COVID-19 testing:
A reflection on test accuracy in the real world

Produced by the ASPHER COVID-19 Task Force

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Executive Summary:

Testing is essential to understand the dynamics of COVID-19 in the population, to plan preventive measures, and to provide the basis for appropriate therapeutic measures. After the identification of the SARS-CoV-2 virus and the clinical definition of COVID-19, there was a rapid development of diagnostic and screening tools. Their accuracy needs to be assessed carefully as the interpretation of results could have profound impacts on individual and public health decision-making. We reviewed the information available on the performance of existing tests to identify the virus (molecular tests) or its immunological expression (serological tests). The major findings and concerns are highlighted below:

Molecular tests for clinical cases and contact tracing

1. Molecular tests - reverse transcription-polymerase chain reaction (RT-PCR) - are essential to confirm a COVID-19 case – to trace contacts, to isolate, and subsequently, to confirm viral clearance, as decisions based on clinical resolution do not seem to match the viral clearance from testing;
2. A meta-analysis cited in this report estimated the pooled sensitivity to RT-PCR as 89% (95%CI: 81-94%);
3. This should lead us to be cautious about the proportion of reported false negatives. The performance of RT-PCR tests, whether for diagnosis or screening, is a very important concern. False positive results will lead only to a degree of inconvenience for the individuals who have positive results. However, a large number of individuals with false negative results allowed back into general

society, or into health and social care, could have a considerable impact and the opportunity for the spread of the virus into susceptible individuals.

4. In the peak period of the pandemic passing through communities, mass testing and contact tracing may not be practicable, and the advice to individuals will be the same regardless of a test result, namely self-isolation.
5. However, there will be a place for reintroduction of testing and contact tracing as numbers reduce, such that countries are looking to reduce lockdown and isolation procedures, and sufficient staffing resources and test kits permit.

Serological tests for case confirmation and advice for contacts and key workers

6. Serological tests are a potential tool that can be used in large scale surveillance efforts to estimate the prevalence of the population ever exposed to the virus, which is expected to be much higher than those diagnosed at any one moment.
7. The presence of antibodies for SARS-CoV-2 does not exclude that the individual may remain infectious due to recent infection, thus, clinical and epidemiological history should also be considered in relation to serological testing;
8. The reliability of serological tests needs to be checked and further development of serological tests is needed. For example, if we are to pursue the idea of an "immunity passport" – this will rely critically on clear evidence of long-lasting immunity;

Serological testing for whole populations

9. Interpretation of test results must be careful and cautious, whether for individuals or whole populations;
10. The positive predictive value is the proportion of all positive tests which are true positive cases. This figure is more reliable when there is a high prevalence of virus circulating in the community. However, as we test more people, we will find large numbers of false positive results, even if the test is thought to have a high

specificity. Our estimates of how much virus there is in communities will be very difficult. *We do not know if the high figures being quoted in some studies represent a high level of asymptomatic infections or a high level of false positive tests.*

The assessment of false positives becomes especially important in this situation - as an overestimate of the degree of a previous infection could allow relaxation of controls too soon.

11. An assessment of the extent of spread would be very important in assessing the level of immunity in the population, and the likely impact of relaxing physical distancing and other lockdown measures.
12. An international consensus on what the population parameters are when the relaxation from lockdown might start would be desirable.
13. Testing alone is not a panacea. Testing must always be considered as part of the range of public health, non-pharmaceutical measures available to respond to the current pandemic;
14. A major effort is needed to transparently communicate to the public the issues of testing effectiveness. The public needs and deserves to understand the issues of testing effectiveness and efficiency including what is meant by sensitivity, specificity, and predictive values.

Background

An emerging respiratory syndrome, later identified as a consequence of an infection by the novel coronavirus, designated Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was first reported in December 2019 in Wuhan, China, causing the coronavirus disease 2019 (COVID-19). After the global spread of the disease, a pandemic was declared on March 11, 2020, by the World Health Organization (WHO). On April 23, 2020, more than 200 countries, territories, or areas were affected, there were over 2 500 000 confirmed cases and more than 170 000 deaths worldwide (1).

In the absence of a vaccine or effective treatment, it is essential to correctly diagnose people at an early stage and isolate those carrying the virus to prevent further transmission. Furthermore, widespread testing is necessary to monitor the epidemic. Presently there are two types of tests available for COVID-19: a molecular test - RT-PCR and a serological test - antibody-based test. These will be analysed separately.

Serological tests are blood-based tests that can be used to identify whether people have been exposed to the pathogen by looking at their immune response. In contrast, the RT-PCR tests currently being used to diagnose cases of COVID-19, indicate the presence of viral material during infection.

A positive RT-PCR has been used to confirm the diagnosis of a suspected COVID-19 patient (2, 3). The European Centre for Disease Control (ECDC) recommends, that patients' de-isolation should be based on the clinical resolution of symptoms and, where testing capacity permits, evidence for viral RNA clearance, taken after two upper respiratory tract samples negative for SARS-CoV-2 have been collected with at least a 24-hour interval (4).

Accuracy

The tests' accuracy is captured by two measures:

- *Sensitivity* – the ability to correctly detect those with the disease, i.e., the percentage of people with the disease that test positive;
- *Specificity* – the ability of the test to correctly detect those who are disease-free, i.e., the percentage of people without the disease that test negative (5).

These measures are only dependent on test performance, while the predictive values depend on the characteristics of the tested population (See examples in Appendix 1).

- The Positive Predictive Value (PPV) is the percentage of patients who test positive that actually have the disease, i.e. the probability of having the disease among those who test positive;
- The negative predictive value (NPV) is the percentage of patients who test negative that actually do not have the disease, i.e. the probability of not having the disease among those who test negative (5).

From an individual and a Public Health point of view, we need to take into account the PPV which is highly variable according to the prevalence of the disease and the specificity of the test. In a population with a low prevalence of the disease (for example 1%), as shown in Appendix 1, a test highly accurate - 99% sensitive and specific -, would mean a PPV of 50%, i.e., 50% of false positives, the chance of having a face when tossing a coin. This is a particular concern in serological tests (later analysed). Tests with lower sensitivity and specificity will mean more false positives.

RT-PCR tests

The accuracy of RT-PCR tests can be compromised at various stages, from sampling to result (6-11), as summarized in Table 1.

