EDCTP Contract N⁰ RIA2020EF-2918

Rapid diagnostics for COVID-19: manufacturable in Africa to increase affordability, improve epidemic preparedness and strengthen local resilience

Deliverable report

Deliverable number	6.7
WP no/title	Clinical study of NAT in COVID-19
Deliverable title	Publication of study report
Responsible partner	**KNUST**
Due date	**Month 20**
Actual submission date	**Month 21**

Delivery Type

R	Report	✓
DEM	Demonstrator, pilot, prototype, plan designs, new or revised health policies etc	
DEC	Websites, patents filing, press & media actions, etc	
OTHER	Other	

Dissemination Level

PU	Public	✓
RE	Restricted to a group specified by the consortium.	

Document Log

Version	Date	Author	Description of Change
1.0	Jan 2022	Samuel Sakyi	

List of content

1 STATUS OF THE DELIVERABLE 2
2 SUMMARY OF THE RESULTS (MAX. 1-2 PAGES)..2
3 DESCRIPTION OF WORK PERFORMED AND OBTAINED RESULTS 2
3.1 PLEASE USE NUMBERED SUBSECTIONS 2

Partner	Contribution to this deliverable
DTU	Provided PathPod with its cartridges
UCAM	Provided Boost2 Bst enzymes

1 Status of the Deliverable

Complete

The Afridx Project evaluated the Reverse Transcriptase Loop Mediated Isothermal Amplification (RT-LAMP) PathPod system from DTU, compared with the gold standard RT-qPCR.

KNUST, KCCR and NMIMR received the PathPod system and cartridges from the DTU and undertook a study of 3559 clinical samples. Data on specificity and selectivity was obtained and divergence between the RT-LAMP and RT-PCR data was evaluated and discussed.

The aim is to replaced the BST and RT enzymes in the PATHPOD with locally expressed enzymes. RT and BST constructs developed by UCAM were expressed in Ghana and tested in a LAMP assay.

Data obtained from the study has been drafted in a manuscript with running title: “LAMP assay as POC alongside locally expressed BST and RT enzymes in low resource settings against standard RT qPCR procedure: A Multicentre Comparative Study.” This manuscript is currently undergoing input by study contributors.

2 Summary of the results

2.1 Pathpod Performance

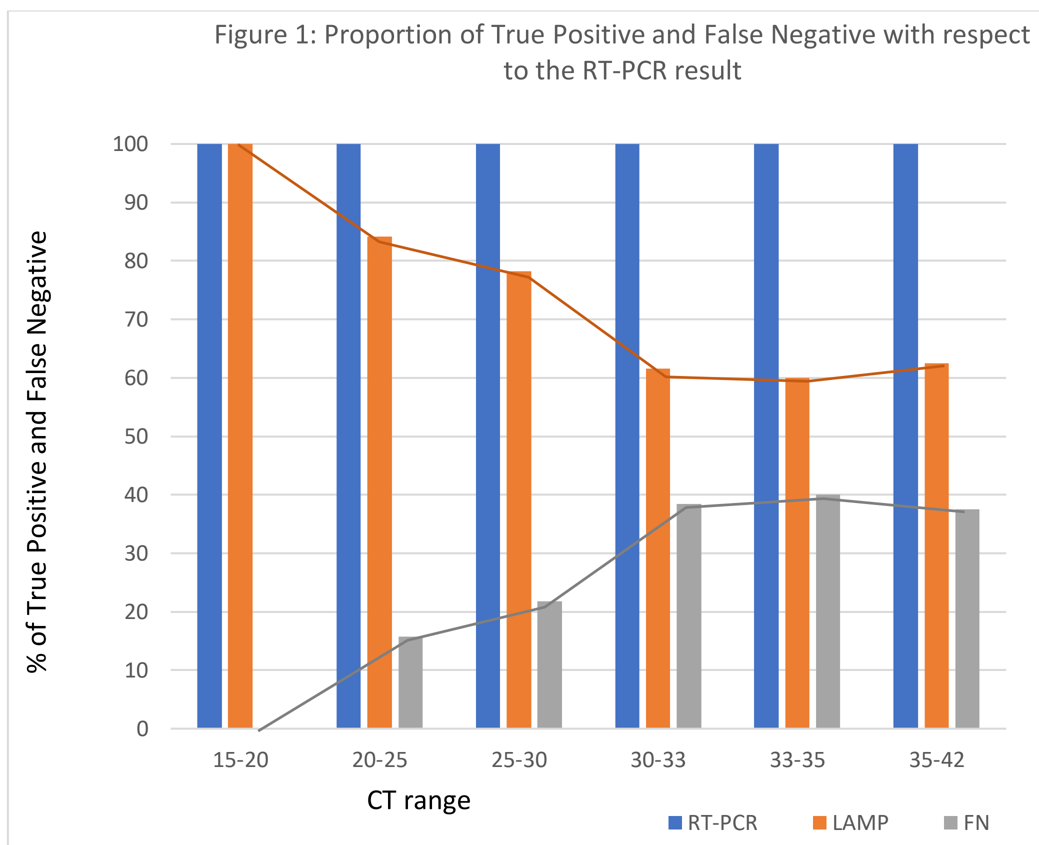
Cartridges for the Pathpod were provided by DTU for circa 4000 samples. Each cartridge contained wells for 10 samples and 2 references. The Pathpod RT-LAMP study was divided into two phases. In the first batch of testing, a total of 1,943 tests was conducted by KNUST, KCCR and NMIMR. In the second batch 1612 samples were tested. All samples were also tested by RT-PCR and a C_T value was obtained for positive samples.

