

UPDATE ON LABORATORY DIAGNOSIS OF SARS-COV-2 (COVID-19)

¹Omoti CE, ²Ahmed AAB

¹Consultant Haematologist, ²Clinical Pathologist, (Microbiology and Virology), Laboratory Administration and Blood Banks, Hail Regional Laboratory, Kingdom of Saudi Arabia.

ABSTRACT

Aim: To show the currently available methods of laboratory diagnosis of the novel corona virus.

Methods: Information was obtained from the WHO/CDC guidelines, internet search, journal articles, Entrez PubMed.

Results: Origin of SARS-CoV-2 was from Wuhan, China. It is a positive single stranded RNA virus belonging to the betacoronavirus. Mode of transmission after review was through droplet, airborne aerosol, direct and indirect contact through fomites. Affected patients may be asymptomatic and symptomatic ones present with fever, cough and shortness of breath. This results from the spike glycoprotein of the virus entering the body through ACE2 receptors to cause a cytokine storm. Challenges of setting up a laboratory testing was divided into preanalytical, analytical and post analytical including using the laboratory assessment tools (LAT); taking note of the risk assessment tools and laboratory bio security risks involved. Type of specimens collected include nasopharyngeal/ oropharyngeal swabs, blood etc. Nucleic acid base testing for the genome of SARS-CoV-2 was recommended.

Conclusion: Covid 19 is caused by SARS-CoV-2 which is transmitted by droplets, aerosols and by contact. Main diagnostic test is by molecular Nucleic acid base from nasopharyngeal swabs. Infection prevention and control measures are key to containing the virus. No vaccine has been produced so far.

Key words: Covid 19, SARS-CoV-2, Nucleic acid base testing, genome.

Introduction

Coronaviruses (CoV) are a large family of viruses that cause illnesses ranging from mild respiratory illness to more severe disease. The corona virus has been in existence since 2002 when it was first discovered in Guangdong province, Southern China.^{1,2} Then a new strain of novel coronavirus (2019-nCoV) was detected in December 2019 in Wuhan city, capital of Hubei province in China.³ This 2019-nCoV became an epidemiological alert in January 16th, 2020 and by Jan 30th, 2020, the WHO with the prevailing upon by the Public Health Emergency International Committee (PHEIC) declared it to be a pandemic with 106 cases and involvement of 19 countries. By February 11th the International Committee of Taxonomy on viruses (ICTV) named this novel 2019 coronavirus as Human coronavirus-19 (HCoV-19) or Severe Acute Respiratory Syndrome

Coronavirus 2 (SARS-CoV-2) which causes the Coronavirus disease (COVID 19 disease).

Modes of Transmission

It was initially thought to be a zoonotic pathogen that can be transmitted to humans through contact with infected animals or animal products, in this case seafoods and later bats. However, rapid increase in the number of cases with the viral infection having no contact with animals suggested an animal-to-animal transmission. The Sars-Cov-2 was reported to be transmitted as droplets, airborne aerosols (liquids or solid particles suspended in air), by direct and indirect contact or through contaminated fomites.^{4,5,6} This person to person spread occurs in community and healthcare settings, with local transmission reported in many countries.⁷ The virus has also been reported to be detected in blood, cerebrospinal fluid, pericardial fluid, placental tissue, urine, saliva, tears and conjunctival

All correspondence to: Prof Caroline E. Omoti, Department of Haematology, School of Medicine, University of Benin, Benin City, Nigeria. Email: caroline.omoti@uniben.edu

secretions. Faecal-oral transmission however was reported to be possible in one patient.⁸

Biology and classification of Sars-Cov-2

The Sars-Cov-2 is a positive –single stranded RNA (+ssRNA) virus with a crown like appearance. It belongs to the order Nidovirales, family Coronaviridae and subfamily Orthocoronavirinae. The virus is classified into four (4) genera namely AlphaCoV- 229E, NL63, BetaCoV- OC43, HKU1 with a subgenus Sarbecoronavirus, DeltaCoV and GammaCoV. The Sars-Cov-2 belongs to the betacoronavirus, subgenus Sarbecovirus and is the largest RNA virus with 30kb in length. It is the 7th corona virus known to infect humans but is distinct from Sars-CoV and MERS-CoV.^{9,10} The MERS-CoV and SARS-CoV are also betaCoV which cause Middle East Respiratory Syndrome and Severe Acute Respiratory syndrome respectively; with the new SARS-CoV-2 making up a total of seven (7) common human CoVs (HCoVs) which have been detected to infect humans. The Sars-Cov, Mers-Cov and Sars-Cov-2 are known to cause Severe Respiratory Disease while NL63, OC43, 229E and Hku1 are known to cause Mild Respiratory Ailments

