

## Sampling of Sars-Cov-2 Covid-19 an Air Using the Impingement Method with Real-Time PCR as Detection Examination

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### ABSTRACT

Examination of airborne microbial for SARS-CoV-2, Coronavirus Disease 2019 (Covid-19) has not been widely developed. Whether this virus is airborne is still an unanswered question. This preliminary study was to evaluate the presence of the Covid-19 in the air. The study was conducted on 4 rooms, which are laboratory rooms used for Covid-19 activities. The impingement which is an active method used for air sampling, while the detection was carried out through a molecular approach using RT real-time PCR. The overall Covid-19 virus was not found in the room being examined. Four rooms gave CT values above the positive control, that is administration room (CT 30.44), BSL room (CT 30.23), BSC (CT 30.69 ) and waiting room (CT 35.61). No found SARS-CoV-2 Covid-19 in air using the impingement method with real-time PCR as detection examination in the room being examination. Preliminary study shown SARS-CoV-2 Covid-19 can not transmitted through the air. Research needs to be done with more sampling as continuation of this study.

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## **INTRODUCTION**

Since its first appearance in 2019, SARS-CoV-2, Coronavirus Disease 2019 (COVID-19) remains a topic of discussion today. Responding to the uncontrolled spread of COVID-19, WHO established the status of a global COVID-19 pandemic on March 11 (Guo et al., 2020; WHO, 2020). Globally, confirmed cases of COVID-19 until December 2020 reached 77.133.709 positive cases, deaths 1.698.851 people with Case Fatality Rate (CFR) of 2,2% (WHO, 2020).

In Indonesia, there were 671.778 positive cases, 20.088 people died with CFR of 2,9% so that Indonesia ranks 29th as the country with the highest confirmed positive cases of COVID-19 globally (Ministry of Health of the Republic of Indonesia., 2020 ; Johns Hopkins University and Medicine., 2020). April 13, 2020 Presidential Decree No. 12 of 2020 was issued regarding the Determination of Non-Natural Disasters Spreading regarding Determination of Non-Natural Disasters Spread of Covid19 as a National Disaster. In response to the presidential decision, the Minister of Health Regulation No. 9 of 2020 concerning Large-Scale Social Restrictions (LSSR) issued. LSSR were carried out on the grounds that the spread of COVID-19 could occur from human through droplets on infected people and spread easily (Anies, 2020 ; Gubernur of Jakarta, 2021). Other countries have imposed lock down policies such as China, Italy and Turkey (Daud et al., 2020). The impact of the policy paralyzes almost all segment of people's lives without exception, starting from economy, social, culture, education, politics, religion, tourism, transportation, etc (Nasution, 2020 ; Made et al., 2020).

Airborne transmission is defined as the spread of infectious agents caused by the spread of droplet nuclei (aerosols) while suspended in the air and moving over long distances. Airborne transmission of Covid-19 can occur during medical procedures that generate aerosols. WHO, together with scientist continues to actively discuss and evaluate whether SARS-CoV-2 can also spread by aerosol (Faridi et al., 2020).

Several studies conducted in health care settings where Covid-19 patients were treated, but which did not undergo aerosol-generating procedures, reported the presence of SARS-CoV-2 RNA in air samples, whereas other studies in hospital settings reported the SARS-CoV-2 RNA in air samples; health and non-health care facilities did not find the presence of SARS-CoV-2 RNA , no study found viable virus in air samples (Bullard et al., 2020 ; Durante et al., 2020) .

Based on this problem, we are interested in conducting research on the detection Covid-19 in the air using impingement method. Impinge equipment has been developed to meet the needs of air sampling equipment, is easy to operate and inexpensive. The main equipment of impinge is a modifies air pump that has been selected into a vacuum pump, equipped with a potentiometer as a means of air velocity and an air flow meter a measure of air velocity. The basis of this technique is to trap air particles as air bubbles are passed through a liquid. The air sample is obtained through a suction pump through a narrow inlet tube in to the liquid medium. The sampling in liquid

media was then followed by a real time PCR to detect virus Covid-19 (Bullard et al., 2020 ; Durante et al., 2020).