Table 1. Causes of inaccuracy with RT-PCR tests.

- specimen collection;
  - sampling *per se*;
  - sampling time;
  - insufficient sample material collected;
  - specimens' source (lower or upper respiratory tract);
- transportation;
- technical and analytical errors that might be increased during a pandemic, and in real time;
  - lack of appropriate laboratory reagents;
  - lack of harmonization;
- different limits of detection depending on the kit used;
- the virus-specific diagnostic window;
- severity of disease.

WHO recommends to "test, test, test", noting that South Korea and Taiwan were having success in limiting infections by doing so (12). However, the message of WHO Director came at a phase in the outbreak when there was no knowledge of asymptomatic carriage and the idea was not to test as much as you can but to test all suspected cases in order to contain the virus and not miss opportunities.

An unpublished report from mass testing of 3000 inhabitants of the city of Vò, in Italy, suggested that identification and isolation of individuals was the key to controlling the

virus (13). It found a high prevalence of asymptomatic positive individuals and strongly supported the arguments for testing, particularly health and social care workers who would be at most risk of the inadvertently spreading of the virus. However, the results need to be formally published and the possibility of false positives, or positives for other coronaviruses is a concern. The fact that a person is labelled negative when he or she actually has the disease (false negative case) tends to occur when the test has a low sensitivity and this creates an easily understood risk, because of the spread of the contagion that this may cause. On the other hand, the fact that a person tests positive when in fact he or she does not have the disease (false positive case associated with low specificity) also causes problems.

Despite the strong calls for testing, many countries are experiencing a shortage of test kits and reagents, and skilled operatives for the PCR machines. RT-PCR testing has been restricted to those with symptoms in general. From the 41 countries with available data, among symptomatic population groups, most of them (n=39) are testing individuals who are hospitalised, followed by testing health and social care workers (n=32) and only one-third of the countries (n=13) are testing individuals with mild symptoms – this varies according to the capacity of testing and the phase of the epidemic (14).

Questions are emerging regarding the accuracy of the currently available tests. They are expected to be very accurate under laboratory conditions, working with known viral samples (15, 16). However, there are reports of symptomatic patients testing positive only on the sixth nasal swab (17) and after 8 days after symptoms onset, with at least two nasopharyngeal and oropharyngeal swabs in between (18). Retrospective studies have demonstrated that the positive rate seems to be low (8, 19). The positive test rate refers to the assessed rate of positive tests results among those who have previously been diagnosed according to specific criteria - known samples containing the virus or confirmed clinical cases.

Reports of the accuracy of tests should be scrutinised carefully, as the technical details presented might not be enough for a proper assessment and sample sizes so far quoted are small leading to unacceptably large confidence intervals. The reports summarized in table 2 (8, 19), come from the first scientists to present data on this issue. Further studies are required, with larger sample sizes and more detailed methods.

The severity of disease and days after onset of symptoms influence the results. The probability of false negatives increases after the first week of symptoms mainly in mild cases (8, 17, 20). Yang et al found the highest positive rates in samples collected within the first 7 days after the onset of symptoms and among severe cases, mainly in sputum, regardless of the severity (88.9% positive rate in severe cases vs. 82.2% positive rate in mild cases) (8). In severe cases, bronchoalveolar lavage fluid (BALF) presents the highest rate of positives (78.6-100.0%) (8). Considering inpatients with COVID-19 from 3 hospitals in Hubei and Shandong provinces and Beijing, China (n=205), the highest positive rates were found among BALF (93%), followed by sputum (72%), nasal swabs (63%), fibrobronchoscope brush biopsy (46%), pharyngeal swabs (32%), faeces (29%), and blood (1%), urine specimens did not test positive (19). Lin et al, considered paired specimens, throat swab, and sputum, for 52 suspect cases, and found higher positive rates for sputum (76.9% vs. 44.2%) (24). The fall of positive rates over time may reflect the course of the illness but there is a possibility of false negative results at any stage. A meta-analysis estimated the pooled sensitivity to RT-PCR as 89% (95%CI: 81-94%), but substantial heterogeneity was present (21).

The US Centers for Disease Control and Prevention recommends nasopharyngeal swab as the preferred choice (22). Although the most widely applied it could mean around 30-40% false negatives, which increases up to 46% when the sample is collected after the first week of onset of symptoms in mild cases (8, 19). Lower respiratory tract samples seem to be more accurate – BALF and sputum sampling (8, 19, 23, 24). However, not all patients present sputum (25) and BALF collection is complex representing higher risk of

aerosol production and therefore added risk to the sampler, and it is painful for patients, and therefore only reasonable for the sickest patients (3, 8). For patients post 14 days, the highest negative rates, either reflecting viral clearance or false negative results, are 21.4 % (BALF) - 53.2% (Throat) for severe cases and 40% (Throat), 57.1% (Sputum) for mild cases. Interpretation of negative results for recovering patients will require the most caution.

Wang *et al* detected live virus in faeces (19) and Zhang *et al* found that patients with a negative oral swab, may present positive anal swabs or blood but (26) the impact of those shedding routes is not clarified.

Table 2. Accuracy of RT-PCR in detecting SARS-CoV-2 in different specimens of COVID-19 patients. Summary of two studies (8, 19).

