LABORATORY GUIDELINES ON THE DIAGNOSIS, MONITORING, SURVEILLANCE AND TESTING STRATEGY OF COVID-19

APRIL 2020

Foreword

These testing guidelines for SARS-CoV-2 were developed in response to the new Coronavirus Disease 2019 (COVID-19). The outbreak started in Wuhan City, Hubei Province in mainland China in December 2019 and has since spread globally, infecting more than 2,0000,000 people and resulting in over 170,000 deaths.

These guidelines was developed to help the health care workers and medical laboratory professionals to understand the role of laboratory testing in the diagnosis, management and control of this global epidemic.

The guidelines describe the testing strategy that is relevant to the Kenyan context, at the current stage of the pandemic in Kenya, with the first case confirmed in Kenya on 12th March 2020. The testing strategy will require to be adapted as we move to the next phase of the pandemic.

In developing these guidelines, we hope that our experience with SARS-CoV-2 will guide us to build robust systems that will respond quickly to future pandemics should they arise just us South Korea, Hong Kong and Singapore managed to keep their curve flat through aggressive testing, a small amount of targeted closures, and voluntary social distancing by citizens, lessons learned from their experience with SARS.

We hope that these guidelines will help demystify the role and use of laboratory testing to all frontline workers and Kenyans working tirelessly to battle this unseen enemy.

Dr Alice Kanyua
Chair, KACP COVID 19 TWG
21st April 2020

Acknowledgement

These guidelines have been developed by a technical committee set up by the Kenya Association of Clinical Pathologists (KACP) to guide clinicians and other healthcare workers in appropriate test choice and testing strategies in the diagnosis, prognosis and management of patients with COVID-19. The KACP executive appreciates all the time and effort of the dedicated members of this committee led by the Chair Dr Alice Kanyua. We hope that these guidelines will fill an important gap in handling the spread of the pandemic in Kenya.

Dr. Geoffrey Omuse
KACP Chair

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Abbreviations

ALT - Alanine aminotransferase

AST - Aspartate aminotransferase

BSC - Biosafety cabinet

BSL - Biosafety level

CBC- Complete blood count

COVID - Corona Virus Disease

CRP- C reactive protein

LDH - Lactate dehydrogenase

PCR- Polymerase Chain Reaction

PRN - pro re nata (when necessary)

PT - Prothrombin time

PPE- Personal protective equipment

SARS COV-2: Severe Acute Respiratory Syndrome Corona Virus 2

WBC - White blood cell count

Introduction

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. Chinese authorities identified a novel coronavirus (COVID 19/SARS CoV2), which has resulted in hundreds of thousands of confirmed human infections in many countries throughout the world including in Kenya. Confirmation of a case requires laboratory confirmation by PCR, and a PCR diagnosis is confirmatory even in the absence of clinical signs and symptoms.

This guidance aims to address the role of the laboratory in diagnosis, clinical management and prognostication, monitoring of viral shedding, and disease surveillance.

In developing this guidance, it is necessary to understand that many aspects of the virus and disease are still not understood. A better understanding will be needed to provide improved guidance. This is an interim guideline that is likely to evolve as more information becomes available.

Guidance On Samples for SARS Cov2 Testing

Laboratory testing is needed to confirm a suspected or probable case of COVID-19/ SARS CoV2. The decision to test should be guided by clinical and epidemiological data which both inform the case definition for suspected or probable cases.

Rapid testing of suspected individuals plays a critical role in clinical case management and outbreak control. Laboratory experts are able to give guidance on proper specimen types, specimen collection, transport, storage and testing.

Specimens:

The virus can be detected from a range of specimens. Of these, respiratory samples have been shown to give the greatest yield, hence they are the preferred samples for SARs CoV2 testing.

At a minimum, a respiratory sample should be collected. For initial testing in an ambulatory patient, an upper respiratory sample, preferably a nasopharyngeal swab (NP) which yields higher viral loads as compared to an oropharyngeal swab (OP) should be collected. If an NP is not available or cannot be obtained, there is limited evidence that an OP, nasal swab or nasal turbinate swab can be collected. Healthcare workers designated to do the collection should be trained on proper techniques of collection of these samples.

In more severe respiratory disease, and when clinically indicated in a mechanically ventilated patient, a lower respiratory tract sample – bronchoalveolar lavage and tracheal aspirates - are acceptable samples and should be transported in viral transport medium. Bronchoscopy should not be performed for the sole purpose of obtaining a specimen for laboratory testing for COVID-19 as there is significant risk associated with specimen collection.

Sputum, in a patient with a productive cough, is also an acceptable lower respiratory tract sample. Sputum should not be induced in a patient who doesn't present with a productive cough.

Specimen collection:

Upper respiratory Tract Samples:

NP and OP samples should be collected using synthetic fiber swabs. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media or universal transport media.

Nasopharyngeal swab:

Insert a swab into the nostril, parallel to the palate.

Swab should reach depth equal to the distance from the nostrils to the outer opening of the ear as shown in the diagram below.

Leave the swab in place for at least 5 seconds to absorb secretions.

Slowly remove the swab while rotating it.

