

TRACKING THE SARS-COV-2 VIRUS THROUGH OUR SEWERAGE SYSTEM

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NATIONAL WATER RESEARCH INSTITUTE OF MALAYSIA (NAHRIM)

Research Centre under Ministry of Environment and Water focusing on research related to water and its environment (water quality, climate change, hydrology, groundwater, oceanography etc)

WATER QUALITY LABORATORY NAHRIM

1) SERVICES: Accredited laboratory under MS ISO 17025 for chemical and microbiology testing.

2) RESEARCH: emerging pollutants including pharmaceuticals, pesticides, plasticizers, microplastics, antimicrobial resistance

KIMIA

- LCMS/MS
- ICP-OES
- GCMS/MS
- UV-VIS
- FIMS
- TDC
- SPE-DEX

MIKROBIOLOGI

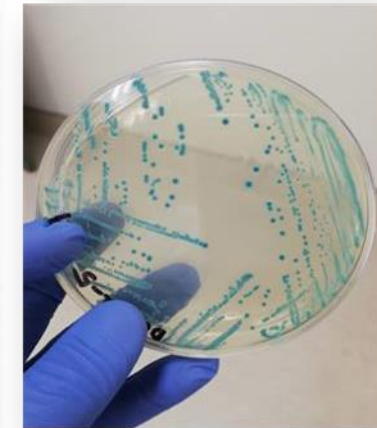
- Automated colony counter
- BSC II
- RT-qPCR
- HPLC
- Microbial DNA Identification: Bacteria and Fungi
- Light Microscope

PERANCANGAN RMK12

- FTIR
- GC FID
- Pyrolysis GCMS
- ICPMS
- Microbial Isolation
- Sequencing
- Genome Extractor

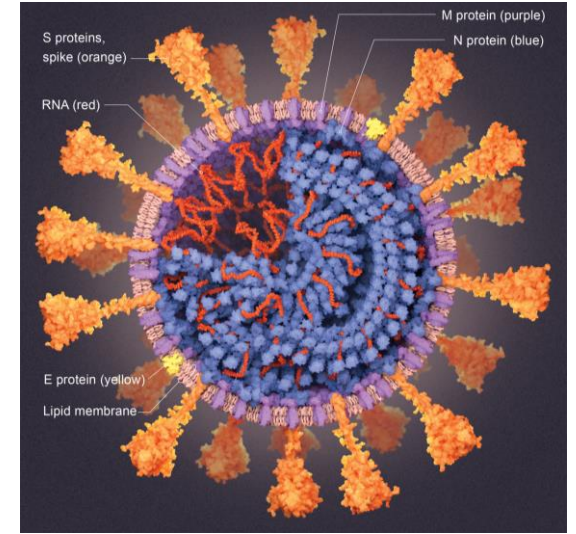
Menampung lebih 80 parameter berkaitan kualiti air (kimia dan mikrobiologi)

KAPASITI PERKHIDMATAN ANALISIS MAKMAL KUALITI AIR SEHIGGA 2020



COronaVirus Disease-2019

- Caused by a novel virus called **S**evere **A**cute **R**espiratory **S**yndrome **CO**rona**V**irus **2** (SARS-CoV-2).
- First COVID case was reported November 2019 in China
- Since then, the disease has become pandemic globally where **~223 millions were infected; ~5.3millions deaths reported (up to 12th September 2021).**



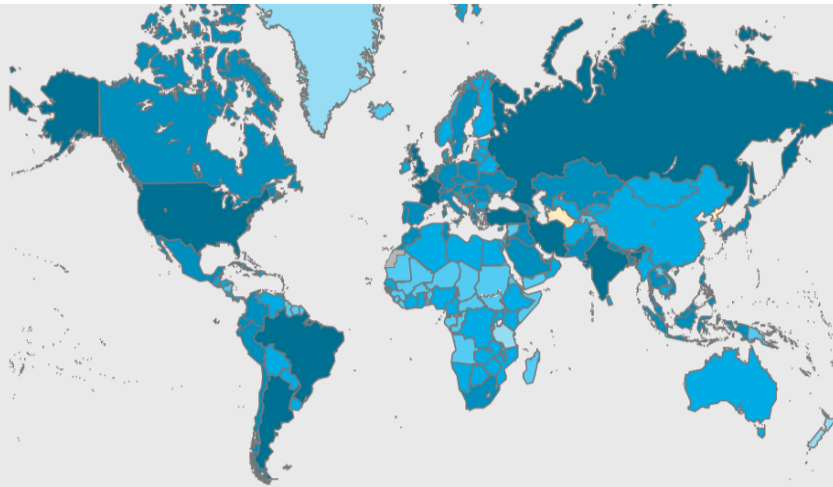
Search by Country, Territory, or Area

Covid-19 Response Fund

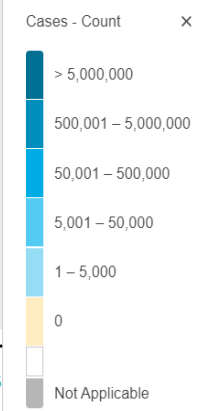
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WHO Coronavirus (COVID-19) Dashboard

[Overview](#) [Measures](#) [Data Table](#) [Explore](#)



Cases	▼
Total	▼
584,433	new cases
223,022,538	confirmed cases
4,602,882	deaths
5,352,927,296	vaccine doses administered



Globally, as of 4:47pm CEST, 10 September 2021, there have been 223,022,538 confirmed cases of COVID-19 including 4,602,882 deaths, reported to WHO. As of 5 September 2021, a total of 5,352,927,296 vaccine doses have been administered.

First Covid-19 case happened in November, China government records show - report

Earliest case detected on 17 November, weeks before authorities acknowledged new virus, says Chinese media

Coronavirus: live updates



COVID-19 may incubate for days after exposure. For example:

Day 0:
Amina was exposed to COVID-19

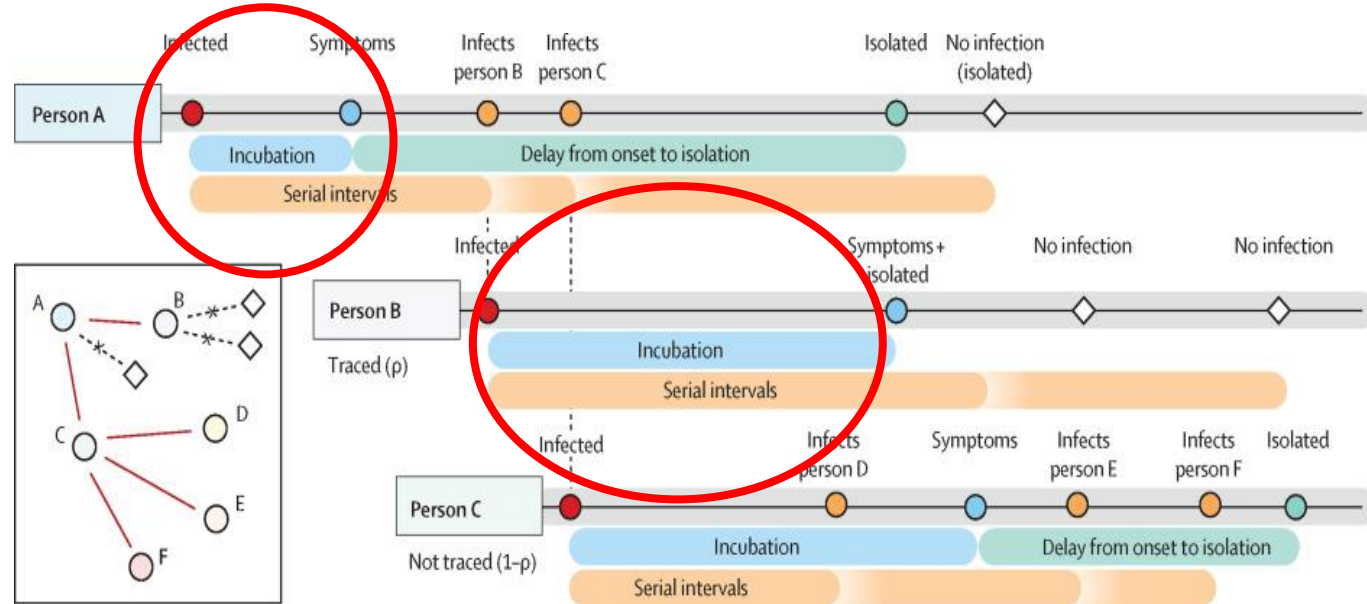
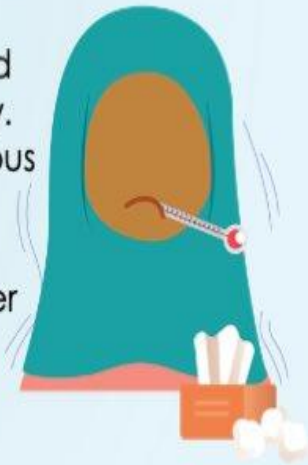


Day 14:
Amina showed symptoms and tested positive for COVID-19.



Day 5:
Amina was tested for COVID-19 and the results were negative

Days 8-9:
Thinking she did not have COVID-19, Amina celebrated Eid with her family. She was contagious 48 hours before symptoms appeared and her relatives were exposed.

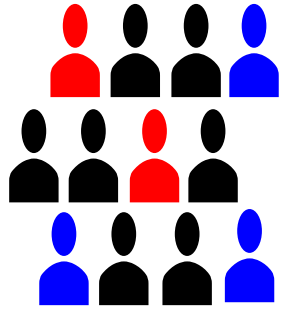


During the incubation period/asymptomatic phase, Amina did not realize she's carrying the virus and continue with her social activities.

Contact tracing started when Amina was tested positive (Person B and C).

How many people involved? Does Amina remember all the person she met? How about Person B and C? their close contact?

COMMUNITY A



Symptomatic patient
Asymptomatic patient



Virus shedding
through faeces
from Day 1
infection



Wastewater
treatment plant

Table 1
Detection of SARS-CoV-2 in human excreta specimens.

Specimen	Country	Method	Positive rate ^a	Remarks	Reference	
Feces or anal/rectal swab	China	qPCR	14/31 (45%)	Day 0–11; $550\text{--}1.21 \times 10^5$ gene copies/mL Pediatric patients; Positive for a mean of 21 (range: 5–28) days Day 3–13	(W. Zhang et al., 2020b)	
		qPCR	8/22 (36%)		(J. Zhang et al., 2020)	
		qPCR	9/17 (53%)		(Pan et al., 2020)	
		qPCR	8/10 (80%)		(Y. Xu et al., 2020a)	
		qPCR	5/6 (83%)		(Jiehao et al., 2020)	
		qPCR	54/66 (82%)		(Ling et al., 2020)	
		qPCR	39/73 (53%)		(Xiao et al., 2020)	
		qPCR	1/1 (100%)		Asymptomatic	(A. Tang et al., 2020a)
		qPCR	41/74 (55%)		Positive for a mean of 27.9 days (range: 8–48)	(Y. Wu et al., 2020a)
		qPCR	12/19 (63%)			(Chen et al., 2020c)
		qPCR	10/10 (100%)			(Lo et al., 2020)
		Cell culture	1/1 (100%)		Culturable virus isolated	(Zhang et al., 2020a)
		qPCR	44/153 (29%)			(W. Wang et al., 2020c)
		Cell culture	2/4 (50%)		Culturable virus isolated	
		qPCR	1/1 (100%)		Day 7	(Holshue et al., 2020)
qPCR	4/8 (50%)		(Young et al., 2020)			
qPCR	8/9 (89%)	Up to 10^8 copies/g-feces	(Wölfel et al., 2020)			
Cell culture	0/4 (0%)	No culturable virus isolated				
qPCR	2/5 (40%)	$6.3 \times 10^5\text{--}1.3 \times 10^8$ gene copies/g-feces	(Lescure et al., 2020)			



DAY 1



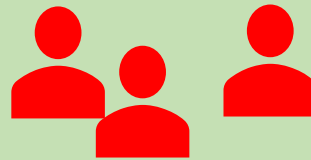
Wastewater samples
taken and tested for
COVID-19

DAY 3-4



Results from
wastewater
surveillance – report to
public health

DAY 5-6



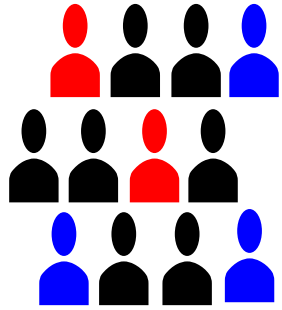
People starts to show
symptoms and go for
testing

DAY 7-8



Patients tested positive
Hospitalization/
quarantine

COMMUNITY A



Symptomatic patient
Asymptomatic patient



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Wastewater
treatment plant

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DAY 1



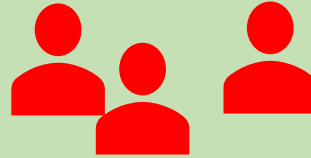
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DAY 5-6



People starts to show
symptoms and go for
testing

DAY 7-8



Patients tested positive
Hospitalization/
quarantine

WASTEWATER BASED SURVEILLANCE/EPIDEMIOLOGY

- A process of monitoring pollutants (chemicals or pathogens) in a population through wastewater
- Widely used for outbreak surveillance like poliovirus, antimicrobial resistance and drugs.
- WBE for COVID-19?
 - Respiratory infection
 - However the virus shed through the fecal and enters the wastewater line
 - Non intrusive surveillance to complement with clinical testing



