

# Clinician guide to COVID-19 diagnostics

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## INTRODUCTION

The novel SARS-CoV-2 virus responsible for COVID-19 has resulted in a worldwide pandemic. To curtail the ongoing pandemic, emphasis has been placed on enhanced testing. Consequently, there has been an exponential rise in the number of diagnostic platforms that are available. This review gives an overview of the diagnostic methods for COVID-19, highlighting key considerations needed when interpreting the test results.

## PHYSIOLOGICAL BACKGROUND

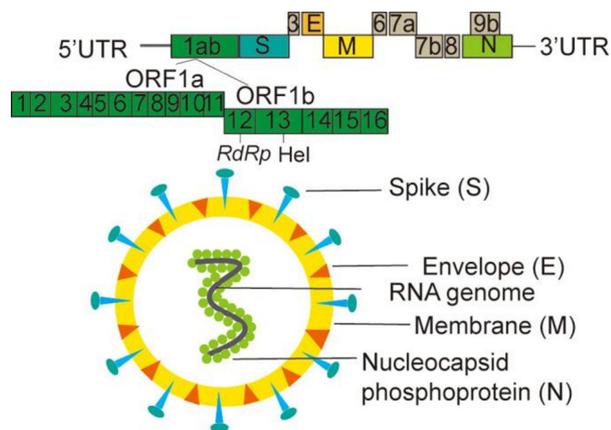
The aims of SARS-CoV-2 testing are to (1) diagnose acute infection in symptomatic patients, (2) screen for and diagnose asymptomatic infection and (3) identify past infection. The detection assays used include nucleic acid amplification tests (NAATs), antigen-based tests and antibody (serological) tests.

NAATs are aimed at identifying the presence of SARS-CoV-2 ribonucleic acid (RNA) in a sample. Reverse transcription polymerase chain reaction (RT-PCR) is the gold standard NAAT used for the diagnosis of COVID-19. In RT-PCR, repeated, automated cycles of heating and cooling (thermocycling) are used to amplify specific targets, that is, segments of the virus' genome. The targets for SARS-CoV-2 include open reading frame 1ab/RdRp, envelope, nucleocapsid (N) and spike (S) genes (figure 1). The amount of virus in a sample is then quantified in real time using fluorescently labelled probes. A test is positive if the fluorescence goes above a certain threshold level. The number of thermocycles required to get over this threshold is termed the cycle threshold (Ct) value. The lower the Ct value, the higher the quantity of viral genetic material in the sample; this is used as a proxy for viral load and infectivity. A 3.3-point increase in Ct value is equivalent to a 10-fold decrease in the quantity of viral

genetic material. Roughly speaking, a Ct value of <35 (across all targets) indicates active infection and strongly correlates with cultivable virus,<sup>1</sup> whereas a Ct value of >35 is considered a low-level positive and suggestive of past infection, very early infection, or a false-positive result. Most commonly used SARS-CoV-2 PCR assays target at least two different genes in the SARS-CoV-2 genome (ie, are multiplex PCRs). This guarantees robustness of the assay if mutation occurs in one of the genomic targets. An assay targeting only one gene would be insufficient should there be mutation in that gene.

Nucleic acid amplification testing for SARS-CoV-2 falls under two categories. Diagnostic testing is performed when a patient develops new symptoms suggestive of COVID-19 infection, such as fever, cough or altered sense of smell. Asymptomatic patient screening, which is part of the management process to reduce hospital outbreaks, is performed on all new admissions, and regularly on inpatients, irrespective of symptoms. Asymptomatic patient screening is also performed if a patient has had significant exposure to another patient or staff member who subsequently tests positive for SARS-CoV-2. Different NAAT platforms are routinely used in hospitals across the United Kingdom, and the decision on what testing platform to use depends on the urgency of the test. For emergency admissions, including those requiring emergency surgery or admission to intensive care, testing is usually performed using a rapid platform, with a turnaround time of less than 90 minutes. For more routine and elective admissions or asymptomatic patient screening, a non-rapid (turnaround time of 4–6 hours) but high-throughput platform is usually used.