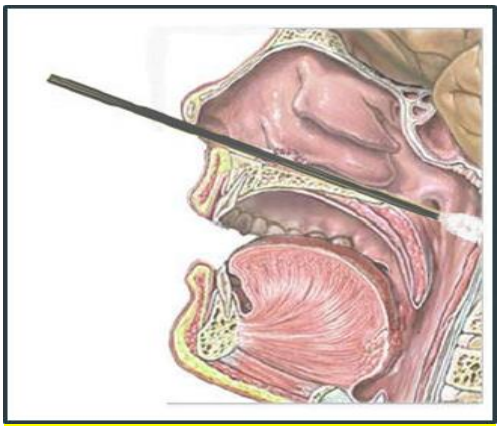

Figure 1:Collection of nasopharyngeal specimen

Oropharyngeal swab:

Swab the posterior pharynx, avoiding the tongue.

Out of the two, NP is the preferred sample type. If both NP and OP swabs are collected, they should be combined in a single tube to maximize test sensitivity and limit use of the testing resources available.

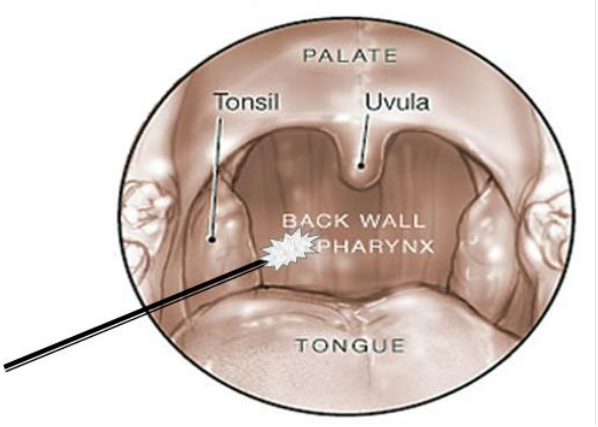

Figure 2: Collection of oropharyngeal specimen

Lower Respiratory Tract Samples

Sputum

Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.
Do not induce sputum production.

Bronchoalveolar lavage, Tracheal aspirate

collect 2-3 mL into a sterile, leak-proof, screw-cap sterile dry container that contains VTM.

Specimen Transport and storage:

Specimens collected for virus detection should reach the laboratory as soon as possible after collection. Correct handling of specimens during transportation is essential.

Specimens should be transported in triple packaging containing a leak proof primary container, a rigid, leak proof, watertight secondary packaging with absorbent material, and a rigid outer packaging to protect the specimen during shipment.

Specimen collection and transport requirements

Specimen type	Collection materials	Storage temperature until testing in-country laboratory	Recommended temperature for shipment according to expected shipment time
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	2-8 °C	2-8 °C if ≤5 days –70 °C (dry ice) if >5 days
Bronchoalveolar lavage	Sterile container *	2-8 °C	2-8 °C if ≤2 days –70 °C (dry ice) if >2 days
(Endo)tracheal aspirate, nasopharyngeal or nasal wash/aspirate	Sterile container *	2-8 °C	2-8 °C if ≤2 days –70 °C (dry ice) if >2 days
Sputum	Sterile container	2-8 °C	2-8 °C if ≤2 days –70 °C (dry ice) if >2 days
Tissue from biopsy or autopsy including from lung.	Sterile container with saline or VTM.	2-8 °C	2-8 °C if ≤24 hours –70 °C (dry ice) if >24 hours
Serum	Serum separator tubes (adults: collect 3-5 ml whole blood).	2-8 °C	2-8 °C if ≤5 days –70 °C (dry ice) if >5 days
Whole blood	Collection tube	2-8 °C	2-8 °C if ≤5 days –70 °C (dry ice) if >5 days
Stool	Stool container	2-8 °C	2-8 °C if ≤5 days –70 °C (dry ice) if >5 days
Urine	Urine collection container	2-8 °C	2-8 °C if ≤5 days –70 °C (dry ice) if >5 days

* For transport of samples for viral detection, use viral transport medium (VTM) containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens. If VTM is not available sterile saline may be used instead (in which case, duration of sample storage at 2-8 °C may be different from what is indicated above).

Figure 3: Specimen collection and transport requirements

Communication with the Lab:

Prior to sending samples, timely communication between the clinical and laboratory staff is encouraged. This in effect minimizes the risk incurred in handling specimens from patients with possible COVID-19, and also ensures proper and timely processing of samples and timely reporting by the laboratory. Specimens should be correctly and clearly labelled and accompanied by a correctly filled case definition form provided by the Ministry of Health (see appendix 1).

Laboratory Diagnostic Testing:

All diagnostic testing should be using a molecular test that detects presence of unique viral ribonucleic acid material in the sample. This is usually based on polymerase chain reaction (PCR). There are a number of PCR assays available, from lab developed tests to commercial open manual tests and closed proprietary tests and platforms. The viral genes targeted so far are the N, E, S, ORF1a and RdRP genes. For a case to be called a laboratory confirmed case, a result showing positive nucleic acid amplification test result for at least two different targets on the COVID-19 virus genome, of which at least one target is preferably specific for COVID-19 virus should be documented.

Laboratories starting testing are encouraged to send the first five positives and the first ten negative COVID-19 samples to the National Influenza Center reference laboratory. For laboratories carrying out COVID-19 testing, only validated protocols and assays should be used. Laboratories planning to start in-house testing for COVID-19 should collaborate with the National Influenza Center for test validation and guidance.

