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An Australian diagnostic microbiology surge response to the COVID-19 pandemic

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ABSTRACT

Diagnostic microbiology services form a critical component of the response to infectious disease outbreaks. Like previous respiratory virus pandemics, the COVID-19 pandemic has placed significant strains on the standing capacity of laboratories around the world. In this case study, we describe the surge response required by our laboratory to meet the fluctuating demand for SARS-CoV-2 in our regional pathology service in Western Sydney, Australia between March and May 2020. While the overall number of SARS-CoV-2 PCR positive cases was relatively low compared to other Australian local health districts, testing numbers were highly unpredictable and changed on a weekly basis as local outbreaks were detected. As with other laboratories, numerous other challenges were also faced during this period, including the requirement to introduce a new and unaccredited diagnostic PCR assay for SARS-CoV-2, local and global shortages of reagents for sampling and sample processing, and a significant institutional SARS-CoV-2 outbreak in our laboratory catchment area. A successful service delivery during this period could only be maintained by a dynamic whole-of-laboratory and organizational response including (1) operational changes to the hours of service and the expansion of diagnostic testing at our laboratory site and other sites within our organization (2) careful management of specialist staff and re-training and recruitment of additional staff (3) changes to laboratory workflows to improve SARS-CoV-2 PCR test turnaround time and to accommodate limits to precious laboratory reagents; (4) clear communication within our laboratory and the NSW Health Pathology organization; and (5) collaborative co-ordination and support by NSW Health Pathology.

1. Introduction

A novel coronavirus (SARS-CoV-2) emerged in China in December 2019 and has subsequently spread around the world (Lu et al., 2020), with a global pandemic declared in early March 2020 (Jin et al., 2020).

SARS-CoV-2 causes a respiratory illness (COVID-19) with symptoms including fever and cough (Guan et al., 2020). Myalgia, expectoration, and dyspnea are less common (Li et al., 2020). Chest computed tomography scanning reveals radiological findings of ground-glass opacity in individuals who develop severe respiratory disease (Guan et al., 2020). While most individuals experience only mild disease, the elderly are particularly susceptible to COVID-19, experiencing higher mortality rates and more severe disease compared to young and middle-aged individuals (Liu et al., 2020). To date, testing around the world has confirmed cases of approximately 27 million cases of SARS-CoV-2 globally (World Health Organisation 2020) with >900,000 deaths associated with this virus. Over half of these cases have been documented in North and South America.

Rapid microbiology diagnostic laboratory testing has been at the forefront of global efforts to diagnose SARS-CoV-2 infections in individual patients, inform contact tracing efforts and local and national governments in developing public responses to the emergence of the different waves of the pandemic. The current gold standard for the laboratory detection of SARS-CoV-2 infection is a real-time reverse-transcriptase polymerase chain reaction (RT-PCR) for detection of SARS-CoV-2 nucleic acid in specimens (e.g., nasal and/or throat swab) collected from patients (D'Cruz et al., 2020). Since its discovery, a range of commercial SARS-CoV-2 specific-RT-PCR assays and diagnostic platforms have been rapidly developed and deployed to meet the significant global demands for COVID-19 testing. Fortunately, these assays have been reported to have high sensitivity and specificity (D'Cruz et al., 2020). A role has also emerged for SARS-CoV-2-specific serology, particularly in understanding the broader seroprevalence of SARS-CoV-2 in the community (Theel et al., 2020). Seroconversion typically occurs within 2 weeks, with the earliest positive results detected by 6 days and the latest by 21 days (Okba et al.,

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2020). A 4-fold increase in IgG antibodies can be detected by Day 20 (Haveri et al., 2020) with a 4-fold rise in SARS-CoV-2 antibodies between acute and convalescent sera collection typically diagnostic of recent infection. Significant interest also exists for the potential use of point-of-care antigen tests for SARS-CoV-2 to allow testing in the community rather than the laboratory, although concern exists over the quality of these assays and the studies evaluating them (Dinnes et al., 2020).

