2 TESTING

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MODULE 2: TESTING

WHAT'S NEW?

Update March 2021

Revised testing criteria

- Advice on the retesting of patients who have had COVID-19 previously.
- Guidance on the use of rapid antigen tests.
- Guidance on testing after vaccination.
- Advice on when a positive test should be discussed for possible sequencing.

KEY RECOMMENDATIONS

PCR-based tests are recommended as the gold standard for the diagnosis of acute COVID-19. Upper respiratory tract samples should be sent for all patients. Sputum or bronchoalveolar lavage (if the patient is intubated) samples should be sent when available.

Rapid antigen tests are less sensitive than PCR-based tests but quick. A positive result confirms the diagnosis of COVID-19, but a negative result should be confirmed by a PCR test if there is a high pre-test probability of COVID-19.

Antibody tests are not recommended for the diagnosis of acute COVID-19, due to very poor sensitivity within the first 1-2 weeks after symptom onset.

WHO SHOULD BE TESTED?

The following categories of people should be tested:

1. **All hospitalised patients**, whether symptomatic or not. Patients with symptoms suggestive of COVID-19 (see Module 1 – Clinical characteristics) must be prioritised so that their results are received within 24 hours.
2. **Outpatients who have symptoms** suggestive of COVID-19. Prioritisation should be given to those at high risk for infection (e.g. healthcare workers) or poor outcomes (e.g. those aged >60 years, those with significant comorbidities), and pregnant women.
3. Individuals who are **close contacts** (see below) of confirmed cases, whether symptomatic or not. (Note: close contacts should only be tested when a minimum of five days has passed since first contact with an infectious individual. Earlier testing is usually futile due to the incubation period.).
4. **Post-mortem** testing – in line with current guidance.

Testing is NOT indicated for the purposes of returning to work/school or sports participation purposes. COVID-19 patients should not be tested prior to de-isolation (see Module 8 – De-isolation criteria).

A **close contact** is defined as a person having had face-to-face contact (≤1 metre) or having been in a closed space with a confirmed COVID-19 case for at least 15 minutes while not wearing a mask (medical or non-medical, as appropriate). This includes, amongst others:

- All persons living in the same household and people working closely in the same environment as a COVID-19 case.
- Healthcare workers or others providing direct care for a COVID-19 case while not wearing recommended personal protective equipment.
- Those sharing the same vehicle (car, taxi, bus, etc.) for >15 minutes with a person with COVID-19.
- A contact in an aircraft sitting within two seats (in any direction) of the case, travel companions or persons providing care, and crew members serving in the section of the aircraft where the case was seated

WHAT TESTING IS INDICATED FOR PATIENTS WITH A PREVIOUS HISTORY OF CONFIRMED COVID-19?

Re-infection with COVID-19 has been confirmed to occur, although it is unknown how common it is and whether different viral variants may differ in re-infection risk. Distinguishing true re-infection from persistent viral shedding is only strictly possible if both samples are available for genetic comparison; this is unlikely in routine practice.

- For symptomatic patients who fulfil the above testing criteria, we recommend repeat testing if 45 days or more have passed since the initial confirmed infection.
- For asymptomatic individuals who fulfil the above testing criteria, we recommend repeat testing if 90 days or more have passed since the initial confirmed infection.

Re-infection before these time points is uncommon but has been described. We suggest discussing such patients with an infectious diseases or virology expert prior to testing.

WHICH TESTS ARE INDICATED FOR THE DIAGNOSIS OF ACUTE COVID-19?

Testing for acute COVID-19 should be by means of either polymerase chain reaction (PCR) assays or antigen tests. Currently, PCR and antigen tests in use in South Africa are capable of detecting all known lineages of SARS-CoV-2, including the B.1.531 ('South African') lineage. While PCR tests remain the gold standard, rapid antigen tests provide a quicker turnaround time and are cheaper. However, they are less sensitive than PCR-based assays.

