Evidence current as of 22/4/2020

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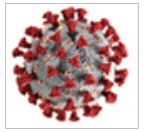

Covid-19
Rapid Review for the EC

Thursday, 23 April 2020

Review 6

Compiled by Dr Luke Bush, Dr Lauren Lai King and Dr Kamlin Ekambaram
Special thanks to Dr A. Parker

The purpose of this regular review, is to present COVID-19 related questions in Emergency Medicine

We accept that available literature on the topics covered in these reviews may be scarce, but shared discussion of novel front line strategies may be a tool to augment our clinical practice and develop future policy

Topic:

- **Diagnostics**

Correspondence with authors

Covid-19

Advice

Patients are most infectious during the 1st week of illness and potentially prior to onset of symptoms.

Early spread via droplet and aerosol is a clear feature of SARS-Cov-2.

This corresponds to the 1st phase of the illness prior to the onset of dyspnoea when those who are going to progress to worsening illness are likely to present to hospital.

Reducing spread of the virus relies on very **early recognition of symptoms, testing** (if available) and appropriate **isolation**.

Complete containment of the virus, in light of its early spread is highly unlikely

Wits Journal of Clinical Medicine - Diagnosis of COVID-19: considerations, controversies and challenges in South Africa

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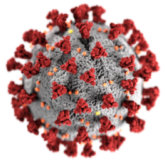
References, resources & comments via QR code

SARS-Cov-2 Diagnostics

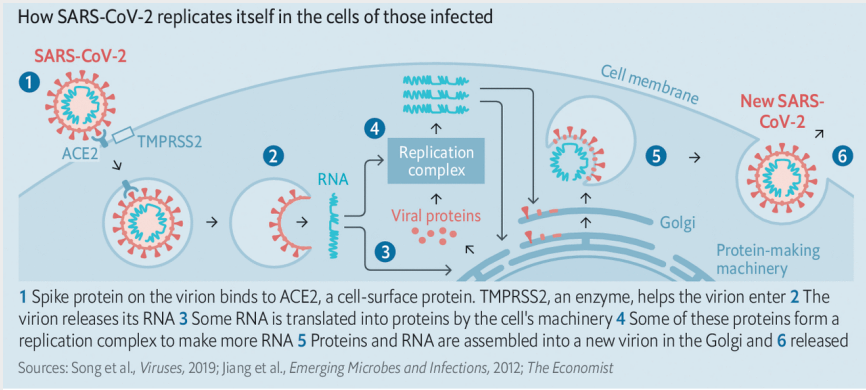


SARS-Cov-2 belongs to the genus Betacoronavirus within the family Coronavirdae(1). It is termed novel as it is a new virus to human populations with a zoonotic origin. Although humans have exposure to other coronaviruses, no one has immunity to SARS-Cov-2 and so we are all at risk of contracting and transmitting the virus.

COVID-19 is the full spectrum of illness caused by infection with SARS-Cov-2. This can range from asymptomatic to a fulminant disease process with a CFR estimated at between 2-4%(2).

Virological analysis of patients with COVID-19

A German team of researchers have shown evidence for active virus replication in the upper respiratory tract and that virus shedding is at it highest, with peak viral loads 1000 times that of SARS, in the first week of illness9(3).



Seroconversion occurred after 7 days in 50% of patients and 14 days in all, but was not followed by a rapid decline in viral load from RT-PCR of sputum. Despite this prolonged viral shedding, no virus was isolated from culture of sputum or nasal and pharyngeal swabs beyond day 8(3).

Further research is required with regard to the immunity that seroconversion provides. At this point it is unclear as to both the duration and level of post-infection immunity.

As there is no immunity against SARS-Cov-2, there is a large pool of people for the virus to infect and further transmit the virus. The fastest way to control spread is by governments enforcing IPC measures including varying degrees of lockdown, physical distancing and encouraging people to take adequate precautions: regular washing of hands, coughing etiquette and not touching ones face.