The laboratories should be aware of the limitations of assays before releasing results, since this will inform the interpretive comment that will go out with the result.

An SOP should be available that outlines the test principle, sample preparation (including nucleic acid material extraction and hybridization, if the assay requires that), quality control and result interpretation based on the assay used.

Result interpretation and sign off:

The laboratory director or designate will ensure that samples are handled by staff that are competent and appropriately trained to run the samples and release the test results.

One or more negative results do not rule out the possibility of COVID-19 virus infection.

A number of factors could lead to a negative result in an infected individual, including:

1. Poor quality of the specimen, containing little patient material (as a control, consider determining whether there is adequate human DNA in the sample by including a human target in the PCR testing).
2. In people who are incubating the infection, the virus is usually not detectable until the onset of symptoms or up to 2-3 days before onset of symptoms.
3. The specimen was collected late in the infection.
4. The specimen was not handled and shipped appropriately.

5. Technical reasons inherent in the test, e.g. virus mutation or PCR inhibition.

Quality Control:

All PCR testing runs should be carried out with at least a positive, negative and an internal control. All laboratories doing testing are encouraged to participate in an external quality assurance program or an inter-laboratory comparison.

Serologic Testing for COVID-19

Serologic testing for diagnosis of SARS-CoV-2

Serological tests for COVID-19 are used to detect the presence of antibodies against SARS-COV-2 (antibody-based tests) in a blood sample of serum. Antibodies are proteins produced by the immune system that recognize specific components of pathogens and target them for destruction; some pathogen-specific antibodies can protect a host from future infection.

The antibody response in SARS-CoV-2 is still being studied. Published studies show that the median day of seroconversion after infection is 15 - 20 days after exposure, and 7 – 13 days after the onset of symptoms. Elderly and more immunocompromised patients may never develop anti-SARS-COV-2 antibodies, or only develop them much later. It is also thought that asymptomatic infected persons may clear the virus quicker than symptomatic individuals, and may therefore have lower antibody titers than infected symptomatic individuals.

Antibody-based serological testing for COVID-19 infection measures exposure to SARS-CoV-2 by detecting presence of antiviral antibodies either due to an active infection or a past infection. This type of testing is only useful later in the course of illness, after the body has initiated an adaptive immune response against SARS-CoV-2 (more than 7 – 10 days), or after recovery. The graph below demonstrates the difference between the level of IgG and IgM antibodies and the virus titre, in relation to the incubation period for COVID-19.

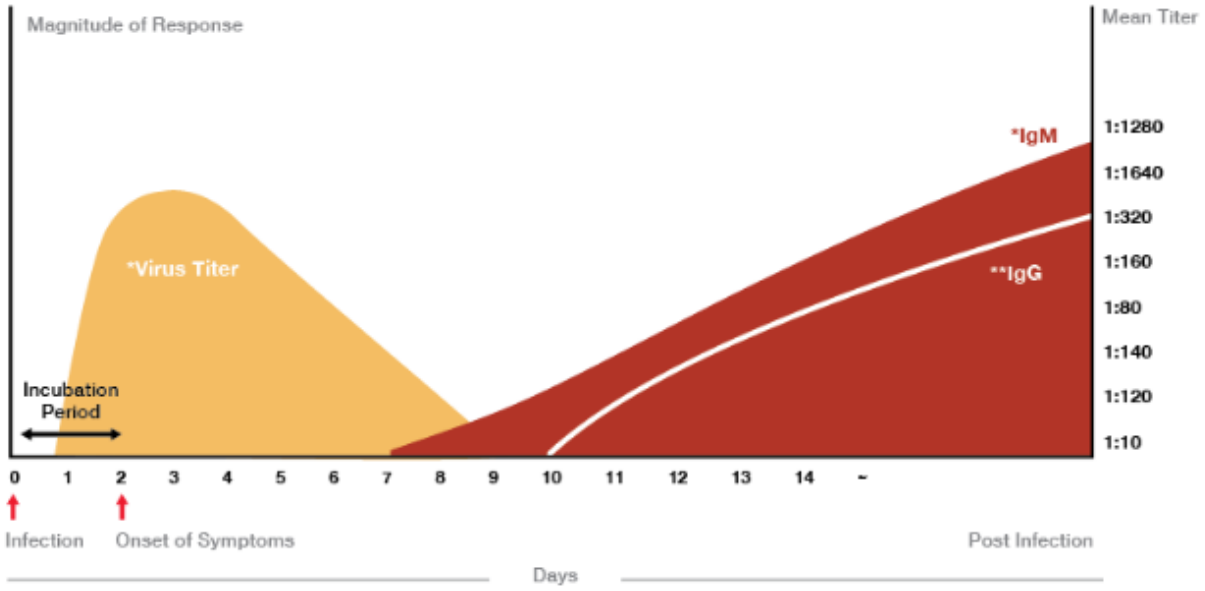

Figure 4: Antibody response in COVID-19 patients - Adopted from Royal College of Pathologists Australia media release 01 April 2020

This limits the utility of serology for diagnosis of COVID-19, and there are important considerations for test result interpretation.

1. A negative test does not exclude active infection as this could be in the early phase of infection before there is a detectable antibody response (window period).
2. A positive test does not provide information on active infection or whether one has cleared the virus.

