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Non-SARS-CoV-2 respiratory viral detection and whole genome sequencing from COVID-19 rapid antigen test devices: a laboratory evaluation study

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Summary

Background There has been high uptake of rapid antigen test device use for point-of-care COVID-19 diagnosis. Individuals who are symptomatic but test negative on COVID-19 rapid antigen test devices might have a different respiratory viral infection. We aimed to detect and sequence non-SARS-CoV-2 respiratory viruses from rapid antigen test devices, which could assist in the characterisation and surveillance of circulating respiratory viruses in the community.

Methods We applied archival clinical nose and throat swabs collected between Jan 1, 2015, and Dec 31, 2022, that previously tested positive for a common respiratory virus (adenovirus, influenza, metapneumovirus, parainfluenza, rhinovirus, respiratory syncytial virus [RSV], or seasonal coronavirus; 132 swabs and 140 viral targets) on PCR to two commercially available COVID-19 rapid antigen test devices, the Panbio COVID-19 Ag Rapid Test Device and Roche SARS-CoV-2 Antigen Self-Test. In addition, we collected 31 COVID-19 rapid antigen test devices used to test patients who were symptomatic at The Royal Melbourne Hospital emergency department in Melbourne, Australia. We extracted total nucleic acid from the device paper test strips and assessed viral recovery using multiplex real-time PCR (rtPCR) and capture-based whole genome sequencing. Sequence and genome data were analysed through custom computational pipelines, including subtyping.

Findings Of the 140 respiratory viral targets from archival samples, 89 (64%) and 88 (63%) were positive on rtPCR for the relevant taxa following extraction from Panbio or Roche rapid antigen test devices, respectively. Recovery was variable across taxa: we detected influenza A in nine of 18 samples from Panbio and seven of 18 from Roche devices; parainfluenza in 11 of 20 samples from Panbio and 12 of 20 from Roche devices; human metapneumovirus in 11 of 16 from Panbio and 14 of 16 from Roche devices; seasonal coronavirus in eight of 19 from Panbio and two of 19 from Roche devices; rhinovirus in 24 of 28 from Panbio and 27 of 28 from Roche devices; influenza B in four of 15 in both devices; and RSV in 16 of 18 in both devices. Of the 31 COVID-19 devices collected from The Royal Melbourne Hospital emergency department, 11 tested positive for a respiratory virus on rtPCR, including one device positive for influenza A virus, one positive for RSV, four positive for rhinovirus, and five positive for SARS-CoV-2. Sequences of target respiratory viruses from archival samples were detected in 55 (98·2%) of 56 samples from Panbio and 48 (85·7%) of 56 from Roche rapid antigen test devices. 98 (87·5%) of 112 viral genomes were completely assembled from these data, enabling subtyping for RSV and influenza viruses. All 11 samples collected from the emergency department had viral sequences detected, with near-complete genomes assembled for influenza A and RSV.

Interpretation Non-SARS-CoV-2 respiratory viruses can be detected and sequenced from COVID-19 rapid antigen devices. Recovery of near full-length viral sequences from these devices provides a valuable opportunity to expand genomic surveillance programmes for public health monitoring of circulating respiratory viruses.

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Introduction

Point-of-care rapid antigen tests have become an important diagnostic modality for infectious diseases, as seen recently for COVID-19.[1] Rapid antigen tests, which detect the presence of pathogen-derived proteins, provide essential information to assist with clinical decisions (eg, the need for quarantining and contact tracing to prevent onward transmission), improve patient triage in the emergency department, and facilitate early administration of antiviral therapies.[1–3] The identification of active infection in

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Research in context

Evidence before this study

Rapid antigen tests have become a common diagnostic modality for infectious diseases, including respiratory viral infections. For SARS-CoV-2, these devices have served as an affordable and timely alternative to nucleic acid-based testing. The ability to recover viral nucleic acid from rapid antigen devices has previously been demonstrated for dengue virus and SARS-CoV-2, including the ability to sequence whole viral genomes for SARS-CoV-2. It is currently unknown whether non-SARS-CoV-2 respiratory viruses can be detected and fully sequenced from COVID-19 rapid antigen test devices. We searched PubMed for studies published between database inception and March 14, 2023, with no language restrictions, relating to respiratory viral detection and sequencing from rapid devices, using the terms "respiratory virus", "sequencing" AND "rapid device" OR "antigen test". Of the identified publications, only one included non-SARS-CoV-2 respiratory viral sequencing from rapid antigen devices, and whole genome sequencing was not done on these viruses.

Added value of this study

In this evaluation study, we show that common respiratory viruses can be detected and sequenced from COVID-19 rapid antigen devices. We apply a capture-based sequencing approach and automated computational pipelines to generate informative genotypic data for these pathogens, including samples with mixed infections.

Implications of all the available evidence

The creative use of these devices provides a valuable opportunity to expand surveillance programmes for public health monitoring of circulating respiratory viruses, including expansion of genomic surveillance in community and health-care settings.

health-care and community settings has contributed significantly to public health responses throughout the COVID-19 pandemic, supported by the ease of use and accessibility of these tests.

