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COVID-19 testing from a public health perspective:

Selection of test, cut-off and sampling scheme should depend on the purpose of testing

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Summary

The available COVID-19 tests are of high to absolute specificity (Sp) but lack in sensitivity (Se), especially for the detection of asymptomatic and early infected individuals. This hampers control efforts. A purpose-specific approach to the selection of tests and cut-offs and/or repeated testing schemes could be used for the effective control of the pandemic with these tests. To assess the efficiency of control programs valid estimates of COVID-19 prevalence/incidence are needed. Such estimates must be based on a proper sampling scheme that will ensure the representativeness of the target population. However, current estimates of COVID-19 incidence/prevalence are based on passive surveillance and are thus subject to bias. They cannot be compared among countries, regions, or even among different time periods within the same country/region. Finally, incidence /prevalence must adjust for the imperfect Se/Sp of the diagnostic process. Diagnostic Se and Sp are population-specific and must be relevant to – if not obtained from – the target population. Se and Sp estimates from a clinical setting or populations that have a different mixture of the various infection stages should not be naively extrapolated to dissimilar settings.

1. Available tests for COVID-19

Currently, two main types of tests are available for COVID-19: (a) tests aiming to detect the virus or particles of it and (b) tests detecting the immune response of the host. Nucleic acid tests that detect the presence of the viral RNA – mostly RT-PCR tests – and antigen tests detecting the presence of a viral antigen (i.e., part of a surface protein) fall within (a) while tests for the presence of antibodies against SARS-CoV-2 fall within (b). International organizations like the World Health Organization (WHO) (1), the European Centre for Disease Prevention and Control (ECDC) (2) and the Centers for Disease Control and Prevention (CDC) regularly provide updated guidance on the development of COVID-19 tests.

The ability of available tests to detect infected individuals depends, among others, on the time of testing. Efforts to effectively control COVID-19 have been hampered by the absence of sensitive tests for the detection of newly infected, asymptomatic individuals that are infectious (3). The probability of a polymerase chain reaction (PCR) test to identify infected individuals is low, one week before the onset of symptoms and gradually increases to peak one week post symptom onset. Antigen tests follow a similar pattern with the probability of being positive peaking with the onset of symptoms. Antibody tests do not turn positive before the second week post symptom onset with immunoglobulin M (IgM) preceding IgG. IgM levels drop significantly until week six post symptom onset, while IgG levels can persist for several months (4).

2. Interpretation of test results

While the interpretation of PCR and antigen tests is more straightforward, antibody tests should be interpreted in connection to clinical signs, history, and the results of other tests, to assess whether an active infection is present or past infection(s) has(ve) occurred.

Interpretation of RT-PCR tests

A positive real time (RT-PCR) test has been used to diagnose a case of COVID-19 since the beginning of the pandemic (5). Positive results are indicative of an “active infection”. The ability of RT-PCR to detect infected individuals varies with sample type with bronchoalveolar lavage, nasopharyngeal and sputum samples being the most sensitive (6,7). Test results should be interpreted according to the pre-test probability and test performance. An RT-PCR test is more likely to be positive within the first week after symptom onset (4), but individuals may test positive for several weeks. A positive RT-PCR test does not necessarily mean infectiousness, as the test cannot distinguish viable virus of viral RNA fragments (2,8). Viral viability can be assessed through virus isolation and culture (2). A viral culture is more likely to be positive within the first eight days post symptom onset and with lower cycle threshold (Ct) values – indirect measures of viral load (8,9).

Interpretation of antigen tests

Antigen tests are available either as laboratory-based or rapid (point-of-care) tests (10). They have lower sensitivity but higher specificity than RT-PCR. Nevertheless, they are less expensive, less time consuming and relatively simpler to handle (11). Rapid antigen tests are sensitive enough to detect cases with low Ct, that is, individuals more likely to be infectious and contributing to the silent spread of COVID-19 (8,11).

Interpretation of antibody tests

A positive antibody test has been assumed indicative of a "past infection" (12). Seroconversion can occur from the second week post symptom onset and peaks between the third and fourth week, remaining stable up to four months (4). Antibody tests are unlikely to identify infectious individuals at an early infection stage and are not useful for efforts to prevent transmission. The utility of rapid serological tests in the clinical setting remains unclear (13,14).