Study (Author, year)	Yang et al., 2020 (8)			Wang et al., 2020 (19)
Location and data	Shenzhen Third People's hospital between Jan 11 and Feb. 03, 2020			Hospitals in the Hubei and Shandong provinces and Beijing, China, from January 1 through February 17, 2020
COVID-19 diagnosis criteria	Guangdong CDC confirmed 2019-nCoV infected patients			based on symptoms and radiology and confirmed by SARS-CoV-2 detection
Severity	According to the guidelines of 2019-nCoV infection from the National Health Commission of the People's Rep. of China			-
Sample size	COVID-19 patients: 213 Specimens analysed: 866			COVID-19 patients: 205 Specimens analysed: 1070
Samples	Collected upon admission and various time-points, thereafter, further divided in 0-7 d.a.o., 8-14 d.a.o. and >14 d-a-o-: - nasal swabs; - throat swabs; - sputum; - BALF			Collected from most patients 1 to 3 days after hospital admission: - pharyngeal swabs; Collected throughout the illness: - blood; - nasal - sputum; - feces; - urine Sampled from patients with severe illness or undergoing mechanical ventilation: - BALF; - fibrobronchoscope brush biopsy
RT-PCR	Performed using a China Food and Drug Administration (CFDA) approved commercial kit specific for 2019-nCoV detection (GeneoDX Co Ltd, Shanghai, China)			Performed using a 2019-nCoV nucleic acid detection kit according to the manufacturer's protocol (Shanghai bio-germ Medical Technology Co Ltd)
Positive criteria	Cycle threshold* value was ≤ 37.0			Cycle threshold* value was < 40
Detection in respiratory samples	d.a.o.	**Severe cases**	**Mild cases**	- BALF (93%) - sputum (72%) - nasal swabs (63%) - fibrobronchoscope brush biopsy (46%) - pharyngeal swabs (32%) - faeces (29%) - blood (1%) - urine (0%)
	0-7	throat: 60.0% nasal: 73.3% sputum: 88.9% BALF: -	throat: 61.3% nasal: 72.1% sputum: 82.2% BALF: -	
	8-14	throat: 50.0% nasal: 72.3% sputum: 83.3% BALF: 100.0%	throat: 29.6% nasal: 53.6% sputum: 74.4% BALF: 0%	
	>14	throat: 36.8% nasal: 50.0% sputum: 61.1% BALF: 78.6%	throat: 60.0% nasal: 54.5% sputum: 42.9% BALF: -	

BALF: Bronchoalveolar lavage fluid
d.a.o: Days after illness onset
*Cycle threshold values are inversely proportional to the amount of target nucleic acid (i.e. lower the Ct higher the viral load).

Recently, on April 10, the director of the Korea Centres for Disease Control and Prevention (KCDC) reported that 91 patients previously cleared of the new coronavirus had tested positive again (27). In the University Hospital of São João, Porto, Portugal, where 2055 patients were admitted between March 2 and April 20 of 2020, from the initial 123 patients that had a negative first negative to document recovery, 52 (42%) presented a positive result in the immediate test performed after at least 24 hours (Tavares M and Severo M, personal communication). Two health care workers from Zhongnan Hospital in Wuhan, China, were reported to test positive after discharge based on 2 negative swabs 24h apart (28). Lan et al followed 4 patients after discharge, all had 2 consecutive negative RT-PCR, and 3 had the CT imaging abnormalities resolved, but all tested positive 5 to 13 days later (29). Similarly, among 209 COVID-19 patients, 4.7 average days after discharge, 9 (4.3%) were RT-PCR positive in throat swabs, 13 (6.2%) RT-PCR positive in anal swabs, and 22 (10.5%) positive in either (30). Data on these cases is missing, epidemiological studies are being conducted, any conclusions at this time would be imprudent, but questions are inevitable: were they cleared from the virus or were there false negatives? Or is the much desired, long-lasting immunity far from being the reality?

Since March 23, 2020, the Institute of Public Health of the University of Porto in partnership with the journal Público and the Institute for Systems and Computer Engineering, Technology and Science, in “Diaries of a Pandemic”, followed 11 125 individuals that voluntarily provide preferentially daily information on risky contacts, symptoms, and testing for SARS-CoV-2. Participants who presented symptoms (cough or fever or dyspnea) were more frequently tested. Considering those with symptoms and recognized contact of risk (n=295) only 24.7% (n=73) did a test, and 27 (37.0%) were positive. This may indicate that the definition of risk is very sensitive (which is expected in a pandemic situation) or the sensitivity of the test itself is not as high as would be desired. On the contrary, among the 8 613 participants who had neither such symptoms nor contacts with suspected or confirmed cases of infection, 187 (2.2%) were tested

and, almost half (48.7%; n=91) were positive, a higher proportion than among the participants with a priori higher risk. This could mean that the individual's perception of risk may not be a good assurance or the false positives among molecular tests are higher than desirable (data not published).

In brief, there are two major points to value in clinical history when RT-PCR tests are concerned:

- whether a clinical suspect case carries the virus, being then considered a confirmed COVID-19 case; and
- whether a confirmed case has recovered – viral clearance. This would then aid a decision to de-isolate.

A negative RT-PCR does not exclude COVID-19 (7, 10, 11, 31, 32), the test result needs to be contextualized, in the presence of clinical symptoms and a suggestive epidemiological link. The accuracy of the different swabs when using RT-PCR cannot be determined with certainty at this point. The sensitivity of nasal swabs seems to be moderate. False negatives have the potential to cause inadvertent circulation of the virus in the community, contributing to missed opportunities to contain transmission. Thus, policies that assume high accuracy of RT-PCR tests should be cautiously reviewed. Despite all theoretical benefits from an epidemiological point of view, massive testing or large scale testing of mild symptomatic individuals requires substantial resources (33). The outcome for these individuals will be the same as if they had not been tested-namely to self-quarantine as part of the lockdown, with no specific treatment being available.

Studies have found that subclinical COVID-19 patients may show chest CT changes earlier than a positive RT-PCR (34-36). However, in low prevalence areas, chest CT screening has low PPV (1.5-30.7%) (21). The American College of Radiology, the Society of Thoracic Radiology and the American Society of Emergency Radiology stated that CT should not be used to screen for as a first-line COVID-19 (37, 38).

RT-PCR and key workers, namely health and care workers

According to the ECDC, among health care workers and other critical infrastructure responders classified as mild suspected or confirmed COVID-19 may end isolation after the resolution of fever for at least 3 days and if eight days from the onset of symptoms have elapsed (4). They recommend that health care workers can return to work under these conditions, using a surgical mask during work hours until 14 days after the onset of symptoms. Stating that where testing capacity allows, they should be considered a priority group for testing. For a clinically recovered patient, two negative RT-PCR tests from respiratory specimens at 24 hours interval, at least eight days after onset of symptoms (4). However, it is not clear if those who had unprotected contact with a COVID-19 case and do not develop symptoms in the following 14 days should be tested. Testing once is unlikely to be sufficient given the issue of false negatives. This is particularly important for health and social care workers. This problem is particularly stressed in connection with the lack of personal protective equipment faced by health care workers in some countries.

