Feasibility of Antigen Test Kits for Massive Screening and Detection of SARS-CoV-2 (COVID-19) Infection

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Introduction:

N and S proteins of SARS-CoV-2 (COVID-19) are the main immunogens among the four structural proteins (E, M, N, and S) [1]. The N protein-IgG ELISA provides a sensitivity of 94.7 % that is significantly higher than that of the S protein-IgG ELISA [2]. Antibodies against N proteins are longer-lived and have greater volume, in comparison to E, M, and S proteins [2]. Approximately, 100 companies are manufacturing or developing rapid antigen tests (RATs), one of the four types (virus isolation with cell cultures, serological testing, RATs, and molecular techniques) [2] for SARS-CoV-2 (COVID-19) detection

[3]. Most RATs for SARS-CoV-2 (COVID-19) detection use a simple-to-use lateral flow test format that commonly used for influenza, malaria, and HIV testing as a sandwich immunodetection [4]. The testing results are interpreted by the operator within 10 to 30 minutes after collecting the respiratory specimen and applying it to the test strip [5]. In comparison to the nucleic acid amplification tests (NAATs), a decreasing sensitivity is found in the trade-off for simplicity of RATs for SARS-CoV-2 (COVID-19) operation [4]. As of September 11, 2020, only three companies submitted documents towards WHO's Emergency Use Listing (EUL) procedure, two tests have been approved by Japan's Pharmaceutical and Medical Devices Agency, and only four tests have received United States Food and Drug Administration (FDA) Emergency Use Authorization (EUA) [6, 7]. In comparison to NAATs in respiratory specimens (nasal or nasopharyngeal swabs), the specificity is consistently high (> 97 %), whereas the sensitivity ranges from 0 to 94 % [8-17]. When the cycle threshold (Ct) values are equal to or less than 25 or the SARS-CoV-2 (COVID-19) viral loads are more than 106 genomic virus copies/mL that frequently present in the pre-symptomatic period (1-3 days before the symptom onset) and the first 5-7 days of the acute COVID-19 illness phase [18-20]. Kweon et al conducted a study on evaluation of the diagnostic accuracy of the two newly developed, point-of-care, RATs, the ichroma COVID-19 AgTM and AFIAS COVID-19 Ag for detecting SARS-CoV-2 (COVID-19) infection by serially collecting 200 nasopharyngeal samples from 38 COVID-19-infected patients and 122 samples from negative control group [21]. The study revealed that both RATs demonstrated the sensitivity of 91.3 % to 100 % for samples with Ct < 25, whereas the specificity was 98.7 % to 98.9 % for AFIAS COVID-19 Ag and 100.0 % for ichroma COVID-19 AgTM [21]. The sensitivity of AFIAS COVID-19 Ag and ichroma COVID-19 AgTM for all targeted genes (E, N, and RdRP) was higher for samples collected before 7-days post-symptom onset than for those collected 8-14 post-symptom onset [21]. Stohr et al studied the sensitivity and specificity of the two self-testing kits (BD Veritor System-BD RAT, n = 1,604 and Roche SARS-CoV-2 RAT, n = 1,611) with lateral flow assay compared to the qtr.-PCR with Ct-value below the Ct-value cut-off demonstrated 78.0 % (95 % CI: 72.5-82.8) and 99.4 % (95 % CI: 99.0-99.6), respectively [22]. A test with the sensitivity of 80 % performed and implemented by at least 70 % of the population once a week was estimated to decrease the reproductive number of SARS-CoV-2 (COVID-19) from the basic reproductive number (R0) of 1.5 to the effective reproductive number (Re) below 1.0 [22].

The WHO has announced the general recommendations for the use of RATs for SARS-CoV-2 (COVID-19) detection as the following : 1) SARS-CoV-2 (COVID-19) RATs that meet the sensitivity of 80 % or higher and the specificity of 97 % or higher, compared to a NAAT reference assay where NAAT prolonged turnaround times preclude clinical utility or is unavailable; 2) Appropriate scenarios for the use of SARS-CoV-2 (COVID-19) RATs (responding to suspected COVID-19 outbreaks in remote settings, semi-closed communities, and institutions where NAAT is not immediately available, supporting outbreak investigations; testing of asymptomatic cases of contacts may be considered even if the RATs is not specifically authorized for this use, due to asymptomatic cases having been shown to have viral loads similar to symptomatic cases; where there is widespread community transmission; and monitoring

