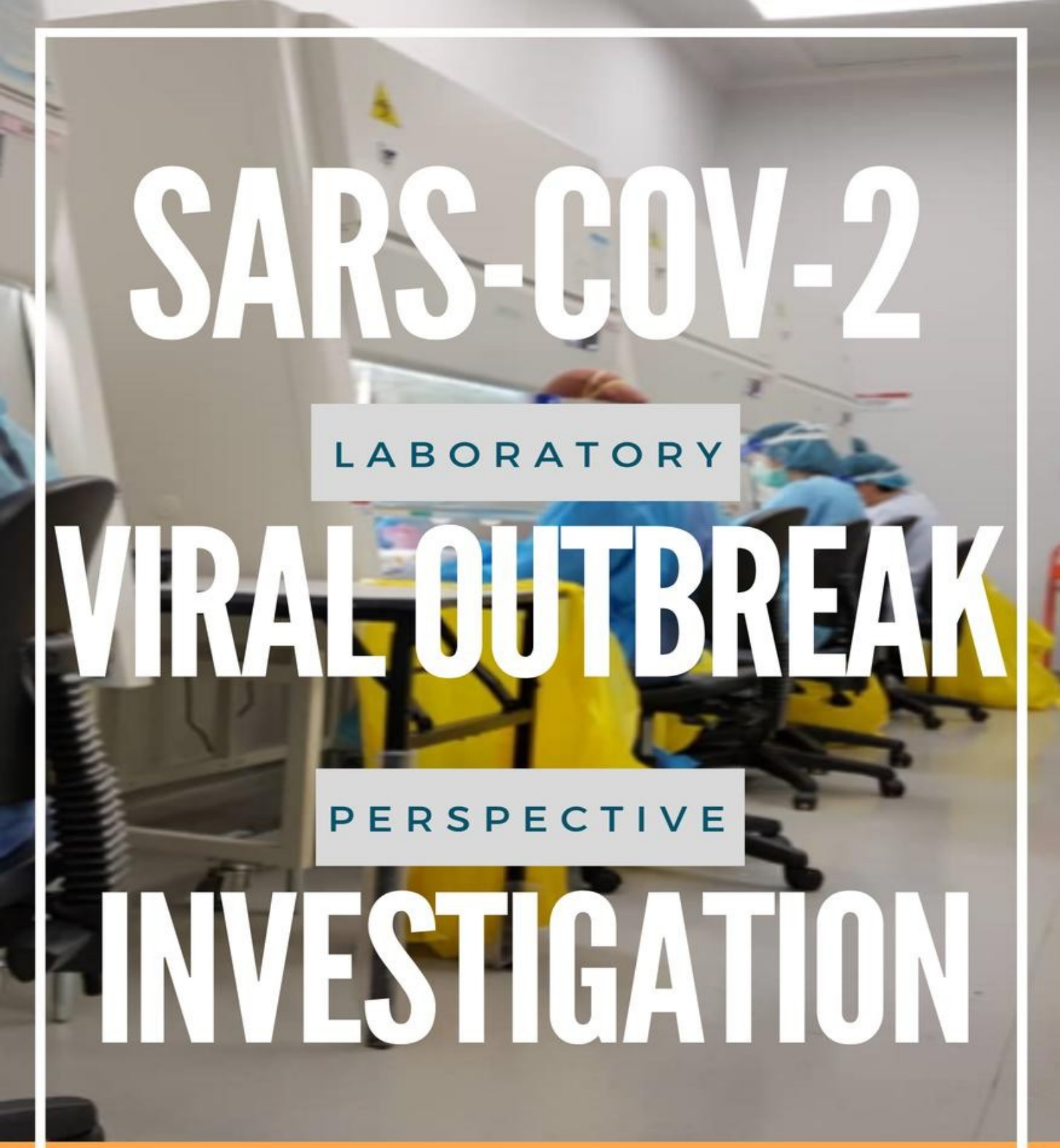

SARS-COV-2

LABORATORY

VIRAL OUTBREAK

PERSPECTIVE

INVESTIGATION

BY

INSTITUTE FOR CLINICAL RESEARCH, NIH MY

CLINICAL UPDATES IN COVID-19

SARS-CoV-2 Viral Outbreak Investigation: Laboratory Perspective

Clinical Updates in COVID-19

Cheng Hoon Chew, Yan Yee Yip, Ming Tsuey Lim, Weng Kiong Lee, Hani Binti Mat Hussin, Ravindran Thayan, Arni Binti Talib, Christopher Kwok Choong Lee, Pik Pin Goh

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SARS-COV-2 VIRAL OUTBREAK INVESTIGATION: LABORATORY PERSPECTIVE

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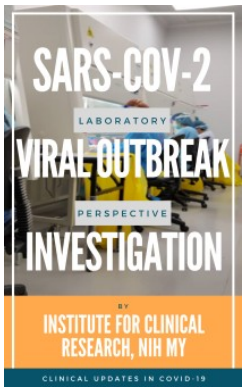
Front cover design by Chew Cheng Hoon.

Written by Cheng Hoon Chew, Yan Yee Yip, Ming Tsuey Lim, Weng Kiong Lee, Hani Binti Mat Hussin, Ravindran Thayan, Arni Binti Talib, Christopher Kwok Choong Lee, Pik Pin Goh



SARS-CoV-2 Viral Outbreak Investigation: Laboratory Perspective

Disclaimer

- This transcript was prepared based on the Clinical Updates in COVID-19 live webinar session on 02/07/2020. The panellists for this webinar are Dr. Hani Binti Mat Hussin, Dr. Ravindran Thayan and Dr. Arni Binti Talib.

- The transcript was prepared by Ms.Yip Yan Yee, Mdm Lim Ming Tsuey, Mr.Lee Weng Kiong and Dr. Chew Cheng Hoon from Institute for Clinical Research, NIH Malaysia.

- This is intended to share within healthcare professionals, not for the public.

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What is the cycle threshold (Ct) in terms of PCR platform?

Dr Ravi, do you know if studies have been done in Singapore to confirm whether their reported 382-nucleotide deletion strain is attenuated?

What is the sensitivity and specificity of rapid antigen test?

Does IMR or other hospital laboratories do heat inactivation of specimens prior to processing? How is it done?

There is still quite an amount of controversy around the world with regards to the use of serology tests. I know we have access to some serology kits now. What is the current situation and what are our experiences with serology tests?

How sensitive is our RTK antibody test?

As we go forward, the numbers (positive COVID-19 cases) now look good. However, we are still worried about the possibilities of a second surge of COVID-19 as we open up the economy, there will be more human-to-human interaction. So, I think we all need to be prepared for it. Going forward, what are the major challenges we have to foresee in laboratory services in terms of preparing for a second surge?

What does it mean by oropharyngeal and nasopharyngeal being 8%?

Final Messages by Panellists

Dr. Arni binti Talib

Dr. Hani Binti Mat Hussin

Dr. Ravindran Thayan

Datuk Dr. Christopher Lee

Speakers' Brief Bio

“Scaling Up National Lab Capacity for COVID-19” by Dr.Hani Binti Mat Hussin, MKAK Sungai Buloh

Introduction

Thank you, Datuk Dr. Chris. I am Dr. Hani from the National Public Health Laboratory. I had been part of the National team for COVID-19 since December 2019. Good afternoon and Assalamualaikum. This afternoon I will be sharing our experience, in coordinating the laboratory services of COVID-19 for the nation. So, my topic for today is 'Upscaling the National Laboratory Capacities for COVID-19'.

