8. Developing and implementing a SARS-CoV-2 testing workflow in a CL2 research laboratory for screening and viral sequencing

8.1 Introduction

Coronavirus disease (COVID-19) is a novel disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged as a serious public health threat in Wuhan, China in December 2019[593,594,567]. SARS-CoV-2 is a highly-transmissible positive-sense, single-stranded RNA coronavirus, which causes a range of symptoms, from a mild fever to severe pneumonia requiring intervention[596]. In addition, SARS-CoV-2 appears to be readily spread by asymptomatic carriers[597]. Due to its transmissibility, SARS-CoV-2 rapidly spread beyond China, and the World Health Organization declared a pandemic on March 11, 2020, at which point there were 118,319 cases and 4292 deaths globally[598]. In the UK, while there was not yet a high burden of COVID-19 cases, the number was increasing, and by March 23rd, when the UK instituted a country-wide lockdown, there were over 300,000 cases globally and 5687 confirmed cases and 281 deaths in the UK[599].

A common diagnostic test for SARS-CoV-2 is real-time reverse-transcriptase polymerase chain reaction (RT-PCR) from a nasopharyngeal swab, which detects viral particles in the sample[600,601]. An alternative to RT-PCR-based assays for hospital-admitted patients is chest x-rays, which have a greater sensitivity but are not feasible for early detection or screening purposes[602]. The principle of an RT-PCR assay is conversion of RNA to DNA using the enzyme reverse transcriptase and subsequent amplification of the DNA. The virus is detected by including SARS-CoV-2 specific primers linked to a fluorescent probe, and the detection is assayed in real time by quantifying the amount of fluorescence from the specific probe[603]. At the outset of the pandemic, SARS-CoV-2 was designated a containment level 3 (CL3) organism in the UK by Public Health England (PHE) based on the lack of treatment and the potential for adverse outcomes from infection[604]. Potential positive samples had to be

handled within a CL3 facility until fully inactivated. Therefore, there was a bottleneck in testing capabilities due to the limitations in CL3 facilities nationwide and the time required to inactivate samples. This had a deleterious effect on the number of samples that could be safely processed, thus limiting the ability to detect and treat COVID-19 patients.

In particular, at the outset of the UK lockdown, limited testing posed a challenge to early detection amongst the healthcare worker (HCW) population, which was vulnerable due to high risk of exposure and elongated contact with COVID-19 patients[593,605–607]. Additionally, due to public health guidance, there was likely a large proportion of healthcare workers self-isolating, thus reducing the number of staff available within hospitals[608–610]. Furthermore, it was desirable to conduct SARS-CoV-2 surveillance within hospitals to mitigate the risk of hospitals becoming outbreak epicentres. However, given the low testing capacity and increasing patient volume in the UK in late March and early April, it was not feasible for hospitals to test HCW in addition to patients. As a result, one possible solution was the utilization of research laboratories proximate to hospitals to increase testing capacity. This could be carried out with strict safety protocols in place and adherence to PHE-approved testing procedures.

Thus, our CL2 laboratory on the Biomedical Campus of the University of Cambridge, proximate to the Cambridge University Hospitals (CUH), began assisting CUH by screening HCW for SARS-CoV-2 from early April 2020. This involved the modification and optimization of existing non-kit viral inactivation protocols to quickly and easily work with patient samples at CL2, bypassing the time-consuming and rate-limiting CL3 inactivation steps[611]. In addition to optimizing and validating CL2 sample extraction procedures using home-made reagents, we liaised extensively with the regional PHE personnel within Addenbrooke's Hospital to ensure standardization of our protocols and results with theirs. We worked closely with clinicians within Addenbrooke's hospital to create a streamlined workflow to obtain samples from HCW, process them and run the diagnostic RT-PCR, and report results directly to the clinicians. We were able to train a designated workforce within our laboratory to run each step of the pipeline and ensure timely result reporting. Finally, we were able to transfer the extract RNA samples from our workflow to other groups within the hospital to enable rapid whole genome viral sequencing. As a result of screening nearly 10,000 HCW over a three-month period, we were able to detect small clusters of infected HCW on hospital wards and found that asymptomatic carriage may play a significant role in COVID-19 transmission within hospital settings.