In both scenarios, a follow-up test that detects presence of the virus (PCR) would be required to confirm or exclude active infection. Also, the anti-SARS-CoV-2 antibodies detected by serology may cross-react with antibody responses to seasonal (non SARS) corona viruses.

Serological testing is therefore not currently recommended for the diagnosis of COVID-19 infections. The gold standard for diagnosis for acutely ill patients remains molecular testing by RT-PCR. The role of serology testing as an add on to molecular testing is still evolving, and the evidence for this will require continuous review.

Serological tests for COVID-19 can also be antigen-based, to detect the presence of viral protein (antigen) in a sample. The appropriate sample for antigen detection is a nasopharyngeal or oropharyngeal swab. Antigen-based tests are still in development and are yet to be evaluated and approved for use in COVID-19 detection.

Serological Testing for COVID-19 in Disease Surveillance

Despite the above-mentioned limitations, serological testing is of value in disease surveillance to determine how widely COVID-19 has spread in a certain population. Their utility for this purpose includes:

1. Determination of the actual proportion of the population that has been exposed and developed immunity, demonstrated by development of IgG antibodies. This provides seroepidemiological evidence on the true extent of the outbreak, and allows for more accurate calculation of the attack rate and case fatality rate. This information can also aid in estimation of possible herd immunity, provided the immunity is long-lasting.
2. Provision of data for studies of the immune response to COVID-19, which will inform development of vaccines and treatment options, including identification of potential donors for convalescent plasma. Paired serum (acute and convalescent) can be useful to retrospectively define cases and determine the antibody response as serological assays become available. The initial sample is collected in the first week of illness and the second ideally collected 2-4 weeks later (optimal timing for convalescent sample needs to be

established). Serum samples can be collected and stored for this purpose where resources allow.

3. Establishment of who has been exposed/infected, and probably recovered, including unrecognised past infection. This can allow for modelling the course of disease transmission to allow for decision-making on appropriate containment measures, including tracing of contacts. This information can also be used to identify potentially immune persons, including essential workers such as health care personnel that may be cleared as safe to return to work.

Rigorous evaluation and validation of antibody-based kits need to be performed before approval and adoption for use in disease surveillance.

Considerations in choosing a serological assay

Identifying the methodology

1. A test for viral proteins (likely a Western blot), with antibodies raised in animals that would allow for detection; this method can be done in resource limited settings, but quality assurance still needs to be done. This would only give qualitative results (positive or negative).

2. An ELISA (Enzyme Linked Immunosorbent Assay) that detects the patient's antibodies to the virus. These would allow for rapid detection of viral proteins, or antibodies to those proteins, using serum alone. This can be used for qualitative and quantitative results, that can be used for follow up of titers to determine antibody response.

Identifying the target

Coronavirus has 4 structural proteins: the spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. In the case of SARSCoV-2, the spike protein appears to be the primary protein interacting with host cells. Hence, the spike protein is likely the protein to which antibodies are raised, but this is not clear at this time. This would thus be the potential target antibody in developing serological tests.

After identification of the target antibody, proper antibody binding, antigen presentation, and protein folding need to be further elucidated. The protein must be presented on the ELISA as it is presented in the body, or the antibodies may not bind and can lead to **false negative results**.

Of note the US FDA does not intend to object to the development and distribution by commercial manufacturers, or development and use by laboratories, of serology tests to identify antibodies to SARS-CoV-2 and as such many unvalidated test assays will be increasingly available in the US market and globally. It is important that due to limitations in diagnosis the following comments are released for all tests done by serologic testing:

1. Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic test should be considered to rule out infection in these individuals.
2. Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
3. Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

Identifying the antibody type

There are three antibody types currently being developed for the detection of humoral response to SARSCoV-2. These are IgG, IgM and IgA. Prolonged detection of IgG titres is a surrogate marker

for the development of immunity against an organism, whereas IgA and IgM titres are usually elevated in the acute convalescent stage.

Samples Other Than Respiratory Specimens

Whereas respiratory specimens are the specimens of choice, additional clinical specimens may be collected as COVID-19 virus has been detected in blood and stool, as had the coronaviruses responsible for SARS and MERS.

The duration and frequency of shedding of COVID-19 virus in stool and potentially in urine is not yet fully understood.

A study looking at the biodistribution of SARS-CoV-2 among different specimens of inpatients with a confirmed diagnosis of COVID 19 found better detection from respiratory tract specimens. The percentage positivity from respiratory tract specimens ranged between 32 to 93%, the percentage positivity from fecal specimens was 29% and 1% from blood specimens.

As such respiratory tract samples are the specimens of choice for diagnosis of SARSCoV-2.

Due to concerns with specimen stability, transport, and appropriate collection materials, self-collection at home or at sites other than designated collection sites staffed by HCPs is currently not recommended

Prognostication of Patients with Covid-19 Based On Laboratory Parameters

From a meta-analysis of epidemiologic data from 30 observational studies of 53,000 COVID-19 patients from China, it was found that elevated level of CRP, LDH and D-dimer, together with reduced level of lymphocytes and platelets were the prominent features of severe cases compared to none-severe cases. Other markers in all cases with disease were elevated ESR, cardiac Troponin I, myoglobin, AST, ferritin, low albumin and low haemoglobin.