Many countries observed marked decreases in non-SARS-CoV-2 respiratory viral infections during the COVID-19 pandemic, owing, in part, to the implementation of public health measures such as stay-at-home orders, social distancing, and mask wearing.[4] The circulation of influenza decreased in many countries, with virtual elimination of common influenza lineages.[5,6] Seasonal epidemics of respiratory syncytial virus (RSV) were also absent in many countries.[7,8] Following the reopening of international borders and reduced use of non-pharmaceutical interventions, respiratory viral infections rebounded, with many rapidly reaching, and in some cases surpassing, pre-pandemic levels.[9,10] In Australia, during the 2022 influenza season, there was almost double the number of laboratory-confirmed influenza notifications compared with the preceding 5-year average, showing a return to pre-pandemic levels.[11]

Clinical and public health interest in the epidemiology of circulating respiratory viruses increased during the COVID-19 pandemic, since co-infection with SARS-CoV-2 and other respiratory viruses has been associated with adverse clinical outcomes[12] and changes to seasonal variation in circulation of respiratory viruses have been observed.[4] Moreover, the importance of genomic surveillance has been highlighted by the early detection of new SARS-CoV-2 variants through global genomic testing throughout the pandemic. Programmes for monitoring non-SARS-CoV-2 respiratory viruses exist at local and international levels, particularly targeting influenza virus and RSV.[13,14] The genomic surveillance of influenza has been used to guide strain selection for seasonal vaccines, identify potential viral reservoirs, and assess pandemic potential of circulating strains, including the recent outbreak of avian influenza in multiple continents.[15–17]

Recovery of SARS-CoV-2 RNA from COVID-19 rapid antigen test devices has been demonstrated by our group and others,[18,19] allowing for the identification of variants through multiplex real-time PCR (rtPCR) and whole-genome sequencing. As such, it is possible that negative COVID-19 rapid antigen tests from symptomatic individuals could carry recoverable nucleic acids of non-SARS-CoV-2 respiratory viruses and represent a practical sample type for the detection and genotyping of these pathogens. Rapid antigen test devices provide a unique sample type with the potential for broad community representation considering that COVID-19 rapid antigen test use is routine in many centres, including in the emergency department to facilitate patient triaging[2] and in care facilities for older adults, in schools, and in regional centres where access to rapid PCR testing is not readily available. Therefore, the use of rapid antigen devices for respiratory virus testing provides the potential for expanded respiratory viral surveillance. In this study, we sought to assess feasibility of detection and whole-genome sequencing of respiratory viruses from negative COVID-19 rapid antigen tests using a collection of samples from patients who were positive for respiratory virus infection and two commercially available rapid antigen devices.

Methods

Study design and samples

In this laboratory evaluation study, we obtained viral culture isolate material and archival nose and throat clinical swab samples from the Victorian Infectious Diseases Reference Laboratory (VIDRL), which is the public health laboratory for the state of Victoria, Australia. Viral culture isolate material for eight viral taxa (adenovirus, influenza A virus, influenza B virus, human metapneumovirus, parainfluenza virus, RSV, and seasonal human coronaviruses [HCoV] 229E and HCoV-OC43) were prepared from stored reference isolates at VIDRL. Archival clinical samples were

collected between Jan 1, 2015, and Dec 31, 2022, from patients with respiratory symptoms in Victoria, whose samples were referred from the community or other health services to VIDRL for testing. All stored samples that previously tested positive by PCR for at least one respiratory virus (adenovirus, influenza A virus, influenza B virus, metapneumovirus, parainfluenza, rhinovirus, RSV, or seasonal human coronaviruses [HCoV-229E, HCoV-NL63, HCoV-OC43, or HCoV-HKU1]) and negative for SARS-CoV-2 (for samples collected from Jan 1, 2020, onwards) were selected for inclusion, with a maximum of 30 samples for any one viral taxon. All archival samples were stored in viral transport media at –80°C. Samples testing negative for respiratory pathogens by PCR collected in the same period were used as negative controls and a gamma-irradiated positive SARS-CoV-2 control (omicron; subvariant BA.1 of the B.1.1.529 variant) was used, prepared as previously described.[18]

This study was approved by The Royal Melbourne Hospital Human Research Ethics Committee as a quality assurance project (QA 2019134). Informed consent was waived for testing of rapid antigen test devices obtained from patients admitted to the emergency department, as these tests were done as part of routine clinical care by the treating clinician.

Outcomes

The primary outcome of this study was the proportion of samples with non-SARS-CoV-2 respiratory viruses detected by PCR from COVID-19 rapid antigen test devices using archival clinical samples. Additional outcomes included PCR detection of non-SARS-CoV-2 respiratory viruses from rapid antigen test devices using viral culture isolate material or a viral culture mix; changes in PCR cycle threshold (Ct) with increased incubation time or use of preservatives and storage media; completeness of genome sequences of non-SARS-CoV-2 respiratory viruses obtained from rapid antigen test devices; and detection of non-SARS-CoV-2 respiratory viruses from clinically collected COVID-19 rapid antigen test devices obtained from the emergency department.

