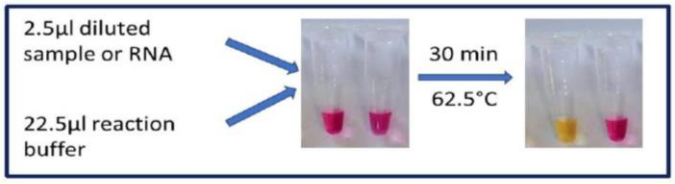
DEPLOYMENT PROPOSAL FOR CoVAMP (COVID-19) RAPID MDx KIT

Problem: The current gold standard for the diagnosis of COVID-19 is a PCR-based test which requires an expensive acquisition of instrumentation and technical skillset, including laborious and time-consuming laboratory workflow that could run for an average of 4-6hours or a number of days depending on logistics.

Solution: We hereby present CoVAMP (COVID-19 colorimetric isothermal AMPlification) – a complementary, cheaper and equally efficient rapid genetic-based test with a shorter turnaround time of about 30mins to 1hour – depending on RNA extraction time per sample prep. The molecular diagnostic test is based on a widely published method of gene amplification which enables nucleic acid tests to be conducted on biological samples at a single isothermal temperature.

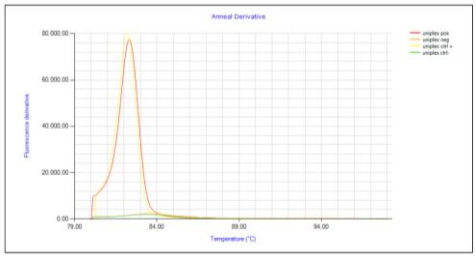
Uniqueness: Colorimetric visual detection of positive samples within 30mins of isothermal amplification – using low cost laboratory materials such an inexpensive water bath or heating block.



Samples of colorimetrically assessed tubes following RT-LAMP (Yellow – POS; Pink – NEG)

Rapid RNA Extraction: The kit also has a rapid 5-min buffer integrated in its components to enable RNA extraction from a crude lysate. While a standard RNA-extraction protocol (e.g. Qiagen) is preferred, to improve the positive predictive value of the kit, the rapid lysis and RNA extraction buffer included in the CoVAMP kit can also enable its use as an RDT for point of care testing.

Integrated Automation: The CoVAMP kit can also be deployed as a fully automated assay which can be integrated on existing instrumentation (qPCR equipment) for quantitative and qualitative analysis – from Cq values and melt curves.



Melt Curves derived from the Uniplex CoVAMP Assay tested in real time using an LC 480 II Roche Instrument. Positive and Negative RNA templates from clinical COVID-19 samples were tested. A synthetic gblock positive control with an ORF1a fragment was also tested alongside a negative control (Di-H20) as the legend shows. Both clinical and synthetic positive samples showed melt peaks at about 82°C while negative controls remained flat.

Product Validation at ACEGID & NRL: Extensive validation exercises were conducted on the CoVAMP kit at ACEGID in May, 2020 while other validation exercises were subsequently conducted at the NCDC National Reference Laboratory in Abuja, Nigeria between July and November 2020. The main conclusions are shared below:

RESULTS AND CONCLUSION

Our result showed that the CoVAMP RDT performed as good as the DaAn gene kit . The CoVAMP RDT was still sensitive for samples with both high and low CT values. Finally, based on our result, I will recommend the CoVAMP colorimetric detection kit because it saves time,(visual examination) more economical (minimal procedure one step heating) and at this volume (1ul)RNA you can detect samples with high or low viral load. Considering the availability and the large number of test required, the reaction volume will save reagents and sample volume. We will keep you updated if there is any development.

Thank you.

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In conclusion, CoVamp kit has shown sensitivities and specificities within the range observed for Isothermal amplification assays. Our observation is that given high integrity kit (kit supplied in Cold-chain) the assay performance might improve beyond the reported values. Kindly find detailed results of the various analyses and pictures of the assays at various incubation times as well as the Gel images.

Sincerely,

Mrs Nwando MBA
Director, Public Health Laboratory Service

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Kit Deployment Specifics:

CoVAMP Kit Versions Validated @NRL	Uniplex (ORF1a Target): 90% Sensitivity Duplex (ORF1a/ORF1b): 71% Sensitivity
Proposed Kit Version to be Deployed (CE-Marked)	Uniplex (Colorimetric & Fluorescence Detection)
Cost Per Test	$12 (Approx. ₦5000)
Proposed Deployment Volume	100,000 tests in 4-8weeks (50 tests per kit box)
Financing Options for Kit Deployment	Eurekan Biotech can advance financing for kit deployment with an official LPO not beyond a 90-day tenor.

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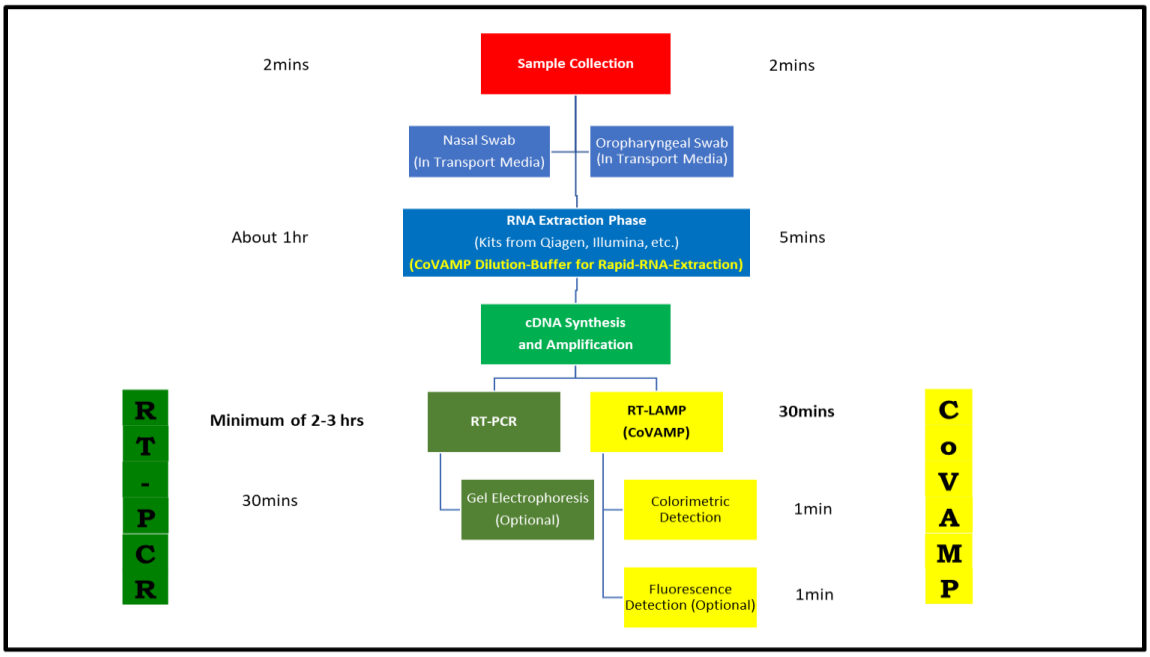
Workflow Comparison of CoVAMP with RT-PCR gold standard



Sample Workflow for CoVAMP Integration on an RT-PCR Equipment

- Same reaction tube to be used for CoVAMP can be used for qRT-PCR in a 30-min run time.

Protocol/Methods:

Please find underneath the program for the Light Cycler 480
LAMP COVID-19_62 program:

LC480 II (Roche) please select following filter combinations:
Excitation filter: 465- Emission filter: 510

Cycles: 55 **Analysis mode:** Quantification

Target (°C)	Acquisition Mode	Hold (hh :mm :ss)	Ramp rate (°C/s)	Acquisitions (per °C)
62°C	Single	00:00:34	4.40	
62°C	None	00:00:01	4.40	

Melting:
Cycles: 1 **Analysis mode:** Melting curves

Target (°C)	Acquisition Mode	Hold (hh :mm :ss)	Ramp rate (°C/s)	Acquisitions (per °C)
99	None	00 :01 :00	4.4	
80	Continuous		0.05	11
40	None	00 :00 :01	2.2	