In Australia, the first confirmed case of SARS-CoV-2 infection occurred in late January in Victoria. Large numbers of cases were attributed initially to returned travellers from countries with high disease prevalence, as well as cruise ship dockings including the Diamond and Ruby Princess Cruise Liners and outbreaks in aged care facilities (COVID-19 National Incident Room Surveillance Team 2020). While early control efforts were initially successful, lapses in the management of returned travelers with apparent concomitant failures to adhere to social distancing and other recommended infection control measures in the community, led to a rapid increase in cases in Victoria, Australia (COVID-19 National Incident Room Surveillance Team 2020). This meant that public health authorities had to enforce an extended period of control measures in this state, while the same measures were relaxed in other jurisdictions. A key foundation of these control measures has been high rates of community testing with >5 million SARS-CoV-2 PCR tests performed in Australia from the commencement of the pandemic to the August 16, 2020. The cumulative testing positivity from these samples was <0.3% (COVID-19 National Incident Room Surveillance Team 2020).

The urgent need to significantly increase diagnostic microbiology testing capacity for SARS-CoV-2 during the pandemic has been a challenge faced by urban and regional pathology services around the world. This experience is not a new one, however. During the H1N1 Influenza A pandemic in 2009, reference laboratories in the New York Metropolitan area experienced a 7.5-fold increase in demand for respiratory virus diagnostic testing as the pandemic spread through this region (Crawford et al., 2010). In response, a range of initiatives were introduced to increase laboratory capacity rapidly, including workforce changes and modifications to the workflows for respiratory virus testing and reporting of diagnostic test results.

In this outbreak study, we describe the surge response introduced by our Australian diagnostic microbiology laboratory to meet the urgent need for SARS-CoV-2 PCR testing with optimized turnaround time, including the local experience, successes, and challenges of instituting and maintaining a new diagnostic platform in the setting of the COVID-19 pandemic.

2. Study setting

Our laboratory service of NSW Health Pathology (NSWHP) Nepean, provides diagnostic services for community facilities as well as 3 hospitals in the Nepean Blue Mountains Local Health District (NBMLHD) situated in Western Sydney, Australia, with a total inpatient bed capacity of 745. The microbiology department routinely provides highly valuable diagnostic service, and houses an efficient, moderately sized molecular laboratory. Prior to the CoVID-19 pandemic this area was operational between 0830 and 1730 with one 100% full time (1.0 full time equivalent; 1.0 FTE) position allocated to molecular screening, filled from a rotation of 10 staff members with appropriate training. Molecular respiratory pathogen detection was performed using the AusDiagnostics Respiratory Pathogens B (16-well), AusDiagnostics Atypical Pneumonia (8-well) and GeneXpert Xpert Xpress Flu/RSV assays, and had an average throughput of 24 specimens per day.

At the beginning of the pandemic all SARS-CoV-2 PCR requests processed by NSWHP Nepean were sent off-site to NSWHP Institute of Clinical Pathology and Medical Research (ICPMR) Westmead until evaluation and commissioning of the AusDiagnostics SARS-CoV-2 panel by the NSWHP Nepean Microbiology Laboratory was completed on March 25, 2020.

2.1. Local experience of SARS-CoV-2 testing during the COVID-19 pandemic

A total of 167 cases were confirmed positive at NSWHP Nepean for SARS-CoV-2 based on PCR testing between January 28 and May 15, 2020 with a total of 19,983 tests performed during the period reviewed in this study (Fig. 1).

Our first SARS-CoV-2 PCR request was received by the NSWHP Nepean Microbiology Laboratory on the 28th of January, 2020. This sample was tested at NSWHP ICPMR Westmead and the result was negative. The first confirmed case of SARS-CoV-2 infection in the NBMLHD occurred on the March 9, 2020 from a close contact of a known COVID-19 case. Both samples were tested and confirmed by an in-house PCR platform at NSWHP ICPMR Westmead between March 1 and 20, 2020, there were a further 11 patients who tested positive, with equal numbers of returned travellers and known close contacts cases in Western Sydney (1 patient with no data recorded). The NSWHP Nepean Microbiology Laboratory commenced testing for SARS-CoV-2 using the AusDiagnostics High-Plex SARS-CoV-2, Influenza and RSV (8-well) assay (hereafter as AusDiagnostics assay) on the March 25, 2020.