## **THEORETICAL REVIEW**

The Virus that causes Covid-19, continues to mutate to form new variants. The latest variant that has been detected is the Omicron variant known as B.1.1.529 (Guo et al., 2020). The variant was first reported in South Africa on 24 November 2021 and has now spread worldwide (Kominfo RI 2021 ; Torjesen , 2021). WHO classifies the SARSCoV-2 virus into two categories, namely, variant of interest (VOI) and variant of concern (VOC). Currently WHO has classified the Omicron variant as VOC. This classification is based on the finding of a large number of mutations in this variant and some of them are worrying. Preliminary studies indicate an increased risk of reinfection in this variant compared to other VOC variants. In addition, this variant also shows rapid growth so that will have an epidemiological adverse impact (WHO, 2021).

The expert predict that this Covid-19 pandemic will last a long time. But of course people cannot live in quarantine forever. Thus, a new normal adaptation of new productive and safe habits of Covid-19 is needed for the community (Ghinai et al., 2020)). Thus, the Decree of the Minister of Health of The Republic of Indonesia No. Hk.01.07/Minister of Health/413/2020 concerning Guidelines for Prevention Control of 3 Coronavirus Disease 2019 (Covid-19), there are several points that the community must comply with during the adaptation of new habits including, wearing masks and maintaining distance when crowds, washing hands using soaps and running water/hand sanitizer increasing body resistance by implementing a clean and healthy lifestyle, using electronic money and preventing point that the is the most concern of health experts is the use of masks in the community, because how to use masks is matter of concern which essential in public health along with the development of science around the use of the masks in general public intervening the spread of Covid-19 which also diverse (Ministry of Health of the Republic of Indonesia, 2020). The modes of transmission Covid-19 are interesting things to discuss. Modes of transmission SARS-CoV-2, including contact, droplet, airborne, fomite, fecal-oral, blood-borne, mother-to-child and animal-to-human transmission. SARSCoV-2 infection generally causes mild to severe respiratory illness and death, while some people infected with this virus never show symptoms. Contact and droplet transmission SARS-CoV-2 can occur through direct contact, indirect contact or close contact with an infected person through secretions such as saliva and respiratory tract secretions or respiratory droplets released when an infected person coughs, sneezes, talk or sing (Liu et al., 2020 ; Luo et al., 2020). Airway droplets have diameter  $>5-10\ \mu\text{m}$  while those with a diameter  $\leq 5\ \mu\text{m}$  are referred to as droplet nuclei or aerosols (WHO, 2014). This transmission can occur when a person is in close contact (within 1 meter) with a person infected who have respiratory symptoms (such as coughing or sneezing) or who are talking or singing. Under these circumstances, respiratory droplets containing the virus can reach the mouth, nose and eyes of a susceptible person and can cause infection. Indirect contact

transmission where there is contact between a susceptible host and a contaminated object or surface (fomite transmission) may also occur (WHO, 2020).

## **METHODOLOGY**

### ***Examination Room***

The room to be examined consist of 4 rooms, consisting of : Covid-19 laboratory administration room, Covid-19 examination laboratory room, BSC for covid-19 and the general patient waiting room

### ***Sampling Air***

The tools used for this research is air sampler (Merck) with the principle of impingement method. The principle of this method is accelerating the air towards the surface of the media. The air sample is obtained through a suction pump which enters a narrow inlet tube into the media flask (Cox et al., 2020).].

In the sampling room, the air sampler is palced in a safe position. A total of 5 mL of Viral Transport Medium (VTM), consisting of MEM/DMEM/HBSS (Gibco), Bovine Serum Albumine 5% in PBS (Sigma) and Penicillin Streptomycin (Sigma) was inserted into the imoinge tube. Then 0.1 mL Of anti-foam (Merck) was added. Shaken until homogen. Tubes and impingement devices are connected by hoses. Then it was turned on for 15 minutes (Ali et al., 2020 ; Chia et al., 2020). The captured sample were examination molecularity.

