COVID-19 diagnostics for Africa

Protocol Number:

Version Number: 1.00

Date: 20.12.2020

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Protocol Summary

Title	COVID-19 diagnostics for Africa
Aim	The aim of the project is to provide reliable diagnostic systems for the confirmation of the clinical diagnosis of COVID-19 in Africa from first infection through to recovery and immunity.
Study Design	This study is a cross sectional experimental which involves the development and evaluation of two low cost point of care (POC) and rapid throughput NAT and SARS-Cov-2 antibody titre tests. This study has been organised into 9 work packages under the broad themes: Project management, development of point of care diagnostics, clinical study and field test, innovation and dissemination.
Samples details	Nose, throat or nasopharyngeal swab, sputum or bronco-alveolar lavage will be collected for diagnostic testing and the Essential Information for Clinical Studies form. Sera from SARS-Cov-2 positive and negative patients will be used to validate a proof of concept Immunoassay.
Number & Name of Site(s):	Three sites. 1. Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Ghana 2. Noguchi Memorial Institute for Medical Research (NMIMR) 3. Kwame Nkrumah Universitry of Science and Technology (KNUST)
Study Duration:	Fifteen months (approx.).
Description of Device:	The total setup consists of innovative portable and price-competitive POC RT-LAMP (PATHPOD from DTU, a novel raw material production process for low resource countries and a proof of concept immunoassay (UCAM) PATHPOD: Nucleic acid test for viral RNA, which uses RT-LAMP (reverse transcription loop-mediated amplification). The PATHPOD system consists of an instrument with integrated heater, optical detection systems, and polymeric injection moulded Lab-on-a-chip cartridges with 12 chambers suitable for on line or on-site rapid detection of detect COVID-19 virus in clinical, environmental and animal samples. The prototype chip with 10 samples and 2 controls is made in a low-cost polymeric material by injection moulding. It can easily be up-scaled for industrial production. The industrial version of the chip can include 32 samples. The PATHPOD Lab-on-a-chip (LOC) system includes integrated sample preparation and real-time reverse transcriptase (rRT) Loop-mediated Isothermal Amplification (LAMP) for rapid and quantitative detection of COVID-19 virus.
Objectives:	*Primary Objective:*

	1.To provide reliable diagnostic systems for the confirmation of the clinical diagnosis of COVID-19 in Africa from first infection through to recovery and immunity <u>*Specific Objectives:*</u> 1.1. To use the front line testing facilities in NMIMR and KCCR, Ghana, as a testbed for a new POC nucleic acid diagnostic emerging from DTU (PATHPOD - detection of virus RNA). <u>*Secondary Objective*</u>: 1. To replace the BST and RT enzymes in the PATHPOD with BST and RT constructs designed by UCAM for low resource production in Africa and evaluate the AfriDX-RT-LAMP alongside the PATHPOD and standard RT-qPCR procedure. 4. To develop an Antibody Titre assay to proof of principle from a UCAM single-chain antibody (scFv) library and produce scFv constructs for low resource production of a lateral flow immunoassay (LFIA) in Africa (AfriMx). 5. To evaluate combined batch testing as methodology adopted from normal veterinary protocols (but not widely used for human testing) and assess accuracy, cost and time benefits in epidemic testing pathways. 6. To draft a plan to produce AfriDx in Africa for the African market, with outline analysis of socioeconomic and healthcare benefits.
Study Participants/Sample Collection	Individuals presenting to KCCR or MNIMR with suspected COVID-19 infection will be assessed by clinical staff and clinical specimens (e.g. nose, throat or nasopharyngeal swab, sputum or bronchoalveolar lavage collected for diagnostic testing. Sera fromnSARS-Cov-2 positive and negative patients will be used to validate a proof of concept Immunoassay.
Statistical Analysis	Data will be analyzed using software Statistical Packages for Social Sciences (SPSS) version 22.0. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy will be calculated using standard formulas. Kappa and McNemar tests will be performed to determine the concordance and discordance between test assays and real-time RT-qPCR-based methods.

1.0 Background and rationale for the study

In January 2020, the World Health Organization (WHO) declared a global health emergency over an outbreak of a new coronavirus (2019-nCoV), which originated in the Chinese city of Wuhan [1]. In February 2020, 2019-nCoV was renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease coronavirus disease (COVID-19) [2]. On 11 March 2020, the WHO characterised the COVID-19 outbreak as a pandemic [2], following alarming levels of infection spread and disease severity.