An influx of new cases was seen following the docking of the Ruby Princess Cruise Ship, a cruise ship which disembarked 2700 passengers into Sydney on the March 19, 2020. The first related case was diagnosed on the March 21, 2020. Cases in the NBMLHD continued to be observed until the April 21, with no deaths documented from this outbreak. During this time, “Fever Clinics” were established in the NBMLHD, prompting a significant increase in the requests for SARS-CoV-2 PCR testing from 43 in the week prior, to 822 and 1516 respectively in the subsequent 2 weeks, with a peak of 3738 requests in the first week of May. These clinics provided access to SARS-CoV-2 PCR testing for the general public who fulfilled the Communicable Disease Network Australia case definition of possible SARS-CoV-2 infection. Following the institution of testing, the number of SARS-CoV-2 PCR requests decreased by 33% as the case rate of COVID-19 decreased over the period of March 25 to April 8 compared with the week prior.

On the April 11, 2020, a healthcare worker in a residential aged care facility was diagnosed with SARS-CoV-2 infection. The facility housed 102 residents at the time the outbreak was confirmed. A screening program was commenced on both symptomatic and asymptomatic residents and staff members, which led to a 5-fold increase in SARS-CoV-2 requests between April 14 and 17, 2020. Two other cases of significant public health concern in the NBMLHD were diagnosed on April 24, one a health care worker in an unrelated aged care facility and another, a customer service employee. All aged care facility residents and staff, as well as contacts of both patients were tested. All SARS-CoV-2 PCR tests were resulted negative. This influx of screening led to another peak in testing, with a new maximum of test requests of 601 on the April 29, 2020.

Contact screening was also required following the diagnosis of SARS-CoV-2 on the April 28, 2020 in a 4-year-old attending a local childcare facility, with 84 adults and children undergoing testing. A local primary school also was exposed to an infected adult, diagnosed on the May 13, 2020, with testing performed on close contacts. All contacts for both exposures were negative. There were no further cases diagnosed after the May 13, 2020.

2.2. Rapid evaluation and implementation of new diagnostic assays for SARS-CoV-2

NSW Health Pathology implemented a “staged” introduction of SARS-CoV-2 PCR testing throughout NSWHP. SARS-CoV-2 PCR testing was commenced at our laboratory using a commercial assay