Samples to be sent are:

- *Upper respiratory tract samples* – A sample from the upper respiratory tract should be sent from all patients. A single site is sufficient. Currently, a nasopharyngeal swab is the preferred specimen, but in patients where this is not possible (e.g. recent nasal

surgery, or severe coagulopathy), an oropharyngeal, nasal mid-turbinate, or anterior nares swab or sputum can be collected instead.[1, 2]

- *Lower respiratory tract samples* – send when available. Lower respiratory tract samples may have a higher sensitivity than upper respiratory tract samples.[1, 3] Sputum, tracheal aspirates, or bronchoalveolar lavage fluid are all acceptable samples. However, sputum induction should not be performed.

Where both upper and lower respiratory tract samples are available, both should be sent.

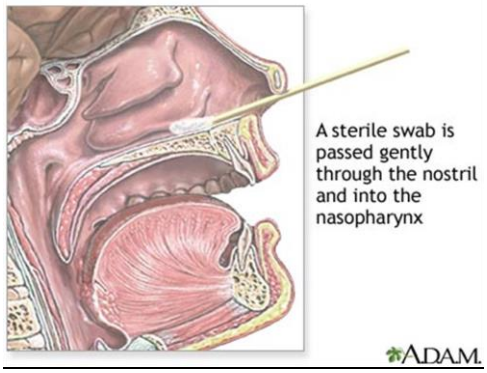
Appropriate personal protective equipment (PPE) should be worn by all healthcare workers when collecting specimens (see Module 9: Infection prevention and control).

COLLECTING SAMPLES FOR SARS-COV-2 TESTING

- Healthcare workers collecting respiratory samples require appropriate personal protective equipment, including eye protection (goggles or visor), gloves, an apron or gown, and an N95 respirator (or equivalent, e.g. FFP2 mask). Meticulous hand hygiene is also essential.
- Collecting a good-quality specimen is vital – see Figure 1.

Collection of a nasopharyngeal specimen

1. Ask the patient to tilt his/her head back slightly.
2. Gently insert swab into the nostril, aiming backwards (not upwards) until a slight resistance is met – about the distance from the nose to the anterior ear. If resistance is met before fully inserted, remove and try the other nostril.
3. Rotate swab 2-3 times and hold in place for 2-3 seconds.
4. Slowly withdraw the swab and put into the specimen tube containing universal transport medium.
5. Break the swab's shaft and close the tube.



Collection of an oropharyngeal specimen

1. Ask the patient to tilt his/her head back and open their mouth.
2. Hold the tongue down with a tongue depressor.
3. Have the patient say "aahh" to elevate the uvula.
4. Swab each tonsil first, then the posterior pharynx in a "figure 8" movement.
5. Avoid swabbing the soft palate or the tongue as this can induce the gag reflex.
6. Break the swab's shaft and close the specimen tube tightly.

Collection of a mid-turbinate specimen

1. Ask the patient to tilt his/her head back slightly.
2. Gently insert swab less that 2cm into the nostril (unless resistance is met at the turnbinates).
3. Gently rotate the swab several times against the nasal wall.
4. Repeat in the other nostril, using the same swab.
5. Withdraw the swab and put it into the specimen tube containing universal transport medium.
6. Break the swab's shaft and close the tube.

Collection of an anterior nares (nasal) specimen

1. Ask the patient to tilt his/her head back slightly.
2. Insert the swab at least 1cm inside the nares.
3. Firmly sample the nasal membrane by rotating the swab and leaving it in place for 10-15 seconds.
4. Sample both nares with the same swab.
5. Withdraw the swab and put it into the specimen tube containing universal transport medium.
6. Break the swab's shaft and close the tube.

Figure 1: Specimen collection

- Appropriate swabs are flocked or spun, and consist of polyester, nylon or rayon material with a plastic or aluminium shaft. Cotton swabs, calcium alginate swabs, and swabs with a wooden shaft are not recommended, as they may contain substances that inactivate SARS-CoV-2 and inhibit PCR testing.

