Scalable and robust SARS-CoV-2 testing in an academic centre

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To the editor:

The emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1–3] has led to a pandemic infecting more than four million people worldwide in less than five months, posing a major threat to healthcare systems. This is compounded by the shortage of available tests causing numerous healthcare workers to unnecessarily self-isolate. Here, we provide a roadmap to show how a European research institute can be repurposed in the midst of this crisis, in collaboration with partner hospitals and an established diagnostic laboratory, harnessing existing expertise in virus handling, robotics, molecular testing and informatics to derive a rapid, high-throughput diagnostic testing pipeline for detecting SARS-CoV-2 in individuals with suspected COVID-19.

Comprehensive and reliable testing is essential to identify the virus in individuals presenting with COVID-19 symptoms in hospital, to guide community interventions that contain the spread, and to perform enhanced surveillance programs of healthcare workers to maintain a workforce to safely deliver care. These requirements have placed an unprecedented demand on the testing capability of all countries. This demand on diagnostic laboratories, coupled with a global shortage of commercial kits and reagents, reduced commercial flights and cargo capacity and international competition for testing resource, has rendered the testing capacity of many countries inadequate to deal with the outbreak effectively.

The pipeline we created is used to detect SARS-CoV-2 from combined nose-throat swabs and endotracheal secretions/bronchoalveolar lavage fluid. Notably, it relies on a series of in-house buffers for virus inactivation and the extraction of viral RNA, thereby reducing the dependency on commercial suppliers at times of global shortage. We use a commercial RT-PCR assay, from Shenzen-headquartered BGI, and report the results using a bespoke online web application that integrates with the national healthcare digital system. This strategy allows the remote reporting of thousands of samples a day in around 24 hours, universally applicable to laboratories worldwide.

The Francis Crick Institute (the Crick) is a biomedical research institute dedicated to the discovery of biology underlying human health. Situated in central London, an epicenter of the UK pandemic, the Crick elected to repurpose its scientific and technical resource to support the immediate healthcare needs of its partner hospital, University College London Hospital, during the outbreak. Providing an end-to-end pipeline for clinical diagnostic testing of COVID-19, would result in increased testing capacity that could meet local demand, and allow new surveillance programs of healthcare workers, to be implemented.

Key to finding a solution was the partnership created between the Crick, a major London healthcare provider together with its clinical virology expertise (University College London Hospitals (UCLH) National Health Services Trust) and a UK Accreditation Service (UKAS)-recognized clinical diagnostic laboratory (Health Services Laboratories; HSL), forming the CRICK COVID-19 Consortium (CCC). This partnership effectively removed the barriers of clinical translation and facilitated rapid implementation of robust end-to-end testing within 10 days under the oversight of an accredited laboratory. Importantly, it also allowed resources and expertise to be mobilised to meet local healthcare needs.

A notable strength of the CCC pipeline is that it allows the testing of a wide variety of swabs that can be either dry or in any proprietary virus transport media (VTM). These are taken at hospital sites or local drive-through stations and submitted to HSL before being transferred to the Crick. Upon arrival, specimens are barcode tracked, then proceed immediately to viral inactivation, automated extraction of viral RNA and RT-PCR to quantify SARS-CoV-2 RNA. Results are accessed through a custom-made online web portal facilitating data to be analysed remotely by a panel of trained reporters, and are returned to the reference laboratory. The speed and precision of the pipeline permits the reporting of thousands of samples/day, adopts processes that are widely used by many research laboratories worldwide, and is free from dependence on supply-chain constraints.

Given the urgent, two-week timeframe set to implement SARS-CoV-2 testing, it was not possible to secure clinical laboratory accreditation for the Crick to an appropriate standard (The International Standards Organisation (ISO) 15189:2012; and the equivalent to College of American Pathologist (CAP) /Clinical Laboratory Improvement Amendments (CLIA) accreditation). As an alternative, the Crick took steps to ensure that the CCC test was evaluated, verified and performed for diagnostic use in an environment that adhered to equivalent international standards.

