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Uganda Virus Research Institute
Performance Evaluation for COVID-19 Diagnostic tests

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Contact:

Professor. Pontiano Kaleebu: **Principal Investigator**

Director, Uganda Virus Research Institute (UVRI)

P.O. Box 49 Entebbe

Email: pkaleebu@uvri.go.ug;
Pontiano.Kaleebu@mrcuganda.org

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Investigators, their affiliations and contacts

Name	**Affiliation**	**Contact**
Pontiano Kaleebu (PI)	UVRI and MRC/UVRI & LSHTM Uganda Research Unit	pkaleebu@uvri.go.ug; pontiano.kaleebu@mrcuganda.org
Julius Lutwama (Co-PI)	UVRI	jlutwama@uvri.go.ug
Robert Downing (Co-I)	UVRI	downingrg@gmail.com
Tom Lutalo (Co-I)	UVRI	tlutalo@uvri.go.ug
Christine Watera (Co-I)	UVRI	cwatera@uvri.go.ug
Jennifer Serwanga (Co-I)	UVRI and MRC/UVRI & LSHTM Uganda Research Unit	Jennifer.Serwanga@mrcuganda.org
Bernard Kikaire (Co-I)	UVRI	bkikaire@uvri.go.ug
Aminah Nalumansi (Co-I)	UVRI	Analumansi@uvri.go.ug
Deogratius Ssemwanga (Co-I)	UVRI and MRC/UVRI & LSHTM Uganda Research Unit	deogratius.ssemwanga@mrcuganda.org
Matthew Cotten (Co-I)	UVRI and MRC/UVRI & LSHTM Uganda Research Unit	Matthew.Cotten@lshtm.ac.uk
John Kayiwa (Co-I)	UVRI	jkayiwa@uvri.go.ug
Henry G. Mwebesa (Co-I)	Ministry of Health	mwebesah@yahoo.co.uk
Isaac Ssewanyana (Co-I)	Central Public Health Laboratory	sewyisaac@yahoo.co.uk
Susan Nabadda (Co-I)	Central Public Health Laboratory	sndidde@gmail.com
Christopher Nsereko (Co-I)	Entebbe Regional Referral Hospital	chrisdoc23@yahoo.com
Moses Muwanga (Co-I)	Entebbe Regional Referral Hospital	fahimmuwanga@gmail.com
Nathan Onyachi	Masaka Regional Referral Hospital	
Mark Jjuuko	Masaka Regional Referral Hospital	

***Masaka and Mbarara RRHs have accepted to collaborate and we wait for the names of people that will coordinate this effort at these hospitals**

List of acronyms, abbreviations and definitions

CE: Conformité Européene/European Conformity
COE: Center of Excellence
CPHL: Central Public Health Laboratory
COVID-19: Corona Virus Disease 2019
EIA: Enzyme Immunoassay
ESC: Evaluation Study Coordinator
EUA: Emergency Use Authorization
Evaluation: aims to quantify the performance of a new protocol in relation to an existing protocol
FDA: Food and Drug Administration
GCLDMP: Good Clinical and Laboratory Data Management Practice
GCP: Good Clinical Practice
GCLP: Good Clinical Laboratory Practice
HIPAA: Health Insurance Portability and Accountability Act
IgA: Immunoglobulin A
IgG: Immunoglobulin G
IgM: Immunoglobulin M
LIMS: Laboratory Information Management System
LSHTM: London School of Hygiene and Tropical Medicine
MoH: Ministry of Health
NAAT: Nucleic-acid amplification test
NDA: National Drug Authority
NIC: National Influenza Center
NPV: Negative Predictive Value
PCR: Polymerase Chain Reaction
PI: Principal Investigator
POC: Point-of-Care
POE: Point-of-Entry
PPV: Positive Predictive Value
REDCap: Research Electronic Data Capture
RDT: Rapid Diagnostic Test
REC: Research Ethics Committee
RNA: Ribonucleic acid
SARS-CoV-2: Severe Acute Respiratory Syndrome (SARS)-Coronavirus
SOP: Standard Operating Procedure
UNCST: Uganda National Council for Science and Technology
UVRI: Uganda Virus Research Institute
Validation: An evidence-based assessment of how a test performs in the laboratory
Verification: the confirmation, through provision of objective evidence that specified requirements have been fulfilled
WHO: World Health Organization

Background

In December 2019, a cluster of cases of severe pneumonia was identified in China, now known to be caused by a newly-identified virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which causes COVID-19 disease [1] . The disease quickly disseminated across China and worldwide, leading to more than four million cases and 333,489 deaths in approximately 188 countries on six continents as of 22nd May 2020 (https://coronavirus.jhu.edu/map.html) [2]. The virus is transmitted efficiently between humans via respiratory droplets and, based on data from all over the world, causes severe disease and a high mortality rate in the elderly, those with chronic co-morbidities, and the immunosuppressed [3].

In Uganda the first case of COVID-19 was diagnosed on 21st March 2020 and by May 27th 2020, two hundred and fifty three (253) cases had been confirmed (https://covid19.gou.go.ug) [4]. The coronavirus SARS-CoV-2 is posing major challenges for healthcare systems and medical institutions worldwide. The ability to rapidly and accurately diagnose infection with the virus is invaluable in curbing the exponential spread of the virus throughout the country.