Similarly, in a systematic review of 11 studies of patients in China, eight reported the rate of abnormal laboratory test results in 426 patients (including 34 children) as follows: increased white blood cell (WBC) count, increased neutrophil count, decreased lymphocyte count, decreased albumin, increased lactate dehydrogenase (LDH), increased alanine aminotransferase (ALT), increased aspartate aminotransferase (AST), increased total bilirubin, increased creatinine, increased cardiac troponin, increased D-dimer, increased prothrombin time (PT), increased procalcitonin and increased C-reactive protein (CRP).

Findings from a study of 127 patients from Wuhan were that a CD3+ lymphocyte count of ≤470/µL , prothrombin time ≥13.5 seconds and procalcitonin ≥0.15 ng/mL were independent prognostic factors for death.

With regards to prognostic laboratory data, Wang et al. examined six laboratory parameters throughout 19 days of hospitalization in 138 patients with COVID-19 infection (33 with severe disease). Significant differences were noted between patients who needed admission to the intensive care unit and those who did not: higher WBC count (1.5-fold), higher neutrophil count (1.7-fold), lower lymphocyte count (0.9-fold), as well as higher LDH (2.1-fold), ALT (1.5-fold), AST (1.8-fold), total bilirubin (1.2-fold), creatinine (1.1-fold), cardiac troponin I (2.2-fold), D-dimer (2.5-fold) and procalcitonin (1.2-fold).

We thus recommend the following tests for risk stratification and follow up for severe COVID-19 illness that requires admission as shown in table 1:

Table 1: Suggested tests for diagnosis and management of COVID-19 patients

No	TEST	TEST FREQUENCY					RATIONALE
		INITIAL	DAILY	ALT. DAYS	PRN	DISCHARGE	
1.	SARS-COV 2 PCR	×				×	-Confirm COVID-19 diagnosis -Determine infectious potential at discharge. Where capacity allows, ensure two consecutive negative test results done at least 24 hours apart
2.	CBC with WBC differential	×	×			×	-Poor prognosis if lymphopenia (<800 cells/µL) or increased neutrophil-lymphocyte ratio (>3.13) or low platelets
3.	U/E/Cr	×	×			×	-Assessing kidney function
4	LFTs	×	×			×	-Assessing liver function -Poor prognosis if ALT or AST increased
5.	Blood gas	×	×		×		-Diagnose ARDS -Monitor ventilation adequacy
6.	Lactate	×	×		×		-Identify end organ dysfunction -Monitor response to septic shock treatment
7.	Glucose	×			×		-Screen for diabetes -Sepsis management
8.	CRP	×		×			-Poor prognosis if >100 mg/L
9.	Bacterial cultures	×			×		-Diagnose co-infection with bacteria as appropriate
10.*	NTproBNP or BNP	×			×	×	-Distinguish respiratory vs cardiac cause of difficulty in breathing -Screen for heart failure -Monitor response to heart failure treatment
11.*	Troponin I / T	×			×		-Diagnose ACS or myocarditis
12.*	Respiratory panel PCR/TB gene Xpert	×			×		-Diagnose co-infection with other respiratory viruses or TB
13.*	D-dimer	×		×			-Poor prognosis if >1 µg/mL
14.*	LDH	×		×			-Poor prognosis if >245 U/L
15.*	Procalcitonin	×		×			-Poor prognosis if ≥0.15 ng/mL

*Optional based on availability

Patients with suspected or confirmed COVID19 can have co-infections including TB, HIV and other comorbidities. It is therefore important for clinicians to always consider additional testing based on differential diagnosis guided by patient history, clinical presentation and regional epidemiology of disease.

Role of Laboratory Tests in Guiding Management and Discharge of Patients

When deciding on criteria for hospital discharge of COVID-19 patients, healthcare facilities should consider several factors such as patient specific factors, discharge destination, the existing capacity of the healthcare system, laboratory diagnostic resources, and the current epidemiological situation.

The challenge in clinical management of suspected cases lies in deciding whether they may be de-isolated or if further isolation and repeat testing are required.

Infectivity

The interval during which an individual with COVID-19 is infectious is uncertain. Most data informing this issue are from studies evaluating viral RNA detection from respiratory and other specimens. However, detection of viral RNA does not necessarily indicate the presence of infectious virus. Both asymptomatic and symptomatic persons may transmit the virus.

Severely ill patients may transmit disease for longer periods of time and the disease may be more infective at the earlier stage of the illness.

The rate of transmission is also determined by location and infection prevention measures underpinning the importance of patient education before discharge.

Discharge

Various national bodies in countries that have experienced local transmission of SARSCoV-2 have differed in guidelines but have largely been based on:

1. The clinical resolution of symptoms.
2. The evidence for viral RNA clearance from the upper respiratory tract
3. Serological tests specifically IgG
4. Empirical timelines if testing is not available or practical.
5. The discharge criteria is based on the evolving epidemiology of the disease.

Early Phase (Few cases) of Epidemic Discharge Strategy:

Clinical criteria (e.g. no fever for > 3 days, improved respiratory symptoms, pulmonary imaging showing obvious resolution of inflammation, no hospital care needed for other pathology, clinician assessment)

Laboratory evidence of SARS-CoV-2 clearance in respiratory samples; 2 negative RT-PCR tests for respiratory tract samples (nasopharynx and throat swabs with sampling interval ≥ 24 hours). Testing at a minimum of 7 days after the first positive RT-PCR test is recommended for patients that clinically improve earlier.