The Crick is partnering with HSL (Analytics) LLC to provide diagnostic PCR testing to UCLH and other NHS Trust customers of HSL. All HSL services are compliant with HTA and MHRA regulatory requirements, where appropriate. The Crick worked with HSL—which already had a clinically validated COVID-19 RT-PCR test against the SARS-CoV-2 nucleocapsid (N) gene —to ensure that the research institute's RT-PCR test against the SARS-CoV-2 ORF1a gene was properly audited and validated. Samples have been regularly exchanged with HSL and the Crick laboratory is also expecting quality control samples from the QMCD EQA (reg. GB396) with whom the CCC is registered.

Advice and oversight was also sought from registered professionals from existing nearby UKAS-accredited medical laboratories; HSL (UKAS 10204); Royal Marsden Hospital and North Thames Genomic Laboratory Hub (UKAS 9839); Great Ormond Street Hospital, North East Thames Regional Genetics Lab and North Thames Genomic Lab Hub (UKAS 7883); Institute of Neurology (UKAS 8045) and an approved UKAS inspector. CCC protocols were either written on demand, or based on existing institutional protocols, to ensure clinical grade testing at the Crick. Guidance from these professionals assisted the compiling of clinical diagnostic Standard Operating Procedures (SOPs) for every stage of the pipeline, including implementing checklists and risk mitigation steps alongside the methods. Additional SOPs were followed for sample storage, disposal of materials, batch certification of reagents and incident reporting. Appropriate risk assessments, training and competency assessment procedures were established and documented. Record sheets were created to document the receipt, batch acceptance testing, and start/end of use dates for key reagents and consumables. An inventory of all key equipment was compiled which, where appropriate, included details of service and calibration records. Systems were established for the control of all key documents (version implementation, distribution and acknowledgement), audit trail (what samples were tested when, by whom, with what equipment and using which consumable/reagent batches), and a record of all incidents/issues (to facilitate appropriate investigation, rectification and recurrence prevention).

Assurance of the pipeline was performed in collaboration with quality assessment provider Genomic Quality Assessment (GenQA; https://www.genqa.org/), following their checklist for non-accredited laboratories, the lab and CCC workflow was inspected by a qualified UKAS assessor against the GenQA guidelines to verify compliance to IS015189 equivalent standard.

To ensure full traceability, samples were barcoded and all processes were recorded using Clarity LIMS software (Illumina). UCLH provided access to barcoded swabs pre-booked onto the Crick's laboratory information management system (LIMS) to enable tracking from sample receipt through to result reporting. In urgent response to the clinical need, formation of these partnerships was vital to drive the speed of pipeline setup in the Crick's central London research laboratory (all key documents are available at https://www.crick.ac.uk/research/covid-19/covid19-consortium.

The CCC pipeline is illustrated in Figure 1. The specific reagents and requirements for each step of the entire pipeline—sample receipt, virus inactivation, RNA extraction, RT-PCR assay for the ORF1a gene, data quality assessment, online web reporting, barcode sample tracking—are available at protocols.io[4].

In response to potential shortages of supplies, we forecasted demand, ordered reagents in large batches, used in-house buffers wherever possible, and an in-house N gene assay was established as a contingency plan.

Several amendments to procedures were implemented to ensure the CCC test performed robustly at the Crick. Although the Crick performed viral inactivation in a containment level 3 suite with trained staff, other research laboratories with only containment level 2 (CL2) facilities may be able to adapt the CCC test model, provided that appropriate risk assessments are carried out or swabs are inactivated before transportation (a containment level 2 procedure is also provided with our protocols). Moreover, other protocols also exist for alternative viral-inactivation methods using heat, further demonstrating the potential for CCC pipeline applicability where availability of guanidinium may be limited.

