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LETTER TO THE EDITOR

Estimating the False-Positive Rate of Highly Automated SARS-CoV-2 Nucleic Acid Amplification Testing

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Molecular testing for infectious diseases is generally both very sensitive and specific (1). Well-designed PCR primers rarely cross-react with other analytes, and specificities seen during test validation are often 100% (2). However, analytical specificities measured during limited validation studies may not reflect real-world performance across the entire testing process. Surprisingly, there is exceedingly little literature on the specificity of high-throughput, sample-to-answer PCR testing, which critically informs debates on future SARS-CoV-2 screening algorithms.

Here, we examined the false-positivity rate of high-throughput, sample-to-answer nucleic acid amplification testing (NAAT) on three commercial assays: the Hologic Panther Fusion, Hologic Aptima, and Roche cobas 6800. Our University of Washington institutional review board (IRB)-approved study specifically used high-frequency testing in asymptomatic cohorts which confirmed any positive result on an orthogonal platform and attempted to recollect the patient/employee and retest within 24 h to confirm the initial positive result.

Our testing cohort comprised 7,242 results from 451 people (median age 27 years [interquartile range [IQR], 23 to 33 years]) repetitively sampled from May to November 2020 using nasal swabs collected by a health care worker. The median number of tests per individual was 10 (IQR 6 to 16), and the median number of days between consecutive tests was 2.1 days (IQR 2 to 5 days). During the study period there were 12 positive tests (0.17%) from 9 individuals (Table 1). Total and positive results by platform were as follows: Panther Fusion, 1,932 (26.7%, six positive); Aptima TMA, 1,526 (21.0%, four positive), and Roche cobas 6800, 3,784 (52.3%, two positive). Eight positive tests (0.11%, five individuals) were considered bona fide true positives based on repeat positives or outside testing and epidemiological data. One positive test had no follow-up testing and could not be adjudicated beyond it testing positive on an orthogonal platform, suggesting it was likely a true positive as well. Three positive tests from three separate individuals did not repeat as positive on a subsequent collection, nor did the original positive specimen test positive on an orthogonal platform (cases 2, 3, and 6). We consider these three tests false positives and estimate the overall false-positive rate of high-throughput, automated, sample-to-answer NAAT to be approximately 0.04% (3/7,242, 95% confidence interval [CI], 0.01 to 0.12%), yielding a specificity of 99.96% (95% CI, 99.88 to 99.99%). Two out of three of our false-positive cases occurred using the Aptima TMA assay with relative light unit (RLU) values of 614 and 615, respectively. The Washington State SARS-CoV-2 test positivity rate was 3.7% to 10.2% during the study period (https://www.doh.wa.gov/Emergencies/COVID19/DataDashboard). The main limitation to this work is that our measured false-positivity rate may not be generalizable outside our lab, and there is a potential that our false positives could constitute low-level blip shedding (3). Only one other study has examined the false-positive

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TABLE 1 Positive results and association with screening testing in this population and their interpretation[a]

Case	Previous result	Days from prior test	First test platform	Repeat positive on orthogonal?	C_T first test primary platform	C_T first test orthogonal platform	Next result	Days to next test	Second test platform	Clinical interpretation
1	Negative	14	cobas	Yes	32.5	34.6	Positive	1	Unknown	True positive[b]
2	NA	NA	Aptima	No	NA	Negative	Negative	1	Aptima	False positive[b]
3	NA	NA	Aptima	No	NA	Negative	Negative	1	Aptima	False positive[b]
4	Negative	2	Fusion	Yes	37.5	34.72	NA	NA	NA	True positive[c]
5	Inconclusive	1	Aptima	Yes	NA	33.7	NA	NA	NA	True positive
6	Negative	5	cobas	No	31.8	Negative	Negative	7	cobas	False positive
7	Negative	0	Fusion	Yes	30.6	29.38	Positive	6	Fusion	True positive
8	Negative	2	Fusion	Yes	17.4	17.93	Positive	1	Fusion	True positive
9	Negative	10	Aptima	Yes	NA	30.4	NA	NA	NA	Unknown

[a]Abbreviations: C_T, threshold cycle; NA, not available.
[b]Testing at an outside lab was performed within 1 day after our result; no C_T value was available.
[c]Treated as a clinical true positive. The individual developed symptoms within 1 day of test and had close contact with a person who was positive.

rate of SARS-CoV-2 testing, which found a similarly low rate (0.1%), albeit for the Thermo TaqPath assay extracted on the KingFisher Flex, which is neither a sample-to-answer platform nor random access (4). Overall, these very low false-positivity rates associated with high-throughput SARS-CoV-2 testing offer confidence in SARS-CoV-2 PCR test results and help laboratorians, epidemiologists, and regulators understand the specificity and positive predictive value associated with high-throughput NAAT.

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