### ***Detection of Real-Time RT-PCR***

Extarction of Covid-19 RNA sample was carried out according to the procedure on the QIAmp DNA Minikit (Intron) extraction kit. The extraction was then detected using the SensiFast probe RT-PCR kit (Bioline) with the following steps : 2x Reaction RT Probe 10.0  $\mu$ L, 10 $\mu$ M Primer/Probe Mix Rp 1,5 $\mu$ L, Enzim RT/RNA Polymerase 0.2 $\mu$ L, RNase Inhibitor 0.4 $\mu$ L, DEPC Tread Water 2.9 $\mu$ L, Aliquot 15.0 $\mu$ L mix RT- PCR 0.2 mL tube. Addition RNA 5.0 $\mu$ L, then mixed and spun during 15 seconds.

This mixture was amplified using an automatic Thermal cycler otomatis (Bio Rad) with the following conditions : 50°C for30 minutes, 95°C for 2 minutes, 95°C for 15 minutes, during 45 cycles and annealing temperature of 58°C for 1 minute.

SARS-CoV-2 viral detection was carried out based on CDC protocols with suggested primers and probes targeting nucleocapsid gene as N1 and N2 regio (Table 1).

**Table 1 : Primers and probes targeting nucleocapsid gene as N1 and N2 regio**

Primers and probe	Nucleotide sequence (5' - 3')	Relative position within genome	Amplicon size (bp)
N1 forward	GACCCCAAATCAGCGAAAT	28303-28322	73
N1 reverse	TCTGGTTACTGCCAGTTGAATCTG	28374-28351	
N1 probe	ACCCCGCATTACGTTTGGTGGACC	28325-28348	
N2 forward	TTACAAACATTGGCCGAAA	29180-29199	67
N2 reverse	GCGCGACATTCCGAAGAA	29246-29228	
N2 probe	ACAATTTGCCCCCAGCGCTTCAG	29204-29226	

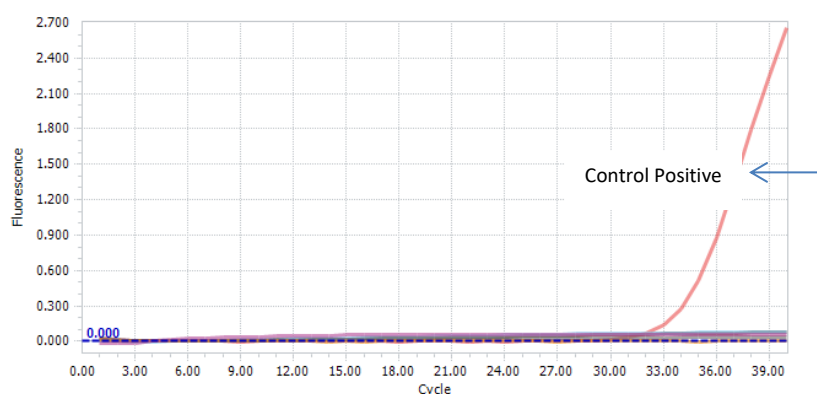
## RESULTS

The choice of rooms to be examined on the basis of Covid19 examination laboratory room consists of 5 indoor and 1 outdoor. Sampling was carried out on different days.

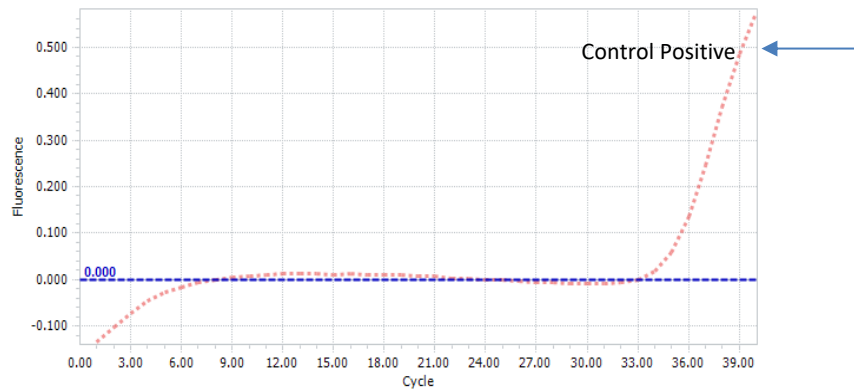
The RNA amplification curve using real-time RT-PCR in the first sampling can be seen in Fig 1, while the second sampling results are shown in Fig 2. The figure shows that the positive control forms a sigmoid curve while the negative control does not form a sigmoid curve.