trends in COVID-19 incidence; 3) Initial introduction of RATs into clinical use; 4) in institutions where confirmed testing with NAAT in not feasible, and indications that RAT results may be incorrect should raise about validity suspicions; and 5) Use of RATS is not recommended in populations or settings with low expected prevalence of SARS-CoV-2 (COVID-19) (for examples; elective surgery, blood donation, screening at point of entry) [4]. The WHO also recommends the selection of RATs for procurement and implementation that includes 1) Quality of available data to validate the test; 2) Reported performance; 3) Manufacturing capacity and further evidence of quality; 4) Manufacturing quality and regulatory status; 5) Distribution and technical support; 6) Storage conditions, shelf-life, and shipping; 7) Availability, completeness, and clarity of instructions for use; 8) Cost of RATs; 9) Contents of RAT kit; and Specimen collection requirements [4]. Additionally, the WHO recommends the implementation considerations that include 1) Strictly following the supplier-recommended procedures; 2) Biosafety requirements for operators must be in place; 3) Each of these RATs has a specifically indicated method for specimen processing after collection; 4) Specimen collection is one of the most critical factors affecting performance of RATs; 5) Use of instrumented detection systems demands additional training requirements and sufficient infrastructure; and 6) Post-market surveillance with regulatory oversight [4]. Several variables may impact on RAT clinical performance that include 1) Pre-analytical influencers (sample type and way of sampling; collection device, transport media, and volume versus direct testing without dilution by transport media; time to test and storage/transport conditions, the time delay before processing); 2) Analytical influencers (Viral load of the specimen and viral load distribution in respective cohort (represented by SARS-CoV-2 (COVID-19) viral RNA copies/mL or Ct), analytical sensitivity and specificity of the PCR reference standard, PCR assay specifics, for examples, different targeted genes (E-/N-/RdRp-gene, etc., and across-laboratory differences (for examples, the definition of a positive specimen starting at Ct < 38 or < 40); and 3) Clinical parameters of the tested individual (days post-symptom onset of sampling, asymptomatic versus symptomatic status, the definition of symptoms " suspicion of SARS-CoV-2 (COVID-19) infection, and severity of symptom [23]. The sensitivity and specificity of the rapid SARS-CoV-2 (COVID-19) antigen (N protein) detection results are demonstrated in Table 1 [2].

Country; Reference	Test; Manufacturer	Specimen Type	Method	Sensitivity; Specificity (%) (95 % CI)
Belgium; [24]	COVID-19 Ag Respi-Strip assay (Coris BioConcept, Gembloux, Belgium)	Nasopharyngeal secretions	Immunochromatographic test	30.2 (21.7-39.9); 100

Belgium; [25]	COVID-19 Ag Respi-Strip assay (Coris Bioconcept Gembloux, Belgium)	Nasopharyngeal secretions	Immunochromatographic test	30 (16.7-47.9); 100
Chile; [8]	SARS-CoV-2 antigen test (Bioeasy Biotechnology Co., Shenzhen, China)	Nasopharyngeal, oropharyngeal swabs	Fluorescence immunochromatographic test	93.9 (86.5-97.4); 100 (92.1-100)
France; [26]	COVID-19 Ag Respi-Strip assay (Coris BioConcept, Gembloux, Belgium)	Nasopharyngeal secretions	Immunochromatographic test	50.0 (39.5-60.5); 100 (91.8-100)
Germany; [27]	SARS-CoV-2 rapid antigen test (Roche, Switzerland)	Nasopharyngeal swabs	Lateral flow assay	70.7 (59.0-81.0); 96 (89.0-99.0)
USA; [28]	Sofia 2 SARS antigen FIA (Quidel Corporation)	Nasopharyngeal swabs	Lateral flow immunofluorescent sandwich assay	80 (68-88); Not available

Table 1: Demonstrating the sensitivity and specificity of the rapid antigen (N protein) detection tests for SARS-CoV-2 (COVID-19)

Recently, the United States Centers for Disease Control and Prevention established an interim guidance for RATs for detecting SARS-Cov-2 (COVID-19) infection in a community setting [29, Figure 1]. If the individual has a low risk of SARS-CoV-2 (COVID-19) infection, a positive RAT result from an asymptomatic individual in a community setting may need confirmatory testing (for examples; a low risk of SARS-CoV-2 (COVID-19) infection would be an individual who is fully COVID-19 vaccinated, or has had a SARS-CoV-2 (COVID-19) infection in the last 3 months, or has had no known exposure to a COVID-19-infected individual within the last 14 days) [29]. If a asymptomatic individual has a high risk

