International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Laboratory Examination SARS Cov2

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Abstract: ***Corona virus disease 2019 (COVID 19) is a novel disease which caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS Cov2). The virus morphology is a spherical virus with envelope (outer layer), membrane, and nucleocapsid. It also has protrusion on the surface called spike. It will make a bond with receptor Angiotensin Converting Enzyme 2 (ACE2) in surface of the host cells. The genetic materials of SARS Covid 19 is single stranded ribonucleic acid which has positive sense characteristic. It consists of many open reading frame (ORF) which contains envelope gen (E), membrane (M), spike (S), nucleocapsid (N), RNA - dependant RNA polymerase (RDRP), and Helicase (Hel). The gold standard of laboratory detection of covid 19 is genetic material detection. Two specific genes target is required to make diagnosis of SARS Cov2. The most specific genes are gene RdRP and gene N for amplification target. Detection using of antigen and antibody of SARS Cov2 needs adequate knowledge about disease timeline and serology marker of SARS Cov2. Antigen testing needs adequate viral load to produce reactive result. Antibody testing needs ± 10 - 14 days after emerging of symptoms to produce reactive result.***

Keywords: SARS Cov2, laboratory test, genetic materials, antigen, antibody

1. Introduction

Coronavirus is a positive - sense single - stranded RNA virus (virus +ssRNA) with genome length 26 - 32Kb. (Lu et al.2020) SARS CoV - 2 is virus RNA withthe mantle. It has astructure like a crownwith electron microscope. Because of this structure, it is called coronavirus. (Brian and Baric 2005)

Corona virus causes infective disease Coronavirus 2019 (COVID - 19) which is spread all over the world and became the health global crisis. The first emergence reported in Wuhan, China in November 2019. This outbreak is the third coronavirus occurrence in last 20 years. The first is Severe Acute Respiratory Syndrome (SARS) in year 2002 - 2003, after that Middle - East Respiratory Syndrome (MERS) 2012. (Shah et al.2020)

The Data fromSatuan Tugas Penanganan COVID - 19 (Corona disaster management) in Indonesia until1 September 2021, positive confirmed case COVID - 19 is 4.100.138 cases, with death report133.676 cases. (Indonesian Gov.2021)

The PathogenesisSARS COV 2 enter from respiratory tract epithelial cell or conjunctiva. The average incubation period is5 - 6 days, with length 1 - 14 days. From the epithelial cilial cell, goblet cell, and then binding with Angiotensin Converting Enzyme 2 Receptor (ACE2). Virus replicates and the clinical manifestation starts. (Kowalik et al.2020) (Rahman et al.2021) Symptoms that arise like fever, myalgia, weakness, and gastrointestinal disease. Some cases will infect lower respiratory tract, which causes sepsis, lung oedema, sepsis Acute Respiratory Distress Syndrome (ARDS), haematology, neurology, cardiovascular, dan renal complication. (Rahman et al.2021)

Virion SARS COV 2

Virion SARS COV 2 classified as β coronavirus. It has round spherical shape with two layers. The outer layer is envelope. The second are the membrane and nucleocapsid. There is a spike in outside layer which can bound with angiotensin converting enzyme 2 (ACE2). The host has this receptor, which makes the virus possible to make penetration. The genetic materials of SARS Covid 19 is single stranded ribonucleic acid which has positive sense characteristic. It consists of many open reading frame (ORF) which contains envelope gen (E), membrane (M), spike (S), nucleocapsid (N), RNA - dependant RNA polymerase (RDRP), and Helicase (Hel). (Kubina & Dziedzic, 2020)

