



Is the Patient Infected with SARS-CoV-2?

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ABSTRACT The U.S. Food and Drug Administration currently uses the nasopharyngeal swab specimen as the reference standard for evaluation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) assays. We propose that the patient-infected status algorithm is a superior way to classify whether an individual is infected or not infected.

KEYWORDS assay, assess, COVID-19, SARS-CoV-2, testing

Determining whether a person is infected or not infected, a patient's infected status, is critical for patient care and evaluating new diagnostic assays (1). When there is an absence of a perfect test, this task can be difficult. The current reference standard used by the U.S. Food and Drug Administration (FDA) to determine whether a person has a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is an RNA-positive nasopharyngeal specimen tested with an existing emergency use-authorized (EUA) PCR assay (2).

A shortcoming of using a single specimen type (nasopharyngeal swab, nasal fluid, oral fluid, etc.) to determine the infected status of an individual for the evaluation of new diagnostic assays is that, often, there is not a single specimen type that is reliably positive (3). In the course of SARS-CoV-2 infection, the earliest anatomic site of infection might be the saliva or oropharynx, followed by the nasopharyngeal mucosa and then the lower respiratory tract (4). By fixing a reference standard to one anatomic site to assess new tests, this may increase the risk of determining that a new assay performs worse than it truly does, typically in the direction that the new assay is less specific because it identifies "unconfirmed infections." Less commonly, the new assay may be found to be less sensitive if the single reference comparator detects clinically insignificant infections beyond the relevant clinical period of infection.

Studies have found that when nasopharyngeal specimens were compared to specimens from other anatomic sites (nasal and oral fluid or saliva), infected persons were missed by tests using nasopharyngeal specimens (5–7). Additionally, in one study that detected SARS-CoV-2 RNA in saliva specimens from 13 asymptomatic persons with 9 nasopharyngeal specimens matched to those samples, 7 nasopharyngeal specimens did not have detectable SARS-CoV-2. All 13 individuals tested positive, however, for CoV disease 2019 (COVID-19) on repeat nasopharyngeal swab testing (5). In a recently published meta-analysis of 37 studies with 2,372 paired specimens comparing salivary and nasopharyngeal specimens, 13 studies found tests using saliva detected a greater number of infected cases than tests using nasopharyngeal specimens (7). Thus, the detection of infection may vary based on the anatomic site of specimen collection as it relates to the duration and time course of infection.

Before the invention of highly sensitive molecular tests, the diagnosis of infection often relied primarily upon culturing the causative infectious agent (bacterium, virus, fungus, etc.) in a clinical laboratory. With new molecular tests that are generally more sensitive than culture or observational methods for certain microorganisms, there is an absence of a reference

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standard. To address that lack of reference standard and reduce overestimation of sensitivity bias resulting from one analytic method, called discrepant analysis, the FDA, nearly 10 years ago, proposed the use of a composite reference standard known as the patient infected status (PIS) algorithm (8). That comparator method is based on the findings of culture (the accepted reference standard) or at least 2 or more nucleic acid detection tests using specimens from at least 2 or more anatomic sites (9–11). The PIS algorithm was used by the FDA as the reference comparator method for the recent approval of new diagnostic devices to detect extragenital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections (12).

To address the concern that the current evaluation of SARS-CoV-2 diagnostic assays may be biased toward decreased specificity, based on the use of a single anatomic site used as a reference comparator, we recommend that the FDA and commercial assay manufacturers adopt the use of the PIS algorithm. In that case, with the use of 3 anatomic site comparator specimens (i.e., nasopharyngeal specimens, nasal specimens, and oral fluid/saliva specimens), at least 2 sites would have to be positive for the reference comparator to be considered positive and at least 2 sites would have to be negative for the reference comparator to be considered negative. Other combinations of results (e.g., 1 positive, 1 negative, and 1 indeterminate or no test) would be considered equivocal or indeterminate.

It is time to advance the evaluation of SARS-CoV-2 testing to catch up with the increasing knowledge about the biology of SARS-CoV-2 infection. At the beginning of the pandemic, it was simple and convenient to select a single specimen type as a reference comparator. Now, however, with new knowledge and better information, the regulatory framework should be updated.