Aetiology/cytokine storm description

Pathophysiology is not fully understood. SARS-CoV-2 binds to the Angiotensin Converting Enzyme 2 (ACE2) receptor in humans through its spike protein.¹¹ Spike glycoprotein receptor binding domain of SARS-CoV-2 has a functional polybasic (furin) cleavage site at the S1-S2 boundary; it confers a potentially binding affinity for ACE2 on host cells compared with SARS-CoV.¹² The virus uses the host transmembrane protease serine 2 (TMPRSS2) for S protein priming and fusion of viral and host cell membrane occurs.¹³ A down regulation of the ACE2 by the SARS-CoV-2 leads to a toxic over accumulation of angiotensin 11, which may induce ARDS and fulminant myocarditis.¹⁴ The production of the excessive immune reaction (cytokine storm) to the virus result from IL-4,6,10 and TNFalpha which acts on a large number of cells and tissues, stimulates acute phase proteins, thermo regulation, bone maintenance and pro and anti inflammatory cells.

Clinical manifestations

According to WHO it classified the COVID-19 disease symptoms into mild, moderate and severe disease.¹⁵ Mild cases of the Covid 19 disease have been associated with fever >38°C, respiratory symptoms of cough, nasal congestion; fatigue, headache, diarrhoea, nausea/vomiting and loss of smell/taste without evidence of shortness of breath and pneumonia; a past history of travel 14 days prior to onset of symptoms or close physical contact with a positive case of COVID 19. Moderate and severe disease are found in adolescents or adults with clinical signs of pneumonia and children with non-severe pneumonia. Factors that have been reported to be associated with prolonged viral shedding include male sex, older age, comorbid HTN, delayed admission after symptom onset or severe illness on admission and use of invasive mechanical ventilation or corticosteroids¹⁶ Widders et al¹⁷ reported no correlation between duration of viral shedding and duration of infectivity.

The WHO and CDC bodies have been the driving organisations that are Involved in the coordination of the pandemic. As the disease has been evolving, guiding principles are brought out regularly in order to contain the spread of the disease. These include

1. WHO interim guidance for laboratory testing (24/3/20)
2. WHO interim guidance for laboratory biosafety related to COVID-19.
3. WHO reference laboratory providing confirmatory testing for COVID-19.
4. Molecular assays to diagnose COVID-19.
5. Guidance for laboratory shipping specimens to WHO reference labs that provide confirmatory testing for COVID-19 virus.
6. Laboratory assessment tools for laboratory implementation of COVID-19 test.
7. Scientific brief: advice on the use of point of care immunodiagnostic tests for COVID-19.

The Guiding principles for lab testing updated as at 27/4/2020, showed that Laboratory Decision to test be based on:

- Clinical factors- History/mildly symptomatic/other causes of infection.
- Epidemiological factors (Local community transmission)
- Priority Assessment in contact tracing and mass screening for areas with high transmission rate.
- Rapid collection, testing of samples with the aim of clinical management and prevention of outbreak.

What are the challenges¹⁸-

These are divided into pre-analytical, analytical and post analytical stages.

- Pre-analytical stage starts from the
 - Proper collection of specimen regarding the time, site, volume of sample taken. This is one of the most important step towards the laboratory diagnosis of the Covid 19 disease. A specimen that is not collected correctly may lead to false negative test results.
 - Safety measures that must be put in place which starts from physician collection of sample, transportation of the specimen, the laboratory reception site and laboratory testing proper.
 - Getting the right capacity and skills of people, proper laboratory space, equipment and chemical reagents.
 - Logistics including putting up robust plans for safe access, safety of HCWs, laboratory consumables and PPE.
- Analytical stage which involves the types of test available whether they are molecular/nucleic acid based or antibody based techniques or point of care immunodiagnosics which have various challenges in the procedures and finally in the reporting of the tests
 - Accuracy and reliability of the tests must be ensured.
- Post-analytical stage which involves the Interpretation of results-Molecular, Serological before they are sent out to

the various submitter requesting the test eg hospital, laboratory.