Procedures

Each viral culture isolate or clinical sample was applied to two COVID-19 rapid antigen test devices—the Panbio COVID-19 Ag Rapid Test Device (Abbott, Abbott Park, IL, USA; referred to as Panbio rapid antigen test device) and Roche SARS-CoV-2 Antigen Self-Test (Roche, Basel, Switzerland; referred to as Roche rapid antigen test device). 130 µL (for Panbio) or 50 µL (for Roche) of viral culture isolate or clinical sample was mixed 1:1 with the relevant antigen test kit buffer before being applied to a rapid antigen test device, as per manufacturer's instructions,[20,21] and allowed to dry for 2 h in a class 2 biosafety cabinet. The test devices were then opened and the membrane strip was removed and incubated in 800 µL of lysis buffer for 10 min in 5 mL tubes. The tubes containing the strips were vortexed and centrifuged for 3 min to pellet suspended fibrous material. 680 µL of the supernatant was aspirated and sampled onto a QIAcube HT (Qiagen, Hilden, Germany) for nucleic acid extraction. Purified nucleic acid material was converted to cDNA for PCR testing (appendix 1 pp 3–4). Each sample was tested in batches of 10–20 alongside a gamma-irradiated SARS-CoV-2 positive control and an archival sample that previously tested negative for respiratory viruses on PCR as a negative control.

See Online for appendix 1

rtPCR and whole genome sequencing

We used an in-house respiratory viral rtPCR panel to confirm the presence of a respiratory viral pathogen in each clinical sample (appendix 1 pp 3–4). We assessed viral recovery from clinical samples through a comparison of the post-extraction rtPCR Ct values with the Ct values of the original sample. For archival clinical samples, we re-extracted nucleic acid from the primary sample on the same day as the nucleic acid extraction from the rapid antigen test devices to determine the baseline Ct value (appendix 1 p 3). Additional internal controls included an RNA virus (bovine viral diarrhoea virus;[22] acting as a reverse transcriptase control) and a human gene target (RNAse P [*RPP30*][23] acting as a process control).

For whole-genome sequencing of archival clinical samples, we pooled respiratory virus-positive samples before library preparation. Each pool comprised a PCR-positive sample for adenovirus, metapneumovirus, parainfluenza virus, rhinovirus, RSV, seasonal coronavirus, and influenza A or B virus (appendix 1 pp 4–5). For each rapid antigen test device brand, we prepared eight pools of the seven taxa, with samples with the lowest Ct values selected, given the higher likelihood of viral genome recovery. cDNA was synthesised (appendix 1 p 5) and indexed libraries prepared using the Twist Total Nucleic Acids Library Preparation Kit for Viral Pathogen Detection and Characterization (Twist Biosciences, San Francisco, CA, USA) following the manufacturer's protocol. Twist hybridisations followed the manufacturer's protocol using the Twist Pan-Viral Panel (Twist Biosciences). We sequenced the samples using a paired-end 150 bp chemistry with a P2 cartridge on an Illumina NextSeq2000 (Illumina, San Diego, CA, USA).

Assessing viral recovery over time and effect of preservatives

We assessed viral recovery from rapid antigen test devices over time using archival clinical samples on both Panbio and Roche rapid antigen test devices, and viral culture isolate material on the Panbio rapid antigen test device. Archival clinical samples were incubated for either 24, 48, or 120 h before device extraction and viral culture isolate material was incubated for 72 or 120 h before device extraction (appendix 1 p 6). In addition, we assessed the effect of device storage temperature (4°C, 20°C, and 36°C) and application of commercially available storage media or preservatives (RNALater [Thermo Fisher

Scientific, Waltham, MA, USA], DNA/RNA Shield [Zymo Research, Irvine, CA, USA], Viral Transport Media [University of Melbourne, Melbourne, VIC, Australia], and Universal Transport Media [Copan Diagnostics, Murrieta, CA, USA]), or nuclease-free water control, to the Panbio rapid antigen test device using a viral culture mix (American Type Culture Collection [Manassas, VA, USA]; appendix 1 pp 6–7). Following application of preservative or storage media, devices were incubated for 72 h or 1 week at either 4°C, 20°C, or 36°C temperature before device extraction.

Clinical rapid antigen test device sample collection

To assess the real-world application of this testing approach, we collected rapid antigen test devices from The Royal Melbourne Hospital emergency department during a 2-week period (between June 19, 2023, and July 2, 2023). We collected and stored these COVID-19 rapid antigen tests, done as part of routine care for patients presenting with respiratory symptoms, in specimen collection bags in a dedicated container at room temperature. We collected devices daily for PCR testing and whole-genome sequencing of positive samples. Test strips were extracted within 24–48 h of collection.

Bioinformatic analyses

For more on the **automated pipeline** see https://github.com/esteinig/cerebro

See Online for appendix 2

For more on the **computation of coverage statistics** see https://github.com/esteinig/vircov

We developed an automated pipeline for handling and analysis of metagenomic enrichment data for this study, encompassing read quality-control; host and non-viral background depletion; alignment-based detection using a non-specific viral database; automated, segment-aware selection of highest coverage references; re-alignment and coverage evaluation; and consensus genome reconstruction (appendix 1 pp 7–9). We conducted subtyping for influenza and RSV using curated whole genome reference databases (appendix 1 pp 8–9).