Study Shows Covid-19 Can Be Detected In A Single Asymptomatic Person Through Wastewater Surveillance



William A. Haseltine Contributor ©
Healthcare

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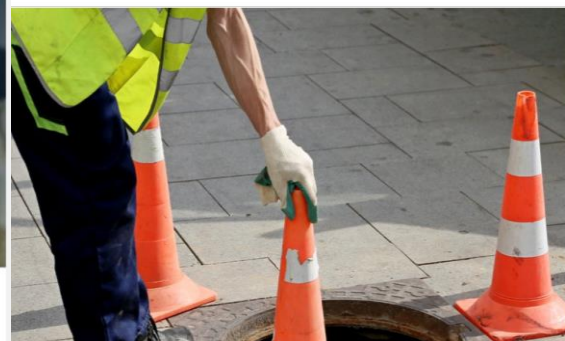
S'pore to double Covid-19 wastewater testing surveillance sites by 2022 from current 200



Poop: The Newest Disease Detection Tool for COVID-19 and Beyond

...eds of efforts around the nation are aiming to turn waste into valuable health data.

er Health News | Aug. 13, 2021, at 11:45 a.m.



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ABOUT RESEARCH WORK WITH US CAREERS EDUCATION NEWS EVENTS

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Monitoring wastewater for COVID-19

Australian researchers have achieved the first step in developing an early warning surveillance system to track COVID-19 prevalence in the community through tracing the presence of the novel coronavirus gene in raw sewage.

CONTACT SHARE

COVID-19 RESEARCH

Researchers from The University of Queensland (UQ) and Australia's national science agency CSIRO have completed a proof of concept study in early April using wastewater samples from two wastewater treatment plants in South East Queensland, representing populations living in the Brisbane region.

Protecting healthcare workers from COVID-19 aerosols

The next step is to build the capacity on a national scale to develop an early warning system to track the incidence of outbreaks in assembly centres, and to assemble and respond in the community base.

As of 1 September the influent of all 323 Dutch municipal waste water treatment plants must be monitored daily on the presence of SARS-CoV-2 RNA gen material and the data must be processed to the national Covid-19 dashboard.

Dutch health minister Hugo de Jonge asked the national health institute RIVM to prepare this large-scale sewer surveillance. The surveillance fits in with the government policy for a regional oriented control of new outbreaks.

Promising results

In recent months, the RIVM national health institute has conducted research at 29 locations on the presence of the virus in sewage water. On a weekly bases samples of influent have been collected at municipal waste water treatment plants across the nation. The locations represent a quarter of the total Dutch population.



Release 2021/06/14

SHIONOGI Announces the Start of the Wastewater Epidemiological Surveillance Service for the Novel Coronavirus in Japan

OSAKA, Japan, June 14, 2021 - Shionogi & Co., Ltd. (hereafter "Shionogi") announced that it starts wastewater epidemiology surveillance service for the novel coronavirus (SARS-CoV-2) to wastewater treatment plants of municipality.

This is an investigative service to confirm the infection status of SARS-CoV-2 in the target area by regularly monitoring the amount of SARS-CoV-2 in wastewater based on wastewater epidemiology. By regularly collecting and monitoring wastewater at the wastewater treatment plants of each local government, it is expected to contribute to grasp the infection status in the target area.

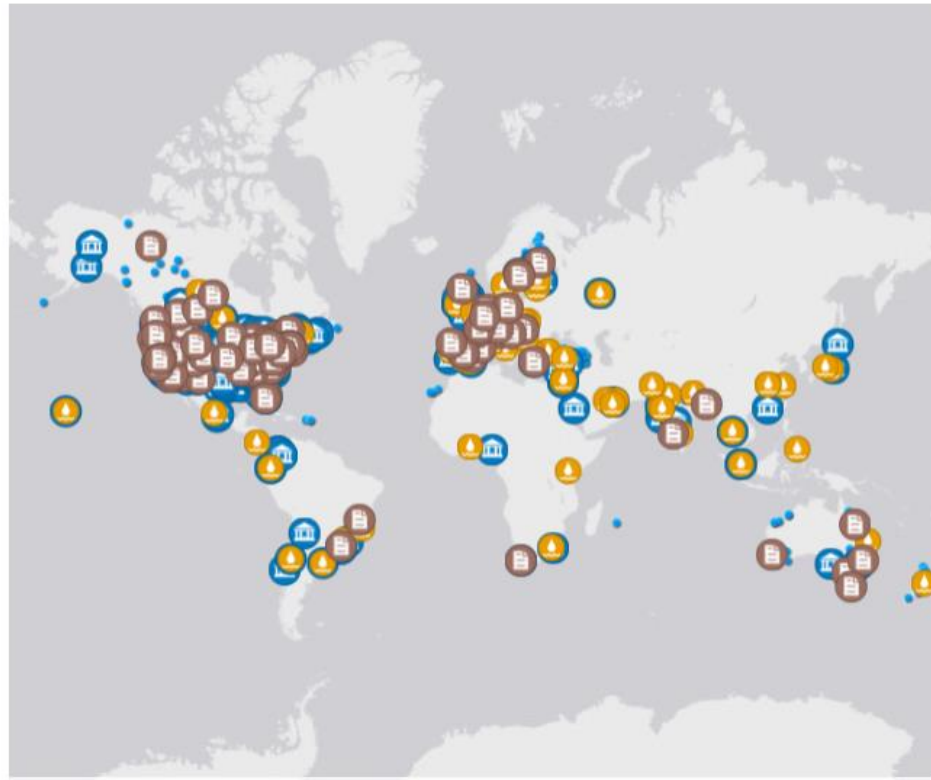
SARS-CoV-2 has been detected in the feces of a significant proportion of infected individuals¹. In the United States and the Netherlands, the amount of SARS-CoV-2 in the wastewater of facilities and cities is regularly monitored to detect epidemics at an early stage and determine convergence^{2,3}. In Japan, there have been fewer reported cases of COVID-19 infection per capita compared to the United States and some European countries and regions; therefore, for social implementation, the challenge had been to develop a virus detection method with increased sensitivity.

<https://www.covid19wbec.org/covidpoops19>

COVID19Poops Dashboard



COVIDPoops19 Summary of Global SARS-CoV-2 Wastewater Monitoring Efforts by UC Merced Researchers



Esri

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Dashboards

 92

Universities

 263

Countries

 55

Sites

 2,691

Please follow [@COVIDPoops19](#) for wastewater SARS-CoV-2 updates. Funded by [CITRIS](#). Country data from [COVID-19 WBE Collaborative](#). Link to our [pre-print](#) for our methods. Want to be added to the Map? Fill out this [form](#).