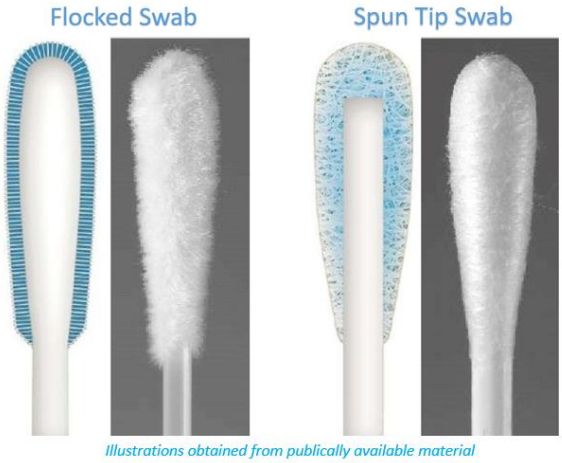


Figure 2: Types of swabs

Transport of specimens

- Nasopharyngeal, mid-turbinate and anterior nares samples should ideally be placed in viral/universal transport medium (UTM) and kept between 2-8°C until they are processed at the laboratory. Due to constraints in the supply of viral/universal transport medium, dry swabs can be sent provided that the sample reaches the laboratory within two days. Dry swabs can be sent at ambient temperature. Please confirm conditions with your laboratory. Some rapid antigen tests require special specimen buffers for transport which may or may not be suitable for subsequent testing by PCR if required.
- Lower respiratory tract samples can be sent in standard specimen containers and do not require viral/universal transport medium.

Table 1: Transport time to testing laboratory

<2 days	≥2 days
Can use dry swab (no transport medium needed) and can be transported at ambient temperature	Transport in UTM, at 2-8°C (using ice packs). If UTM is not available, normal saline can be used as an alternative.

Samples should be submitted with a laboratory request form containing complete and accurate patient information needed for the case linelist:

- Name and surname
- Sex
- Date of birth
- Address
- Cell phone number
- ID or passport number

REPEATING THE PCR TEST

PCR tests may produce false-negative results due to factors such as poor sampling technique, suboptimal specimen storage (e.g. unavailability of viral/universal transport medium, or specimen not stored at cold temperatures), the site the sample is obtained from, and the time point at which the swab is taken (viral loads are usually highest early on in the disease course). If a high clinical suspicion for COVID-19 persists despite an initial negative test, repeat testing should be considered in consultation with an infectious diseases expert, particularly in hospitalised patients for whom management might be significantly altered. However, it is equally important to maintain a broad differential diagnosis and to consider alternative diagnoses (see **Error! Reference source not found.**).