Statistical analysis

Differences in Ct value shift between viral taxa (seasonal coronaviruses *vs* non-seasonal coronaviruses) for archival clinical samples were compared using Mann-Whitney U test. A separate analysis was performed to assess differences between baseline and post-extraction PCR Ct value by normalising for RNAse P (appendix 1 p 9). Wilcoxon signed-rank test was used to assess the effect of temperature, preservatives, and storage duration on virus detection, with PCR-negative samples attributed a Ct value of 45. We modelled the relationship between respiratory viral rtPCR Ct and genome coverage using simple linear regression using the lm function in R (3.6.1), with adjusted R^2 reported. The linear regression model was assessed to ensure assumptions of homoscedasticity, normality of residuals, and linearity between outcome and covariate were met. p values less than 0·05 were considered statistically significant. Analyses were performed using R (3.6.1) or GraphPad Prism (9.5.1).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

As a proof-of-concept, we first tested the recovery of viral culture isolates for eight respiratory viruses following extraction from rapid antigen test devices. Compared with baseline PCR Ct value (ie, for the primary viral culture isolates), the PCR Ct value increased for extracted samples from both Panbio (mean Ct 27·4 [SD 3·2], an increase of 5·2) and Roche (mean Ct 27·9 [SD 4·8], an increase of 5·9) rapid antigen test devices, although this varied between taxa (appendix 1 p 21). We used the same viral culture isolate material to assess viral recovery at two additional timepoints, 72 h and 120 h. All viral taxa were detected at later timepoints, 72 h and 120 h, except for influenza B virus and seasonal coronaviruses (appendix 1 p 22). Recovery of influenza A virus was reduced at both 72 h (Ct value increase of 7·4 from baseline) and 120 h (Ct value increase of 5·7).

We also assessed respiratory viral recovery from rapid antigen test devices using 132 archival, respiratory virus-positive, clinical nose and throat swab samples, eight of which had two viral targets detected, yielding a total of 140 positive viral targets. We also included 20 negative control samples. 100 (71%) of 140 viruses were detected from clinical samples after extraction from rapid antigen test devices (appendix 2 p 1). Recovery was similar for both device types, being 89 (64%) of 140 for Panbio and 88 (63%) of 140 for Roche rapid antigen test devices. As seen for culture isolate material, respiratory viral recovery varied between viral taxa. Recovery was highest for adenoviruses (six of six for both devices), RSV (16 of 18 for both devices), and rhinovirus (24 of 28 for Panbio and 27 of 28 for Roche rapid antigen test devices). Recovery was lower for parainfluenza (11 of 20 for Panbio and 12 of 20 for Roche rapid antigen test devices), human metapneumovirus (11 of 16 for Panbio and 14 of 16 for Roche rapid antigen test devices), influenza A virus (nine of 18 for Panbio and seven of 18 for Roche rapid antigen test devices), seasonal coronaviruses (eight of 19 for Panbio and two of 19 for Roche rapid antigen test devices), and influenza B virus (four of 15 for both devices; table 1; figure 1). Overall, respiratory viral recovery was lowest for seasonal coronaviruses, with an increase in Ct value observed in device-extracted samples compared with baseline (mean Ct 33·9 [SD 5·1], an increase of 10·0 cycles from baseline for the Panbio rapid antigen test devices and mean Ct 35·7 [SD 3·3], an increase of 16·4 cycles from baseline for the Roche rapid antigen test devices; appendix 1 p 10). No targets were detected in the 20 negative clinical samples (appendix 1 p 10).

Respiratory viral recovery and detection were dependent on the viral load of the baseline sample. For samples with Ct values of less than 30, viral genetic material was recovered from 79 (82%) of 96 Panbio and 72 (75%) of 96 Roche rapid antigen test devices. For samples with Ct values of 30 or

	Samples			Panbio rapid antigen test			Roche rapid antigen test			RNAse P process control				
	Total	Detected in Panbio	Detected in Roche	Viral target Ct (baseline)*	Viral target Ct	Change in viral target Ct	Viral target Ct (baseline)†	Viral target Ct	Change in viral target Ct	RNAse P Ct (baseline)	RNAse P Ct (Panbio)	Change in RNAse P Ct (Panbio)	RNAse P Ct (Roche)	Change in RNAse P Ct (Roche)
Influenza A	18	9	7	26·4 (2·8)	31·1 (2·6)	4·7 (1·9)	26·4 (2·5)	31·1 (2·2)	4·7 (3·3)	27·0 (2·9)	29·8 (2·4)	2·8 (1·6)	30·1 (2·3)	3·1 (1·3)
Influenza B	15	4	4	22·1 (2·6)	29·8 (4·6)	7·7 (3·0)	23·1 (4·1)	28·8 (0·7)	5·7 (4·1)	28·0 (3·4)	31·3 (3·6)	3·3 (1·8)	31·9 (3·7)	3·9 (2·8)
Parainfluenza	20	11	12	25·8 (2·8)	30·9 (4·8)	5·1 (2·9)	26·9 (4·0)	31·3 (4·7)	4·4 (2·4)	25·9 (3·3)	28·6 (3·1)	2·7 (0·8)	29·7 (3·1)	3·8 (0·7)
Adenovirus	6	6	6	26·0 (5·5)	28·5 (5·3)	2·5 (0·7)	26·0 (5·5)	30·0 (5·6)	4·0 (0·3)	26·7 (2·9)	29·3 (3·0)	2·6 (0·4)	30·3 (3·1)	3·6 (0·7)
Human metapneumovirus	16	11	14	25·1 (3·0)	29·1 (3·0)	4·0 (0·9)	27·1 (4·8)	30·1 (4·3)	3·0 (1·9)	27·6 (2·4)	30·6 (2·3)	3·0 (0·8)	30·5 (2·3)	2·9 (0·6)
Seasonal coronavirus	19	8	2	23·9 (4·0)	33·9 (5·1)	10·0 (3·9)	19·3 (1·7)	35·7 (3·3)	16·4 (5·1)	26·9 (1·9)	29·7 (2·0)	2·8 (0·4)	30·6 (1·9)	3·7 (0·7)
Respiratory syncytial virus	18	16	16	26·5 (5·5)	30·0 (4·9)	3·5 (1·6)	26·6 (5·6)	29·7 (4·9)	3·1 (1·2)	27·6 (2·8)	30·0 (2·6)	2·4 (0·8)	30·2 (2·2)	2·6 (0·9)
Rhinovirus	28	24	27	26·0 (4·1)	29·9 (4·2)	3·9 (0·8)	26·7 (4·3)	30·7 (4·9)	4·0 (1·5)	27·5 (2·8)	31·2 (2·7)	3·7 (0·9)	30·4 (2·7)	2·9 (1·0)
Negative	20	..	..	..	..	..	..	..	..	26·8 (1·9)	30·7 (2·7)	3·9 (0·9)	30·5 (3·1)	3·7 (0·8)
Total	160	89 of 140	88 of 140	25·7 (4·1)	30·4 (4·3)	4·7 (2·7)	26·4 (4·6)	30·5 (4·5)	4·1 (2·8)	27·1 (2·7)	30·2 (2·8)	3·1 (1·1)	30·5 (2·7)	3·4 (1·3)