The coordinators for COVID-19 laboratory services are led by our Deputy DG YB Dr.Hishamshah Bin Mohd Ibrahim. The team consists of Dr.Arni Bt Talib, the National Head of Pathologist Services, Dr.Hani Binti Mat Hussin (myself) and Dr.Mawaddah binti Ghazali from Medical Division, MOH and our hero Dr.Ravindran Thayan from IMR.

I will be sharing on the timeline of our national/country laboratory capacity for COVID-19, the laboratory preparedness and a few limitations especially in the initial phase.

COVID-19 pandemic in Malaysia

The COVID-19 pandemic in Malaysia is part of the ongoing worldwide pandemic of coronavirus caused by SARS-CoV-2. The virus was confirmed to have reached Malaysia in January 2020, when it was detected among travellers from China arriving via Singapore on 25 January, following the outbreak of COVID-19 in Hubei, China.

On the 24th of January 2020, the National Public Health Lab (MKAK) received 8 samples from close contacts to the positive case in Singapore. They were travellers from China. We received the samples from Johor Bahru Public Health Laboratory. On 25th of January 2020, 3 out of the 8 samples turned out to be positive for SARS-CoV-2.

Reported cases remained relatively low and were largely confined to imported cases, until localized clusters began to emerge in March; with the largest cluster was linked to a religious gathering held in Sri Petaling, Kuala Lumpur in late February and early March, leading to a massive spike in local cases and an exportation of cases to neighbouring countries.

This is a great challenge for our laboratory in terms of laboratory testing capacity. However, we managed to upscale on the laboratory real time PCR testing capacity. Furthermore, lately there are other methods of laboratory testing like Rapid Test Antigen (RTK Ag) and the Rapid Test Antibody (RTK Ab) which we use for follow up on Day 13.

Timeline National/Country Laboratory Capacity for COVID-19 (RT-PCR)

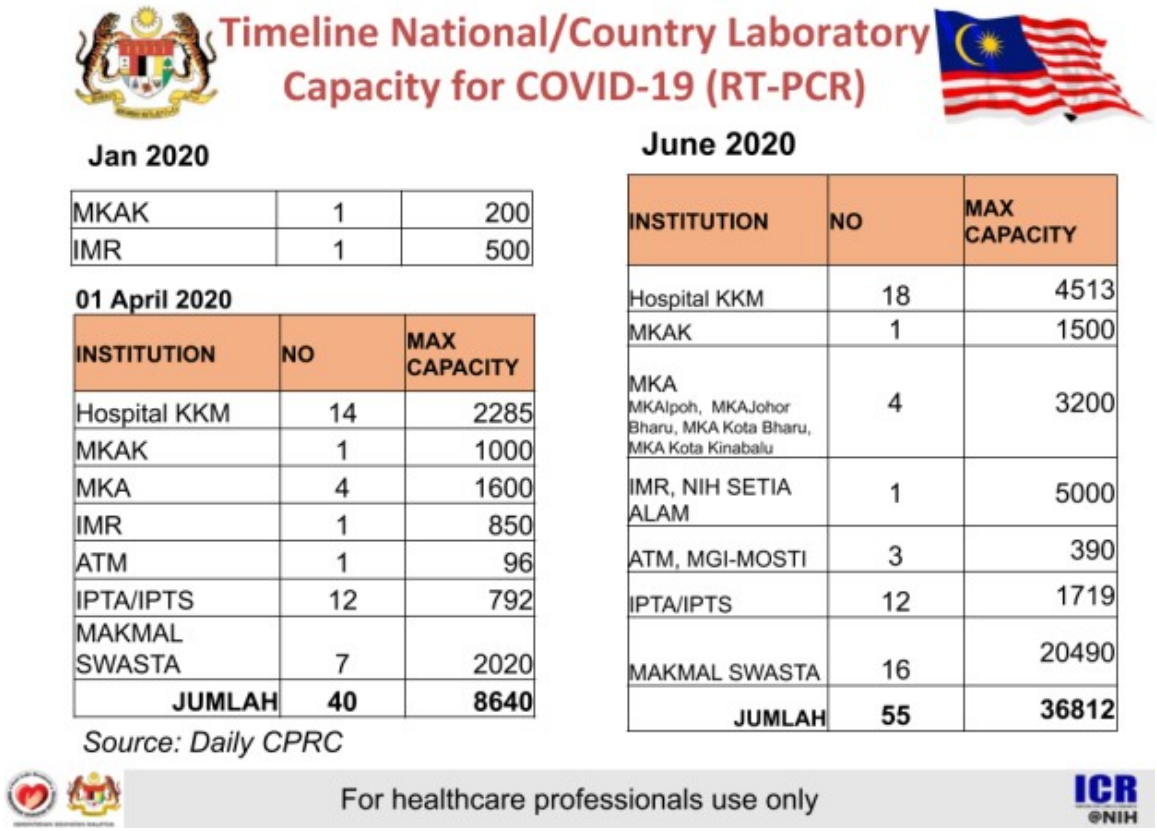


Diagram 1: Malaysia's laboratories testing capacities from January 2020 until June 2020.

The next is the timeline of National or Country Laboratory Capacity. In January 2020, there were 2 laboratories: the National Public Health Laboratory (MKAK or NPHL) and the Institute for Medical Research (IMR) doing RT-PCR for COVID-19 with capacity of about 700. By mid of February, we upscaled the RT-PCR for COVID-19 testing capacity by recruiting MOH hospital laboratories, all 4 other regional public health laboratories, laboratories from the universities, military hospitals and the private sector. So, in early April, a total of 40 laboratories are ready to do COVID-19 testing with a maximum capacity of 8640. And now, our COVID-19 testing capacity is 36,812 by 55 laboratories.

Laboratory Preparedness for SAR-CoV-2 Viral Outbreak

We increased our laboratory preparedness for SAR-CoV-2 during the pandemic by:

- ❏ Strengthening Surveillance Activity For COVID-19
- ❏ Enhancing Country Laboratory Testing Capacity For COVID-19
- ❏ Reviewing Laboratory Test Protocol and Validation
- ❏ Strengthening Laboratory Biosafety and Biosecurity
- ❏ Enhancing Laboratory Information System (SIMKA)
- ❏ Reviewing, Preparing and Expanding Central Procurement
- ❏ Scientific Data Contribution

Strengthening Surveillance Activity for COVID-19

Ongoing Influenza like Illness (ILI) Surveillance Program - Upon alerted by unusual cases of respiratory illness of unknown aetiology reported in Wuhan, China in December 2019, a close monitoring of ILI Surveillance activities were heightened to support early response and rapid case detection towards potential outbreak of COVID-19 in Malaysia

Now, we still continue the close monitoring and surveillance for both ILI and Severe Acute Respiratory Infection (SARI) activities with the purpose to detect any transmission of SAR-CoV-2 in the community. We do this by expanding the sentinel sites (in health facilities) throughout all the states.

Enhancing Country Laboratory Testing Capacity for COVID-19

The technical training has been intensified and expanded beyond Ministry of Health facilities such as universities and private sectors. The training was conducted by IMR using an in-house primer developed by Dr.Ravi. These trainings cover pre-analytical and analytical requirements of the testing; specimen collection, packaging and transportation and safe use of Personal Protective Equipment (PPE) as well as relevant technology transfer (RT PCR) for COVID-19. In early stages, we communicated with the World Health Organization (WHO) especially for the support on the reagent, primer and probes.