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REFERENCES

- Ronn MM, Mc Grath-Lone L, Davies B, Wilson JD, Ward H. 2019. Evaluation of the performance of nucleic acid amplification tests (NAATs) in detection of chlamydia and gonorrhoea infection in vaginal specimens relative to patient infection status: a systematic review. *BMJ Open* 9:e022510. <https://doi.org/10.1136/bmjopen-2018-022510>.
- Food and Drug Administration. 2020. Molecular diagnostic template for commercial manufacturers. Food and Drug Administration, Silver Spring, MD. <https://www.fda.gov/media/135900/download>.
- Mallett S, Allen AJ, Graziadio S, Taylor SA, Sakai NS, Green K, Suklan J, Hyde C, Shinkins B, Zhelev Z, Peters J, Turner PJ, Roberts NW, di Ruffano LF, Wolff R, Whiting P, Winter A, Bhatnagar G, Nicholson BD, Halligan S. 2020. At what times during infection is SARS-CoV-2 detectable and no longer detectable using RT-PCR-based tests? A systematic review of individual participant data. *BMC Med* 18:346. <https://doi.org/10.1186/s12916-020-01810-8>.
- Dos Santos WG. 2020. Natural history of COVID-19 and current knowledge on treatment therapeutic options. *Biomed Pharmacother* 129:110493. <https://doi.org/10.1016/j.biopha.2020.110493>.
- Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, Warren JL, Geng B, Muenker MC, Moore AJ, Vogels CBF, Petrone ME, Ott IM, Lu P, Venkataraman A, Lu-Culligan A, Klein J, Earnest R, Simonov M, Datta R, Handoko R, Naushad N, Sewanan LR, Valdez J, White EB, Lapidus S, Kalinich CC, Jiang X, Kim DJ, Kudo E, Linehan M, Mao T, Moriyama M, Oh JE, Park A, Silva J, Song E, Takahashi T, Taura M, Weizman OE, Wong P, Yang Y, Bermejo S, Odio CD, Omer SB, Dela Cruz CS, Farhadian S, Martinello RA, Iwasaki A, Grubaugh ND, et al. 2020. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. *N Engl J Med* 383:1283–1286. <https://doi.org/10.1056/NEJMc2016359>.
- Kojima N, Turner F, Slepnev V, Bacelar A, Deming L, Kodeboyina S, Klausner JD. 19 October 2020. Self-collected oral fluid and nasal swab specimens demonstrate comparable sensitivity to clinician-collected nasopharyngeal swab specimens for the detection of SARS-CoV-2. *Clin Infect Dis* <https://doi.org/10.1093/cid/ciaa1589>.
- Bastos ML, Perlman-Arrow S, Menzies D, Campbell JR. 12 January 2021. The sensitivity and costs of testing for SARS-CoV-2 infection with saliva versus nasopharyngeal swabs: a systematic review and meta-analysis. *Ann Intern Med* <https://doi.org/10.7326/M20-6569>.
- McAdam AJ. 2000. Discrepant analysis: how can we test a test? *J Clin Microbiol* 38:2027–2029. <https://doi.org/10.1128/JCM.38.6.2027-2029.2000>.
- Food and Drug Administration. 2011. Draft guidance for industry and Food and Drug Administration staff; establishing the performance characteristics of in vitro diagnostic devices for *Chlamydia trachomatis* and/or *Neisseria gonorrhoeae*: screening and diagnostic testing; availability. Food and Drug Administration, Silver Spring, MD. <https://www.federalregister.gov/documents/2011/05/11/2011-11532/draft-guidance-for-industry-and-food-and-drug-administration-staff-establishing-the-performance>.
- Food and Drug Administration. 2015. Nucleic acid amplification assays for the detection of *Trichomonas vaginalis*—class II special controls guideline for industry and Food and Drug Administration staff. Food and Drug Administration, Silver Spring, MD. <https://www.fda.gov/medical-devices/guidance-documents-medical-devices-and-radiation-emitting-products/nucleic-acid-amplification-assays-detection-trichomonas-vaginalis-class-ii-special-controls>.
- Doernberg SB, Komarow L, Tran TTT, Sund Z, Pandori MW, Jensen D, Tsaliak EL, Deal CD, Chambers HF, Fowler VG, Evans SR, Patel R, Klausner JD. 2020. Simultaneous evaluation of diagnostic assays for pharyngeal and rectal *Neisseria gonorrhoeae* and *Chlamydia trachomatis* using a master protocol. *Clin Infect Dis* 71:2314–2322. <https://doi.org/10.1093/cid/ciz1105>.
- Food and Drug Administration. 2019. FDA clears first diagnostic tests for extragenital testing for chlamydia and gonorrhea. Food and Drug Administration, Silver Spring, MD. <https://www.fda.gov/news-events/press-announcements/fda-clears-first-diagnostic-tests-extragenital-testing-chlamydia-and-gonorrhea>.