D1.8 – Mid-term Report

Project Information

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Contributors	DTU, KNUST, KCCR, NMIR, UCAM
Reviewers	Lisa Hall (UCAM)
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2.0	25/05/2021	Lisa Hall	Revisions and corrections

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1 Executive Summary

This document is the deliverable "1.3 : Midterm Report" of the European & Developing Countries Clinical Trials Partnership (EDCTP) funded project "AfriDx: COVID-19 Diagnostics for Africa" (AfriDx). The Midterm Report summarizes the progress, achievements and challenges encountered so far by the project.

Notable accomplishments from the project so far includes the creation of the project handbook from work package 1, the arrival of the PATHPOD system and chips at partner institutions in Ghana and successful training on its use under work package 2, the design of BST and RT constructs from UCAM and progress of tests adapting those to the PATHPOD at DTU under work package 3. In work package 4 among others, an initial trial comparing the ability of the PATHPOD system to detect samples previously tested by PCR as positive or negative suggest good concurrence but some differences between PATHPOD and PCR, that are being further investigated. Work package 5 reports the incorporation of a fluorescent protein (mCherry) into screened scFVs against hIgG and hIgM as a first step to a self labelling immunoassay, while work package 6 reports the development of the AfriDx protocol and SOPs and regulatory approvals. Data collected in work package 7 indicates that there should be freedom to operate in Ghana for the core technologies used in AfriDx and AfriMx while work package 8 reports the development of the AfriDx Manufacturing course handbook and SOPs. Work package 9 reports the development of the "Plans for Exploitation and Dissemination", the launching of the AfriDx website and multiple information dissemination channels amongst others.

The midterm report is structured into five sections. Beyond this executive summary, the Introduction (section 2), provides some background information into the aims and objectives of the AfriDx project. Section 3 gives the status of each work package with respect to deliverables, some details about the work in progress. Section 4 gives a general overview of the progress of deliverables in the project that have been completed, that are in progress and those for which work is yet to commence. Section 5 highlights specific challenges the project has faced so far and how they were resolved.

2 Introduction

AfriDx is a multi-institutional high impact research programme to provide sustainable, low cost and reliable tools for correct clinical diagnosis of COVID-19 in Sub-Saharan Africa. Working directly with partner institutions in Ghana, a unique aspect of the project is the inclusion of, and training of front local line professionals and partnering with African business to deliver the aims and objectives of the project.

The aim of the project is to provide reliable diagnostic systems for the confirmation of the clinical diagnosis of COVID-19 in Africa from first infection through to recovery and immunity, with:

- RNA viral testing enabled for point of care (POC)
- Prrof of principle Antibody titre testing for POC
- Sample to result time of less than one hour
- >90% sensitivity and specificity
- Compatibility for distributed production at low cost in Africa

The objectives are:

- To use the front-line testing facilities in NMIMR and KCCR, Ghana, as a testbed for a new POC nucleic acid diagnostic emerging from DTU (PATHPOD - detection of virus RNA), that is currently under clinical test in Europe.
- To replace the BST and RT enzymes in the reagent mixture in PATHPOD chips with BST and RT constructs designed by UCAM for low resource production in Africa and evaluate the AfriDX-RT-LAMP to use in the PATHPOD and compare with standard RT-qPCR procedure
- To develop an Antibody Titre assay to proof of principle from a UCAM single-chain antibody (scFv) library and produce scFv constructs for low resource production of a lateral flow immunoassay (LFIA) in Africa (AfriMx).
- To evaluate combined batch testing as methodology adopted from normal veterinary protocols (but not widely used for human testing) and assess accuracy, cost and time benefits in epidemic testing pathways.
- To draft a plan to produce AfriDx in Africa for the African market, with outline analysis of socioeconomic and healthcare benefits.

The aim is that the novel diagnostics developed by this project, will be able to be produced in Africa at a cost affordable to Africa and will allow early and reliable diagnosis of COVID-19 by minimally trained laboratory personnel in hospitals and health clinics, or in POC environments like drive-through laboratories and by epidemic response teams. It is anticipated that this will lead to increased throughput of samples tested and triage-able at POC. Apart from the direct benefit to clinical diagnosis of COVID-19, socioeconomic long-term independence from external grant-aid to supply diagnostics and development of a local manufacturing infrastructure would emerge.

The AfriDx project began in October 2020 and in this report, we present the status of each of the nine (9) work packages, progress, achievements, and challenges experienced in the first eight (8) months (mid-term) of the project.

3. Work Package Summaries

3.1.1. WP 1: Project Coordination

The **Project Coordination** Work Page has three deliverables that are due to be completed by the time of publishing the midterm report. These are:

- D1.1 Project Handbook and communications/documentation tools for file-sharing and discussion – due in Month 5
- D1.2 The Advisory Board – due in Month 6
- D1.3 The Midterm Report – due at the end of Month 8

3.1.2 Description of WP1 deliverables completed or in progress

D.1.1: The Project Handbook and communications/documentation tools for file-sharing and discussion

D1.1 comprised of four elements:

i) Project Handbook: which serves as a signposting document, containing contact information of consortium members, information about work packages and deliverables.

ii) Shared Dropbox: To facilitate the distribution of files without emailing

iii) Zoom Meetings: includes the monthly All hands meetings (involving all partners), training meetings and work package specific meetings which can be scheduled on request.

iv) Google groups email: A general shared email account was created and trialed by project partners but was decided to not be an effective means of group communication.

D1.2 The Advisory Board

Suggestions from the AfriDx advisory board were collected from members of the consortium. The suggested individuals have been approached with an invitation to the AfriDx board. The AfriDx Advisory Board member names have been confirmed and have been published on the website following acceptance received from the prospective members.

D1.3 The Midterm report

This midterm report is written with contributions from all partners as summaries of work done so far in all work packages. The contribution of other project partners are as documented in the work packages below.

3

The AfriDx project utilizes the output from another EU funded project, CORONADX (https://coronadx-project.eu/) lead by DTU. This includes

- the PATHPOD devices fabricated at DTU,
- cartridges filled with ready-to-use reagents for SARS-CoV-2 detection
- the LAMP assay was developed, optimized and adapted to PATHPOD

The performance evaluation of the PATHPOD system was previously executed through the CORONADX project.

In addition to the PATHPOD system, UCAM has shared a crude and silica attached BST enzyme it developed with DTU. Preliminary studies on the adaptation of the *BST* constructs/enzyme to the PATHPOD system has been conducted at DTU.

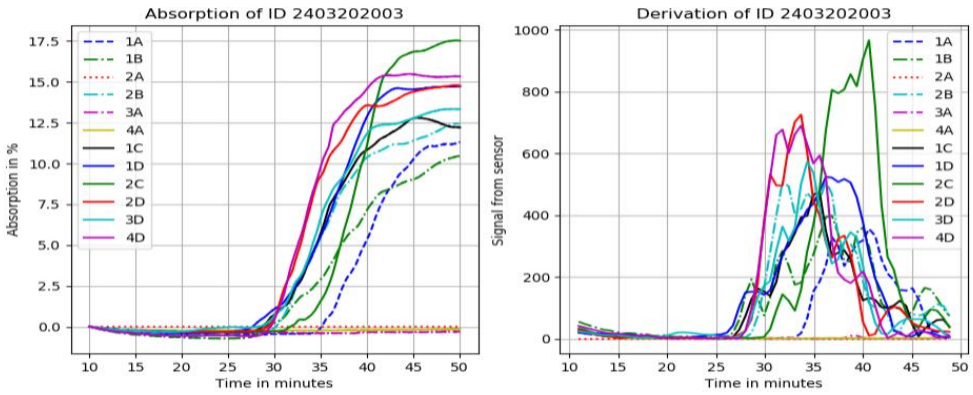
A brief description of the of the QC and LAMP assays adaption to the PATHPOD are as outlined below:

a. DTU has fabricated 5 PATHPOD systems in synergy with another EU funded project CORONADX (https://coronadx-project.eu/**)**.
b. DTU also fabricated 500 Cartridges necessary for use in PATHPOD for testing SARS-COV-2 in AfriDX project. The assembled cartridges are QC tested. Approximately, 1% of the chips injection molded per batch production was QC tested and the results of the QC tested were satisfactory.
c. In the first batch 275 cartridges were filled with gelified LAMP reagents optimized in the CORONADX project.
d. A novel RT-rLAMP assay suitable for the PATHPOD system developed in the CORONADX project was adapted in AfriDX project.
e. The turbidimetry method is used as detection method in the PATHPOD. The volume of the RT-rLAMP reaction is 30 µL.
f. The sensitivity of the PATHPOD system was initially determined with commercial SARS-CoV-2 plasmid control supplied by Integrated DNA technologies.
g. A serial 10 fold dilution of a commercial plasmid DNA that contain SARS-CoV-2 genome fragment with known concentration (copies/µl according to manufacturer) was prepared.
h. The tested concentrations were between 24000 copies/reaction and 15 copies/reaction.
i. PATHPOD could detect pure plasmid DNA around 30-50 copies per reaction within 40 minutes
j. The sensitivity was 100% for 300 and 150 copies (6 repetitions each) and 44% for 120 copies. 6 repetitions of 75 copies also gave 100% true positive results. This infers that the LOD of PATHPOD is between 75-150 copies/reaction.
k. Specificity of the RT-rLAMP assay was tested with a total of 12 different bacteria and viruses that included *Escherichia coli*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniea*, *Staphylococcus aureus*, Avian Influenza Virus, SARS-CoV control (bat-SL-CoVZC45), MERS CoV control (KNIH/002_05_2015), and Paramyxovirus as non-specific targets along with a positive control (n-CoV Plasmid DNA with known number of copies) and negative control in the experiment.