Reviewing Laboratory Test Protocol and Validation

Protocol development and validation for testing of unknown pathogens was also revised to support laboratory testing for COVID-19. Series of technical trainings focusing on molecular testing were conducted in house. The training was expanded to all regional Public Health Laboratories and completed by early January 2020.

Strengthening Laboratory Biosafety & Biosecurity (NPHL)

Next, strengthening laboratories biosafety and biosecurity for NPHL followed by all public health laboratories and later to all the laboratories.

Through Institution of Biosafety and Biosecurity (IBBC) team has look into the in-house biosafety and biosecurity requirement and practices of the laboratory, with reference to WHO and CDC guidelines which focused on

- Adequate training on handling high risk organism,
- Transportation requirement of the biological material,
- Procedures relating to the laboratory waste etc.

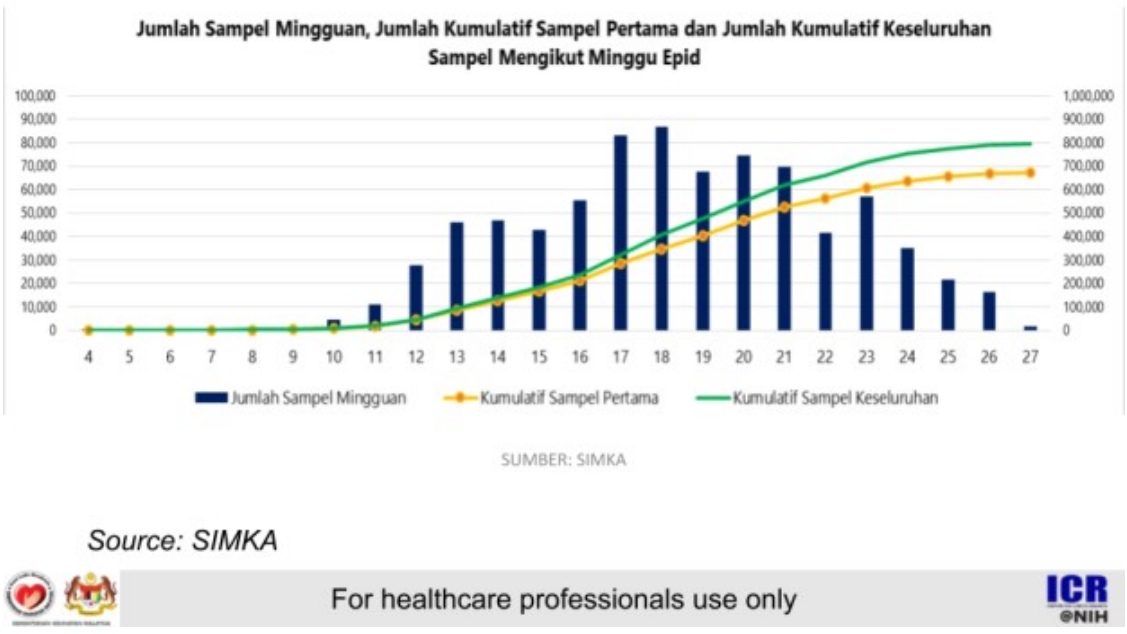
Enhancing Laboratory Information System (SIMKA)

NPHL has developed Sistem Makmal Kesihatan Awam (SIMKA) an in-house web-based Laboratory Information System (LIS) to support data collection for disease outbreak and surveillance program, developed by in-house Information and Communication Technology (ICT) programmer of NPHL.

SIMKA Outbreak was launched on the 16th of Mac 2020. Initially, the system was used by all regional public health laboratories and later was mandated by the MOH as the main system to be used for centralized data collection for the country by all laboratories including MOH facilities and also private and universities.

In addition to laboratories, the mandate also goes to all frontliners using Rapid Test Kit (RTK) to use SIMKA to capture data collection on RTK activities on site.

To further enhance system utilization, new modules – Online Ordering Test and user dashboard have been incorporated into the system. Currently, it is in the process to be integrated with a system under the Crisis Preparedness Response Centre (CPRC), MOH - eCOVID.



Source: SIMKA

Diagram 2: Graph generated from SIMKA showing total samples according to weeks (included first sample, repeated samples)

Diagram 2 is an example of what we managed to compile from all the laboratories including the private laboratories. However, there are some limitations on the details we can get from the private laboratories. From the data collected, we can customize and monitor specific variables. For example, the special target groups such as close contacts or religious groups. We also managed to monitor laboratory turnaround time, the positivity rate etc. The information generated help in the policy making.

Scientific Data Contribution (NPHL)

NPHL is equipped with a high containment laboratory - Biosafety Level 3 (BSL3) Laboratory, Virus Isolation laboratory, Electron Microscope Suites, Molecular Laboratory, genomic sequencer and trained laboratory personnel. This allows NPHL to enhance its approaches handling COVID-19.

The scientific data contribution mainly comes from IMR and I will share the NPHL scientific contribution. NPHL is the first laboratory that identified and confirmed the presence of SARS-CoV-2 virus in 3 out of 8 contact samples received from the first cluster (on the 24th of January 2020). The positive samples identified were then cultured in Vero E6, MDCK, MK2 and A549 cell lines which showed presence of Cytopathic Effect (CPE) in the Vero E6 cell line. The result was reconfirmed by Real Time Polymerase Chain Reaction (PCR).

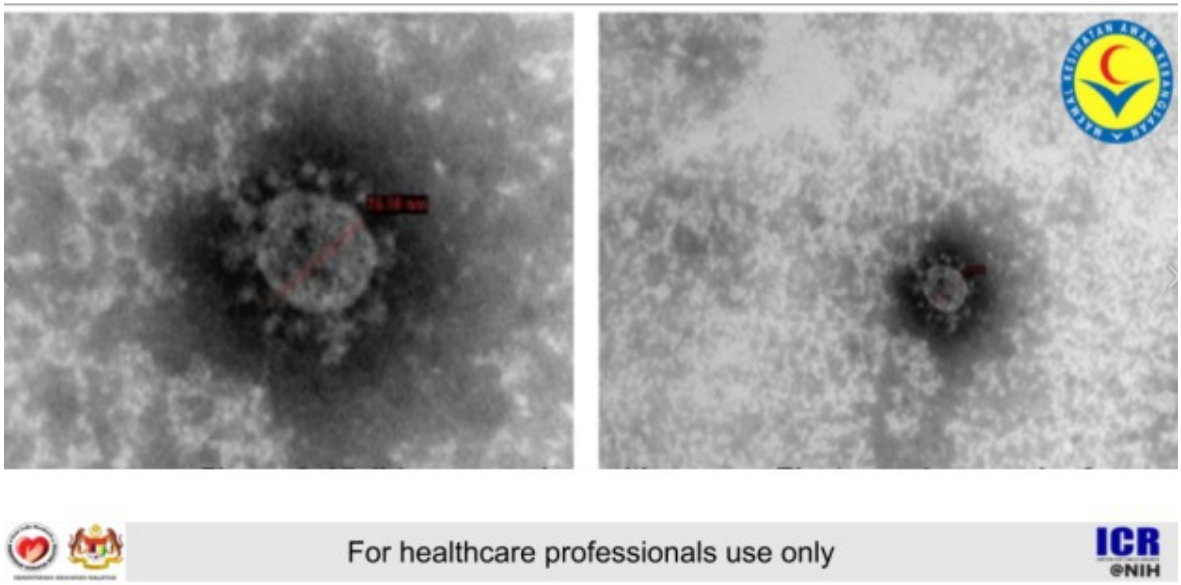


Diagram 3 - Transmission Electron Microscope (FEI, Tecnai spirit G2 120kv) showed visible coronavirus with crown.

