ISRCTN29409638 https://doi.org/10.1186/ISRCTN29409638

Assessment of the accuracy of a new test for COVID-19

Submission date
26/01/2022

Recruitment status
No longer recruiting

Registration date
17/02/2022

Overall study status
Completed

Last Edited
11/02/2022

Condition category
Infections and Infestations

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[_] Results
[_] Individual participant data
[_] Record updated in last year

Plain English summary of protocol

Background and study aims
The COVID-19 pandemic has impacted all facets of life in the world. With rising infection rates, a timely diagnosis is important to limit the spread of COVID-19. The standard test used in most countries is a PCR test which is very expensive, needs to be performed by highly qualified staff and the results can take a day to several days. New affordable tests are needed that can be carried out in local health centres and district hospitals in low- and middle-income settings that cannot perform PCR. One such test is called the LAMP test for COVID-19, which a team at Kamuzu University of Health Sciences (KUHes), Blantyre compass have developed and would like to assess how well it works in real life. They want to learn about people's experience and expectations of the LAMP test for COVID-19 and what they can do improve their experience of the LAMP test. The researchers also want to know about the experiences of health workers when using the test because this knowledge might help them to learn how to better use this LAMP test for COVID-19 in this community.

Who can participate?
Adults and children of any age presenting with COVID-19 symptoms which include fever (a temperature 38.0°C or over measured at the day of presentation at a health facility or reported within the previous 24 hours) AND one of: cough, sore throat, nasal congestion, myalgia (muscle ache), fatigue, loss of taste or smell, poor feeding, vomiting or lethargy/drowsiness). Asymptomatic contacts of COVID-19 patients can also participate.

What does the study involve?
This study will involve the collection of nasopharyngeal (throat behind the nose) swabs by a laboratory technician. The swabs will be taken to the laboratory for the LAMP test for COVID-19 and PCR testing. Participants also answer a questionnaire that will take about half an hour.

What are the possible benefits and risks of participating?
To ensure participant safety, all samples will be collected by qualified laboratory staff collecting swabs as per standard of care with the appropriate professional registration. Swab collection may be uncomfortable but it is safe. Participants may share very personal and confidential information and may feel uncomfortable talking about some of the topics. The participants do not have to answer any questions or take part in the survey if they do not wish to do so.

Participants do not have to give any reason for not responding to any question, or for refusing to take part in the survey. There will be no direct benefit to participants other than knowing the result of their COVID -19 test as per standard procedure, but their participation is likely to help the researchers to find out more about how to improve COVID-19 testing and management in their community in Blantyre, Malawi.

Where is the study run from?
1. University of North Carolina (UNC) (USA)
2. Kamuzu University of Health Sciences (KUHes) (Malawi)

When is the study starting and how long is it expected to run for?
July 2021 to July 2022

Who is funding the study?
NIH Fogarty International Center (USA)

Who is the main contact?
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Contact information

Type(s)
Principal Investigator

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Additional identifiers

EudraCT/CTIS number
Nil known

IRAS number

ClinicalTrials.gov number
Nil known

Secondary identifying numbers

P.08/21/3366

Study information

Scientific Title
Validation of a real-time SARS-CoV-2 loop-mediated isothermal amplification assay in Blantyre, Malawi (SARS_LAMP)

Acronym
SARS_LAMP

Study objectives
To evaluate the point of care testing (POCT)/near patient SARS-CoV-2 reverse-transcriptase loop-mediated isothermal amplification (RT-LAMP) assay that can be utilised in Malawi in comparison to SARS-CoV-2 RT-PCR, the gold standard PCR.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Approved 30/08/2021, the College of Medicine Research and Ethics Committee (COMREC; College of Medicine, P/ Bag 360, Chichiri Blantyre 3, Malawi; +265 (0)888118993; comrec@medcol.mw), ref: P.08/21/3366

Study design
Diagnostic test accuracy study

Primary study design
Observational

Secondary study design
Cross sectional study

Study setting(s)
Hospital

Study type(s)
Diagnostic

Participant information sheet
Not available in web format

Health condition(s) or problem(s) studied
COVID-19 (SARS-CoV-2 infection)

Interventions
This study will be conducted at the Kamuzu University of Health Sciences (KUHes), Blantyre campus. Standard methods will be used to evaluate diagnostic test accuracy. The gold standard

is RT-PCR. The researchers will develop a SARS-CoV-2 loop-mediated amplification assay (LAMP) which is very rapid (around 30 minutes), highly sensitive (detecting CT values <30) and also specific (>95%) as it uses 4-6 specific primers targeting the N gene.