3. Test Strategies for COVID-19

In this report we focus on test strategies from a Public Health and Epidemiological perspective. Test strategies for case confirmation and follow-up at the individual level and within a clinical context will not be discussed.

Test strategies for COVID-19 should depend on the purpose of testing. Selection of test type, cut-off and sampling scheme – one-time vs. repeated testing, individual vs pooled testing – should be determined once an objective is set. Currently, most of the testing strategies aim to identify individuals with an active infection and are usually performed when a person has signs or symptoms consistent with COVID-19, or when a person is asymptomatic but had a recent, known, potential exposure to SARS-CoV-2. On the other hand, screening for COVID-19 aims to identify infectious individuals who are asymptomatic and without known or suspected exposure to SARS-CoV-2. Screening is performed to identify persons who may be infectious so that measures can be taken to prevent further spread of the virus.

Testing to control the spread of COVID-19 through early identification of infectious individuals should, ideally, be based on an active surveillance scheme and a test that that would be sensitive in the detection of asymptomatic individuals. In the absence of sensitive tests, a repeated testing scheme can be used to increase the sensitivity of the whole diagnostic process. For example, rapid antigen tests that are of moderate to low sensitivity, especially at the early infection stages, can be used in a repeated testing scheme to overcome the lack of sensitivity, a strategy that has also been recommended by ECDC (15).

Testing to estimate the prevalence of COVID-19 infection in a target population – whether this is cumulative or point prevalence – must be based on a random sampling scheme to ensure representativeness. Ideally, a test and cut-off that minimizes the overall misclassification rate (i.e., false negative and false positive) must be selected and the estimated prevalence should adjust for the Se and the Sp of the diagnostic process. Pooled testing can also be an option especially in the case of low-prevalence populations (16).

4. Cut-off selection depending on the purpose of testing

To serve the different objectives of the various testing strategies, tests should not have a fixed cut-off. The cut-off must be subject to change.

During the COVID-19 pandemic RT-PCR tests have been used to diagnose, screen, and estimate the prevalence/incidence of the infection, in populations with different prevalence. RT-PCR are qualitative tests with a quantitative component – (Ct) values. An optimal cut-off (Ct value) should be selected according to the purpose of testing to reduce the probability of misclassification and associated costs. A cost-benefit analysis of the diagnostic test can be used to define the optimal cut-off, using the misclassification cost term (MTC). MTC takes into account the cost of incorrect classifications – the cost of false negatives (CFN) and the cost of false positives (CFP) – the expected prevalence of infection in the target population (p) and the Se and Sp of the test (17–19).

As the ratio of CFN/CFP increases, for any given prevalence, the Ct cut-off must increase (more sensitive tests) to achieve the minimum misclassification cost (20). The Ct cut-off will also increase when the prevalence of the infection increases (20). When an RT-PCR test is used to perform screening to prevent transmission the CFN is expected to be higher because a FN result contributes to further transmission of the infection. Then a more sensitive test should be used to reduce the misclassification cost.

In a similar manner – and perhaps a more straightforward way – the selection/modification of cut-offs for antigen tests should be subject to change depending on the target population (i.e., low or high prevalence population) and the objective (i.e., lowered cut-offs to improve the Se of detecting infectious but asymptomatic individuals, even at a cost in Sp). An example of using MCT to identify appropriate cut-offs is presented in appendix 1.

5. Sampling to estimate COVID-19 prevalence

Nonresponse, selection bias and non-probability sampling do not allow for valid estimation of COVID-19 prevalence and incidence. Most of the existing sampling and testing schemes for COVID-19 are essentially carried out in a passive surveillance setting. Thus, the current crude estimates of population prevalence (i.e., the number of cases out of the number of tested samples) are subject to ascertainment bias, whereas a large proportion of infected individuals are asymptomatic and not tested. Prevalence estimates are not comparable between different time periods and/or among countries since the intensity of sampling and criteria for testing greatly vary between areas under different jurisdiction as well as over time (21).