Reviewing, Preparing and Expanding Central Procurement

This is the main part. The NPHL reviews, prepares and expands the central procurement. We conduct disaster procurement to ensure uninterrupted laboratory supplies to all laboratories under MOH. That means NPHL is in charge of central purchasing for all the hospital laboratories and public health laboratories including IMR with the help of the finance department in MOH.

Limitation

We had a few limitations but this was in the early phase. Firstly, there was a lack of local suppliers and unavailability of local production for laboratory supplies has caused a significant delay in securing adequate supply for the laboratories. Furthermore, the major laboratory supply producing countries are also affected by COVID-19 outbreak which makes the situation worse. For example, Korea, Germany and also Italy.

Secondly, during the early phase of pandemic, there was a sudden increase in workload beyond the maximum laboratory capacity and the limited capacity of outsourcing laboratories, has caused delay in laboratory Turnaround Time (TAT) for testing. This occurred after Epid week 12 where we had a cluster in a religious group at Sri Petaling. However, we managed to overcome the limitation by upscaling the country laboratory capacity.

Few words before I end. *We test, we manage and we must win*. So that's all. Pass back to Datuk Dr Chris.

Slide presentation: https://cutt.ly/mpPwPBT

Video: https://youtu.be/2enmIG6cSl0

“IMR’s Role in Viral Outbreak Investigation: COVID-19 Menace” by Dr.Ravindran Thayan, Infectious Diseases Research Centre, IMR, NIH

Thank you very much Datuk Chris. A very good afternoon to everyone, Datuk Chris, Dato Goh, ICR members for organizing this webinar for us to share our experiences.

Introduction

I will be talking about the laboratory preparedness of IMR in the face of COVID-19, laboratory procedures of testing of COVID-19, coordination of laboratory services for COVID-19, escalation of COVID-19 testing in Malaysia and also shared some selected research findings we had so far.

COVID-19 (Circulation Worldwide) as of 01 July 2020

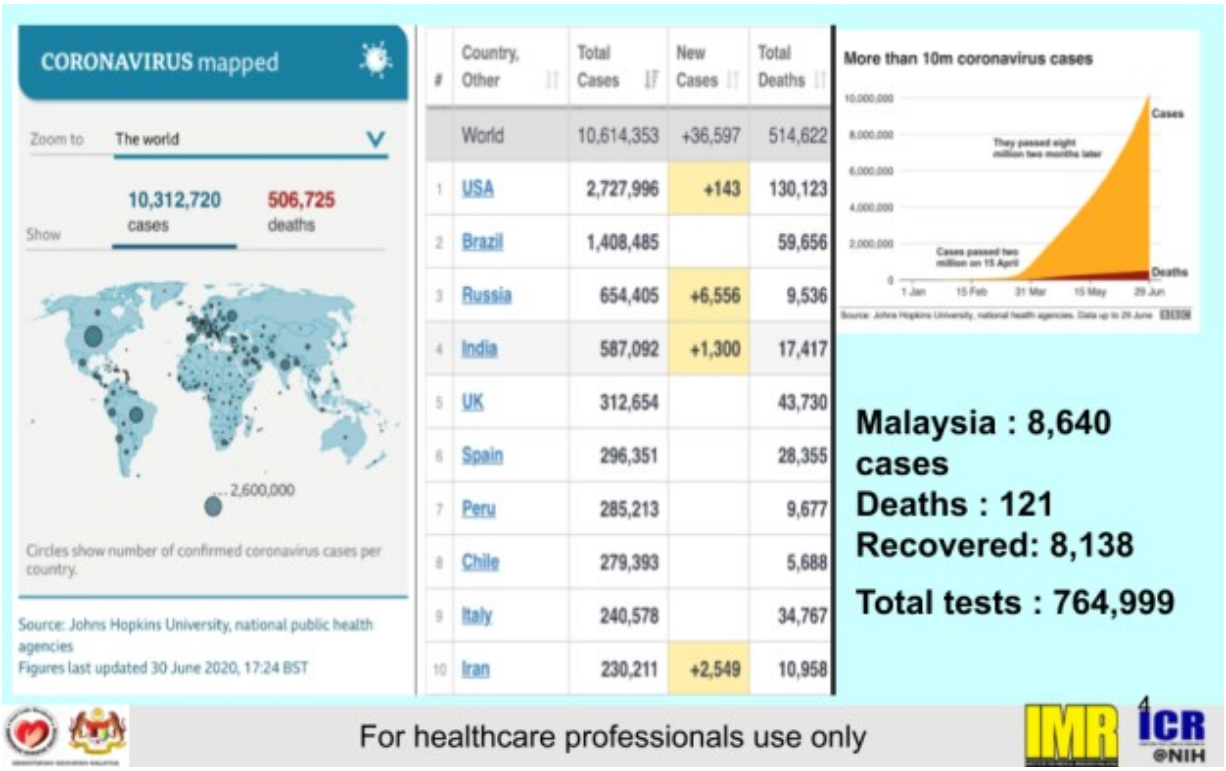


Diagram 4: An overview of COVID-19 cases as of 1st July 2020

Since yesterday, there have been more than 10 million cases (Diagram 4). We did not have any case yesterday. So far, we had 8640 cases. The death remained at 121 and 8138 recovered. We had tested nearly 800,000 cases.

Laboratory Preparedness

The Virology Unit, IDRC, Institute for Medical Research (IMR), Ministry of Health Malaysia is the National Reference Laboratory for viral infections especially for novel, emerging and re-emerging viral Infections.

Back in 2009 or eleven years ago, we had a pandemic influenza outbreak. We had learned a lot of lessons from it. We had decentralized molecular testing and at the same time we had developed our own capacity to detect viral infections especially those of public health importance including viral haemorrhagic fever such as Ebola, Marburg, Lassa, Crimean Congo Haemorrhagic fever virus, novel coronaviruses, Yellow fever, West Nile Virus, Rift Valley Virus, Rabies and others.

How do we go about it? We basically looked at the polymerase chain reaction (PCR). Of course, we don't have the virus. We have the synthetic apex virus which we used as positive control.

In late December 2019, there were rumours or news on the possible emergence of new coronavirus in China. We revisited our standard operating procedure (SOP) for the detection of coronavirus using pan-coronavirus reagents which could detect all coronaviruses. We were not sure whether it can be of use for this new coronavirus.

Fortunately, the scientists from China had shared the full genome sequencing of COVID-19 in a common sharing database (GISAID and Genbank). IMR is the WHO National Influenza Centre which enables us to access to the genomic materials. We have officers who are able to design and synthesize primers and probes specific to COVID-19. This enabled us to order reagents and it arrived quite soon at that point of time, within a week.

Laboratory Testing

The reagents for identification of COVID-19 arrived at our laboratory on 21 Jan 2020. At the same time, Prof. Dr. Jamal from University Malaya Medical Centre (UMMC) had provided inactivated positive control (which belongs to the same family) as positive control for the tests. The positive control was SARS-CoV-1 which shared 85% similarities with SARS-CoV-2.

