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LETTER TO THE EDITOR

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Rapid antigen detection alone may not be sufficient for early diagnosis and/or mass screening of COVID-19

To the editor,

Currently, coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still the biggest public health crisis faced by the world. In spite of the several vaccines that have been approved for emergency use, ongoing generation of various SARS-CoV-2 variants is challenging to the efficacy of these vaccines in preventing COVID-19. High transmissibility of SARS-CoV-2 and the high proportion of presymptomatic and asymptomatic cases who are contagious, highlight the importance of early diagnosis and mass screening of COVID-19, which enables timely and appropriate interventions to prevent further spread of the virus.[1,2] Due to high sensitivity and specificity, the reverse-transcription quantitative polymerase chain reaction (RT-qPCR) was widely used as the gold standard for diagnosing COVID-19 with upper respiratory tract specimens. Given the challenge in detection capacity for mass screening for presymptomatic and asymptomatic cases, and the requirement for frequent testing and monitoring of high-risk contacts during the COVID-19 pandemic, simple, fast, sensitive, and inexpensive point-of-care testing (POCT) assays are largely encouraged. Rapid antigen test and viral RNA detection by isothermal amplification assays (e.g., reverse-transcription loop-mediated isothermal amplification [RT-LAMP]) are two kinds of POCT approaches that are suitable for mass screening of SARS-CoV-2 in resource-rich and resource-limited settings.

Recently, seven papers published in J Med Virol evaluated the performance of nine rapid antigen testing devices on SARS-CoV-2 detection (Table 1).[3–9] Two-thirds of these rapid antigen tests had overall sensitivities (30.8%–68.9%) below the WHO recommended standard of ≥80%. One rapid antigen test (COVID-VIRO®) showed an excellent sensitivity of >90% even for the samples with a low viral load (>32 C_t values in RT-qPCR),[3] one (COVID-19 Ag ECO Test) had an overall sensitivity of 82.0%,[4] and one (Panbio™ COVID-19 Ag Rapid Test) showed variable sensitivities (75.0%–100%) in three different investigations.[4–6] After excluding one study with very small samples size (n=44) that showed very high sensitivity (100%),[4] we recalculated the sensitivity of the Panbio™ Ag Test using the data from two different investigations,[5,6] and obtained a 76.3% (216/283) (Table 1). In spite of having a significantly positive correlation of antigen testing with SARS-CoV-2 viral culture assays,[10] it is clear that the vast majority (6/7) of tested rapid antigen assays have substantially lower sensitivity than the WHO recommended standard, especially for asymptomatic cases.[6,8] The low sensitivity of these antigen testing assays is mainly due to low detection capacity for samples with low viral load (<100 000 RNA copies/ml or C_t < 30). Therefore, it is of concern whether the antigen detection alone is sufficient for early diagnosis and/or mass screening of COVID-19 in the fight against the pandemic.

Transmissible SARS-CoV-2 persists during incubation and acute phases of COVID-19 (Figure 1), and presymptomatic and asymptomatic COVID-19 individuals are the main source of the transmissible virus. The majority of the infections were found to be acquired via silent transmission from presymptomatic and asymptomatic individuals.[1,2] To find the presymptomatic individuals, as well as asymptomatic individuals whether they are infectious or not, is crucial for the containment of COVID-19 (Figure 1). High proportion of asymptomatic and presymptomatic cases with high transmissibility of SARS-CoV-2 highlight the importance of mass and/or contact-based screening for presymptomatic and asymptomatic individuals, which enables timely and appropriate interventions to prevent silent transmission.

The incubation phase represents the early stage of infection, during which the rapidly replicating virus is highly transmissible, but the viral load (or antigen level) might be relatively low (Figure 1). The major challenge for rapid antigen testing is that its low detection sensitivity for low viral load samples will miss a large proportion of presymptomatic and asymptomatic COVID-19 individuals during the incubation phase (Figure 1), and these missed SARS-CoV-2 carriers will enlarge the silent transmission chain.[1,2] Therefore, the vast majority of the rapid antigen testing alone should be cautiously recommended for early diagnosis and mass screening of COVID-19 because of its low sensitivity.

As a promising POCT technique, RT-LAMP has comparable detection sensitivity with RT-qPCR, but significantly shorter sample-to-result time (about 30 min vs. about 4 h for RT-qPCR), easier operation, and less dependent on sophisticated equipment.[11] In particular, we and other groups developed direct probe-based SARS-CoV-2 RT-LAMP assays that can detect clinical samples at a level of over 1000 copies/ml (unpublished data),[12] enabling the finding of most presymptomatic and asymptomatic COVID-19 individuals (Figure 1). Although RT-qPCR is considered as the golden standard for SARS-CoV-2 detection, high dependence on molecular laboratory (sophisticated equipment with professionals) and long sample-to-result time (about 4 h) limit its capacity in mass screening or community-based testing of SARS-CoV-2. As a good alternative, direct probe-based RT-LAMP assay or other nucleic acid amplification (NAA)-based POCT strategies should be recommended to use alone or together with rapid antigen test in mass screening or community-based testing of SARS-CoV-2.

