Opinion Paper

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Making sense of rapid antigen testing in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostics

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Abstract: Although the most effective strategy for preventing or containing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreaks relies on early diagnosis, the paramount and unprecedented number of tests needed to fully achieve this target is overwhelming worldwide testing supply and capacity. Molecular detection of SARS-CoV-2 RNA in nasopharyngeal swabs is still considered the reference diagnostic approach. Nonetheless, identification of SARS-CoV-2 proteins in upper respiratory tract specimens and/or saliva by means of rapid (antigen) immunoassays is emerging as a promising screening approach. These tests have some advantages compared to molecular analysis, such as point of care availability, no need of skilled personnel and dedicated instrumentation, lower costs and short turnaround time. However, these advantages are counterbalanced by lower diagnostic sensitivity compared to molecular testing, which would only enable to identifying patients with higher SARS-CoV-2 viral load. The evidence accumulated to-date has hence persuaded us to develop a tentative algorithm, which would magnify the potential benefits of rapid antigen testing in SARS-CoV-2 diagnostics.

Keywords: coronavirus; COVID-19; diagnosis; immunoassay; virus.

Introduction

The current coronavirus disease 2019 (COVID-19) pandemic, sustained by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread across the planet, causing over one million fatalities so far [1]. According to the Johns Hopkins University COVID-19 Dashboard website [2], as of November, 2020, the virus has infected over 50 million people, following an exponential epidemic trend that is very unlikely to reverse soon.

It is now universally agreed that the most effective policy for preventing or containing local outbreaks of SARS-CoV-2 encompasses a strategy of early diagnosis and timely isolation of infected and infectious cases. Nonetheless, the paramount and unprecedented number of tests that are needed to fully achieve these targets is largely overwhelming the worldwide testing supply and capacity. According to a recent survey from the American Association of Clinical Chemistry (AACC) [3], over 60% of worldwide laboratories which responded to the questionnaire declared facing serious problems in obtaining reagents and test kits for routine SARS-CoV-2 diagnostics. This alarming scenario has been reemphasized by some recent publications, underlining that achieving a goal of which everyone who needs a test shall have results within 24 h is far from being met [4], and that urgent testing is dramatically failing due to low throughput and long turnaround time of molecular tests [5]. Moreover, it has been noted that failure to efficiently test medical staff and their family members, implies that physicians, as well as other frontline healthcare workers, are forced into long self-isolation, being unable to provide the necessary care to patients with COVID-19 and other diseases, or continue to work without knowing their COVID-19 status, potentially putting their colleagues, patients, and families at risk of infection [6]. If one considers that the need to provide a proportionate number of tests will increase in parallel with an apparently relentless pandemic progression, the situation may even worsen in the future, such that reliable strategies and alternatives to address the significant imbalance between demand and supply of conventional nucleic acid amplification tests (NAATs) must be urgently planned.

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State of the art of rapid antigen tests for SARS-CoV-2 diagnostics

Beside molecular detection of SARS-CoV-2 RNA in nasopharyngeal swabs, which is still considered the reference diagnostic approach according to the guidelines of the World Health Organization (WHO), the identification of SARS-CoV-2 proteins in upper respiratory tract specimens and/or saliva by means of rapid immunoassays is emerging as a promising screening approach, and has also been recently endorsed by the US Centers for Disease Control and Prevention (CDC). Several rapid tests have already received some forms of authorization for clinical use by the US Food and Drug Administration (FDA) and/or

by the European Community (CE), as summarized in Table 1. This technology has many advantages compared to NAATs, such as its availability as a point of care (POC) diagnostic test, the absence of requiring skilled personnel and dedicated instrumentation for performing the test, lower costs, and a short turnaround time, whereby test results can usually be delivered within 5–30 min (Table 1). The widespread use of rapid antigen tests could hence be an important resource in overcoming the current shortage of molecular tests. However, rapid antigen tests, despite their unquestionable advantages, have some notable shortcomings, the first of which is indeed a lower sensitivity compared to NAATs. As recently highlighted by a systematic literature review published by the Cochrane COVID-19 Diagnostic Test Accuracy Group [7], the

Table 1: Manufacturers' declaration on rapid antigen tests for coronavirus disease 2019 (COVID-19) diagnostics approved for clinical use by the US Food and Drug Administration (FDA) and/or by the European Community (CE).