The officers from IMR had then successfully optimized the real time RT-PCR for the detection of COVID-19 by 22 January 2020. We had a confirmed case from China who entered Singapore and then proceeded to travel to Malaysia. We were able to provide the reagents to MKAK (National Public Health Laboratory), Sungai Buloh to test contacts of a confirmed case in Singapore on 25th January 2020.

Preparations of Staff

We started with a routine briefing for all the staff involved in the testing for COVID-19. We taught them on the correct donning and doffing of personal protective equipment (PPE). The staff includes officers and support staff within IDRC as well as staff from other centres in IMR were trained before they were included in the COVID-19 testing work.

SARS-CoV-2 is something new. So, we collected baseline serum from all staff before they get involved in COVID-19 testing. Then we collected the serum after 3 to 4 months for comparison pre- and post-service.

Laboratory Procedures for Testing COVID-19

At the same time, WHO shared a protocol based on Corman/Drosten 2020 (Corman/Drosten earlier developed a protocol for SARS-CoV-1) on the 17th January 2020 which utilized real time RT-PCR platform to detect COVID-19. The protocol included two gene targets:

- "E gene" = screening target (First line screening assay: E gene assay)
- "RdRp Gene" = confirmatory target (Confirmatory assay: RdRp gene assay)

The interpretation of the result depends on the test kit that was used. Some test kits have Ct values of 38 as cut off point and some kits have Ct values at 40. Any Ct values below 38 - 40 can be considered as positive whereas Ct values above 40 are considered as negative. We also have to look at the curve whether it is a sigmoid curve. However, if the initial sample (first time) has Ct values between 38-40, we will suggest repeating the test with fresh NPS. The Ct values between 38 and 40 indicate low viral count (<10) and we were not able to confirm whether it is truly infective or not. Therefore, we will request for a second sample.

Moving forward, WHO also shared that in places with high prevalence of COVID-19 virus, we do not need to do two target genes. If there is a need to use a single target gene (with only a confirmatory target), then the laboratory has to perform "correlation study" to compare those samples with presence of two target genes and re-test with the single target gene.

Reporting of Results

All results must be reported immediately to Crisis Preparedness and Response Centre (CPRC) Ministry of Health Malaysia. This is because after testing, there is a need to isolate infected patients and to conduct contact tracing. In addition, results are also reported simultaneously to requesting hospitals.

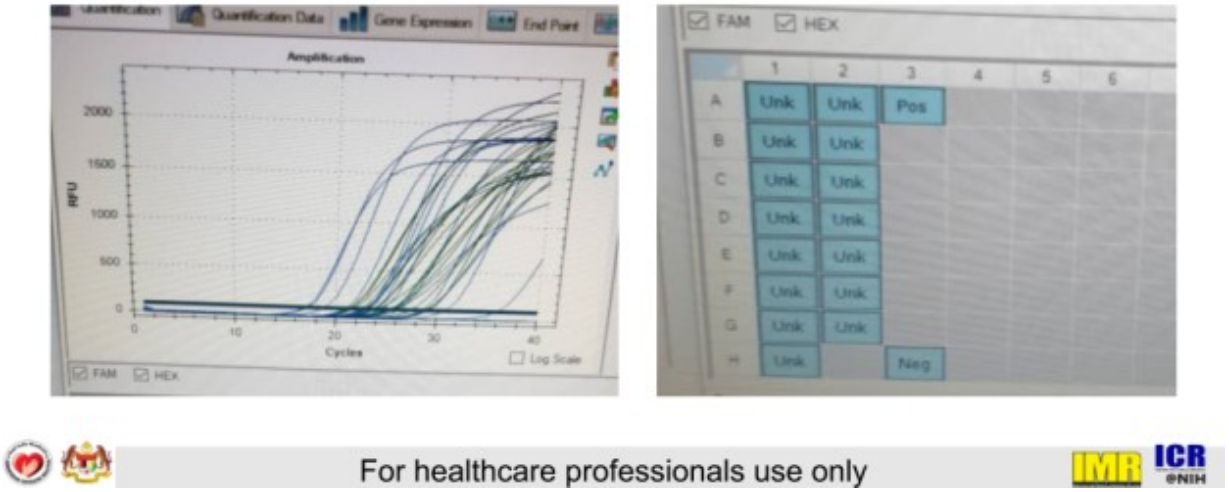


Diagram 5: Testing result

The diagram 5 showed a typical sigmoid curve and this is how it looks like. We have an online reporting system based in MKAK called SIMKA where everyone has to report in the same database.

Training for MOH Hospitals and Public Health Laboratories

The Virology Unit, IMR had conducted training on laboratory testing for COVID-19 to all state hospital laboratories except Kangar as well as five public health laboratories, on 30 January 2020 to enable decentralization of laboratory testing for COVID-19.

At the initial stage (first two weeks), they sent the detected positive cases to IMR to be reconfirmed to ensure accurate reports at that point of time. IMR also provides COVID-19 External Quality Assessment Project (EQAP) to all MOH testing laboratories to ensure competency in them.

To increase the capacity of testing, we evaluated the capability of private laboratories. This was done because we (IMR and MKAK Sg Buloh) couldn't cope with a large number of samples. Also, the samples needed to be tested immediately so that we can trace and contain them. Because of this, we wanted more facilities to be able to test.

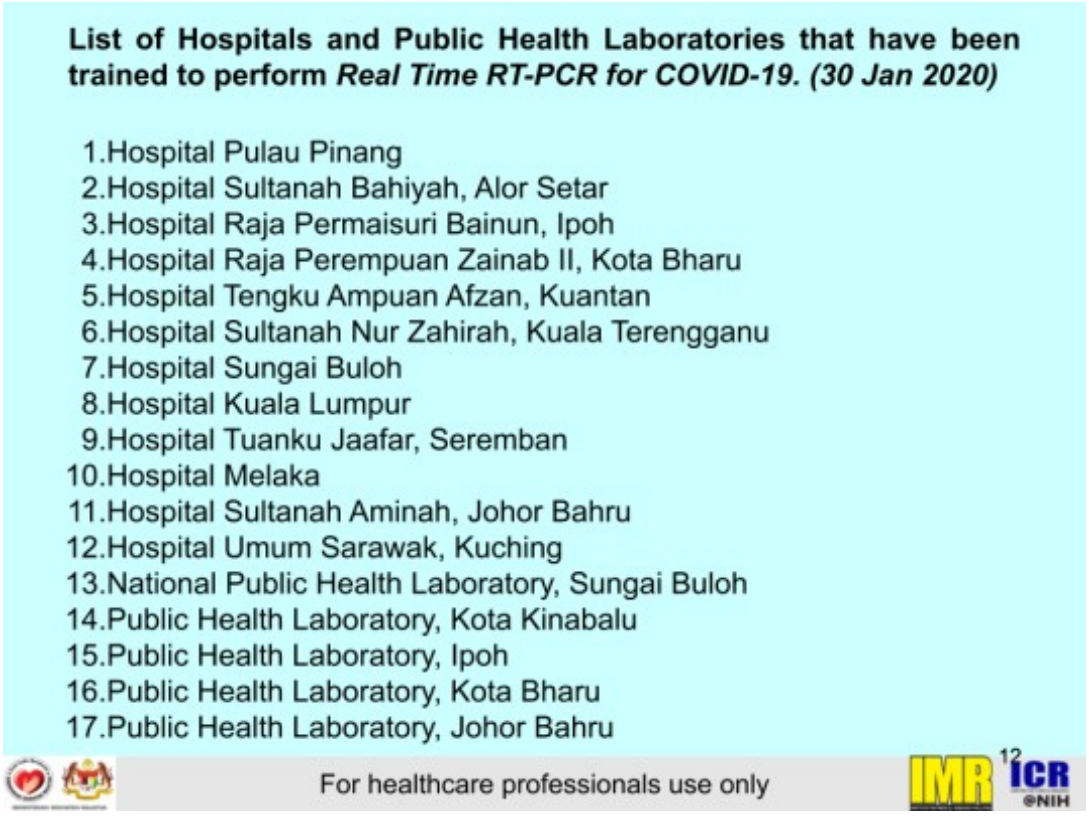


Diagram 6: List of Hospitals and Public Health Laboratories That Have been trained to perform Real Time RT-PCR for COVID-19 (30 Jan 2020)