There is a need for population surveys that will be based on a representative sample of individuals in order to provide valid estimates for the number of active and recovered cases (22). In line with these, the WHO recently released a population-based, age-stratified, seroepidemiological investigation protocol for COVID-19.

Simple random sampling is the golden standard for cross-sectional prevalence studies. However, the method of recruiting participants should reflect the objective, feasibility, and resources available. WHO protocol recommends a convenient sampling method for recruitment of the participants (23). Participants can be recruited over all age ranges and different age ranges may be applied depending on the objectives. A cluster sampling scheme may improve the logistics and deal with feasibility constraints. Finally, there is an issue with different and hard to reach populations that must also be addressed (24). In such cases, a different response rate is expected, and the sampling scheme must adjust for the anticipated non-response.

6. Need for population-specific diagnostic accuracy estimates

The diagnostic Se and Sp are population-specific characteristics that depend on the distribution of the various infection stages within each population and the potentially varying distribution of viral strains – that can be of different virulence – among populations. Hence, Se and Sp estimates cannot be simply extrapolated from one population to another. While diagnostic evaluation studies are time-consuming and resource-demanding, valid statistical methods exist for the rapid evaluation of diagnostics without the need for a reference test. Such methods can and have been used (25,26) to provide COVID-19 Se and Sp estimates in different settings. Their application in a broader context will facilitate estimation of population-specific Se and Sp.

Importantly, Se and Sp estimates that are obtained from a clinical setting are not relevant to the Se and Sp estimates of the same tests in the general (and mainly asymptomatic) population.

7. Estimation of COVID-19 true prevalence

True prevalence estimates should adjust for the Se and Sp of the diagnostic process. Methods for true prevalence estimation have existed for over fifty years (27) but are not often applied. The apparent prevalence (*ap*) of disease is the proportion of individuals testing positive to the test that is used. The number of test-positive individuals, *y* out of the *n* sampled individuals can be assumed to follow a binomial distribution:

$$y \sim Binom(n, ap)$$

If the diagnostic *Se* and *Sp* of the test are known, *ap* can be expressed as a function of the true prevalence (*p*) of disease/infection and the *Se* and *Sp*:

$$ap = pSe + (1 - p)(1 - Sp)$$

Subsequently, estimates of the true prevalence can be obtained using standard frequentist approaches whereas a Bayesian estimation framework is also available and allows for the capture of the hierarchical structure of populations (28,29).

Nevertheless, true prevalence estimates will only be valid if the accuracy (Se & Sp) of the test is relevant to the target population. Otherwise, it may not be wise to estimate and report true prevalence in place of the apparent prevalence (30).

8. Testing infrastructures for COVID-19 surveillance

Depending on the strategy, testing can be applied in different settings and circumstances. Point-of-care (POC), rapid diagnostic tests are used in various settings, such as:

1. Physician offices
2. Urgent care facilities
3. Pharmacies
4. School health clinics
5. Long-term care facilities and nursing homes
6. Temporary locations, such as drive-through sites managed by local organizations

Laboratory tests for COVID-19 are implemented in:

1. Clinical laboratories
2. Public Health laboratories
3. Hospital-based laboratories (Public or Private)
4. Research and Reference laboratories

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Appendix 1

The misclassification cost term (MCT) takes into account the relative cots of false positive and false negative test results (CFP and CFN, respectively), and the expected prevalence of disease (P) in the target population in order to determine the optimal cut-off. MCT is given by:

$$MCT = \frac{CFN}{CFP}[P * (1 - Se)] + (1 = P)(1 - Sp)$$

The optimal cut-off is the one that minimizes MCT. In figure 1, we have a ROC curve from hypothetical data of an antigen test. In figure 2, the MCT is plotted for different combinations of $CFN/_{CFP}$ and expected prevalence (*P*) in the target population.

Figure 1. ROC curve from hypothetical data of an antigen test.