The project will be conducted in two stages. The initial phase will be the validation of the LAMP assay followed by an assessment of performance, acceptability, usability and cost-effectiveness in comparison to conventional RT-PCR testing.

Methodology
The study is cross-sectional in that the subjects are only evaluated at one timepoint but it includes mixed methodology (both qualitative and quantitative components).

Study place
The study will take place at KUHes, Blantyre Campus , Queen Elizabeth Central Hospital and Blantyre DHO and their health centres and testing sites. Blantyre is a high burden district for COVID-19 hence it will be easier to reach the sample size. The KUHeS Blantyre campus pathology department is already undertaking COVID-19 testing. The KUHes pathology team has already been working with the principal investigator on LAMP assays for other infectious diseases, hence the laboratory team are familiar with LAMP.

Study population
Patients presenting with symptoms suggestive of COVID-19 will be offered SARS-CoV-2 LAMP assay testing and those whose participants/caregivers who give consent will be entered into the study.

Study period
The project will be conducted in two stages.

Phase I
This is a laboratory-based validation of the SARS-CoV-2 LAMP assay.

The SARS-CoV-2 LAMP LAMP assay
The LAMP assay will use SARS-CoV-2 LAMP primers targeting N gene (9, 10), freeze-dried OptiGene IS0-DR004 mastermix with reverse transcriptase RT50 (OptiGene mastermix IS0-DR004-RT50). The researchers will test LAMP mastermixes using known positive residual SARS-CoV-2 clinical extracts plus a series of Negative Template Controls (NTCs). In the first instance, the researchers will pre-heat extracts to 95°C for 5 min before analysis. They will run assays on a GENIE II instrument at 65°C for 30 minutes followed by standard melt temperature (Tm). They will record time to positivity (TT) and specific Tm in °C. The chosen run temperature of 65°C reflects that recommended by Zhang et al. (2020) and is also the optimal reaction temperature for OptiGene mastermix IS0-DR004-RT50. There will be no manufacturing performance data as the researchers are not using a commercial LAMP assay.

The project will evaluate and compare both LAMP with and without an RNA isolation step so the researchers will have accuracy data on both LAMP assays in comparison to the gold standard. For the actual application and clinical use, the expectation is that LAMP without RNA isolation will be accurate and sensitive enough for identifying people who have SARS-CoV-2 viral loads with Ct <30 and with RNA extraction, the results will be similar to the gold standard. The OptiGene mastermixes with reverse transcriptase will be procured from OptiGene Limited. The researchers will evaluate one or more published SARS-CoV-2 LAMP primers which will be

procured from the Integrated DNA technologies in the UK. All reagents will be freeze-dried ambient. The researchers at KUHes, Blantyre Campus and the Queens University Belfast team have experience in sending LAMP reagents to Malawi.

The researchers used the OptiGene mastermix IS0-DR004-RT50 for optimisation and development of an HIV LAMP assay. So far they have sent LAMP consumables in bulk and have not yet experienced stock out. Some of the investigators at KUHes have sent samples to South Africa and Queen's University Belfast for sequencing so the researchers will know during this project how good the LAMP assay is at identifying the SARS-CoV-2 variants present in Malawi including the Alpha variant B.1.1.7, the Beta variant B.1.351 / 501Y.V2, the Gamma variant P.1 and the Delta variant B.1.617.