The Laboratory Assessment Tools (LAT) implementations

- Purpose-designed to assess capacities of existing labs or new ones to be able to test for the Sars-Cov-2 with the aim of making sure the labs have the facility to implement.
- Target audience: National Health authorities, Multilateral agencies, NGOs and laboratory managers

LAT tools according to the WHO include

1. WHO interim guidance for laboratory testing (updated, 22/3/20)

Tracking of the virus, understanding epidemiology of community transmission spread of the virus and dissemination of information is key to help suppress the outbreak of the disease. The information received as regarding the total number of cases, positive and negative confirmed cases and numbers of deaths all put together in tracking the virus and knowledge of local community transmission are sent to each of the central bodies of Centre for disease control in each country onwards to National bodies. A high number of cases recorded in the initial wave of the pandemic infection with community transmission led to the department of Infection, Prevention and Control been called in to assist in the following ways:

- a. Early recognition of symptoms and disease in controlling the outbreak by encouraging HCWs to have a high index of suspicion; identification of the source of infection including rapid diagnosis, isolation facilities made available and new ones urgently built; contact tracing and stringent infection control measures; and source control using the visual and respiratory triage of passengers arriving at point of entry, thermal check for passengers in suspected cases and immediate referral to designated places for management.¹⁹ Post signage is also put in place to remind symptomatic patients and alert HCWs.
- b. Application of Standard Precautions (consistent use of recommended PPE, Hand hygiene with soap and alcohol based disinfectant, respiratory

hygiene or cough etiquette as important preventive measures.

c. Implementation of additional precautions: Fit tested particulate respirator (N95). The "N" means "Not resistant to oil" and it has a high filtration efficiency. The N95 can be safely decontaminated without undermining functional integrity only two or three times, a government study showed.²⁰ The Powered Air-Purifying Respirator (PAPR) is a type of respirator, loose fitting face piece used to safeguard workers against contaminated air. It provides 150% more protection than an N95 mask. Also has an advantage of reduced heart, lung and heat stress.

d. Administrative control of procedures, policies, principles including putting up an IPC infrastructure and building of quarantine facilities if not available, regular training of dedicated staff, Biosafety issues all put together to safeguard environment and human population

e. Environmental and engineering controls which involves ensuring environmental ventilation and cleaning with disinfection procedures being carried out. Physical separation of at least (6ft) 1m distance was to be maintained between each suspected cases with persons wearing facemask or cloth face covering.

General guidelines for collection and handling of CoV specimen

Every specimen is considered to be potentially infectious and must avoid any form of contamination. The aim is to try and minimise risks to health care workers including the laboratory staff by strict adherence to standard protocols.

- GOOD COMMUNICATION by way of information between the attending physician and the laboratory staff involved must take place either by direct communication or by electronic means.
- PROPER COLLECTION OF SPECIMEN using the appropriate PPE under aerosol generating procedure; single polyester or synthetic fiber swabs with plastic or wire shafts to collect the sample; calcium alginate swabs or swabs with wooden shaft should not be used as it may inhibit

the PCR testing. The sterile swabs for upper respiratory specimen collection can be packaged in 2 ways: individually wrapped which is the preferred one and bulk packaged.²¹

- PACKAGING of specimen requires a basic triple packaging system which involves primary receptacle (watertight leak-proof receptacle with enough absorbent material), secondary packaging (enclose and protect the primary receptacle with enough material to absorb all liquid in case of breakage) and outer packaging (secondary packaging is placed in an outer shipping packaging with suitable cushioning material. This protects contents from outside influences, such as physical damaging while in transit).²² The sample is placed in the recognised pressurised, leak-proof specimen plastic biohazard bag that have a separate sealable pocket for the specimen to be put into a tube containing 3 ml of the Universal Transport Medium (UTM) or Viral Transport Medium (VTM). Patients complete biodata with a clearly written laboratory request form are then delivered by hand whenever possible.
- TRANSPORT SHIPMENT of specimen must follow the International Air Transport Association ((IATA, updated, 19/3/2020) of using dry ice. The purpose of the document explains process required for shipment of the specimen by Category B for infectious substances with a consigned shipped number of UN 3373. The Sars-CoV-2 transport system falls into the classification requirements for Category B which is defined as an infectious substance carried in a form that when exposure occurs it is not capable of causing permanent disability, life threatening or fatal disease in an otherwise healthy humans or animals The maximum quantity for a package containing Category B infectious substances is 4L (liquids) or 4kg (solids). Laboratory booking form must be filled

including the email to courier plus shipping laboratory invoice, export and import permit