While this period from late March through early July was highly disruptive to my PhD, it was an opportunity to be involved in the local COVID-19 response and use my expertise and training to contribute to COVID-19 epidemiology, diagnostic testing, and sample workflows. Specifically, a core group of lab members tested pre-existing PHE RT-PCR assays to recapitulate results in our laboratory. We tested a number of RT-PCR master mix reagents to optimize test sensitivity, specificity, and costs using a pre-existing set of known positive SARS-CoV-2 samples. I was one of the leads on this work. We then established and optimized a standardized set of protocols to manage incoming swabs from HCW, including determining appropriate handover procedures to maximize our sample throughput and minimize our turnaround times. Five lab members were trained on hospital sample management software to directly register samples received in the lab and update test results. One of the greatest complexities was maintaining effective communication with the clinical team and associated research nurses to anticipate and manage sample numbers with quick turnover.

8.2 A blueprint for the implementation of a validated approach for the detection of SARS-CoV-2 in clinical samples in academic facilities.

In this study, we established and implemented a set of procedures to perform diagnostic testing for SARS-CoV-2 in a CL2 laboratory. We validated and optimized the RT-PCR protocol used in the Cambridge University Hospitals diagnostic lab, developed a sample workflow from the hospital to our lab, and created a comprehensive system of space and personnel to manage each step of the sample processing and SARS-CoV-2 testing[612]. A pdf version of the paper can be found here.

8.3 Screening of healthcare workers for SARS-CoV-2 highlights the role of asymptomatic carriage in COVID-19 transmission.

For this study, we performed SARS-CoV-2 testing using the protocols and workflows discussed in section 8.2. The HCW swab results we reported to CUH clinicians were combined with collated information from HCW about their symptoms to determine the rate of symptomatic and asymptomatic infections amongst a subset of the hospital staff. The extracted viral RNA from SARS-CoV-2 positive cases were additionally used for viral genome sequencing[613]. A pdf version of the paper can be found here.

8.4 Effective control of SARS-CoV-2 transmission between healthcare workers during a period of diminished community prevalence of COVID-19.

The work in this study followed on from that discussed in section 8.3, in which we continued testing symptomatic and asymptomatic HCW for SARS-CoV-2. The period captured in this study was from April 25^{th} to May 24^{th}, 2020, during which 3388 swabs were obtained from HCW. We used our pre-established protocols and workflows to extract RNA and perform RT-PCR on these samples, finding 34 positive tests, a 1.0% positivity rate across asymptomatic and symptomatic testing initiatives. From our testing, we were able to detect a small ward-based outbreak of cases, enabling a rapid response from the hospital for additional screening and cleaning measures[614]. A pdf version of the paper can be found here.

8.5 Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: a prospective genomic surveillance study.

This study was a genomic analysis of SARS-CoV-2 cases in hospital settings within CUH and East of England. Viral sequencing was conducted using nanopore sequencing on samples from hospital patients and healthcare workers. Our involvement in this study was the RNA extraction and SARS-CoV-2 testing of HCW samples. Any positive RNA samples were transferred for nanopore sequencing[615]. A pdf version of the paper can be found here.

8.6 Secondary pneumonia in critically ill ventilated patients with COVID-19.

As part of our SARS-CoV-2 RT-PCR diagnostic testing, we also assisted CUH by providing early results for patients receiving mechanical ventilation in the intensive care unit (ICU) based on RNA samples extracted in a CL3 laboratory. During a period of high volumes of SARS-CoV-2 testing within the hospital, we were able to rapidly run RT-PCR tests and report results. Any positive samples were subsequently assayed for secondary pathogen signatures using a TaqMan multi-pathogen array card. By doing this, it was possible to assess associations between COVID-19 positivity and ventilator-associated pneumonia (VAP). While this was a small study with a few patients, it was possible to determine that there was a higher rate of VAP within the COVID-19 positive patients[616]. A pdf version of the paper can be found here.