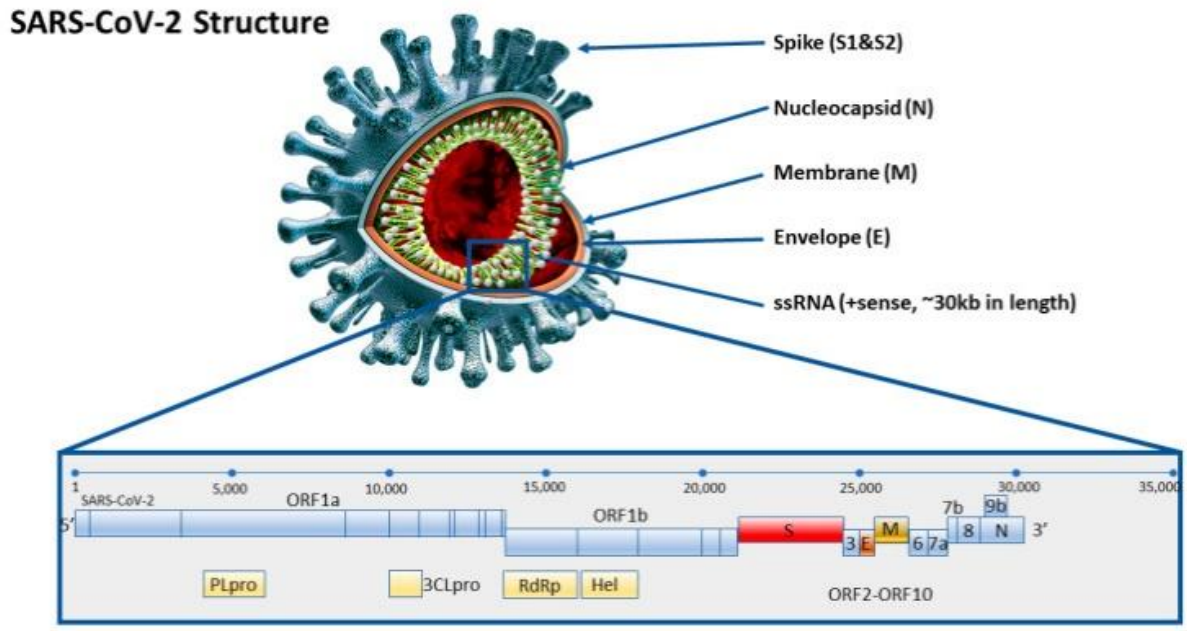

Figure 1: SARS Cov2 Structure. (Kubina & Dziedzic, 2020)

Laboratory examinations for SARS COV 2
Until now the choice for detecting SARS COV 2 are genetics materials, antigen detection, or antibody detection.

Genetic materials detection
Genetic material is the gold standard for diagnosis of SARS COV 2. The general technique for SARS COV 2 is the amplification using reverse transcription process. The most used technique is real time Reverse Transcriptase Polymerase Chain Reaction (rt - RT - PCR). International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) suggests using RdRP gen and N gen for amplification target, because it is the most specific genetic materials, moreover gene E is for filtering diagnosis. (Adeli & Bohn, 2021)

The polymerase chain reaction (PCR) is a technique based on the exponential amplification of nucleic acids by the thermostable Thermus aquaticus (Taq) polymerase.
The PCR process most typically consists of three steps:
1) A denaturation step at 94 or 95C,
2) Primer annealing to the single stranded DNA (ssDNA) strands at 55 - 65C
3) A primer extension at 72C

By repeating these steps for a number of times, usually 30 to 40 cycles, the resulting DNA target sequence will be amplified exponentially. Based on this simple principle, a classical PCR amplification reaction will consist of three phases:

1) Exponential amplification: At every cycle, the amount of product is doubled (assuming 100% reaction efficiency). The reaction is very sensitive and specific.
2) Linear phase (levelling off): The reaction slows down, due to decreasing activity of the Taq DNA polymerase and the consumption of reagents such as dNTPs and primers.
3) Plateau phase: The reaction has ended, and no more nucleic acids are synthesized due to the exhaustion of reagents and the Taq DNA polymerase. (Patrinos et al.2017)

Moreover, World Health Organization suggests 2 gen targets for diagnosis. (WHO, 2020)

There are many things can influence the result of the test. In analytic steps target gene differences, target primer usage, and also mutation in target gene. It can cause differences in laboratory examination result. Moreover, preanalytical phase also has influences the result. Contamination, impure inhibition substance, delivery, cold chain system, and gathering specimen timing. (Lippi, Simundic, and Plebani 2020)