a)


CORONADX
CORONADX
CORONADX

b)


Absorption of ID 2403202003
Absorption in %
Time in minutes
Derivation of ID 2403202003
Signal from sensor
Time in minutes
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1B
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c)

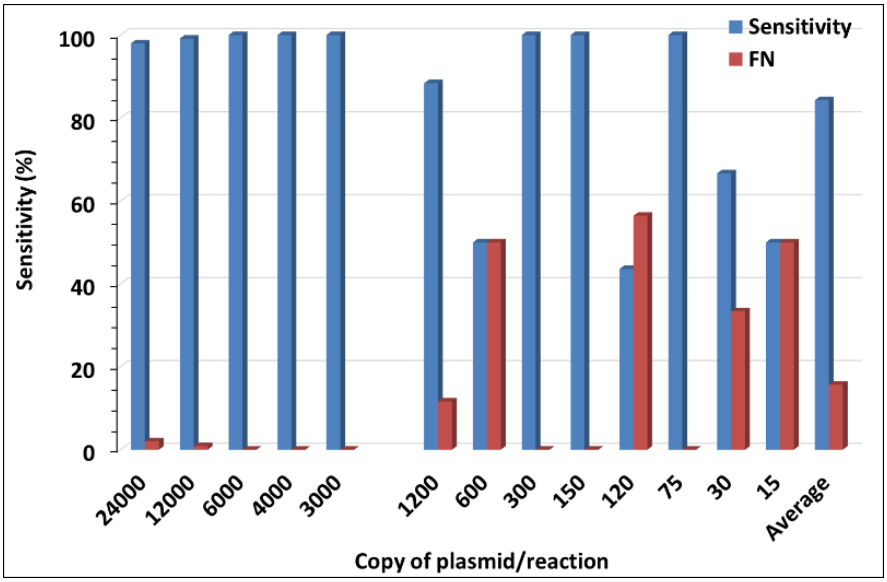


Figure 1: PATHPOD System QC and LAMP assay optimization. A) The PATHPOD devices and ready-to-use cartridge for SARS-CoV-2 testing B) Sensitivity of the RT-rLAMP to detect SARS-CoV-2 using the PATHPOD system C) Sensitivity of the PATHPOD system

Four deliverables are expected to be completed by the time of publishing the midterm report. These are:

- D2.1 Define end-user needs including the number of NAT systems and chips required for clinical testing, completed in month 6 by KCCR and DTU
- D2.2 PATHPOD systems and chips delivered for clinical testing in Ghana, completed in month 8 by DTU and all partners in Ghana
- D2.3 Remote training course for use of PATHPOD system and chips, completed in month 6 by DTU
- D2.4 Workflow for PATHPOD use in Ghana to detect COVID-19, anticipated to completed in month 9 by DTU

3.2.2. Description of WP2 deliverables completed or in progress

D2.1: Definition of end-user needs for deployment of PATHPOD in Ghana

KCCR currently receives and conducts about 600 tests daily. Between KCCR and KNUST the goal is to have capacity to conduct 4000 tests. To define the number of NAT and chips needed, the pooling method of testing at NMIMR was adopted although this testing regime is more appropriate under low positivity rate. In addition, DTU will also evaluate current testing workflows and resources.

Five hundred (500) cartridges were requested by partners in Ghana, with the excess 100 serving as backup for optimization and SOP validation. KCCR and KNUST also procured 2 fridges to store the cartridges and extracted RNA samples. In response to request, DTU has prepared 5 PATHPODs with 250 ready-to-use cartridges for Ghana. Upon receiving feedback from the first testing round a second batch with 250 cartridges will be sent. Based on the workflow and feedback on the performance of PATHPODs at Ghana necessary adaptations will be made.

D2.2 PATHPOD systems and chips delivered for clinical testing in Ghana

Five PATHPODs and 250 ready-to-use cartridges (First batch) were shipped from DTU to NMIMR for further distribution to KCCR and KNUST. On March 21, 2021, 3 PATHPODS (numbered 25, 26 and 30) were transported from NMIMR to KNUST. Subsequently, on May 10, 2021, three sets of 42 chips and additional 7 chips (for training purposes only) were transported from NMIMR to KNUST, Kumasi. KNUST and KCCR received three sets of PATHPOD systems and 133 chips.

D2.3 Remote training course for use of PATHPOD system and chips

The PATHPOD quick user guide, standard operating procedures and instruction video for training were prepared by DTU and shared with AfriDX project partners by depositing in the shared dropbox folder.

On April 13, 2021 (Month 7) a virtual training session was conducted via zoom on how to use the PATHPOD system. Although specifically planned for the Ghanaian partners, all partners participated and provided some feedback on the training materials and instruction videos. An updated version of training materials was prepared incorporating feedback received. A follow-up training session was conducted on May 12, 2021, with a live laboratory demonstration of the use of the PATHPOD system transmitted via zoom from DTU. The partners at KNUST and KCCR also replicated the use of the PATHPOD system and chip to test a real sample immediately after demonstration also on zoom. The training session was successfully executed**.**

D2.4 Workflow for PATHPOD use in Ghana to detect COVID19

The preparation of this deliverable is still in progress; the first phase and review of the data obtained is anticipated to be completed by month 9; a further shipment of cartridges will then allow the second phase to be undertaken, which will take into account feedback from the data in phase 1.

3.3.1 WP 3: Local Manufacturing of Biologicals

This work package has two deliverables expected to have been completed by the midterm report. These are

- D3.1 Engineered Boon2-BST and accompanying expression and purification SOP, completed Month 6
- D3.2 Engineered Boon2-RT and accompanying expression and purification SOP, anticipated to be completed by the end of Month 8.

The design of the Boon2-BST is completed and synthesized. A functional, permanently immobilized iteration of the enzyme has been characterized and delivered to DTU as part of Deliverable number D3.1 (see section 3.3.3). Training modules for protein expression and purification in both documentation and video formats have also been made available to all partners in the shared drobox. In addition, a functional reverse transcriptase has been recombinantly synthesized and tested for activity DTU. However, a final iteration of the enzymes (that can be eluted off the silica) will be finalized by month 8. At that time, the protein will be characterized for its ability to self-cleave off the silica and remain functional.

3.3.2 Description of WP1 deliverables completed or in progress

Summary of WP3 results

Multiple silaffin-tags were tested to determine their adhesiveness to unmodified silica gel microparticles. Of the peptides tested, the 3 amino-acid truncated R5-tag (Δ3R5), the unmodified Car9 peptide and the Car9-[R4Q] peptide were observed to have the highest affinity to silica, as shown in Table 1. In all 6 cases, once the peptide was electrostatically bound to the silica gel particles, incubating the silica at temperatures of up to a maximum of 60°C did not dissociate the proteins off the silica.

Table 1: Fusion proteins were immobilised at a ratio of 250 µL of cell lysate (normalised via mCherry fluorescence) to 1.25 mg of 63µm silica gel.

Silaffin-Tag	Amino Acid Sequence	Immobilisation Percentage
R5	SSKKSGSYSGSKGSKRRIL	66 ± 5
Δ11R5	KGSKRRIL	85 ± 2
Δ3R5	KSGSYSGSKGSKRRIL	97 ± 5
Car9	DSARGFKKPGKR	95 ± 5
Car9-[R4Q]	DSAQGFKKPGKR	96 ± 8
Car9-[K8Q]	DSARGFKQPGKR	88 ± 7

The final design of the Boon2-BST is comprised of 5 individual components synthetically linked together through protein engineering. As shown in Figure 2, the independent constituents are: a silaffin affinity-tag, a photocleavable protein (PhoCl), a fluorescent protein (mCherry), a

histidine (His)-loop, and a *Geobacillus stearothermophilus* (BST) DNA polymerase. This combination of proteins and tags would allow for silica-based purification (silaffin tag + PhoCl), visual tracking (mCherry), DNA amplification for isothermal nucleic acid diagnostic assays (BST 2.0 DNA polymerase), and a secondary purification option for additional enzyme characterisation (His-loop).

Figure 2: Schematic of proposed fusion protein.

Preliminary testing using a non-PhoCl iteration of the Boon2-BST showed very good activity from the enzyme, even when compared to the commercial reagent, as shown in Figure 3 and 4. Due to the large quantity of protein produced, the untreated synthetic BST2.0 construct was able to react faster and produce a stronger fluorescent signal than its commercial counterpart.