Company	Device	Time	LoD	Sensitivity	
				Overall	Specific conditions
Abbott Diagnostics ^a	BinaxNOW COVID-19 Ag Card	~15 min	22.5 TCID ₅₀ /swab	0.97 (95%CI, 0.85–1.00)	Ct _≥ 33: 0.83 (0.36–1.00)
Amedica SA ^b	Amela Covid-19 Antigen test	~30 min	N/A	N/A	–
ArcDia International Ltd ^b	mariPOC SARS-CoV-2	~20 min	N/A	0.92 (95%CI, N/A)	–
Becton Dickinson ^a	BD Veritor System for Rapid Detection of SARS-CoV-2	~15 min	140 TCID ₅₀ /swab	0.84 (95%CI, 66–95)	–
Beijing Kewei Clinical Diagnostic Reagent ^b	Kewei COVID-19 Antigen Rapid Test Kit	~15 min	N/A	0.85 (95%CI, N/A)	–
Beijing Savant Biotechnology ^b	SARS-Cov-2 Antigen Fluorescence Rapid Detection Kit	~15 min	50 ng/mL	N/A	–
Coris BioConcept ^b	COVID-19 Ag Respi-Strip	~15 min	N/A	0.60 (95%CI, 0.48–0.71)	Ct _{<} 25: 0.77 (0.61–0.88)
Liming Bio-Products ^b	Strongstep COVID-19 Antigen Rapid Test Device	~15 min	N/A	0.73 (95%CI, 0.60–0.83)	–
LumiraDx UK Ltd ^a	LumiraDx SARS-CoV-2 Ag Test	~12 min	32 TCID ₅₀ /swab	0.98 (95%CI, 0.92–1.00)	–
PCL Inc ^b	PCL COVID19 Ag Rapid FIA	N/A	N/A	N/A	–
Quidel Corporation ^{a,b}	Sofia SARS Antigen FIA	~15 min	113 TCID ₅₀ /swab	0.97 (95%CI, 0.83–1.00)	–
RapiGEN Inc ^b	Biocredit COVID-19 Ag	5–8 min	N/A	0.90 (95%CI, 0.79–0.97)	–
SD Biosensor Inc	Standard F COVID-19 Ag FIA	~30 min	7.81 × 10 ^{1.2} TCID ₅₀ /mL	N/A	–
SD Biosensor Inc	Standard Q COVID-19 Ag FIA	15–30 min	1.25 × 10 ^{3.2} TCID ₅₀ /mL	0.97 (95%CI, 0.91–0.99)	–
Shenzhen Bioeasy Biotechnology ^b	Bioeasy 2019-nCoV Ag	~15 min	N/A	N/A	–
Sugentech, Inc ^b	SGTi-flex COVID-19 Ag	~20 min	530 TCID ₅₀ /swab	0.80 (95%CI, 0.61–0.91)	–
Verify Diagnostics ^b	COVID-19 Antigen Rapid Test Device	~15 min	N/A	0.80 (95%CI, 0.74–0.86)	–

Ct, cycle threshold; LoD, limit of detection; N/A, not available (not found); TCID, tissue culture infective dose. ^aUS Food and Drug Administration – Emergency Use Authorizations for Medical Devices (FDA-EUA); ^bCE.

diagnostic sensitivity of many of these POC antigen SARS-CoV-2 tests seems acceptable in respiratory specimens with high viral load (e.g., typically >80% with cycle thresholds of 25 or lower), whilst the positive rate in samples with low viral load (e.g., cycle thresholds >25/30) is always <80%, being observed between 8 and 72%. This data was confirmed by another recent investigation published by Liotti et al. [8], showing that the percent positivity agreement with NAATs of a commercially available rapid antigen test is >95% in upper respiratory tract specimens with high viral load (i.e., cycle thresholds <25), but dramatically declines to 20–40% in samples with cycle thresholds ≥ 25 . Similar evidence has also been published by Porte and colleagues [9], who also found that the diagnostic sensitivity of a rapid antigen test was almost dependent on viral load (i.e., 100% in nasopharyngeal samples with cycle thresholds ≤ 25.1 vs. 72% in those with cycle thresholds >25.1), as well as by Nalumansi et al., who also showed that rapid antigen test results were more frequently positive in nasopharyngeal swabs with lower cycle thresholds (i.e., 92% with cycle thresholds ≤ 29 vs. 55% with cycle thresholds >29) [10].

Manufacturers' claims, when available or easily retrievable, are also in keeping with this evidence, since the limit of detection (LoD) and diagnostic sensitivity of some FDA- or CE-approved kits are substantially modest (Table 1). Notably, the analytical and diagnostic performance of some of these tests cannot be found and/or are neither reported in the relative information sheets, thus calling for great caution in routine usage without appropriate analytical and clinical validation.

Taken together, these findings would lead us to conclude that both the diagnostic windows and the so-called “grey zone” will be different when using rapid antigen tests as opposed to NAATs, as illustrated in Figure 1. Briefly, the diagnostics window of conventional molecular tests is broader and allows for identification of patients infected with lower viral loads, thus the chance of obtaining false negative results would be contained over a shorter period of time during the acute SARS-CoV-2 infection as compared to rapid antigen diagnostics.