RT PCR
The RT PCR protocol currently used in Malawi uses the Omega Biotek Mag-bind Viral RNA/DNA 96 kit extraction kit, 2019-nCoV CDC EUA Kit PCR primers, which are standard CDC assay and Quantabio qScript XLT 1-step RT-qPCR ToughMix Low ROX PCR Master Mix. Two platforms are used, either the Rotorgene Q or QuantStudio 7. Like most RT-PCR assays, it is expected to have 95% sensitivity and specificity. It can be hard to exactly define these for the gold standard test (s) which by definition should be out-performing other tests. Details of this assay including performance data (FDA emergency use approval) are available on the FDA website.

For SARS-CoV-2 testing, there is an increasing view that RT-PCT is too sensitive, with many positives reflecting residual RNA - not infectious positives. So if using RT-PCR, using a viral load (Ct) cutoff to define clinically relevant positives (with low load positives just reported as "reactive") is becoming common. The clinically relevant cutoff (Ct) is below 30. In this study, Ct >30 will be reported as "reactive" (low viral load/non-infectious), and Ct <30 will be considered positive/potentially infectious. If the assay is deemed acceptable, which the researchers anticipate from the literature, they will conduct a phase II of the study.

Phase II
This will be prospective and is the phase where the LAMP assay will be used as the diagnostic assay for patient care.

Processes for recruitment of study participant
Participants (n=600 to 700) will be recruited by the research team from KUHes clinic, QECH (hospital COVID-19 surveillance) Blantyre DHO offices and their health centres e.g. Ndirande, Bangwe and Limbe (community COVID-19 surveillance). The research team will liaise with these teams at KUHes clinic, Blantyre DMO, Blantyre DHO and QECH Medicine department to identify participants who have symptoms suggestive of COVID-19 who come for testing at any of these sites. The participants will be approached to participate in the study through regular Department medicine, DHO surveillance team and Health Centre procedures. Participants interested in taking part in the study will then give informed consent in a private room. The participants will undergo standard of care testing for COVID-19 by laboratory technicians. The participants will have additional nasopharyngeal swabs taken by the same laboratory technicians for SARS-CoV-2 LAMP testing.
Study nurses assisted by research assistants will collect data on demographic details, the time the swabs were taken, sample arrival time in the KUHes laboratory, processing time and turnaround time of results. RT-PCR results from the QECH laboratory of KUHes lab will also be recorded by the study team.
The study team will conduct approximately 50 interviews with the participants to assess the acceptability of the SARS-CoV-2 LAMP assay and 200 interviews to collect costing data. Data on the usability of the SARS-CoV-2 LAMP assay will be collected from all laboratory technicians who

will be doing LAMP testing using both open-ended and closed questions as well as the Likert scale.

The researchers will assess the turnaround time for gold-standard PCR testing, which is a few days in the developed world, while in LMICs sample collection to receiving test results, which is clinically useful, sometimes takes 3 to 7 days. The turnaround time for LAMP is only a few hours. The researchers will conduct a costing and cost-effectiveness analysis of the two testing strategies using turnaround time (TAT) of results as the outcome measure.

Cost comparison SARS-CoV-2 LAMP assay and RT PCR Rotorgene Q or QuantStudio 7 (PCR)
The researchers will estimate health facility costs and economic costs borne by carers and hence will evaluate costs both from a provider and societal perspectives. For the health facility costs, intervention-specific variable costs will be estimated using the ingredients approach while shared overhead costs such as waste management and utilities will be apportioned by using the annual expenditure for a hospital, allocating it to the laboratory using an allocation basis (estimated proportion of lab to hospital area), multiplying that by a proportion of the cost attributable to the study (e.g. proportion of lab electricity that a machine will use for testing) and multiplying that by the proportion of the study period to the financial year (number of study days/365).