COVID-19 testing at Uganda Virus Research Institute

The Uganda Virus Research Institute (UVRI) is the national reference laboratory for viral diseases and is one of the mandated centers for validating COVID-19 diagnostic assays and for providing external quality assurance (EQA) (*Ref. approved national lab response plan*). As of 16th May 2020, more than 67,000 naso-pharyngeal swabs have been received and tested at UVRI using the nucleic acid amplification tests (NAAT) for possible COVID-19 infection. The COVID-19 pandemic is still considered to be in its infancy in Uganda with limited community spread 0.03% (unpublished community surveillance data) and only samples from suspected and probable cases and their close contacts plus those who self-report as being at risk and those passing through points-of-entry (POE) at borders are being received and tested at UVRI. The Ministry of Health situation report of 15th May, 2020 records ninety-three percent (149/160) of COVID-19 cases as imported, 5% as local transmissions (8/160) while 2% have an unknown chain of transmission (3/160; ref sitrep #87).

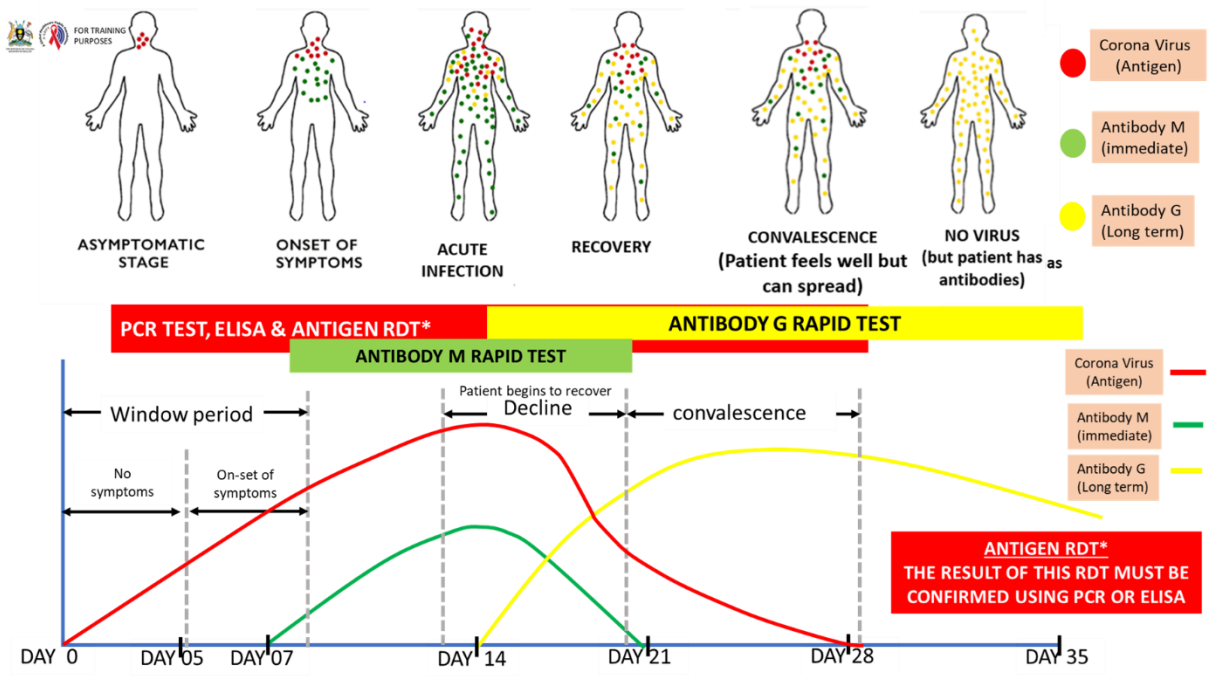
UVRI uses NAAT for the diagnosis of COVID-19 infection. The molecular test kit being used in this emergency, is based on the Berlin protocol, and is approved for use by the World Health Organization (WHO) [5] and recommended by the Uganda Ministry of Health. UVRI has the capacity to test approximately 3000 samples per day and has plans to increase this capacity. However, with the exponential growth in numbers of confirmed cases observed in other countries, Uganda has made a decision to decentralize COVID-19 testing to high-risk areas such as POE where laboratory capacity has been assessed, validated and certified to conduct testing (see national lab COVID-19 response manual). The Ministry of Health has developed a decentralized testing plan to target mobile lab testing (ref MoH draft) and molecular testing through the upcountry Centers of Excellence (COE). With this approach, potential testing labs will first be assessed using the WHO recommended, country-customized risk assessment tool before conducting additional validation processes on lower ranked facilities. The UVRI assumed the mandate for validation and certification of qualifying labs using the national COVID-19 validation standard operating procedure (SOP now in draft).