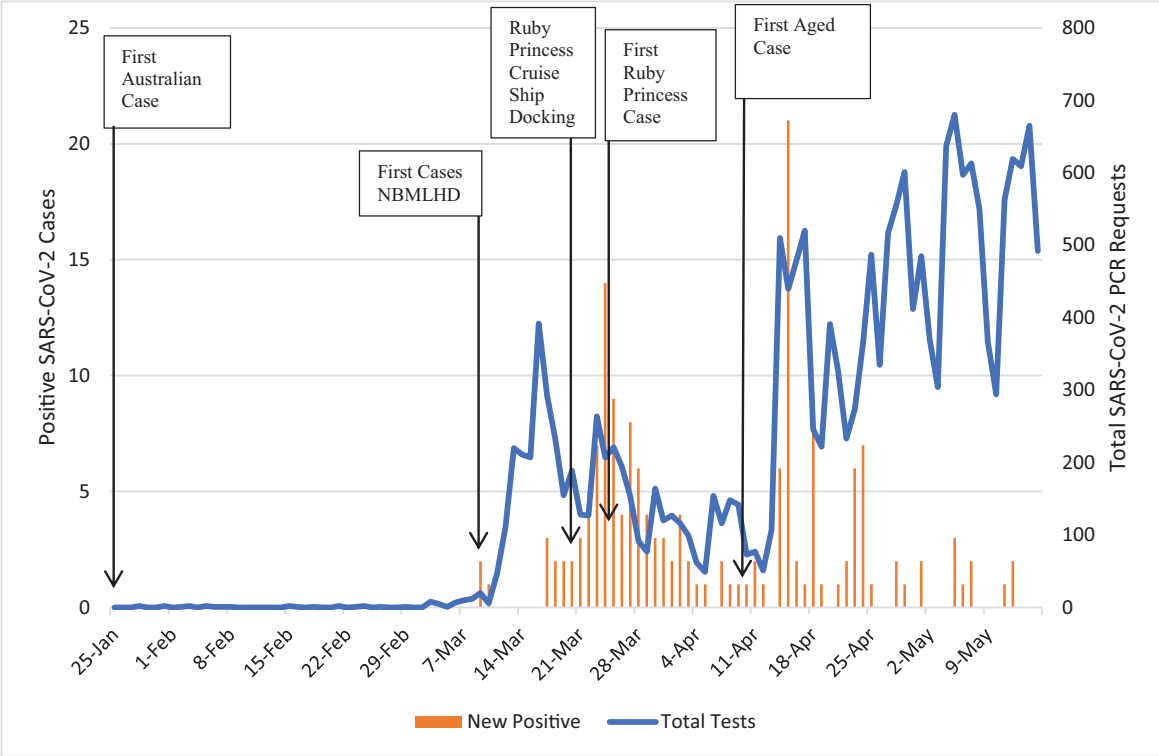

Fig. 1. Timeline of SARS-CoV-2 PCR screening requests and positive cases in the Nepean Blue Mountains Local Health District between January 25 and May 19, 2020.

developed by AusDiagnostics Pty Ltd on March 25, 2020. This assay uses the SARS-CoV-2 ORF1a and ORF8 genes as targets to detect viral RNA using an existing molecular diagnostic platform. Between the March 10 and 20, 2020, the AusDiagnostics assay underwent an internal evaluation process, whereby 218 patient samples and 13 culture cell supernatants were tested and compared with the previously evaluated in house platform. This comparison revealed high concordance (99.1%) and an improved limit of detection for SARS-CoV-2 compared to the in-house assays (unpublished observations). The assay was approved for use by the Therapeutic Goods Administration on the March 22, 2020 (Kirkland and Frost, 2020).

In late April 2020, the GeneXpert Xpert Xpress SARS-CoV-2 cartridges were supplied to NSW Health Pathology sites, which provided the benefit of rapid laboratory bench turnaround time (approximately 1 hour) from sample receipt to diagnostic test result. The testing kit had been approved by the Therapeutic Goods Administration on March 22, 2020. A local verification was performed to supplement an initial state-wide verification, prior to the commencement of testing on the April 25, 2020. These individual rapid GeneXpert SARS-CoV-2 PCR tests were constrained by global supply chain issues and their use has been restricted to cases that require emergency results for therapeutic purposes or cases with large potential public health implications. A clinical vetting process by each laboratory was implemented at each local health district site to ration the use of the small vendor allocated GeneXpert cartridges.

2.3. Dealing with supply shortages and outsourcing of less urgent clinical specimens

During the first few months of the COVID-19 pandemic were both local and national shortages of supplies that were critical to SARS-CoV-2 PCR based testing. This included flocked swabs, viral transport media (VTM), and extraction reagents required for SARS-CoV-2 PCR sample preservation and testing.

The NSWHP Public Health Pathology division and the NSWHP Incident Management Team (for COVID-19) recognized the need to invest in staff, equipment and stock to facilitate a rapid increase in PCR requests. NSWHP recruited specific staff to manage surge capacity options and the regulatory requirements. The Principal Veterinary Virologist at the Department of Primary Industries veterinary laboratory in South-West Sydney, Elizabeth Macarthur Agricultural Institute (EMAI), was approached by NSWHP staff and the veterinary viral testing system was adapted to enable testing for human COVID 19 patient samples, under human diagnostic testing protocols and pathologist supervision. This cooperative arrangement provided test surge buffering for the state pathology laboratories when under pressure from local cluster and outbreak test surges.

Chemical reagents, including the NUCLISENS® easyMAG® Extraction Buffer 1 required for RNA extraction had been depleted worldwide prior to commencement of SARS-CoV-2 testing at NSWHP Nepean in March 2020. It was predicted that, by early April 2020, there would be sufficient local RNA extraction reagent for only 3000 specimens if processed individually. To conserve reagents, sample pooling was evaluated and instituted, whereby up to 4 samples were able to be processed using the same amount of reagent as one specimen. Despite maximizing samples tested by pooling, reagent supply continued to be depleted due to worldwide shortages, and testing at NSWHP Nepean reached capacity. SARS-CoV-2 PCR testing was sent off-site to EMAI from the April 21, 2020 as per the surge capacity agreement detailed previously. All inpatient and health care workers samples were kept on-site at NSWHP Nepean to maintain short turnaround times for these critical samples. This service was further utilized during periods where on-site testing was unavailable due to failure of critical equipment and/or to conserve rapidly depleting reagents.