Serology: appearance of specific IgG when an appropriate serological test is available.

If testing is not readily available, use clinical criteria and 14 days of transmission-based precautions at the destination of the patient.

Late Phase (Many Cases) of Epidemic Discharge Strategy:

This would be declared under the guidance of the Ministry of Health based on local epidemiology as some areas are more likely to have higher disease burden than others.

If the number of patients increases beyond the capacity of the facilities, discontinuation of transmission-based precautions is not mandatory in determining whether to discharge patients. Once clinical criteria are met, patients may be discharged.

Clinical criteria such as no fever for > 3 days, improved respiratory symptoms, pulmonary imaging showing obvious resolution of inflammation, no hospital care needed for other pathology determined by clinician assessment.

Patients will then spend 14 days in quarantine or isolation centers and where resources allow they should have an RT PCR at the end of the 14-day period. If negative they can be discharged home based on national guidance, they will need to practice transmission-based precautions in their homestead.

Discontinuation of Transmission Based Precautions:

The decision can be guided by a test based or a non-test-based strategy based on availability of resources.

Test-based strategy:

Resolution of fever without the use of fever-reducing medications and

Improvement in respiratory symptoms (e.g., cough, shortness of breath), and

Negative results of an authorized SARSCoV-2 molecular assay for detection of SARS-CoV-2 RNA from at least two consecutive nasopharyngeal swab specimens collected ≥24 hours apart (total of two negative specimens).

Non-test-based strategy:

At least 3 days (72 hours) have passed since recovery defined as resolution of fever without the use of fever-reducing medications and improvement in respiratory symptoms (e.g., cough, shortness of breath); and,

At least 7 days have passed since symptoms first appeared or 14 days from last possible exposure.

Monitoring Viral Shedding

Whereas SARS-CoV-2 virus has been detected in respiratory, fecal, urine and blood specimens, diagnosis of infection is currently anchored on detection of the virus from respiratory samples through Nucleic Acid Amplification Tests (NAAT). Upon confirmation of infection, subsequent testing for detection of viral RNA serves to

a. Monitor disease progression

b. Establish eligibility for discharge upon recovery

SARS-CoV-2 virus can be detected from upper respiratory specimens about 2 days prior to onset of symptoms. The duration during which the virus is detectable during the course of the disease is variable but appears to reflect on the severity of the disease. On average, the virus is detectable from upper respiratory samples for 1-3 weeks from the onset of symptoms.

The median duration of viral shedding has been documented as 19 days in those with severe disease and approximately 24 days for critical cases. Patients with mild disease exhibit viral shedding for approximately 7-12 days from onset of symptoms. Non-survivors have been observed to demonstrate viral shedding up to the time of demise.

Viral RNA has been detected in fecal specimens in up to a third of patients from day 5 following onset of symptoms, and has been noted for up to 4-5 weeks in moderate cases. However, it is still unclear whether this correlates with the presence of infectious virus. While live virus has been cultured from stool in some cases, the role of fecal-oral transmission is not yet well understood but is not thought to be a driver of COVID-19 transmission. Proper hygiene and sanitation practices must be adhered to.

Laboratory Testing Strategy

Considerations

The global spread of COVID-19 has dramatically increased the number of suspected cases and the geographic area where laboratory testing needed to be implemented. The increased COVID-19 molecular testing has placed a great strain on available testing resources leading to shortages of molecular testing reagents for COVID-19 globally as well as shortages of equipment needed to collect specimens such as NP swabs and transport media.

It is recommended that each country assesses its risk and rapidly implement the necessary measures at the appropriate scale and prepare for a testing and clinical care surge to reduce both COVID-19 transmission and economic, public health, and social impacts.

Table 2: Strategy for laboratory testing based on transmission scenario

	No cases	**Sporadic cases**	**Cluster of cases**	**Community transmission**
Transmission scenario	No reported cases	One or more cases, imported or locally acquired	Most cases of local transmission linked to chains of transmission	Outbreaks with the inability to relate confirmed cases through chains of transmission for most cases, or by increasing positive tests through sentinel samples (routine systematic testing of respiratory samples from established laboratories)
Public health aim	Stop transmission and prevent spread	Stop transmission and prevent spread	Stop transmission and prevent spread	Slow transmissions, reduce case numbers, and end community outbreaks. Reduce health, social and economic impact of the outbreak. Minimize disruptions in health care for non-COVID-19 illness
Testing strategy	Test all individuals meeting the suspected case definition.	Test all individuals meeting the suspected case definition Testing of contacts and those in quarantine also recommended	Test all individuals meeting the suspected case definition. Testing of contacts and those in quarantine also recommended	If diagnostic capacity is insufficient, implement **prioritized** testing and measures that can reduce spread (e.g. isolation). · People at risk of developing severe disease and vulnerable populations · Symptomatic health workers (including emergency services and non-clinical staff), regardless of whether they are a contact of a confirmed case · The first symptomatic individuals in a closed setting (e.g. schools, long term living facilities, prisons, hospitals) to quickly identify outbreaks and ensure containment measures.

There exists a significant risk of the testing capacity being overwhelmed especially during the phase of community transmission. In such circumstances, alternative testing strategies should be considered.