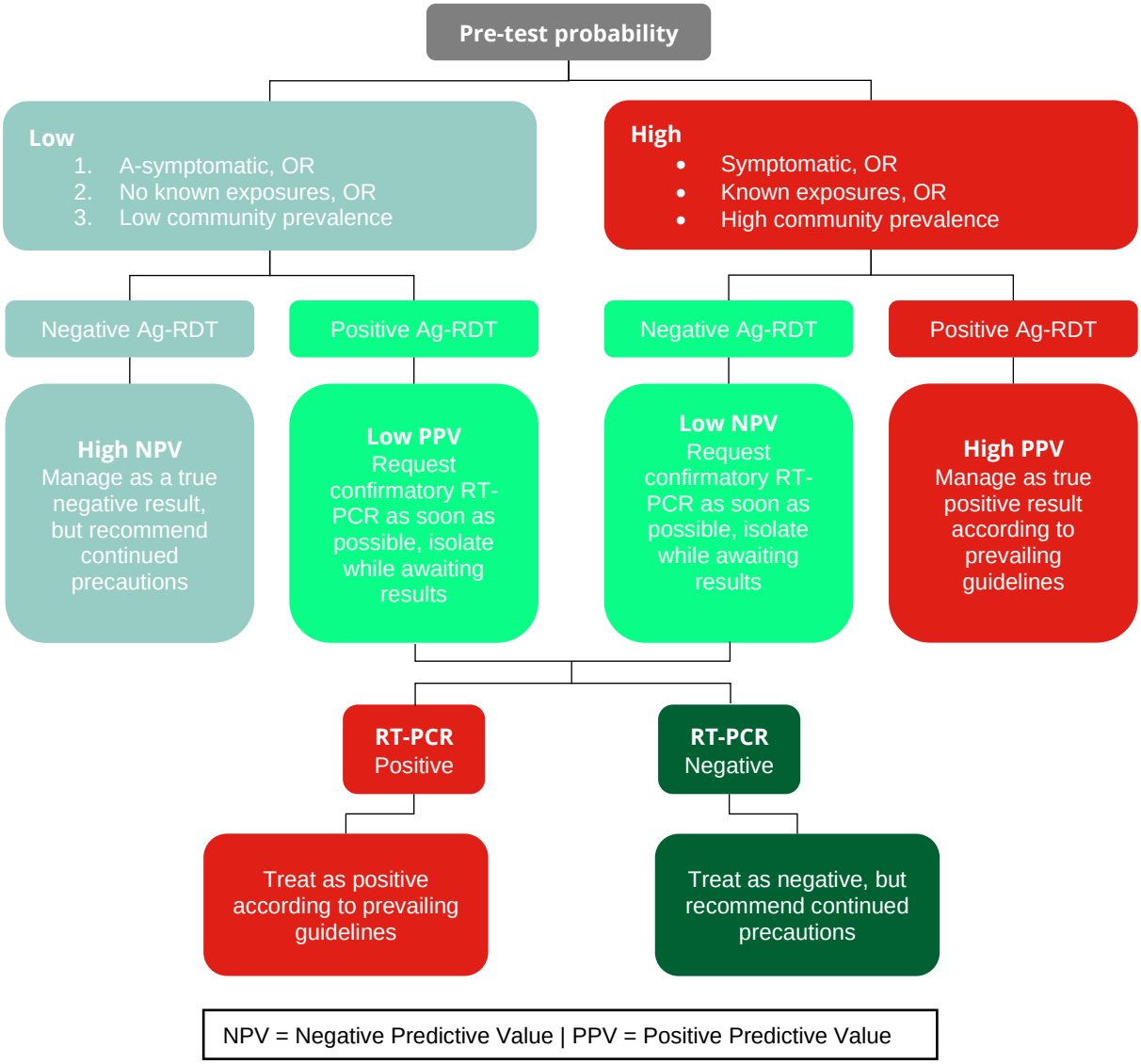


Figure 3: Testing protocol

A single positive PCR test is sufficient proof of COVID-19. There is no role for repeat 'confirmatory' PCR testing on patients who test positive despite the absence of symptoms, as PCR have excellent specificity, and asymptomatic and pre-symptomatic COVID-19 patients are well described.
'Equivocal' or 'indeterminate' results need to be interpreted in the light of clinical circumstances. A repeat sample obtained a few days later often helps to resolve doubtful cases.

RAPID ANTIGEN TESTS

Rapid antigen tests are considerably less sensitive than PCR tests.[4]
Thus false-negative results are more common. In addition, the rate of false-positive results is also higher than with PCR-based testing. The interpretation of a rapid antigen test result therefore depends on the pre-test probability of a patient having COVID-19. Patients with a high pre-test probability include:

- Patients with symptoms suggestive of COVID-19 (see above).
- Patients with a high-risk exposure (known to have been in close contact, as defined above, with a patient who had COVID-19).

Patients with a high pre-test probability:
A positive antigen test result confirms infection, but a negative result should be followed by a PCR test. While a considerable proportion (up to 30% and more)

Differential diagnoses

The differential diagnosis of suspected cases includes influenza (remembering the seasonality), both conventional and atypical bacterial pneumonias, and in patients with HIV and a CD4 count <200 cells/mm^3 (or equivalent immunosuppression), *Pneumocystis jirovecii* pneumonia (PJP).

Malaria as the cause of an acute febrile illness (typically with headache, rigors and malaise) must always be considered in persons residing in or travelling from malaria-transmission areas. If malaria is diagnosed, effective treatment must not be delayed (see adult and paediatric standard treatment guidelines on malaria).