Antigen-based tests detect the presence of viral antigen or protein. Most antigen tests probe for the N or S proteins of



**Figure 1** Structure of SARS-CoV-2 showing genomic organisation. Yuan X *et al.*<sup>24</sup> ORF, open reading frame; RdRp, RNA-dependent RNA polymerase; UTR, untranslated region.

SARS-CoV-2 using different techniques—lateral flow or Enzyme-linked immunosorbent assay (ELISA). Antigen-based tests are not routinely used as point-of-care tests in hospitals and are beyond the scope of this paper. However, they have a role in asymptomatic testing of healthcare workers and in the community.

SARS-CoV-2 serological tests are aimed at identifying antibodies (immunoglobulins A, G, M or a combination of these) directed against SARS-CoV-2-specific antigens, usually the S and N proteins. There are also multiplex serological assays that simultaneously measure antibodies directed against several SARS-CoV-2-specific antigens. Serological testing is primarily used to identify individuals who are very likely to have had previous SARS-CoV-2 infection. They can be used to support the diagnosis of acute infection where there is high suspicion of a false-negative PCR test. They also have a role in the assessment of patients with the multisystem inflammatory syndrome temporally associated with SARS-CoV-2 (PIMS-TS). Available SARS-CoV-2 serological assays include ELISA (the most commonly used), chemiluminescence immunoassays and lateral flow.

## CLINICAL SCENARIOS

### Question 1: If children have a positive SARS-CoV-2 PCR test, does that mean they have COVID-19?

A positive PCR test indicates the presence of SARS-CoV-2 in the tested sample. SARS-CoV-2 can be detected in upper respiratory tract samples several days prior to symptom onset and can remain detectable several weeks later. PCR cannot differentiate between viable (replicating) and non-viable (non-replicating) virus. Thus, it is crucial to correlate Ct values (where available) with the clinical picture and pretest probability of acute infection, that is, highly suggestive symptoms or a history of these in the preceding week, or recent significant contact with an infected person.

A positive SARS-CoV-2 PCR result with a low Ct value, even in the absence of symptoms, is strongly

suggestive of acute infection and high risk of transmission.<sup>1</sup>

A positive SARS-CoV-2 PCR result with a high Ct value could represent very early, resolving, or past infection, a false-positive result, or could be due to poor sample quality. A single PCR result provides only snapshot information and does not give insight into the trajectory of the illness. Therefore, repeat testing to monitor the Ct value trend is useful. An increase in Ct value on repeat testing would suggest resolving infection. In this scenario, serological testing can be helpful; detection of SARS-CoV-2-specific antibodies would be consistent with past infection. A decrease in Ct value on retesting would be indicative of evolving acute infection, even in the absence of symptoms. Notably, in a South Korean study, presymptomatic children had remained symptom free for a median of 2.5 days (range 1–25) before displaying any symptoms despite a positive test.<sup>2</sup> A negative result on retesting would indicate resolved infection, or an initial false-positive result. Uncommonly, false-positive results may occur due to sample contamination and cross reaction with other genetic materials that can be amplified by the PCR test. The false-positivity rate in the UK has been estimated to be between 0.8% and 4.0%.<sup>3</sup> Nonetheless, due to the potential infection control implications, the child should be isolated while awaiting results of repeat testing.

### Question 2: If children have a negative SARS-CoV-2 PCR test, does that mean they do not have COVID-19?

Generally speaking, a negative PCR test means that SARS-CoV-2 RNA was not detected in the tested sample. This can be due to a true absence of viral genetic material in the sample or that the amount of virus in the sample is too low to be detected (ie, a false-negative result). In a systematic review of 34 studies (with only 2 studies involving children) enrolling over 12 000 patients with COVID-19, false-negative PCR rates were variable, ranging from 1.8% to 58.0%.<sup>4</sup>

