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CORRESPONDENCE

Performance evaluation of Roche and Abbott Panbio multiplex SARS-CoV-2 and influenza A/B rapid antigen tests

To the Editor,
The clinical presentation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for the ongoing coronavirus disease 2019 (COVID-19) pandemic, is similar to that of influenza illness and distinguishing the two infections based on symptom combinations is challenging.[1] Currently, real-time polymerase chain reaction (RT-PCR) assays are the primary means of distinguishing these infections. In the Southern Hemisphere, including Australia, the re-opening of international borders in early 2022 led to the first influenza season in 2 years. The peak of the influenza season in Australia in 2022 was predicted to coincide with continuing widespread transmission of SARS-CoV-2 (predominantly the Omicron variant) on a background of low immunity to influenza due to lack of both vaccination and natural infection in the preceding 2 years.

Respiratory sample collection for infection diagnosis can be uncomfortable and testing fatigue is a recognised consequence of the SARS-CoV-2 pandemic.[2] Therefore, simultaneous testing for multiple pathogens on a single sample helps mitigate these challenges. Rapid antigen testing (RAT) for SARS-CoV-2 has been in widespread use in Australia, including home self-testing. At the time of our study, no multiplex RATs that include simultaneous testing for influenza and SARS-CoV-2 had been approved for use in Australia by the Therapeutics Goods Administration.

There is currently a paucity of peer reviewed published performance data for multiplex SARS-CoV-2/influenza RATs. A study of combo RAT QuickNavi-Flu+COVID19 Ag (Denka Co Ltd, Japan) published in June 2022 for SARS-CoV-2 detection in 1,510 nasopharyngeal samples, found the sensitivity and specificity of the antigen test were 80.9% and 99.8%, respectively.[3] However, during the study period, influenza viruses were not detected by QuickNavi-Flu+COVID19 Ag or reference real-time RT-PCR and therefore the influenza component of this combo antigen test was not able to be evaluated. Two manufacturer sponsored trials of Panbio COVID-19/Flu A&B Rapid Panel are registered on ClinicalTrials.gov; however, results for these are currently pending.[4] Accordingly, we sought to evaluate the analytical performance characteristics of two multiplex SARS-CoV-2/influenza RATs, including performance with the locally dominant SARS-CoV-2 and influenza strains circulating in Victoria, Australia.

We performed an analytical evaluation of the SARS-CoV-2/FluA and Flu B combination Rapid Antigen Test (Roche, Australia) and Panbio COVID-19/Flu A&B Rapid Test Cassette (Abbott, Australia). To evaluate the analytical sensitivity of each multiplex antigen kit we used a dilution series of representative isolates for influenza A, influenza B, and irradiated SARS-CoV-2.[5] Omicron BA.2 was the chosen SARS-CoV-2 variant as this was dominant in circulation in Victoria at the time of testing. Influenza A H3N2 (A/Darwin/726/2019) and H1N1 (A/Victoria/2455/2019), and influenza B (B/Victoria/28/2020) vaccine isolates were kindly provided by the World Health Organization Collaborating Centre for Reference and Research on Influenza (WHO CCRI).

Each stock of SARS-CoV-2, influenza A and influenza B virus was serially diluted 10-fold in viral transport media (VTM) from 10^{-1} to 10^{-5}, equivalent to a range of viral loads between 8 $\log_{10}$ copies/mL to 4 $\log_{10}$ copies/mL. Briefly, each virus dilution was mixed with kit-supplied assay buffer in equal volume. The number of drops applied to each test cassette and incubation time were followed as per the manufacturer's instructions for use. Each dilution was tested in quadruplicate and limit of detection (LOD) was determined by the last dilution where all four replicates returned a positive result. Results were interpreted by at least two independent readers, with a third reader used if any discrepant results were obtained.

