PROTOCOL

LoCKamp

A study to evaluate the performance of a Lab-on-Chip LAMP device for rapid SARS-CoV-2 diagnosis

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Protocol authorised by: Professor Jerry Nolan, Principal Investigator and Dr Despina Moschou Laboratory Chief Investigator

Date: 14th October 2021

Signature:  **Dr Despina Moschou**

1. CI and Research Team Contact Details

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Details of Sponsor:

The University of Bath is the research sponsor for this study.

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This protocol describes **LoCKamp-Pilot** and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the trial. Problems relating to this trial should be referred, in the first instance, to the Principal Investigator.

This trial will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd Edition). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

Abbreviations

RUH	The Royal United Hospital
UoB	University of Bath
PPI	Patient and public involvement
LoC	Lab-on-Chip
LAMP	Loop-mediated Isothermal Amplification
AE	Adverse Event
AR	Adverse Reaction
SAE	Serious Adverse Event
SOP	Standard Operating Procedure

Keywords: Lab-on-Chip, SARS-CoV-2, infection, diagnosis, point of care device, LAMP

Table of Contents

1. Introduction

Ramping up reliable testing and diagnosing COVID-19 potential spreaders as soon as possible is the only viable solution for managing this pandemic in the mid- and long-term, without having to paralyze our society and economy. Nonetheless, there is a limit to testing laboratories and reagents. But what if we could multiply the testing sites, minimizing the time-to-result to few minutes and reducing the reagent consumption? Miniaturized microchips like the PI's published work on microPCR devices have proven this capability [1-2], implementing on few cm^2 microchips DNA amplification protocols (PCR and LAMP) at few µL-scale samples within 5-10 minutes.

Several approaches for point-of-care testing are being pursued. They are broadly grouped in two categories: a) lateral-flow diagnostics able to detect SARS-CoV-2 antigens in patients and b) molecular tests, able to confirm a current infection by identifying the virus genetic sequence. The lateral-flow tests currently used can't inform us reliably on whether the tested person is carrying the virus and thus may spread it further. Molecular tests are required to this end. Nonetheless, DNA amplification is a much more complex laboratory process, which can't be technically implemented without any electronic instrumentation. The main Point-of-Care technology proven to perform molecular diagnostics is the cepheid GeneXpert, used for several years for infectious disease identification. The instrument costs around £2,000 and the COVID-19 reagent cassette around £5; the main issue with deploying this system or any of the similar ones (e.g. Diagnostics for the Real World), is the overreliance on company output in providing their preloaded test cassettes and a time-to-

result in the order of 1.5 hours. In the proposed approach, reagents from any provider can be used following the two different explored protocols (RT-PCR and LAMP). Moreover, owing to the miniaturization of the assays; much lower reagent consumption is anticipated (at least 10-fold compared to the GeneXpert), along with sample throughput comparable to the central laboratory tests, amplifying sequentially on the same chip multiple patient samples within a few minutes.
In order to address this technology gap, we have been developing a LAMP-based LoC rapid diagnostic system, whose clinical validation is the main objective of this pilot study [3-4].

Work to date

For the last two years, Sotirios Papamatthaiou (MEng and Post-Doctoral Research Assistant) and Despina Moschou at the University of Bath have been working on technological solutions for improving the accurate rapid diagnosis of SARS-CoV-2 outside a laboratory setting. This includes the design and testing of an isothermal amplification (LAMP) Lab-on-Chip device and the accompanying portable instrumentation.

More recently, the Bath team proven the functionality and accuracy of their system. The system is low-cost to produce and gives diagnosis of SARS-CoV-2 with PCR-standard accuracy within 10 minutes. See figure 1 below:

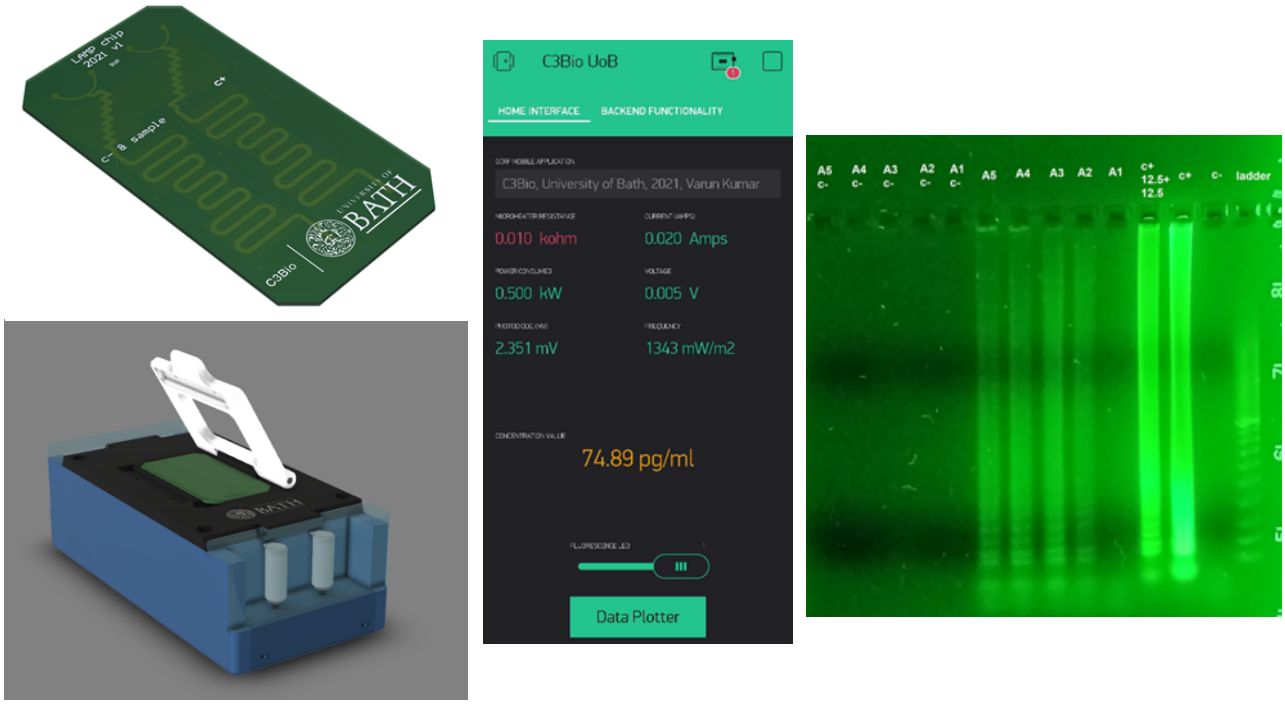

Figure 1: LoCKAmp diagnostic system, LoC device, mobile phone app and verification of on-chip protocol.

Our publications on the subject of rapid molecular diagnostic LoCs include:

1. Papamatthaiou, S, McConnell, W, Sampath Kumar, VK, Zupancic, U, Lee-Emery, E, Di Lorenzo, M, Reboud, J, Cooper, JM, Estrela, P & Moschou, D 2021, 'LAB-ON-PCB TECHNOLOGY FOR HANDHELD, SAMPLE-IN-ANSWER-OUT SARS-CoV-2 DIAGNOSTIC', Paper presented at Miniaturized Systems for Chemistry and Life Sciences(µTAS 2021), 10/10/21 - 14/10/21.
2. Despina Moschou, Nikolaos Vourdas, George Kokkoris, George Papadakis, John Parthenios, Stavros Chatzandroulis, Angeliki Tserepi, All-plastic, low-power, disposable, continuous-flow PCR chip with integrated microheaters for rapid DNA amplification, Sensors and Actuators B: Chemical, Volume 199, 2014, Pages 470-478

3. IEEE Healthcare Summit, 6 Oct 2021, Keynote presentation: Lab-on-PCB technology and the COVID-19 outbreak case study.
4. https://www.theengineer.co.uk/lockamp-covid-lab-on-chip-bath-university-covid/

2. Study aim and objectives

2.1 Study Aim

To test the correlation of LoCKamp diagnostis results from hospitalized patients with SARS-CoV-2 infection verified by the clinical standard PCR testing and commercial lateral flow tests. Explore any potential limitation/advantage of LoCKamp in identifying asymptomatic patients, patients on ventilation and explore any correlation with other clinical COVID-19 symptoms.