Another adaptation made in the CCC test is to use a series of home-made buffers for automated RNA extraction which circumvents dependence on reagents that may be in short supply during a pandemic. At the time of writing, the most important bottleneck in performing PCR tests for COVID-19 detection is the shortage of kits for RNA extraction. We developed an in-house RNA extraction protocol using magnetic silica beads from G-Biosciences, and we have also validated our assay with SeraSil Mag 400 beads (GE Healthcare/Cytiva), which can serve as a reliable substitute. RNA extraction using silica is based on the protocol developed by Boom *et al.*[5] over 30 years ago. In the Boom method, concentrated guanidinium thiocyanate serves as virus and RNase inactivation agent and promotes binding of nucleic acids to silica. We have tested RNAclean XP SPRI magnetic beads (Beckman) and found them compatible with our virus inactivation solution; viral RNA could be purified following manufacturer's recommendations. Moreover, protocols exist for the production of either type of magnetic beads from inexpensive and accessible starting materials[6]. Therefore, we designed a pipeline that uses common reagents and is automatable on widely available liquid handling platforms allowing its implementation in a large number of biomedical laboratories with suitable robotic platforms that can be re-progammed for this use. The reagents can also be utilised for manual RNA extraction where liquid handling platforms are unavailable. The universal applicability of this approach could allow a resilient response to future critical events even in countries where particular resources may be limited.

Selection of an appropriate PCR assay for detection of SARS-CoV-2, the BGI kit, was based on (i) our accredited laboratory having a ready set of validation data and experience with the US Food and Drug Administration (FDA) emergency use authorised assay and (ii) a guaranteed supply chain for the assay kit in the face of falling demand in China, and growing demand in the United States (for US suppliers). The primers used in the CCC test target SARS-CoV-2 ORF1a, enabling detection of full-length genomic and antigenomic RNA, whereas the N gene assay also targets the abundant subgenomic RNAs. With many mutations having been reported in the ORF1 region of SARS-CoV-2, it was paramount to adequately assess false positive and false negative rates. The verification steps of the CCC pipeline allowed us to compare the BGI kit with the in-house developed N gene assay. Overall, the diagnostic sensitivity of the CCC test is 92.86% with a specificity of 100% and a high degree of accuracy in the detection of SARS-CoV-2. The N gene assay is slightly more sensitive than the CCC assay at the limits of detection. When performed in duplicate, we observed a discordant rate of 1.1% (95 samples of 8433 samples). To improve the accuracy of true positive reporting (and reduce the chance of reporting false positive tests), the assay is performed in duplicate and discordant samples are recommended for re-testing at source. The full verification is documented on Figshare[7].

As sample timing and adequacy are likely to be more important determinants of false negatives than qPCR sensitivity[8], we have chosen a test that also includes a control for cellular RNA (beta-actin), which serves as a partial proxy for sample adequacy. Although high sensitivity at the assays limits of detection could impact identification of low levels of viral shedding beyond the assay's limits of detection, these samples are unlikely to be producing infectious virus[9]. BGI PCR therefore exhibits adequate sensitivity for current clinical algorithms, in which testing for symptomatic healthcare workers is performed within a specific timeframe.

The high-throughput RT-PCR assay carried out at the Crick in 96-well plate format has the potential to screen thousands of samples per day and can be scaled up to 384-well format with further optimisation. Since conception of the CCC, we have performed over 14,000 tests; starting with one batch of 39 samples on the first day of live testing and scaling up to around 500-1000 samples per day. 1000 samples/day is delivered on a pipeline operated by 44 members of Crick scientific staff working a 10 hour (staggered) shift. Competency training was conducted for staff to work on virus inactivation, RNA extraction, RT-PCR and result reporting. This is far less than our maximum capacity for CCC testing, and throughput is limited by logistic and operational limitations within the community and partner hospitals e.g. local swabbing capacity. Our partner laboratory HSL has capacity for 1000-1250 samples per day, but due to limitations on supply chain, their testing is reserved for hospital inpatients. Conversely, the CCC pipeline created capacity to test a new population that had hitherto been unable to access testing, namely asymptomatic and symptomatic healthcare workers, and self-isolating keyworkers. Indeed, we now provide the healthcare worker testing for UCLH NHS trust and North Central London Hospitals. We believe that this approach fulfilled a critical gap in the existing testing infrastructure, and one that has major impact on the safe delivery of healthcare during the COVID-19 pandemic.