Diagram 7: Locations of Public Laboratories that can Perform Real Time RT-PCR for COVID-19 in Malaysia

Training for 17 laboratories was done on 30th of January 2020 (Diagram 6). Diagram 7 showed the location of all the laboratories with molecular testing. We also want to include more hospitals like Sibu and Tawau in future. Currently they may not have the service at their laboratories. We planned to give these hospitals a GeneXpect for the detection of SARS-CoV-2 virus.

Coordination of Laboratory Services of COVID-19, Ministry of Health

Diagram 8: Team behind the coordination of laboratory services of COVID-19 in MOH Malaysia.

The coordination of laboratory services of COVID-19 consist of Dr.Hishamshah Ibrahim, the advisor and the members are Dr.Arni Talib (HKL), Dr.Hani Mat Hussin (MKAK), Dr.Mawaddah Ghazali (MOH) and Dr.Ravindran Thayan (IMR). The role of this committee is to coordinate lab services like providing suggestions, purchasing of reagents/equipment, setting-up assays, monitoring reagents, evaluation of kits, testing laboratories etc.

Accreditation of Private Laboratories to perform COVID-19 rRT-PCR

The first engagement with private laboratories and universities was held in early March in MKAK Sg Buloh. We invited them for training and asked whether they can offer testing.

Both IMR and MKAK are involved in the accreditation of private laboratories to perform COVID-19 rRT-PCR. Initial stage, they have to show that their laboratories are able to detect the SARS-CoV-2 screening gene target and confirmation gene target, show their sigmoidal curve and testing report. Then we will provide the known positive samples for testing as the first line of evaluation. Once the laboratory has shown that they can detect the positive samples and provide the graph (basic model), then IMR will provide the blinded samples (some are positive and some are negative) to see if they can detect according to our Ct values. This is the second line of evaluation. Once the laboratories started to provide COVID-19 testing service, they also had to send the first 5 positive and negative COVID-19 samples to IMR to see correlation. This is the third line of evaluation. After they passed all three evaluations, these laboratories will be listed in the Laboratories Guidelines Annex 4a [1]. The private laboratories listed in Annex 4a is to notify people who want to send their samples to the accredited private laboratories. This will help to increase the testing capacity in the country.

Escalation of COVID-19 testing in Malaysia

The escalation of COVID-19 testing service was started from February 2020 onwards. The maximum capacity now is 36,812 tests per day. There are 56 laboratories involved in the upscale of the laboratory testing:

- 6 from IMR and National Public Health Laboratories,
- 18 from MOH hospital laboratories,
- 2 from ATM,
- 12 from universities,
- 1 from MOSTI and
- 17 from private laboratories.

Escalation of Testing in IMR

In the early days, our IMR laboratory in Jalan Pahang was only able to do 800 to 1,000 tests per day because the equipment was mainly manual extraction and shortage of staff. From 5th May 2020 onwards, we have increased the capacity to 5,000 tests per day in the new IMR laboratory in NIH. The escalation of testing was possible with the automation, more equipment and increase in manpower.

Data entry to the system is one of the important steps. For example, if we have 1,000 request forms, we need manpower to enter the data into the system.

Diagram 9: An overview of what happens in IMR when the sample arrived.

When we receive samples, we will process the samples. One important step we have to do is to inactivate the samples to reduce risk for the staff working in IMR. Then, PCR extraction and we have staff who validates the result before releasing the result into SIMKA where the users (e.g. hospital staff, district health officers) can trace the result. The result is available online, in real time.

Selected Research Findings

Study on best clinical samples for COVID-19 detection

I am sharing some of the research findings that IMR managed to do in this small window of time. We were looking for the best clinical samples for COVID-19 detection. Of course, based on recommendation, the best sample would be from the lower respiratory tract as it is not easy to obtain sputum from many of the patients. The combination of nasopharyngeal swab (NPS) + oropharyngeal swab (OPS) gave 51.6% of positivity followed by NPS alone (19.1%) and OPS (16.5%). The best is always the combination of NPS + OPS.

rRT-PCR Ct values and infectivity

We know that some of them were having long or months of positivity and we couldn't discharge them. At that point in time, we also looked at the rRT-PCR Ct values and infectivity. We worked closely with the Infectious Disease (ID) team in Hospital Sungai Buloh. We looked at those patients with Ct values from 32 to 40 on Vero-E6 cells. Ct values is a proxy for viral load. We found that samples with Ct values above 34 had no growth in Vero-E6. This means that there was no risk of infectivity. Therefore, patients with these Ct values can be discharged. However, we have guidelines on discharge criteria. This is to show that the patients who were sent home and after two week they came back with positive Ct values of 38 were actually not infective.

Local COVID-19 strains

We did some full genome sequencing. We have sequenced and shared up to 15 COVID-19 isolates in the GISAID database. This includes the viruses isolated from the first wave and second wave. When we looked at the phylogenetic analysis of the samples of SARS-CoV-2 full-genome sequences, we found that most of the local COVID-19 strains were from strains B and C.

Strain B can be divided into B ancestral strains and B derived strains. The B ancestral strains came from Wuhan and it is closely related to Wuhan strain. Initially, it was B ancestral strain and it passed on to a lot of people. Because it is a RNA virus, it will mutate as it passes from person-to-person becoming B derived strain. From the first generation, it can mutate to the second generation and to the third generation and so on. We will see a lot of mutations. We also see a distinct branching out of strain C, which was mainly from Europe and Singapore and also some local cases from Sri Petaling gathering.

We are fortunate as we didn't find any distinct mutation such as D614G mutation which is based on spike protein. There are some papers looking at D614G mutation. This gene mutation has been associated with high transmissibility. Some papers reported antibodies found in patients who had been infected with earlier forms of the SARS-CoV-2 pathogen failed to neutralize the D614G mutated strain.

Mutations in Spike Protein of Malaysian SARS-CoV-2 Strains

Nucleotide position in Spike protein	Amino acid change	Strain ID	Role	Type of mutation
G1600T	V534I	IMR_WC2665	Unknown	Active mutation
C2367T	-	IMR_WC2665, IMR_WC2423, IMR_WC1453, IMR_WC1018, IMR_WC2453	Unknown	Silent mutation
Deletion	Del QTQTN	IMR_WC1098	Possible rapid transmission or just a random mutation caused by cell passaging	Deletion

Table 1: Mutations in Spike Protein of Malaysian SARS-Co-V-2 Strains

We are looking at the mutations of SARs-CoV-2 spike protein. Active mutation means there are amino acid changes. Silent mutation is without any amino acid change and it does not cause any kind of structural change.

