UNHRO
Uganda National Health Research Organisation

Uganda Virus Research Institute

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REPUBLIC OF UGANDA

Our Ref: GC/127/20/04/771

April 20, 2020

UVRI REC APPROVAL NOTICE

To: Prof. Mathew Cotten, Principal Investigator

Re: Application Number: **"Local sequencing of SARS-CoV-2 from Uganda Covid-19 Disease cases"**

Type: [✓] Initial Review

I am pleased to inform you that at the REC Meeting 03 convened on April 16, 2020 the UVRI REC voted to approve the above referenced application.

Approval of the research is for the period of April 20, 2020 to April 20, 2021.

As Principal Investigator of the research, you are responsible for fulfilling the following requirements of approval:

- All co-investigators must be kept informed of the status of the research.
- Changes, amendments, and addenda to the protocol or the consent form must be submitted to the REC for re-review and approval **prior** to the activation of the changes. The REC application number assigned to the research should be cited in any correspondence.
- Reports of unanticipated problems involving risks to participants or other must be submitted to the REC. New information that becomes available which could change the risk: benefit ratio must be submitted promptly for REC review.
- Only approved consent forms are to be used in the enrollment of participants. All consent forms signed by subjects and/or witnesses should be retained on file. The REC may conduct audits of all study records, and consent documentation may be part of such audits.
- Regulations require review of an approved study not less than once per 12-month period. **Therefore, a continuing review application must be submitted to the REC eight weeks prior to the above expiration date of** April 20, 2021 **in order to continue the study beyond the approved period.** Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study, at which point new participants may not be enrolled and currently enrolled participants must be taken off the study.

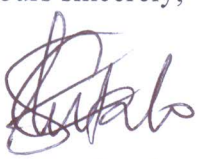
the study beyond the approved period. Failure to submit a continuing review application in a timely fashion may result in suspension or termination of the study, at which point new participants may not be enrolled and currently enrolled participants must be taken off the study.

- You are required to register the research protocol with the Uganda National Council for Science and Technology (UNCST) for final clearance to undertake the study in Uganda.

The following is the document approved in this application by UVRI REC:

- Protocol Version 1.1 dated 16 April 2020

Yours sincerely,



Dr. Tom Lutalo
Chair Person, UVRI REC
C.C Secretary, UVRI REC

Study Protocol

Study title: Local sequencing of SARS-CoV-2 from Uganda COVID-19 cases

Short title: Local SARS-CoV-2 sequencing

Principal Investigator (PI)	Matthew Cotten, MRC/UVRI & LSHTM Uganda Research Unit
Investigators	John Kayiwa, UVRI Pontiano Kaleebu, MRC/UVRI & LSHTM Uganda Research Unit Henry Mwebesa, Uganda Ministry of Health Julius Lutwama, UVRI Deogratius Ssemwanga, MRC/UVRI & LSHTM Uganda Research Unit Jonas Lexow, MRC/UVRI & LSHTM Uganda Research Unit
Sponsor	London School of Hygiene and Tropical Medicine Through MRC/UVRI & LSHTM Uganda Research Unit
Funding	MRC/ UKRI

Protocol number:

Study site: Uganda

GCP Compliance: The study will be conducted in compliance with ICH-GCP as applicable and all other applicable regulatory requirements.

Declaration of Confidentiality

The information contained herein is confidential and therefore are provided in confidence as a potential examiner or investigator. It is understood that this information will not be disclosed to others without the written permission of the Sponsor, except to the extent necessary as required by applicable regulations and law.

Signature page

By my signature below, I hereby confirm that I will conduct the study described in this protocol in compliance with ICH/GCP and the version of such protocol agreed to by the applicable regulatory authorities and approved by all Institutional Review Board and Ethical Committees.

Sponsor (Geoffrey Kimbugwe, MRC/UVRI & LSHTM Uganda Research Unit)

_____

Signature Date

Principal Investigator (Matthew Cotten, MRC/UVRI & LSHTM Uganda Research Unit)

_____

Signature Date 25March2020

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Abbreviations/acronyms

CoV	Coronavirus
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
COVID-19	Coronavirus Disease 2019
EC	Ethics Committee
GCP	Good Clinical Practice
GCLP	Good Clinical Laboratory Practice
LSHTM	London School of Hygiene and Tropical Medicine
MoH	Ministry of Health
MRC	Medical Research Council
REC	Research Ethics Committee
SARS	Severe Acute Respiratory Syndrome
UK	United Kingdom
UKRI	UK Research and Innovation
UMIC	Uganda Medical Informatics Centre
UNCST	Uganda National Council of Science and Technology
Unit	MRC/UVRI & LSHTM Uganda Research Unit
UVRI	Uganda Virus Research Institute

1.0 Background

The current coronavirus pandemic is a novel challenge for healthcare systems worldwide. The virus is currently spreading at alarming rates in Asia, Europe and North America, and it is essential that African countries prepare for the arrival and local transmission of SARS-CoV-2 and develop effective response mechanism. The African MRC Units have a critical role to play in researching the epidemic but also in supporting local health systems in this response. In Uganda, COVID-19 diagnostics are performed at the Uganda Virus Research Institute (UVRI, Entebbe). Close to 48 SARS-CoV-2 positive cases have been identified to date after the first

positive case identified on the 22nd March 2020. Given the rapid spread of the virus globally, there is a high risk for further positive cases to be detected in Uganda over the next few weeks.

