Clinical Infectious Diseases

INVITED COMMENTARY

Validating combination throat-nasal swabs for COVID-19 tests would improve early detection, especially for the most vulnerable

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Individuals with immunocompromise and other vulnerable groups at high risk for severe disease continue to rely heavily on COVID-19 testing. This reliance often includes screening contacts prior to in-person interactions, to prevent the risk of exposure to individuals with presymptomatic or asymptomatic infections. Even in the absence of symptoms or known exposure, individuals with immunocompromise may also test themselves regularly to identify early infection and quickly initiate treatment. For this population—approximately 7 million people in the U.S. with primary immunodeficiencies or immunosuppressive treatment for cancer, transplants, or autoimmune disorders[1]—tests that detect early infection with high sensitivity are essential.

Among COVID-19 tests, the low cost, direct-to-consumer sale, and rapid results of at-home antigen rapid diagnostic tests (Ag-RDTs) make them an attractive and increasingly used diagnostic modality both for high-risk individuals and the general population.[2,3] While the United States Food and Drug Administration (FDA) has long been open to throat swab

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specimens for COVID-19 testing, all at-home Ag-RDTs are currently only authorized for use with self-collected nasal swabs.[4] However, nasal-swab Ag-RDTs have been demonstrated to have low to moderate (~50-80%) clinical sensitivity to detect infected individuals, especially when those individuals are asymptomatic and/or in the early stage of infection,[5] when transmission of SARS-CoV-2 often occurs.[6]

Several cross-sectional studies have demonstrated that Ag-RDTs exhibit higher clinical sensitivity when a combination of nasal (anterior nares) and throat (posterior oropharynx plus palatine tonsils) swabbing is used, compared with nasal-swab-only (**Fig 1A**). A small study in Nova Scotia evaluated the use of combined nasal and throat swabbing for two separate Ag-RDTs (Panbio COVID-19 Ag Rapid Test Device, BTNX Rapid Response COVID-19 Antigen Rapid Test) among asymptomatic individuals.[7] Among 62 and 40 infected individuals respectively, a 24% (Panbio) and 18% (BTNX) improvement in clinical sensitivity was observed by combining nasal-swab and throat-swab Ag-RDT results over nasal-swab-only Ag-RDT results. This study also demonstrated a 13% increase in clinical sensitivity by testing a single combined throat-nasal swab compared with nasal swab alone among 38 infected individuals. A separate, large study of 827 infected individuals in Copenhagen recently demonstrated that combined nasal-swab and throat-swab Ag-RDT results improved clinical sensitivity by upwards of 16% over nasal-swab Ag-RDT results alone.[8]

Longitudinal viral load data suggests that infection stage influences the magnitude of the benefit of combined throat-nasal swab Ag-RDT compared with nasal-swab-only Ag-RDT. Daily viral loads quantified from prospectively collected nasal and throat swabs by individuals with incident SARS-CoV-2 infection revealed that virus often presents in the throat days before the nose.[9] A simplified representation based on available data[9,10] for the typical presentation of viral loads in the throat and the nose during early infection illustrates how the benefit of adding throat swabs to nasal swab Ag-RDTs is expected to be greatest during the first few days of infection (**Fig 1B**). Indeed, based on quantitative viral-load measurements in the throat and nose during the first four days of incident infection, we predicted[5] that a combined throat-nasal swab Ag-RDT would have approximately 25% greater clinical sensitivity than a nasal-swab-only Ag-RDT (**Fig 1C**). This prediction was similar to the benefits observed in the later studies performed in Nova Scotia[7] and Copenhagen.[8] Additionally, supplemental data from Copenhagen shows that the benefit of combined throat-nasal swab Ag-RDT results over nasal-swab-only Ag-RDT decreased with time from symptom onset among individuals for whom healthcare workers collected specimens, from 32% on the first day of symptoms to 13% thereafter.[8]

The benefit of combined throat-nasal sampling extends to molecular COVID-19 tests as well. Among 14 individuals with naturally acquired incident SARS-CoV-2 infection, 10 (71%) had viral loads above 1000 copies/mL in throat swabs for at least a day before viral loads in the nose rose to over 1000 copies/mL.[9] For many individuals, the delay was longer: over a third of participants (5 of 14) had virus in the throat at least three days before the nose, and up to 7 days for one individual.[9] In a separate study of individuals who underwent intranasal inoculation with