Fixed costs will be annuitized using the standard formula,
=
1 – (1 +)
–

where is the initial capital outlay, is the annual sum which at interest rate for years will be equal to the initial capital outlay (25). Useful lives, , of capital items will be collected from different sources and an interest rate of 3% was used. Capital items will include equipment and staff training.
Household costs will include costs incurred by patients and guardians in the process of seeking care, i.e. transportation, food, purchase of health passport, consultation, registration, laboratory test, medicines, gifts to health workers and under the table payments. The study will randomly select one out of every three people coming for COVID-19 testing and screening. The study will estimate the opportunity cost of time that participants and their companions will spend travelling to health facilities and waiting to get the COVID-19 test. 2013 earnings data for the occupations represented in the household cost survey will be adjusted for inflation using annual consumer prices indices for 2013 and 2018, multiplied by the times that each occupation category spent and converted into US dollars using a 2021 exchange rate of 1$ = MK805. Based on the simple random sampling, a weight of 3 will be used to inflate the costs and an average patient cost will be calculated.

Cost-effectiveness analysis
Cost per test will then be estimated by dividing the total cost of each testing strategy by the sample size of n= 700. The standard formula used for the incremental cost-effectiveness ratio (ICER) will be used i.e. the difference in costs for the two strategies divided by the difference in their turnaround times. The turnaround time of results will be calculated from the time that a sample will be taken from a patient to the time test results will be given to the patient and will be measured in hours.
The researchers will conduct a deterministic sensitivity analysis by extending the study period to a year (365 days). They will also conduct a probabilistic sensitivity analysis by making 1000 Monte Carlo simulations and calculating the probability that a strategy could be effective at different threshold ratios. For each simulation, random draws will be made from the Gamma

distribution for cost data parameterized as (,) in Microsoft Excel where = 2/ 2 and = , 2/ and the normal distribution (μ, 2) for turnaround times. Cost data will be categorized as equipment, human resources, lab consumables, overheads and transport for each strategy. When a draw is taken for each of these categories for each strategy, Microsoft Excel will be used.

Data sources
The researchers will use the Government of Malawi Integrated Financial Management Information System (IFMIS) expenditure data for the 2020/2021 financial year for the Blantyre district health office and Queen Elizabeth Central Hospital to approximate overhead costs. Unit cost data will be provided by the Principal Investigator, while data on waste management contracts will be obtained from the Ministry of Health. Data on the useful lives of various equipment will be obtained online and from other published literature. Data on household costs incurred in the process of seeking care will be collected through a costing survey questionnaire. TAT data will be compiled by laboratory staff at both centres of the study. Consumer price index data will be obtained from the Reserve Bank of Malawi website. Open Data Kit will be used to collect and manage data, and Stata-14 (StataCorp, Texas, USA) will be used for analysis.

Acceptability of SARS COV-2 LAMP assay to participants
The acceptability of the SARS COV-2 LAMP assay thus whether it is appealing, likeable, welcome and approved by participants in comparison with SARS-CoV-2 RT-PCR. This will be assessed quantitatively using descriptive structured questionnaires and Likert scale surveys. Analysis will be conducted using STATA 14 (StataCorp, Texas, USA). Descriptive statistics such as proportions will be calculated from the structured questionnaires. Overall median scores and 95% confidence intervals will be calculated from the Likert scale surveys.

Usability of the SARS-CoV-2 LAMP assay by laboratory technicians
Usability of the SARS-CoV-2 LAMP assay in comparison to SARS-CoV-2 RT-PCR by laboratory technicians will be assessed quantitatively using structured questionnaires and Likert scale surveys. All analyses will be done using Stata-14 (StataCorp, Texas, USA). The data collected will be useful in the determination of and guidance of future use guidelines in Malawi.

Study procedures and processes
Initially a pilot study will be conducted to set up the workflow, trial the CRFs, finalise standard operating procedures, train staff and allow staff to familiarise themselves with the study. This way any unforeseen problems can be addressed and improved prior to the commencement of the main study. The PI, study nurses or research assistants will identify participants and invite them/their caregivers to participate in the study. After obtaining consent, participants will be enrolled into the study and assigned a unique code. A nasopharyngeal sample will be collected from the patients at the time of recruitment by the study nurse or laboratory technicians who will take it to the laboratory to be tested with LAMP by laboratory technicians at KUHes, Blantyre campus. Standard of care SARS-CoV-2 PCR testing as part of routine care for COVID-19 testing will take place by hospital staff. The results and turnaround times of LAMP versus PCR will be compared.