Table 2

Details of reported molecular detection of SARS-CoV-2 in wastewater.

Sampling location		Water type	Virus detection methods			Detection results		Reference
Country	State/city		Virus concentration method	qPCR assay ^a	Sequence confirmation	Positive rate	Maximum concentration (copies/L)	
Australia	Brisbane, Queensland	Untreated wastewater	Electronegative membrane-direct RNA extraction; ultrafiltration	N_Sarbeco NIID_2019-nCoV	Direct sequence of qPCR products (Sanger + MiSeq)	2/9 (22%)	1.2×10^2	(Ahmed et al., 2020)
The Netherlands	Amsterdam, The Hague, Utrecht, Apeldoorn, Amersfoort, Schiphol, Tilburg	Untreated wastewater	Ultrafiltration	CDC N1, N2, N3 E_Sarbeco	Not done	14/24 (58%)	Not available	(Medema et al., 2020)
USA	Massachusetts	Untreated wastewater	PEG precipitation	CDC N1, N2, N3	Direct sequence of qPCR products (Sanger)	10/14 (71%)	$>2 \times 10^5$	(F. Wu et al., 2020b)
France	Paris	Untreated wastewater	Ultracentrifugation	E_Sarbeco	Not done	23/23 (100%)	$>10^{6.5}$	(Wurtzer et al., 2020)
		Treated wastewater	Ultracentrifugation	E_Sarbeco	Not done	6/8 (75%)	$\sim 10^5$	
USA	Bozeman, Montana	Untreated wastewater	Ultrafiltration	CDC N1, N2	Re-amplification by regular PCR followed by Sanger sequencing	7/7 (100%)	$>3 \times 10^4$	(Nemudryi et al., 2020)

SURVELAN COVID-19 DI DALAM SISTEM KUMBAHAN DI MALAYSIA



KEMENTERIAN
ALAM SEKITAR
Ministry of Environment and Water



KEMENTERIAN ALAM SEKITAR MEMPELOPORI KAJIAN GENETIK COVID-19 DALAM SISTEM PEMBETUNGAN

BERITA - Nasional
Rabu, 22 April 2020 | 10:49am



COVID-19 : Kajian sistem kumbahan boleh bantu kenal pasti kawasan berisiko

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KUALA LUMPUR : Ramai penyelidik di peringkat antarabangsa sudah mula mengkaji sekiranya air kumbahan dalam sistem pembentungan boleh menjadi 'amaran awal' untuk virus pandemik COVID-19 kembali ke dalam komuniti.

Malaysia sendiri melalui Kementerian Alam Sekitar Dan Air mengambil pendekatan proaktif untuk menjalankan kajian mengenai risiko COVID-19, sama ada ia boleh merebak melalui kaedah itu.

OBJECTIVES:

To detect genetic fragments of SARS-CoV-2 in the selected sewerage system as an early warning system for an outbreak.