2.2 Study Objectives:

Feasibility of study design with a view to a larger trial:

- Practicalities of sample collection and transfer to LoCKamp system
- Sample preparation protocol for virus inactivation and RNA release.
- A PCR and lateral flow test for the presence of SARS-CoV-2 in nasopharyngeal swabs.
- Correlation of technology result against standard tests

3. Study design

3.1 Recruitment

Consenting patients with SARS-CoV-2 positive PCR result, at time of admission to the hospital.

3.2 Study design

A pilot study to test the practicalities of undertaking a larger diagnostic accuracy study of the LoCKamp system. Feasibility issues will include: ability to recruit and patient acceptability; ability to warn of possible false positive/negative testing results; correlation of LoCKamp malfunction with usability and system acceptability; ability to take samples and clinician acceptance.
When patients arrive in RUH and test positive for SARS-CoV-2 with the standard PCR test, they will be screened for inclusion/exclusion in the study based on the criteria following and they will be given the option to participate in the study. Should the patients agree to participate, they will be given the attached consent form and accompanying information and have the protocol explained to them. Consent to this study is voluntary; participants will be offered written information and the opportunity to ask any study-related questions before signing written consent.
For the consenting patients a nasopharyngeal swab will be collected by the research nurse and placed in 15mL plastic tubes containing 3mL NaCl 0.9% serum, labelled with patient ID and reference number and date, which will blind the academic team from name / address / identification

information about the patient The patients will then proceed to have their standard care, in the clinic. All collected samples will be stored in fridge at 5C, to be collected by a designated study team member on Mondays between 15.00-17.00 on a weekly basis for the duration of the study. On Monday mornings, right before being collected by the UoB team on Monday afternoon, half of the collected samples will be inactivated on site using commercialy available Inactivating Viral Transport Medium and half by thermal treatment in a water bath at 95oC for 1 minute.
The inactivated samples will then be transported to the University of Bath laboratory for analysis and data collection. Laboratory staff at UoB will be blinded to all clinical data. The study team at UoB will run the inactivated samples in LoCKamp, lateral flow tests and standard RT-PCR in parallel. There will be no blinding at hospital sites, with treatment decisions made independently, and will be irrespective of any UoB test results. Patients will be followed-up, at 6 weeks, with a structured telephone interview.
Retrospective analysis of which patients have been ventilated and admitted in ICU, in the six-week period following sample donation, will be undertaken by the clinical team on an individual patient basis. The date on which sampling occurred will be recorded.

3.3 Sample processing

All samples will be taken using a standard method:

- *Week 1* will be a pilot, where details of the laboratory SOP will be finalised and then used in all subsequent weeks.
- *Weeks 2-12*, (subject to changes in SOP following the pilot) the sample will be analyzed within 2 hours of arrival at the UoB lab (bags not immediately analysed will be stored at -20^{o}C), and three sample aliquots of 500 µl will be created in 1 ml sterile Eppendorf tubes. One will be used for RT-PCR analysis, one for lateral flow testing and one for LoCKamp. Results will be recorded for all three techniques.

- The response of LoCKamp to the sample will be electronically recorded and logged via the system app, by a member of the research team.

    - Once the response has been recorded, the sample and bag will be safely disposed of. The inactivated virus sample will be disposed of via the sewerage system; the collection bag and LoC via the autoclavable waste stream.

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3.4 PPI invitation

All patients recruited to the study will be asked if they would like to be involved in helping with their views on a future, follow-up research study, evaluating the acceptability of diagnostic technology at home or community, the technology to be tested in the study and study concept. In an additional section of the consent form, participants will be asked if they would be happy to be contacted for further PPI at a later stage. This will involve being asked about their involvement in the study and future collaboration in designing the next stage of the study.

3.5 Data security

Screening logs will be kept at the RUH. Paper data collection forms (CRFs) will be stored in a locked cabinet at the research office. Source data will be stored in accordance with the NHS code of confidentiality.

Research staff will ensure that the participants' anonymity is maintained through protective and secure handling and storage of patient information at the trial centre. The participants will be identified only by a patient ID number on the CRF and database. All documents will be stored securely and only accessible by trial staff and authorised personnel. Data will be collected and retained in accordance with the Data Protection Act 2018.

Data identified by the participant's unique study number will be entered directly into the database by the research staff.

Study documents (paper and electronic) will be retained in a secure location during, and after, the trial has finished. All essential documents, including patient records and other source documents will be retained for a period of 5 years following the end of the study. Where study-related information is documented in the hard copy medical records – those records will be identified by a 'Do not destroy before dd/mm/yyyy' label where the date is 25 years after the last patient's last visit. Where electronic records are in use, trust policy will be followed.

4. Participant entry

4.1 Inclusion Criteria

- Patients with SARS-CoV-2 positive PCR test
- Adult >18yrs
- Attendance at hospital
- Consent gained for study

4.2 Exclusion Criteria

- Consent not gained for study
- Child < 18 yrs
- Adult without mental capacity to consent

5. Data collection and analysis, sample size, statistical analysis and results

5.1 Data collection and analysis

All data and samples will be anonymised and labelled with the allocated study number, along with storage details. Study data will be stored on paper CRF forms kept in a secure location in the RUH Research Office, and investigators will only have access to their own site's data. No identifiable personal data will be sent to the laboratory site.

Correlation will be sought between LoCKamp results with standard PCR and lateral flow tests.

Demographics, clinical details and outcomes in addition to screening criteria will be collected. Data from the clinical record including temperature and any other known COVID-19 symptoms will be analysed using descriptive statistics; relationships between variables will be investigated using correlation matrices.

5.2 Sample size

The study will aim to recruit 100 patients

5.3 Results

Results will be publicly disseminated by publication in the medical-scientific literature and presentation at COVID-19 and diagnostics specific conferences.

6. Safety Reporting

6.1 Adverse events or reactions (AE/AR)

As the primary outcome is an *ex vivo* outcome – the patient is otherwise having standard care and the study has no effect on patient care.

An ***adverse event*** is any untoward medical occurrence in a subject to whom a medicinal product/medical device/intervention has been administered, including occurrences which are not necessarily caused by, or related to, that product.

6.2 Serious adverse events or reactions (SAE/SAR/SSAR/SUSAR)

An ***adverse event, adverse reaction*** or ***unexpected adverse reaction*** is defined as serious if it:

(a) results in death,
(b) is life-threatening,
(c) requires hospitalisation or prolongation of existing hospitalisation,
(d) results in persistent or significant disability or incapacity, or
(e) consists of a congenital anomaly or birth defect.

This is a non-interventional study and thus considered low-risk. We do not anticipate any SUSAR in this observational study. All collected data will be stored in University of Bath secured server systems, following all approved protocols for researcher access and processing.

6.3 Reporting Procedures

Adverse events will be recorded and reported in accordance with Royal United Hospital's (RUH) Research Safety Reporting SOP.

6.4 Non serious AEs

All such events, whether expected or not, should be recorded.

6.5 Serious AEs

An RUH Research Related SAE/SUSAR Initial Report form should be completed by the local investigators and submitted to the sponsor (UoB) within 24 hours of an SAE occurrence. However, relapse and death, and hospitalisations for elective treatment of a pre-existing condition, do not need reporting as SAEs.

All SAEs should be reported to the Research Ethics Committee where in the opinion of the Chief Investigator, the event was:
A non-IMP SUSAR- an SAE that occurs in a non-IMP trial and is:

- "Related" – that is, possibly, probably or definitely resulted from administration of any of the research procedures, and
- "Unexpected" – that is, the type of event is not listed in the protocol as an expected occurrence.

Within 24 hours of a member of the research team becoming aware of a *serious adverse event* the sponsor must be notified.

Reports of related and unexpected SAEs should be submitted as soon as possible, ideally within 24hours of the Chief Investigator becoming aware of the event, using the template provided in the RUH Research Safety Reporting SOP.

6.6 (Serious) Breaches

The Investigator and the research team have a responsibility to ensure that the research is conducted in accordance with the Protocol and Good Clinical Practice. Where there is a breach, this must be assessed by the Investigator and reported to the Sponsor within 24 hours of becoming aware of the event (unless it is the Sponsor that has identified the breach). Any non–serious breaches will be filed in the Investigator Site File and Trial Master File.

For serious breaches, the Ethics committee **must be notified within 7 days of the breach being identified**. Where UoB are the Sponsor, the R&I department will liaise with the research team in order to make the required notification.