Data collection
Study staff will also conduct interviews using standardised questionnaires with participants /caregivers to record the age of the participants and COVID-19 related data, health care costs, societal cost including transport costs will also be analysed to assess the costing and cost-effectiveness of LAMP. Sample collection time, processing time and data on the ease of use of the LAMP instrument and assay will be extracted from the questionnaires administered to laboratory staff. All participants or their caregivers will be assessed on the acceptability of LAMP as POCT.

Data will be entered into case report forms (CRF) which will be piloted prior to use. An electronic Case Report Form (eCRF) will also be designed and used.

Sample size calculation, data management and analysis
As of 09/09/2020, KUHes, Blantyre Campus had completed 3,967 (approx. 600 per month) SARS-CoV-2 PCR tests with 459 positives, a positivity rate of 459/3,967 (11.5 %). The minimum sample size required for sensitivity and specificity analysis of diagnostic studies {for both null (Ho) and alternative (Ha) hypotheses} is estimated by fixing the values of the power and the type I error (22). Assuming prevalence =5%, Ho=0.5, Ha= 0.8, power =0.8, and P value= 0.04, 400 retrospective samples will be required for Phase I (22). For Phase II, all persons being tested over a 1-2 month period will be included in the real-time cost analysis and turnaround time analysis. Approximately 600 persons were tested per month in the second wave of the COVID-19 pandemic in Malawi, an adequate number for this phase of the analysis. Open Data Kit will be used to collect and manage data and Stata-15 (StataCorp, Texas, USA) for analysis. The test accuracy and corresponding 95% CI for sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value and negative predictive value will be calculated. Medians and the corresponding interquartile ranges will be calculated for TAT and mean with standard deviation for costs. Receiver operating characteristic curve (ROC curves) accuracy of the SARS-CoV-2 LAMP assay in predicting COVID-19 disease will be assessed using area under the curve (AUC).

Informed consent (both verbal and written consent)
Participants and caregivers of participants meeting the inclusion criteria will be provided with information about the study verbally and using posters by the research assistant at the COVID-19 testing area and in the outpatients department (OPD). Those willing to take part in the study will see a nurse in a room in OPD who will obtain consent and pre-counsel the family. The consent form and assent forms will be in clear, simple language with both spoken and written information about the study in Chichewa and English. Those willing to participate in the study will go through a thorough informed consent process before any study-specific procedure is conducted. Two original ICFs will be completed, dated and signed personally by the participants and caregivers of participants and by the study nurses. If the participant/caregiver of the participant is unable to write, s/he can thumbprint. The participant/caregiver of the participant will be given one signed original form, the second original will be kept by the study staff.

Potential discomfort/hazard
This project will collect nasopharyngeal swabs. Sample collection procedures will be explained to each participant prior to informed consent being taken. To ensure participant safety, all samples will be collected by qualified laboratory staff collecting swabs as per standard of care with the appropriate professional registration. Nasopharyngeal and oral-pharyngeal swab collection may be uncomfortable but is safe.

Participant compensation
This is a small study with a small budget. Using the Malawi research remuneration rates, the compensation has now been converted into monetary terms to cover for minimal discomfort (MK2,000) and time commitment which include travel (MK300) =Total MK2,300.

Confidentiality
All study data will be kept under lock, in the study office, with access restricted to authorized trial staff. All laboratory specimens, including stored specimens, data collection tools, and administrative forms will be identified by the patient’s unique study number. Names will not be used on any of these documents. All databases will be secured with password-protected access

systems. The investigator will analyse the results and write reports whilst ensuring the anonymity of the patients.

Intervention Type
Other

Primary outcome measure
Calculated using a 2 by 2 table (LAMP assay versus PCR) at a single timepoint (day of recruitment):
1. Sensitivity
2. Specificity

Secondary outcome measures
Measured at a single timepoint (day of recruitment)
1. Turn-around time (TAT) calculated from sample collection to the time test results are available
2. Cost per test calculated from all health services costs incurred per test

Overall study start date
12/07/2021

Completion date
19/07/2022

Eligibility

Key inclusion criteria
Patients presenting with symptoms suggestive of COVID-19 will be offered SARS-CoV-2 LAMP assay testing and those participants /caregivers who give consent will be entered into the study.