Covid-19

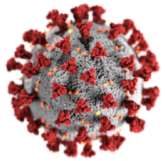
Diagnostics

Sampling

RT-PCR samples are taken from naso/oropharynx (sputum and tracheal aspirates will also yield results). Specimens are collected on dacron or polyester flocked swabs and placed into a viral transport media to release viral RNA from the swabs into solution. Transport at 2°C-8°C

The quality of the sample collected will affect the accuracy of the RT-PCR test

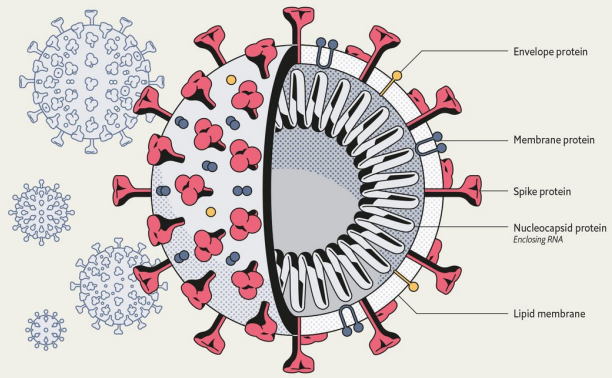
Serology tests are performed on blood either as a sample sent to the laboratory for an ELISA or rapid diagnostic test that can be done at point of care



Culture

Viral culture isolates live virus from specimens and suggests that there is virus replication from the source tissues.

Utility
Research
Genome mapping



RT-PCR

Reverse transcription polymerase chain reaction isolates fragments of SARS-Cov-2 from a clinical sample. This is done through the addition of specific primer and probe sets (assay) that enable transcription of fragments of viral RNA to DNA and amplification and marking of this DNA. As amplification and marking occur, real time (r)RT-PCR machines detect these fluorescent markers.

Utility
PCR tests are the **diagnostic** gold standard and newer tests are rapid and able to detect low levels of virus within specimens. They allow for the earliest diagnosis of COVID-19. The primer and probe sets are designed to be both sensitive and specific.

Serology

A serology test is performed on blood to detect antibodies as a result of exposure to COVID-19 or the SARS-Cov-2 antigens themselves. There are many different serological testing techniques available but the most likely to have an impact and be widely used is a Rapid Diagnostic Test (RDT).

Utility
A RDT will be useful for **screening** of the population to determine who has had infection. Antibody testing is not currently useful in the acute illness but as tests are developed some are showing promise at detecting IgM within the 1st week. At this point it is unclear as to both the duration and level of post infection immunity.

Coronavirus disease 2019 (COVID-19) Quick Reference for Health Workers

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

References, resources & comments via QR code

Covid-19

Advice

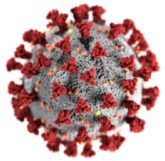
RT-PCR tests are the **diagnostic test** of choice as they are able detect low levels of virus within specimens and newer tests have reduced the processing time

The accuracy of these PCR tests depend on the quality of the sample collected and when during the course of illness the sample is taken

A **positive** PCR is likely a true positive

A negative PCR does not exclude SARS-Cov-2 and if symptoms are consistent with COVID-19, patients should be treated and given advice as if they are positive

RT-PCR

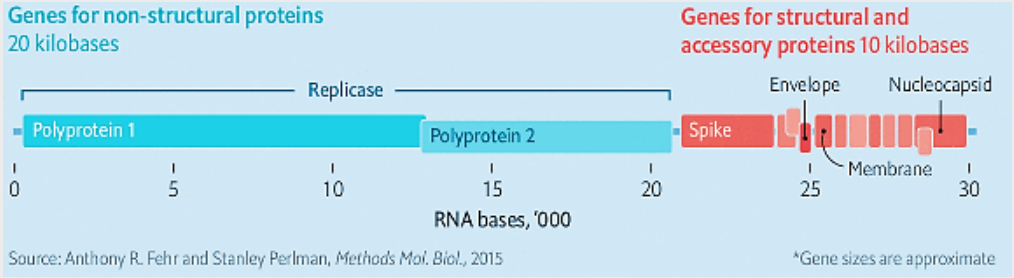


There are a number of primer sets and probes (assays) that have been developed and these can be tested for their **sensitivity in the laboratory** using a fixed concentration of viral RNA (positive controls).

The **limit of detection (LOD**) is the lowest possible concentration of SARS-CoV-2 that can be detected in-vitro, so that a test with a low LOD would be considered more **sensitive**.

Specificity refers to whether the test recognizes only SARS-CoV-2 RNA and not other closely related pathogens or human RNA transcripts.

Different assays can be optimised to improve sensitivity and their specificity improved by ensuring that they do not share sequence similarity with other viruses. The most common target is the nucleocapsid phosphoprotein (N protein), although other stable regions of the genome are also targets.



In-vitro sensitivity vs real world experience: each assay reports a LOD and declared specificity. Although most assays report a test accuracy of >90% (test performance characteristics), this does not reflect real world experience, where there are many reports of false negative rates in the region of 30%.

WHY?