To establish the CCC pipeline at the Crick, we made use of category 3 equipment, liquid handling platforms and RT-PCR instruments that were available within our institute and simply repurposed them for COVID-19 testing. The barcoding equipment and tool tracker were already used with our LIMS system. Only a limited amount of additional protective equipment was procured for buffer preparation and the pipeline can be potentially scaled up further with minimal additional equipment. A rate limiting step preventing the CCC pipeline to proceed at full capacity is the global availability of swabs. Additional testing regimens are being considered which would circumvent the dependency on viral swabs.

Medical laboratory accreditation is held to the standard of ISO 15189:2012 across the world, with the exception of the USA, which operates to CLIA certification/CAP accreditation. Laboratories are assessed for compliance to ISO or CLIA/CAP standard by a national awarding body; in the case of the United Kingdom this body is UKAS. While the process of acquiring accreditation, and the typical assessment time span and rules for extending existing ISO or CLIA/CAP scope to partnering institutions will vary between countries, any research institution seeking to establish clinical testing should seek clinical accreditation wherever possible. While pursuing this process, our approach has been to implement processes in line with international accreditation standards, and those processes remain under the supervision of our partner accredited laboratory (HSL). We have also regularly sought advice from GenQA and are in the process of implementing their recommendations in an agreed timeframe to comply with the standards required to meet ISO151890.

Health information systems, such as the EPIC electronic medical record used at UCLH, interface with LIMS, such as WinPath, to enable sample barcodes to be associated with patient hospital numbers. The pipeline set up at the Crick uses a custom-made reporting web application compatible with remote reporting. This allows multiple trained reporter personnel to access anonymised data through a portal from home, which is particularly advantageous in a pandemic. As a result, the CCC pipeline is capable of an accelerated turn-around for results of 2500-3000 samples in approximately 24 hours.

The potential advantages of implementing a clinical diagnostic pipeline in research laboratories are clear: a substantial increase in capacity for testing, and the ability to adopt flexible and agile approaches to testing in the face of global constraints. Our experience at the Crick in implementing mass-scale testing within the CCC has taught us invaluable lessons for the wider academic community: first, diagnostic testing to clinical standards can be successfully achieved through partnership and guidance from a clinical diagnostic laboratory; second, the choice of techniques and approaches should be adapted to the local resource, and staff expertise, already existing within a research laboratory; and third, the scale and implementation of testing should be aligned with the healthcare needs and demands of the local population.

Acknowledgements

This work was supported by the Francis Crick Institute that receives its core funding from Cancer Research UK (FC001169, FC001078), the UK Medical Research Council (FC001169, FC001078), and the Wellcome Trust (FC001169, FC001078). C.S. is Royal Society Napier Research Professor and is also funded by the Breast Cancer Research Foundation (BCRF). E.N. receives research funding from the NIHR, MRC, GSK and H2020. S. Gandhi is an MRC Senior Clinical Fellow.The authors wish to thank Heather Ringrose for support with the Hamilton liquid handling workstation.

Competing Interests statement

C.S. receives grant support from Pfizer, AstraZeneca, BMS, Roche-Ventana, Boehringer-Ingelheim, Ono Pharmaceutical and has consulted or received an honorarium from Pfizer, Novartis, GlaxoSmithKline, MSD, BMS, Celgene, AstraZeneca, Illumina, Genentech, Roche-Ventana, GRAIL, Medicxi, and the Sarah Cannon Research Institute. C.S. is a shareholder of Apogen Biotechnologies, Epic Bioscience, GRAIL, and has stock options in and is co-founder of Achilles Therapeutics.

M.Hubank has received laboratory support, consultancy fees, or advisory board membership from Guardant Health, Roche Diagnostics, Boehringer-Ingelheim, Bristol Myers Squibb, and AstraZeneca.

Editor's note: This article was peer-reviewed.