The internal controls (IC) the standard that the sampling has cells and the real-time PCR reaction is running. Virus gene (Fam) was carried out to monitor the presence of virus in the control and sample which was examined while sampling control (CY5) was carried out to determine whether the sample contained the virus (Fig 1 and 2)

Fam (Virus gene)



### VIC (Sampling control)



### CY5 (Internal control)

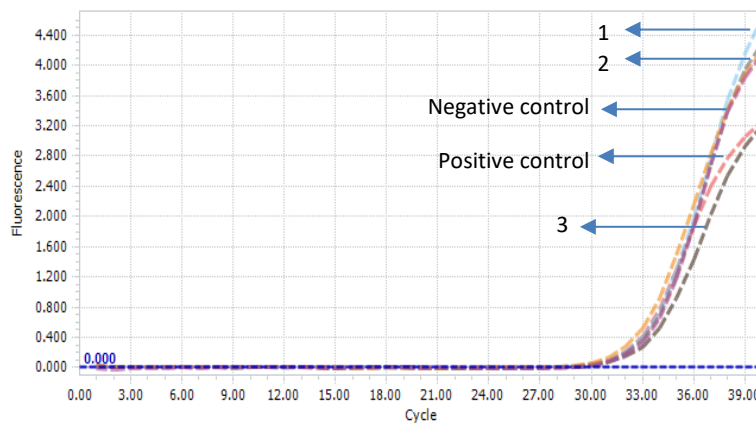
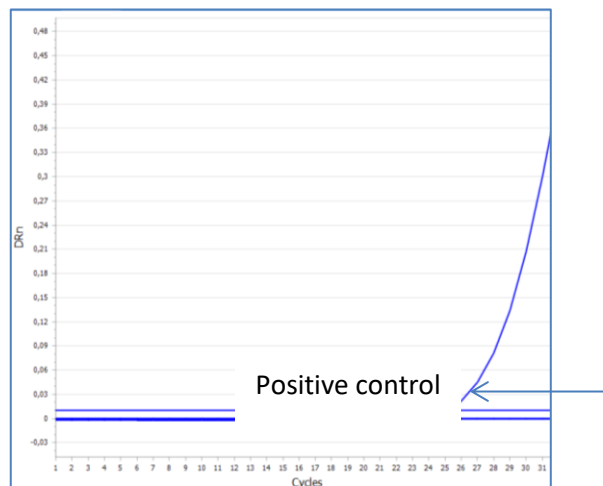
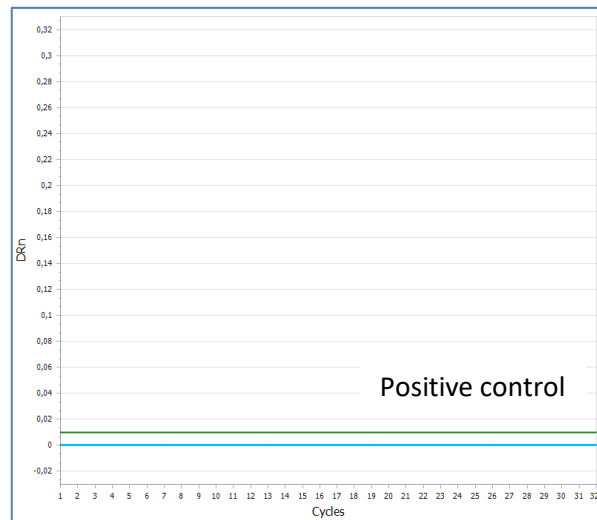


Figure 1 : Covid19 RNA detection results using real-time RT-PCR. The horizontal line is the real-time RT-PCR cycle. The vertical line is the fluorescent signal (cycle threshold/Ct). C+: positive control. C-: negative control. Room number : 1. Administration room, 2. BSL room 3. BSC.