As we are simultaneously using RT-PCR as a screening and a diagnostic test, it must present both high sensitivity and high specificity. Tests with higher sensitivity or a combination of tests to increase sensitivity are needed. However, tests, or combinations of tests, which increase sensitivity also tend to decrease specificity; as more true positives are found, so too are more false positives. The performance of RT-PCR tests, whether for diagnosis or screening, is a very important concern. False positive results will lead only to a degree of inconvenience for the individuals who have positive results. However, a large number of individuals with false negative results allowed back into general society, or into health and social care, could have a considerable impact and the opportunity for the spread of the virus into susceptible individuals. National aspirations, as with the UK government, to create ‘a national effort for testing, to build a mass-testing capacity for the UK’ (39) need to be considered more cautiously, if they are not

to liberate large numbers of infectious people back into the workforce, and into the health service. Test limitations must be carefully discussed in policy decision making because finally it may be the case that 'a bad test is worse than any test' (39).

Rapid diagnostic tests based on antigen detection

Some companies have been developing rapid diagnostic tests based on antigen detection (40) – these detect the presence of viral antigens of the SARS-CoV-2 virus in a sample from the respiratory tract, usually qualitative tests (positive/negative result). These tests are similar to RT-PCR in the sampling process and purpose - used to identify acute or early infection, as the antigens detected are expressed only when the virus is actively replicating, however, the antigen tests present rapid results - within 30 minutes and are easier to perform (41). Although some companies claim that the tests are highly reliable (42-44), more evidence in the performance of the tests is needed. At the moment, WHO does not recommend to use of these tests on patient care, encouraging further studies (41). However, WHO does not exclude that if they "demonstrate adequate performance, they could potentially be used as triage tests to rapidly identify patients who are very likely to have COVID-19, reducing or eliminating the need for expensive molecular confirmatory testing" (41), being a major advantage.

Serological testing: individual and population level

There are three major types of serological test (45): rapid diagnostic tests, the most frequently used for population-based studies in the context of COVID-19, most frequently test for patient antibodies (IgG and IgM) and are typically a qualitative (positive or negative) assay that can be used at the point of care. The enzyme-linked immunosorbent assay (ELISA) can be qualitative or quantitative and generally lab-based and test COVID-19 patients' antibodies (IgG and IgM). Neutralization assays take patient antibodies to prevent viral infection of cells to demonstrate the blocking of virus replication.

Several rapid tests have been developed (46, 47). On April 1, 2020, the ECDC reported that there were over 60 rapid SARS-CoV-2 antibody tests (48), and on April 16, 2020, already more than 100 were listed on the Foundation for Innovative New Diagnostics (49), only considering those CE-Marked. However, until now, few studies on the accuracy of those tests are available. Li et al developed a rapid test that considers both IgM and IgG and presented a sensitivity of 88.7% and a specificity of 90.6% (50). Hoffman et al evaluated the COVID-19 IgG/IgM rapid test described in Li et al and found a sensitivity of 69% IgM and 93.1% for IgG and a specificity of 100% for IgM and 99.2% for IgG (51).

In March the American Food and Drugs Administration (FDA) allowed the "developers of certain serological tests to begin to market or use their tests...without prior FDA review if certain conditions outlined in the guidance document are met" (52). We should not be naive enough to believe there is no commercial imperative and potential conflict of interest inherent in the development and adoption of new tests. On the 8th of April

2020, WHO recommended to use these new tests only in research settings, encouraging further studies (41).

At the individual level, serological testing has been seen as a non-invasive complement to RT-PCR. Immunoglobulin M (IgM) is the first antibody to appear in the blood, thus at the early stage of infection IgM seems to be a good addition to RT-PCR diagnosis (53), as the antibody response increases when viral load decreases (26, 54). Padoan *et al* found that after day 11 since fever onset, all patients tested positive for IgG, while IgM positivity varied from 50% to 88% (55). Similarly, Zhang *et al* found that IgM positivity varied from 50% to 81% while IgG increased from 81% to 100%, being both higher later in the illness course (26). Zhao et al found that combining RNA and viral antibodies increased the sensitivity from 67% to 99% (RNA: 67.1%, Antibodies: 93.1%, IgM: 82.7%, IgG: 64.7%, RNA plus antibodies: 99.4%) (56). Lou et al found the sensitivity of total antibody detection was higher than the IgM or IgG, at different times after onset, reaching both the highest sensitivity more than 14 days after onset, 100.0%, 96.7%, and 93.3%, respectively (54). Severe cases, in general, had earlier IgM response and higher IgM and IgG levels than milder ones (57).

At the population level, serological surveillance aims to understand the true scale of the population exposed to the SARS-CoV-2 and to adjust public health measures to the local context. An early report from a German town estimated that 14% of inhabitants were considered immune (58). This raises several hypotheses: a possibility of overestimated prevalence due to cross-reactivity with other coronavirus antibodies, a much higher prevalence than previously thought, or that the town is for unknown reasons unrepresentative of the usual dynamic of the infection. A study in 3330 inhabitants of Santa Clara County, California, estimated that 1.5% presented specific antibodies to the virus; this was 50 to 85 times more than the confirmed cases (59), but still, it meant that only 3% of the population had been exposed. In Munich, Germany, the Division of Infectious Diseases & Tropical Medicine at the Medical Centre of the University of

Munich intends to start a cohort with 3000 randomly selected households, to determine antibodies against SARS-CoV-2, repeated several times over 12 months (60).

The "immunity passport"?

The interest surrounding the rapid tests has been because they may be the key to obtain or prescribe an "immunity passport": those who test positive are immune – and therefore are out of risk - and those who test negative are not immune – and therefore considered to be at risk. This is of particular interest to decide whether health and social care workers can go back to work (61). However, this might cause more harm than good, considering the currently available evidence on test performance and our current knowledge on the immunological response to the virus (62). Even if the available tests are reliable, at the moment, there is no evidence regarding the long-lasting immunity that a past exposition to SARS-CoV-2 may confer.

Before large scale serological testing, guidelines must be discussed and made available to all who will have access to rapid tests. The presence of antibodies for SARS-CoV-2 does not exclude that the individual may remain infectious due to recent infection, thus, clinical and epidemiological history should also be considered in serological tests. Most of the currently available rapid tests detect IgM and IgG if IgM is detected it could mean that there is a current acute infection. How to proceed in this case should be acknowledged: should a RT-PCR test be performed? Should the local health entities be informed? However, IgM may be detected for different reasons, pregnant woman and people with autoimmune diseases tend to test positive, who should undergo a molecular test in these situations should be clearly stated. If the test is not accompanied by accurate and efficient counselling, comprising an explanation of the test result. A false positive may confer an inaccurate sense of security, and lead to neglecting measures of physical distance and individual protection, particularly critical among health workers who are at higher risk.