Data are n or mean (SD), unless specified. Ct=cycle threshold. *Mean (SD) PCR Ct value of baseline primary samples calculated only for samples detected on Panbio rapid antigen test devices. †Mean (SD) PCR cycle threshold value of baseline primary samples calculated only for samples detected on Roche rapid antigen test devices.

Table 1: **Detection of respiratory viruses from rapid antigen test devices and mean change in Ct using archived clinical samples**

more, viral recovery was lower at ten (23%) of 44 for Panbio and 16 (36%) of 44 for Roche rapid antigen test devices (table 2). For the eight samples with two respiratory viruses detected in the primary sample, both targets were detected in five of eight from Panbio and six of eight from Roche rapid antigen devices (appendix 1 p 23).

We did metagenomic sequencing on recovered nucleic acid from archival clinical samples (appendix 2 p 2). In total, we used 56 matched samples derived from Panbio and Roche rapid antigen test devices, with genomic sequences of target respiratory viral taxa detected in 55 (98%) of 56 samples from Panbio and 48 (86%) of 56 samples from Roche rapid antigen test devices. These results included detection of viral sequences from seven of eight samples for influenza A; eight of eight for influenza B (including two device-extracted samples that were not detected on rtPCR); and 16 of 16 for RSV (figure 2; appendix 1 p 15). We found sequences from two viral species of the same genera in four of 16 sample pools for adenovirus (adenovirus species B and C) and two of 16 sample pools for rhinovirus (rhinovirus species B and C). Most (98 [87·5%] of 112) of the viral genomes were more than 90% complete, with mean genome completeness of 86·7% (SD 35·0) for influenza A virus, 99·4% (SD 0·3) for influenza B virus, and 99·9% (SD 0·1) for RSV. Respiratory viral genome coverage was proportional to the relevant viral Ct value (adjusted R^2=0·54, p<0·0001; appendix 1 p 16), with higher coverage for samples with lower Ct values. We subtyped influenza A virus, influenza B virus, and RSV genomes. Influenza A/H3N2, influenza B/Victoria, influenza B/Yamagata, RSV-A and RSV-B were recovered, with broadly matching subtypes between Panbio and Roche rapid antigen test devices across all methods of subtyping (appendix 1 p 24; appendix 2 pp 3–4). Global strain types for influenza viruses were consistent between consensus genomes recovered from the two devices for each method (appendix 2 p 5).

We evaluated the stability of viral nucleic acid through PCR and whole-genome sequencing using archival clinical samples applied to rapid antigen devices incubated for up to 120 h. PCR detection of influenza A and influenza B viruses decreased over time with length of incubation (appendix 1 p 11, 25) and detection was improved by whole-genome sequencing up to 120 h (appendix 1 p 17; appendix 2 p 6). In addition, refrigeration at 4°C or the use of the preservative, RNALater, had improved viral detection at 72 h and 1-week timepoints compared with no preservative (nuclease-free water control) or non-refrigerated storage conditions, particularly for influenza B (appendix 1 pp 12–13; appendix 1 p 18; appendix 1 pp 26–29).

To assess real-world application, we tested rapid antigen test devices collected from patients who had reported respiratory symptoms at The Royal Melbourne Hospital emergency department. Over the 2-week period, 31 samples were collected, including five test devices that were positive for SARS-CoV-2. Of the 26 COVID-19-negative rapid antigen test devices, six had a respiratory virus detected on PCR—one was positive for influenza A virus, one was positive for RSV, and four were positive for rhinovirus (appendix 2 p 7). Only SARS-CoV-2 was detected by PCR from the five positive rapid antigen test devices. Sequencing of the 11 positive samples recovered near-complete genomes for influenza A, RSV, and the five SARS-CoV-2 samples (appendix 1 p 19, appendix 2 p 8). Sequence coverage was lower for the four rhinovirus samples. Subtyping was possible on all 11 samples (appendix 2 p 8).