There are the examples of gene target and primer differences in Table 1. (Arena et al.2021)

Source	Primer/Probe Name	Target Gene	Sequence	Lenght	Genomic Region *
China CDC, China	Forward (F)	ORF1ab	CCCTGTGGGTTTTACACTTAA	21	13,342–13,362
China CDC, China	Reverse (R)	ORF1ab	ACGATTGTGCATCAGCTGA	19	13,442–13,460
China CDC, China	Fluorescence probe (P)	ORF1ab	CCGTCTGCGGTATGTGGAAAGGTTATGG	28	13,377–13,404
China CDC, China	Forward (F)	N	GGGGAACTTCTCCTGCTAGAAT	22	28,881–28,902
China CDC, China	Reverse (R)	N	CAGACATTTTGCTCTCAAGCTG	22	28,958–28,979
China CDC, China	Fluorescence probe (P)	N	TTGCTGCTGCTTGACAGATT	20	28,934–28,953
Institut Pasteur, France	nCoV_IP2- 12669Fw	RdRp	ATGAGCTTAGTCCTGTTG	18	12,690–12,707
Institut Pasteur, France	nCoV_IP2- 12759Rv	RdRp	CTCCCTTTGTTGTGTTGT	18	12,780–12,797
Institut Pasteur, France	nCoV_IP2- 12696bProbe(+)	RdRp	ATGTCTTGTGCTGCCGGTA	19	12,719–12,737
Institut Pasteur, France	nCoV_IP4- 14059Fw	RdRp	GGTAACTGGTATGATTTCG	19	14,080–14,098
Institut Pasteur, France	nCoV_IP4- 14146Rv	RdRp	CTGGTCAAGGTTAATATAGG	20	14,167–14,186
Institut Pasteur, France	nCoV_IP4- 14084Probe(+)	RdRp	TCATACAAACCACGCCAGG	19	14,105–14,123
Institut Pasteur, France	E_Sarbeco_F1	E	ACAGGTACGTTAATAGTTAATAGCGT	26	26,269–26,294
Institut Pasteur, France	E_Sarbeco_R2	E	ATATTGCAGCAGTACGCACACA	22	26,360–26,381
Institut Pasteur, France	E_Sarbeco_P1	E	ACACTAGCCATCCTTACTGCGCTTCG	26	26,332–26,357
US CDC, USA	2019-nCoV_N1-F	ORF9b	GACCCCAAAATCAGCGAAAT	20	28,287–28,306
US CDC, USA	2019-nCoV_N1-R	ORF9b	TCTGGTTACTGCCAGTTGAATCTG	24	28,335–28,358
US CDC, USA	2019-nCoV_N1-P	ORF9b	ACCCCGCATTACGTTTGGTGGACC	24	28,309–28,332
US CDC, USA	2019-nCoV_N2-F	ORF9b	TTACAAACATTGGCCGCAAA	20	29,164–29,183
US CDC, USA	2019-nCoV_N2-R	ORF9b	GCGCGACATTCCGAAGAA	18	29,213–29,230
US CDC, USA	2019-nCoV_N2-P	ORF9b	ACAATTTGCCCCCAGCGCTTCAG	23	29,188–29,210
US CDC, USA	2019-nCoV_N3-F	ORF9b	GGGAGCCTTGAATACACCAAAA	22	28,681–28,702
US CDC, USA	2019-nCoV_N3-R	ORF9b	TGTAGCACGATTGCAGCATTG	21	28,732–28,752
US CDC, USA	2019-nCoV_N3-P	ORF9b	ATCACATTGGCACCCGCAATCCTG	24	28,704–28,727
National Institute of Infectious Diseases, Japan	NIID_2019-nCOV_N_F2	N	AAATTTTGGGGACCAGGAAC	20	29,142–29,161
National Institute of Infectious Diseases, Japan	NIID_2019-nCOV_N_R2	N	TGGCAGCTGTGTAGGTCAAC	20	29,280–29,299
National Institute of Infectious Diseases, Japan	NIID_2019-nCOV_N_P2	N	ATGTCGCGCATTGGCATGGA	20	29,239–29,258
Charité,Germany	RdRP_SARSr-F2	RdRp	GTGAAATGGTCATGTGTGGCGG	22	15,431–15,452
Charité,Germany	RdRP_SARSr-R1	RdRp	CAAATGTTAAAAACACTATTAGCATA	26	15,505–15,530
Charité,Germany	RdRP_SARSr-P2	RdRp	CAGGTGGAACCTCATCAGGAGATGC	25	15,470–15,494
Charité,Germany	E_Sarbeco_F1	E	ACAGGTACGTTAATAGTTAATAGCGT	26	26,269–26,294
Charité,Germany	E_Sarbeco_R2	E	ATATTGCAGCAGTACGCACACA	22	26,360–26,381
Charité,Germany	E_Sarbeco_P1	E	ACACTAGCCATCCTTACTGCGCTTCG	26	26,332–26,357
HKU,HongKongSAR	HKU-ORF1b-nsp14F	ORF1b	TGGGGTTTTACAGGTAACCT	20	18,778–18,797
HKU,HongKongSAR	HKU-ORF1b-nsp14R	ORF1b	AACACGCTTAACAAAGCACTC	21	18,889–18,909
HKU,HongKongSAR	HKU-ORF1b-nsp141P	ORF1b	TAGTTGTGATGCAATCATGACTAG	24	18,849–18,872
HKU,HongKongSAR	HKU-NF	N	TAATCAGACAAGGAACTGATTA	22	29,145–29,166
HKU,HongKongSAR	HKU-NR	N	CGAAGGTGTGACTTCCATG	19	29,236–29,254
HKU,HongKongSAR	HKU-NP	N	GCAAATTGTGCAATTTGCGG	20	29,177–29,196
NationalInstituteofHealth,Thailand	WH-NICN-F	ORF9b	CGTTTGGTGGACCCTCAGAT	20	28,320–28,339
NationalInstituteofHealth,Thailand	WH-NICN-R	ORF9b	CCCCACTGCGTTCTCCATT	19	28,358–28,376
NationalInstituteofHealth,Thailand	WH-NICN-P	ORF9b	CAACTGGCAGTAACCA	16	28,341–28,356

