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Controlling the Spread of SARS-CoV-2 by Broadening the Diagnostic Strategy

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has put the entire world to the test. It is a fact that we urgently need reliable clinical tests for rapid detection. The gold standard in the diagnostics of SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19) is the molecular detection of viral RNA via real-time reverse transcriptase PCR (real time RT-PCR); interpretation of results can be sometimes tricky and need repetition. We have rapid tests based on the detection of viral antigens, but these often-present non-optimal sensitivity. To date, available techniques are expensive and not readily available for point-of-care applications. Every diagnostic test encompasses specific targets: Antigen-detection diagnostic tests (Ag-RDTs) detect proteins produced by replicating virus, RT-PCR nucleic acids. In our opinion, combining different virological diagnostic tools with clinical manifestations, especially in public health local management where it is more difficult to determine isolation and quarantine for cases and suspected cases of COVID-19, can make the pandemic containment more effective and efficient. This emerging infection should push the scientific community to ponder on the best use of the current knowledge of molecular biology, which must correlate with the clinical presentation, to optimize resources and take consequent decisions grounded on evidence.

As common in virology, the gold standard in the diagnostics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19) is the molecular detection of viral RNA via real-time reverse transcriptase PCR (real time RT-PCR). The CDC (Centers for Disease Control and Prevention) diagnostic panel for upper and lower respiratory specimens is a real time RT-PCR based on the TaqMan technology (https://www.fda.gov/media/134922/download), with primers and probes on two different regions of the virus nucleocapsid (N) gene (N1 and N2). An additional primer/probe set for the human RNase P transcript (RP) is included, to control the quality of the complementary DNA (cDNA) obtained from clinical specimens. A no-template control is finally added to check for contamination, an occurrence far from being rare when running a PCR with high number of cycles (45 in this case).

Interpretation of results can be sometimes tricky and need repetition: (1) when only one between N1 and N2 is positive, (threshold cycle, Ct<40) (inconclusive test), (2) when both viral targets and human control have Ct>40, thus are negative (invalid result). Moreover, the detection of viral RNA does not necessarily imply the presence of intact viral particles, especially in case of high threshold cycles, e.g. close to 40, not easy to distinguish from background contamination. Indeed, when performing real time-PCR for research, it is common not to consider amplicons with such Ct, for the difficulty of attributing biological or pathological meanings. Finally, how should we consider the case of a sample negative for one viral target, with the other showing a Ct close to 40?

These issues reinforce some considerations that have already been posed on the diagnostics of SARS-CoV-2. It is a fact that we urgently need reliable clinical tests for rapid detection. To date, available techniques are expensive and not readily available for point-of-care applications [1].

We have rapid tests based on the detection of viral antigens, but these often present non-optimal sensitivity [2]; also, we have the real time RT-PCR on nasopharyngeal swab, the main diagnostic tool. However, it was set up as a research technique and may be difficult to transpose for diagnostic purposes, especially with the numbers that we all unfortunately know. It is likely that its limits may undermine a diagnostic flow in most cases based exclusively on it, without any positive data being confirmed by other means, e.g. through detection of viral proteins (see for example the case of AIDS, where confirmatory Western Blots are usually performed [3]). This would be desirable, at least in the case of inconclusive or invalid results, especially in the presence of high Ct.

Other critical issues are the criteria for defining suspected and confirmed cases [4], and those for discharging patients from isolation [5]. We have assisted to a big change in these criteria at the end of the first European wave (May 2020), proclaiming not necessary using diagnostic tests (PCR or antigenic) for defining cases or suspected cases, and for discharging patients from isolation or quarantine. Indeed, as prolonged viral RNA detection and viral shedding upon resolution of symptoms imposes changes in containment strategies rules.

The underlying rationale balances risks and benefits in a context where we do not have enough evidences yet to fully assess risk of viral transmission and where real time RT-PCR is not the method to defining such risks.

Among the molecular tests, those based on saliva showed an optimal agreement with the nasopharyngeal ones [6]. Numerous evidences are accumulating in favor of these tests: lower variability of mRNA into the specimen, early positive results compared to nasopharyngeal swab (therefore advantage for identifying presymptomatic/asymptomatic), early viral clearance [7].

Antigenic tests, commonly referred to as “rapid”, also entered the diagnostic flow [8].

Every diagnostic test encompasses specific targets: Antigen-detection diagnostic tests (Ag-RDTs) detect proteins produced by replicating virus, RT-PCR nucleic acids. Sensitivity of Ag-RDTs is highly variable (0-94%), but they are highly specific (>97%). According to WHO, they are optimally used in patients with supposed high viral loads, corresponding to first days of symptoms onset. Consequently, if a negative result is obtained in a patient for at least 3 days upon symptoms ended, a low viral load is likely present, and can be discharged. It is not recommended to use Ag-RDTs in people with more than 5-7-days history of symptoms, according to supposed low viral loads that could lead to false negative results.

In our opinion, combining different virological diagnostic tools with clinical manifestations, especially in public health local management where it is more difficult to determine isolation and quarantine for cases and suspected cases of COVID-19, can make the pandemic containment more effective and efficient.

If we want to assess a diagnosis of COVID-19 in a symptomatic patient, we must define the time of symptoms onset. In the first 5 days we can use Ag-RDTs, if this window has passed, we need to use real time RT-PCR.

For mass screening in cohorts where a positive case has been identified, saliva test could be used. The same test could be selected for discharging from quarantine a close contact of confirmed case by definition asymptomatic.

For a confirmed case to exit isolation, at least 3 days without any symptoms should be observed and the use of Ag-RDTs recommended for two reasons: (1) the aim is to determine if a patient is at risk of transmitting infection, hence the need to know if he/she had viral protein produced from a virus in active replication; (2) available data suggest that RNA detection by RT-PCR- remains positive for longer time in comparison to the risk of transmitting the virus.

SARS-CoV-2 has put the entire world to the test. Efficient diagnostics as containing strategy is an essential and winning weapon against pandemics, while we wait for large-scale and efficient vaccination programs. This emerging infection should push the scientific community to ponder on the best use of the current knowledge of molecular biology which must correlate with the clinical presentation, to optimize resources and take consequent decisions grounded on evidence.

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