For better interpretation of a negative SARS-CoV-2 PCR result, consideration should be given to the analytical and clinical sensitivity of the platform used, as well as the pretest probability of infection. Clinical sensitivity is a measure of the ability of a test to identify those who are infected. The clinical sensitivity of a platform depends on a variety of factors including the sample type and quality, and sampling time in relation to symptom onset. The Medicines and Healthcare products Regulatory Agency states that laboratory-based SARS-CoV-2 NAATs in use in the UK have to demonstrate a minimum clinical sensitivity and specificity of 95%.<sup>5</sup> Analytical sensitivity is the ability of a testing platform to detect a target viral nucleic acid in a given specimen. For SARS-CoV-2 testing platforms, the measure for analytical sensitivity is the limit of detection (LoD). The LoD is the lowest concentration of SARS-CoV-2 viral particles that can be detected in

**Table 1** Manufacturer reported LoD for the Food and Drug Administration-approved nucleic acid amplification tests

Test	Company	LoD (viral copies/mL)
Alinity mSARS-CoV-2 assay	Abbott	100
BioFire COVID-19 Test	BioMérieux	380
BioFire Respiratory Panel 2.1	BioFire Diagnostics	300
BioFire COVID-19 Test	BioFire Defence, LLC	330
Xpert Xpress SARS-CoV-2	Cepheid	250
Xpert Omni SARS-CoV-2	Cepheid	400
Panther Fusion SARS-CoV-2	Hologic	0.01*
Aptima SARS-CoV-2 assay	Hologic	0.01*
Qiasat-Dx Respiratory	Qiagen	500
Aries SARS-CoV-2 assay	Luminex	1000
NxTAG CoV Extended Panel Assay	Luminex	5000
Simplexa COVID-19 direct assay	DiaSorin Molecular	242
2019-nCoV Real-Time RT-PCR Dx Panel	CDC assay	1000
ePlex Respiratory Pathogen Panel 2	GenMark Diagnostics	250
e-Plex SARS-CoV-2 test	GenMark Diagnostics	10
COVID-19 RT-PCR	LabCorp COVID-19	6250

Adapted from COVID-19 infographic <https://csb.mgh.harvard.edu/covid>.

\*Denotes LoDs reported as median tissue culture infectious dose.

LoD, limit of detection; RT-PCR, reverse transcription PCR; TCID<sub>50</sub>, tissue culture infectious dose of virus that will produce infection in 50% of cell cultures inoculated in vitro.

≥95% of repeat measurements and can be reproducibly distinguished from negative samples.<sup>6</sup> There are several PCR diagnostic platforms in use with varying LoD (table 1), in part due to difference in the PCR target and in the chemistry of the assay. Higher LoD increases the risk of false-negative results. Notably, a 10-fold increase in LoD could increase the false-negative rate by 13%.<sup>6</sup>

SARS-CoV-2 PCR testing is typically performed on specimens obtained from the upper or lower respiratory tract. Several factors can affect the accuracy of viral RNA swabs, including the sample type, site of collection and sample quality. In one study involving 205 patients with COVID-19, bronchoalveolar fluid specimens showed the highest rate of positivity (93%), followed by sputum (72%), nasal swabs (63%) and pharyngeal swabs (32%).<sup>7</sup> Using nasopharyngeal samples for the detection of SARS-CoV-2 is considered standard of care. However, the process is poorly tolerated. Mid-turbinate swabs offer ease of collection, are better tolerated and are increasingly gaining wider acceptance but could be falsely negative, particularly in the context of low viral loads. One study involving 40 patients with COVID-19 showed a higher rate of positivity using nasopharyngeal swabs compared with mid-turbinate swabs (76% vs 46%, respectively;

p value=0.001).<sup>8</sup> In the same study, patients whose nasopharyngeal swabs were PCR positive with a Ct value of >30, often tested negative by mid-turbinate swab. Poor quality samples with low genetic material could give a false-negative result. Saliva is also increasingly being used as an alternative to nasopharyngeal swabs. Meta-analysis studies show that saliva is an inferior sample compared with upper respiratory tract swabs.<sup>9 10</sup>

A crucial consideration around a negative PCR result is the timing of sampling since this can affect the clinical sensitivity of the test. In very early stages of SARS-CoV-2 infection, virus levels can be lower than the LoD of the testing platform used and can give a falsely negative result. Notably, in an analysis of seven studies, estimated rates of false-negative PCR results were 100% on day of exposure, 38%, 20% and 66% on days 5, 8 and 21, respectively, from onset of symptoms.<sup>11</sup> Repeat testing should be performed where the pretest probability of an acute infection is high but the PCR result is negative.