We observed in one case where a deletion occurred at 5-amino acid but we couldn't see any change in that particular strain of virus. Sometimes deletion can result in the "disappearance" of that particular virus or the virus just "disappear". Most importantly, the most frequently reported mutation D614G in the spike protein was not found in any of the Malaysian strains. Other reported mutations such as S943P, V367F, V483A were also not found in our strains.

That is all. Thank you for your attention.

Reference

1. Ministry Of Health, Malaysia. Annex 4a - SENARAI MAKMAL YANG MENJALANKAN UJIAN RT-PCR BAGI COVID-19. Updated until 2020 June 15. http://covid-19.moh.gov.my/garis-panduan/garis-panduan-kkm/Annex_4a_Agihan_Makmal_Ujian_16062020.pdf

Full COVID-19 guidelines are available here: http://covid-19.moh.gov.my/garis-panduan/garis-panduan-kkm

Slide presentation: https://cutt.ly/8pS4T7X

“Hospital's Laboratory Services during COVID-19” by Dr.Arni Binti Talib, Head of the National Pathology Services in Malaysia and the Head of the Department of Pathology at Kuala Lumpur Hospital.

Introduction

I jumped into this group much later. I think I joined late in February or sometime in early March. Basically, just to fill up the gap between the health side and IMR side because there are many testing centres which are also from the hospital laboratories. They have been roped in to upscale the testing. They needed someone to become the ears and the eyes (*i.e.* liaison person) for the hospital laboratories. This was when I started to join the group.

Basically everything has been done by Dr.Hani and Dr.Ravi. They have conducted training, but there were some issues with regards to interpretation of tests and troubleshooting of technicalities. Therefore, my role at that time was to organise CME sessions for them, together with Dr.Hani and Dr.Ravi. We discussed issues related to interpretation and the shortfalls in terms of the quality of testing.

We had a few CME sessions and we also have our own WhatsApp group for discussion. The 12 testing centres have done PCR testing activities for influenza as well as MERS-CoV, so we are not new to this testing. All of these testing centres have clinical microbiologists and good scientific officers. There were not many issues, but we just have to make sure the running is smooth, and there is no gap in between. This is because they are not there at the receiving end.

Different laboratory information systems in hospital laboratories

These testing centres have their own systems for them to report to. It was quite a difficult time to get all the data in place - the reports on RT-PCR testing result whether they have detected or not detected COVID-19. The best is to have one system, that was when I decided that we probably should use Sistem Informasi Makmal Kesihatan Awam (SIMKA). There was a lot of hassle in the beginning. Eventually, we managed to put our data entry into SIMKA, which was good. Now, we have a unified data system. Dr. Hani can analyse the data and we can get national data. After that the university and the private laboratories also joined in, and we made sure that all data is entered into one system.

Difficulty in getting equipment and reagents during pandemic

The other thing that we do together is the pooled procurement for all our reagents. At that time, we need to upgrade our laboratories, we need to upgrade our equipment as well. As Dr. Ravi has stated earlier, when there is a pandemic, there is a lot of demand, and the supply is low. It was really stressful, it felt like we had money but we weren't able to get the reagents and equipment, and we weren’t able to move forward. There are a lot of alternative plans that we have to put in place to make sure that the testing can be carried out well.

Stock inventory management during pandemic

We also keep stock of our inventory. Dr.Mawaddah Ghazali from the Medical Development Division has done this part very well. She has been monitoring the stock of all our reagents really well. By doing so, we would know which hospital laboratories are out of stock of reagents, who can redistribute the reagents. We can also coordinate testing, because in some areas, especially in the red zone, there are many testing that need to be done, and some of the laboratories are overloaded. So, we divert the testing to other laboratories which are less loaded. We did this for areas in East Malaysia, we make sure there is a transport of specimens from Sabah and Sarawak to Klang Valley, so that we can help out with our colleagues in East Malaysia as well. There is a need to have strategic planning for the whole system. We have to think of how to do better and to be innovative each time.

Setting up a mini laboratory in KLIA

We also helped out Crisis Preparedness and Response Centre (CPRC) in setting up a mini laboratory in KLIA so that they can provide testing for people who are returning home to Malaysia, so that home surveillance can take place faster and those who are tested COVD-19 positive can be diverted to the hospital as soon as possible.

That is all from me, thank you Datuk Chris.

Q&A Session

What is the cycle threshold (Ct) in terms of PCR platform?

Dr. Ravi: Cycle threshold (Ct) value in terms of PCR platform is in-built into the machine. What they actually measure is the fluorescence signal. During the test, there would be something in which we call a "scope." When the probe binds to our target, that is when amplification will occur, as the signal gets boosted more, it would be captured by a detector in the machine. The threshold is in-built in the machine, which will minus the background, and as it passes the section threshold, it would become positive. This is how we would evaluate, but having said that, it has to be a sigmoidal, S-curve. This is where we would evaluate if it is positive, or not positive.

Let's say, if the Ct value is 40, above 40 would be negative. Having said that, if the Ct value is between two types of samples, what we usually think would be the higher load would be, the lower Ct value, the higher viral load. The Ct value of 50 is the higher viral load which is translated to a higher risk of transmission. As compared to someone with a Ct value of 58, it may not be similar. 34 and above, there is no means of surviving (the virus does not survive). That would give us an indication.

Datuk Dr. Chris: This is just for clarification, as when we changed the guidelines for patients on discharge. Some people did not understand this part very well. It is just a fine tuning of positive, negative results.

Dr Ravi, do you know if studies have been done in Singapore to confirm whether their reported 382-nucleotide deletion strain is attenuated?

Dr. Ravi: 382-nucleotide deletion is a huge deletion, whether it is attenuated or not, we are not sure. We do have deletion, but our deletion is very short, it is about 50 nucleotides in one particular case, which results in 5 amino acid deletions. I'm not too sure, because 382-nucleotide deletion is huge, hence I am not too sure if it is attenuated or not. Having a 382-nucleotide deletion is very unusual hence more data is needed.

What is the sensitivity and specificity of rapid antigen test?

Dr. Ravi: We have conducted the evaluation at IMR. Basically, our take is that at one point, there were not many kits with good sensitivity. At one point, our cut off point was 80%. There was only 1 kit that managed to reach that high sensitivity level. Sensitivity is the ability to pick the sample correctly (those who have COVID-19) while specificity is the ability picking those without COVID-19, (or picking other coronavirus or those with other respiratory diseases). We would have the kits evaluated, currently 1 is being evaluated, but there would be more. When we do evaluation of rapid tests, we need to have patients in the hospital, but currently, we do not have many patients in the hospital. We have to optimise spike samples and we are currently doing it really well, we have tested 2 kits so far.

Does IMR or other hospital laboratories do heat inactivation of specimens prior to processing? How is it done?

Dr. Ravi: To ensure less exposure, we use heat inactivation. For example, for Ebola, the temperature heating up recommended is at 65°C. For SARS-CoV-2 specimens, we use 65°C to ensure it would not affect the quality of testing.

We conducted initial testing and did a comparison. Using the same samples, one with heat inactivation while one without heat inactivation, we compare Ct values and make sure it is identical. The reason why we conduct comparison is to make sure that the initial stages of processing samples are being inactivated, so when it comes out from the subsequent extraction, we can opt for surgical masks as PPE. This is because at one point in time, we also had problems with PPE supply, hence we only use PPE during processing samples, but when the sample goes to the PCR, surgical masks are sufficient.