Discussion

In this study, we demonstrated the feasibility of detection and genomic sequencing of non-SARS-CoV-2 respiratory viruses from two commercially available COVID-19 rapid antigen test devices. All eight viral taxa, representing the most common causes of human respiratory viral infection,

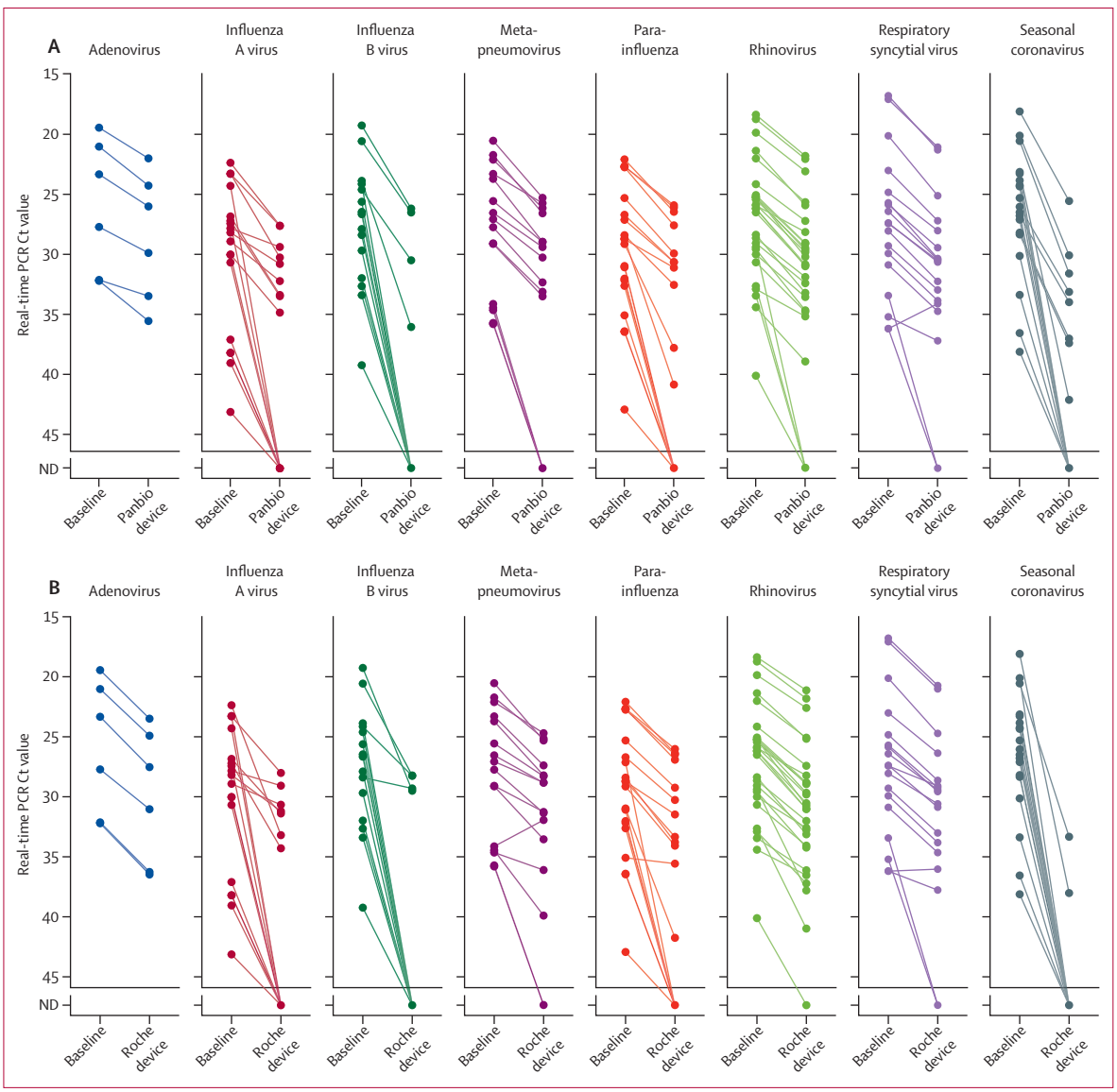

Figure 1: **Respiratory viral nucleic acid recovery from COVID-19 rapid antigen test devices**
Comparison of PCR detection and Ct values between baseline and device-extracted samples from Panbio (A) and Roche (B) rapid antigen test devices. Ct=cycle threshold.

were detected by PCR from device-extracted samples. PCR test concordance was high between rapid antigen device-extracted samples and the primary sample, particularly for samples with Ct values of less than 30. Furthermore, we recovered near full-length viral sequences in device-extracted samples, highlighting the potential for using rapid antigen test devices to supplement existing public health surveillance programmes for respiratory viruses.

PCR concordance between device-extracted samples and primary samples varied across the different respiratory viruses tested. Adenovirus, RSV, and rhinoviruses had high test concordance, and detection was lowest for seasonal coronaviruses and influenza B virus. There was reduced recovery of seasonal coronaviruses, influenza A, and influenza B viruses following incubation for longer than 24 h using both clinical samples and viral culture isolates, which might relate to viral particle stability. Disruption of viral particle envelopes probably occurs during interaction with the device extraction buffer due to the presence of surfactant (eg, Tween-20 present in the Panbio rapid antigen test device buffer).[20] Degradation of the viral envelope might affect subsequent viral nucleic acid stability, thereby reducing recovery of nucleic acid material, as observed for influenza A and B viruses. Conversely, reduced activity of surfactant on non-enveloped viruses (adenovirus and rhinoviruses), in addition to greater environmental stability of non-enveloped viruses, might explain their improved detection from rapid antigen test devices. Non-enveloped viruses are more resistant to drying or desiccation,[24] which can facilitate improved recovery during the device extraction