KOLABORASI BERSAMA



KEMENTERIAN
ALAM SEKITAR DAN AIR
Ministry of Environment and Water



Suruhanjaya Perkhidmatan Air Negara



WASTEWATER BASED SURVEILLANCE FOR COVID-19

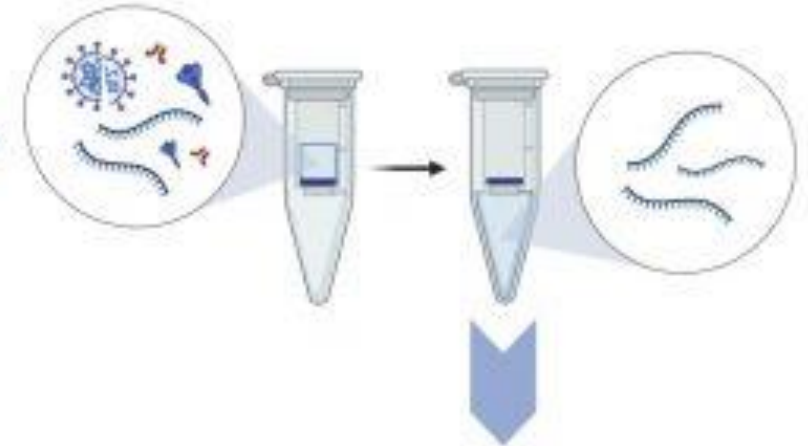
COVID-19 prevalence



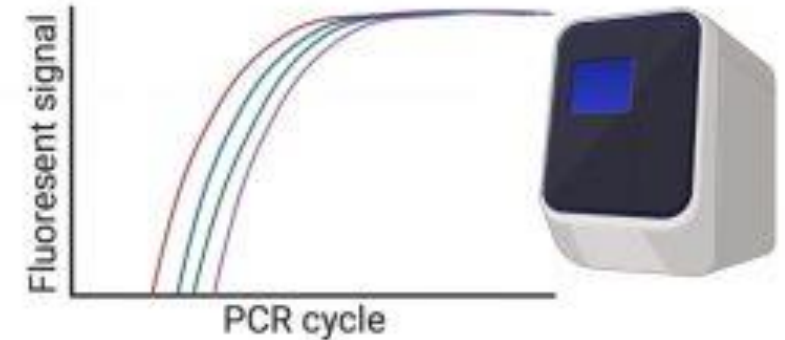
Virus concentration



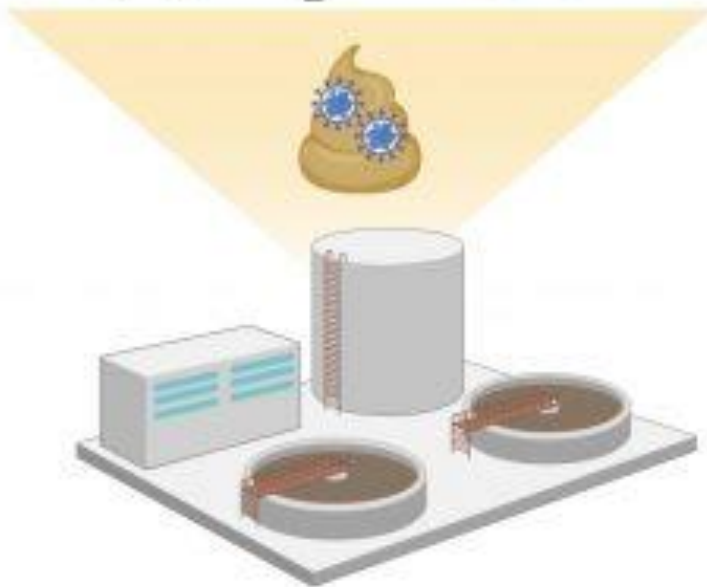
Viral RNA extraction



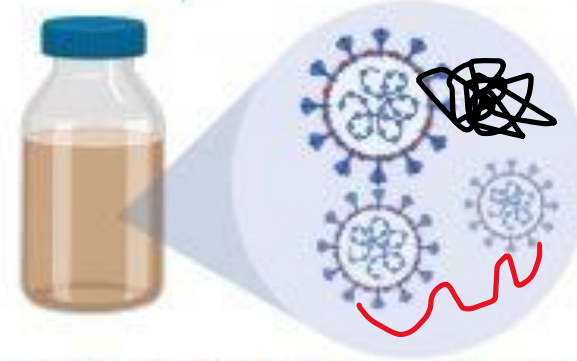
Quantitative PCR



Detection & quantification of SARS-CoV-2 RNA



Wastewater treatment plant



SARS-CoV-2 in wastewater

SAMPLING LOCATION

- Two sewage treatment plants in Klang Valley
- Hospital Sungai Buloh

SAMPLE TYPE

- Untreated influent
 - Sludge
- Treated effluent
- Composite sample

FREQUENCY

- Twice a month for 9 month continuously

LABORATORY TESTING