- STORAGE of samples at 2-8°C for ≤ 48 hours for testing and if it is going to be delayed further, samples should be stored at -70°C.
- SPECIMEN MUST BE CORRECTLY LABELLED WITH DIAGNOSTIC REQUEST FORM. Information is sent through each country's Health Electronic Surveillance Network (HESN). The request form must be properly filled with the medical record number (MRN)/ patient ID, CDC number, clinical history and contact phone number for sharing of results, Date of specimen collection, time and type of specimen collected.

2. WHO interim guidance on bio safety and testing with the purpose of guiding HCW. The document is produced for the Strategic Use of Diagnostic Testing in Outbreak Control. Samples for diagnostic testing with indeterminate results are taken to the National Health Lab (NHL)

3. WHO reference laboratories are those that have been recognised as confirmatory laboratory tests after the first 5 positives and first 10 negatives COVID 19 samples has been validated. This usually applies to those laboratories with no testing capacity and those with limited experience in Sars-CoV-2 testing.

4. Specimen types and Priority

- A. Upper respiratory tract specimen
Nasopharyngeal/oropharyngeal specimen; nasopharyngeal wash/aspirate; Deep nasal swab/nasal mid-turbinate (NMT).
- B. Lower respiratory tract specimen which provides a better yield of detection of the virus.
Sputum (if produced) into a collection cup or sterile dry container; Bronchoalveolar lavage, endotracheal aspirate, pleural fluid, lung biopsy (severe disease) collecting 2-3ml into a sterile, leak proof screw cap. .
- C. Additional clinical specimens including Blood (serum/plasma); Stool (duration,

frequency and shedding of the virus are unknown); Urine

D. Tissue specimen (autopsy material)-lung tissue.

Most reliable specimen used for the RT-PCR test performed were from the nasopharyngeal swabs or other upper respiratory tract specimens, including throat swab or more recently saliva.²³

A study of 205 patients with confirmed COVID-19 infection, RT-PCR positivity was reported to be highest in bronchoalveolar lavage (93%), followed by sputum (72%), nasal swabs (63%) and pharyngeal swabs (32%).²⁴

5. Bio safety Guidelines for handling samples

- Biological risk assessment of the specimen must be taken into cognisance, including identification (acceptable/ unacceptable if it is in the correct plastic biohazard bag and in the approved viral transport medium), probability of occurrence and severity of adverse effect on human health and environment.
 - Laboratory bio security risk (unauthorised access, loss, theft of reagents, diversion/intentional release of results must be well guarded)
 - Risk assessment tools include pathogenicity, virulence and infectious dose, availability of prophylaxis, communicability and stability in the environment.
 - Laboratory PPE and materials available for decontamination of work surfaces and equipment.
 - Testing for the Sars-CoV-2 is limited to qualified laboratory staff and use of the Class II bio safety cabinets of Bio Safety Level (BSL-3) is recommended.

Laboratory diagnosis of Sars-CoV-2

- 1) Detection of SARS-CoV-2 is Nucleic acid based (Nucleic Acid Amplification Test) using the real time Reverse Transcriptase Polymerase Chain reaction (rRT-PCR). This helps to detect current infection with the Sars-CoV-2 genome. The targeted gene's on SARS-CoV-2 are Envelope (E), Nucleocapsid (NC), Spike (S),

ORF and RdRp (RNA dependent RNA polymerase, a charite primer/probe which has a slightly lower sensitivity may be due to a mismatch in the reverse primer).²⁵ The three most common mutations are seen in the ORF1, ORF8 and NC gene's.²⁶

b) Isothermal nucleic acid amplification assays (updated 27th March, 2020). c) GeneXpert, self enclosed system integrating nucleic acid extraction, amplification and detection when available can also be useful.

-Serum (rRT-PCR)-detection of virus and not antibody.

2. Immunological tests:

Serological antibody IgG and IgM testing by ELISA technique. Result can be received within less than an hour; but the result does not inform one of previous infection or present infection as the lag time of infectivity takes about 1-3 weeks before antibodies are formed. Also antibodies cross reactivity occurs with presence of Sars CoV and other related viruses. Hence, for proper Diagnostic accuracy, testing of paired samples with initial PCR and 2weeks later with ELISA may be done.