Table 3: Situations and management alternatives if testing capacity is overwhelmed

Situation	Alternative testing strategy if system overwhelmed/ testing not possible
Suspected case, mild, with no risk factors	Register as a suspected case, home isolate according to WHO guidelines, and do not test.
Suspected case requiring admission in a health care facility regardless of severity.	Strongly recommended to test. If testing is not possible, implement isolation measures warding against nosocomial transmission (thus no cohort isolation possible); test and treat other possible causes according to local guidelines.
Symptomatic health care worker identified as a contact	Strongly recommended to test
Symptomatic health care worker with no known COVID-19 contact I	In areas with COVID-19 community transmission, test
Increased number of suspected cases in a specific demographic group (potential cluster).	Test a subset of the suspects
Contact tracing in areas of community transmission	Quarantine contacts for 14 days, in lieu of testing. If symptomatic, assume COVID-19 and extend quarantine.
Patients hospitalized for severe disease in settings with no testing capacity.	Consider as suspected case and take precautions as if COVID-19 positive, treat for treatable local diseases.

Laboratory testing for COVID-19 for purposes of disease surveillance

In order to monitor the full extent of the circulation of SARS-CoV-2 in the general population, WHO recommends implementing testing for COVID-19, as resources allow, via existing national

sentinel surveillance sites for influenza-like illness (ILI) and severe acute respiratory infection (SARI).

Within the existing surveillance systems, the patients selected for testing for COVID-19 should preferably be representative of the population and include both mild and severe illnesses.

Priority for COVID-19 testing should be given to influenza-negative specimens. It is recommended to test at least 50 to 100 specimens for COVID-19 per week, as resources allow, that are negative for influenza. Depending on availability of resources, specimens positive for influenza may also be tested for COVID-19 to detect possible co-infections.

Laboratory Biosafety

Both the Centers for Disease Control and Prevention (CDC) and the World Health Organization released documents to provide interim guidelines for the collection, handling, and testing of clinical specimens that might contain SARS-CoV-2. Since little scientific data regarding handling this pathogen exists this far, the guidelines that have been prepared take into consideration the recommendations previously published on how to handle specimens that might contain severe acute respiratory syndrome coronavirus (SARS-CoV) or Middle Eastern respiratory syndrome coronavirus (MERS-CoV).

Prior studies of other coronaviruses showed lower concentration of the virus was noted in the nonrespiratory specimens (stool, urine, and blood), and that these specimens did not pose a major risk to the laboratorian while using standard bloodborne pathogen biosafety level (BSL)–2 precautions.

Recently a study by Wang et al also showed that low titer SARS-CoV-2 RNA was present in 29% of fecal specimens and 1% of blood specimens and that none of the urine specimens had detectable viral RNA from patients infected with COVID-19.

As a standard for any laboratory biosafety program, the first step to consider is to identify hazards by performing a biological risk assessment. All laboratories should perform a site-specific and activity-specific risk assessment to identify and mitigate risks, resulting in an adequately trained workforce to safely perform the tasks as assigned in the laboratory. Risk assessments and mitigation measures are dependent on:

1. The procedures performed right from sample collection, sample reception, sample preparation and clinical testing
2. Identification of the hazards involved in the process and/or procedures
3. The competency level of the personnel who perform the procedures
4. The laboratory equipment and facility
5. The resources available

Follow Standard Precautions when handling clinical specimens, all of which may contain potentially infectious materials. Standard Precautions include hand hygiene and the use of personal protective equipment (PPE), such as laboratory coats or gowns, and gloves.

Routine diagnostic testing of specimens, such as the following activities, can be handled in a BSL-2 laboratory using Standard Precautions:

- Using automated instruments and analyzers
- Processing initial samples
- Staining and microscopic analysis of fixed smears
- Examination of bacterial cultures
- Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues
- Molecular analysis of extracted nucleic acid preparations

For procedures with a high likelihood to generate aerosols or droplets, use either a certified Class II Biological Safety Cabinet (BSC) or additional precautions to provide a barrier between the specimen and personnel.

Examples of these additional precautions include: Personal protective equipment (PPE) – double gloves, fit-tested N95 respirator or surgical mask if N95 respirator is not available, face shield, or other physical barriers, like a splash shield; centrifuge safety cups; and sealed centrifuge rotors to reduce the risk of exposure to laboratory personnel.

Procedures that concentrate viruses, such as precipitation or membrane filtration, can be performed in a BSL-2 laboratory with unidirectional airflow and BSL-3 precautions, including respiratory protection and a designated area for donning and doffing PPE. The donning and doffing space should not be in the workspace. Work should be performed in a certified Class II BSC.

Non-propagative diagnostic laboratory work (for example, sequencing, nucleic acid amplification test [NAAT]) should be conducted at a facility using procedures equivalent to Biosafety Level 2. Inactivation methods should be done, before transferring the specimens to other areas for further manipulation, such as PCR analysis. Inactivation of SARS-CoV-2/COVID-19 virus can be achieved through a number of methods, such as heat inactivation, chemical inactivation and use of solvents. Facilities are advised to carry out validation of the inactivation in-house, or to adopt a well-established inactivation procedure validated by others. Propagative diagnostic work should be conducted in a BSL 3 laboratory.

Routine laboratory practices and procedures for decontamination of work surfaces and management of laboratory waste should be followed.