- Water Quality Laboratory, NAHRIM



STEP 1

SAMPLE PRETREATMENT

- Samples were preserved in ice before transported to laboratory.
- Kept at chiller (~4°C) upon arrival. Best to process ASAP or within 24 hours
- (OPTIONAL) Inactivation: preheat at 60°C, 1 hours

STEP 2

SAMPLES CONCENTRATION

- Various method for virus concentration were optimized:
 1. **Membrane filtration + MgCl**
 2. Membrane filtration
 3. Membrane filtration + acidification
 4. Amicon ultracentrifuge

STEP 3

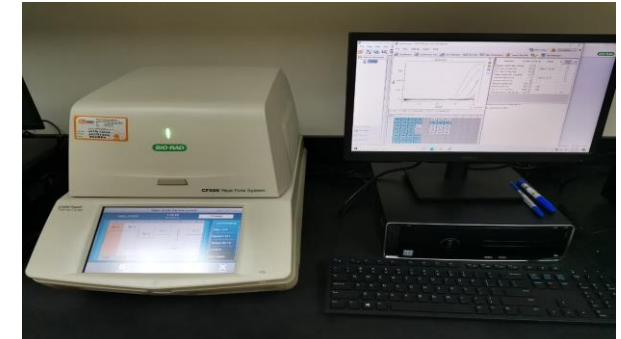
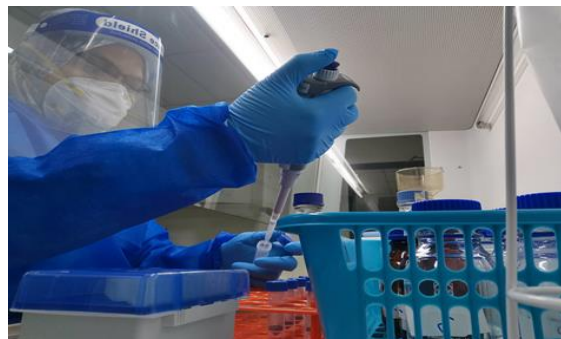
RNA EXTRACTION

- Concentrated samples were the used in RNA extraction using Power Water Rneasy Kit

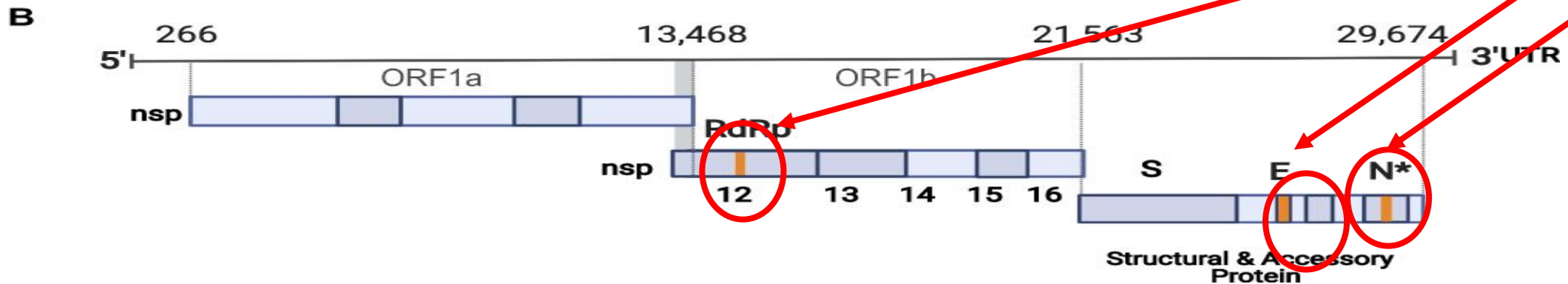
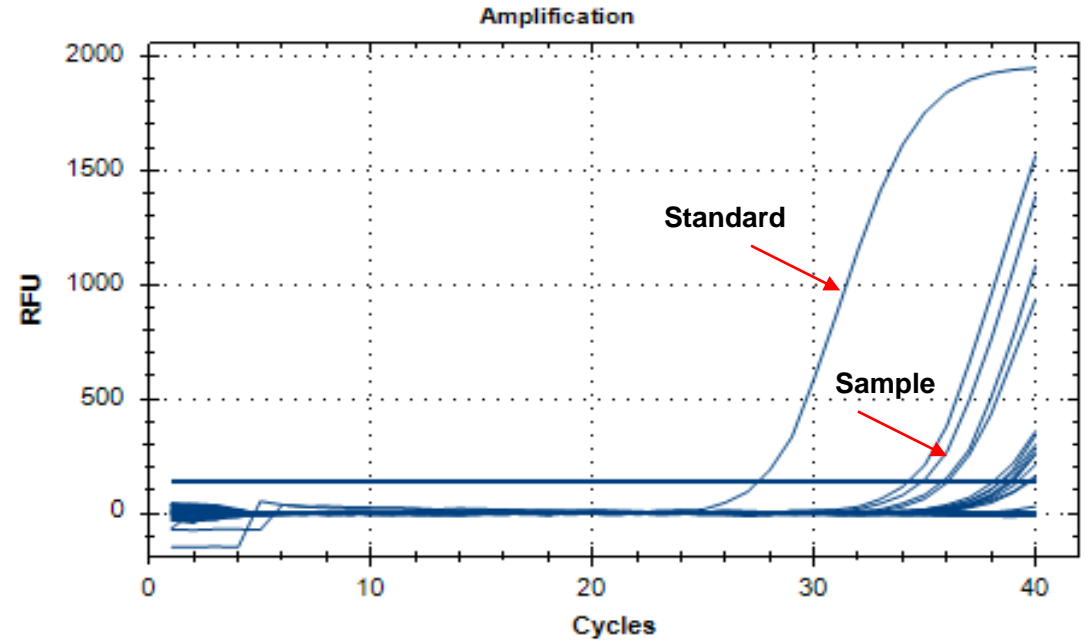
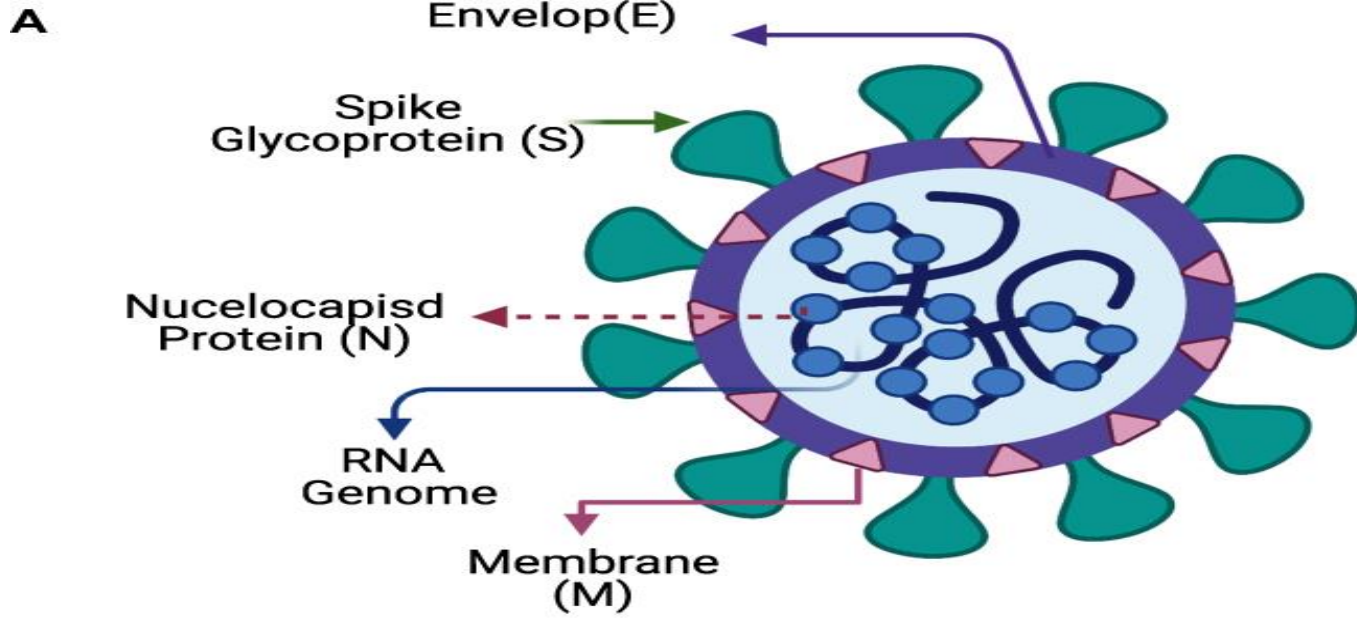
STEP 3