Clinical use of rapid antigen testing in SARS-CoV-2 diagnostics

Based on these conclusions, the usage of rapid antigen tests would appear theoretically unjustified, since the lower diagnostic performance (especially the higher false negative rate) may leave many asymptomatic or mildly symptomatic

Figure 1: Detectability of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection with conventional nucleic acid amplification tests (NAATs) or rapid antigen testing. NAAT, nucleic acid amplification tests; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

COVID-19 patients undiagnosed and not isolated from the community. Nonetheless, it is important to consider that the real infectivity and efficiency of viral transmission in asymptomatic patients with low SARS-CoV-2 viral load, as detected by NAATs but not with rapid antigen tests (e.g., cycle thresholds ≥ 30), is still a matter of open debates. Although there is no doubt with respect to the potential for transmission by asymptomatic or pre-symptomatic SARS-CoV-2 infected individuals [11], it is also been demonstrated that the attack rate of subjects with no symptoms is lower compared to symptomatic COVID-19 patients. In support of this evidence, a recent meta-analysis published by Buitrago-Garcia et al. concluded that asymptomatic and pre-symptomatic subjects with SARS-CoV-2 infection have 65% and 37% lower risk, respectively, of transmitting the virus compared to symptomatic patients [12]. Despite asymptomatic subjects appearing to have similar or an even longer durations of viral shedding compared to those with symptoms [13], another meta-analysis has recently concluded that these subjects may not be very infective, especially during the later stage of their infection [14]. Although most studies included in the final pooled analysis were of low-to-moderate quality, it could be concluded that the identification of SARS-CoV-2 RNA does not always reflect the presence of viable and transmissible viral particles [14], as also supported by evidence published by Jeong et al. [15]. It is also noteworthy that reliable evidence now exists that the severity of COVID-19 illness seems to be directly dependent on the volume of viral particles loaded [16]. Therefore, even if asymptomatic or presymptomatic subjects with SARS-CoV-2 infection are still capable of transmitting the virus, a lower viral inoculation would be

Figure 2: Tentative algorithm for making sense of rapid antigen testing in coronavirus disease 2019 (COVID-19) diagnostics.

associated with a much higher likelihood of developing only mildly symptomatic or even totally asymptomatic illness [17]. Another aspect that needs to be highlighted, is that the likelihood of obtaining a positive viral culture for SARS-CoV-2 peaks between 0 and 3 days from symptom onset, whilst it dramatically falls afterward, so that the risk of secondary attack 6 days after the symptom onset was reported as being very close to zero [18].

Conclusions

According to the last WHO ad interim guidance for SARS-CoV-2 antigen testing [19], these methods could be used for early detecting positive cases, where molecular/reference assays are unavailable or laboratory services are overloaded, and shall be specifically used in settings where molecular testing is not immediately available, for outbreak investigations, for monitoring trends in disease incidence within the community, as well as during widespread community transmission, thus enabling to timely detect and isolate SARS-CoV-2 positive cases [19]. The minimum diagnostic performance of these tests encompasses at least 80% diagnostic sensitivity and 97% diagnostic specificity, respectively, whilst all positive samples should undergo confirmation with laboratory-based molecular tests [19]. Notably, the use of rapid antigen tests is discouraged where disease prevalence is low or when confirmatory molecular testing is unfeasible. Finally, the data used for validating the test must be of sufficient quality [19].

The evidence accumulated to-date would hence persuade us to develop a tentative algorithm, aimed at defining the role of rapid antigen testing in COVID-19 diagnostics (Figure 2), based on test kits that have successfully passed clinical validation and display sufficient analytical sensitivity. Briefly, rapid antigen-based tests may be useful for targeted population screening (as indicated by the WHO) [19], with timely isolation from the community when asymptomatic patients with high viral loads are identified, or for emergency testing of subjects with potential COVID-19 symptoms, thus enabling rapid detection of patients with symptomatic SARS-CoV-2 infection displaying higher viral loads, who incidentally are the most infective, and who may need early triage and clinical management [20]. For those testing negative, the likelihood of active SARS-CoV-2 infection with high viral load is predictably low, and thereby clinical surveillance and/or repeated testing if/when suggestive COVID-19 symptoms appear, seems an overall feasible strategy. Irrespective of test results, reinforced adoption of measures such as face masking, social distancing and hand hygiene will still be unavoidable for preventing virus transmission.

Since the vast majority of SARS-CoV-2 infected individuals (i.e., over 85%) now fails to report COVID-19 specific symptoms [21], this approach seems a feasible strategy for avoiding to segregate an immense number of asymptomatic people whose secondary attack rate is limited [22, 23], thus preserving testing resources for SARS-CoV-2 positive subjects who carry instead higher risk of household or community virus transmission, as recently highlighted by Qian et al. [24]. This strategy would also be in keeping with the allegation of Mina and colleagues [25], according to whom widespread use of rapid and relatively inexpensive antigen tests, although characterized by lower analytic sensitivity compared to conventional molecular assays, shall be regarded as a valuable support for massive SARS-CoV-2 testing. Nonetheless, analytical and clinical validation of antigenic assays, including accurate estimation of the upper limit of cycle thresholds after which the diagnostic sensitivity becomes poor, remains a preliminary and virtually unavoidable step for making sense of rapid antigen testing in COVID-19 diagnostics.

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