Nucleic acid testing (NAT) is the most reliable method to identify the causative agent (viral RNA), but these are expensive and usually require well-resourced laboratories. It is also not suitable for deployment outside a sophisticated laboratory environment with necessary equipment and skilled technicians (biosafety level 2 or above) [3]. In Ghana, there are national testing facilities in NMIMR and KCCR, with a combined capacity to process 4500 tests/day and now further regional testing is being established. The cost of testing (>$10/test) in Ghana/Africa is not sustainable for long term testing. Detection of raised antibody titres is an alternative assay, but it first becomes useful later in the disease pathway and into recovery to identify people (including asymptomatic individuals) who have had the disease and are now showing immunity. This test is not sensitive early in infection. Together a NAT and immunoassay give a powerful tool for managing the spread of infection and 'herd immunity', as well as providing the best information for care of the patient. As such this study seeks to develop and evaluate new low-cost point of care (POC) and rapid throughput NAT and SARS-Cov-2 antibody titre tests in Ghana.

The advantages of the suitcase lab are:

A. Easy to be implemented in low resource settings.
B. Rapid time to result around 30 minutes including the extraction procedure.

The AfriDx consortium seeks to contribute to diagnosis of COVID-19 infection and patient management and public health preparedness in Africa, in response to the epidemic. It recognizes that Africa's demographics (1.3 billion population), its high prevalence of poor health and densely populated overcrowded urban areas present an enormous challenge to an under-resourced health service and may function to spread the virus suddenly and rapidly.

Figure 1: *PATHPOD device and Suitcase lab for SARS-CoV-2 detection*

2.0 Aim and study objectives

Aim

The aim of the project is to provide reliable diagnostic systems for the confirmation of the clinical diagnosis of COVID-19 in Africa from first infection through to recovery and immunity, with:

- RNA viral testing enabled for point of care (POC)
- Antibody titre testing for POC
- Sample to result time of less than one hour
- >90% sensitivity and specificity
- Compatibility for distributed production at low cost in Africa

Hypothesis

Test assays are as sensitive and specific as real-time RT-qPCR-based molecular methods.

Primary objective

To provide reliable diagnostic systems for the confirmation of the clinical diagnosis of COVID-19 in Africa from first infection through to recovery and immunity

Specific Objectives

The objectives are:

- To use the front line testing facilities in NMIMR and KCCR, Ghana, as a testbed for a new POC nucleic acid diagnostic emerging from DTU (PATHPOD - detection of virus RNA), that is

currently under clinical test in Europe and will be seeking Emergency Use Authorization (EUA) in the next 6 months.

Secondary Objective:

- To replace the BST and RT enzymes in the PATHPOD with BST and RT constructs designed by UCAM for low resource production in Africa and evaluate the AfriDX-RT-LAMP alongside the PATHPOD and standard RT-qPCR procedure
- To develop an Antibody Titre assay to proof of principle from a UCAM single-chain antibody (scFv) library and produce scFv constructs for low resource production of a lateral flow immunoassay (LFIA) in Africa (AfriMx).
- To evaluate combined batch testing as methodology adopted from normal veterinary protocols (but not widely used for human testing) and assess accuracy, cost and time benefits in epidemic testing pathways.
- To draft a plan to produce AfriDx in Africa for the African market, with outline analysis of socioeconomic and healthcare benefits.

3.0 Study design

This study is a cross sectional experimental which involves the development and evaluation of two low cost point of care (POC) and rapid throughput NAT and SARS-Cov-2 antibody titre tests. This study has been organised into 9 works packages under the broad themes: Project management, development of point of care diagnostics, clinical study and field test, innovation and dissemination. The diagnostic performance of PATHPOD, a point of care viral detection system developed by DTU and adapted for SARS-Cov-2 viral assays will be evaluated with viral nucleic acid extracted from sputum, oral and nasal pharyngeal swabs. This evaluation will be conducted at KNUST, KCCR and NMIMR with assistance from DTU and TATAA using samples collected for both symptomatic patients and asymptomatic individuals, identified through contact tracing or through testing of healthcare workers. A comparative data with the currently used laboratory-based RT-qPCR will be obtained for the innovative RT-LAMP (PATHPOD) to produce information on sensitivity and specificity. After this initial evaluation of the PATHPOD, the low-cost systems (Boon-BST and Boon-RT production) developed at UCAM for expression and purification of the DNA polymerase Bst and a reverse transcriptase (RT), the enzymes used for the RT-LAMP assay in the PATHPOD system will be employed for local production these reagents. If successful, this

locally produced reagents in Africa (AfriDx-RT-LAMP) will be tested on a subset of samples collected compare results to the existing PATHPOD system.

The high-affinity single-chain variable fragment (scFv) developed by UCAM (Proof of Concept Serological Test Development) for COVID-19 immunoassays will also be adapted in this study. The resulting proof of concept AfriMx system will be tested on a small number of serological samples that will be collected from individuals who test positive and negative for SARS-CoV-2. Boon-scFV production protocols and design principles for scFV immunoassays will be transferred and disseminated through training activities in Ghana (Training and Capacity Building).

Sample size and statistical analyses

NMIMR, KCCR are supporting the frontline of SARS-Cov-2 testing in Ghana using RT-qPCR. Their capacity is ~1500 tests/day. We envisaged a total of about 4000 tests from KNUST and KCCR.

Data will be analyzed using software Statistical Packages for Social Sciences (SPSS) version 22.0. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy will be calculated using standard formulas. Kappa and McNemar tests will be performed to determine the concordance and discordance between test assays and real-time RT-qPCR-based methods.

4.0 Research participants

Individuals suspected COVID-19 infection and presenting to KCCR, or KNUST and NMIMR with will be assessed by clinical staff and clinical specimens (e.g. nose, throat or nasopharyngeal swab, sputum or broncho-alveolar lavage collected for diagnostic testing upon giving their consent.

5.0 Study procedures

The overall study will be performed, after obtaining regulatory and IRB approvals as applicable in the country and the participating institutions. According to the study design, the diagnostic performance of PATHPOD, a point of care viral detection system developed by DTU and adapted for SARS-Cov-2 viral assays will be evaluated with viral nucleic acid extracted from sputum, oral and nasal pharyngeal swabs. This evaluation will be conducted at KNUST, KCCR and NMIMR with assistance from DTU and TATAA using samples collected for both symptomatic patients and asymptomatic individuals, identified through contact tracing or through testing of healthcare

workers. A comparative data with the currently used laboratory-based RT-qPCR will be obtained for the innovative RT-LAMP (PATHPOD) to produce information on sensitivity and specificity. After this initial evaluation of the PATHPOD, the low-cost systems (Boon-BST and Boon-RT production) developed at UCAM for expression and purification of the DNA polymerase Bst and a reverse transcriptase (RT), the enzymes used for the RT-LAMP assay in the PATHPOD system will be employed for local production of these reagents and testing. If successful, this locally produced reagents in Africa (AfriDx-RT-LAMP) will be tested on a subset of samples collected compare results to the existing PATHPOD system.

The high-affinity single-chain variable fragment (scFv) developed by UCAM (Proof of Concept Serological Test Development) for COVID-19 immunoassays will also be adapted in this study. The resulting proof of concept AfriMx system will be tested on a small number of serological samples that will be collected from individuals who test positive and negative for SARS-CoV-2. Boon-scFV production protocols and design principles for scFV immunoassays will be transferred and disseminated through training activities in Ghana (Training and Capacity Building).

Nucleic acid extraction and RT-LAMP

Viral RNA will be extracted and purified from swab using the heating method.

Virus lysis

Samples will be collect from patient using the E-swabs according to standard protocol specified by WHO, CDC or ECDC. Each sample will be well labelled and stores at 4 oC for 5 min. The tube together with the sample will be vortex briefly to release the virus on the swab into the solution. The swab will be remove, and the solution heated at 95 0C for 5 min in a heat block or water bath to inactivate virus. The sample can be loaded on the chip or stored at - 20 °C until.

Isothermal amplification (RT-LAMP)

The isothermal amplification will be conducted using the PATHPOD device.

Figure 2. The amplification cycle of LAMP showing forward, loop and backward primers.

The amplification depends on binding of the recombinase to oligonucleotide primers. The complex then scans the template DNA for the corresponding sequence and initiates 5´-strand invasion of the oligonucleotide at the site of homology. The strand invasion is stabilized by the single strand binding protein. The extension of the primer ensues via a strand displacing DNA polymerase

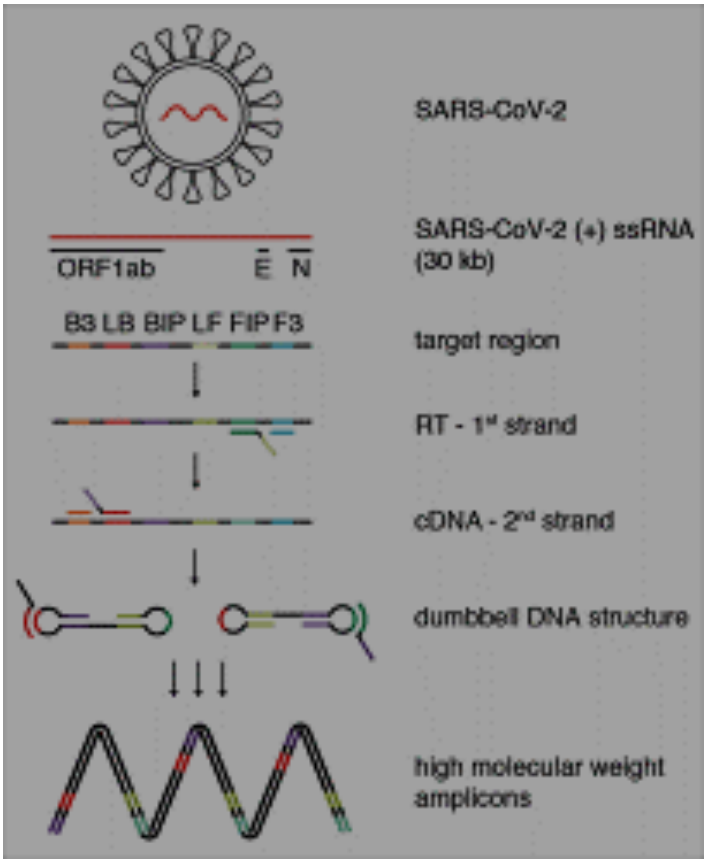

Table 1: Study Time Frame

Activities	M 1	M 2	M 3	M 4	M 5	M 6	M 7	M 8	M 9	M 10	M 11	M 12	M 13	M 14	M 15
Testing Strategy															
Development of study protocol and supporting documents															
Development of comparative study operational procedure and training															
Institutional and Regulatory Approvals:															
Sample collection and testing															
Data Management and Analysis															
Local Production of Boon-RT and BST in Ghana for use in RT-LAMP assay															
Reporting of study findings															
Demonstration of COVID-19 antibody detection via AfriMx immunoassay in Ghana															

6.0 Recruitment

Participants of this study include individuals suspected of contracting Covid-19 and are presenting to KCCR or KNUST and NMIMR. Their clinical specimens (e.g. nose, throat or nasopharyngeal swab, sputum or Broncho alveolar lavage collected for diagnostic testing upon obtaining an informed consent.

7.0 Consent process

The study will be explained to prospective participants who cannot read whereas those who can read and comprehend will be given the participant information leaflet from the CHRPE/IRB, that contains details of the study. Individuals who agree to take part in the study will have to give their consent by appending signature or thump printing the consent form.

8.0 Risks

Apart from minimal discomfort arising from sample collection, we do not anticipate any sever risk to the participants.

9.0 Mitigation of risks

The samples will be obtained by qualified clinicians/laboratory personnel. This will help in mitigating the risk.

10.0 Benefits

Even though there is no direct immediate benefit to the participant, successful completion of this study will be of use to the nation, continent and world at large. This will be achieved in the cost effective and rapid turnaround time of the device, not compromising on the sensitivity and specificity.

Again, all publications and relevant data will be made Open Access, training and capacity building activities aim to transfer knowledge and technology to the Ghanaian partners such that they are able to continue independent use and development of the technologies and engage in further international collaborations.

11.0 Study and safety monitoring

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the process and data generated. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Site monitoring will be conducted to ensure that the human subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet the regulatory guidelines.

Regular monitoring will be performed to ensure all the procedures are performed as per the approved protocol and the study is conducted as per ICH good clinical practice guidelines.

12.0 Managing and reporting adverse events

The study procedures do not involve intervention process to any study participants. We, therefore, do not envisage any adverse event. However, clinicians will be at the site of recruitment and sample taking to assist should any such event arise.

13.0 Managing and reporting unanticipated problems or protocol deviations

The investigator will report any deviation from an approved protocol or any study-related procedures to the PI by close of working day. Any serious deviations or unanticipated problems (e.g., a breach in confidentiality, changes at site that affect the conduct of the research, sample specification, etc.) will be managed and reported to the reviewing IRB/ethics committee and others as per their mentioned guidelines.

14.0 Confidentiality and data management

All sites will maintain the confidentiality of data collected from study participants as per the procedure defined in the consent forms according to regulations by the IRB/ethics committee. All samples will be labeled with random numbers by the site investigator.

Data obtained cannot be linked directly to any individual. All the data will be entered in a Microsoft Access database and kept safely with the PI. In reporting findings, by way of publication, unique codes will be used and not names.

15.0 Study costs

The study-related costs including pay for procedures associated with the study shall be covered by European and Developing Countries Clinical Trials Partnership (EDCTP). No costs will be incurred to any individual whose samples will be considered in the study.

16.0 Care for injury

We do not anticipate research-related injuries. Researchers handling participants and samples are encouraged to strictly comply by good laboratory practices. However, clinicians will be onsite to handle any of such unforeseen circumstances.

17.0 Compensation

We will compensate participants for the transportation costs and the time required to participate in this study. The minimum amount of GHS 20.00 will be given to the participants depending on the distance travelled. However, extra transportation cost incurred beyond GHS 50 will be reimbursed to participants.

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