RT qPCR

- Primer Assay: N1, N2, E
 - Instrument: CFX 96 (Biorad, USA)



SPECIFIC REGION FOR SARS-COV2 IDENTIFICATION IN RT-QPCR



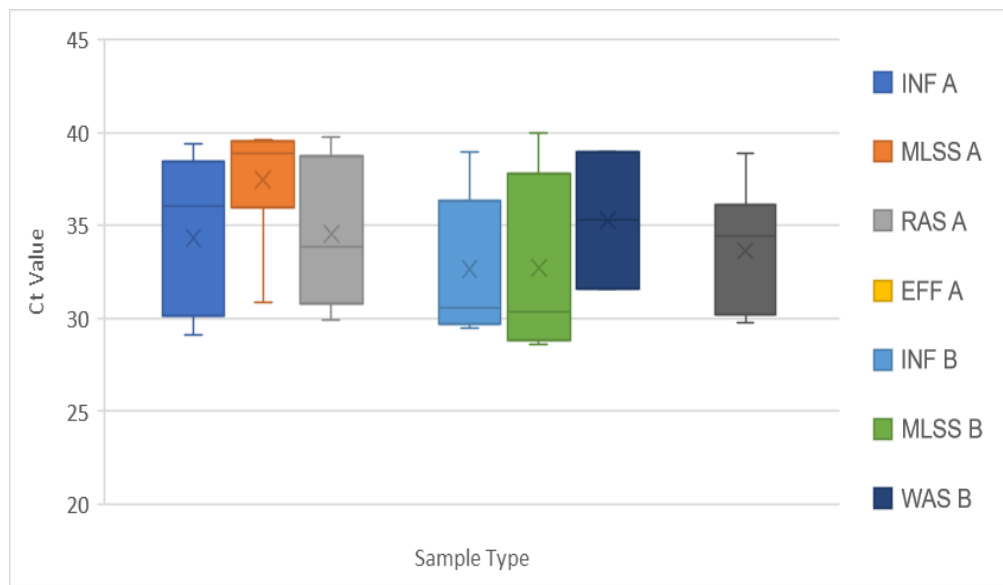


Figure 1. Box plot on Ct values for each samples type

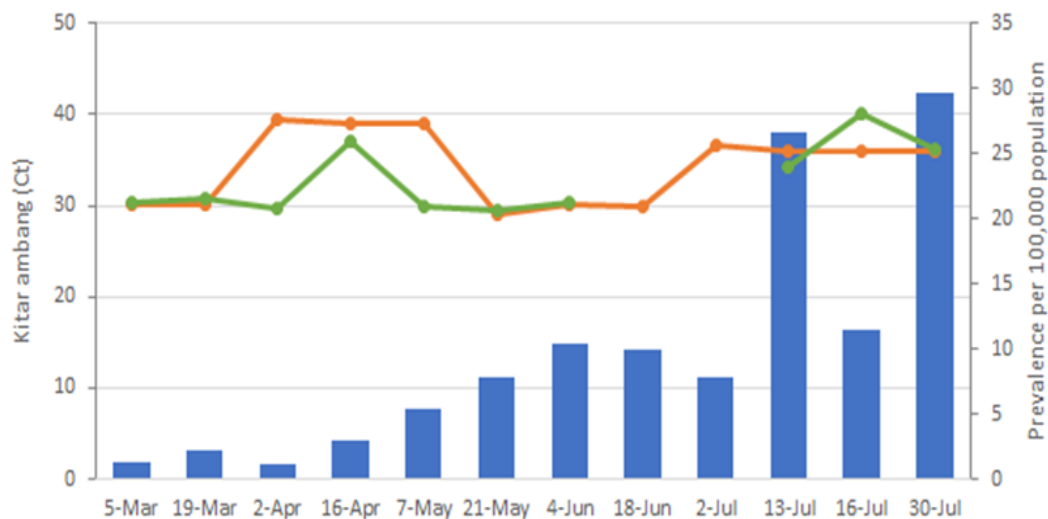
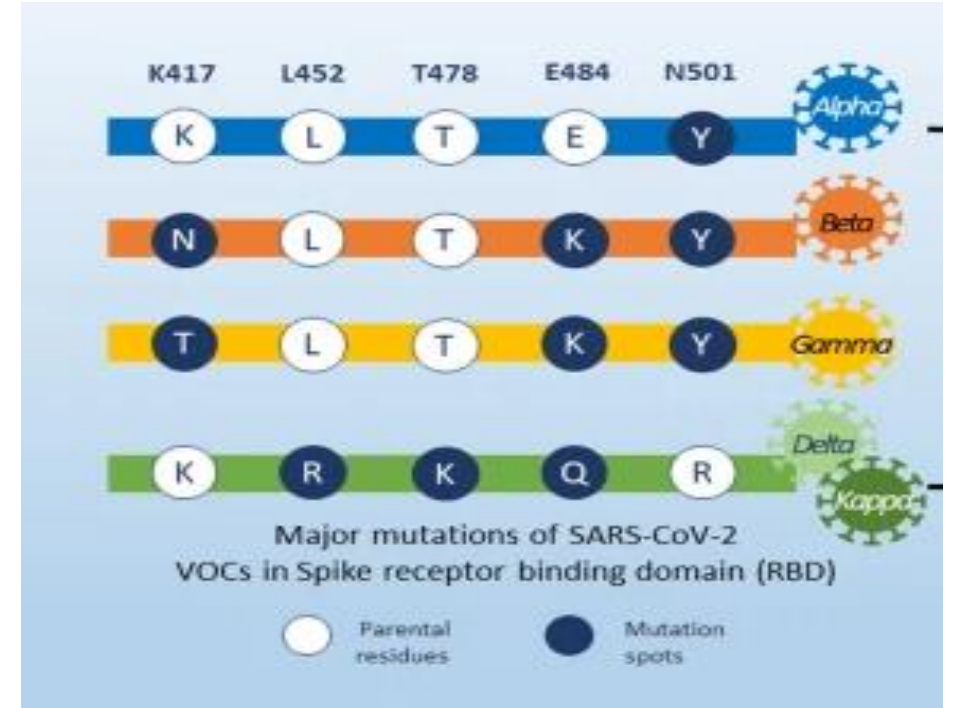
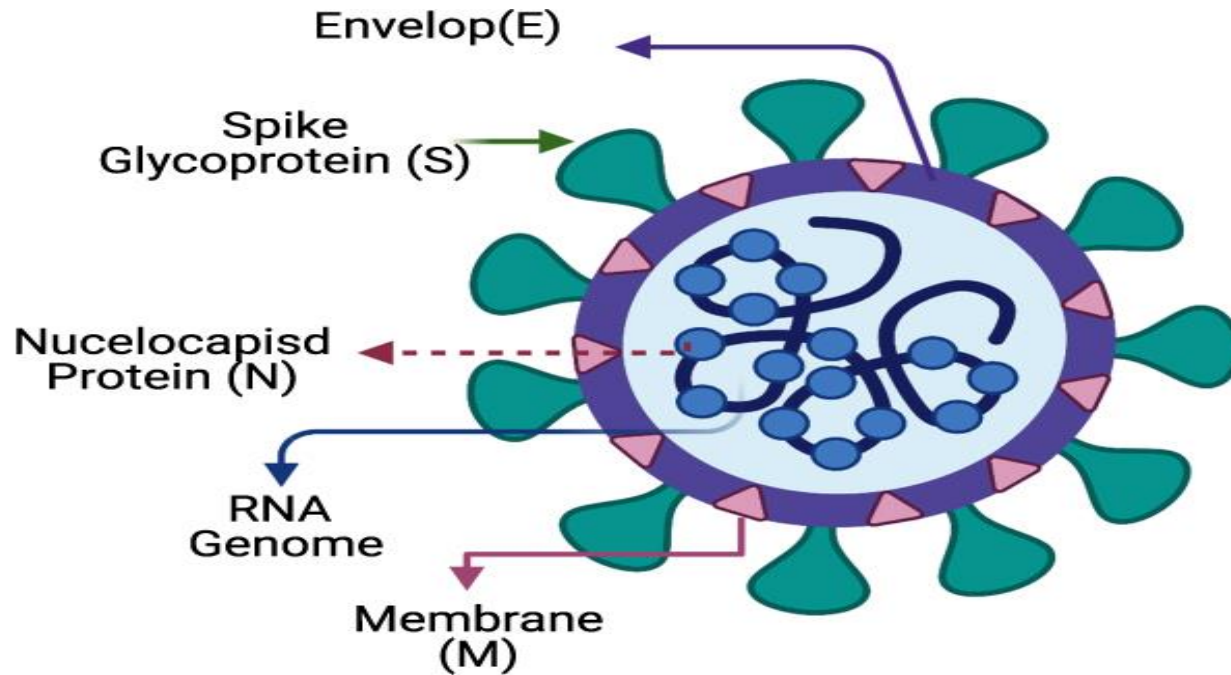


Figure 2. Ct values correlation with active cases reported

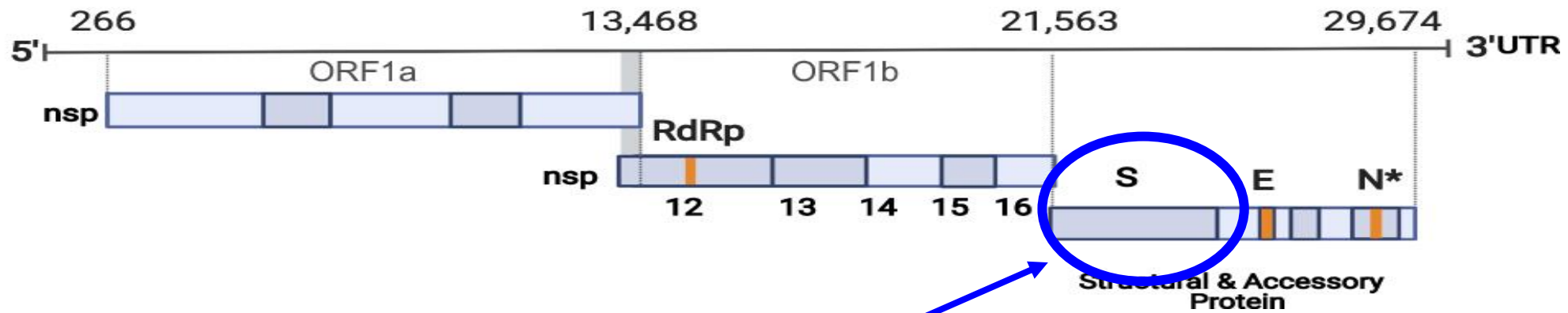
- Samples were considered positive if cycle threshold (Ct) value is <40.
- Samples from influent, MLSS and RAS were detected positive with Ct value range around Ct 30- 39 (Figure 1).
- On average, Ct value for influent is 33.6 (± 3.9), MLSS is 34.3 (± 4.6) and RAS 34.7 (± 3.9) (Figure 1).
- No signals were detected from effluent samples; indicating the treatment process is adequate.
- Higher Ct values (40) indicating a weak signals/low presence of RNA fragments in the samples. It also may indicates the virus is not in active state or low in viral load.
- Ct values obtained did not show any significant trend when compared with active cases reported.