3. Point-Of-Care Immunodiagnostic assay is a Rapid test kit that is used after it has been validated. It has a small plastic cartridge which acts by the lateral flow mechanism. Provides the advantage of fast time to get results and low cost detection of SARS-CoV-2 provisional diagnosis; but they suffer from poor sensitivity based. Monoclonal antibodies and rapid antigen testing given the variability of viral loads, antigen detection may miss cases due to low infectious burden or sampling variability. Disadvantages of POC include

*Specimen must be collected when the virus is replicating

* It does not give the information of whether the person has been recently infected or not and this may lead to

*Misinterpretations of results and IgM responses are non specific

*Do not detect SARS-CoV-2 itself

Other adjunct COVID 19 tests

4. Viral isolation and culture is NOT ATTEMPTED with indirect fluorescent as debate is still ongoing regarding manipulation of the virus.

Fresh samples
↓ inoculated
Vero-E6 cells (culture)
↓
Cytopathic effect (96hrs)
↓
SARS-CoV-2 NA

5. Viral detection of RSV, influenza: does not rule out SARS-CoV-2

6. Detection of indicators of inflammation related biomarkers.

- CRP-2° infection
- Procalcitonin-normal
- Ferritin, D-dimer-poor prognosis
- Lymphopenia-poor prognosis
- IL-4,6,10, TNF- α ,IFN γ increased levels progress to cytokine release syndrome acute respiratory disease syndrome.

7. Chemistry- LFT \uparrow : commonly typical of community acquired pneumonia, increase LDH, serum proteins

8. Flow cytometry: CD4+ and CD8+

Safety measures in the laboratory for PCR specimen

- Class II BSL 3 cabinet work procedure. There are 4 biological safety levels and BSL 3 is the one recommended by the WHO/CDC. This is applicable for production facilities where work is performed with agents that may cause serious or potentially lethal disease through inhalation to the personnel and may contaminate the environment.
- Negative air pressure room is used to contain airborne contaminants within a room preventing cross contamination, designed to help prevent the spread of disease to another.²⁷ It requires a minimum of 12 air changes of exhaust per hour and a required maintenance of minimum 0.01inch in water column negative pressure differential to the adjacent door. Doors should be self-

closing and the room should be "well-sealed" including sealing of the ceiling and gaskets around items which enter into the room e.g. electric sockets, gas supplies, etc. Temperature control should be within the room and there should be 1/2 " gap under the door. Exhaust ducts are labelled "Caution - negative pressure isolation room. Permanent room pressure monitor provides instant notification if the pressurisation fails or fluctuates. Monitors need to be able to accurately and reliably measure a negative pressure of -0.001 WC. The Alarm will sound when room pressurisation drifts to less than the monitors reference pressure value; Negative pressure value should be at least 0.006 WC

- Transport medium
- Universal Transport Medium (UTM) tube are used as liquid for the swabs/specimen. According to WHO/CDC the Eagle minimum essential medium should be used.

Components of the VTM

- ✓ Prepared locally,
- Add 10g veal infusion broth and 2g bovine albumin fraction V to Sterile distilled water 400ml.
- Add 0.8 ml Gentamicin-sulfate solution (50mg/ml) and 3.2ml Amphotericin B(250ug/ml)
- Sterilise by filtration.

Document of Centre Disease Control Prevention (CDCP) on preparation of VTM

- Reagents required for VTM (aseptic technique & sterility maintained) are:
- Hanks Balanced Salt Solution (HBSS) 1x with Ca and Mg ions, no phenol red, 500ml bottle
- Sterile, heat inactivate fetal bovine serum (FBS)
- Gentamicin sulfate (500mg/ml)
- Amphotericin B 250ug/ml (Fungizone)
- Sheep blood agar plate or equivalent QC plate

- Sterilise by filtration and Disinfectant (70% ethanol)
- Record Lot Information In Laboratory Controlled Book
- Assign Laboratory Appropriate Id Eg Lot No

Components of materials present in kit for the PCR testing

.Proteinase solution (PASE)

.RNA Internal control (RNA IC) to monitor entire sample preparation and amplification process. If the IC is not positive then the whole test procedure is repeated.

.Master Mix Reagent (MMR) 1 and 2, a 2x concentrated solution of DNA polymerase, dNTP and all other components which reduce contamination arising from pipetting steps required for PCR to prevent preparation error.