1. COVID-19 patients: adults and children of any age presenting with COVID-19 symptoms which include:
1.1. Temperature ≥38.0°C measured at presentation or reported within the previous 24 hours AND one of the below
1.2. Cough
1.3. Abnormal sounds on chest auscultation (crackles, reduced breath sounds, bronchial breathing, wheezing)
1.4. Clinical signs of dyspnea (chest indrawing, nasal flaring, grunting)
1.5. Signs of respiratory dysfunction: tachypnoea for age or decreased oxygen saturation (<92% in room air)
1.6. Signs of reduced general state: poor feeding, vomiting or lethargy/drowsiness
1.7. Loss of taste/smell preceding respiratory symptoms
1.8. Sore throat, nasal congestion, myalgia, fatigue
2. Asymptomatic contacts of COVID-19 patients

Participant type(s)
Patient

Age group
Adult

Sex
Both

Target number of participants
600

Key exclusion criteria
1. Inability to obtain informed consent
2. Alternative non-infectious diagnosis that explains clinical symptoms

Date of first enrolment
28/09/2021

Date of final enrolment
19/07/2022

Locations

Countries of recruitment
Malawi

Study participating centre
Kamuzu University of Health Sciences (formerly known as College of Medicine)
Mahtma Ghandi Road
Blantyre
Malawi
Chichri Blantyre 3

Study participating centre
Queen Elizabeth Central Hospital
PO Box 95
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Sponsor information

Organisation
Fogarty International Center

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Sponsor type
Government

Website
http://www.fic.nih.gov/Pages/Default.aspx

ROR
https://ror.org/02xey9a22

Funder(s)

Funder type
Government

Funder Name
Fogarty International Center, Grant #D43TW009340

Alternative Name(s)
Fogarty, Fogarty at NIH, John E. Fogarty International Center, John Edward Fogarty International Center, NIH John F. Fogarty International Center, NIH's Fogarty International Center, NIH Fogarty International Center, Fogarty International Center at NIH, Fogarty International Center, U.S. National Institutes of Health (NIH), Fogarty International Center AT THE NATIONAL INSTITUTES OF HEALTH, FIC

Funding Body Type
Government organisation

Funding Body Subtype
Research institutes and centers

Location
United States of America

Results and Publications

Publication and dissemination plan
The study will be registered with the Research Support Centre prior to the start at KUHes. Upon completion of the study report, the results of the study will be submitted for publication in peer-

reviewed journals by the principal investigator and posted in a publicly accessible database of clinical study results. Authorship of any publication will be based on the uniform requirements for manuscripts submitted to Biomedical Journals. The study results will be communicated to stakeholders - KUHes, MoH (QECH) locally and national level, at the regional level, the biennial international conference of the African Society for Laboratory Medicine (ASLM); and UNC /Fogarty internationally, through dissemination meetings, conferences/seminars, and local, regional or international workshops.
To study participants/caregivers, the researchers will use language-appropriate information sheets. Investigators will present results at the CoMREC annual dissemination conference and other relevant conferences. Public access to the participant-level dataset of main study results and statistical code will be made available. A copy of the final report and any published paper(s) or abstracts of papers read at conferences out of the research findings will be submitted to the College of Medicine Research and Ethics Committee (COMREC), College of Medicine Library, the Health Sciences Research Committee (through the COMREC Secretariat) and the University Research and Publication Committee (URPC) (through the COMREC Secretariat).

Intention to publish date
19/01/2023

Individual participant data (IPD) sharing plan
The datasets generated and/or analysed during the current study will be published as a supplement to the results publication. The data-sharing plans for the current study are unknown and will be made available at a later date.

IPD sharing plan summary
Published as a supplement to the results publication, Data sharing statement to be made available at a later date