	Total samples			Panbio		Roche	
	Total	Ct <30	Ct ≥30	Detected Ct <30 in primary sample	Detected Ct ≥30 in primary sample	Detected Ct <30 in primary sample	Detected Ct ≥30 in primary sample
Influenza A	18	10	8	8	1	7	0
Influenza B	15	11	4	4	0	4	0
Parainfluenza	20	11	9	11	0	10	2
Adenovirus	6	4	2	4	2	4	2
Human metapneumovirus	16	11	5	11	0	11	3
Seasonal coronavirus	19	15	4	8	0	2	0
Respiratory syncytial virus	18	13	5	13	3	13	3
Rhinovirus	28	21	7	20	4	21	6
Total	140	96	44	79/96	10/44	72/96	16/44

Data are n or n/N, unless specified. Ct=cycle threshold.

Table 2: **Detection of respiratory viruses from rapid antigen test devices stratified by baseline Ct value (Ct<30 or Ct≥30)**

process. The reason for the poor recovery of influenza B virus compared with other enveloped viruses from both rapid test devices is unclear; however, in a study assessing virus stability on banknotes, the duration of influenza B virus infectiousness was shown to be as short as 1–2 h in the absence of respiratory mucus.[25]

Improvement in storage conditions, including the use of DNA or RNA stabilisers into the rapid antigen test device might improve viral nucleic acid stability and its subsequent extraction from rapid antigen test devices. We showed that storage of the test device at 4°C and application of RNALater improved recovery of viral nucleic acid material. These approaches represent a practical means for improving the recovery of viral nucleic acid material from test devices and might improve detection from samples with lower starting viral material (Ct value >30). Future work is required to assess the stability of clinical samples across a range of baseline Ct values.

Genomic surveillance has been instrumental in the public health response during the COVID-19 pandemic, providing a means of characterising circulating variants and allowing early detection of emerging variants of concern. WHO, through the development of the Global Influenza Surveillance and Response System Plus, aims to further develop integrated surveillance systems for respiratory viruses, including influenza viruses, RSV, and other respiratory viruses with pandemic potential,[26] which is dependent on robust genomic testing with high sample representation of circulating viruses in the community. The devastating outbreak of highly pathogenic avian influenza in poultry and wild birds in Europe in 2022, and its rapid geographical spread,[17] highlights the need for large-scale surveillance efforts for early detection of viruses with pandemic potential. Our study provides a proof-of-concept that rapid antigen test devices can be used as an additional sample type for respiratory virus sequencing, including the ability to accurately subtype circulating influenza viruses and RSV. As an example, we were able to successfully subtype influenza B/Yamagata from a rapid device using archival samples, a lineage now thought to be extinct.[5] We also demonstrated ability to obtain near-complete genomes from pooled samples, providing an approach to sequence on a larger scale with greater efficiency and lower cost.

We propose a potential model to expand respiratory virus surveillance using rapid antigen test devices: test devices can be collected at sentinel sites with high device use, including emergency departments, ambulatory centres, regional hospitals, and community care facilities for older adults. Devices could be stored in optimised conditions, either refrigerated or with the application of a preservative before storage, and transported to dedicated public health laboratories able to perform surveillance PCR testing and whole-genome sequencing. Expanding respiratory virus molecular testing to rapid antigen test devices has dual advantages of increasing community representativeness of surveillance sampling and, in outbreak situations, where there might be heightened use of rapid antigen test devices, allowing upscaling of pathogen tracking to assess transmission and the emergence of new variants.

There are several limitations to our study, including the small sample size and use of archival clinical samples from clinician-collected nose or throat swabs, rather than patient self-collected samples. We aimed to closely simulate the rapid antigen device self-testing method by following manufacturer instructions regarding sample input volumes and duration of incubation time before reviewing results. In addition, we demonstrated the feasibility of test collection through the emergency department, with successful detection and sequencing of non-SARS-CoV-2 respiratory viruses, including influenza A and RSV. Storage and transport conditions might negatively influence virus recovery when applied in the clinical setting. Indeed, PCR testing at 120 h after application of virus material to rapid antigen test devices had reduced ability to detect influenza A virus, influenza B virus, and seasonal coronaviruses compared with earlier testing at 2–3 h. However, optimised storage conditions and the addition of preservatives might be a promising and practical approach to improve viral nucleic acid recovery. Further work is required to assess the performance of storage conditions using clinical samples,