.SARS-CoV-2 Positive control.

.Buffer Negative control.

.Target 1- SARS-CoV-2

. Target 2- pan SarbeCoV- HCoV,229E, NL63, OC43, HKU1

Result interpretation for RT-PCR

- In information flows, electronic requesting and reporting should be the accepted standard; indicators required are number of cases reported, patients tested, patients who tested positive, number of tested suspected cases per 100,000 population.
- If the target gene is positive and the IC is negative, report target gene as positive.
- Inconclusive/indeterminate result are sent to NHL
- Repeat test if negative if there is a high level of suspicion.
- Single negative test does not rule out the infection
- Samples with high cut off value rule out contamination
- Negative results are to be interpreted in correlation with clinical findings, history and diagnostic procedures.
- Positive results indicate infection with SARS-CoV-2 and co-infection with other viruses may not be ruled out.

- Positive results reflect only the detection of viral RNA and does not necessarily indicate the presence of a viable virus.²⁸ Presumptive positive results should be reported back to the testing laboratory/clinician for patient sampling.
- PCR positivity declines more slowly in sputum and may still be positive after nasopharyngeal swabs are negative.
- Valid-Batch is valid if no flags appear for any controls.
- Invalid- If the batch is invalid repeat testing of the entire batch.
- Validation of results is performed automatically by the cobas software based on neg and pos control performance.

Result interpretation for serological test

- COVID-19 disease can be detected indirectly by measuring host immune response to SARS-CoV-2 infection since exposure and post symptoms onset
- Serologic-Depends on when infected & Timing
Positive: Protective step is taken towards management; but it does not tell if due to past or present infection. False labelling may occur.
- Negative result shows that the patient is not infected at that time
It does not rule out Sars-Cov-2 infection
Follow up testing with molecular diagnostic test
Ab test should not used as a sole basis to diagnose.
- Serological diagnosis may be an important tool to understand the extent of COVID-19 in the community and identify individuals who are immune that may result in herd immunity.

Limitations

- ✓ Cobas SARS-CoV-2 CAN ONLY BE USED WITH CONTROL

- ✓ SAMPLE COLLECTION, STORAGE & HANDLING>RESULTS
- ✓ USED ONLY FOR NP/OP SWABS.
- ✓ ALSO AFFECTED BY PATIENTS FACTOR-SYMPTOMS, INFECTION
- ✓ MUTATIONS IN TARGET REGION CAN AFFECT PRIMER
- ✓ FALSE NEGATIVE/INVALID RESULTS >INTERFERENC (IC)

Drugs proposed²⁹

- Avigan (favipiravir)
- Hydroxychloroquine
- Remdesiver

*No vaccine-immunity passport/risk free certificate

WHO Recommendations

- ✓ Give attention to the diagnosis
- ✓ Preventive measures (PPE, Face masks N95, FFP2, Hand hygiene, respiratory etiquette etc)
- ✓ Environmental cleaning and disinfection
- ✓ Physical distances (2m)
- ✓ Government: promoting, amending laws, Staff training
- ✓ Quarantine facilities
- ✓ Scientists, medical workers and pharmaceutical organizations: vaccine
- ✓ Timely disease surveillance

Conclusions

- COVID 19/SARS-CoV-2 is positive ssRNA genome
- Transmission is by droplets/contact
- Challenges in specimen pre to post analytic stage.
- WHO guidelines as category B transportation.
- Types of specimens.
- Main diagnostic test is Molecular testing.
- Rapid immunosassays is fast/low cost but suffer from poor sensitivity based
- No vaccines
- Monoclonal Abs are under preparation

Sources of information for the laboratory diagnosis

- CDC guidelines: <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>
- World Health Organization. Infection prevention and control of epidemic- and pandemic-prone acute respiratory infections in health care. Geneva: World Health Organization; 2014. available from: <https://apps.who.int/iris/bitstream/handle/10665/1sequence=1>
- Nigeria Centre for Disease Control: National Strategy to Scale up Access To Coronavirus Disease Testing In Nigeria. info@ncdc.gov.ng
- www.facebook.com/nigeria.ncdcgov
- <https://www.ncdc.gov.ng>
- Saudi centre for Disease Prevention and control and Laboratory diagnosis of COVID 19 in suspected Cases.