Non-infectious causes of dyspnoea and/or fever should also be considered, such as pulmonary emboli, myocardial infarction and heart failure.

For patients with severe disease who require admission, appropriate tests may include:

- HIV test (if status unknown).
- Full blood count + differential.
- Blood culture.
- Nasopharyngeal and/or oropharyngeal swabs for detection of viral and atypical bacterial pathogens.
- Chest radiography.
- Sputum for MCS and *Mycobacterium tuberculosis* detection (GeneXpert MTB/RIF Ultra).
- Urine for lipoarabinomannan (LAM) if HIV positive.
- Beta-D-glucan and expectorated sputum/tracheal aspirate for PJP if HIV positive and clinical suspicion of PJP (without inducing sputum).
- Malaria antigen and smears (if residing in or travelling from malaria-transmission areas).

For patients with mild disease who do not require admission, a more limited workup may be appropriate. Depending on the specific presentation, test may include:
HIV test (if status unknown).
Sputum GeneXpert MTB/RIF Ultra if patient is HIV positive and is coughing (would fulfil case definition for TB), or if HIV negative and in close contact with TB patients.

of PCR-positive samples may be missed by rapid antigen tests, these are usually samples with low viral loads (high Ct values on PCR) and thus from patients unlikely to be highly infectious currently.

Patients with a low pre-test probability:
A negative result can be assumed to be a true negative, whereas a positive result should be followed by a confirmatory PCR test to exclude a false-positive result.

ANTIBODY TESTS

Antibody-based (serological) tests should not be used for the diagnosis of acute COVID-19. These tests are insufficiently sensitive early in the disease course (before sufficient antibodies have been produced which often takes two weeks or longer).[5, 6]

- Antibody-based tests may have a role in other scenarios, such as for seroprevalence surveys, or the diagnosis of Multisystem Inflammatory Syndrome in Children (MIS-C).

What testing is indicated for patients after vaccination against COVID-19?

- No vaccine is 100% efficacious, and this is even more true in the context of new viral variants; vaccines may protect against severe infection and death rather than infection *per se*. Vaccinated patients fulfilling testing criteria should be tested by PCR or antigen tests as needed.
- Positive PCR or antigen test results following vaccination (possible SARS-CoV-2 vaccine failures) should be discussed with the virology lab in order that viral sequencing is considered (see below).
- Antibody tests must be interpreted with caution in patients who have been vaccinated against COVID-19. Antibody tests targeting the spike (S) protein are expected to give positive results after vaccination (all current vaccines elicit antibodies against the spike protein), whereas antibody tests targeting the nucleocapsid (NC) will not be affected by vaccination.
- Antibody testing to ascertain immune status following COVID-19 or vaccination is not recommended currently due to many uncertainties regarding the interpretation of test results.

When should positive results by PCR or by rapid antigen test be discussed with the laboratory so that sequencing of the virus can be considered?

To facilitate surveillance for new lineages of SARS-CoV-2, which requires sequencing of the viruses involved,[7] we recommend discussing the following scenarios with the virology laboratory or directly with the Network for Genomic Surveillance in South Africa.

- Possible SARS-CoV-2 vaccine failures (breakthrough infections in vaccinated individuals).
- Prolonged shedding with high viral loads (i.e. low Ct values) in immunocompromised hosts.[8]
- Sudden increases in the COVID-19 caseload, increasing frequency of 'unusual' cases (e.g. in terms of disease presentation, patient groups affected, etc.) or the development of case clusters.[9]

- Possible animal-to-human spread.[10]
- Clinical suspicion of a change in the performance of diagnostic assays (PCR, antigen or antibody assays).
- Suspected cases of importation from another country, especially countries known to harbour virus variants of concern or countries with little available information.
- In addition, where most or all cases are diagnosed by rapid antigen test, provisions need to be made for a certain proportion of positive samples to be referred for possible surveillance sequencing. Please observe national or regional specific advice/guidance.

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