Positive predictive value - vitally important in population estimates

As mentioned previously, the PPV varies widely with the prevalence of the disease in the population tested, and consequently also the false positive does. Considering the prevalence of COVID-19 in the different Italian regions estimated by Signorelli *et al* (63) and the specificity and sensibility of the antibody tests estimated by Li *et al* (50), if we test the entire population of Lombardy and Sicily regions – which have the highest and lowest prevalence, the estimated results are expressed on figure 1 and 2, respectively.

In a low prevalence setting, as Sicily (Figure 2), if all the population was tested, the estimated PPV would be of 3.2%, this means that, of the 483 878 who would test positive, only 15 523 had the disease and 468 355 would be false positives, i.e., 96.8% would be wrongly considered immune, and therefore will be at risk and could be an opportunity to further spread the virus if allowed to back into general society, or into health and social care, could have a considerable impact and the opportunity for the spread of the virus into susceptible individuals.

The PPV would increase with more accurate tests, however, the prevalence of the disease is the main reason for lower PPV and, consequently, higher rates of false positives (See examples in Appendix 1). As we test more people, we will find larger numbers of false positive results, even if the test is thought to have a high specificity. Our estimates of how much virus there in communities will be very difficult. *We do not know if the high figures being quoted in some studies represent a high level of asymptomatic infections or a high level of false positive tests.* Evidence-based policymaking should be individualized and take into account the different variables that may affect the reliability of the test result.

Mass surveillance results - a need for caution

There might a case for mass surveillance to understand the spread of the virus and immunity in the community (64). Such surveillance is being committed to, in some countries (45, 60).

A population-based assessment could be anonymous and done on samples taken for other purposes, or through a random sample, as in Spain. An assessment of the extent of spread would be very important in assessing community immunity, and the likely impact of relaxing physical distancing and other lockdown measures. An international consensus on what the population parameters are when the relaxation from lockdown might start would be desirable. However, the assessment of false positives becomes especially important in this situation - as an overestimate of the degree of previous infection rates could allow relaxation of controls too soon. Table 3 presents the major characteristics obtained after real-world studies using RT-PCR, chest CT and serological testing. Sensitivity rates ranged from 67.1%-97% and specificity ranged from 25%-90.6%. So, at present, one needs to carefully assess the performance of the tests to make appropriate inferences both at an individual or a population level, while expecting that better performing tools are available.

Interpretation of test results must be careful and cautious, whether for individuals or whole populations. We show the possible approaches and considerations in the decision trees (Appendix 2) – highlighting that, at the moment, WHO does not recommend the use of rapid tests on the clinical practice, however with the increased availability of CE-Marked tests, we found it imperative to discuss the implications of those test results, on the light of the current evidence.

Figure 1. The Lombardy region – an example of a region with a high prevalence of the disease.

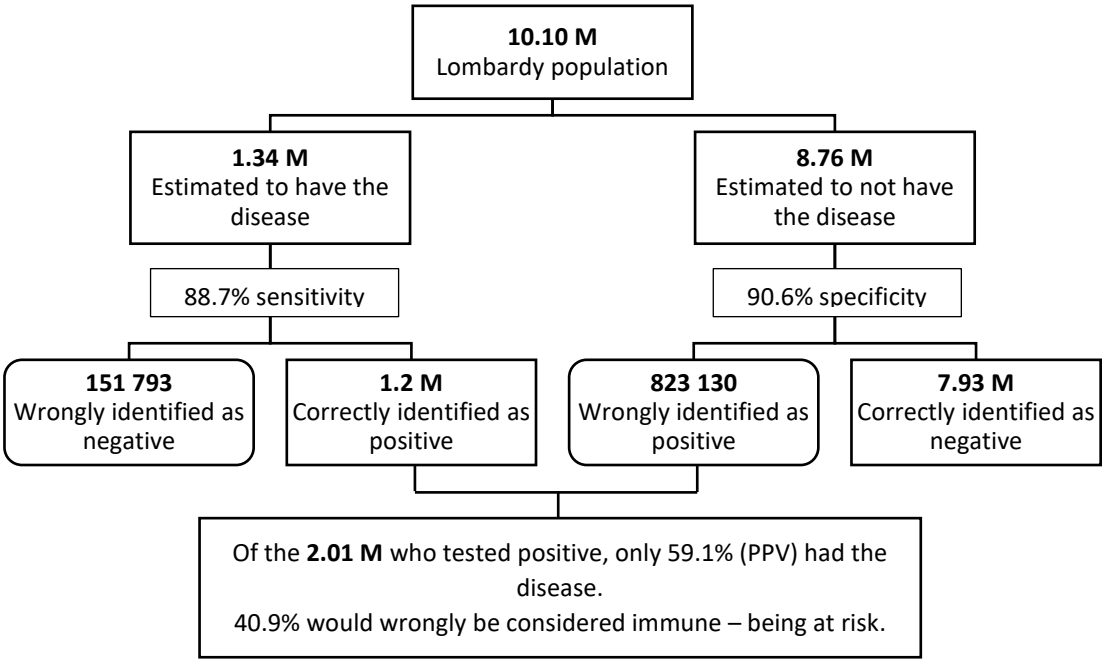

Figure 2. The Sicily region – an example of a region with a low prevalence of COVID-19.

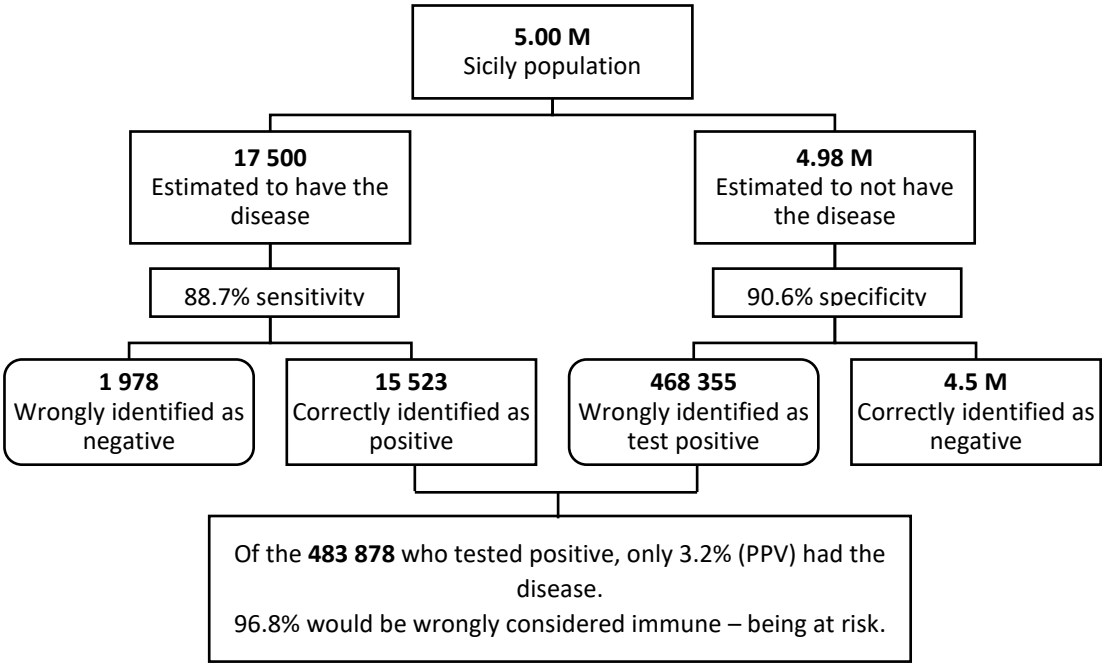

Table 3. Real-world testing: RT-PCR and serological test studies.

Study (authors)	Ai, Yang (35)	Long, Xu (36)	Zhao, Yuan (56)	Caruso, Zerunian (65)	Li, Yi (50)
Objective	Performance of chest CT using RT-PCR as reference	A retrospective study of patients with diagnosed COVID-19, and both chest CT and RT-PCR at initial presentation	Investigate the dynamics of total Ab, IgM, and IgG against SARS-CoV-2 in serial blood samples from confirmed COVID-19 patients	Performance of chest CT using RT-PCR as reference	SARS-CoV-2 IgG-IgM combined antibody test
Place of study	Wuhan, China	China	Shenzhen Third People's Hospital, China	Rome, Italy	Multiple hospitals, China
n (%)	n total: 1014 RT-PCR positive: 601 (59%) chest CT: 888 (88%)	n total: 36	n total: 173	n total: 158 RT-PCR positive: 62 (39%) chest CT: 102 (64%)	n total: 525 RT-PCR positive: 397
Clinical specimens (sample)	throat swab	N.A	respiratory tract swabs	nasopharyngeal and oropharyngeal swabs	blood
Time until results	N.A.	N.A.	N.A.	N.A.	<15 min
Sensitivity (95%CI)	97% (95-98)	chest CT: 97.2% RT-PCR: 83.3%	RNA: 67.1% (59.4-74.1) Ab: 93.1% (88.2-96.4) IgM: 82.7% (76.2-88.0) IgG: 64.7% (57.1-71.8) RNA+Ab: 99.4% (96.8-100.0)	97% (88-99)	88.7%
Specificity	25% (22-30)	N.A.	N.A.	56% (45-66)	90.6%

Conclusions

1. The World Health Organization recommended a strong commitment from all countries to “test, test, test” whenever suspected. However, the concern about test performance remains. Decision-makers, the health community, and the general public need to understand the limitations of testing. This applies to its use with individuals in diagnosing the illness and predicting recovery. It also applies to national assessments of population exposure and immunity.

2. Testing in the early phase of the pandemic response is part of a comprehensive response of which lockdown and isolation are the most significant elements. Testing and contact tracing will have an important role supporting other non-pharmaceutical measures in the de-escalating phase.

3. We believe the levels of false negative reporting for RT-PCR are such that reliance on this test to allow health workers and other key workers to return to work is not without considerable risk if strict adherence to universal protection measures is not respected. The ECDC recommends two negative RT-PCR results before permitting people to return to work. We believe this is prudent, but it does still carry the risk that individuals tested who test negative might still be carrying the virus and be infectious.

4. There are questions about the relevance of population serology approaches because of concerns particularly with the positive predictive value of the available tests, particularly in phases or populations with a low prevalence of infection.

Their importance to estimate the level of population exposure is probably more relevant than its interest for individual decisions, such as an "immunity passport".

5. The role of testing, both RT-PCR and serology, in the second wave, de-escalation and recovery phases, in each country, needs continued research and a truly concerted collaborative international effort to address the recognized gaps in our understanding.

6. Testing must always be considered as part of a range of public health, and non-pharmaceutical measures available for response to the current pandemic.

7. Interpretation of test results must be careful and cautious, whether for individuals or whole populations. We show this in the decision trees shown in appendix 2.

8. A major effort is needed to communicate transparently to the public the issues of the effectiveness of testing. The public needs and deserves to understand the issues of testing effectiveness and efficiency including what is meant by sensitivity, specificity, and predictive values.

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Appendix 1

We calculated the predictive values, considering different sensitivity and specificity values, in different prevalence scenarios.

For example, considering a 10 000 population, with a disease prevalence of 10%, using a test with a sensitivity of 65% and specificity of 90%. The two-entrance table would look like this:

	COVID-19 positive	**COVID-19 negative**	
Test positive	650	900	1550
Test negative	350	8 100	8450
	1 000	9 000	10 000

And the predictive values would be estimated as follows:

PPV = 650/1550 = 0.4193 ≈ 41.9%

NPV = 8100/8450 = 0.9585 ≈ 95.9%

	Se: 99% Sp: 99%	**Se: 99% Sp: 90%**	**Se: 90% Sp: 99%**	**Se: 90% Sp: 90%**	**Se: 80% Sp: 90%**	**Se: 90% Sp: 80%**	**Se: 65% Sp: 90%**	**Se: 90% Sp: 65%**
	Positive Predictive values							
Prevalence: 1%	50.0%	9.1%	47.6%	8.3%	7.4%	4.3%	6.2%	2.5%
Prevalence: 5%	83.0%	34.3%	82.6%	32.1%	29.6%	19.1%	25.5%	11.9%
Prevalence: 10%	91.7%	52.4%	90.9%	50.0%	47.0%	33.3%	41.9%	22.2%
Prevalence: 20%	96.1%	71.2%	95.7%	69.2%	66.7%	52.9%	61.9%	39.1%
2	Negative Predictive values							
Prevalence: 1%	100.0%	100.0%	99.9%	99.8%	99.8%	99.9%	99.6%	99.8%
Prevalence: 5%	99.9%	99.9%	99.5%	99.4%	98.8%	99.3%	98.0%	99.2%
Prevalence: 10%	99.9%	99.9%	98.9%	98.8%	97.6%	98.6%	95.9%	98.3%
Prevalence: 20%	99.7%	99.7%	97.5%	97.3%	94.7%	97.0%	91.1%	96.3%

Se = Sensibility; Sp = Specificity.