For the child with symptoms suggestive of acute SARS-CoV-2 infection whose initial PCR test is negative, repeat testing is recommended. SARS-CoV-2 PCR positivity has been reported in some cases in as short as 24 hours after an initial negative test.

One question that could arise is the role of multiple sampling from different sites in the respiratory tract to improve clinical sensitivity. Although lower respiratory tract samples provide the highest virus yield, in the majority of children, this would involve undergoing a general anaesthetic and bronchoscopy, which are not without their risks, and a repeat nasopharyngeal swab should be done in the first instance.

### Question 3: If children have a negative SARS-CoV-2 antibody test, does that mean they haven't had COVID-19?

In general, a negative SARS-CoV-2 antibody test makes previous COVID-19 infection less likely; however, it does not rule it out since false-negative results can occur. When interpreting SARS-CoV-2 antibody result, various factors should be considered, including the (1) performance of the platform, (2) timing of sampling, (3) type of test performed (ie, IgG, IgM or total antibodies), and the target of the testing platform (ie, S or N protein).

There are several commercial serological assays now available with varying sensitivities and specificities (table 2). One recent evaluation of SARS-CoV-2 serological assays reported sensitivities and specificities ranging from 81% to 99% and from 94% to 99%, respectively.<sup>12</sup>

A meta-analysis of ELISA assays measuring either IgM or IgG demonstrated a pooled sensitivity of 84% and specificity of 97%–99%.<sup>13</sup>

SARS-CoV-2 specific antibody levels rise at different times after symptom onset. One study in adults

## Interpretations

**Table 2** Summary of performance of COVID-19 serological assays following laboratory evaluation by PHE<sup>15</sup>

Company (total number of samples used for PHE validation)	Roche Elecsys Anti-SARS-CoV-2 (93 convalescent, 472 negative samples)	Ortho Clinical Diagnostics VITROS Immunodiagnostic (93 convalescent, 490 negative samples)	Ortho Clinical Diagnostics VITROS Immunodiagnostic (100 convalescent, 491 negative samples)	Beckman Coulter Access (100 convalescent, 499 negative samples)	Siemens Atellica-IM (100 convalescent, 499 negative serum samples)
<b>Assay target (type of assay)</b>	<b>Total antibody (electrochemiluminescent)</b>	<b>IgG (chemiluminescent)</b>	<b>Total serology (chemiluminescent)</b>	<b>IgG (chemiluminescent)</b>	<b>Total antibody (chemiluminescent)</b>
Specificity (manufacturer)	99.8 (99.7 to 99.9)	100 (99.1 to 100.0)	100 (99.1 to 100.0)	99.8 (99.4 to 99.9)	99.82 (91.59 to 99.98)
Sensitivity (manufacturer)	100 (88.1 to 100.0) (samples taken ≥14 post-PCR confirmation)	90.0 (76.3 to 97.2) (samples taken >15 days after symptom onset)	100 (92.7 to 100.0) (samples taken >8 days after symptom onset)	100 (93.8 to 100.0) (samples taken >18 days from positive PCR test)	100 (91.59 to 100.0) (samples taken ≥14 post PCR confirmation)
Specificity (PHE)	100 (99.1 to 100.0)	99.7 (98.6 to 100.0)	99.5 (98.2 to 99.9)	99.3 (97.8 to 99.8)	100 (99.1 to 100.0)
Sensitivity ≥14 days after symptom onset (PHE)	86.1 (76.5 to 92.8)	79.7 (69.2 to 88.0)	91.8 (83.3 to 96.6)	76.5 (66.0 to 85.0)	89.4 (80.8 to 95.0)
Sensitivity of the assay at ≥21 days after symptom onset (PHE)	86.7 (76.8 to 93.4)	81.3 (70.7 to 89.4)	93.5 (85.5 to 97.9)	79.2 (93.8 to 100.0)	92.4 (84.2 to 97.2)
Company (total number of samples used for PHE validation)	Euroimmun Medizinische Labordiagnostika AG (93 convalescent, 499 negative samples)	Abbott (96 convalescent, 760 negative samples)	DiaSorin LIAISON (100 serum, 472 negative samples)		
<b>Assay target (type of assay)</b>	<b>IgG (ELISA)</b>	<b>IgG (chemiluminescent)</b>	<b>S1/S2 IgG (chemiluminescent)</b>		
Specificity (manufacturer)	99.6 (No CI)	99.6 (99.1 to 99.9)	98.5 (97.6 to 99.1)		
Sensitivity (manufacturer)	94.4 (no CI) (≥10 days after symptom onset)	100 (95.9 to 100.0) (≥14 days after symptom onset)	97.4 (86.8 to 99.5)		
Specificity (PHE)	99.0 (97.5 to 99.7)	100 (99.1 to 100.0)	97.7 (95.8 to 99.0)		
Sensitivity of ≥14 days after symptom onset (PHE)	73.4 (62.3 to 82.7)	93.9 (86.3 to 98.0)	69.4 (58.5 to 79.0)		
Sensitivity of the assay at ≥21 days after symptom onset (PHE)	74.7 (63.3 to 84.0)	93.5 (85.5 to 97.9)	71.4 (60.0 to 81.2)		