*Site numbering uses Wuhan - Hu - 1/2019 as reference (access. MN908947.3).

Antigen SARS COV2 Detection

Generally, antigen SARS Cov2 examined with immunochromatography method. This method requires shorter duration rather than other methods. World Health Organization (WHO) states antigen SARS COV2 used must have sensitivity ≥80% and specificity ≥97% than rt - RT - PCR. And also Antigen can be detected for specimen which has result of rt - RT - PCR with cycle threshold (Ct) ≤25 or number of genomes $\geq 10^6$ copies/millimetre. (WHO 2021)

Cochrane database for systematic reviews review that antigen tests do not appear to perform as well in asymptomatic populations compared to symptomatic populations for detecting infection. It means some antigen tests are accurate enough to replace RT - PCR when used in people with symptoms. This would be most useful when quick decisions are needed about patient care, or if RT - PCR is not available. Antigen tests may be most useful to identify outbreaks, or to select people with symptoms for further testing with PCR, allowing self - isolation or contact tracing and reducing the burden on laboratory services. (Dinnes et al.2020)

Antibody SARS COV2 Detection

Generally, there is two formats available (immunochromatography and enzyme immunoassay) for antibody SARS COV2 Detection. However, the weakness of antibody test is the timing for detection is too slow when symptoms appear rather than genetic materials or antigen detection. Antibody can be detected in blood for 10 - 14 days after symptoms appear. (Lippi, Horvath, and Adeli 2020)