Prior to the outbreak, UTM swabs (Copan Diagnostics, Murrieta, CA) were a main product used for viral PCR testing. In the initial period of the outbreak however, demand for these swabs outweighed the supply, with shortages on a local, state and national level. It was recognized that centralizing the process of procurement by NSW Health Pathology for products in serious short supply was critical. Local production of VTM was also investigated by NSW Health

Pathology Public Health Pathology division (NSWHP PHP). Again, the established relationship with the Department of Primary Industries state reference testing laboratory, "Elizabeth Macarthur Agricultural Institute" was used to create a viral media manufacturing capability that could produce a well-characterized and validated VTM for SARS-CoV-2, as recently described (Kirkland and Frost, 2020). NSW Health Pathology also established resilient international and national supply chains for dry swabs and then assembled appropriate kits to supply to the NSWHP sites including NSWHP Nepean until commercial supplies returned.

2.4. Strategies to improve turnaround time at different testing sites

Between January 28 and March 25, 2020, all SARS-CoV-2 PCR requests processed by NSWHP Nepean were sent to NSWHP ICPMR Westmead, with 4,273 tests were performed at and 104 SARS-CoV-2 positive results (2.4% positivity) identified at this facility. Between March 25 and the May 15, 2020, 9,998 SARS-CoV-2 tests were performed at our laboratory (or NSWHP Nepean) with 160 positives identified (1.6%). There were 5712 SARS-CoV-2 tests between April 21 and May 15, 2020 sent offsite to EMAI as part of the surge response detailed in the previous section. Of these 13 samples (0.2%) were identified as positive.

At the onset of testing, laboratory capacity for SARS-CoV-2 PCR testing was estimated to be 150 individually processed specimens per day. The AusDiagnostics assay primarily used for SARS-CoV-2 PCR testing consisted of 8 wells and had the ability to process 24 single samples at one time. The theoretical turnaround time (TAT) from time of receiving a specimen at the laboratory specimen reception to verification of result was approximately 4 to 6 hours (including 115−160 minutes of hands on-time), divided between a number of labor-intensive steps: (1) the manual receipt of samples in the Sample Reception Management section; specific worksheets and labels are then manually generated and applied to samples and associated tubes used through the screening process. Samples are then organized into racks for nucleic acid extraction (40−70 minutes); (2) manual pipetting of each sample while using the NucliSENS® easyMAG® (bioMérieux Australia, Norwest, Australia) nucleic acid extraction kits (65−90 minutes); (3) manual preparation of the AusDiagnostics PCR reaction tubes (35−90 minutes); (4) automated amplification steps using the High-Plex machine with user transfer of plates between amplification steps (80−90 minutes); and (5) interpretation and reporting of results (5−10 minutes). As the outbreak evolved, a 2 to 3 hours TAT was maintained for samples from highly suspicious COVID-19 cases requiring urgent clearance.

Turnaround times for SARS-CoV-2 PCR testing changed over the period of testing described (Fig. 2). Initially, TAT was >24 hours as samples from the NBMLHD had to be transported to NSWHP ICPMR Westmead. TAT during high demand periods was >24 hours. The longer TAT was due to samples requiring additional transport to NSWHP ICPMR Westmead (located 26 km from NSWHP Nepean), and the high demand testing across NSW. TAT, however, decreased once testing was moved on site. As a result of increasing issues with reagent shortages, sample pooling commenced on the March 26, 2020. Up to 4 patient samples were combined by adding 50 μL of each sample to a single well for nucleic acid extraction and the eluted products tested together. If a well of pooled samples was positive, each patient sample was re-extracted from the primary sample and tested separately. Following the introduction of this measure, testing capacity increased to over 600 specimens per day.

The process of referring samples off-site to the Department of Primary Industries, state reference testing laboratory at EMAI, required approximately 1 week to embed and enabled surge testing to occur. Although the EMAI laboratory had a rapid turnaround time with large capacity, due to packaging and transport required for referral of samples to EMAI, the overall TAT average increased (Fig. 2). Inpatient and critical results remained onsite for processing at NSWHP Nepean to optimize time to results, while "Fever clinic" and other outpatient samples were sent off-site. These critical samples were reported on average 5.8 hours faster when compared with samples sent off-site for routine testing, with the most apparent effect on TAT in late April to early May 2020.