Sensitivity and specificity are presented as percentages and confidence intervals in brackets.

CI, confidence intervals; PHE, Public Health England; S, spike protein.

showed that SARS-CoV-2 IgM can be detected as early as 5 days (median of 5–17 days), whereas SARS-CoV-2 IgG is detected later, approximately 1 week (median 6–14 days) from illness onset.<sup>14</sup> The same study showed that SARS-CoV-2 IgM and IgG levels peak in the third week of the illness; however, while a decline in SARS-CoV-2 IgM was observed, SARS-CoV-2 IgG levels were maintained at 4 weeks after

symptom onset.<sup>14</sup> Also, seroconversion rates were high and SARS-CoV-2 IgG was detected in over 90% of individuals at 2 weeks and 100% at 4 weeks from symptom onset.<sup>14</sup> A Cochrane review<sup>11</sup> including >150 000 samples of which 50% were from patients with confirmed COVID-19 reported a maximum sensitivity (days from symptom onset in bracket) of 96% (22–25), 100% (>35), 75.4% (15–21) and 88.2%

(15-21) for combined IgG and IgM, IgA, IgM and IgG tests, respectively. Furthermore, across four serological assays evaluated by Public Health England (PHE), sensitivity of over 98% was observed, when tested 30 days from diagnosis.<sup>15</sup> So, an antibody test performed less than 4 weeks from primary infection might not show evidence of IgG seroconversion.

Furthermore, individual variability in the longevity of antibodies has been reported. Antibody levels can fall lower than the LoD of the testing platform the longer the interval since the primary infection, resulting in a false-negative result.

SARS-CoV-2 serology test result can also be influenced by the nature of the immune response to the infection. An individual's immune response can be heavily skewed towards a particular antigen (ie, N or S protein). Interestingly, one study showed that children predominantly generated SARS-CoV-2 IgG antibodies specific for the S protein.<sup>16</sup> Hence, serological testing can be falsely negative if the target antigen in the testing platform is different from that to which the individual has developed antibodies. Second, the degree of antibody response following COVID-19 has been shown to correlate positively with disease severity,<sup>17</sup> and a more rapid decay of antibodies or lack of a detectable antibody response has been reported in adults with mild or asymptomatic infection.<sup>18</sup> The extent to which these findings can be extrapolated to children who tend to have a milder disease phenotype compared with adults and how this might impact on the results of serological testing is unclear.