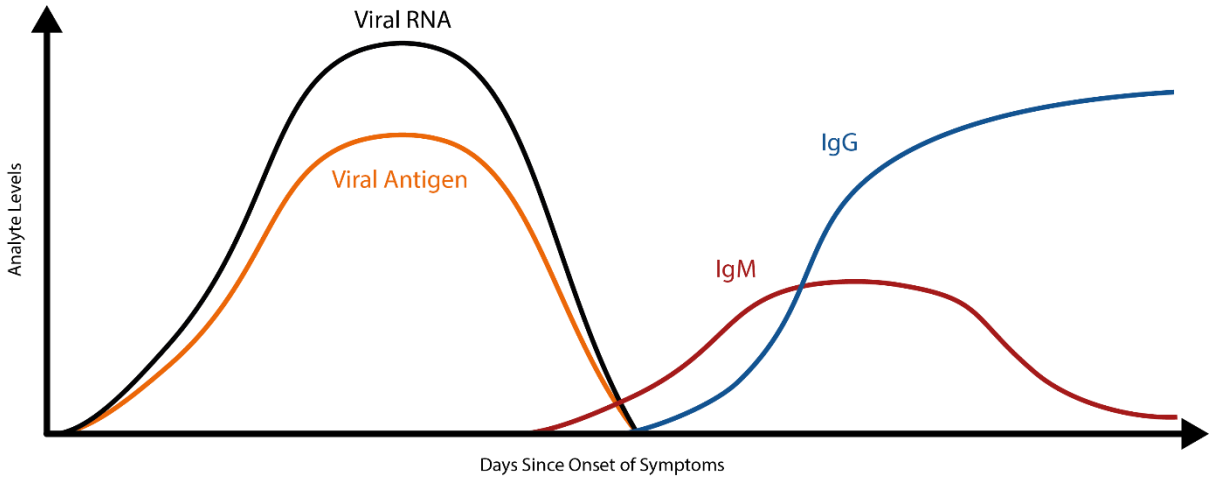
Rationale for COVID-19 test evaluation

The two major methods for diagnosing viral infection are NAAT and immunoassays. NAAT has been invaluable in COVID-19 case-confirmation and guidance on the need for isolation. However, while it is fast and sensitive, NAAT suffers from some inherent drawbacks that limit its use to diagnosis during the acute phase of the infection. Drawbacks include the need for high-quality specimens that contain a sufficient amount of intact virus yet viral load in the respiratory tract has been shown to vary considerably between individuals [6]. The test protocol is complex and expensive and is mainly suited to large, centralized diagnostic laboratories. While NAATs in the laboratory typically take 4-6 hours to complete, the average time it takes to ship clinical samples to UVRI, carry out the test and return results to patient care centers is 24-48 hours at best [7]. There have been reports of probable cases testing negative after multiple swabs which potentially exposes health care workers to the risk of infection [7, 8]. New molecular tests are being offered on the market which may be cheaper to set up and use but they must first undergo verification [9]. It is planned that all new molecular testing kits and immunoassays introduced to Uganda must undergo an in-country laboratory verification at UVRI, or another MOH-recommended laboratory, before being recommended to the MOH and to the National Drug Authority (NDA) for use in the country. WHO advises that before tests are recommended, they should be evaluated/verified in appropriate populations and settings [10]. Unlike molecular techniques that are already in widespread use in the world, there is no immunoassay which has been recommended for use in Uganda. To better understand the transmission dynamics of COVID-19 and to develop countermeasures against it, it is essential that the country prepares for the use of antigen and antibody-based immunoassays.

Unlike molecular techniques (NAATs) that detect nucleic acids (RNA and DNA), immunoassays detect the presence of specific immunoglobulins or viral antigens. These assays take on a wide range of different formats, but essentially consist of an antigen or antibody, immobilized on a surface (most often on a micro-titre plate or paper strip), which capture virus-specific antibodies or antigens from a patient sample (blood, sputum, urine etc.). By adding a further reporter protein, it is then possible to detect a virus-specific immune signal or a viral antigen to confirm the presence of acute or past viral infection. Antigen and antibodies are considerably more stable than RNA, which makes them less susceptible to degradation during transport and storage, thereby reducing the chance of false-negative results.

One of the biggest advantages of antibody immunoassays over NAATs and antigen immunoassays is their ability to detect past infections. Once a patient has recovered from COVID-19 and virus is cleared from the body, viral RNA and antigen are no longer detectable in the respiratory tract, leaving only a short window during the acute stage of infection in which COVID-19 can be detected. This works well for diagnosis of ongoing infections but gives no indication of whether a patient has had an infection historically and what their immune status is (i.e. if they have immunity to COVID-19 or are still susceptible to infection). Unlike RNA, antibodies are long-lasting and can persist in the bloodstream for many years after infection. As such antibody immunoassays enable us to identify retrospectively, patients that have had a viral infection. The type of antibody, IgM or IgG and its relative level can also be used to indicate the stage of infection and to estimate the time since exposure for contact tracing. However, as immunological data continues to emerge, it is becoming apparent that the body's antibody response to COVID-19 is slower than might be expected. While data is limited at this point, it appears the initial IgM antibody response doesn't peak until ~9 days after initial infection and the IgG antibody response doesn't peak until day ~11 [11, 12].





UVRI Capacity to evaluate NAATs and diagnostic immunoassays

UVRI is set to validate molecular assays and to evaluate/verify immunoassays (EIAs and rapid diagnostic tests - RDTs) as the country prepares itself for using both molecular and serological diagnostic tests. The evaluation of the immunoassays will be conducted against test kits approved by WHO or FDA under emergency use authorization/listing (EUA/L) or with the CE-mark (European Conformity). UVRI will evaluate and recommend the best performing COVID-19 NAATs, EIAs and RDTs, both for antibodies and antigens, to the MOH and the NDA for use in the country. UVRI, together with the MOH will also train laboratory technicians in the country and staff at POC facilities on how to use the recommended test kits. UVRI has a number of laboratories where evaluation of different kits will be conducted. For the COVID-19 NAATs, the National Influenza Center (NIC) Laboratories, in the Department of Arbovirology, Emerging and Re-Emerging Infectious Diseases, will carry out the evaluations. There are 6 regular laboratory technologists in the NIC (4 from UVRI and 2 from

Makerere University, Walter Reed Program). The Department, however, has other programs with another 6 technologists who may all be involved given the increasing demand for evaluation of test kits. For the evaluation of serologic diagnostic kits both the immunology and virology laboratories will be involved. They both have the personnel and equipment needed for such evaluations.

This proposal documents standard evaluation procedures that will be followed at UVRI for commercial diagnostic kits intended for the detection of acute and past COVID-19 infection. The evaluation of new NAATs, EIAs and RDTs (kits under evaluation) on the market determines the accuracy of the test in comparison with a 'gold-standard' test. Performance characteristics to be considered include; - sensitivity, specificity, negative and positive predictive values, accuracy, reproducibility and precision, as well as the ability to distinguish new infections from prior exposure. In addition, several operational characteristics of the test kits will be assessed including the suitability of the test for use in laboratories and/or in testing settings with limited infrastructure.

The presence of viral nucleic acids in biological specimens is highly indicative of an ongoing infection. Thus, PCR-based, WHO/FDA-approved NAATs for the detection of viral nucleic acids have become the gold-standard for viral disease detection. Multitudes of clinical tests, including POC tests (for example molecular-based POC tests like GeneXpert which takes 45 minutes to run), are increasingly becoming available for current or previous exposure to COVID-19 infection based on the detection of antibody or antigen. These tests, especially POC tests enable the rollout of diagnostic testing to additional sites beyond UVRI. The GeneXpert is currently being rolled out at some border posts following evaluation at UVRI. Prior to the rollout for use in the country, UVRI evaluates the performance of all new WHO or FDA-approved RDTs plus those from the private sector identified by the MOH or the NDA, to determine their testing performance in comparison to the 'gold-standard'.

Overall objectives

The overall objectives of this performance evaluation are:

- To verify the performance characteristics of new on-the-market NAATs and antigen RDTs,
- To establish well-characterized COVID-19 sera/plasma reference panels to support evaluation efforts and quality assurance.
- To evaluate the performance characteristics of new, on-the-market, EIAs and RDTs (IgA, IgG and IgM),
- To train laboratory and POC staff in biosecurity and the required technical skills to perform COVID-19 diagnostic testing,

Specific Objectives:

- To confirm whether or not a new NAAT complies with the manufacturer's specifications,
- To determine the performance equivalence of new on-the-market NAATs, including POC devices, using the Berlin protocol NAAT as the gold standard (or any other comparatively/equally as good WHO/FDA approved NAAT),
- To determine the performance equivalence of new on-the-market antigen tests against the gold-standard NAAT,

- To determine the sensitivity and specificity of new on-the-market diagnostic EIAs and RDTs against a gold-standard method,
- To evaluate the operational characteristics of new on-the-market immunoassays. Operational utility characteristics that may be considered include;
    - ease of performance
    - specimen type utility
    - inter-reader variability
    - reaction endpoint stability
    - suitability for use in facilities with limited infrastructure (no/limited electricity, no/limited clean water)
- To document SOPs and train laboratory staff in performing COVID-19 testing using recommended diagnostic tests,
- To establish a repository of well-characterized COVID-19 sera/plasma and swabs/extracted RNA to support future evaluation and verification of immunoassays.
- To establish a cohort of COVID cases in the country whose samples and demographic characteristics will be used to help understand the dynamics of human immunity development to SARS CoV-2 infection in order to inform the clinical interpretation of the various diagnostic assays.
- To document the performance characteristics of various diagnostic assays against the patient's symptoms and signs.

Study Design

These will be laboratory-based, cross-sectional evaluations using samples collected from consented active cases in selected referral hospitals, convalescent cases after discharge, and non-cases. In addition to the evaluation panels expected from WHO, samples will be collected and received at UVRI following the procedures described in the documents below (Appendices I-II):

i) UVRI SOP LB-CVD-001 for collecting, processing and storing COVID-19 blood specimens,

ii) The MOH "Coronavirus Disease-2019 (COVID-19) Preparedness and Response Plan - Laboratory manual"

Selection of the diagnostic test to be evaluated

The kits to be evaluated will be identified by UVRI management/MOH and/or the NDA and will have FDA/WHO EUA/EUL or will carry the CE mark or other standard mark. The evaluation will obtain regulatory approval by national bodies before evaluation [13]. Locally-manufactured kits identified by the MOH/NDA or kits from collaborating institutions like Makerere University, LSHTM, CDC and University of Glasgow (and approved for evaluation by the MOH) will also be considered for evaluation.

Sample size consideration

This will depend on the number of available samples from patients with confirmed infection identified by the WHO-approved NAAT. For the NAAT evaluations, it is recommended that between 20-50 reactive samples and 20-50 non-reactive samples are tested in parallel (evaluated kit and reference). However, the actual numbers involved

will be agreed upon by the study team depending on the availability of samples from confirmed cases identified in the country. The negative and positive controls of one kit must be included in the panel of specimens to be tested on the other kit. For now, the confirmed COVID-19 cases in Uganda are 253 from whom multiple samples (nose and throat swabs plus whole blood) have been and will continue to be collected. For a confirmed case, a schedule of 7 days interval is followed to collect additional samples until discharge. Once the patient has been discharged samples will be collected after 7 and 14 days and thereafter every month until the study team decides to exit the convalescent case which period should not exceed a year after discharge.

For a non-case only those who have not beed exposed will be enrolled in this study. Between 5 and 60 mls (depending on the schedule) of whole blood will be collected. During the convalescent stage, 60 mls of whole blood will be collected. A schedule of procedures is summarized in table 1 below.

For the immunoassay diagnostic tests, a minimum of 50 reactive samples and 50 non-reactive samples will be used for each evaluation. The 50 non-reactive samples will preferably be picked among those samples archived during the period when COVID-19 had not affected the world population. Borderline reactive samples (low positive on EIA) will also be included in the evaluation as part of the positive specimen panel. UVRI also expects to receive well-characterized positive panels from WHO and Africa CDC to enhance the evaluation process. Depending on the information for use included with each kit, and evolving literature, additional alternative sample types such as saliva and sputum may be collected outside the routine OP/NP swabs.

Table 1: Schedule of procedures and sample collection

Case and Non-Case Hospital stay till discharge								Cases Only after discharge															
Sample Type	Tube type	Tubes Collected	D0	D7	D14	D21	D28	Tubes Collected	W1	W2	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Serum	SST (8.5 ml)	2	17	17	17	17	17	3	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5	25.5
Plasma, Cells, Pellet	ACD (8.5 ml)	2	17	17	17	17	17	3	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5	24.5
	EDTA (10 ml)	1	10	10	10	10	10	1	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Nasal Swab				X																		
CBC clinical test			X		X at discharge																	
Total blood volume (ml)			44	44	44	44	44		60	60	60	60	60	60	60	60	60	60	60	60	60	60
Non-Case till discharge																						
Serum	SST (8.5 ml)	2	17	17	17	17	17															
Total blood volume (ml)			17	17	17	17	17															

Ethical Considerations

This proposal will be submitted to the UVRI's Research Ethics Committee (REC) and the Uganda National Council for Science and Technology (UNCST) for ethical review. The evaluation will be initiated after obtaining approval from the ethical regulatory bodies and permission from the MOH. Data collection will follow the national guidelines for research involving humans. Specimens will be unlinked to personal identifiers so that they cannot be traced to individual patients. It is only the PI, the study coordinator and the data analyst that will be able to link personal identifiers to the specimens, but confidentiality will be paramount. For convalescent cases, consent to collect new demographic data and to store samples for future use will be sought. The study objectives will be explained to all participating individuals.

Biosafety guidelines used

Use of personal protection equipment and Good Clinical Laboratory Practice (GCLP) plus Good Clinical Practice (GCP) are enforced at UVRI.

Use of a mandated laboratory

The UVRI is a national Centre of Excellence (COE) for viral diagnostics, surveillance, and research. It is mandated by the Uganda MOH for the evaluation of diagnostic assays. UVRI has also been identified by Africa CDC to serve as a Center of Excellence for Evaluation of COVID-19 diagnostic kits. The laboratories at UVRI considered for the evaluation include the Arbovirology, HIV Reference Lab, EPI-Lab, Virology, NIC, Viral Hemorrhagic Fever and Immunology laboratories. The Arbovirology laboratory will continue to identify COVID-19 reactive and non-reactive samples using NAAT on naso- or oral-pharyngeal samples received from the isolation centers in the country. The virology laboratories will perform the required EIA and RDT comparative assays supported by the immunology laboratory using available samples.

The following issues are key in minimizing error and maximizing the value of these evaluations:

- The Principal Investigator (PI) or designee will be responsible for training the laboratory technicians in the evaluation protocol and in the performance of each assay undergoing evaluation,
- Only those personnel that have received specific training for these evaluations will be employed in the evaluation,
- All kits will be stored according the manufacturer's recommendations,
- All tests will be performed according to the instructions on the product insert provided by the manufacturer,
- Accurate record keeping is crucial to the success of the evaluation. The PI will be responsible for ensuring that all data required for the evaluations are recorded on the agreed data collection sheets and are accurate and up to date. A linkage to the National LIMS will be established to share requested demographic and clinical data needed for national aggregates by the Ministry of Health.
- Line managers will review all test data for accuracy and validity before it is included in statistical analyses. Individual kits' pass and fail criteria will be determined by the study coordinators at the beginning of the evaluations,
- It is important to plan work in advance and follow SOPs as prepared and controlled by the PI and evaluation study coordinator (ESC),
- To reduce the risk of adding an incorrect specimen to a test device/well, before starting the test run, the operator will prepare experimental plan worksheets; and label all tubes, dilution vessels, test devices or plates with the specimen's unique number. Dedicated technicians will cross-check each other's experimental plans and labeling prior to commencement of the testing,
- Because objective, machine-generated, permanent results for simple/rapid diagnostic tests are not feasible, it is essential that the PI or designee emphasizes, to the operator performing the tests, the need for accurate recording of results and record keeping. In addition, photographs of the RDT/molecular POC results will be taken.,

- In most colorimetric assays, eventually strong signals will surpass the linear range of the test amplification. Once this happens, wells/tests cannot be accurately compared. Therefore, recording the assay development duration (from substrate addition to stop solution), and the duration from stopping to reading the EIAs will be critical, and will thus be documented so that only valid assays are included in the comparative analyses. Likewise, the time-lapse before reading of RDTs will be documented,
- To minimize the risk of error, the RDT results will be read and recorded independently by three trained certified operators, blinded to each other's reading,
- To allow immediate correction of erroneous recording of RDT results (rather than differences in visual interpretation), the PI or designee will assess the results as soon as possible to allow him/her to return to the original test device to investigate apparent discordant readings OR check the photographs taken,
- The use of electronic images (photographs) will be decided at the beginning of the evaluation by the study coordinating team.

Safety

HIV, hepatitis B and hepatitis C and many other viruses are transmissible by blood and body fluids. Therefore, all types of specimens (including venous and capillary whole blood, serum/plasma, oral fluid, etc.) will be handled as potentially infectious. Appropriate universal precautions to minimize exposure to infectious hazards will be taken at all stages from the collection of specimens to the disposal of used materials from the laboratory. UVRI has safety guidelines that laboratory staff carefully follow for all procedures. Compliance with all local, institutional and international statutory requirements for health and safety will be required. All personnel involved will undergo a retraining in Good Clinical Laboratory Practice (GCLP) and Good Clinical Practice (GCP).

Storage of test kit reagents

All reagents will be stored as indicated in the manufacturer's 'instructions for use'. Some reagents may not need refrigeration. Calibrated thermometers will be placed at each location where reagents and specimens are stored, i.e. ambient, refrigerator and freezer to track the storage temperatures. Temperatures will be recorded and monitored daily. The lot numbers of the test kits received/used and their expiry dates will be recorded on the individual run worksheets.

Sequence of testing

Each serological kit under evaluation will be used strictly in accordance with the 'instructions for use' issued by the manufacturer.

Reference serological panel

A well-characterized serological reference panel of positive and negative specimens will be established. Negative specimens from low-risk donors will be tested on 3 (or two, depending on availability of EIAs) CE-marked SARS-2 CoV-19 IgG EIAs and on 1 IgM EIA to confirm their negative status.

Since most RDT have both IgM and IgG, we will also attempt to have 3 IgM EIAs. If availability of the 3 EIAs proves difficult we plan to use two orthogonal tests [14].

Positive specimens will be from convalescent patients who had been infected with SARS-2 CoV-19, confirmed by NAAT and will be tested on 3 EUA/EUL and/or CE-marked SARS-2

CoV-19 IgG EIAs. Samples from patients with early/acute stage infection will also be used to evaluate the performance of IgM-based kits. Negative specimens to be included in the reference serological panel will be negative on all 3 EIAs each IgG and IgM whilst positive specimens to be included in the reference serological panel will be positive on all 3 EIAs each, IgG and IgM. In case there are no available EIA IgM kits we will opt to use two of these. Ideally the convalescent specimens will be those taken right before discharge and 2 -6weeks after discharge while early/acute will be those taken at a time a patient is still PCR positive. Individual samples will be uniformly mixed, aliquoted in many replicates and stored at -20°C ±5°C prior to use. To avoid repeated freeze-thaw cycles, a new aliquot will be used for each test.

Immunological profiling of specimens in the reference panel

The immunological profile of available specimens having sufficient volumes will be characterized on the available EIAs namely:
Anti-SARS-CoV ELISA **IgG** (Euroimmun)
Novel Coronavirus COVID-19 **IgG** ELISA (Epitope)
SARS-CoV-2 **IgG** Assay (Abbott)
Novel Coronavirus COVID-19 **IgM** ELISA (Epitope)
Platelia SARS-CoV-2 Total Ab (**IgA/IgM/IgG**) (Bio-Rad)
Definitions:
A negative specimen will be negative on all assays
A positive early/acute specimen will be positive on the IgM EIA and on the IgA/IgM/IgG assay
A positive convalescent specimen will be positive on all 3 IgG EIAs, positive on the IgA/IgM/IgG EIA and negative on the IgM assay
For evaluation/verification, the RDTs (IgM/IgG in the main) will be used to test negative, positive early/acute and convalescent specimens.

Detection limit testing

Detection limits will be compared by testing positive samples of various positivity levels on EIA (from weak to strong); tested undiluted and diluted from 1:5 to 1:1600. Frequencies of endpoint dilutions for each kit will be tabulated and compared, as described elsewhere [15]. For the NAAT tests we will assess the lowest detection limits using viral RNA copies/ml.

Recording and Interpretation of Results

All EIA test results will be captured electronically directly from the plate reader and imported into a certified data management application (eg MS Access or REDCap) for further data analysis. For subjectively-read assays (RDTs), the intensity of the band/line/spot and the time intervals between starting the test and reading the result will be entered on the data collection sheet. The intensity rating system will be scored as described in Table 2 below. Visual interpretation of results of subjectively read assays will be made independently by three readers (without the knowledge of the other sets of results and blinded to the reference result for the specimen) and entered onto the data collection sheets. These results will be compared with those of the operator carrying out the assay so that any potential transcription or reading errors may be identified and rectified immediately. Should recording errors be identified, both the original and corrected result will be recorded, dated and initialed by the reader. When the three readers interpret the results differently from each other, the consensus will be recorded as that interpretation which occurs two out of three times. In cases where all three interpretations are different e.g. Reader A scores 0, Reader B score 1, Reader C scores 3, the test will be repeated

and if the inconsistency happens again then the result will be recorded as indeterminate. It is also planned for the 3 observers to use the photograph taken of the RDT to reach a consensus.

Table 2: Scoring Index Used at UVRI*

Scoring index	Intensity reading scale 1	Intensity reading scale 2
0	Negative	Negative
1	Very Weak but positive	Positive
2	Medium to Strong Reactive	Indeterminate
3	Indeterminate	Invalid
4	Invalid**	

*The scoring index will be determined before study initiation. It may/will depend on the kit insert instructions.
** including no control line/band/dot/spot visible, or obviously defective test device, no flow, debris present.

Data Management

The UVRI departments of Information Technology (IT) and Epidemiology and Data Management play a leading role in the development, updating and application of the UVRI Laboratory Information Management System (LIMS) whose key functions include sample management, integrated data-capture applications and electronic data exchange. UVRI has a licensed copy of the Research Electronic Data Capture (REDCap) software that departments utilize. The data capture applications developed at UVRI are used for building and managing both offline and online surveys and databases following Good Clinical and Laboratory Data Management Practice (GCLDMP)in a **HIPAA** compliance environment. Data capture applications developed promote electronic transfer of laboratory results to multiple-step password-protected databases. The LIMS is managed by 2 data managers, a database developer (Cisco-certified) and a team of 4 data-entry clerks. The data management system is connected to the data server in the IT section. Apart from REDCap, other software used for application development and operations include MySQL, MS Access, MS Excel and STATA 15 for analysis. All tools and applications used are password-protected and there is scheduled back-up of databases and programs. Paper records are stored in a well-protected documentation center with lockable file cabinets managed by a records officer. The documentation center is also used for storage of consent and study forms.

The UVRI has data protection and data sharing policies which shall be followed in management of any data accruing from this study.

Evaluation of RDTs (Ag) for SARS-CoV-2

It is planned that when the swab from a suspect case returns a NAAT-positive test result a further swab is used to validate the Ag RDT test. A response team on standby will be deployed to the isolation center where the index case is held. The response team will move with a UVRI laboratory staff who will carry the Ag RDT. The UVRI staff will take a new swab and test it on the Ag RDT under evaluation. A further swab will be taken for NAAT confirmation in the event the Ag RDT under evaluation returns a negative result. Around 10 UVRI staff will be trained on how to use the Ag RDT test under evaluation and be ready to be deployed to any part of the country. The Ag RDTs will also be tested on SARS-CoV-2 low risk NAAT-negative individuals.

Analysis of Results

Criteria for test result acceptance and invalid test runs/devices

All tests will be performed according to the manufacturer's instructions. For each kit, manufacturer's protocols will be used to set up-front test acceptance or failure criteria. Test runs will be regarded as invalid for various criteria guided by the manufacturers' instructions that may include weak or no signal; saturated signals, high backgrounds, poor replicate data, inconsistent assay-to-assay results. Tests will be declared valid only if the reference negative and positive controls (provided by the manufacturers) included on each test plate (for EIAs) yield the expected results. For visual interpretations (RDTs), samples on which analysts disagree will be regarded as indeterminate.

Invalid runs/devices

The number of invalid test runs will be recorded as the proportion (percentage) of invalid runs out of the total number of runs performed for clinical specimens only. The number of invalid devices (if rapid diagnostic test or other format) will be recorded as the number of invalid test devices as a percentage of the total number of devices used with clinical specimens.

Inter-reader variability

The inter-reader variability will be calculated when assay result readings are performed without any objective reading instruments i.e. RDTs. Three persons will independently interpret each test result. The inter-reader variability will be expressed as the percentage of specimens for which initial test results are differently interpreted (i.e. reactive or non-reactive or indeterminate) by the independent readers. The photographs taken will also be used to reach a consensus among the readers.

Performance characteristics

The following strategies will be used to calculate the performance characteristics for each assay under evaluation and is closely linked to the reference panel testing results

Table 3: 2 x 2 Table for Calculation of Performance Characteristics

	Reference testing results			
Results of assay under evaluation		Positive	Negative	Total
	Positive	a (true positives)	b (false positives)	a+b
	Negative	c (false negatives)	d (true negatives)	c+d
	Total	a + c	b + d	a+b+c+d

Sensitivity

Sensitivity is the ability of the assay under evaluation to detect correctly, specimens that contain the antibody or antigen (reference results positive). Therefore, sensitivity is the number of true positive specimens identified by the assay under evaluation as positive (a), divided by the number of specimens identified by the reference assays as positive (a+c), expressed as a percentage.