### FAM (Virus gene)



### VIC (Sampling control)



### CY5 (Internal control)

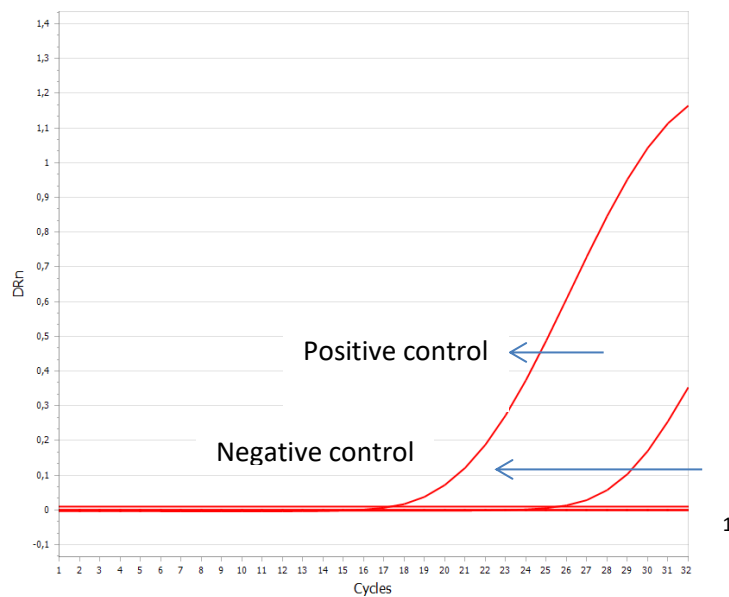


Figure 2 : Covid19 RNA detection results using real-time RT-PCR. The horizontal line is the real-time RT-PCR cycle. The vertical line is the fluorescent signal (cycle threshold/Ct). C+: positive control. C-: negative control. Room number :1. Waiting room.

The results of the real-time RT-PCR showed that of 4 rooms are negative results. The first sampling showed that 3 rooms gave CT values above the positive control, which was >30.02 (Table 2). Meanwhile, in the second sampling, 1 rooms were examined according to the negative control and 1 room above the positive control (35.61). This research shown that all the rooms examined gave negative results (Table 3).

**Table 2 : Covid19 RNA detection results in the first sampling using real-time RT-PCR**

No	Room	PCR Examination			Result
		Virus Gene (Fam)	Sampling Control (VIC)	Internal Control (CY5)	
1	Administration room	-	-	30.44	Negative
2	BSL room	-	-	30.23	Negative
3	BSC	-	-	30.69	Negative
4	Positive control	30.1	33	30.02	Positive
5	Negative control	-	-	30.53	Negative

**Table 3 :Covid19 RNA detection results in second sampling using real-time RT-PCR**

No	Room	PCR Examination			Result
		Virus Gene (Fam)	Sampling Control (VIC)	Internal Control (CY5)	
1	Waiting room	-	-	35.61	Negative
2	Positive control	35.02	-	27.36	Positive
3	Negative control	-	-	0	Negative

## DISCUSSION

The room being examined is the Covid-19 examination laboratory room, which consists of 4 rooms. Administration room is an option because this is where the results are processed to be reported to patients. The Covid-19 and BSC examination laboratory rooms were chosen because where the viral RNA extraction process is carried out.

The impingement method chosen because it is the right method for sucking air through the pipe and into the liquid. All the particles in the air will be trapped in the liquid. This tool generally works at liquid flow rate 12.5 L/min with 20 mL of collecting fluid volume for 20 minutes. In this study we used 5 mL VTM for 15 minutes. Longer sampling times can result in excessive evaporation and death of dissolved microorganisms (Verreault et al., 2022).