Antibody detection after vaccination still in research. The result after vaccinated is appearance of antibody which is capable of neutralisation of antigen and prevents the infection. Neutralization antibody detected with Plaque reduction neutralization test (PNRT) which involved live virus. And there is an alternative using surrogate virus neutralization test (sVNT) which is using replacement molecule of virus. Tests are simply measuring qualitatively or semi - quantitatively the presence of IgG, IgM and/or IgA but do not address the functionality of the antibody response elicited by a SARS - CoV - 2 infection. Functional assays like virus neutralization tests are essential to address specific questions related to protective immunity after vaccination or natural infection. However, these type of assays are operated based on inhouse protocols and lack standardization across laboratories. (Meyer et al.2020)

Usage of genetic material, antigen, and antibody laboratory test

Genetic material detection is indicated for establishing diagnosis for acute patient with symptoms (0 - 14 days), clinical examination for asymptomatic patient, mild

symptoms with exposure history in SARS Cov 2, and also person with special purpose for examples: surgery, travel, hospital discharge, and back to work. Antigen test can be used for alternative from genetic materials test if accomplish the sensitivity and specificity requirements in case the genetic materials test cannot be available in short time period. (Lippi, Horvath, et al.2020)

For diagnostic purpose World Health Organization recommends Diagnostic flow diagram for the detection of acute SARS - CoV - 2 infection in individuals with clinical suspicion for COVID - 19 (Figure 1). (WHO 2021)

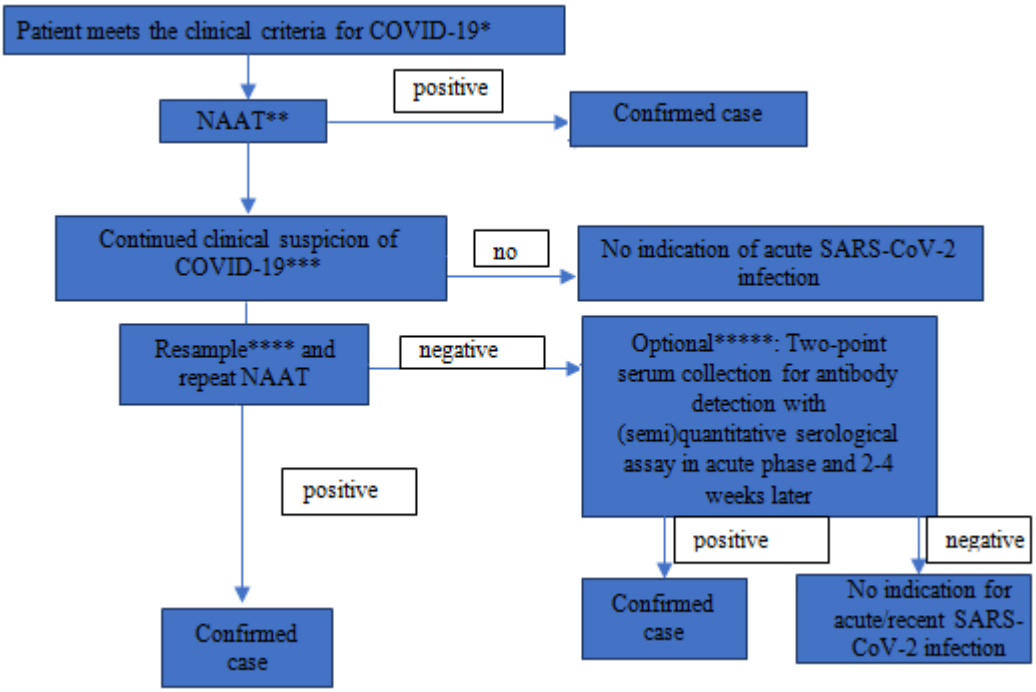

Figure 1: Diagnostic flow diagram for the detection of acute SARS - CoV - 2 infection

*Clinical management of COVID - 19 (Interim Guidance), World Health Organization.

** If antigen detection would be incorporated into the testing algorithm, how this needs to be done depends on the sensitivity and specificity of the antigen test and on the prevalence of SARS - CoV - 2 infection in the intended testing population. For more information see section below on "Rapid diagnostic tests based on antigen detection" and the specific guidance Interim guidance on antigen - detection in diagnosis of SARS - CoV - 2 infection using rapid immunoassays.