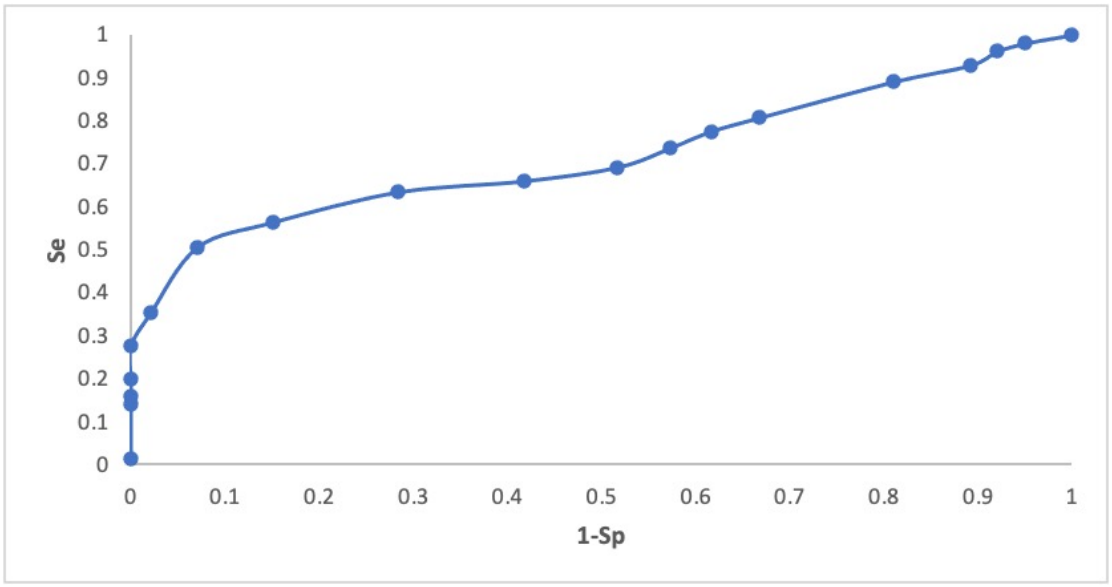
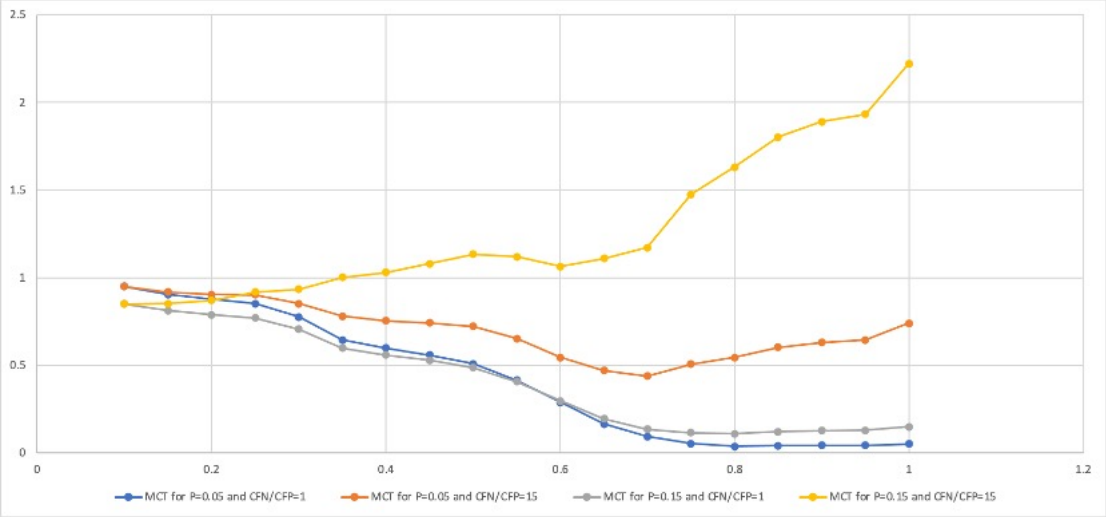


Figure 2. MCT estimates at each cut-off for different (a) relative costs of false positive and false negative results and (b) expected prevalence in the target population.



MCTs are minimized with increasing $^{CFN}/_{CFP}$ (i.e., lowered cut-offs are needed when the cost of a false negative result is assumed to be higher compared to a false positive result) and with increasing prevalence (i.e., lowered cut-offs are needed when the expected prevalence in the r=target population is higher).