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

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A joint venture between The University of Melbourne and The Royal Melbourne Hospital

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Background

- Clinical presentation of severe acute respiratory syndrome coronavirus (SARS-CoV2) is like influenza (1). Distinguishing between infections is challenging without molecular testing
- Access to Polymerase Chain Reaction (PCR) molecular testing has reduced, and is only available through General Practice referral or patient screening in hospital
- Point of care testing (POCT) using COVID-19 rapid antigen tests (RATs) has improved access to cheap and effective community diagnostics.
- Multiplex RATs, that target both influenza and SARS-CoV-2, have recently been included on the ARTG and provide a flexible POCT option for symptomatic respiratory illness
- Unlike COVID-19 RATs, there is little peer reviewed literature assessing the analytical performance of multiplexed antigen tests (2, 3)
- Here, we present the findings of an analytical assessment of 2 commercial multiplex SARS-CoV-2/influenza RATs

Table 1. 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

RSV: respiratory syncytial virus, HRV: human rhinovirus, hCoV: human coronavirus, hAdV3: human adenovirus 3, HSV1: herpes simplex virus 1, CMV: cytomegalovirus, HPIV3: human parainfluenza virus 3, Ct: Cycle threshold, ND: Not Detected

Results

Analytical LoD and specificity assessment

- Both kits were able to consistently detect BA.2, H1N1, H3N2 and B/VIC isolates at viral loads between 7.1 and 7.6 log_{10} copies/mL (Figure 1A), equivalent to Ct values between 17.3 and 19.5 on an in-house derived RT-qPCR assay
  - BA.2, H1N1 and B/VIC were all detectable at 6.1 to 6.5 log_{10} copies/mL (Ct 24.1-26)
- No viruses were detected by either kit above a 10^3 dilution (6.1-6.6 log_{10} copies/mL)
- All kits did not detect any non-SARS-CoV-2/influenza viruses in the distractor panel (Table 1), resulting in 100% (95% CI 62.8-100%) specificity

Combination testing

- Simulated co-infections, tested by combining strong (+++) and weak (++) dilutions of each virus returned results as expected (Figure 1B)
  - A strong band was observed for high-tire virus dilutions and a faint band for lower viral load respectively
- No inhibition was observed for viruses included in low titre (++) samples
- For the B/VIC (+++) / BA.2 (++) sample, a faint false-reactive band was observed at the influenza A mark on the test cassette, but not in the reciprocal or other combinations
- For the sample dilution where all viruses were included at the highest viral load, no inhibition was observed

Read the full article here

References

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Methods

Multiplex RATs and virus isolates

- *Antigen tests assessed in this study:* Roche SARS-CoV-2/FluA & FluB combination Rapid Antigen Test; Panbio COVID-19/FluA & FluB Rapid Test Cassette
- *Virus isolates:* Influenza vaccine isolates: A H3N2 (A/Darwin/726/2019) and H1N1 (A/Victoria/2455/2019); B (B/Victoria/28/2020); Gamma-irradiated Omicron BA.2. This isolate was the dominant variant at the time of testing

Analytical assessment

- Limit of detection (LoD) was assessed using 10-fold serial dilutions of each virus between 4-8 log_{10} copies/mL, tested in quadruplicate
- For co-detection experiments, viruses were pooled in combinations of strong (+++) and weak (++) dilutions ranging between 7.5-6.5 log_{10} copies/mL (A/H3N2), 7.1-6.1 log_{10} copies/mL (BA.2) and 8.6-7.6 log_{10} copies/mL (B/Vic). Weak dilutions were defined as the last dilution where all replicates were positive
- Specificity was assessed against a panel of viruses that would be commonly found in the respiratory tract. All samples were tested in duplicate
- For all testing, each virus was added to kit buffer at an equal ratio of 1:1, mixed thoroughly by inversion, and applied to each test device according to manufacturer instructions

Quantitative and reverse-transcription PCR

- Mean cycle threshold (Ct) for each virus was calculated from triplicate real-time RT-qPCR assays. Gene targets were 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
- Viral RNA copies/mL were quantified by droplet digital PCR (ddPCR) using the same primer sets and performed as previous (4)