*** Continued clinical suspicion can, for example, be the absence of another obvious etiology, the presence of an epidemiological link, or suggestive clinical finding (e. g. typical radiological signs).

**** The selection of specimen type will depend on the clinical presentation, see section "Specimens to be collected". Increasing the number of samples tested will also increase the sensitivity of testing for COVID - 19. More than two samples might be needed on some occasions to detect SARS - CoV - 2.

***** For interpretation of serology, see section "Implementation and interpretation of antibody testing in the clinical laboratory". Serology cannot be used as a standalone diagnostic for acute SARS - CoV - 2 infections and clinical management.

The clinical laboratory testing SARS Cov2 based on symptoms timeline (Figure2) (Lippi, Horvath, et al.2020)

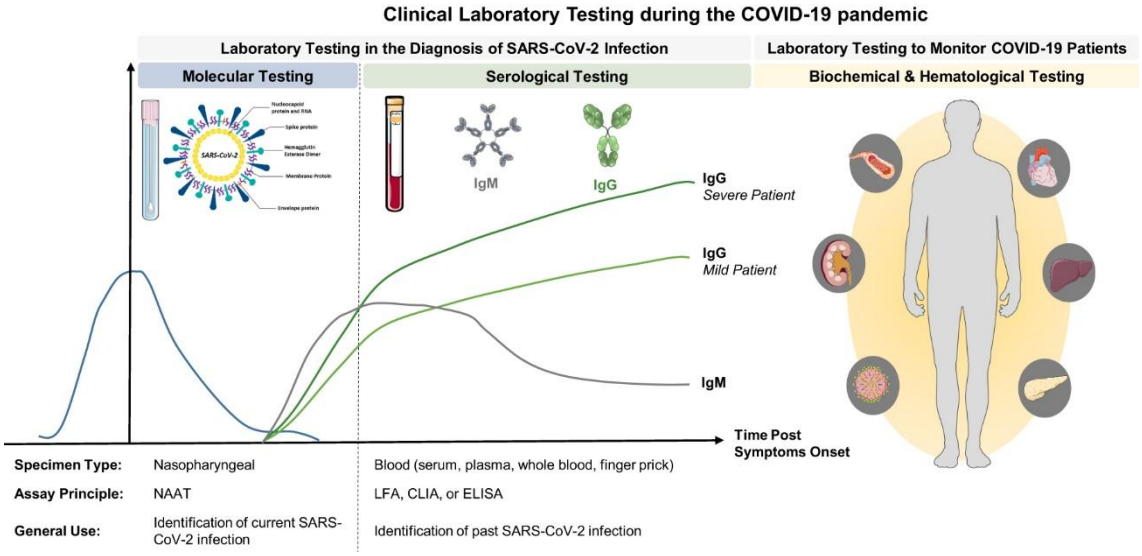

Figure 2: Overview of the role and types of clinical laboratory testing during the COVID - 19 pandemic. (Lippi, Horvath, et al.2020)

2. Conclusion

Corona virus disease 2019 is a new disease which caused by virus SARS Cov2 which is variant of corona virus. Diagnosis needs many variations of laboratory examinations. The gold standard is detection of genetics materials. Detection using of antigen and antibody of SARS Cov2 needs adequate knowledge about disease timeline and serology marker of SARS Cov2. The laboratory examination of SARS Cov2 also influenced by pre analytics and post analytics factors. Antigen tests are accurate enough to replace RT - PCR when used in people with symptoms, but negative antigen test result cannot rule out the possibility of SARS Cov2. Furthermore, there are still many researches needed for understanding the detail of this SARS Cov2 Virus.

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Author Profile

Clinical Pathology enthusiast

Yoelius Wijaya S is Medical Doctor graduated from University of Trisakti, Jakarta. Working in Bungsu Hospital Bandung. Laboratory examination has the critical role in medical diagnosis. It manages the timing of the disease. Also, the detail of drug treatment of some diseases. Laboratory examination can recognize in detail the course of the diseases. The clinician has many benefits from it. Detecting the disease early, prompt therapy, and giving prognosis.