Additionally, immunosuppressed patients do not always mount an adequate antibody response following infection and may display a small (or absent) rise in antibody titre that is below the LoD of the testing platform. Evidence is awaited as to whether this holds true with COVID-19. The correlation between poor antibody response and reinfection is unknown.

**Question 4: If children have a positive SARS-CoV-2 antibody test, does that mean they have been infected?**

A positive SARS-CoV-2 antibody test is indicative of an immune response to the virus. The specific epitopes within the N and S proteins that are targets of antibody tests are highly specific to SARS-CoV-2; therefore, cross reactivity with other coronaviruses is unlikely. The commercially available serological tests have been shown to demonstrate high specificity (all eight kits analysed had a specificity of 98.5%–100.0%) following independent evaluation by PHE (table 2). Thus, a positive antibody test using these routinely used platforms suggests a very high likelihood of previous COVID-19 infection. Of note, samples from children under 10 years were not included in the PHE validation process; therefore, the performance of the serological assays in this group is unknown. Nonetheless, previously infected children, even those who did not develop any

symptoms, have been shown to develop detectable antibodies.<sup>19</sup>

A positive SARS-CoV-2 IgM result may indicate recent infection. Generally, in the second week of infection, the clinical sensitivity of NAATs performed on upper respiratory tract samples starts to decline, whereas the clinical sensitivity for serological tests is increasing. A positive SARS-CoV-2 IgM test in this time can be used to support the diagnosis of acute COVID-19 infection, particularly where the PCR test has not worked. A positive SARS-CoV-2 IgG test indicates past infection. Particularly, SARS-CoV-2 IgG antibody test was positive in 87% of patients with PIMS-TS.<sup>20</sup>

**Question 5: if children have a positive SARS-CoV-2 antibody test, are they immune?**

For SARS-CoV-2, this remains a topic of research. Generally, an antibody level that is more than three times the cut-off level for positivity is regarded as a good response. It is still unknown how long antibodies against SARS-CoV-2 persist and whether these are protective against reinfection. Recent results indicate that children exposed to SARS-CoV-2 have antibodies detectable for at least 2 months, and the mean antibody titres increased over this time period.<sup>19</sup>

Virus-specific neutralising antibodies are antibodies that bind to a virus and block its entry into the host cell. Their presence is commonly considered to correlate with protection against reinfection by the same pathogen. Virus neutralisation tests are laborious and time-consuming, and are currently mainly used for assay validation and research. Plaque reduction neutralisation test (PRNT) is considered gold standard to measure antibody neutralisation. The Liaison S1/S2 IgG assay has a good concordance with detection of neutralising antibodies exhibiting 94.4% positive agreement to the PRNT.<sup>18</sup> The S protein and its antigens are the main antigen target of neutralising antibodies. Wajnberg *et al* found that neutralising antibody titres against the S protein persisted for at least 5 months after infection in adults.<sup>21</sup> In the same study, children demonstrated reduced neutralising activity compared with adults.<sup>21</sup>

There is a lack of scientific data at this time to determine if SARS-CoV-2 neutralising IgG antibodies provide long-term immunity to the virus or if they protect patients against reinfection. Reassuringly, there has been over 85 million COVID-19 cases worldwide, and very few reported reinfections. A study involving healthcare workers showed no confirmed reinfections in those who had COVID-19 in the first wave, compared with 2.9% with no previous history of COVID-19.<sup>22</sup> It is also possible that people exposed to SARS-CoV-2 are protected against future infection regardless of whether they have measurable antibody titres or not. Memory B and T lymphocytes can mediate long-term immunity to infection even in

the face of waning antibody titres.<sup>23</sup> Thus, there are reasons to be optimistic that prior exposure to SARS-CoV-2 does lead to protective immunity.

### BOTTOM LINE

SARS-CoV-2 test results should not be interpreted in isolation. The clinical picture, including pretest probability, sample quality, timing of sampling and performance of the diagnostic platform used, should be considered. Close liaison with the clinical virologist and the infection control team is necessary to ensure that appropriate information is communicated with families and to ensure implementation of appropriate infection control measures.

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