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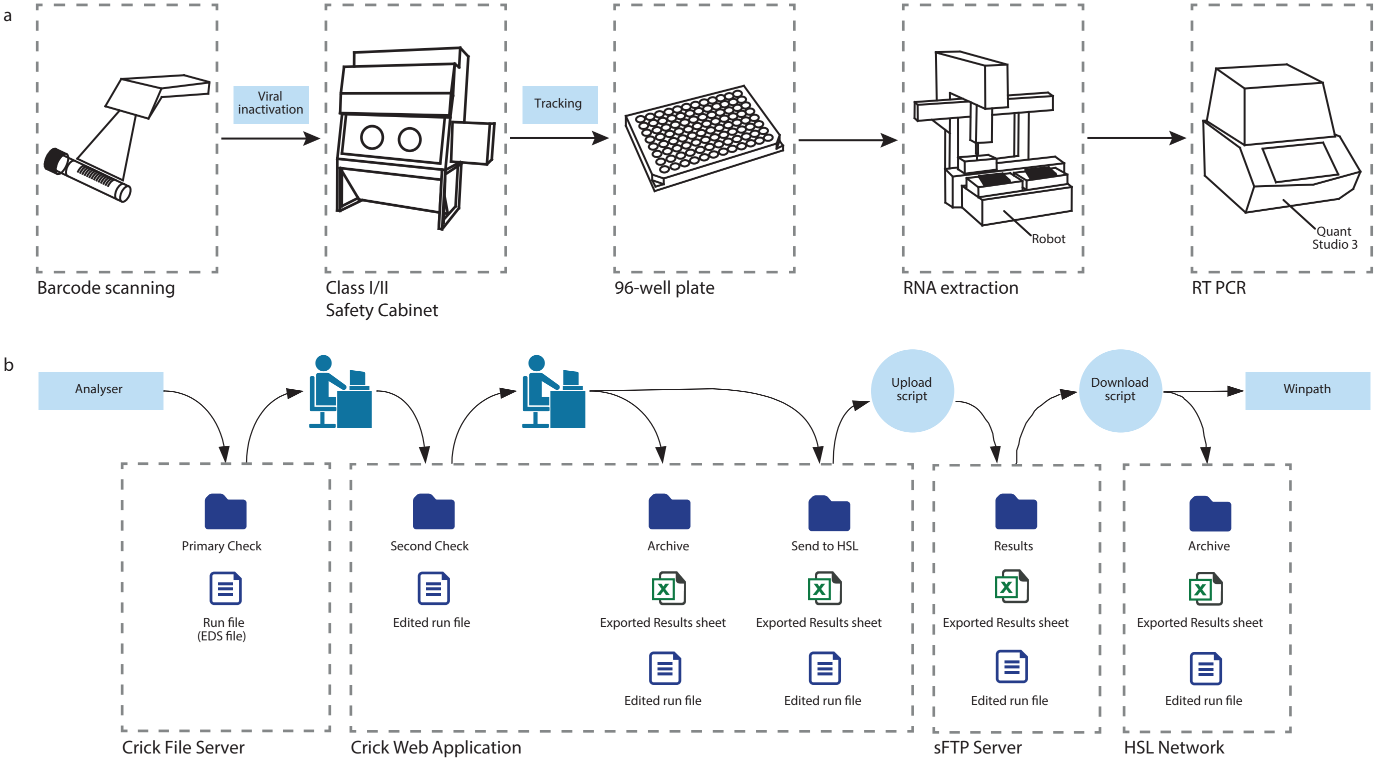
Figure 1. Schematic of the CCC test and reporting pipelines. (a) Specimen barcodes are scanned at sample reception, before viral inactivation in a Class I or II safety cabinet, processing through RNA extraction using an in-house protocol and RT-PCR testing using a commercial kit (BGI). The number of samples processed through the pipeline per day has ranged from 39 (one plate) to a maximum of 1270 (14 plates). (**b**) CCC reporting pipeline. Test results are reported continuously through a custom-made remote web application, allowing remote clinical scientists and pathologists working outside of the institute to authorise reports, in line with the established SOP.

Table 1. Major issues encountered while setting up the CCC pipeline and information on how these issues were dealt with.


a
Viral inactivation
Tracking
Robot
Quant Studio 3
Barcode scanning
Class I/II Safety Cabinet
96-well plate
RNA extraction
RT PCR
b
Analyser
Upload script
Download script
Winpath
Primary Check
Run file (EDS file)
Second Check
Edited run file
Archive
Exported Results sheet
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Archive
Exported Results sheet
Edited run file
Crick File Server
Crick Web Application
sFTP Server
HSL Network