TABLE 1 Sensitivities and specificities of nine rapid antigen testing assays for SARS-CoV-2 detection

Rapid antigen tests	Patient information/disease status	Sample size	Sensitivity	Specificity	Reference
COVID-VIRO®	Symptomatic/asymptomatic	248	96.7%	100%	[3]
Abbott Panbio™ COVID-19 Ag Rapid Test COVID-19 Ag ECO Test	Symptomatic	44 68	100% 82.0%	94.0% 98.0%	[4]
Abbott Panbio™ COVID-19 Ag Rapid Test BioSpeedia COVID19 Speed-Antigen Test	Symptomatic/presymptomatic/asymptomatic	401	75.0% 65.5%	NA 100%	[5]
Abbott Panbio™ COVID-19 Ag Rapid Test	Children: Symptomatic/asymptomatic	744	82.4%	100%	[6]
CoV-Ag Rapid Test Cassette (BioRad) GSD NovaGen COVID-19 Ag Rapid Test (NovaTec) Aegle CoV-Ag Rapid Test Cassette (LumiraDx)	NA	199	62.7% 61.9% 64.0%	100% 85.7% 100%	[7]
AgPOCT (Roche)	Symptomatic/asymptomatic (symptomatic) (asymptomatic)	2375 (1539) (836)	68.9% (69.5%) (62.0%)	99.6% (99.5%) (97.6%)	[8]
COVID-19 Ag Respi-Strip (Coris BioConcept)	NA	50	30.8%	100%	[9]
Abbott Panbio™ COVID-19 Ag Rapid Test[a]	Adult/children; Symptomatic/presymptomatic/asymptomatic	1145	76.3%[a]	NA	This study

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]The sensitivity was recalculated based on the data from References [5] and [6].

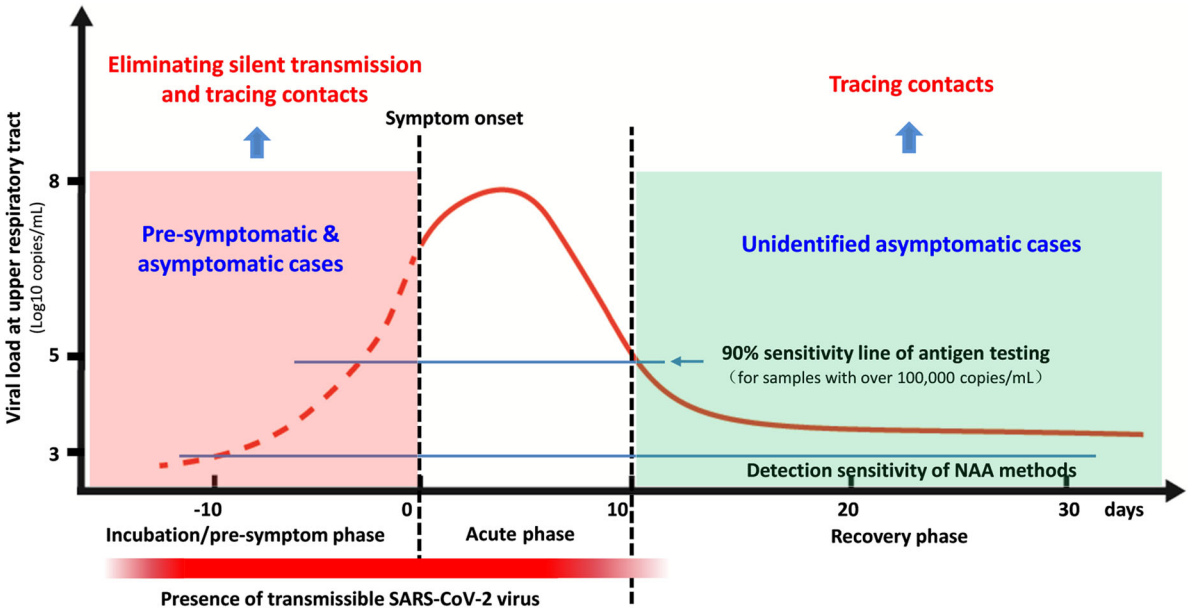

FIGURE 1 SARS-CoV-2 viral load dynamics at upper respiratory tract during COVID-19. SARS-CoV-2 viral load at the upper respiratory tract varies largely during the course of COVID-19, and peaks within about 4 days after symptom onset (or about 2 weeks after initial infection in asymptomatic cases). Symptomatic and asymptomatic individuals share similar viral load dynamics during the course of COVID-19, which is divided into three phases, incubation/pre-symptom, acute, and recovery phases. SARS-CoV-2 viral load is substantially lower during incubation/pre-symptom and recovery phases than the acute phase, and transmissible SARS-CoV-2 virus persists from initial infection up to about 10 days after symptom onset or two to three weeks since initial infection in asymptomatic cases

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CONFLICT OF INTEREST

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Chiyu Zhang: conceptualization, supervision, validation, visualization, writing – original draft, writing – review and editing. **Zhenzhou Wan**: conceptualization, investigation, funding acquisition, writing – original draft. **Yongjuan Zhao**: investigation, visualization, writing – original draft. **Renfei Lu**: investigation. **Yajuan Dong**: investigation. All authors have accessed verified the underlying data.

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