To assess specificity each kit was tested against a panel of quantified non-SARS-CoV-2 viruses previously prepared from VIDRL's collection. Briefly, duplicate samples were prepared for respiratory syncytial virus (RSV), human rhinovirus (HRV), adenovirus 3 (hAdV3), seasonal human coronaviruses 229E and OC43, human herpes simplex virus 1 (HSV1), cytomegalovirus (CMV) and parainfluenza virus 3. Each isolate was diluted 1/100 in VTM equivalent to a cycle threshold (Ct) value of $\leq$25 by an in-house respiratory RT-PCR assay.[6]

To simulate performance of each kit for detecting SARS-CoV-2 and influenza coinfection, different combinations of BA.2, influenza A H3N2 and influenza B were prepared. H3N2 (A/Darwin/726/2019) was selected as this was the major influenza A strain circulating in Australia at the time of the study. Combinations were prepared at a 1:1 ratio of high copies/mL ('strong') and low copies/mL ('weak') and applied to each cassette. A weak dilution (++) was defined as the last dilution where all four replicates tested positive for a given virus. For BA.2 and A/H3N2 this equated to 7.1 and 7.5 $\log_{10}$ copies/mL respectively, and 8.6 $\log_{10}$ copies/mL for B/VIC. A strong dilution (+++) was defined as the dilution that preceded, equating to 6.1 and 6.5 $\log_{10}$ copies/mL for BA.2 and A/H3N2, and 8.6 for B/VIC respectively. An 'all strong' sample was also prepared by adding all three viruses at a 1:1:1 ratio for the strongest dilutions described above. Although these combinations may not all reflect common viral loads in clinical coinfections, combinations were tested to check for any perturbation to test performance that might arise from strongly positive samples. Samples were mixed, applied to the test cassette, and incubated as per the manufacturer's instructions.

Mean Ct values representing viral load for each virus were calculated from triplicate real-time RT-PCR assays targeting the SARS-CoV-2 nucleocapsid (N) gene influenza A/H1N1 pdm09 Hemagglutinin (HA) gene, A/H3N2 Matrix protein 2 (M2) and influenza B nucleoprotein (NP) gene (Table 1). All assays were performed using the 2X Precision FAST qPCR Master Mix and amplification conditions of 95°C (2 min);

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Table 1 Primer and probe sequences for influenza and SARS-CoV-2 real-time PCR (RT-PCR) and droplet digital PCR (ddPCR) assays

	Forward	Reverse	Probe
SARS-CoV-2 N	CACATTGGCACCCGCAATC	GAGGAACGAGAAGAGGCTTG	FAM-ACTTCCTCAAGGAACAACATTGCCA
A/H1N1 pdm09 HA	GGAAAGAAATGCTGGATCTGGTA	ACCCTTGGGTGTCTGACAAGTT	6FAM-CAGTCCACGATTGCAAT-MGB
A/H3N2 HA	GCAACAGGRATGMGRAATG	CATTTTCTATGAAACCYGCKATTG	ATATGCCTCTAGTTTGTTTCTCT
B/VIC NP	ATGGATACAAGTCCTTATCAACTCTGC	TTCATTAARACGCTCGAAGAG	HEX−ccaTctTctTcaTcctccact− BHQ1

HA, hemagglutinin gene; N, nucleocapsid gene; NP, nucleoprotein.

×45 95°C (5 s), 60°C (30 s). Viral RNA copies/mL were quantified by droplet digital PCR (ddPCR) using the same primer sets and performed as previously.[6]

Overall, both multiplex kits were able to consistently detect BA.2, H1N1, H3N2 and B/VIC isolates at dilutions equivalent to viral loads between 7.1 and 7.6 $\log_{10}$ copies/mL (Fig. 1A), equivalent to cycle threshold (Ct) values of 17.3−19.5 on the in-house derived RT-PCR assay. BA.2, H1N1 and H3N2 were consistently detectable by both kits at 6.1−6.5 $\log_{10}$ copies/mL (Ct values 24.1−26.0). None of the kits detected the respective virus at Ct values of 29.5 (BA.2), 27.5 (A/H1N1), 29.4 (A/H3N2) or 27.7 (B/VIC). For specificity testing, reactivity was not observed against individual dilutions of the various non-SARS-CoV-2 or influenza viruses (Table 2), resulting in a specificity of 100% [95% confidence interval (CI) 62.8−100].

Simulated coinfections, tested by combining strong and weak dilutions of each virus isolate, returned results as expected (Fig. 1B) i.e., a strong detection for isolates included in high viral load, and a faint band for isolates with lower viral load. No inhibition of detection was observed for isolates that were included in low quantity. Interestingly, however, a faint false-reactive band was observed for influenza A in the influenza B/VIC (+++)/BA.2 (++) combination, but was not observed in the reciprocal sample, nor for any other combination.