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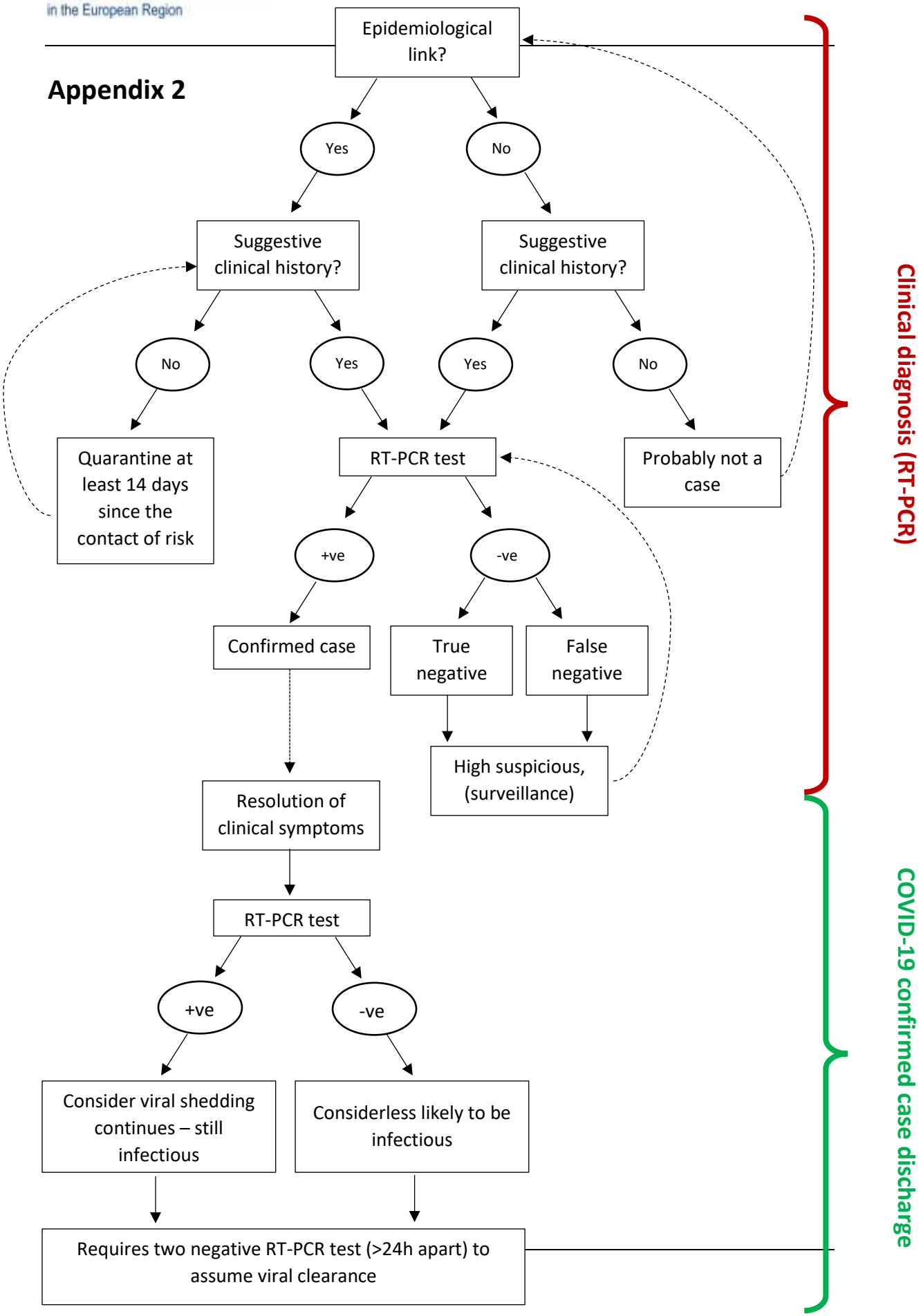
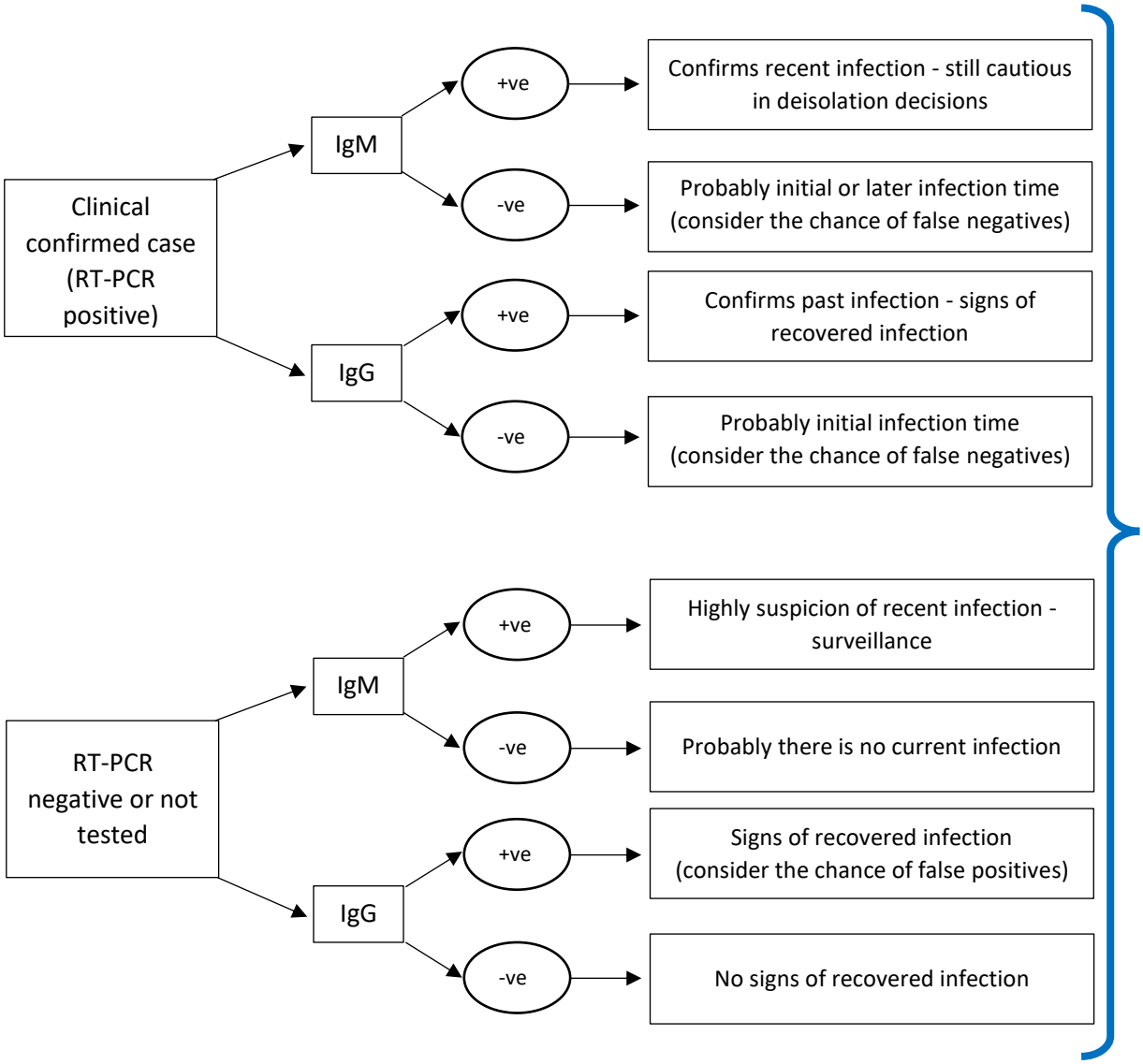
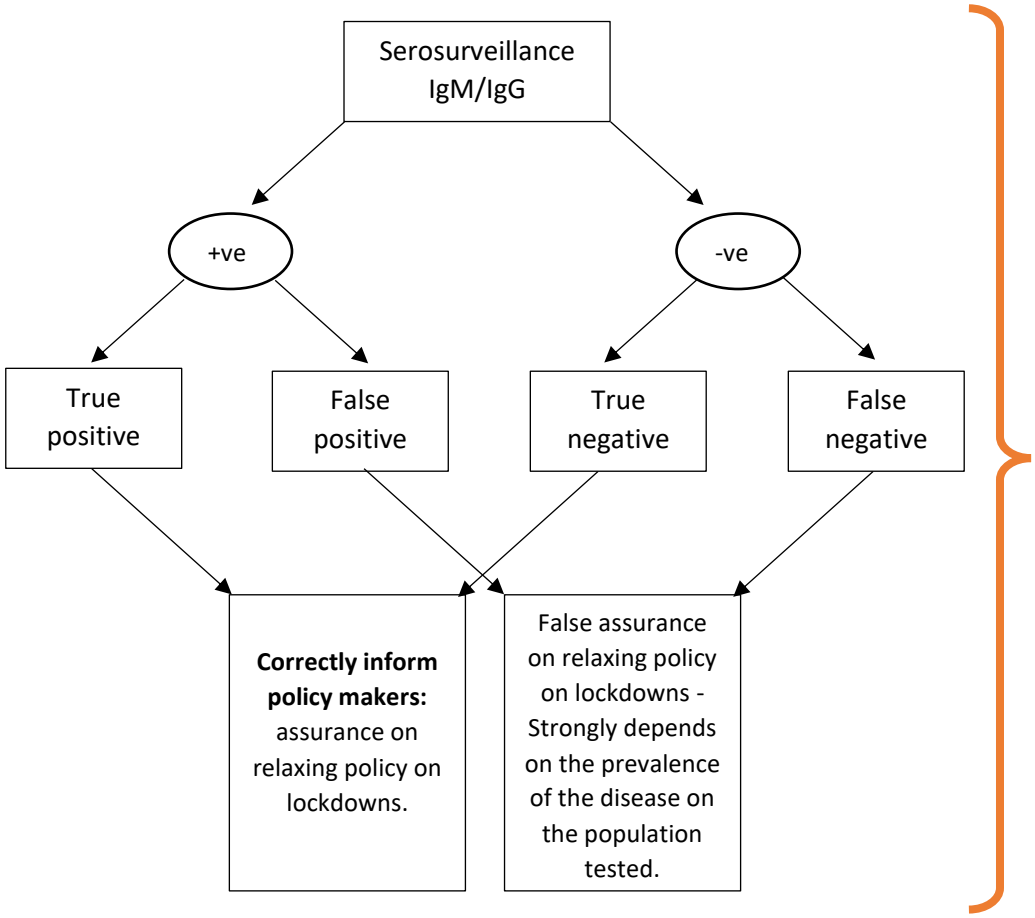
Appendix 2




Clinical confirmed case (RT-PCR positive)
IgM
+ve
Confirms recent infection - still cautious in deisolation decisions
-ve
Probably initial or later infection time (consider the chance of false negatives)
IgG
+ve
Confirms past infection - signs of recovered infection
-ve
Probably initial infection time (consider the chance of false negatives)
RT-PCR negative or not tested
IgM
+ve
Highly suspicion of recent infection - surveillance
-ve
Probably there is no current infection
IgG
+ve
Signs of recovered infection (consider the chance of false positives)
-ve
No signs of recovered infection
Clinical Follow-up: serological testing (individual approach)


Serosurveillance IgM/IgG
+ve
-ve
True positive
False positive
True negative
False negative
Correctly inform policy makers: assurance on relaxing policy on lockdowns.
False assurance on relaxing policy on lockdowns - Strongly depends on the prevalence of the disease on the population tested.
Serological testing (population approach) - seroprevalence