The PathPod achieved ~60% sensitivity overall, but specificity fell from 80% in the first batch to 24% in the second batch. Both batches of cartridges came from the same production run and analysis of

the data to ascertain the key factors influencing the change are ongoing but appear to be related to contamination or adverse storage effects.

		Real-time RT PCR			Assay Performance			
		Positive (n, n %)	Negative (n, n %)	Total samples (n, n%)	Sensitivity (95% CI)	Specificity (95% CI)	Positive Predictive Value (95% CI)	Negative Predictive Value (95% CI)
RT-LAMP	Positive	203	279	482	0.63 (0.57-0.68)	0.77 (0.75-0.80)	0.42 (0.38-0.47)	0.89 (0.87-0.91)
	Negative	121	956	1077				
	Total samples	324	1235	1559				

The assay performs fairly well compared to the gold standard (RT-qPCR). The PathPod assay accurately detected 63% of the positive cases and 77% of the negative cases. The data showed that the FNs could be related to the copy number of virus in the sample, with a strong correlation with RT-PCR C_T value and true positive/false negative LAMP outcome. Figure 1 shows the decrease in RT-LAMP True Positives and increase in False Negatives with increase in C_T.



Upon further analysis of false positives and false negative samples it was observed that the Sars-Cov-2 target gene used had an effect on the sensitivity and specificity compared with the RT-PCR.. This was unsurprising since the Pathpod used a specific gene target, whereas different RT-PCR targets were compared. Subsequently, after adjusting for these confounders, to enable a better matched comparison, showed 92% sensitivity with the PATHPOD with negative samples and ~90% sensitivity with positive samples.

Further examination of the data was able to reveal patterns of response attributable to factors including:

- initial copy number combined with assay sensitivity
- primer-dimer formation during storage
- non template amplification
- contamination or other user introduced error

This information was valuable in establishing factors such as operator or device error, which can lead to future improvements.

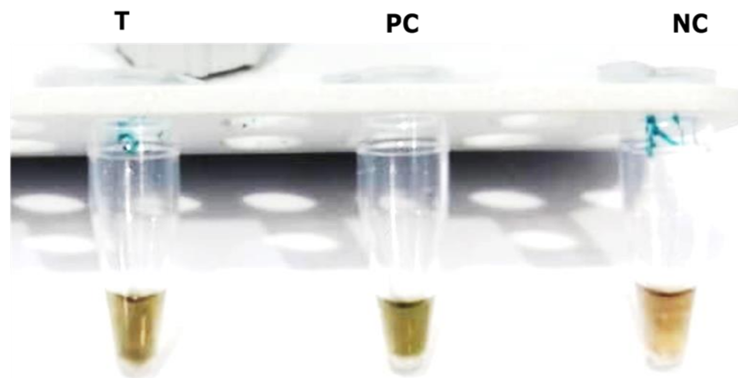
One factor which contributed to the reduced specificity for the second batch of testing, was likely to have been the extended shelf-life of the cartridges. By the end of the study, the remaining cartridges were >1year old. It is well known that cold-chain failures and multiple excursions to high and low temperatures will compromise the RT-LAMP reagents. However this is challenging to guarantee for materials that are shipped between continents. For this reason alternatives were sought to enable local on demand production of RT and BST, the core enzymes for the RNA conversion and DNA amplification.