VARIANT DETERMINATION – SPIKE PROTEIN

A



B



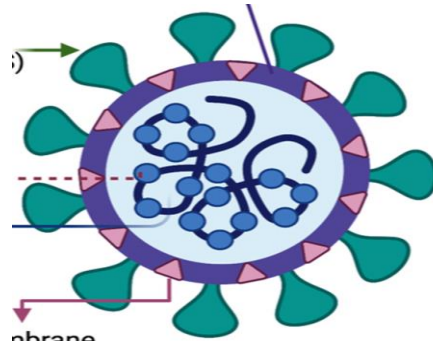
Variant Region

CAN WE GET COVID-19 FROM WASTEWATER?

Results obtained from RT-PCR only can give information on the presence of the virus (Yes or No)

Fragments of RNA (N genes, E genes) were detected using RT-PCR

Spike protein fragments were amplified for variant detection

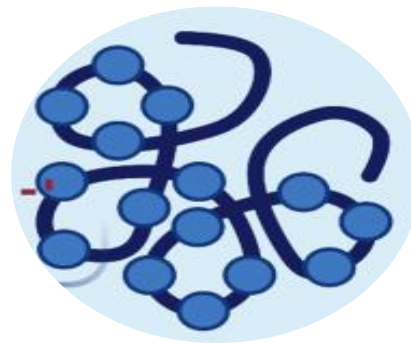
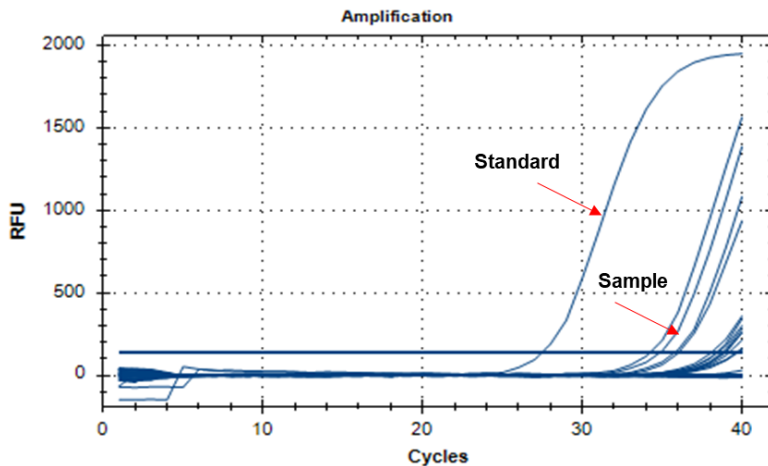


Viable Virus??

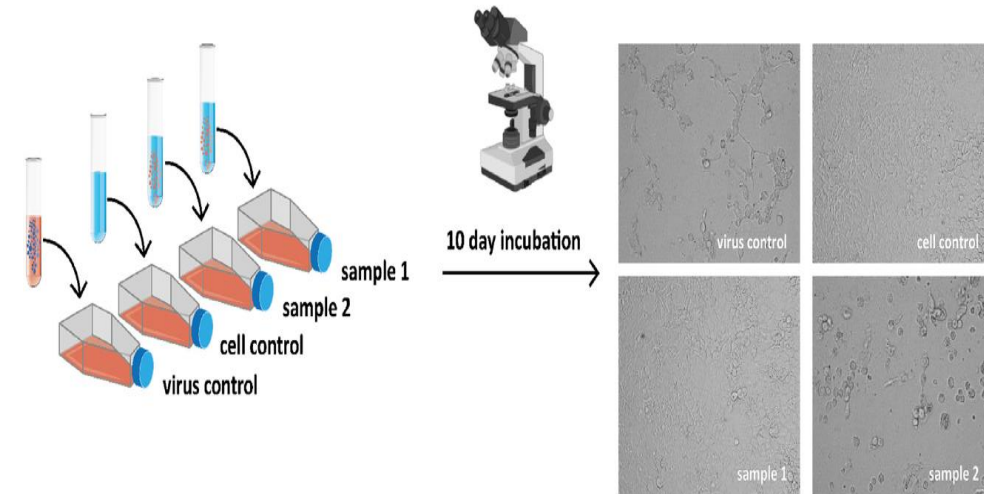
To date, no reports on transmission of COVID-19 from wastewater given the characteristic of the virus as an enveloped virus.

Cell culture assays were carried out to look at the infectivity rate of wastewater samples. (in a Biosafety Level 3 laboratory)

OR



RNA fragment?



SUMMARY

WBE as early warning tools for outbreak detection?

- Presence of SARS-CoV-2 in the sewerage system : **YES**, it is detected in untreated samples
- Do we know the dominant variants: **YES**, one of the samples showing dominant in delta variant
- Transmissible to human: **NO** strong evidence on the transmission

Further to explore:

- Correlation on numbers of people infected in a community (virus are shed differently from each people) –interdisciplinary group of experts in environmental microbiology, wastewater engineering, and public health
- Selection of testing sites and availability of testing laboratory.
- Sensitivity of testing. 1 over 1million people?
- Different sewerage treatment technologies?
- Cost and benefit



Science never solves a
problem without creating ten
more.

George Bernard Shaw

ACKNOWLEDGEMENT

- **Ministry of Environment and Water (KASA)**
- **Institut Penyelidikan Air Kebangsaan (NAHRIM)**
- **National Water Services Commission (SPAN)**
- **Indah Water Konsortium Sdn. Bhd. (IWK)**
 - **Universiti Teknologi Malaysia (UTM)**
- **Delft University of Technology (TU Delft)**
- **GeneSeq Sdn Bhd. (variant sequencing)**