7. Regulatory issues

7.1 Authorisations and Research Governance Statement

The study will be performed subject to favourable opinion/ authorisation/permission from all necessary regulatory and other bodies. This includes, but is not limited to, REC, HRA, NHS trusts.

OTHER CENTRES/LABS will ensure all necessary approvals are in place for their site. The trial must be submitted for capacity and capability review at the RUH. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the trial. The trial will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions. This study will be conducted in accordance with: Good Clinical Practice and the Research Governance Framework for Health and Social Care.

7.2 Consent

Consent to this study is voluntary; participants will be offered appropriate written information and the opportunity to ask any study related questions before signing written consent.

7.3 Confidentiality

All data and samples will be anonymised and labeled with the allocated study number along with storage details. Once testing and photography is completed, the sample will be destroyed.

7.4 Indemnity

This is a University of Bath-sponsored research study. The University of Bath has arranged Public Liability insurance to cover the legal liability of the University as Research Sponsor in the eventuality of harm to a research participant arising from management of the research by the University. This does not in any way affect an NHS Trust's responsibility for any clinical negligence on the part of its staff (including the Trust's responsibility for University of BATH employees acting in connection with their NHS honorary appointments).

The University of Bath holds Professional Indemnity insurance to cover the legal liability of the University as Research Sponsor and/or as the employer of staff engaged in the research, for harm to participants arising from the design of the research, where the research protocol was designed by the University.

The University of Bath's Public Liability and Professional Indemnity insurance policies provide an indemnity to our employees for their potential liability for harm to participants during the conduct of the research.

7.5 Sponsor

The University of Bath will sponsor the study

7.6 Funding

The EPSRC Impact Acceleration Account is providing funding for this study, from 1 March 2021. The trial is expected to last for 3 months.

7.7 Monitoring and Audit

The study will be monitored in accordance with the RUH's Monitoring SOP. All trial-related documents will be made available, on request, for monitoring and audit by the RUH, the relevant Research Ethics Committee and for inspection by the Medicines and Healthcare products Regulatory Authority or other licensing bodies. The monitoring plan will be developed and agreed by the sponsor.

7.8 Trial Management

The day-to-day management of the trial will be coordinated by Ms Carrie Demetriou.

8. Publication policy

Peer-reviewed scientific journals, internal report and BAUS conference presentations.

9. References

1. Papamatthaiou, S, McConnell, W, Sampath Kumar, VK, Zupancic, U, Lee-Emery, E, Di Lorenzo, M, Reboud, J, Cooper, JM, Estrela, P & Moschou, D 2021, 'LAB-ON-PCB TECHNOLOGY FOR HANDHELD, SAMPLE-IN-ANSWER-OUT SARS-CoV-2 DIAGNOSTIC', Paper presented at Miniaturized Systems for Chemistry and Life Sciences(μTAS 2021), 10/10/21 - 14/10/21.

2. Despina Moschou, Nikolaos Vourdas, George Kokkoris, George Papadakis, John Parthenios, Stavros Chatzandroulis, Angeliki Tserepi, All-plastic, low-power, disposable, continuous-flow PCR chip with integrated microheaters for rapid DNA amplification, Sensors and Actuators B: Chemical, Volume 199, 2014, Pages 470-478

3. IEEE Healthcare Summit 6 Oct 2021, Keynote presentation: Lab-on-PCB technology and the COVID-19 outbreak case study.

4. https://www.theengineer.co.uk/lockamp-covid-lab-on-chip-bath-university-covid/

5. Appendices

Appendix 1: LoCKamp Study Pathway

Screening and consent

Patient found SARS-CoV-2 PCR positive -> identified to research team -> Screened for inclusion/exclusion criteria -> If eligible patient consent to study and given study ID

Taking the samples

Nasopharyngeal swab taken by research nurse.

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Swab placed in 15mL plastic tubes containing 3mL NaCl 0.9% serum, labelled with patient ID and reference number and date.

Sample storage and transportation

All samples to be stored at 5 C when not being transported or analysed in laboratory at UoB

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Half of weekly samples inactivated by Inactivating Viral Transport Medium and half inactivated by water bath at 95oC for 1min; every Monday 09.00-14.00

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Inactivated samples to be collected on Mondays between 15.00-17.00 and transported directly to the University of Bath

Processing

Data collection and follow up