2.2 Local bio-manufacturing of biologicals

The local bio-manufacturing of biologicals component of the AfriDx project entails using the engineered Boon 2 BST plasmids, designed by UCAM to express and produce the protein on site for the LAMP amplification.

The plasmid was shipped from UCAM to KNUST (since this is DNA, it is more robust to shipping that the protein). The boon-2 BST was successfully expressed at KNUST and applied in the RT-LAMP protocol to detect SARS-Cov-2.

Replacement of the commercial polymerase with locally produced Boon2-Bst polymerase was done using 2µL of locally expressed and purified Boom 2.0 BST DNA polymerase in a SARS-CoV-2 LAMP assay according to SARS-CoV-2 LAMP assay protocol using tris-based storage buffer. Figure 2 provides a visual of the assay, showing the development of a green coloration is the sample compared with the pink/orange of the negative control and similar to the positive control.



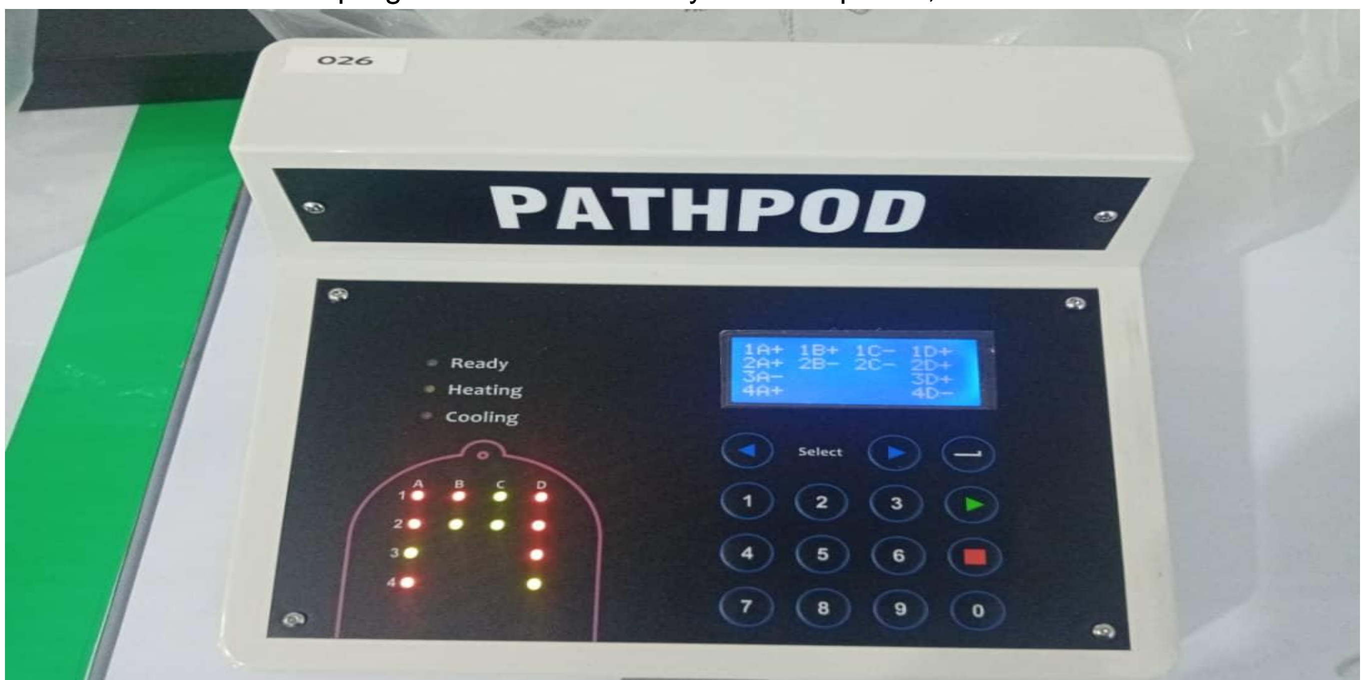
T:Test = LAMP mastermix + SARS-CoV-2 +ve control + 5ul Bst SiB DNA polymerase
PC: Positive control = LAMP mastermix + SARS-CoV-2 +ve control + commercial Bst DNA pol
NC: Negative control = LAMP mastermix + nuclease free water + commercial Bst DNA pol