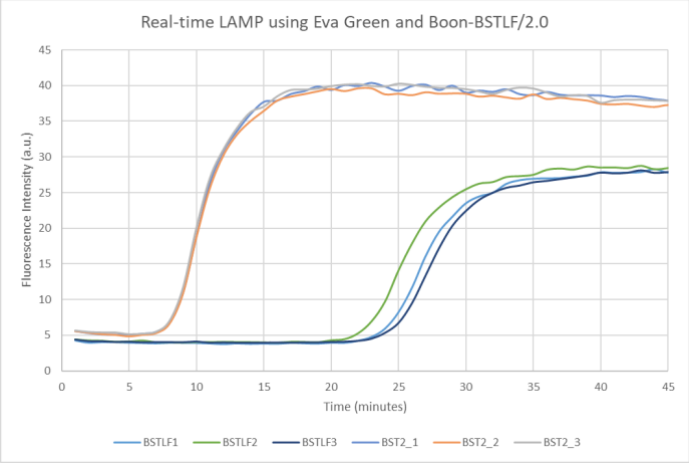


Figure 3: 1 µL of cell lysate was used for the synthetic Large Fragment BST (BSTLF) and BST2.0 DNA polymerase. The rise in fluorescence signal (produced by Eva Green) is indicative of DNA amplification.

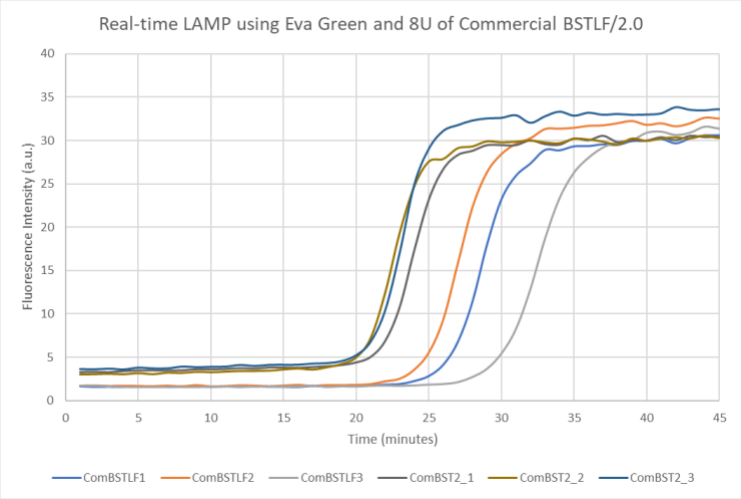


Figure 4: A standard 8U of enzyme were used for each commercial assay. The rise in fluorescence signal (produced by Eva Green) is indicative of DNA amplification.

The enzyme was also shown the be active when immobilised to silica, as shown in Figure 5.

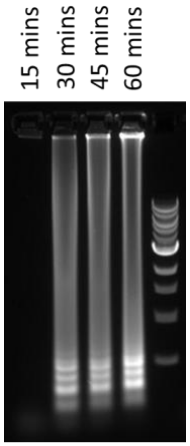


Figure 5: Gel electrophoresis of LAMP reactions using 0.2 mg of silica with immobilised BST2.0 at various time points. The illuminated smear represents a positive signal indicative of LAMP-based DNA amplification.

Finally, recombinantly expressed reverse transcriptase was found to be active in solution, but still requires further cloning to test its compatibility with the desired silaffin-tag and fluorescent protein additions.

Description of work performed and obtained results

As of this report we have synthesised and compared various iterations of silaffin-tags, synthesised and characterised a fusion-construct with BST2.0 DNA polymerase, and lastly synthesised a BST2.0 DNA polymerase construct with the photocleavable protein

Silaffin-Tag Assessment

A total of 2 native silaffin-tags and multiple unique mutated variants of said tags were tested for both their affinity and desorption properties. As was shown in Table 1, truncating the native R5 improved the peptide's affinity to silica, with the shorter truncation resulting in the greatest affinity. These results, in tandem with *ab initio* modelling, suggest that intramolecular binding is interfering with the native R5's ability to bind to silica. Truncating the N-terminal may disrupt these interactions, improving the peptides binding capabilities through the increased availability of the positively charge amino acids. As for the Car9 peptide, the R4 amino acid appears to have little impact on the tag's affinity to silica, despite its positive charge. On the other hand, the K8 amino acid does appear to play in some role with regards to silica affinity, and could be a potential mutation location for the synthesis of a reversable silaffin-tag.

All 6 cases were tested to observe if heat could be a viable pathway for desorption of the immobilised protein. Going up to a maximum temperature of 60°C, there was no measurable desorption of the Boon2-BST. The proteins adhered to silica through silaffin tags were not reversible using quick low-cost methodologies.

Despite not being able to introduce a reversibility characteristic to the silaffin-tags, the **Δ3R5-tag** (that was generated in the process) was a drastic improvement over the native **R5-tag.** Thus, the tag was carried over to all the latter protein constructs synthesised.

Δ3R5-mCh-H10-BST2.0

The first engineered Boon2-BST was stable, activity, and simple to produce. A both a standard expression protocol of 1 mM IPTG with a 4.5-hour incubation at 37 °C and a 10-hour autoinduction protocol at 37°C was enough to produce the protein. As shown in Figure 6, the confirmation of Boon2-BST expression was visualized through its pink appearance.

Figure 6: Cell pellet from a BL21 (DE3) culture without protein expression (left) and with protein expression (right).

As for silica immobilisation, the Δ3R5-tag does not appear to be hampered by the large size of the fusion protein. Incubating unmodified silica gel with the raw cell lysate revealed high affinity of the Boon2-BST, shown in Figure 7, and some degree of selectivity, as shown in Figure 8.

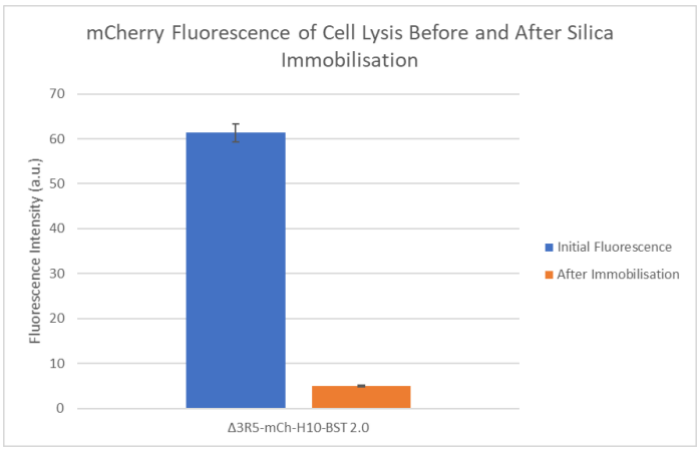


Figure 7: The mCherry fluorescent signal of the cell lysate drastically decreases after silica gel exposure. This indicated that the Boon2-BST is being removed from the lysate solution by the silica.

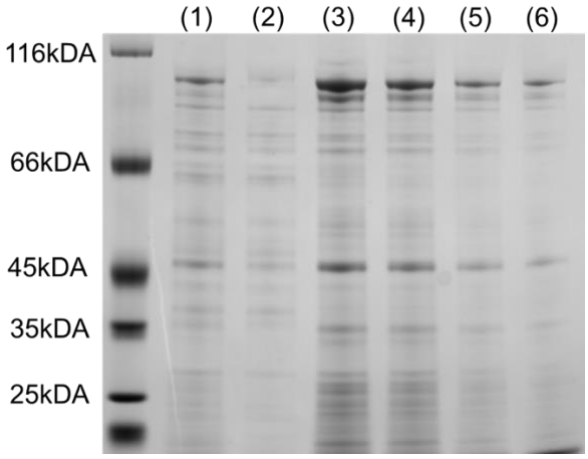


Figure 8: SDS-Page gel highlighting silica absorption and selectivity. The band for the Boon2-BST (98 kDa) is visible in the cell lysate (lane 1), but significantly less visible after silica absorption (lane 2). Lanes 3 to 6 are the absorbed proteins from 0.2 mg, 0.1 mg, 0.04 mg, and 0.02 mg of silica, respectively. The strong presence of the Boon2-BST band in the silica sample further emphasises the strong affinity of the silaffin-tag.

Lastly, the quantity of immobilised protein necessary for nucleic acid amplification (within a 1 hour timeframe) was tested by varying the quantity of protein absorbed silica per reaction sample. As shown in Figure 9, if at least 0.1 mg of silica was used, a positive reaction can be visualised via gel electrophoresis at the 45-minute mark.