SARS-CoV-2, 10 of 18 (55%) participants with sustained infection had detectable virus in the throat for at least one day before virus was detectable in the nose by PCR.[10] Notably, replication-competent (infectious) virus was successfully cultured from throat swabs prior to nasal swabs in 12 of these 18 individuals (67%). These data suggest if nasal swabs alone are used, even molecular COVID-19 tests with high analytical sensitivity (low limits of detection down to 1000 copies/mL) could yield false negative results for individuals who may be capable of transmitting SARS-CoV-2.[11] Analyses of paired viral load dynamics from the cohort with naturally acquired infection suggested that using combined throat-nasal swabs rather than a nasal-swab-only swab with a high analytical sensitivity molecular COVID-19 tests would result improve clinical sensitivity by over 40% during the first days of SARS-CoV-2 infection.[9] However, because a subset of individuals may present with rising viral loads in the nose before the throat, combination throat-nasal swab tests are likely to yield higher clinical sensitivity than throat swabs alone. Indeed, the current Infectious Diseases Society of America Guidelines on the Diagnosis of COVID-19[12] recommend against the use of throat swabs alone for both molecular diagnostic tests[13] and Ag-RDTs.[14]

Cross-sectional analyses of participant populations later in infection (after the first few days) are unlikely to observe the benefit of combining throat-nasal swabbing on Ag-RDT clinical sensitivity. For example, reanalyzing viral loads between days 0 and 12 of infection from our study[5] cross-sectionally predicted only a marginal benefit (6%) for combined throat-nasal swab Ag-RDT over the observed clinical sensitivity of a nasal-swab-only Ag-RDT (43%). This small, predicted benefit is similar to that observed in a later cross-sectional study of 96 infected individuals in San Francisco.[15] In that study, a combined throat-nasal swab Ag-RDT increased clinical sensitivity from 54% (nasal-swab only Ag-RDT) to 59%.[15] We note that the high PCR-positivity rate (83%) among the 115 participants screened may suggest a study population skewed towards later infection. The clinical sensitivity of a combined throat-nasal swab Ag-RDT may also be influenced by throat swab specimen collection technique,[16] or if a test designed for use with nasal swabs exhibits lower analytical sensitivity when used with throat swabs.[12,17]

Maximizing the clinical sensitivity of COVID-19 tests—both Ag-RDTs and molecular diagnostic tests—for early detection is paramount, particularly given surges in emerging variants with potential for evasion of humoral immunity.[18] To improve performance, Ag-RDTs and molecular COVID-19 tests need to be analytically and clinically validated by manufacturers for use with combination throat-nasal swab specimens, including clinical-validation studies on (at least) symptomatic patient specimens. This combination throat-nasal swab test could use a single swab sampling both the throat and the nose, or (to address consumer hesitancy) separately collected swabs from the nose and throat which could be placed into the same elution media. Based on past FDA flexibilities offered for the validation of COVID-19 tests for Emergency Use Authorizations (EUAs) (**Supplemental Table**), the FDA is likely to accept non-inferiority studies, perhaps even only on symptomatic patients (historically ~30 positives and 30 negatives required for the EUA). For clearance, the FDA may accept evaluation of the combined throat-

nasal swab against a standard single swab, and showing in at least symptomatic patients that the combination swab is not inferior (has equivalent or better sensitivity) on the requisite number of positive patients, usually 120 positive patients and 500 negative patients for an over-the-counter test. The best way for developers to determine what the FDA expects is through the Q-Submission process,[19] which is a no-charge FDA submission. The developers can ask their questions of the FDA and receive a response within 70 calendar days.[19] Although it may not be required for test validations, it would be particularly useful for studies to include populations for whom early detection is most impactful, such as the immunocompromised and those residing in congregate settings (e.g., skilled nursing facilities, dormitories). These populations would demonstrate just how useful combination throat-nasal swabs are for populations at high risk of transmission or severe disease. We also suggest studies to investigate whether the use of combined throat-nasal swabs provide similar benefit for diagnostic testing of other upper respiratory viral infections, such as influenza and respiratory syncytial virus.

FOOTNOTES:

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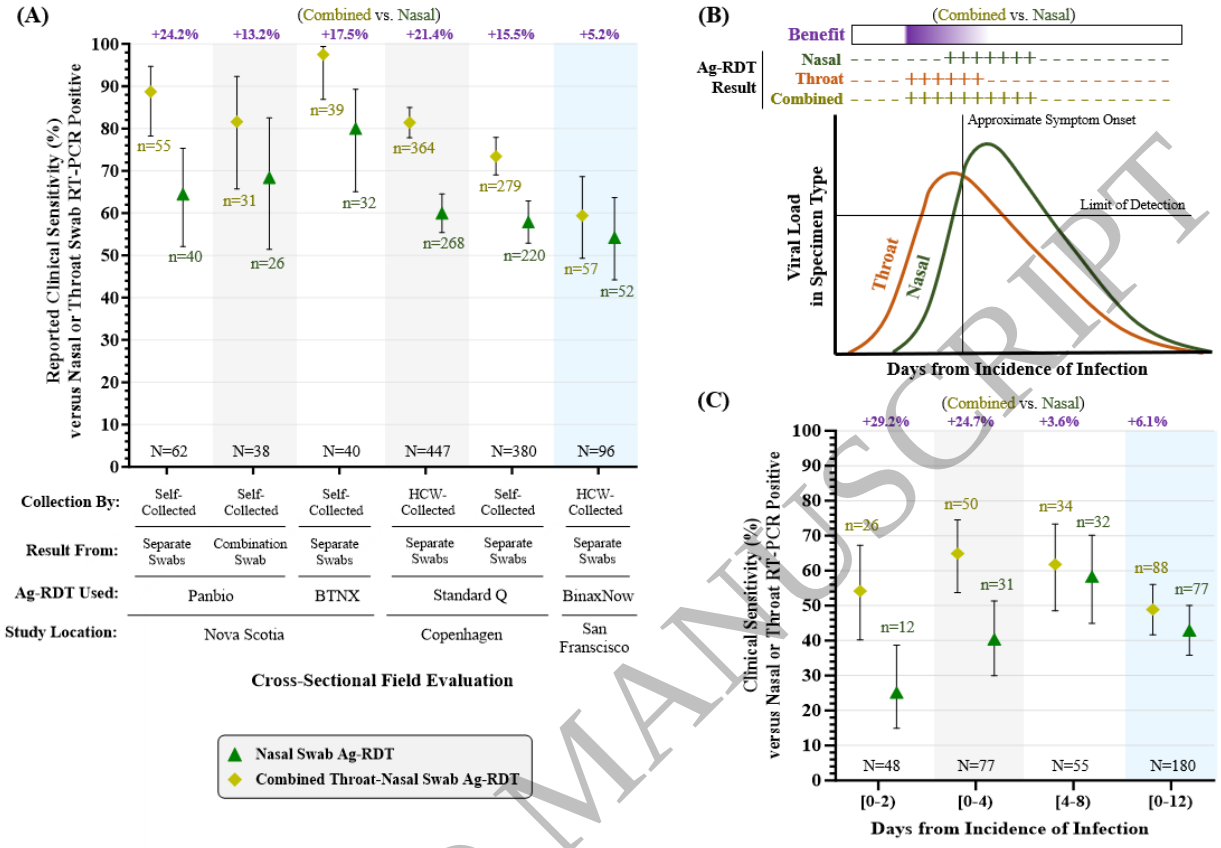

FIGURE 1. (A) Summary of studies reporting the clinical sensitivity of combined throat-nasal swab antigen rapid diagnostic tests (Ag-RDTs) compared with nasal-swab-only Ag-RDTs. The difference between clinical sensitivity of combined throat-nasal swab Ag-RDT results over nasal-swab-only Ag-RDT results alone is shown in purple. Data are reproduced from cross-sectional field evaluations in Nova Scotia,[7] Copenhagen,[8] and San Francisco.[15] These field evaluations had slight differences in design, which we highlight: 'HCW-collected' refers to nasal and throat swabs specimen collection performed by a by a healthcare worker, whereas 'Self-collected' refers to collection by the study participant. 'Separate Swabs' refers to designs where test results represent the composite outcome of testing a nasal swab and a throat swab, separately, whereas 'Combination Swab' refers to designs where the test result was determined by directly testing a single swab that had sampled both the nose and throat. **(B)** Conceptual schematic depicting the typical presentation of longitudinal SARS-CoV-2 viral loads in nasal and throat swab specimens from the incidence of infection, based on data from a study of individuals with naturally acquired infection in Los Angeles[9] and individuals inoculated with SARS-CoV-2 in London.[10] The hypothetical nasal, throat, and combined throat-nasal swab Ag-RDT results are expected based on this typical presentation of viral loads, to illustrate why the increased clinical sensitivity of a combined throat-nasal swab Ag-RDT over a nasal-swab-only Ag-RDT would be greatest during early in infection and wane during later infection. The horizontal line indicates

the limit of detection for Ag-RDTs. **(C)** Clinical sensitivity of combined throat-nasal swab Ag-RDT (inferred from viral loads) and nasal-swab Ag-RDT results (participant reported) during different periods of infection, based on data from a nasal-swab Ag-RDT field evaluation with paired viral load quantification in Los Angeles.[5] Blue shading in panels A and C highlight how cross-sectional evaluations that include timepoints late in the infection may underestimate the benefit of a combined throat-nasal swab Ag-RDT over nasal-swab-only Ag-RDT.

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