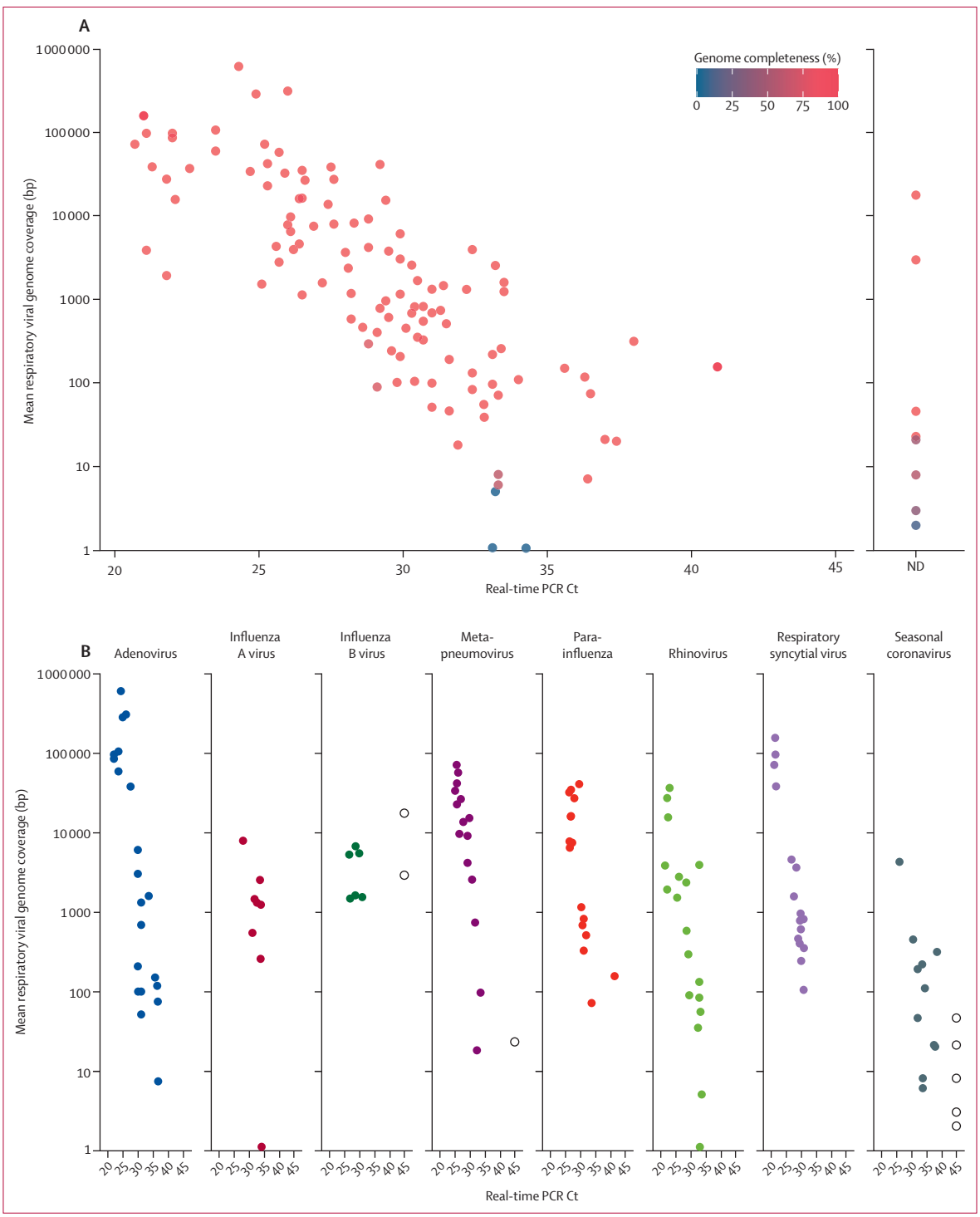

Figure 2: **Respiratory viral genome coverage from COVID-19 rapid antigen test devices**
(A) Mean respiratory viral genome coverage and genome completeness (bases in the genome represented by one or more reads as a portion of all bases in the genome) by Ct value. (B) Mean respiratory viral genome coverage by viral taxa. White circles indicate viral targets not detected on real-time PCR from device-extracted samples. Ct=cycle threshold. ND=not detected.

given the improvement in viral detection that was observed using viral culture isolates. Our approach of testing the rapid antigen test device strips rather than the device test kit buffer also improves feasibility, given the ease of transporting test devices compared with test kit buffers, which are often disposed of soon after testing and cannot be adequately

sealed for transport to prevent contamination or spillage. Finally, we were unable to directly compare the performance of Panbio and Roche rapid antigen test devices, given the difference in sample input volume might account for any observed differences in PCR Ct values across the two devices.

Detection of non-SARS-CoV-2 respiratory viruses from rapid antigen devices and the ability to recover near full-length viral sequences from these devices provides a valuable opportunity to expand surveillance programmes for public health monitoring of circulating respiratory viruses, including genomic surveillance. The feasibility of this process has already been shown at our centre through routine sequencing of SARS-CoV-2 from rapid antigen test devices and through successful detection and sequencing of non-SARS-CoV-2 respiratory viruses from clinical samples obtained from the emergency department. Our study provides a novel means for implementing broader public health monitoring of respiratory viruses, increasing access to samples with wider community representativeness. An expanded testing programme of respiratory viruses from rapid antigen test devices might facilitate early detection and characterisation of emerging viruses.

Contributors

MAM, GT, ES, GB-S, GEM, CKL, and DAW conceived and designed the study. MAM, GT, ES, and DAW prepared the manuscript. MAM, GT, ES, GB-S, MZ, GEM, JP, LC, IGB, TT, JD, CKL, and DAW contributed to the design and optimisation of experimental methods and preparation of clinical samples. Experiments were conducted by GT, GB-S, MZ, IS, MLT, SC, JM, JO, JP, GLP, MK, KS, JF, and TT. All authors had full access to all the data in the study and accepted responsibility for the decision to submit for publication. Underlying data for the study were accessed and verified by MAM, GT, and ES.

Declaration of interests

We declare no competing interests.

Data sharing

All consensus viral sequence data generated in this project are available at Bioproject (PRJEB67729) and the relevant code is available at https://github.com/esteinig/cerebro.

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