Lessons learnt from other countries suggest that rapidly sequencing SARS-CoV-2 positive samples provides important information for controlling the epidemic. SARS-CoV-2 accumulates nucleotide changes at a slow but persistent rate and these changes are fingerprints that allow investigators to follow the virus movement globally and locally. If sufficient sequence data are available from all locations, the sequence of any new infection help identify close viral variants in the world or locally and this information informs public health measures on the effectiveness of quarantines and travel restrictions. Furthermore, the nucleotide changes need to be monitored to identify changes that might alter the virus pathogenicity or impair the ability to detect the virus with standard diagnostics. The methods were used to successfully control Ebola virus in West Africa [1][2] and to follow MERS coronavirus in the Middle East [3]. The Unit has well-trained laboratory staff, robust procedures and excellent facilities which could support the national effort to prevent and contain an outbreak in Uganda. We are applying for permission to use these method to help understand and control COVID-19 in Uganda.

2.0 Rationale

The Unit intends to prepare for supporting the national diagnostic capacity by offering up to 2,500 tests per month over the next 4 months. Diagnostic activities are fully embedded in the Ministry of Health (MoH) case detection programme and are initially not a research activity *per se* but part of routine clinical care. Full virus genome sequences would be generated from a subset of positive samples, the resulting data would support the international effort in understanding the regional movement of the virus and would monitor for virus changes that might influence diagnostics or the pathology of the virus.

Valuable Additional information on the virus and its movement through the community and regionally can be obtained from computational analysis of viral genomic sequences combined with the large set of SARS-CoV-2 sequences available from the global outbreak. Currently (22 March 2020) >1100 sequences are publicly available on GISAID (https://www.gisaid.org/). These sequence data are important for determining the source of the infection, for monitoring changes in the virus and as a validation of other diagnostic tools. Sequences can be generated locally from the same samples collected for diagnostics. Given the rapid pace of the virus spread, the lack of therapy or vaccine, it is essentially the viral sequence data are generated rapidly and locally without sending samples to laboratories outside of the country.

It is important that Sub-Saharan Africa and the East African sub-region are represented in the global sequencing effort. Leveraging the Unit's high-performance computing cluster, referred to as Uganda Medical Informatics Centre (UMIC), is a unique opportunity to ensure data from the region will become available and is not missed.

3.0 Hypothesis

No formal hypothesis is to be tested.

Objectives

To document the transmission of COVID-19 in Uganda using full viral genome sequencing.

3.0 Study methods

3.1 Study design

The study will be nested within the national COVID-19 outbreak surveillance which will be supported by MRC/UVRI & LSHTM Uganda Research Unit.

3.2 Study site

The study will be carried out in Uganda and samples will be analyzed at the diagnostic and research laboratories at the Uganda Virus Research Institute and MRC/UVRI & LSHTM Uganda Research Unit in Entebbe.

3.3 Study duration

Given the outbreak nature of the study, the study team proposes to conduct the study until such a point when the sample size has been attained and or up to the end of the outbreak whichever happens first.

3.4 Study population

Viral RNA samples that tested positive during the diagnostic testing at UVRI will be sent to be sequenced at MRC/UVRI & LSHTM Uganda Research Unit. There is no direct interaction with the study population.

3.5 Sample size

There is no *a priori* sample size targeted for this study. A planned subset of approximately 80 SARS-CoV-2 positive samples will undergo viral genomic sequencing undertaken although this number may change as the outbreak evolves and if additional support can be organized. As we have seen the other outbreaks, a more detailed viral genome sequence database provides much more information for tracking the virus through the population.

3.6 Sampling procedures:

Testing will be done at UVRI as part of the national case detection effort, as supported by the Unit. Where positive cases are identified, a number of viral RNA isolates from these samples will be used for research, namely for sequence analysis. There is no additional sampling involved in this research.