The accuracy of PCR is significantly affected by the **quality of the sample** and when during the course of the illness the sample is taken. The RT-PCR is able to detect very low levels of virus but if there is no virus in the specimen or the specific viral RNA for that assay has degraded, then the test will be negative despite the individual tested having the virus.

Viral mutations: as the virus mutates, targets for some of the assays change and so regular review of the viral genome is important so that assays can be adjusted accordingly.

References, resources & comments via QR code

Covid-19

Advice

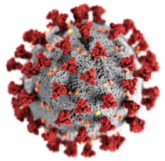
When they become available RDT/POC test are likely to have a significant role to play

ELISA and possibly improved POC tests, when combined with RT-PCR may improve our diagnostic accuracy during the acute phase of illness, thereby reducing the number of false negatives

They will enable widespread screening and surveillance of the population

They can be used to screen healthcare workers, particularly those who have had asymptomatic infection or people at risk who may already have had infection and no longer need to quarantine themselves

Serology



Serological tests performed on blood or other fluid detect either antibodies as a result of exposure to COVID-19 or SARS-Cov-2 antigens themselves. They are not currently available in our setting but should become available at some point.

Immunoglobulins to SARS-Cov-2 (IgM and IgG) rise in the first week of illness and can be tested for by various serological techniques. Some of these techniques are laboratory based ELISA, but RDT are being developed and may have good diagnostic performance.

Some early research has shown IgM ELISA to be more sensitive than RT-PCR at 5.5 days from symptom onset, and that if ELISA is used in conjunction with RT-PCR that the combined sensitivity can be significantly improved (1).

The table adjacent suggests that this may be closer to 8 days. This discrepancy may be the result of different assays used with differing test characteristics. Both studies suggest that it is possible to use serology early in the disease with limited sensitivity, and that the sensitivity improves into the second week.

Diagnostic Test Sensitivity in the Days After Symptom Onset†

	Days after Symptom Onset		
SARS-CoV-2 Test	1–7	8–14	15–39
RNA by RT-PCR	67%	54%	45%
Total Antibody	38%	90%	100%
IgM	29%	73%	94%
IgG	19%	54%	80%

Adapted from: Zhao J et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clin Infect Dis*. 2020 Mar 28.

A combined IgM and IgG RDT, designed as a lateral flow immunoassay, had a sensitivity of 88% and specificity of 90% in specimens collected from 397 PCR confirmed COVID-19 patients and 128 negative patients at eight different sites(2). There are some limitations with this study and these values still suggest that 10 people out of 100 would have a false positive result and a similar number a false negative.

Further research is required to give guidance as to when and how RDTs are used and to clearly define both the duration and level of post-infection immunity.

Understanding the test characteristic for the test at your disposal is important when advising patients further.

References, resources & comments via QR code

This is the same as RT-PCR

Tests for SARS-CoV-2/COVID-19 and Potential Uses

Type of Test	Measure	Value	Beneficiary
Nucleic acid amplification test for viral RNA *(nasopharyngeal swab, oropharyngeal swab, sputum, bronchoalveolar lavage fluid, others)*	Current infection with SARS-CoV-2	• Inform individual of infection status so they can anticipate course of illness and take action to prevent transmission • Inform patient management and actions needed to prevent transmission • Inform actions needed to prevent transmission	• Individual • Healthcare or long-term care facility • Public health
Antibody detection	Past exposure to SARS-CoV-2	• Detect susceptible individuals (antibody negative) and those previously infected • Identify individuals with neutralizing antibodies • Facilitate contact tracing and surveillance	• Identify those potentially immune to SARS-CoV-2 (if tests can detect protective immunity, individuals could be returned to work) • Healthcare facilities: Experimental therapy • Public health

Robin Patel et al. mBio 2020; doi: https://doi.org/10.1128/mBio.00722-20

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Frequently asked questions

Please note these answers are not exhaustive and your response may also depend on the specifics of your local disease burden, local policies and testing availability.

What is an accurate test?

An accurate test is where with a high degree of certainty, you can say that either someone has or doesn't have a disease. This requires the test to be highly sensitive (in this case picking up the virus) and highly specific (not confusing the virus with other viruses, antigens or antibodies). Each test will have its own test characteristics in the laboratory, but the true value of the test is how it performs outside the lab. To assess the test accuracy, we use the positive predictive value (PPV), how sure we are the patient has the disease and the negative predictive value (NPV), how sure we are they don't.

Why can't I rule out COVID-19 with a PCR test?