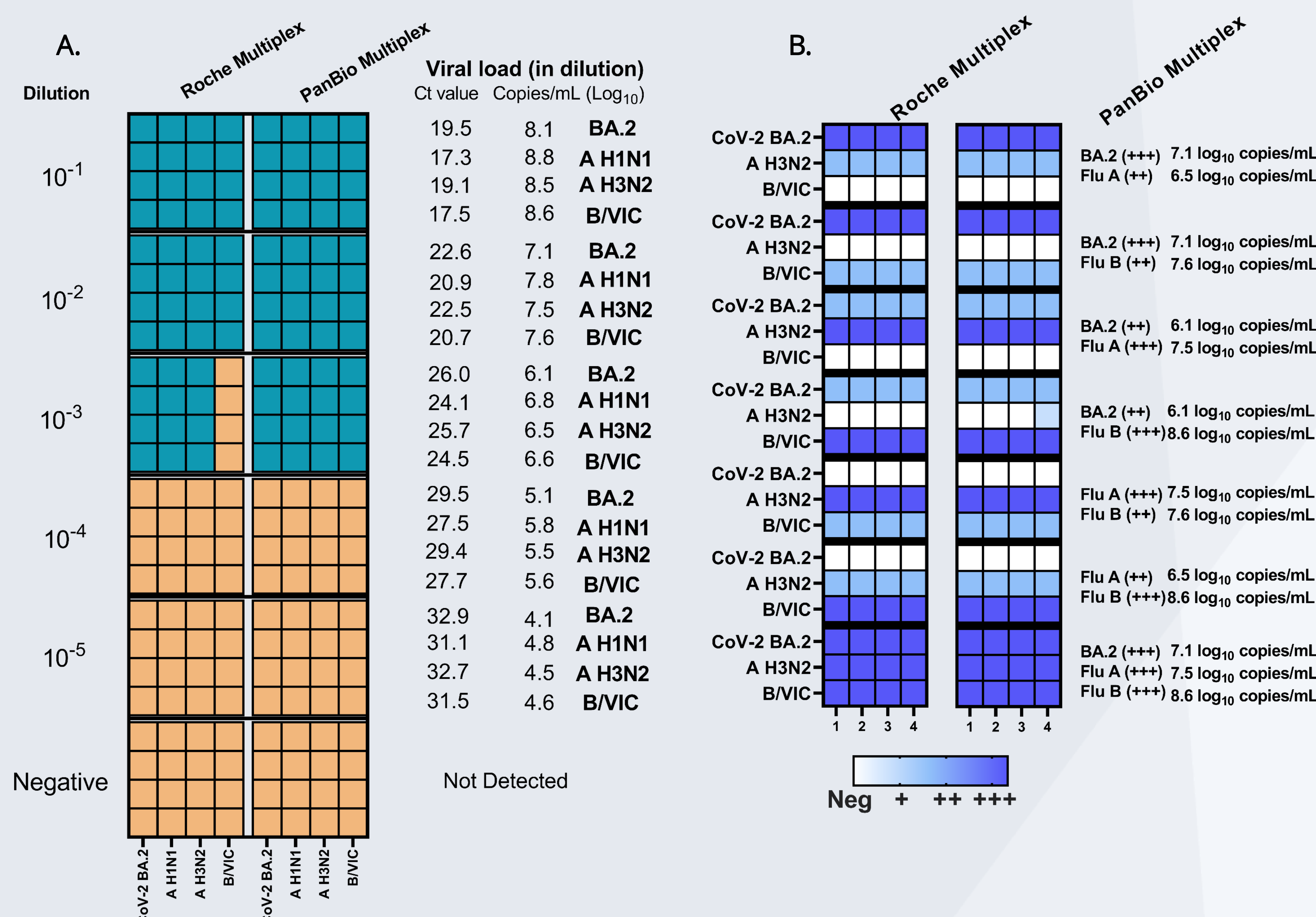

Figure 1. Analytical sensitivities of two lateral flow SARS-CoV-2 and Flu A/B rapid antigen tests against clinical SARS-CoV-2 Omicron BA.2 and influenza A and B vaccine isolates. Antigen kits were tested against 10-fold dilutions of each virus in quadruplicate, for limit of detection (A). A negative 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 a gradient from low (+) to high (+++). The blue gradient depicts degree of positivity and white boxes represent a negative (Neg) result. Ct, cycle threshold.

Summary

- The findings from this report highlight the analytical performance of two commercial antigen tests that co-detect influenza and SARS-CoV-2 in a single sample
- Both kits can detect viral loads in samples that are comparable to those that would be observed in clinical presentations (5, 6). Combined with high specificity, these results are indicative of acceptable performance for routine use where current generation COVID-19 RATs are commonplace
- Although not new technology, antigen tests have enhanced accessibility to rapid, cheap and effective diagnostics that can be performed in the home, thus revolutionizing the test, treat and isolate model, and can enable access to appropriate treatment options for vulnerable populations
- Dependencies on, and familiarity with, antigen tests as the primary means of diagnosing SARS-CoV-2 infection presents an opportunity to expand to include influenza. With influenza cases on the rise (7), accessing POCT devices that can differentiate between respiratory illness with confidence can play a vital role in subsequent winter seasons

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