3.7 Quality control/quality assurance

The study will be undertaken in compliance with GCP and GCLP requirements as applicable, and several steps will be undertaken to ensure the accuracy and reliability of the study data. These will include the selection of qualified study staff, review of the protocol and study procedures with all the study personnel before undertaking any study-related activities as well as providing guidelines for the handling of samples and conducting of the laboratory procedures, including adhering to the safety code of the research site. The accuracy and completeness of the study documents will be reviewed by designated quality control/quality assurance personnel and any discrepancies will be resolved by the responsible study staff or as appropriate.

3.7 Sample and data sharing between UVRI and the MRC Unit

The study samples comprise extracted nucleic acid from COVID-19 positive samples identified as part of the UVRI COVID-19 diagnostic activities. The UVRI scientist will provide extracted RNA in suitable quatities and quality for next generation sequencing. The MRC/UVRI & LSHTM Uganda Research Unit scientists will convert the RNA in nucleic acid preparation to DNA, amplify the material using primers that recognize SARS-CoV-2 sequences and determine the viral genome sequence using established methods (primarily Oxford Nanopore

MinION sequencing but possibly also Illumina methods). Residual nucleic acid material will be stored until the project termination to allow repeat sequencing if necessary. The resulting SARS-CoV-2 sequence data will be shared with all collaborators and in close consulation with the UVRI and Uganda MoH, will be communicated to the global outbreak community using GISAID, Virological.org and eventually peer-reviewed publications. Scientists who participated in the effort will be recognized with co-authorship or acknowledgement.

4.0 Laboratory methods

As part of the COVID-19 diagnostic process, real-time polymerase chain reaction is used to identify the presence of the COVID-19 virus SARS-CoV-2 [4]. If positive, additional information on the virus can be obtained by determining the viral genomic sequences. This information is important for determining the source of the infection, for monitoring changes in the virus and as a validation of other diagnostic tools. The sequence data will be added to international open source collections (GenBank [5], GISAID [6], NEXTStrain [7], CoV-GLUE [8]) to help investigators globally track the virus movement and changes.

Once a sample is identified as SARS-CoV-2 positive by PCR, extracted nucleic acids will be converted to double-stranded DNA, amplified using PCR with SARS-CoV-2 specific primers and then ligated to sequencing adapters and subjected to MinION sequencing to determine SARS-CoV-2 genomic sequences [9]. The laboratory and computational methods required for these processes are well established with more than 1100 SARS-CoV-2 sequences currently available from the outbreak, many using the MinION sequencing method.

5.0 Data management

Our full genome SARS-CoV-2 sequences (including raw data) will be made publicly available on our project website, in GenBank, GISAID, Virological.org or CoV-GLUE as soon as the data have passed our quality controls. The GLUE SARS-CoV-2 website is currently available at [10] for public use and will be extended with new analyses and tools as they become available during the project. We will use social media (institute websites and twitter) to communicate new findings.

6.0 Statistical analysis plan

The sample collection, diagnostics and sequencing are dictated by emergency measures required to control the outbreak. A rigorous statistical analysis is not immediately possible.

7.0 Ethical considerations

Ethical clearance will be obtained from the Uganda Virus Research Institute Research Ethics Committee, Uganda National Council of Science and Technology and the London School of Hygiene &Tropical Medicine Ethics Committee prior to undertaking the study, as applicable.
Confidentiality will be ensured by not capturing personal identifiers such as name, date of birth or telephone contacts during the research process and all collected data will be used for the purposes of this study. The research component is working with anonymised viral samples only.

We will protect the identity of the patient providing the sample in the following methods. Samples and the resulting sequence data will bear only a unique identifier numbers. Essential metadata collected as part of the diagnostics (patient age, gender, travel history, disease

symptoms, outcome, date of collection, sample collection location) will be linked to a sample identification number, however these data will be stored separately and securely.

During the sequencing a small amount of human sequence data may be detected. This is minimized by using SARS-CoV-2-specific amplification primers during the sequence process. Finally, any human sequences present in the final raw read data will be removed computationally before public release of the data. Established methods of identifying and removing human sequences from next generation sequencing data are available and will be used [11].

Previous studies, UNCST has required that for samples collected as part of surveillance, sample sources must be traced to provide consent for use of their samples in research. We would like to request a waiver of this requirement for the following reasons. 1. No human biological samples will be analysed. The sequencing/research component will focus solely on viral RNA obtained from prior diagnostic efforts. 2. All human identifying sequences will be removed from the data before public release. 3. Upholding this requirement will expose the study team to COVID-19 infection risk. 3. The attention associated with revisiting positive patients may risk stigmatizing the patient.

8.0 Limitations

The evident potential limitation of the study include failure to obtain the target sample size more so of the subset as the study team cannot determine the extent of the number of incident cases. To mitigate this, the study team will enrol consecutively all incident cases in Uganda as and when they arise including those that would have occurred prior to study start.

9.0 References

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