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Appendices

Appendix 1: Case reporting form

MINISTRY OF HEALTH
Division of Disease Surveillance and Response

Case investigation form for 2019 Novel Coronavirus (2019-nCoV)

Date of reporting to national health authority:
[_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_] Reporting Health Facility: _____
Sub Country:_____ Country:_____
Detected at point of entry ▫ No ▫ Yes ▫ Unknown If yes, date [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]

Section 1: Patient information

Unique Case Identifier (used in country): _____
Date of Birth: [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_] or estimated age: [__][__][__] in years
if < 1 year old, [_][_] in months or if < 1 month, [_][_] in days
Sex at birth: ▫ Male ▫ Female
Place where the case was diagnosed: Health Facility: _____
Admin Level 1 (County):_____ Admin Level 2 (Sub County):_____
Patient usual place of residency: Country: _____
Admin Level 1 (province):_____ Admin Level 2 (district): _____

Section 2: Clinical information

Patient clinical course

Date of onset of symptoms: [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_] ▫ Asymptomatic ▫
Unknown Admission to hospital: ▫ No ▫ Yes ▫ Unknown
First date of admission to hospital:
[_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_] Name of hospital:_____
Date of isolation:
[_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_
Y_] Was the patient ventilated: ▫ No ▫ Yes ▫ Unknown
Health status (circle) at time of reporting: Stable / Severely ill / dead / unknown
Date of death, if applicable: [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]

Patient symptoms (check all reported symptoms):

▫ History of fever / chills	▫ Shortness of breath	▫ Pain (check all that apply)
▫ General weakness	▫ Diarrhoea	*( ) Muscular ( ) Chest*
▫ Cough	▫ Nausea/vomiting	*( ) Abdominal ( ) Joint*
▫ Sore throat	▫ Headache	
▫ Runny nose	▫ Irritability/Confusion	

▫ Other, specify _____

Patient signs:

Temperature: [_][_][_] ▫°C / ▫ F
Check all observed signs:

▫ Pharyngeal exudate	▫ Coma	▫ Abnormal lung X-Ray findings
▫ Conjunctival injection	▫ Dyspnea / tachypnea	
▫ Seizure	▫ Abnormal lung auscultation	

▫ Other, specify: _____

Underlying conditions and comorbidity (check all that apply):

- ▫ Pregnancy (trimester:_____)
- ▫ Post-partum (< 6 weeks)
- ▫ Cardiovascular disease, including hypertension
- ▫ Immunodeficiency, including HIV
- ▫ Diabetes
- ▫ Renal disease
- ▫ Liver disease
- ▫ Chronic lung disease
- ▫ Chronic neurological or neuromuscular disease
- ▫ Malignancy
- ▫ Other, specify: _____

Section 3: Exposure and travel information in the 14 days prior to symptom onset (prior to reporting if asymptomatic)

Occupation: (tick any that apply)

▫ Student ▫ Health care worker ▫ Other, specify: _____
▫ Working with animals ▫ Health laboratory worker

Has the patient **travelled** in the 14 days prior to symptom onset? ▫ No ▫ Yes ▫ Unknown

If yes, please specify the places the patient travelled:

	Country	City
1.	_____	_____
2.	_____	_____
3.	_____	_____

Has the patient **visited any health care facility (ies)** in the 14 days prior to symptom onset? ▫ No ▫ Yes ▫ Unknown Has the patient had **close contact**[1] with a person with acute respiratory infection in the 14 days prior to symptom onset?

▫ No ▫ Yes ▫ Unknown

If yes, contact setting (check all that apply):

▫ Health care setting ▫ Family setting ▫ Work place ▫ Unknown ▫ Other, specify: _____

Has the patient **had contact with a probable or confirmed case** in the 14 days prior to symptom onset? :

▫ No ▫ Yes ▫ Unknown

If yes, please list unique case identifiers of all probable or confirmed cases:

Case 1 identifier_____ Case 2 identifier_____ Case 3 identifier. _____

If yes, contact setting (check all that apply):

▫ Health care setting ▫ Family setting ▫ Work place ▫ Unknown ▫ Other, specify: _____

If yes, location/city/country for exposure: _____

Have you visited any **live animal markets** in the 14 days prior to symptom onset? ▫ No ▫ Yes ▫ Unknown

If yes, location/city/country for exposure: _____

Section 4: Laboratory Information

Specimen collection *(To be completed by the health facility)*

a. Was specimen collected? ☐ 1=Yes 2=No

If no, why? .....

b. Date(s) of specimen collection: [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]

c. Specimen type: ☐ NP Swab ☐ OP Swab ☐ Serum ☐ Sputum ☐ Tracheal Aspirate

Other (specify): _____

d. Date specimen send to the lab: [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]

(To be completed by the confirming lab)

e. Name of confirming lab: _____

f. Please specify which assay was used:_____ Sequencing done?: ▫ Yes ▫ No ▫ Unknown

g. Preliminary lab results: _____

h. Date of laboratory confirmation: [_D_][_D_]/[_M_][_M_]/[_Y_][_Y_][_Y_][_Y_]

[1]Close contact' is defined as: 1. Health care associated exposure, including providing direct care for nCoV patients, working with health care workers infected with novel coronavirus, visiting patients or staying in the same close environment of a nCoV patient. 2. Working together in close proximity or sharing the same classroom environment with a with nCoV patient. 3. Travelling together with nCoV patient in any kind of conveyance. 4. Living in the same household as a nCoV patient

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