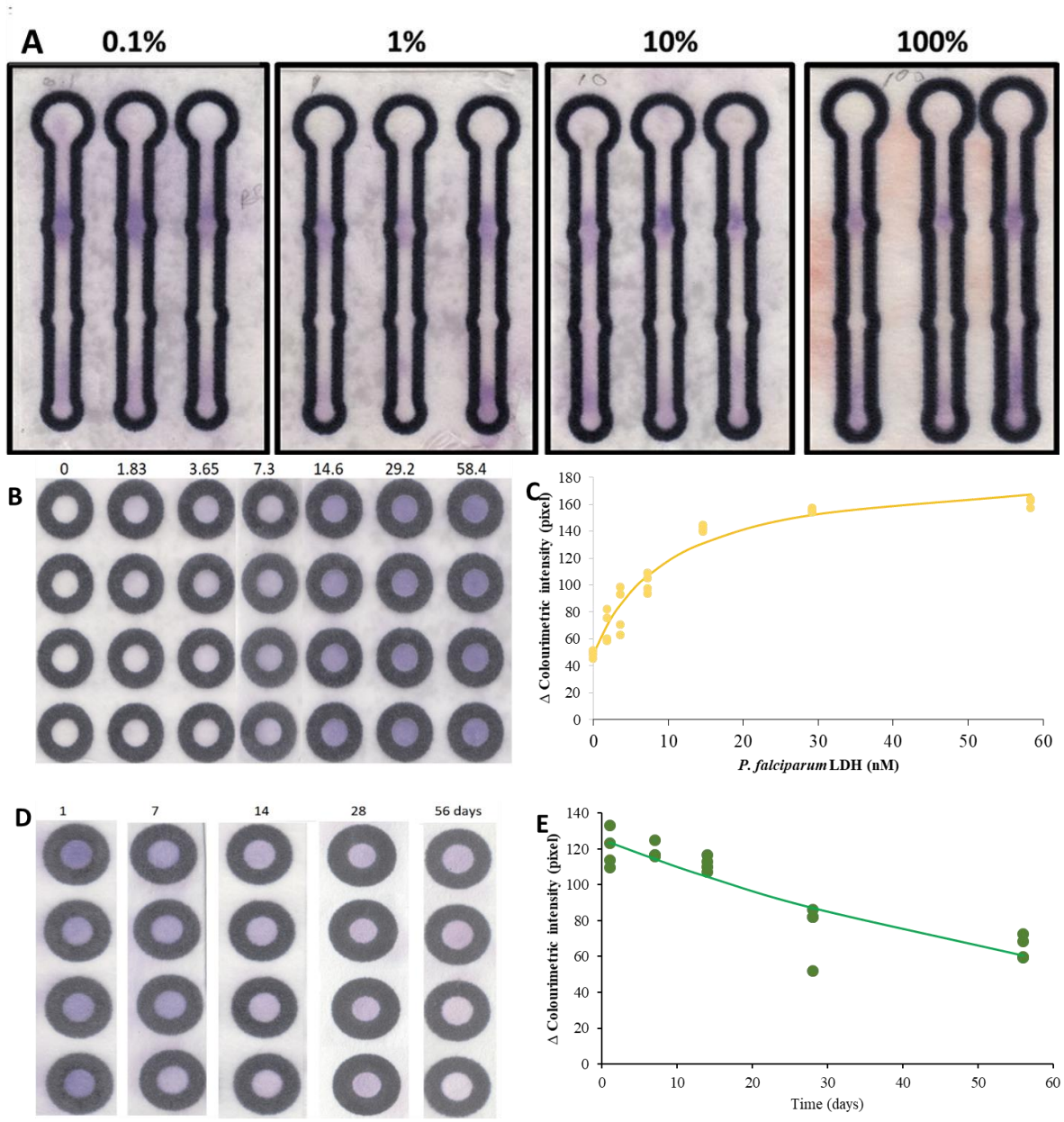


**Figure 2: Detection of PfLDH using aptamer-based (rLDH7 aptamer) in two paper-based colorimetric biosensors. (A**) Aptamer-based µPAD for detection of r*Pf*LDH spiked into blood using APTEC platform**.** Photographs of the colorimetric response of rLDH7 µPADs in the presence of 29.2 nM r*Pf*LDH in varying concentrations of blood lysate. (**B**) Image of the Paper-based aptamer tethered enzyme capture (APTEC) showing dose-dependent response of the diagnostics to a range of PfLDH concentration. (**C**) Colorimetric intensity of paper-based aptamer tethered enzyme capture (APTEC) showing dose-dependent response of the diagnostics to a range of PfLDH concentration. (**D**) Image of stability of the biosensor over a period of 56 days at 7 days interval from the first day. (**E**) Colorimetric intensity of the biosensor over a period of 56 days at 7 days interval from the first day.

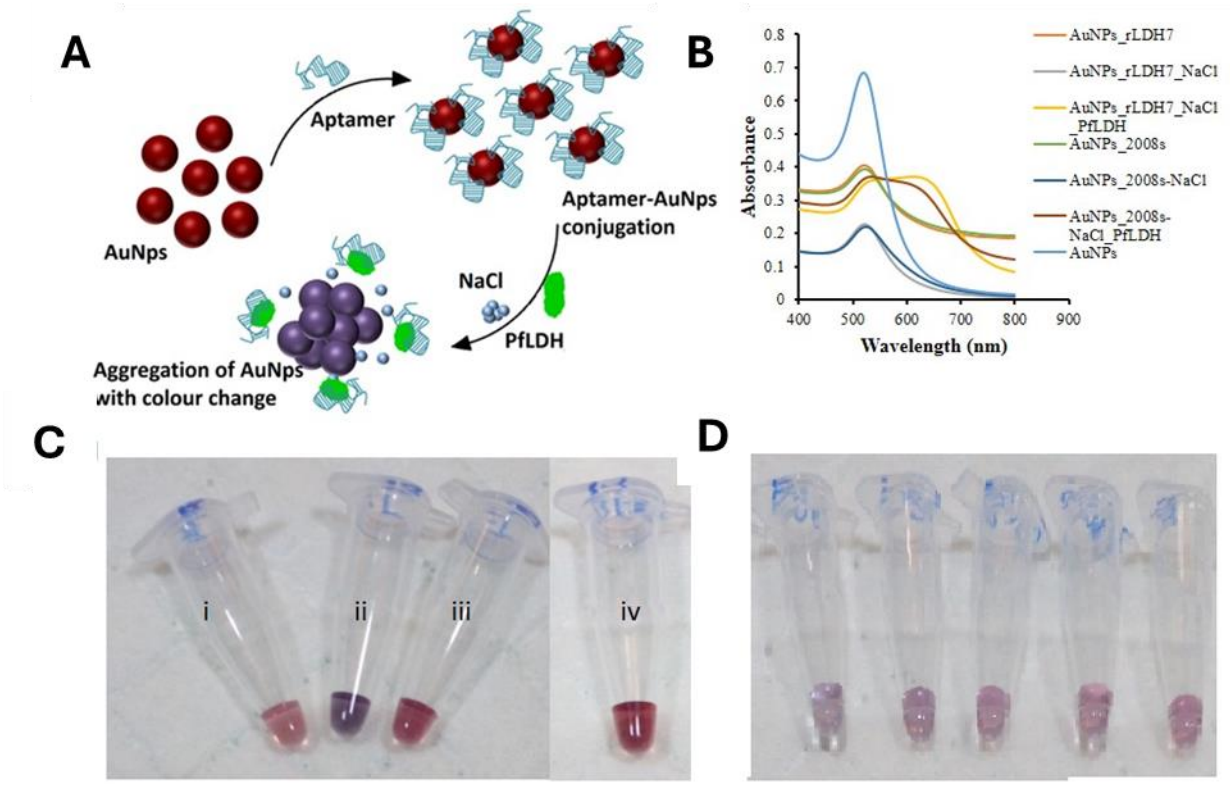


**Figure 3:** (**A**) Schematic of ligand exchange assay illustrating the colorimetric response of aptamer-rPfLDH interaction leading to aggregation of AuNPs upon addition of NaCl solution. (**B**) Spectra of 2008s or rLDH7 aptamer conjugated to AuNPs and the complex of aptamer-rPfLDH leaving AuNPs naked to react with NaCl. **(C**) Image showing the colorimetric responses of ligand exchange assay for signal specificity. (i) Detection of BSA with rLDH7-AnNPs. (ii) Colorimetric response of LEA upon addition of rPfLDH to rLDH7-AuNPs colloidal suspension. (iii) Validation of nonspecific interaction of rPfLDH with AuNPs. (iv) Detection of HSA with rLDH7-AnNPs. Colorimetric responses were observed in all after addition of 137 mM NaCl (PBS). (**D**) Colorimetric response based on different concentration of PfLDH in buffer.

**Technology Readiness Level (TRL) - 5**

The aptamer-based microfluidic device (Figure 2) had been successfully tested in recombinant PfLDH, spiked blood sample and parasitized blood sample. *Plasmodium falciparum* blood cultures of ~6% parasitemia and fresh non-parasitized blood were tested at the Center for Chemico- and Biomedicinal Research, (Rhodes University, South Africa). In rPfLDH, the diagnostic device showed limit of detection (LOD) of 106 ng/ml (an average of 1000 parasite/µl), far below an average concentration (3900 $ng.ml^{-1}$) of PfLDH (i.e. approximately 22000 parasite/µl) in infected blood samples (Jang *et al*. 2013). It also demonstrated a turn-around time of 7 min and retained sensitivity at cold and room temperature at 56 days of storage. The device exhibited significant analytical performance when compared to commercialized product in parasitized blood sample with sensitivity of approximately 1000 parasites/µl of blood (Ogunmolasuyi *et al*. 2022; Figure 1). The limitation stemmed from interference of blood haemoglobin with the actual sensor signal, which was addressed during USAID project.

**Overall Objectives**

Commercialization of aptamer-based microfluidic device for rapid colorimetric detection of malaria at the point-of-care in urban and low-resource settings.

**Objectives**

- Product refinement by iterative design and fabrication of microfluidics paper analytical device possessing improved geometry. Separately, µPAD will be coupled with nitrocellulose (Figure 1) as a mitigation step to develop lateral flow ligand exchange assay.
- Intellectual property filing and protection. The new geometries and processes will be protected through patent and design filing.
- Lab-based validation in spiked samples to ensure performance before clinical testing.
- Preparing scale-up manufacturing at Biosensor and Diagnostic Laboratory, Department of Biotechnology, Federal University of Technology, Akure. We will set up a fabrication facility with guidance from Professor Julien Reboud (University of Glasgow, UK; Reboud et al. 2019) and Professor Janice Limson (Rhodes University, South Africa) who has extensive experience in scaling up microfluidics and RDTs, with pilot runs at low volumes and negotiations with supply chain.
- Clinical testing targeting a gender-balanced, statistically powered cohort of about 300 symptomatic and asymptomatic individuals. Estimation of the sensitivity (>99%) and specificity (>95%) of the diagnostic device before fielding at the POC. PCR assay and existing rapid diagnostic test will be used as gold standard, with performance established by statistical significance.
- Customer engagement. Working with CodixBio Ltd, we will develop a market entry strategy. We will build a regulatory approval dossier (inc. CE marking) for *in vitro* diagnostics. This may eventually require WHO registration, but initially may be sold by CodixBio Ltd. This will lead to product branding decisions.

**Commercialization strategy**

**Product refinement (construction of paper microfluidic device) 9 months**

µPADs of new geometries will be fabricated as illustrated in Figure 1 using Whatman chromatography paper, 1CHR, (GE Healthcare Life Sciences, NY, USA). The 3D design of the sample loading zone consists of two layers of a microzone compressed into the sample loading area of the µPADs. Each of the microzones (sample loading) is 7 mm diameter hydrophilic area surrounded by hydrophobic barrier (black region) created by screenprinting into the pores of the Whatman paper at 120 degrees Celsius for 10 min. The aptamer agent is immobilized at the detection zones using carbodiimide chemistry and streptavidin-biotin interaction. In Figure 1B, a 3D µPADs connected to 3 mm width nitrocellulose will be used for ligand exchange assay with integrated AuNPs conjugated to aptamer as shown in Figure 1B. In both cases, the device in laminated to enable portability and ease-of-use at POC. This is a low-cost colour-based diagnostics prepared to ensure >95% sensitivity and 99% specificity needed for rapid mapping and periodic surveillance of malaria in the endemic region in Nigeria.

**Field evaluation and implementation of diagnostic device (9 months)**

Following scale up and construction of the diagnostic device, 750 diagnostic devices will be produced for clinical evaluation and implementation in collaboration with the FUTA Health Centre and Key of David Specialist Hospital, Akure (Dr Tolulope E. Ariyo), who will also guide the ethics approval Statistically-powered cohort of about 500 malaria patients (sample size calculator: https://sample-size-calculator-69531.firebaseapp.com), including pregnant men, women and underage children (>5yr)

among vulnerable populations will participate to determine specificity (>99%) and sensitivity (>95%) of the device in real samples.

**Branding of products**

For effective commercialization processes and distribution of product entity, our product is branded as M-Qdiks.

**Commercial viability**

This device is expected to show higher performance in the laboratory and clinic for detection of malaria, while showing stability of >80% at extreme conditions of temperature and humidity at $100^{th}$ day. This product will facilitate prompt healthcare delivery in Africa, where malaria is a burden to the public health, specifically to those in the limited resources environment who lacked access to basic healthcare delivery. I have identified and held meetings with the management of a newly inaugurated biosensor company in Lagos (Codix Biosensor Ltd.) to enable collaboration and commercialization of research products. Codix Biosensor Ltd. (International Standard Organization [ISO] certification) holds the World Health Organization franchise to develop and commercialize diagnostic products in Nigeria and West Africa. Our partnership is a clear path to distribution of the product in Nigeria and West Africa diagnostic market. As recognised in the **Presidential Initiative for Unlocking the Healthcare Value Chain (PVAC)**, nearly all African nations are committed to the importation of basic diagnostics for testing pregnancies or diseases, because in that respect, no local production to take on our healthcare services. Cost implication of importation (tariffs) is a major loophole in African economy and local production can stop it, retain our FOREX and create jobs for relevant individual within the system. Additionally, local manufacture will reduce the cost effect on consumers and increase accessibility and approachability in the event of emergency.

**Intellectual property protection**

This product is targeted towards prompt healthcare delivery in Nigeria and other African nations and will be protected or patented according to intellectual property law in Nigeria to create manufacturing and commercialization of this work.

## MONTHLY GANTT CHART

A portable, quantitative diagnostic for point-of-care malaria testing

PROJECT NAME: COMMERCIALIZATION OF MALARIA DIAGNOSTIC DEVICE

| Task | Task | Assignments | Start Date | End Date | New Duration (months) |
|---|---|---|---|---|---|
| 1 | Project kick off meeting | Research group | 05/01/2026 | 05/01/2026 | 0 |
| 2 | Planning & assignments | Research group | 06/01/2026 | 06/01/2026 | 0 |
| 3 | Purchase and arrival of equipement and consu | Martin/Mary/Adeyem | 06/01/2026 | 1/21/2026 | 0.5 |
| 4 | Design & Fabrication of µPAD | Julien/Martin | 1/21/2026 | 20/02/2026 | 1 |
| 5 | PfLDH detection: validation Malstat reagent assa | Martin/Catherine | 20/02/2026 | 20/03/2026 | 1 |
| 6 | Aptamer-AuNPs conjugate/aggregation assay | Mary/Samuel | 20/03/2026 | 20/04/2026 | 1 |
| 7 | Immobilisation of aptamer/antibody at µPAD | Julien/Martin | 20/04/2026 | 20/05/2026 | 1 |
| 8 | Characterisation of µPAD | Julien/Martin | 20/05/2026 | 20/06/2026 | 1 |
| 9 | Analysis of biosensor performance and stabili | Julien/Martin | 20/06/2026 | 20/09/2026 | 3 |
| 10 | Production of µPAD for clinical testing | Julien/Martin | 20/09/2026 | 06/10/2026 | 0.5 |
| 11 | POC application of µPAD | Julien/Martin/Samuel | 06/10/2026 | 06/01/2027 | 3 |
| 12 | Traveling to villages begins | Martin/Samuel/Mary | 06/01/2027 | 06/04/2027 | 3 |
| 13 | Sample collection and analysis | atherine/medical ad | 06/04/2027 | 06/07/2027 | 3 |
| 14 | Market entry (Codix) | Codix Team | 06/07/2027 | 06/01/2028 | 6 |
| 15 | Others activities | Research group | Monthly | | |
| 16 | Weekly meetings | Research group | Weekly | | |
| 17 | Workshop/conferences | Research group | Workshop | 03/11/2026 | |
| 18 | Summary of final reports | Research group | 03/07/2027 | 02/02/2028 | |
| 19 | Manuscripts writing/Publications | Research group | 03/10/2027 | 03/05/2028 | |

Remarks: Eighten months research to commercilaize diagnostic product after disbursment of fund by NASENI. Market entry will be conducted by Codix Team.

**Outcomes**

At the end of the project, we will have achieved a portable, quantitative and stable diagnostics that meet the need of Nigerian populace, particularly the vulnerable and those in extreme poverty that cannot afford basic medical needs to stay healthy. We anticipate that the product will be on the market within 6 months after the completion of the programme.

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