of SARS-CoV-2 (COVI-19) infection, a negative RAT result may need confirmatory testing (for examples; a high risk of SARS-CoV-2 (COVID-19) infection would be an individual who is not fully COVID-19 vaccinated, or has not had a SARS-CoV-2 (COVID-19) infection in the last 3 months, or has had close contact or suspected exposure to SARS-CoV-2 (COVID-19) within the last 14 days) and should consider performing serial RATs every 3-7 days for 14 days [29]. A suspected COVID-19-symptomatic individual with a positive RAT result can be interpreted that the individual is infected with SARS-CoV-2 (COVID-19) [29]. For fully COVID-19 vaccinated individual with a positive RAT, the healthcare providers should inform the public health authorities and ideally, a separate specimen would be collected and sent to a laboratory for public-health-purpose viral sequencing [29]. A positive RAT result in a symptomatic individual with a low risk of SARS-CoV-2 (COVID-19) infection (for examples; for examples; a low risk of SARS-CoV-2 (COVID-19) infection would be an individual who is fully COVID-19 vaccinated, or has had a SARS-CoV-2 (COVID-19) infection in the last 3 months, or has had no known exposure to a COVID-19-infected individual within the last 14 days) may need confirmatory testing [29]. A symptomatic individual with a negative RAT result should be performed serial RATs every 3-7 days for 14 days, in addition to confirmation with a laboratory-based NAAT [29]. In a symptomatic individual with a negative RAT result, the negative RAT result may not need confirmatory SARS-CoV-2 (COVID-19) testing [29]. If a symptomatic individual with a negative RAT result had not any known exposure to SARS-CoV-2 (COVID-19), the individual do not need to quarantine as well as for asymptomatic individual with a negative RAT result who had been fully COVID-19 vaccinated, or had a SARS-CoV-2 (COVID-19) in the last 3 months, or have has close contact or suspected exposure to a COVID-19-infected individual within the last 14 days [29].
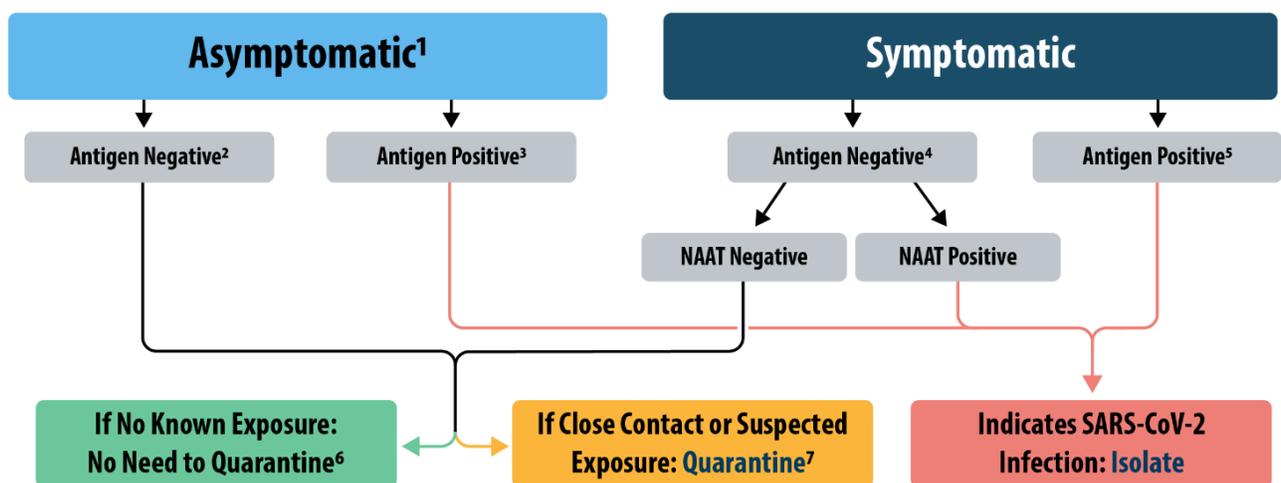
Figure 1: Demonstrating RATs algorithm for community setting

(**Source:** United States Centers for Disease Control and Prevention. Interim guidance for antigen testing for SARS-CoV-2. Updated on May 13, 2021. Available at: https://www.cdc.gov>lab>antigen-tests-guidelines (accessed on July 31, 2021))

Currently, which SARS-CoV-2 (COVID-19) antigens are most appropriate for serological testing and whether the direct detection of viral antigens in the clinical specimens is a more sensitive-rapid-accurate-immunological diagnostic method for SARS-CoV-2 (COVID-19) remain unknown. S1 subunit protein of SARS-CoV-2 (COVID-19) is the S protein as an antigen for the serological diagnosis of SARS-CoV-2 (COVID-19) infection, whereas the S2 subunit protein plays a significant role in the cross-reactivity when the whole S protein is utilized as an antigen. Combinations of both S and N proteins have revealed improvement of the laboratory results through multiantigen protein arrays in comparison to each individual protein antigen. RATs become useful diagnostic tool for the SARS-CoV-2 (COVID-19)-early detection.

In conclusion, they should be used in conjunction with the molecular methods and further urgent studies should be focused on strategies to improve the accuracy and sensitivity and post-implementation evaluation of the diagnostic accuracy.

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