A rule out test requires that we are sure that a patient doesn't have the disease i.e. a very high NPV. For this to be done we need a test that is as close to 100% sensitive as possible. In this Pandemic we also want a test that is accurate early in the disease process, allowing us to limit spread of the disease through isolation and contact tracing. RT-PCR has the right test characteristics to make this assessment. Unfortunately in practice the NPV is not high enough for us to rule out COVID-19. Ensuring proper sampling technique and perhaps combining with RDTs (IgM serology) when they become available may improve the NPV.

My patient tested negative. How should I manage them and what advice should I give them?

If your patient has presented with symptoms consistent with COVID-19 and they test negative, remembering that potentially 1 in 4 patients may be a false negative, Sars-Cov-2 infection cannot be excluded. If they are well enough to be sent home then you would give them the same 'safety-net' advice that you would give to a COVID-19 positive patient that you are sending home or even someone who has tested and is going home to isolate and await the result.

If your patient is admitted to the hospital and they test negative, then other causes in their differential should be sought (not everything will be COVID-19). If they are ill and despite further investigation for their illness, the likely diagnosis is still COVID-19, then a repeat PCR and discussion with the ID team is warranted. When other testing modalities (serology) become available they may aid diagnosis as may a CT chest if it is available.

What does a false positive mean for my patient?

There are few tests that are 100% accurate. Often by increasing the sensitivity of a test (making sure we don't miss any virus) we may decrease the specificity by potentially picking up other viruses RNA (RT-PCR), or other antigens or antibodies that cross-react with the test (serology). This means that a patient can test falsely positive. Some labs may run a second test with a different assay to confirm the first. **Why is this important?** In hospital isolation means removing patients from their families and if they are falsely positive, potentially exposing them to hospital acquired COVID-19 and other risks. A false positive serological test (screening) may give a patient a false sense of security, by believing they have immunity, potentially putting them at increased risk of contracting SARS-Cov-2 and further spreading the virus – this is particularly concerning with some RDT self-test kits that may have poor sensitivity and specificity.

Frequently asked questions

Please note these answers are not exhaustive and your response may also depend on the specifics of your local disease burden, local policies and testing availability.

Why are patients testing positive again after recovering from illness?

As this point in time there isn't a clear answer. There are suggestions that these could be repeat infections or re-activation, but it is important to note that RT-PCR identifies parts of the viral RNA and not live virus. Studies to date have not cultured live virus in patients with repeat a positive PCR. It has also been shown that there is prolonged shedding of viral RNA, particularly in sputum. Combined with knowing that the sensitivity of the RT-PCR decreases over time, some of the repeat positives may have followed false negatives. Whilst this aspect is important to note, we are unsure of its significance and further research is required.

Does a positive PCR test mean that my patient is infectious?

A positive RT-PCR test confirms that there is viral RNA in the specimen. The duration that the patient has been ill will influence how infectious they are. What we know is that within a week of developing symptoms most patients are beginning to seroconvert (develop antibodies). As a result viral loads decrease, and they become less infectious. Those who are more ill may take longer to clear the virus, but for those well enough to be at home, they are likely no longer infectious somewhere between day 10-14 post onset of symptoms.

When can someone be discharged who is admitted or come out of isolation?

Many centres in Europe are discharging their patients from hospital after a combination of clinical, virological and immunological features. These include >3 days no fever and clinical improvement, with two negative PCR tests 24 hours apart and IgG if testing available. In their setting this has made sense to reduce spread, but in many countries the lack of bed availability will mean patients who can isolate at home, will need to be discharged. At 14 days post onset of symptoms you are unlikely to be infectious and this is the recommendation as to when to stop self-isolation. Patients requiring respiratory support may be infectious for slightly longer and so once clinically improving, the recommendation is: continued isolation for a further 14 days.

My patient tested negative but has now come back unwell? Another cause for their illness should be investigated, whilst at the same time taking all the necessary precautions as if they had tested positive originally. Keep in mind that many respiratory and cardiac illnesses can present with the same features, but if there is still a high index of suspicion for COVID-19 then a repeat CXR and PCR is warranted. A discussion with your ID team with regard to further investigation and management may also be necessary.

When will there be a rapid test available and what will their impact be? There are already rapid tests being produced, but are not yet available here. Their sensitivity and specificity vary widely but the most accurate are in the region of 90%. IgM starts appearing fairly early in the disease process and a combination of RDT and RT-PCR in the acute phase, may have the ability to improve our diagnostic accuracy. RDTs will allow us to screen large numbers of people for disease. When available and if consistently accurate, these tests may be done on patients arriving at hospitals, for reasons other than COVID-19 and allow us to cohort those, who test negative, and be less concerned about those who test positive as they have likely recovered from Covid-19 and are not infectious (IgG positive, IgM negative).