Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Response

Dear Editor,

We welcome Stanley's correspondence about the performance of the AusDiagnostics Research Use Only (RUO) SARS-CoV-2 assay. In our evaluation [1], four samples where SARS-CoV-2 was detected by the AusDiagnostics RUO assay were not confirmed using the World Health Organization's (WHO) recommended nucleic acid tests targeting the E, RdRp, M, N, ORF 1b and ORF1ab regions of the SARS-CoV-2 genome in our laboratory [2].

In Australia, as of 17 May 2020 there have been 7045 confirmed cases and 98 deaths from COVID-19. Widespread testing, together with Australia's geographic advantage, border control, social distancing and public health messaging measures have all contributed to limit the number of infections in the country, including in the state of New South Wales (NSW). NSW has one of the highest testing rates in the world at 4523 tests administered per 100,000 population [3]. SARS-CoV-2 nucleic acid testing has been available in NSW since early January 2020, and over 365,000 tests have been performed as of 17 May 2020 in public and private laboratories using either in-house developed or commercial diagnostic assays. Prior to intended use, such assays should be validated to ensure they are fit for purpose, particularly when testing for novel pathogens with pandemic potential.

The AusDiagnostics RUO assay is a real-time, nested, intercalating-dye based PCR assay. Probe-based assays (such as our in-house developed, real-time PCR [RT-PCR] assays based on the WHO recommended targets) are more specific than intercalating dye-based assays as non-specific PCR products and primer dimers may generate fluorescent signals. Analysis of melting curves may help identify these non-specific amplicons, provided that the assay's target(s) are specific for SARS-CoV-2 and no other amplicons that are generated dissociate at the same temperature. As the primers used in the AusDiagnostics RUO assay are not publicly available, we were not able to verify the manufacturer's claims of the correct melting temperature. False positive results of other AusDiagnostics assays using the same methodology have been previously attributed to incorrectly calibrated equipment and poor quality power supply [4].

We reported that the sensitivity, specificity, positive predictive value (PPV) and negative predictive value of the AusDiagnostics RUO assay was 100 %, 92.16 %, 55.56 % and 100 %, respectively when compared to our RT-PCR assay. Even if the specificity of the AusDiagnostics RUO assay was 99 % (i.e. a 1% false positive rate), given the current prevalence of COVID-19 infection in NSW of 0.84 %, the calculated PPV of the assay would be 54.15 %, which is concordant with our findings. Cohen et al. also estimated false positive rates of up to 7% in commercial diagnostics assays detecting SARS-CoV-2 [5].

Whilst SARS-CoV-2 whole genome sequencing (WGS) may have resolved the issue of the four false positive samples, quality consensus sequences are often difficult to obtain from samples with RT-PCR cycle threshold values > 30 [6,7]. Partial sequencing of the SARS-CoV-2 genome was considered less informative without knowledge of the AusDiagnostics RUO assay's target(s) and not attempted as it may not be able to distinguish closely related coronaviruses compared to WGS. Furthermore, false positive RT-PCR results have also been reported from commercial kits that have been contaminated with SARS-CoV-2 sequences [8].

Since our manuscript was written, AusDiagnostics Pty Ltd now offer several diagnostic assays for the detection of SARS-CoV-2, including the AusDiagnostics SARS-CoV-2, influenza and RSV 8-well assay (catalogue number 20,081). Of note, the reported sensitivity and specificity for this dual target assay is 100 % and 100 % for the "a" target and 97.7 % and 99.4 % for the "b" target, respectively [9]. This suggests that during their evaluation, there were samples where the "a" but not "b" target was detected, despite the "b" target having a reported lower limit of detection (175 copies/mL vs 2150–4325 copies/mL). The reported specificity of 99.4 % for the "b" target also implies that false positive results were encountered. The assay's instructions for use however, recommends that detection of either one of the two targets is sufficient to call a result positive.

As Stanley correctly points out, it is important that diagnostic assays have high sensitivity in order to detect all persons with suspected COVID-19 and limit further transmission of infection. However, it is equally important that assays do not misdiagnose a person with COVID-19 infection. Unnecessary quarantining and contact tracing, delays in treating the true illness, exposure and acquisition of infection from other patients with confirmed COVID-19 in a nosocomial setting, wastage of personal protective equipment and potential requirements for second-tier confirmatory testing are all potential negative consequences of a false positive result.

Declaration of Competing Interest

No conflicts of interest declared

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