If the collection time is extended it will increase the evaporation of the liquid and can inactivate the trapped microorganisms. Imposition of microorganism cells into the liquid can cause cell damage. Efficiency will decrease sharply if used for more than 30 minutes because the collecting liquid which has a low viscosity can be evaporated easily. To reduce this weakness, a biosampler has been designed with a liquid collection of oil in the form of non-evaporating heavy white mineral oil which is able to collect air for reduce this

weakness, a biosampler device has been designed with non evaporating oil collection fluid for 4 hours. This has the advantage of being used in air that has few particles so that a large volume of air sample is required (Verreault et al., 2022).

What happened at the beginning of this research experiment was that at the time of sampling, the collecting medium appeared to be foamy so that the liquid came out irregularly. To remove foam on the medium we added 0.1 mL of anti-foam solution as recommended by Pan et al (2019) (Pan et al., 2019). This gave significantly better side effects than no anti-foam.

Analysis using real-time PCR allows for observations during the reaction. The presence of amplified DNA can be observed on the graph that appears as a result of the accumulation of fluorescence from the probe. In real-time PCR, the observation of the results no longer requires the electrophoresis step, so that agarose gel is no needed and the use of Ethidium Bromide (EtBr) which is carcinogenic compound is no longer needed. The result positive when the fluorescent signal is significantly above the baseline. For each reaction, a positive control was used and distilled water was used as a negative control. The result showed that all sample gave negative results. The result of this study are similar to the research of Faridi et al (2020) on the measurement of SARS-CoV2 in the patient rooms and Cheng et al (2019) on air and environmental sampling for SARS- CoV-2 around hospitalized patients with coronavirus disease 2019.

The most important factor in air sampling is the accuracy of the volume of air inhaled. No single sampling procedure is suitable for indoor bioaerosol sampling. Bioaerosols have different properties in air. The nature of the bioaerosol is related to the particle size therefore selecting the appropriate sampling procedure should be based on the particle size and type of bioaerosol. For sampling certain types of viruses in the air, various types of sampling devices, sample pumps, sampling volumes, sampling times, culture media and incubation conditions are used. Each sampling method can give different results for sampling and affecting different types of viruses (Leung et al., 2016 ; Lindsley et al., 2020).

The selection of the sampling procedure should be made on the basis of preventing damage to microbial agent. In this case, it is necessary to minimize microbial stress during the sampling period. In air sampling of RNA viruses, maintaining the viability of the virus is not mandatory, but maintaining the integrity of its nucleic acid is very important because nucleic acids can be rapidly degraded during sampling (Verreault et al., 2022).

In addition to the sampling procedure, the sampling conditions play a key role in accurate sampling and detection of the target virus. In viral air sampling, various factors such as ventilation, air movement, relative humidity, temperature, activity and number of laboratory personnel during sampling, sampling flow rate, sampling time and sampling medium, can affect the results (Cox et al., 2020). Therefore in order to determine the appropriate and standardized protocol for the sampling and detection of SARS-Cov-2, environmental conditions during sampling should be considered. These

sampling conditions can only be controlled in the laboratory to provide constant conditions to investigate the effect of each parameter on sampler performance.

In the sampling of SARS-Cov-2 in the laboratory, other factors to be considered include, the noise level generated by the sampling pump, the movement of air, the wearing of a protective mask that prevents the release of their respiratory droplets at the sampling site, the time required to and air sampling. This makes it difficult to determine sampling conditions and identify a specific and appropriate protocol for determining SARS-Cov-2 in the air a health facility environment.

## **CONCLUSIONS AND RECOMMENDATIONS**

Preliminary study shows SARS-CoV-2 Covid-19 cannot be transmitted through the air. Research needs to be carried out with more samples taken as a continuation of this research and the impingement method needs to be reconstructed.

## **FURTHER STUDY**

The authors declare that all experiments have been examined and approved by the appropriate ethics committee was obtained from all relevant authorities.

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