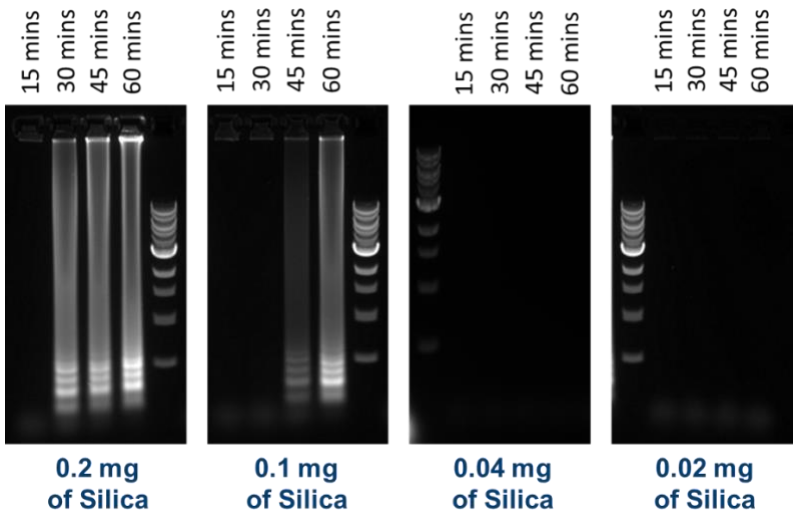


Figure 9: 2% agarose gels highlighting the activity of the immobilised protein. As expected, increasing the quantity of enzyme improves the reactivity of the assay. At lower silica quantities, there is a decrease in likelihood for the formation of a polymerase, primer, DNA triplex.

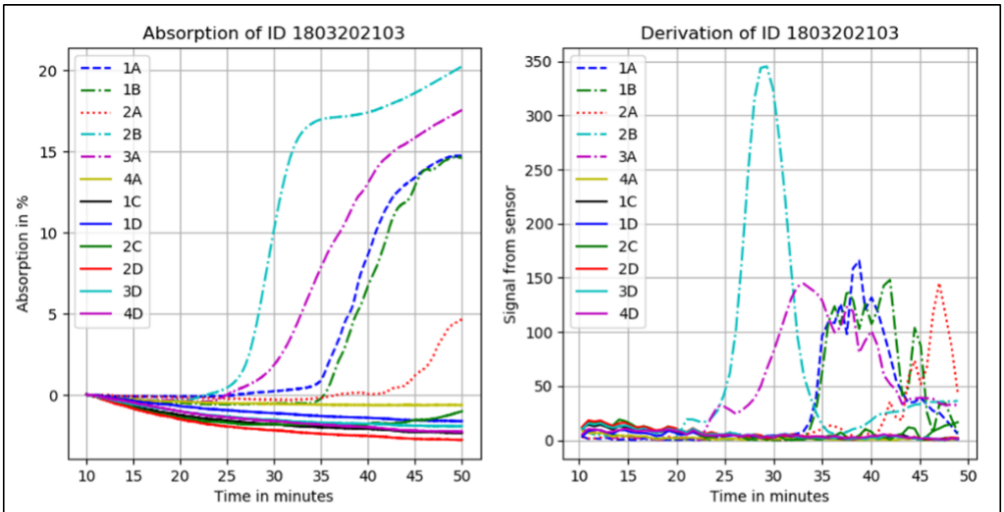
Testing of the BST at DTU

DTU performed preliminary studies on the adaptation of *BST* constructs/enzyme developed by UCAM in the PATHPOD. The *BST* constructs/enzyme were supplied in non-immobilized free form and immobilized on the silica beads. LAMP efficiency of both enzymes was initially tested in conventional thermal cycler and compared with standard *Bst* enzyme from New England Biolabs. SARS-COV-2 plasmid control was used as template (500 copies, 100 copies, 50 copies, 20 copies) in the presence of 12 units of enzyme and SYTO 9 as intercalating dye for fluorescence. The *BST* constructs/enzyme both under free form and under immobilized conditions performed better than standard *Bst* detecting up to 20 copies/reaction in several repetitions (Table 1). Initial experiments were performed to adapt UC-B and UC-S in the PATHPOD. Both UC-S and UC-B resulted in false negative results in the PATHPOD. The control enzyme from NEB gave positive amplifications (Fig. 10).

Table 2 Comparison of LAMP efficiency of *BST* constructs/enzyme with standard *Bst*

	500 copies/reaction Tt (min)	100 copies/reaction Tt (min)	50 copies/reaction Tt (min)	20 copies/reaction Tt (min)	Ns Tt (min)
NEB	9.16	11.12	No Ct	No Ct	No Ct
			No Ct	No Ct	No Ct
			No Ct	27.99	No Ct
			No Ct	No Ct	No Ct
			No Ct	No Ct	No Ct
				No Ct	No Ct
					No Ct
UC-S	13.52	21.76	19.18	28.82	No Ct
			12.92	30.22	No Ct
			17.66	20.44	No Ct
			27.99	29.45	No Ct
			16.45	33.09	No Ct
				17.95	21.95
					21.8
UC-B	7	8.27	24.78	12.04	32.9
			14.99	12.78	49.08
			28.54	21.94	40.58
			9.53	13.68	47.18
			37.29	9.9	28.06
				8.45	48.33
					No Ct

*NEB: New England Biolabs,
*UC-S: UCAM *BST* constructs/enzyme in solution form,
*UC-B: UCAM *BST* constructs/enzyme immobilized on the silica bead



1A: 6K-NEB	1B: 600-NEB	1C: 6K-UC-S	1D: 600-UC-S
2A: 60-NEB	2B: RNA (1:10)-DTU	2C: 60-UC-S	2D: RNA (1:10)-UC-S
3A: RNA (1:100) NEB	SARs-CoV-2 plasmid control Copies/reaction		3D: RNA (1:100)-UC-S
4A: NC-DTU			4D: NC-UC-S

Figure 10: Adaptation of UC-B and UC-S to the PATHPOD

Δ3R5-PhoCl-mCh-H10-BST2.0

A few characterisation tests were performed in UCAM with the photocleavable iteration of the Boon2-BST. Similar to the non-photocleavable version, protein expression would produce a pink pellet. However, unlike the previous iteration, the expression protocol required 0.5 mM IPTG at 18 °C for 16 hours. Optimisation of the expression protocol is still underway.

In addition, the Δ3R5-tag continued to provide high affinity to silica with a good level of selectivity, as shown in Figure 11. The elution of the enzyme through the utilisation of the photocleavable protein, is still ongoing.

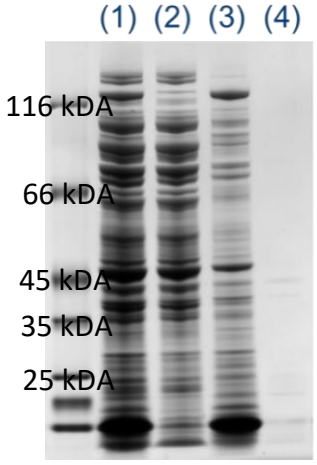


Figure 11: SDS-PAGE gel highlighting the presence and silica absorption of Δ3R5-PhoCl-mCh-H10-BST2.0 (126 kDa). The PhoCl-Boon2-BST band is present in the cell lysate (lane 1), not present in the lysate after silica exposure (lane 2) and is once again present on the silica itself (lane 3). Fortunately, exposing the immobilised protein to ambient indoor light is not enough for cleaving to occur, as there is no protein present the silica sample supernatant (lane 4). This also means extra safety procedures are not required to minimise pre-mature release.

Lastly, the presence of the photocleavable protein, aside from the general increase in the protein molecular weight, was determined though visualising the green fluorescent signal. As shown in Figure 12, whether it be in the *E. coli* or on the surface of the silica gel, the presence of green fluorescence indicated that the photocleavable protein folded correctly. In addition, the strong location overlap between the mCherry fluorescence (red) signal and the green signal suggest that the proteins are attached to one another.

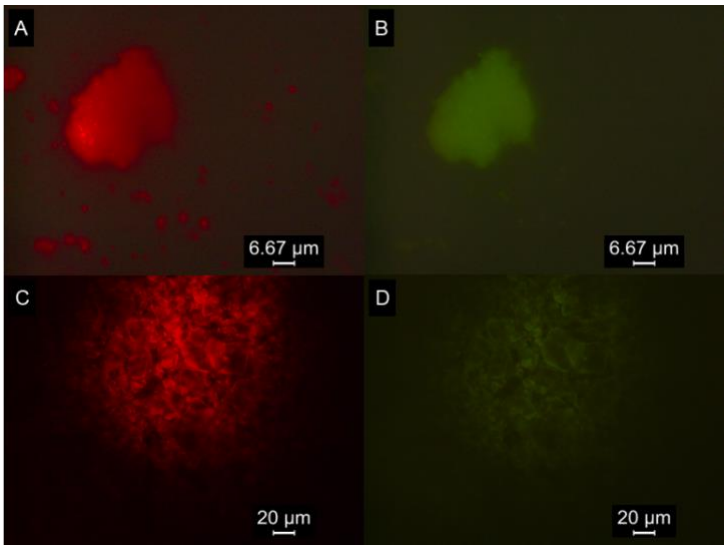


Figure 12: Fluorescence microscopy of E.coli from cell lysate (A, B) and of silica gel after protein immobilisation (C, D).

Reverse transcriptase

A reverse transcriptase was expressed in BL21 (DE3) *E. coli* using a standard IPTG expression protocol. Various lysis methods were tested ranging from using only lysozyme to sonication with subsequent thermal elution. It was determined that lysozyme with protease inhibitor was the best approach for enzyme activity, as shown in Figure 13.

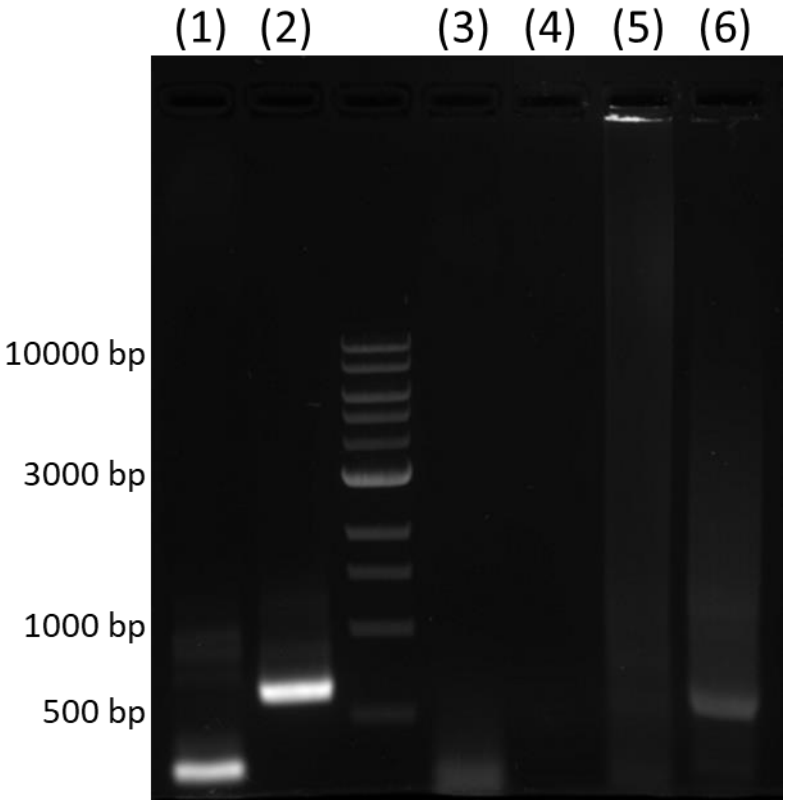


Figure 13: PCR activity test of reverse transcriptase (lysed under different conditions) with a desired amplicon size of 240 bp (1,3,5) or 607 bp (2,4,6). Lanes (1,2): Lysozyme with protease inhibitor. Lanes (3,4): Lysis re-suspension buffer followed by a concentration step and a buffer exchange step. Lanes (5,6): Same as Lanes 3 and 4 but with a thermal elution and filtering step to remove aggregated contaminant protein (prior to the concentration step).

With an expression and purification protocol determined, cloning of reverse transcriptase to the silaffin-tag and mCherry backbone and subsequent testing are currently underway.

3.4.1. WP 4: Testing Strategy

The Work package 4, **Testing Strategy** has two deliverables expected by the mid term report. These are:

- D4.1 Description of model for pooled samples at low, medium, and high prevalence of COVID-19, completed Month 8.
- D4.2 Dataset for limit of detection and report on recommendations for clinical study design, completed month 8.

3.4.2. Description of WP4 deliverables completed or in progress

D4.1 Description of model for pooled samples at low, medium, and high prevalence of COVID-19

A theoretical model for pooling of samples for testing was developed with the assistance of the DTU Section for Statistics and Data Analysis. The model was applied to four scenarios with prevalence of p = 0.1, 0.01, 0.001 and 0.0001, respectively (10%, 1%, 0.1% and 0.01%, respectively). This corresponds to high, medium, low and very low prevalence, respectively. The model calculates the optimal number of samples in a pool as well as the total number of tests needed to determine the results of e.g. 100 samples. It can thus be directly seen how many tests are required. Pooling of samples is not very efficient at high prevalence, but pooling becomes more and more efficient at low prevalence.

D4.2 Dataset for limit of detection and report on recommendations for clinical study design

At KNUST and KCCR data collection to determine the testing strategy is currently in progress. One Hundred (100) archived samples from COVID-19 suspected cases will be used for optimization of all procedures prior to field testing. The optimization experiments will include validating the quick extraction (boiling method) procedure in comparison to standard RNA extraction method (e.g., Qiagen RNA extraction kit) and testing the effect of pool samples on assay sensitivity.

Forty (40) archived COVID-19 RNA extracts have been tested using the PATHPOD as shown in Figure 14. From the results obtained, the PATHPOD detected 17 positive extracts compared with 16 detected by PCR as well as 23 negative extracts compared with 24 by PCR.

Fresh clinical samples tested with the PATHPOD will be compared to results from PCR tests before commencing field work

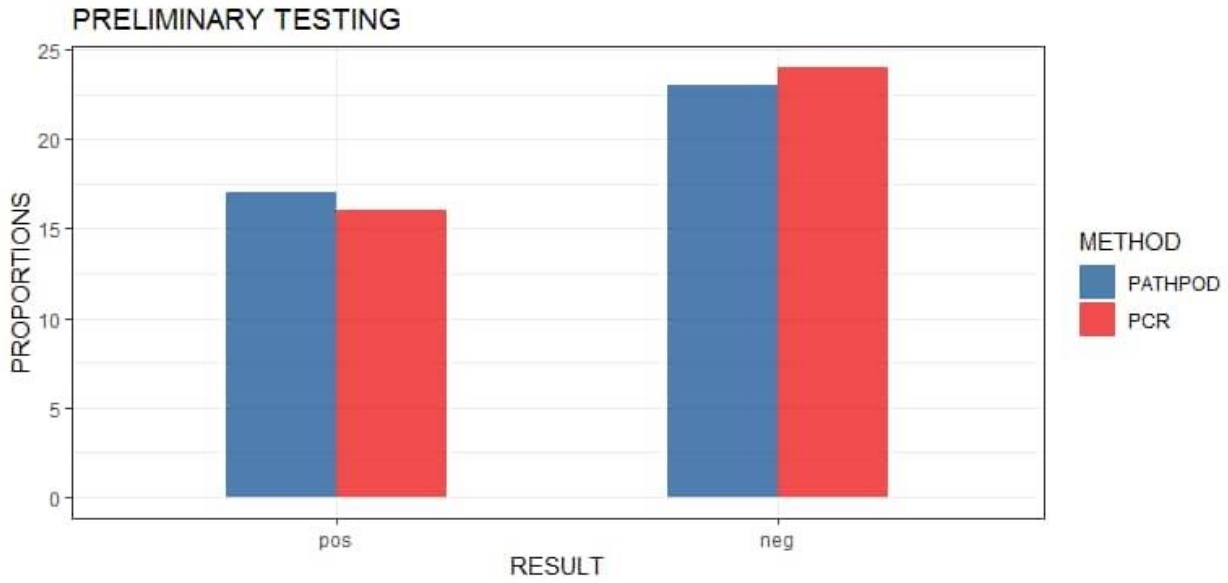


Figure 14: Comparing PATHPOD and PCR results

Table 3: Preliminary results (Diagnostic accuracy with archived samples)

PathPod LAMP	RT-PCR		Sensitivity	Specificity	PPV	NPV	N
	pos	neg					
pos	14	1	0.88 (0.64-0.98)	0.96 (0.81-0.99)	0.93 (0.70-0.99)	0.93 (0.77-0.99)	43
neg	2	26					

3.5.1. WP 5: Proof of Concept Serological Test Development

The Work package 5, **Proof of Concept Serological Test Development** has two deliverables expected to be completed by the mid term report. These are:

- D5.1 Modified and characterised high affinity scFv, anticipated to be completed by Month 8.
- D5.2 Purified covid coat protein fragments, anticipated to be completed month 8.

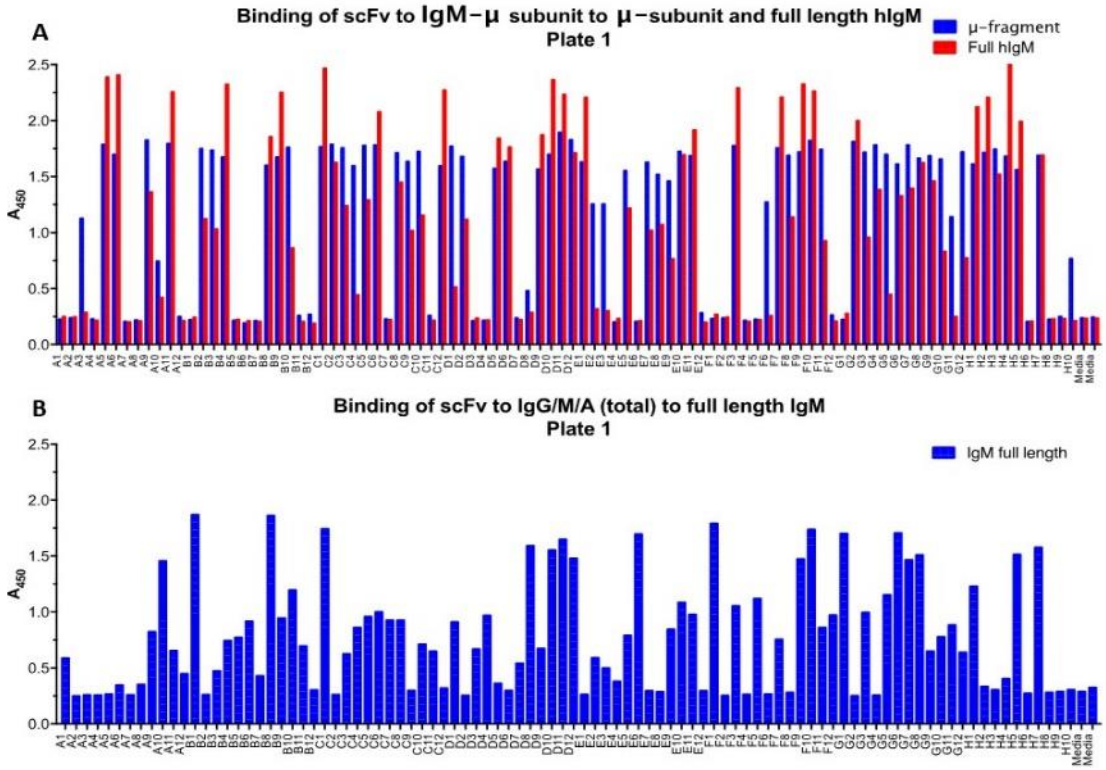
Completed tasks in WP5 include the following:

i) Screening existing single chain fragment antibody library (scFv) for high-affinity and highly specific His-tagged scFvs against human IgM and immunoglobulins.

ii) Designing a 'generic antigen' containing concatenated epitopes covering the following antigens: Covid 19 spike protein; nucleocapsid protein; membrane protein and Orf8 accessory protein. Cloning is now in progress.

3.5.2. Description of WP4 deliverables completed or in progress

The scFvs have been tested for specificity and affinity. cDNAs for these scFvs have been sequenced and cloned into expression vectors for use in Africa (See Figure 15 A-D). In addition, suitable fluorescent tags (eg mCherry) are currently being incorporated into these scFvs. Finally, the 'generic antigen' (see above) will form the basis of a universal antigen for use in Covid ELISA and/or lateral flow tests.



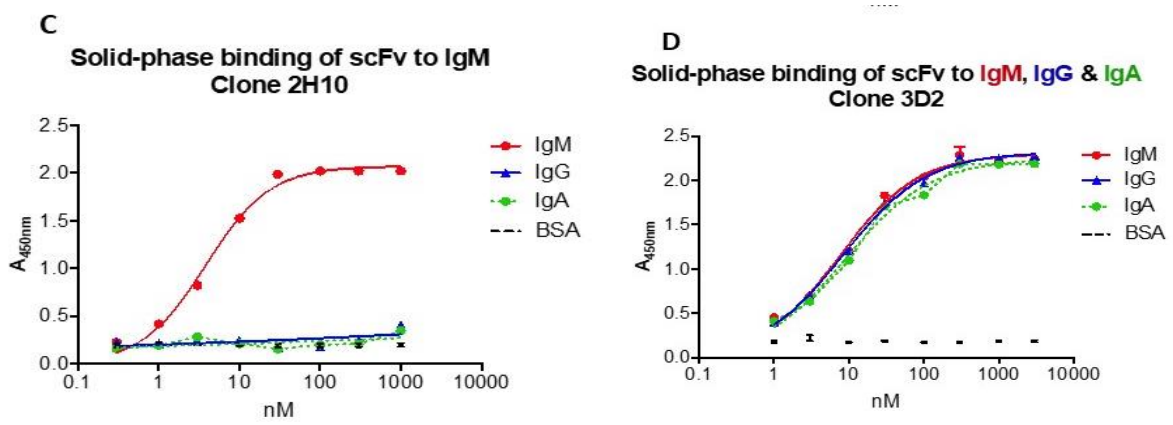


Figure 15: Examples of scFvs screening and characterization. Screening of scFv library for binders to (A), human whole IgM and human IgM µ-fragment. (B), double selection for scFv binders to all classes of human immunoglobulin. (C), selected scFvs from (A) and (B), tested for binding in ELISA format.

These components are now being tested for assembly into a lateral flow assay, with the self indicating scFvs.

3.6.1 WP 6: Clinical Study of NAT in COVID-19

The work package 6 **Clinical Study of NAT in COVID-19** has four (4) deliverables expected to have been completed by the mid term report. These are:

- D6.1 Study protocol and supporting documents completed, completed in month 6
- D6.2 Confirmation of study set up, recruitment and training of staff, completed in month 6
- D6.3 Institutional and regulatory approvals obtained, KNUST approvals obtained, UCAM approval in advanced stages
- D6.4 Provide clinical data obtained to date for mid term report, completed in month 8

3.6.2 Description of WP6 deliverables completed or in progress

D6.1 Study protocol and supporting documents

KNUST and KCCR developed the study protocol and standard operating procedures as adapted from the AfriDx protocol and proposal respectively. These among other documents such as participant information leaflet, informed consent receipt of payment and the completed ethics application form were submitted to the IRB of KNUST. A copy of the study protocol has been published on the AfriDx website:

https://afridx.ceb.cam.ac.uk/files/afridx_protocol_v1.0.pdf

D6.2 Confirmation of study set up, recruitment and training of staff

Approvals were obtained from the appropriate authorities at the study sites (KCCR, NMIMR, PHRL-Kumasi and AGAHF). And interviews were conducted leading to the recruitment of two Research Assistants to assist on the project.

Figure 16: Research assistants at KNUST, KCCR and NMIR working through workflow of the NAT system

D6.3 Institutional and regulatory approvals obtained

The Committee on Human Research Publication and Ethics (CHRPE) in KNUST initially gave a conditional approval for the study but later gave a final approval after addressing the recommended issues. Ethical approval application from the University of Cambridge is in the advance stages and is anticipated to be completed in Month 9. A copy of the ethical approval from KNUST has been published on the website:

https://afridx.ceb.cam.ac.uk/files/ethical_approval_for_afridx_project.pdf

D6.4 Provide data for mid-term report

Data reported for Midterm report is as reported in Section 3.4.2

Figure 16: Nucleic Acid Testing system: a) Cold room storage of chips at NMIMR. b) Storage of chips at 4^{o}C and storage of PATHPOD at KNUST. c) Chips and PATHPOD on the working bench. d) PATHPOD displaying

3.7.1. WP 7: Route to Market

Work package 7: **Route to market** is partially up to date, but the timelines for the deliverables have been adjusted to accommodate the departure of TATAA from the project and their replacement by AVOMA. There is one deliverable anticipated to be completed in month 9 at the time of publishing the midterm report:

- D7.1 Report and dataset on legal and regulatory landscape for PATHPOD and similar IVDs in Ghana, anticipated to be completed in month 9.

The goal of D7.1 was to generate a report and dataset on legal and regulatory landscape for PATHPOD and similar *in vitro* diagnostics (IVDs) in Ghana. It is partly completed as information from the Ghanaian patent office to complete the IP analysis is expected arrive in month 9.

The current analysis indicates that there should be freedom to operate in Ghana for the core technologies used in AfriDx and AfriMx. There are underlying international patents on enzymes that are planned for use in the test, but the underlying RT-LAMP technology is now in the public domain due to patent expiry. Data collected from the African Regional Intellectual Property Organization (ARIPO) suggests that the relevant patents which are still active in the US and other jurisdictions were not filed or are not in force in the African nations whose databases are accessible via ARIPO. As Ghana has not made their patent database searchable, we await final confirmation from the Registrar General's Department. On this basis, AfriDx is not considering major changes to the current design of the IVDs in order to ensure freedom to operate although the use of enzymes that are fully in the public domain would be preferable to facilitate adoption in more parts of the world.

The regulatory landscape in Ghana for IVDs is relatively simple as there is one National Regulatory Authority (NRA), the Medical Devices Department (MDD) of the Food and Drugs Authority. However, due to the lack of experience at the Ghana FDA with regulation of locally manufactured products as opposed to imported products, there is likely to be a longer turnaround time on approval. In addition, to address the regional market it would be advantageous for a legal manufacturer of the AfriDx or AfriMx test kits to undergo World Health Organisation Prequalfication (PQ) as this will aid in gaining regulatory approval elsewhere in the continent and also provide access to technical assistance from the WHO Local Production Assistance Unit and the PQ inspection and audit teams, who would conduct gap analyses and report on remedial actions needed to meet PQ standards.

Work has begun to map the process in more detail for a future deliverable, D7.3 (Regulatory information compiled for IVD use in Ghana and the wider opportunities in sub-Saharan Africa).

3.7.2. Description of WP7 deliverables completed or in progress

3.7.2.1. Searches of international patent database

Patent landscape for AfriDx technologies
The following information summarizes the patent landscape for core AfriDx technologies. We have excluded searching for patents specifically on SARS-CoV-2 diagnostics as patent

applications are typically published no earlier than 18 months after submission so very few will be publicly accessible.

Table 4: Core molecular diagnostic technologies used in the AfriDx project.

Technology	Key Patents	Comment on AfriDx plans
Recombinant Bst DNA Polymerase	Production of Bst-LF US 5830714 A (Priority Apr 17, 1996) Status: Expired Bst 2.0 from NEB US 9157073 B1 (Priority: Aug 31 2012) Status: Active in US and Europe, not found in Africa	The original DTU PATHPOD uses Bst 2.0 which is patented in some jurisdictions but no evidence could be found of a patent being in force in Africa. One goal of WP3 is to move towrds using Bst-LF which is in the public domain.
Recombinant Reverse Transcriptase	There are many public domain RTs with expired patents including the MMLV derivative sold as Superscript III US 7056716 B2 (Priority: Mar 15 2000) Patents are still in force for later MMLV derivatives and also RTs that were reverse engineered from DNA polymerases such as RTX.	AfriDx is currently using RTX which is patented but is exploring alternatives such as MMLV and Tth RT. No RTs were found with active patents in the ARIPO database.
Loop mediated isothermal amplification	LAMP foundational technique US 6410278 B1 (Priority Nov 9, 1998) Status: Expired Detection of loop-mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation was published by the same inventors in 2001.	AfriDx is using a turbidity-based method which is now in the public domain. If this was to change then there would be active patents covering fluroescent, colorimetric and probe-based readout technologies but there is no evidence that they are in force in Africa.

Diagnostic patent landscape in Africa

The full database of the African Regional Intellectual Property Organization (ARIPO) was searched for any patents related to molecular biology which were then further screened for relevance to AfriDx. 29 potential patents of interest were identified but all could be broken down into the following categories:

- Sample Prep 4 Patents
- Disease Specific 12 Patents

- Device 6 Patents
- Strain Engineering 1 Patent
- Unrelated 6 Patents

None were directly related to technologies being used by AfriDx or would have potential to block freedom to operate. This assessment was made based on the technical content but was not subjected to scrutiny by a patent attorney.

Searches of Ghanaian patent database

Patents in Ghana are registered via the Registrar General's Department within the Ministry of Justice and Attorney General. UCAM have requested records directly from the Registrar, to analyze those relevant to IVDs and therefore ascertain the patent landscape for IVDs in Ghana.

This will indicate likely freedom to operate for the commercialization of PATHPOD and related IVDs but will not constitute legal advice and subsequent commercialization partnerships would still need to undertake due diligence.

Non-patent intellectual property rights or existing contractual obligations

Non-patent intellectual property rights or existing contractual obligations impacting the use of the PATHPOD system and/or local productions of enzymes (UCAM) are largely Material Transfer Agreements (MTAs) that govern the use of materials such as cell lines and plasmids.

These MTAs are issued by DTU and UCAM and contain a non-commercial use clause. If a licensing arrangement were to go ahead then a commercial MTA would need to be negotiated although as most materials are DNA, there is nothing to stop a manufacturer synthesizing that DNA de novo whereby it would not carry restrictions. For this reason, without patent protection on the expression and use of the DNA sequences, MTAs increase transactional costs but are weak instruments for controlling use of materials in this context.

3.7.2.2 Regulatory landscape for PATHPOD and similar IVDs in Ghana

IVDs in Ghana are regulated by the Medical Devices Department (MDD) of the Food and Drugs Authority under the Food and Drugs Act, PNDCL 3058 (1992), the Food and Drugs (Amendment) Act, 1996, Act 523 and the Public Health Act, 2012.

Medical devices are classified in Ghana into Class I, II, III, and IV, following the European system. COVID diagnostics are classified as Class IV as per Appendix IV Part 2 of the Guideline of Medical Device Registration:

“An IVDD that is intended to be used to detect the presence of, or exposure to, a transmissible agent is classified as Class II, unless (a) it is intended to be used to detect the presence of, or exposure to, a transmissible agent that causes a life-threatening disease if there is a risk of propagation in the Ghanaian population, in which case it is classified as Class IV;”.

As SARS-CoV-2 is a transmissible agent that causes a life-threatening disease and there is a risk of propagation in the Ghanaian population, COVID-19 tests would be classified as Class IV medical devices if sold as a test kit for professional or self-administered use. However the PATHPOD can be broken down into different components that are being produced, potentially by different manufacturers.

3.7.2.3 Regulatory process for PATHPOD and similar IVDs in Ghana

In order to register, a dossier of information (Table 2) and a fee are submitted to the Ghana FDA along with a fee. Applications typically take a minimum of six months to process.

Table 4: Risk classification and information to be submitted to regulator for PATHPOD devices.

AfriDx Component	Risk Class	AfriDx Manufacturing Plan	Regulatory Documents Required	Comments
PATHPOD device	I	Outsourced		
Enzymes and/or antibodies	II	Local manufacturing	Control of starting materials Source of materials – proteins, etc. Method of manufacture and purification Characterisation Specification and COAs	
Buffers	II	Local manufacturing	Control of starting materials Source of materials Method of manufacture and purification Characterisation Specification and COAs	
Controls and calibrators	IV	Outsourced, in country formulation	Control of starting materials Source of materials Method of manufacture and purification Characterisation Specification and COAs	
Primers	II	Outsourced	Control of starting materials Source of materials Method of manufacture and purification Characterisation Specification and COAs	
Full SARS-CoV-2 RT-LAMP Kit	IV	Local finishing and packing	*In addition to component level info* *Manufacturing Process:*	Approval certificate needed from FDA

			Release specification Shelf-life Specification *Finished Product:* Specificity Sensitivity Accuracy Stability - Justification of Shelf-life	for any clinical trials.
SARS-CoV-2 AfriMx rapid test strip	IV	Local assembly and packing	*In addition to component level info* *Manufacturing Process:* Release specification Shelf-life Specification *Finished Product:* Specificity Sensitivity Accuracy Stability - Justification of Shelf-life	

The applicants must also adhere to the "Essential Principles applicable to IVD Medical Devices" in the Ghana FDA Guidelines.

20. Chemical, physical, and biological properties

20.1 The IVD medical devices should be so designed and manufactured to ensure the characteristics and performance referred to above. Particular attention should be paid to the possibility of impairment of analytical performance due to incompatibility between the materials used and the specimens and/or analyte (measurand) to be detected (such as biological tissues, cells, body fluids and microorganisms), taking account of its intended purpose.

20.2 The IVD medical devices should be so designed, manufactured and packaged to minimize the risk posed by contaminants and residues to the persons involved in the transport, storage and use of the devices and to patients, taking account of the intended purpose of the device.

20.3 The IVD medical devices should be so designed and manufactured to reduce, as far as reasonably practicable and appropriate, the risks posed by substances that may leach or leak from the IVD medical device. Special attention should be given to substances which are carcinogenic, mutagenic or toxic to reproduction.

20.4 IVD medical devices should be so designed and manufactured to reduce, as far as reasonably practicable and appropriate, risks posed by the unintentional ingress or egress of substances into or from the IVD medical device taking into account the device and the nature of the environment in which it is intended to be use

How to best deliver on these will be further developed in D7.3 (Regulatory information compiled for IVD use in Ghana).

4.8.1. WP 8: Training and Capacity building

The work package 8: **Training and Capacity building** is up to date; one deliverable anticipated by Month 8 has been completed:

- D8.1 AfriDx Manufacturing course handbook and SOPs

4.8.2. Description of Deliverable completed or in progress

D8.1 AfriDx Manufacturing course handbook and SOPs

The manufacturing handbook covers both the production and purification SOPs for the Engineered Boon2-BST (WP3). Both autoinduction and standard IPTG protocols have been provided and discussed. Protocol for silica-immobilisation based purification has also been provided.

Each protocol is comprised of the necessary materials, individual steps, and any calculations to assist in either upscaling or downscaling the process to meet the user's needs.

The manufacturing handbook has been published on the website:
https://afridx.ceb.cam.ac.uk/files/d8.1_engineered_boon2-bst_manufacturing_handbook.pdf

4.9.1. WP 9: Communication and Dissemination

The **Communication and Dissemination** work package is up to date; all deliverables anticipated by Month 8 have been completed. All deliverables anticipated from this work package are expected to be completed well in the timeline allocated by the project. Three deliverables have been completed under WP9, namely:

- D9.1 Visual identity and Website - completed Month 4
- D9.2 Plans for Exploitation and Dissemination of Results (PEDR) – completed Month 7
- D9.3 "AfriDx Facts of Myth", posts and explanatory short articles about the AfriDx testing strategy – completed Month 7.

4.9.2. Description of Deliverable completed or in progress

D9.1 Visual identity and Website

D9.1 was comprised of three main results

(i) The AfriDx identity Logo: which describes several aspect of the project graphically, such as the shape outline of the SARS-Cov-2, the map of Africa and rings representing the unity of the African Nations (Figure 1). This logo was commission from SpaceCafe in Ghana by Samuel Sakyi.

(ii) The AfriDx Website : which is the main instrument for disseminating all public deliverables from the AfriDx projects as well as news updates. This website is hosted under the University of Cambridge webservices and went live for the first time on February 18, 2021. Details about the objectives of the project, work packages as well as partners of the project are provided on the website. The full URL is https://afridx.ceb.cam.ac.uk/

(iii) The AfriDx Social Media channels: which are created to enable public outreach for this project, quick sharing of information and targeting specific sections of the public.
The AfriDx Twitter handle (@afri_dx) aims to connect with and disseminate information to research groups and institutions with influence relevant to the goals of the project.
The AfriDx Facebook page (@AfriDx) aims to connect with non-Twitter users, especially members of the African public, where Facebook is more popular.
The AfriDx LinkedIn company page (@AfriDx) aims at reaching a commercial audience.

Similar information is shared on all social media platforms but target different audiences

Figure 17: AfriDx logo

D9.2 Plans for Exploitation and Dissemination of Results (PEDR)

D9.2 is a public deliverable that documents the strategy for information dissemination from the AfriDx project and the exploitation of expected results.

AfriDx information dissemination
The plan for information generated by AfriDx comprises:

- A predefined target audience which cuts across all levels of society.
- Continuous dissemination of information throughout project, with contributions from all partners
- Information management to ensure AfriDx is portrayed in a good light
- Dissemination could be in form of press releases, news items, journal publications, conference presentation, workshops etc.
- Tool to be used include the AfriDx website, social media, outreach activities and more.
- Online presence activity analytics to be checked periodically.

AfriDx exploitation plans

The plan for exploitation of the results of the AfriDx project is as follows

- Exploitable results of AfriDx expected are direct application of the PATHPOD system to COVID testing in Ghana, technical "know-how" from Cambridge leading to local manufacture of reagents in Ghana, open access educational resources relevant to AfriDx, AfriMx.
- A fast-track business plan initially using some of AfriDx funding to supporting business, but with the expectation that business will become independent.
- Seek out a local manufacturer with suitable standards of production in levels
- Direct training by all AfriDx partners on technology and accessibility of knowledge resources
- Follow-on work to further develop the proof-of-concept immunoassay as well as investigations about IP and regulatory issues potentially influencing this.

This deliverable is accessible on the PEDR page of the AfriDx Website

D9.3 "AfriDx Facts of Myth", posts and explanatory short articles about the AfriDx testing strategy

D9.3 is a public deliverable which features blog-like short articles (approx. 500 words) that describes the methods used in the AfriDx project in simple and accessible language (excluding a much as possible, technical terms). Two articles have been published in the section of the website that will host these articles. The articles are:

- Antigen, PCR, Lamp by Dushanth Seevaratnam, UCAM
- 2019 Novel Coronavirus Disease (COVID-19): Possible role of point-of-care diagnostics in preventing the outbreak by Vinayaka Aaydha, DTU

4. Overall Status of Deliverables

The overall status of the AfriDx deliverables is as presented in Table 5. About 70% of deliverables expected at the time of the midterm report has been completed and those outstanding are advanced in work towards its completion. It is anticipated that project will be completed within the time frame budgeted.

Table 5: AfriDx Deliverable Status. Greens indicate deliverables completed, yellows, work on deliverables advanced, no colour indicates work not yet started.

No.	Deliverable Name	Lead	Type	Level	Month
2.0	Preparation for import of materials to Ghana. Submission to FDA (and export from EU)	KCCR	OTHER	CO	0
2.1	Define end-user needs including the number of NAT systems and chips require for clinical testing of	KCCR	R	CO	1
4.1	Description of model for pooled samples at low, medium and high prevalences of COVID-19	DTU	R	PU	1
1.1	Project handbook and communications/documentation tools for file-sharing and discussion.	UCAM	R	CO	2
6.1	Study protocol and supporting documents completed	KNUST	R	PU	2
6.2	Confirmation of study set up, recruitment and training of staff	KNUST	R	CO	2
6.3	Institutional and regulatory approvals obtained	KNUST	R	PU	3
2.2	PATHPOD systems and chips delivered for clinical testing in Ghana	DTU	DEM	CO	3
9.1	Visual identity pack and website	(Select)	DEC	PU	3
3.1	Engineered Boon2-BST and accompanying expression and purification SOP.	UCAM	DEM	PU	4
9.2	Publication of Plan for Exploitation and Dissemination of Results (PEDR).	UCAM	R	PU	4
9.3	AfriDx Fact or Myth? posts and explanatory short articles	DTU	DEC	PU	4
2.3	Remote training course for use of PATHPOD system and chips	DTU	R	CO	5
2.4	Workflow for PATHPOD use in Ghana to detect COVID19	DTU	DEM	CO	5
5.1	Modified and characterised high-affinity scFv	UCAM	DEM	PU	5
5.2	Purified covid coat protein fragments	UCAM	DEM	PU	6
8.1	AfriDx Manufacturing course handbook and SOPs	UCAM	R	PU	6
1.2	List of initial Advisory Board members on website	UCAM	R	PU	7
1.3	Midterm Report	UCAM	R	PU	8
6.4	Provide data for mid term report	KNUST	R	CO	8

3.2	Engineered Boon2-RT and accompanying expression and purification SOP.	UCAM	DEM	PU	8
4.2	Dataset for limit of detection and report on recommendations for clinical study design	KNUST	R	CO	8
7.1	Report and dataset on legal and regulatory landscape for PATHPOD and similar IVDs in Ghana	UCAM/ AVOMA	R	PU	10
3.3	Application of Boon-enzymes in RT-LAMP protocol to detect SARS-Cov-2	KNUST	DEM	PU	9
6.5	Comparative sample testing completed	KNUST	R	CO	10
9.4	Video interviews with partners and stakeholders	UCAM	DEC	PU	10
1.4	Inaurgral Advisory Board meeting	UCAM	Other	PU	11
3.4	Demonstrate the BOON-RT and BST Production at 1-2 L	UCAM	DEM	CO	11
6.6	Record of data collection and analysis	KNUST	R	CO	12
7.2	Commercialization plan for the sub-Saharan Africa	AVOMA	R	CO	12
8.2	AfriDx and AfriMx user course handbook and SOPs	NMIMR	R	PU	12
3.5	Engineered Boon2-scFV and accompanying expression and purification SOP.	UCAM	DEM	PU	14
1.5	EDPTC progress reports and financial reports	UCAM	R	CI	15
5.3	AfriMx protocol and SOP	UCAM	R	PU	15
5.4	Testing pre-screened clinical serum samples with AfriMx	KNUST	DEM	PU	15
6.7	Publication of study report	KNUST	R	PU	15
7.3	Regulatory information compiled for IVD use in Ghana, in anticipation of future filing.	TATAA	R	CO	15
8.3	AfriDx-KIT DNA Collection, user course handbook and SOPs	UCAM	R	PU	15
9.5	AfriDx policy briefs in electronic and printable format	UCAM	DEC	PU	15

5. Challenges encountered, and strategies used to overcome them

Two major challenges the project has faced so far are

- Delays in the arrival of PATHPOD system and cartridges at partner institutions in Ghana
- Withdrawal of original commercialization partner, TATAA from the project

The full PATHPOD system was expected to arrive in Ghana by Month 3. They however arrived in two installments in Month 6 (PATHPOD) and Month 8 (Chips) respectively. This was due to some challenges with the transportation and compliance with the Ghanaian customs service and the courier company. However, by cooperative communications between DTU, Ghana and the external parties involved, these challenges were addressed. The team has also worked very hard to complete outstanding deliverables caused by the delay in the arrival of the PATHPOD system, so this challenge is not expected to impede the progress of this project or affect the timeline.

In view of the uncertainty about cold storage during transportation, and the untested use of PATHPOD in the Ghanaian environment, the clinical trials were divided into two phases, with the first shipment of chips completed. The second shipment has been rescheduled to allow time to assess the data from the first results in the trial and make any necessary modifications. This extends the time period and delays the deliverable for the trial but does not affect the overall project.

The withdrawal of TATAA from the project has also been managed effectively by the recruitment of AVOMA as a replacement. The process of registering AVOMA with EDCTP is currently in progress. AVOMA joining the project also has a secondary advantage which is their experience conducting business in Sub-Saharan Africa. This advantage will be exploited in moving the AfriDx results into the African market. No expected negative consequence is anticipated by the replacement of TATAA with AVOMA. However, the activities that were involving TATAA have been adjusted to accommodate the entry of AVOMA. Although this delays one deliverable, it will not impact the activity of planning of a route to market.