Fig 2: SARS-CoV-2 LAMP assay using locally produced and purified boom 2.0 BST DNA polymerase using the unmodified protocol

3 Methodology

3.1 PathPod

The PATHPOD was used according to the operating instructions.
After short vortex of sample, 6 µl of sample and/or controls were added directly into the center of the well. the yellow paper from the PCR film was removed and placed over the film chip. The film was sealed properly to the chip using a soft roller ready for processing in the PATHPOD system. Using the pathpod keyboard, sample ID was entered and COV assay was initiated. The cartridge was inserted to run the program. When the assay was completed, the result was read.



3.2 BOON-BST Production

Boon 2 BST bacterial glycerol stock was incubated overnight (12 hr. – 16 hr.) at 37 °C/225 rpm, next morning LB broth containing kanamycin was incubated with the overnight culture at 37 °C/225 rpm until the A_{600} of 0.6 – 0.8. After reaching an OD600 OF 0.6 – 0.8, protein production was induced ith IPTG and the culture returned to the incubator (37°C/225 rpm) for 4 hours. After 4- 5 hours, the culture was aliquot into centrifuge tubes and centrifuged at 3750 rpm for 30 min. After centrifugation, the supernatant was decanted and the pellet suspended with 1mg/ml of lysozyme in 1x PBS (pH 7.2) at RT for 30 min. During the lysis incubation, 60 uM silica gel was suspended with 1x PBS (pH 7.2), vortexed and inverted to assist in suspending silica. Cell lysate was mixed with the silica for 30 min (vortexing the samples every 10 min). After a final vortex, the samples were centrifuged at 7.5k rpm for 5 min. The silica immobilized Boon2-BST was suspended at a ratio of 5 ul (1x PBS) per 1mg of Silica. The immobilized Boom2-BST was then collected and stored in tris-based buffer at 4°C for both short- and long-term storage.

3.3 SARS-CoV-2 LAMP testing using Boom 2.0 BST DNA polymerase produced locally at KNUST

2µL of locally produced and purified Boom 2.0 BST DNA polymerase was used to setup the SARS-CoV-2 LAMP assay according to the SARS-CoV-2 LAMP assay protocol.

22.5 µL colorimetric mastermix was place a nuclease-free 0.2 ml PCR tube. 2.5 µL sample was added into test tubes to obtain a final reaction volume of 25.0 µL. One positive control (Twist RNA control and one non-template control (NTC) containing 2.5 µL nuclease-free water was prepared as controls. The final reaction RT-LAMP samples was incubated at 65°C for 45 min. Positive samples turned to a clear green whilst negative samples remained red compared to the negative control. Sample that were neither red nor clear green but has taken a yellowish to brownish (“khaki”) color, were considered inconclusive and the RT-LAMP assay of this sample were repeated.

Footnote:

Master mix: 10x iBuffer 5 and 5 M betaine were pipetted into a nuclease-free 1.5 ml Eppendorf reaction tube. The mixture was mixed thoroughly by vortexing until the solution appears homogeneous. 25x ErioT/MY was added and mixed to obtain a dark red solution. 100 mM $MgSO_4$ solution and 10 mM dNTP solution was also added and mix well to obtain a green solution. 10x primer set stock was added and mixed thoroughly by vortexing on ice. GspSSD2.0 LF DNA polymerase and RTx reverse transcriptase were also added and vortexed to obtain the colorimetric master mix.