Here we present the findings of the first analytical assessment of two commercial SARS-CoV-2 and influenza

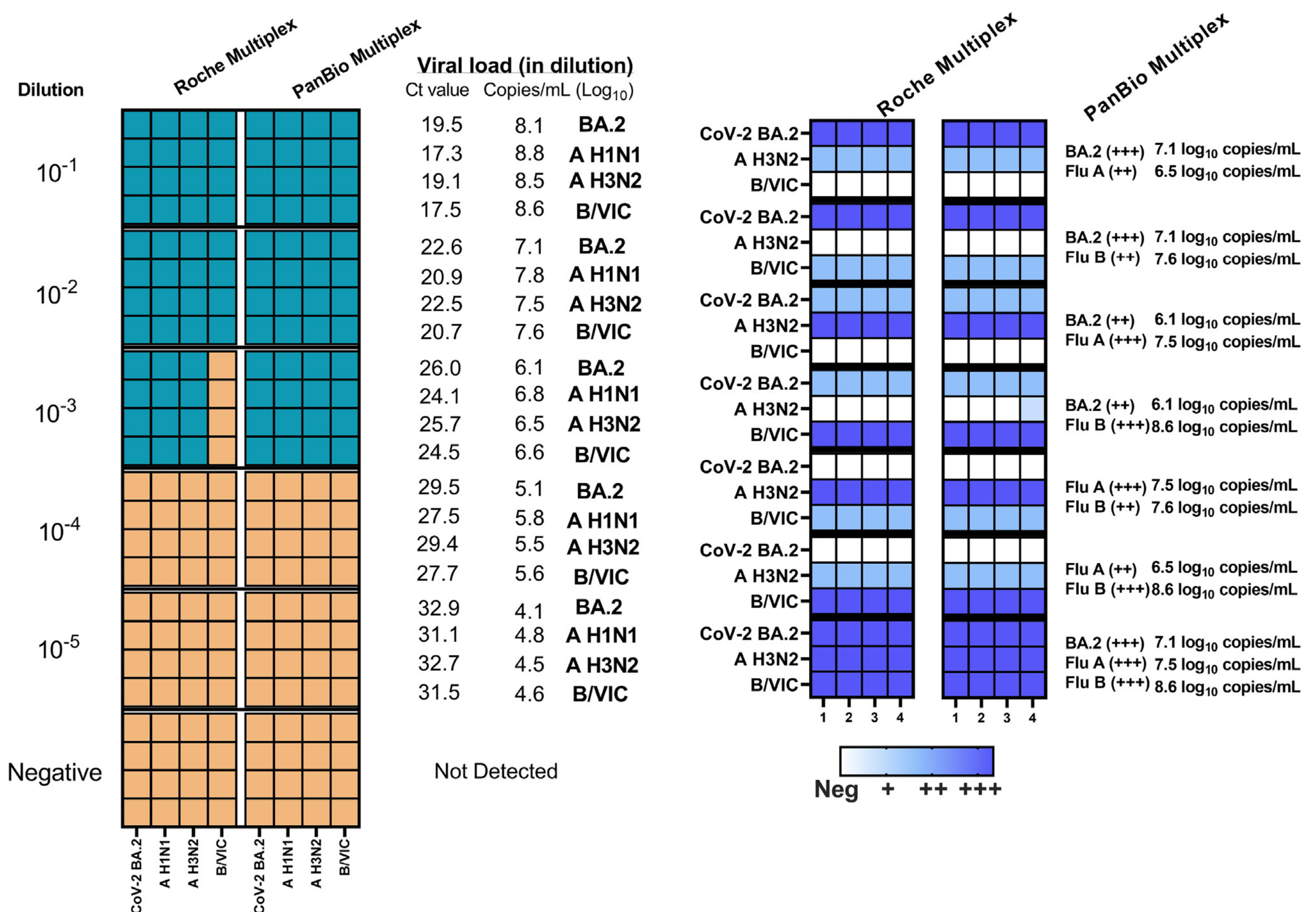

Fig. 1 Analytical sensitivities of two lateral flow SARS-CoV-2 and Flu A/B rapid antigen tests against clinical SARS-CoV-2 Omicron BA.2 and influenza A and B vaccine isolates. Antigen kits were tested against 10-fold dilutions of each virus in quadruplicate, for limit of detection (A). A negative control sample was also tested in quadruplicate. Blue boxes signify a positive test result in a single replicate and orange indicates a negative test result. Simulated coinfection evaluation (B) was performed by testing different combinations of viral load in a single sample and tested in quadruplicate. Combinations of viral load (copies/mL) are represented as high (+++) and low (++). The blue gradient depicts degree of positivity and white boxes represent a negative (Neg) result. Ct, cycle threshold.