Sensitivity = a/a+c

Specificity

Specificity is the ability of the assay under evaluation to detect correctly, specimens that do not contain the antibody or antigen (reference results negative). Therefore, specificity is the number of true negative specimens identified by the assay under evaluation as negative (d), divided by the number of specimens identified by the reference assays as negative (b+d), expressed as a percentage

specificity = d/b+d

Positive Predictive Value (PPV)

The probability that when the test result is positive that the specimen does contain the antibody/antigen. PPVs will be calculated using the formula

PPV = (prevalence)(sensitivity) / (prevalence)(sensitivity)+(1-prevalence) (1-specificity) or

PPV = a/a+b

Negative Predictive Value (NPV)

The probability that when the test is negative that a specimen does not contain the antibody/antigen. NPVs will be calculated using the formula

NPV = (1-prevalence) (specificity) / (1-prevalence) (specificity) + (prevalence)(1-sensitivity) or

NPV = d/c+d

Of Note: The probability that a test result will accurately determine the true infection status of a person being tested varies with the prevalence of the infection in the population from which the person comes. In general, the higher the prevalence of the infection in the population, the greater the probability that a person testing positive is truly infected (i.e., the greater the positive predictive value [PPV]). Thus, with increasing prevalence, the proportion of individuals testing false-positive decreases; conversely, the likelihood that a person whose test result is negative is truly uninfected (i.e., the negative predictive value [NPV]), decreases as prevalence increases. Therefore, as prevalence increases, so does the proportion of individuals testing false-negative.

Recommended sensitivity and specificity

The cut-off for sensitivity and specificity for recommended EIAs and RDTs will be discussed by the study teams. Recommended specificity is planned to be 99% and sensitivity 98% but this will be decided before the evaluation, taking into consideration the number of samples/panels available for the evaluation.

Reproducibility

Reproducibility is the ability to produce essentially the same diagnostic result, under different conditions (different operators, test batch, different laboratories and/or different intervals of time). For this evaluation we intend to test the same sample after different intervals of time.

Verification of NAATs

Verification will be performed whenever the MOH wants to introduce a new validated commercial NAAT with defined performance (from manufacturer) for routine use or where a previously-approved (WHO/FDA approval) and validated method is modified and needs to be verified before use in Uganda. The UVRI will verify the NAATS ability to achieve acceptable results.

Equivalence tests for NAATs

The laboratory will test the hypothesis that the results of the kit undergoing verification do not differ from the results of the reference kit by more than 2%.

To test for equivalence

Hypothesis test 1

H_o: The difference is less than or equal to the lower limit for equivalence between the kit under evaluation and the standard kit.

H_1: The difference is greater than the lower limit for equivalence between the kit under evaluation and the standard kit.

Hypothesis test 2

H_o: The difference is greater than or equal to the upper limit for equivalence between the kit under evaluation and the standard kit.

H_1: The difference is less than the upper limit for equivalence between the kit under evaluation and the standard kit.

Other documented information PPV, NPV, time involved, specimens required, information required, any limitations on lab output.

Procedures for developing a reference panel of well-characterized sera/plasma specimens from COVID-19 cases and controls

NAAT will be used to confirm acute SARS CoV-2 infection in persons with suspected COVID-19.

Blood specimens from NAAT-confirmed cases will be collected at intervals from patients during the acute and convalescent stages of infection.

NAAT-confirmed cases will include those patients with an equivocal/borderline positive result as well as patients with a strong positive result using a WHO-approved molecular test (Berlin Protocol).
A control will be a person at low risk of exposure to the virus and having no evidence of SARS CoV-2 nucleic acid present in their specimen.
Convalescent specimens from cases with NAAT-confirmed infection will be tested on different IgA, IGG and IgM EIAs to confirm the presence of anti-SARS CoV-2 IgG and/or anti-SARS CoV-2 IgM. We will also include samples from acute/early patients when still NAAT-positive to evaluate IgM.
Specimens from controls will also be tested on the same EIAs to confirm the absence of anti-SARS CoV-2 IgG and/or anti-SARS CoV-2 IgM.
Characterized specimens will be archived and used as a gold-standard panel in all future evaluations of SARS CoV-2 RDTs.

Technician's appraisal

The technical aspects of the assay under evaluation will be assessed by the technician who performed the testing. These assessments, along with other selected assay characteristics, contribute to an overall appraisal of each assay's suitability for use in the laboratories. To enable comparison between assays, a scoring system will be used to rate specified operational characteristics.

Laboratory staff performing the kit evaluations will respond to a questionnaire investigating the suitability of the RDTs kits for routine use which will be approved by the UVRI REC. This survey will contain questions covering ease of use criteria, with preset answers represented by a scale of 1 (lowest rating) to 5 (highest rating). The questions will focus on ease of interpretation of procedures, reagents characteristics, ease and time of execution and result interpretation. Overall rating for each question will be assigned using the mode obtained from all the scores reported by the laboratory and summed for each kit. In addition, the questionnaire will also include two open questions requesting advice and recommendations for improvements of each kit. Where two kits have equivalent performance characteristics, the one which is easier to use, cheaper, faster or requires a more easily obtainable sample will be preferred.

Report Preparation

The data analysis and report drafting will be carried out by the study team under the supervision of the project PI. The study team that includes the laboratory team, data management and statistician will check for accuracy and completeness of the report. The PI will verify the report before sharing with the MOH.

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