2.5. Monitoring of SARS-CoV-2 positive patients for viral clearance by PCR testing

During the study period, NSW Health policy required repeated testing of health care workers and in-patients at Day 10 of illness, provided that they were asymptomatic within the previous 72 hours. Testing of samples from these patients, resulted in the detection of an additional 110 positive SARS-CoV-2 PCR test results. For the 29 individuals with multiple positive SARS-CoV-2 PCR samples tested on the AusDiagnostics assay, Ct values were compared and there was trend of overall increasing Ct over time (data not shown).

2.6. Communication of SARS-CoV-2 PCR test results

Results from the AusDiagnostics assay were manually entered into the laboratory information system (Cerner Millennium PathNet, Cerner Corporation) by Medical Laboratory Technicians and Scientists. Positive results were then notified to the medical microbiologist on call, who subsequently notified the attending doctor. In the initial phase of testing, negative results were delivered to patients attending the Fever Clinics manually via telephoning patients, with a small task force enlisted comprising of 2 junior medial officers. This was continued until the March 13, 2020, when testing numbers increased to more than 200 per day, and a semiautomated text message service was developed.

2.7. Changes to laboratory practice including opening hours, optimization of scientific staff rostering and training of addition laboratory staff in molecular diagnostics methodologies

Prior to the COVID-19 pandemic, the microbiology laboratory operated from 06:30 to 22:30 with the molecular section operating from 0830 to 1730 with a 1.0 FTE position dedicated to this section, filled from a roster of 10 qualified staff. On commencement of SARS-CoV-2 PCR testing, rostering hours were adjusted so that additional staff members, starting at 0630 and another at 14:00, were available to extend the operation of the molecular section to match the operating hours of the bacteriology section. Sample extraction was performed in the evening for sample analysis the following morning, which meant sample processing was still occurring even when the laboratory was not physically staffed. There was a significant increase in overtime worked in the study period, with a >1,000% increase compared with the same period in the previous year (270 hours in March−May 2020 versus 18 hours in March−May 2019). There was a 39% decrease in the hours taken in annual leave compared with the same period in the previous year. Due to the increased overtime and likelihood of ongoing demand for SARS-CoV-2 PCR testing, increased staffing levels were approved and positions for 2 additional 1.0 FTE laboratory technician positions were advertised for dedicated SARS-CoV-2 testing. All existing microbiology staff members were also trained in molecular diagnostics and were assessed via the standard laboratory competency process to increase the pool of available laboratory staff that could be rostered to perform SARS-CoV-2 PCR sample processing and testing.

2.8. Other changes to standard laboratory practice

Prior to the SARS-CoV-2 pandemic, the Nepean Laboratory performed molecular testing for respiratory pathogens that included the