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Table 2 Specificity panel of non-SARS-CoV-2 or influenza respiratory viruses

	RSV	HRV	hCoV 229E	hCoV OC43	hAdV3	HSV1	CMV	HPIV3
Log_{10} copies/mL (Ct value)	6.2 (25.0)	6.7 (23.0)	6.6 (25.0)	7.6 (19.0)	7.9 (19.0)	6.6 (23.0)	5.7 (24.0)	7.3 (23.0)
Roche	ND	ND	ND	ND	ND	ND	ND	ND
PanBio	ND	ND	ND	ND	ND	ND	ND	ND

CMV, cytomegalovirus; Ct, cycle threshold; hAdV3, human adenovirus 3; hCoV, human coronavirus; HPIV3, human parainfluenza virus 3; HRV, human rhinovirus; HSV1, human herpes simplex virus 1; ND, not detected; RSV, respiratory syncytial virus.

multiplex RATs against SARS-CoV-2 BA.2 Omicron, influenza A and influenza B. The removal of global travel restrictions in 2022 saw a significant increase in the transmission of influenza and other respiratory pathogens that had largely been absent throughout the COVID-19 pandemic. Early detection to appropriately isolate and treat symptomatic individuals presenting with influenza-like illness (ILI) is important to ongoing management of respiratory infections and protection of healthcare infrastructure.

Both kits were able to detect all four viruses between 7.1 and 7.6 log_{10} copies/mL. BA.2 and influenza A H1N1 and H3N2 were also detected at between 6.1–6.7 log_{10} copies/mL. The Panbio multiplex kit was also able to detect influenza B/VIC at 6.6 log_{10} copies/mL. Similar to concentrations used in our study, viral loads for infected individuals typically range between 5 and 10 log_{10} copies/mL for SARS-CoV-2, depending on sampling site,[5–7] and 4–8 log_{10} copies for influenza A and influenza B.[8] Because viral load may be a determinant of infectivity for individuals infected with influenza or SARS-CoV-2, understanding assay performance is necessary for consideration of their use in population testing. Further, as global travel recommenced other respiratory pathogens like RSV and adenovirus have re-emerged, contributing to the number of presentations with ILI. Thus, assay specificity is also an important feature of RATs, particularly when considering simultaneous detection of pathogens in a single specimen. Both kits were challenged with a panel of 'distractor' viruses and did not show any cross reactivity.

The 2022 winter 'flu season' in Australia coincided with increasing SARS-CoV-2 Omicron cases that placed growing pressures on the healthcare systems. More than 200,000 influenza cases and more than 10 million SARS-CoV-2 cases were reported by 30 August 2022.[9,10] In our setting, influenza H3 A/Darwin/726/2019-like is the dominant strain, as is B/Victoria A/Austria/1359417/2021-like.[9] Co-infections either with influenza A and B, or SARS-CoV-2 and influenza have also been reported and, although presentations are low compared to mono-infections, additional challenges may arise that complicate the treatment and prognosis of at-risk populations.[11,12] Indeed, we showed that analytical testing of SARS-CoV-2 (BA.2 Omicron) and influenza A/H3N2 and B/VIC in different combinations can be co-detected in a single specimen and suggests a likely usefulness in a real world setting. RATs for SARS-CoV-2 have played a significant role in the early detection of SARS-CoV-2 for rapid isolation and treatment of infected individuals and are currently the primary means of community diagnosis in Australia. In contrast to standalone SARS-CoV-2 RAT, many of the current generation multiplex kits require nasopharyngeal rather than deep nasal sampling, likely due to the traditional requirement of this sampling method for influenza and incorporating nasal sampling of the multiplex RAT would likely increase community usage. Nevertheless, it is anticipated that multiplex kits could play a vital role in subsequent winter seasons for detecting influenza and SARS-CoV-2 infection including during the soon anticipated northern hemisphere winter.

Both multiplex RAT devices studied detect BA.2 and influenza A and B isolates at viral loads observed in clinical infection and are able to detect combination SARS-CoV-2 and influenza simultaneously in a single specimen. Data in this study reflect analytical testing and monitoring of RAT performance in a clinical setting and are recommended to inform appropriate ongoing deployment.

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