We also did risk assessments, and we have done the virus isolation from both the samples with and without heat inactivation. The viral culture from the heat inactivated sample did not grow. We also did PCR to ensure that inactivation does not damage the virus and the pick-up rate. It is identical, so no worries.

There is still quite an amount of controversy around the world with regards to the use of serology tests. I know we have access to some serology kits now. What is the current situation and what are our experiences with serology tests?

Dr. Ravi: Serology tests will only tell us about exposure. That's why we do not use it for screening. It would be helpful to look at prevalence. There are 2 types of kits, one is the rapid antibody test, and the ELISA assay. This will tell us about exposure. Usually, IgM peaks around Day 8 while IgG peaks around Day 14. When we are looking at the samples, some of them have RTK antibodies that have very good specificity and sensitivity. More importantly, looking at people who have neutralizing antibodies, it is important to have a neutralizing antibody test.

Previously, when we did this, we had to grow the virus in the BSL-3 lab. Professor Wang Linfa of Duke-NUS Medical School, Singapore has developed the cPASS kit, in which its test ability was to detect neutralizing antibodies, using a BSL-2 lab (no need to grow the virus). The studies we are looking into in IMR include longitudinal studies, to see how long the protective antibodies would last, as well as to look at the seroprevalence (collaboration with NHMS). Therefore, RTK antibodies will only tell us exposure, but more importantly we need to see if the antibodies are protective antibodies or not, we need to do a special cPASS test.

Datuk Dr. Chris: RTK antibodies, of course, it shows seroprevalence. We haven't tested everyone in Malaysia and this will be a good time to look at how widely spread it is because we know a large proportion, up to 85-90% of the population may be asymptomatic.

Dr. Arni: At present, our Ministry of Health (MoH) are already using the rapid antibody test. However, for those who are negative on day 1, the test has to be repeated on day 13 before they are discharged from home surveillance order. This is part of the public health policy.

How sensitive is our RTK antibody test?

Dr. Ravi: If confirmed positive, the sensitivity of RTK antibody test can go up about 90%. Having said that, looking at papers from other countries, we are not sure how many people have the antibodies and how sensitive it is in the population. Papers that came from Hubei, China or Milan, Italy, the RTK antibody test is about 10% in a high caseload setting. We are not sure if the virus is so clever in hiding as it does not expose to the immune system. There are other things that we need to learn as well.

Datuk Dr. Chris: Sure, it also depends on how long after the infection, we do the RTK antibody test. It may affect the result as well.

As we go forward, the numbers (positive COVID-19 cases) now look good. However, we are still worried about the possibilities of a second surge of COVID-19 as we open up the economy, there will be more human-to-human interaction. So, I think we all need to be prepared for it. Going forward, what are the major challenges we have to foresee in laboratory services in terms of preparing for a second surge?

Dr Arni: Well, going forward for a surge, we must make sure that all our laboratories are fully stocked and able to carry out the COVID-19 tests immediately when there is a surge. They should look into the quality of the testing. Quality of the testing is important, because by doing so, whatever test results that we produce are reliable. We are already setting up the quality committee to make sure that the laboratories comply with the standards and with the quality assurance programme. We make sure that these laboratories also take part in the quality assurance programmes in the future.

Other than that, we need to increase the capacity in those areas which we think we can by opening up more laboratories, but these laboratories probably will aim for point-of-care testing types rather than full molecular testing because it is not cost effective to have many molecular laboratories. It is definitely not easy to maintain these molecular laboratories.

What does it mean by oropharyngeal and nasopharyngeal being 8%?

Dr. Ravi: The 8% OPS + NPS indicated that 8% of positive samples in total cumulative samples received and tested at that point of time. Looking at the data here, this was conducted in April 2020. We have almost 1246 positive samples in April 2020, out of all the samples obtained, 649 were combinations of OPS+NPS, in which slightly more than 51%. If we look at nasopharyngeal samples alone, this comprises about 41% while oropharyngeal samples alone comprise about 20%. I would like to clarify that 8% indicates overall.

Datuk Dr. Chris: It is good to clarify. Otherwise they will think that the test is so insensitive. Having said that, a negative test does not mean the patient is not infected. It is not just the technical part of PCR, also how the specimen is taken. Good specimen taking, better result. It also depends on what point (phase) of infection, the sample was taken.

(After the webinar, Dr Ravi edited the slide on OPS and NPS percentage to put a clearer picture. The transcript of Dr.Ravi's presentation no longer has the "8% OPS + NPS" as we used the updated slide for this eBook.)

Final Messages by Panellists

Dr. Arni binti Talib

Well, I think we all have to persevere and keep on our toes all the time, and I think increasing knowledge is important. There is a lot more about COVID-19 that we don’t know. We need to learn up to see what other countries are doing, to improve our testing.

Dr. Hani Binti Mat Hussin

I think the laboratory is the core component of COVID-19 and all infectious diseases. We have to be ready at all times.

Dr. Ravindran Thayan

For us to be there and here, everyone has contributed. Laboratory is one component for us to combat together. It is a very good lesson learned. We need to work together, and this can be used as a model in the future for other viral outbreaks. The pandemic is going to be with us and we have to get used to it.

Datuk Dr. Christopher Lee

As a parting word, I think it is important that yesterday we have no local transmission and we have one imported COVID-19 case. It is a good reminder for us that the virus is still out there. Dr.Tedros from WHO earlier this week declared the worst has yet to come. Obviously, he is looking at the global perspective. We, Malaysia can never be safe without other countries being safe. We are too closely connected. I urge all of us to continue to keep on our toes. I am sure our laboratory staff will continue doing their good work and provide the support as we go forward. So, with that, I hope all of us have a beneficial time this afternoon.

Speakers’ Brief Bio

Dr. Hani Binti Mat Hussin is the Director of National Public Health Laboratory, Sungai Buloh since January 2018. Dr. Hani completed her medical degree from Universiti Sains Malaysia (USM) in 1988 and obtained a Master of Public Health from University of Malaya (UM) in 1997. She was the director of Public Health Laboratory (MKA), Kota Bharu before she was promoted as director of National Public Health Laboratory (MKAK) in the year 2018.

Dr. Ravindran Thayan is a Molecular Virologist by training and is currently Head of Virology Unit, Infectious Diseases Research Centre, Institute for Medical Research (IMR). He is also the Director of WHO National Influenza Centre as well as Coordinator for the WHO National Polio Laboratory. He has been with Virology Unit IMR since 1992 and has been involved in viral outbreak investigation including COVID-19, Polio virus and Measles in 2019, Rabies in 2018, Zika in 2016, Ebola, MERS-CoV and Avian influenza in 2014 and Pandemic Influenza H1N1 in 2009 among others. He is also member of several expert panels including IMR, MOSTI, CREST R&D Grant application as well as Viral Expertise Panel.

Dr. Arni Binti Talib, Head of the National Pathology Services in Malaysia and the Head of the Department of Pathology at Kuala Lumpur Hospital.

Click the link below to view the panellists' information and details of the webinar:

https://clinupcovid.mailerpage.com/resources/w0x5z8-sars-cov-2-viral-outbreak-investi

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Institute For Clinical Research, National Institutes of Health (NIH), Ministry of Health Malaysia, Block B4, No.1, Jalan Setia Murni U13/52, Seksyen U13, 40170 Shah Alam, Selangor Darul Ehsan, Malaysia.

Phone: 603-3362 7700 Fax: 603-3362 7701 Email: contact@crc.gov.my