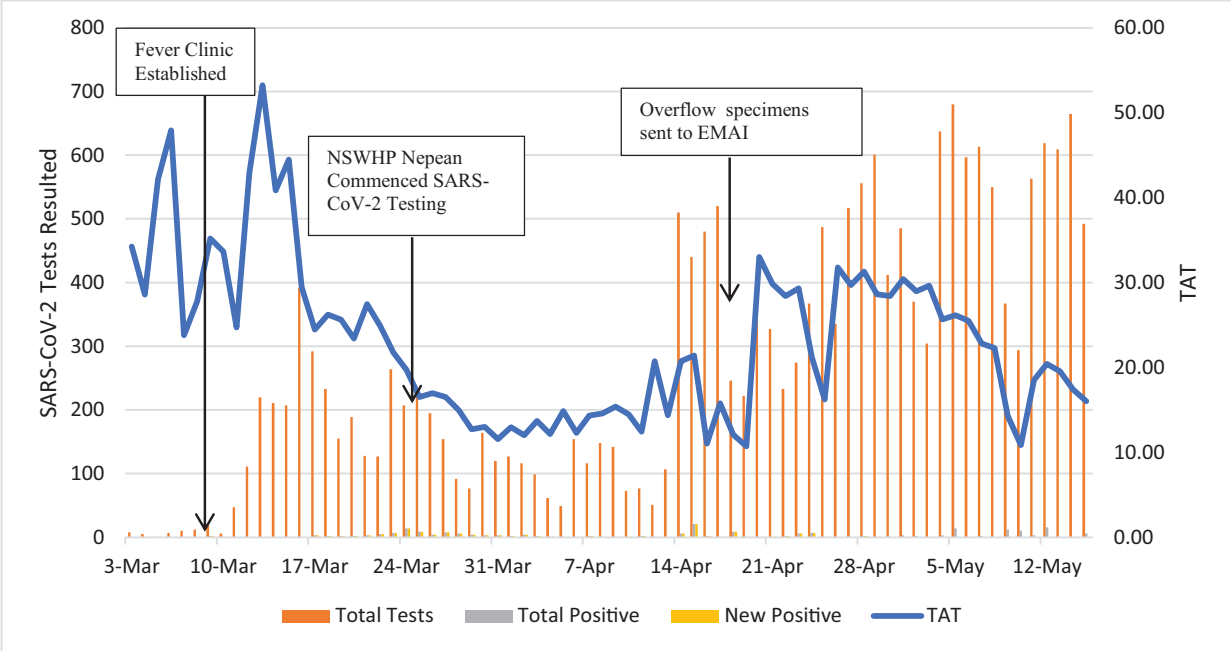

Fig. 2. SARS-CoV-2 PCR testing TAT at different accredited testing sites between March and May 2020.

Xpert Xpress Flu/RSV (GeneXpert System®, Cepheid, Sunnyvale, CA), AusDiagnostics High-Plex Respiratory Pathogens B 16-well (AusDiagnostics Pty Ltd, Mascot, Australia) and AusDiagnostics High-Plex Atypical Pneumonia 8-well assays (AusDiagnostics). The average testing performed was 34 specimens per day prior to the current pandemic, with peak testing numbers at 70 per day during seasonal influenza outbreaks. There was a 309% increase in all molecular testing from March to May 2020 when compared to the same period in 2019 (18,242 tests versus 4,464). To compensate for the significant increase in molecular testing while maintaining the same staffing levels, there were certain limitations enforced on other microbiological tests. This included limiting respiratory viral PCR testing to inpatients only and reduction of multiresistant organism screening. Reduction in elective surgery led to less pre-operative and operative samples referred for bacterial culture. In the period of March to May 2020, there was a 13% reduction in bacterial cultures (20,457 versus 23,535) processed when compared to the same period in 2019.

2.9. Dealing with failure of critical equipment and reagent shortages

An issue encountered which required contingency planning occurred on the April 28, 2020, when the laboratory's only automated nucleic acid extractor failed. SARS-CoV-2 PCR samples were sent off site for processing, while the machine underwent maintenance. TAT was increased by 1.5 hours due to this issue, and was resolved within the day due to prompt technical support

3. Conclusion

Surge response in a microbiology laboratory poses a certain set of unique challenges in the setting of a pandemic. The benefits of having an organized state-wide public pathology network including access to reference laboratories and collaboration with state veterinary laboratory to provide extra capacity cannot be overstated. Demand for SARS-CoV-2 PCR testing over the period described changed significantly and unpredictably on a day-to-day basis as local outbreaks of COVID-19 were detected and public health agencies moved to increase community testing in response. These issues were compounded by the requirement to introduce a test with a high degree of public health importance in a short timeframe as well as resource limitations of both physical materials and personnel. Despite this, rapid and accurate testing was able to be performed, with turnaround times generally maintained below 24 hours. The planning and implementation of off-site pathology testing significantly improved the capacity to respond to surges in testing demand and provided redundancy in the event of local machine breakdowns. Rapid decision making and problem solving in this crisis led to agile and flexible provision of results and an improved public health response for NSW Health Pathology.

Author contributions

Rebecca Sparks: Conceptualization, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing; **Rifky Balgahorm:** Methodology, Validation, Writing – Review and Editing; **Catherine Janto:** Investigation, Writing – Review and Editing; **Adam Polkinghorne:** Formal analysis, Writing – Original Draft, Writing – Review & Editing; **James Branley:** Writing – Review and Editing, Supervision, Project administration; **Harsha Samarasekara:** Formal analysis, Conceptualization, Writing – Review and Editing, Supervision.

Declaration of competing interest

The authors report no conflicts of interest relevant to this article.

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