References

1. Zhong NS, Zheng BJ, Li YM, Xie ZH, Chan KH et al. Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in Feb 2003. *Lancet* 2003;362:1353-1358.
2. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003;348: 1953-1966.
3. Ren LL, Wang YM, Wu ZQ, Xiang ZC, Guo L, Xu T et al. Identification of a novel coronavirus causing severe pneumonia in human: a descriptive study. *Chin Med J* 2020;133:1015-1024.
4. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;395:497-506.
5. Chan JF, Yuan S, Kok KH, To KKW, Chu H, Yang J et al. A familiar cluster of pneumonia associated with 2019 novel coronavirus indicating person-to-person transmission was reported in Lancet. *Lancet* 2020;395:514-523.
6. World Health organisation. Modes of transmission of virus causing COVID-19: implications for IPC precaution recommendations. 2020.
7. Li Q, Guan X, Wu P et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med* 2020; 382:1199-1207.
8. Xiao F, Sun J, Xu Y, Li F, Huang X, Li H et al. Infectious SARS-CoV-2 in feces of patient with severe COVID-19. *Emerg Infect Dis* 2020; 26(8). <https://doi.org/10.3201/eid2608.200681>
9. Zhu N, Zhang D, Wang W et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;382:727-733.
10. Lu R, Zhao X, Li J et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020;395: 565-574.
11. Yan R, Zhang Y, Li Y et al. Structural basis for the recognition of the SARS-CoV-2 by full-length human ACE2. *Science* 2020;367:1444-1448.
12. Chen Y, Guo Y, Pan Y et al. Structural analysis of the receptor binding of 2019-nCov. *Biochem Biophys Res Commun* 2020;525:135-140.
13. Hoffmann M, Kleine-Weber H, Schroeder S et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020; 181:271-280.
14. Hanff TC, Harhay MO, Brown TS et al. Is there an association between COVID-19 and the rennin angiotensin system: a call for epidemiologic investigations. *Clin Infect Dis* 2020 Mar 26[Epub ahead of print].
15. WHO: COVID-19 disease severity. World Health Organisation. <https://www.who.int/publications-detail/clinical-management-of-covid-19>

16. Xu K, Chen Y, Yuan J et al. Factors assessed with prolonged viral RNA shedding in patients with COVID-19. *Clin Infect Dis* 2020 April 9 [Epub ahead of print]
17. Widders A, Broom A, Broom J. SARS-CoV-2: the viral shedding vs infectivity dilemma. *Infect Dis Health* 2020 May 20 [Epub ahead of print]
18. Tang YW, Schmitz JW, Persing DH, Stratton CW. Laboratory diagnosis of covid 19: Current issues and challenges. *J Clin Microbiol* 3rd April, 2020. Open access article.
19. Harbi ISA, Gupta SK. Use of visual triage in the early identification and isolation of acute respiratory infection cases for the control of Hospital outbreak/ infection in reference to Middle East Respiratory syndrome-coronavirus (MERS CoV). *International J of Med and Public Health*. 2019;9:8-12.
20. <https://www.medpagetoday.com>
21. Centers for Disease Control and Prevention. Coronavirus Disease 2019; Specimen collection, [cdc.gov](https://www.cdc.gov)
22. Transport of infectious substances: A guidance document produced by the Department for transport the Civil Aviation Authority and the maritime and Coastguard Agency. Revision 3: November 2006
23. Nandini S, Jeremiah SS, Ryo A;. Interpreting diagnostic tests for SARS-CoV-2. *JAMA* 2020; 323:2249-2251
24. Wang W, Xu Y, Gao R et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA* 2020;323:1843-1844.
25. Nalla AK, Casto AM, Huang MW et al. Comparative performance of SARS-CoV-2 detection assay using seven different primer/probe sets and one assay kit. *J Clin Microbiol* 2020. doi.10.1128/JCM.00557-20.
26. Khailai RA, Safdar M, Ozaslan M. Genomic characterization of a novel SARS-CoV-2. *Science direct*. <https://doi.org/10.1016/j.genrep.2020.100682>.
27. <https://www.ash.org/compliance>.
28. Wolfe R, Corman VM, Guggemos W et al. Virological assessment of hospitalized patients with COVID-19. *Nature* 2020;581: 465-469.
29. [https://www.who.int/internal-publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-\(ncov\)-infection-is-suspected](https://www.who.int/internal-publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected)