INA-RESPOND

INDONESIA RESEARCH PARTNERSHIP ON INFECTIOUS DISEASE

KEMENTERIAN KESEHATAN REPUBLIK INDONESIA

NEWSLETTER

May 2020

Comic Corner

Efficacy vs. Effectiveness ~ Explanatory vs. Pragmatic: Thinking for the Best Design to Fit the Objective

TRIPOD and INA-PROACTIVE Studies' Updates

From Our Sponsors

High-Throughput Drug Discovery and the Success of Remdesivir

Science Corner

Diagnostic Aspect of COVID-19 (part I)

NATIONAL INSTITUTE OF HEALTH RESEARCH AND DEVELOPMENT
MINISTRY OF HEALTH REPUBLIC OF INDONESIA
2020


عيد مبارك
Eid Mubarak
A special month for all muslims in the world

INA-RESPOND newsletter

content

May 2020 Edition | issue #80

FEATURES

MASTHEAD

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INA-RESPOND Newsletter

TRIPOD & INA-PROACTIVE Study Updates

By: Eka Windari R., Lois E. Bang, Maria Intan Josi, M. Ikhsan Jufri, Venty Muliana Sari

STUDY UPDATES

INA102

PARTICIPANT STATUS

Per 05 May 2020, the total ongoing participants in the TRIPOD study are 52 out of 490 enrolled participants. From those 52 ongoing participants, 37 are still on TB treatment while 15 are waiting for a 6-month post-treatment visit. Two hundred and seven participants have completed the study, while 231 participants are terminated early (including death). Therefore, there are still 10.6 % of participants from the total enrolled participants in the follow-up status. From the uploaded CRFs, all participant form site have been completed the study. At the same time, there are 1 participant from site 550 (RSUP dr. Wahidin Sudirohusodo Makassar) who still need to be followed up, 21 participants from site 560 (RSUP dr. Kariadi Semarang), 5 participants from site 570 (RSUD dr. Soetomo Surabaya), 8 participants from site 580 (RSUP dr. Sardjito Jogjakarta), 16 participants from site 590 (RSUP Persahabatan Jakarta), and 1 participant from site 600 (RSUP dr. Adam Malik Medan).

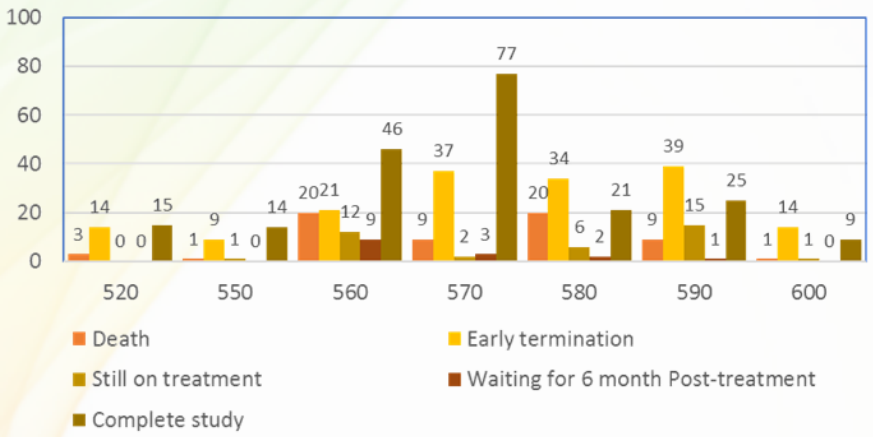

Figure 1.Participant status per site based on uploaded CRF per 5 May 2020

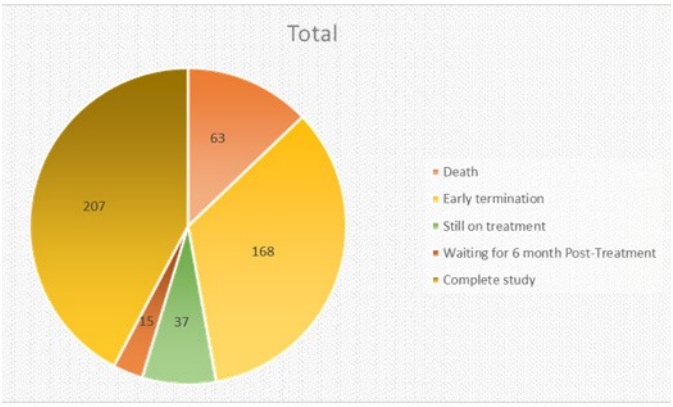

Figure 2. Total participant status based on uploaded CRF per 5 May 2020

TRIPOD MANUSCRIPT

The authors for the TRIPOD manuscript has been selected. In the near future, a meeting with NIH will be performed to initiate the progress. The following are several manuscripts that being planned: a) focus on the baseline findings; b) treatment outcome and the related affected factors; c) related factors of TB and DM co-morbidity. The authors will be sorted according to enrolled participants. A discussion will be set up during the Clinical Research Protocol Writing Workshop.

Site number	Site name	Author
520	RS Sanglah Denpasar	dr. I Gede Ketut Sajinadiyasa, Sp.PD
550	RSUP dr. Wahidin Sudirohusodo	Dr. dr. Irawaty Djaharuddin, SpP(K)
560	RSUP dr. Kariadi	dr. Banteng Hanang Wibisono, Sp.PD-KP
570	RSUD dr. Soetomo	dr. Tutik Kusmiati, SpP (K)
580	RSUP dr. Sardjito	dr Bambang Sigit Riyanto, SpPD-KP, FINASIM
590	RSUP Persahabatan	dr. Diah Handayani, SpP
600	RSUP H Adam Malik	Dr. dr. Bintang YM Sinaga, M.Ked(Paru), Sp.P(K)

INA104

INA-PROACTIVE activities have been halted since 15 March 2020. The temporary halt of screening, enrollment, and follow-up activities of the study has been extended until further notice. However, to avoid any missed visits, subjects who have reached the maximum window period can still do their follow up visits to the site. According to our study data per 13 May, a total of 4,282 subjects had been enrolled, which consisted of 4,096 adults and 186 pediatrics from a total of 7,290 subjects screened. Detailed information is shown in Figure 1.

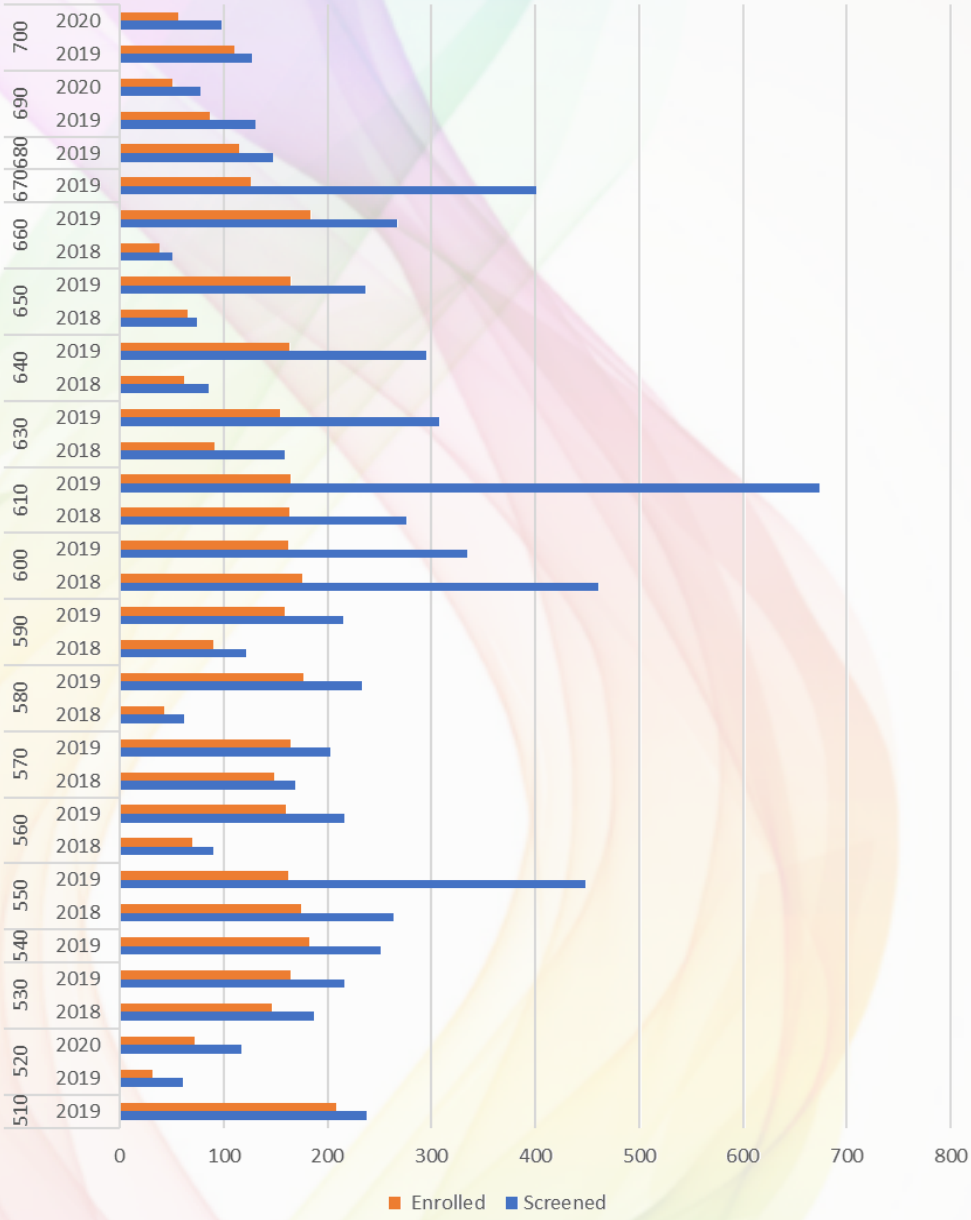

Figure 1. All Site Number Screened vs Enrolled

Regarding study data, some sites are still working on completing potential missing logs, including the completion of study disposition status, especially for the cause of death. The Data Management team is also assisting one of the sites on data entry. A regular bi-weekly teleconference is conducted with all sites' Research Assistants, including one-on-one teleconference if needed.

Remote monitoring was done this month for the following sites:

- 4 May : Remote 5th SMV for Site 610 (Tangerang Hospital)
- 6 May: Remote 2nd SMV for Site 670 (Zainoel Abidin Hospital)
- 12 – 13 May : Remote 2nd SMV for Site 680 (Soedarso Hospital)
- 14 – 15 May : Remote 2nd SMV for Site 690 (Abepura Hospital)
- 15 May : Remote 4th SMV for Site 530 (Cipto Mangunkusumo Hospital)

Site 520 (Sanglah Hospital) study team has asked the permission of INA-RESPOND Secretariat to continue the site's enrollment activities to meet the enrollment goal by the end of June. Some of the site study teams are also enthusiastic and hoping the pandemic crisis will end soon, and INA-PROACTIVE activities will be back to normal.

INA-RESPOND Newsletter

HIGH-THROUGHPUT DRUG DISCOVERY AND THE SUCCESS OF REMDESIVIR

By: Aaron Neal

FROM OUR SPONSOR

The serendipitous discovery of penicillin over 90 years ago launched a wave of research leading to treatments for even the most lethal infections. "It is time to close the book on infectious diseases, and declare the war against pestilence won." Though it is unlikely that U.S. Surgeon General Dr. William H. Stewart ever said or believed this [1], others at the time held a true sense of optimism that the problem of infectious diseases had finally been solved. Unfortunately, all of us at NIAID and INA-RESPOND know all too well that the war against infectious diseases continues to rage on. While drugs, vaccines, and public health practices have helped us fight and even eradicate some infectious diseases, SARS-CoV-2 has been a particularly challenging adversary. One of the biggest concerns with this and other viruses is the lack of known therapeutics to treat patients while scientists work toward a protective vaccine. Thankfully, last month brought much-needed positive news when the NIAID-sponsored Adaptive COVID-19 Treatment Trial (ACTT) found clear evidence that remdesivir could improve COVID-19 patient outcomes [2], leading the U.S. FDA to issue an Emergency Use Authorization (EUA) on May 1 [3]. Now that remdesivir will be part of the standard of care for advanced COVID-19 in the U.S. and other countries, it is worth understanding how the drug works and how it was discovered.

Remdesivir, also known as GS-5734, is a ProTide, or a prodrug that is metabolized into the adenosine nucleotide analog GS-441524 [4]. Nucleoside and nucleotide analogs are powerful tools for combatting viral infections since they interfere with a critical weak point during the virus lifecycle– genome replication. Drugs like ribavirin, which treats the RNA viruses RSV and hepatitis C virus, and acyclovir, which treats the DNA viruses HSV and VZV, are also nucleoside analogs already on the market. Each drug possesses a similar mechanism of action where the molecule is mistakenly incorporated into the viral genome during RNA or DNA replication, resulting in catastrophic errors, synthesis inhibition, and non-viable viruses. In the case of remdesivir, tri-phosphorylated GS-441524 is incorporated by the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) at very high rates, which results in premature viral RNA synthesis termination [5]. Importantly, the nucleoside analog specifically inhibits RdRp and not human or mitochondrial RNA polymeras-

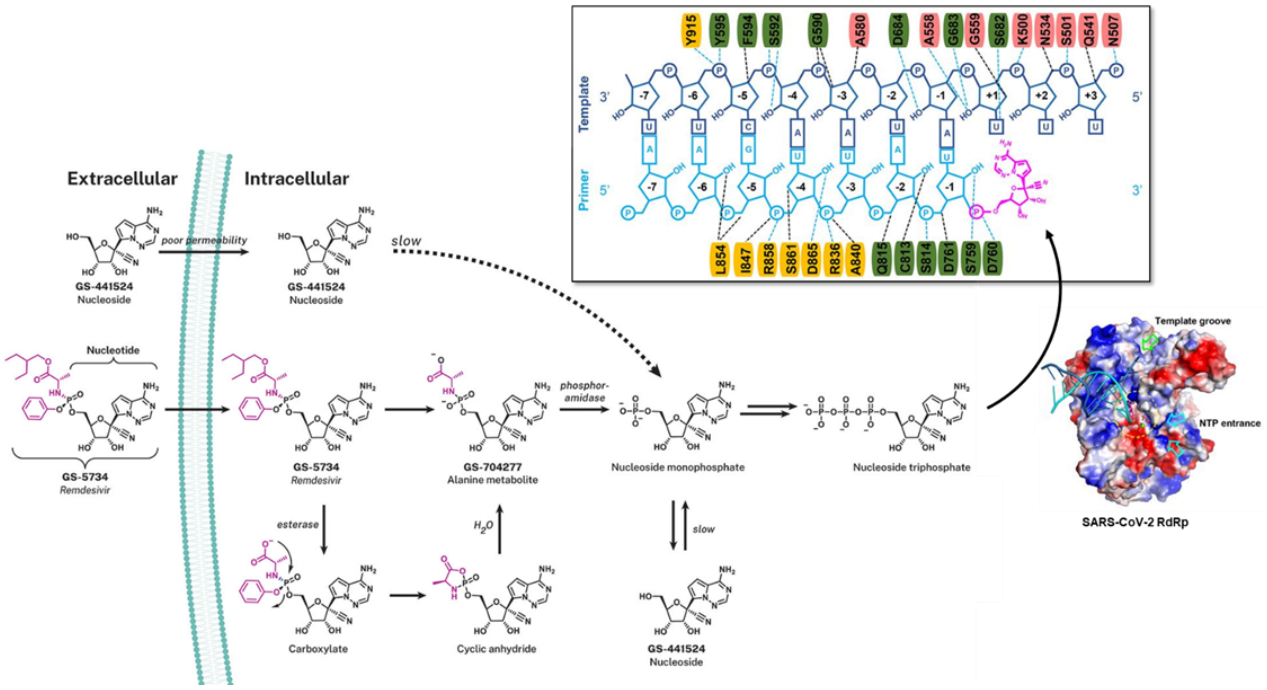

Figure 1. Mechanism of action of remdesivir (adapted from [7] and [8]).

es, possibly due to their increased proof-reading capabilities or molecular structures. Though tri-phosphorylated GS-441524 is a potent and specific inhibitor of RdRps, GS-441524 alone is chemically polar and poorly cell permeable, rendering it less effective.

Furthermore, the molecule must be tri-phosphorylated to become inhibitory, which is a slow process limited by the mono-phosphorylation step. To overcome these barriers, the prodrug remdesivir was designed by attaching a phosphoramidate group to GS-441524 (Figure 1). This increased the cell permeability of the molecule and produced a ready-to-go, mono-phosphorylated form of GS-441524 after the initial metabolism steps [6].

It is exciting to have a successful and well-understood treatment for COVID-19 finally, but the development of drugs like remdesivir for emerging viral diseases is sometimes less elegant and intentional than it may seem. When remdesivir was first created in 2009, long before the recent outbreaks of Ebola, Zika, and SARS-CoV-2, it failed to treat its intended target of hepatitis C [9]. Years later, remdesivir was included in a diverse library of ~1000 nucleoside analogues screened against selected emerging viruses as part of a collaboration between Gilead, the U.S. CDC, and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) [4]. It was during this in vitro screen that remdesivir and its unmodified form GS-441524 demonstrated potency against a panel of RNA viruses across different viral families, leading to the further development of remdesivir as a therapeutic for emerging viral diseases. Promising in vivo results against ebolaviruses led to the first compassionate use of remdesivir in an Ebola patient in October 2015 [10]. However, a subsequent NIAID-sponsored clinical trial in the Democratic Republic of the Congo showed that the drug was an inferior Ebola treatment compared to other available therapeutics [11]. Interest in remdesivir remained, though, leading to rapid in vitro and in vivo studies of its efficacy against coronaviruses, including SARS-CoV-2 [12-13], thus setting the stage for human trials around the world.

Though the complete details of the collaborative drug screen that identified remdesivir have not been published or released, high-throughput drug screens often follow the same general approach. Most experiments begin with a specific pathogen of interest that is capable of being cultured in vitro. Human cell lines are then infected with the pathogen in the presence of a potential therapeutic molecule at physiologically relevant concentrations. After incubation, the viability of the human cells and/or the pathogen is measured to infer if the molecule was protective by preventing cell death and/or killing the pathogen. While this approach can be used to screen any number of compounds, it is most powerful when scaled-up significantly. Rather than only testing a carefully selected handful of compounds that are predicted to be therapeutic, thousands of compounds and randomly generated derivatives of compounds can be blindly tested simultaneously to see what is cytoprotective. A 2015 study looking for new antimalarial drugs and drug combinations demonstrated the power of large-scale screens by testing 2,317 single compounds in 13,910 combinations of different concentrations against three distinct Plasmodium falciparum

parasite lines, producing 728,216 data points [14]. Results from the screen identified valuable new synergistic and antagonistic antimalarial combinations, unique strain-specific variability in antimalarial effectiveness, and vital parasite pathways to target with future drugs.

At the NIH, the National Center for Advancing Translational Sciences (NCATS, https://ncats.nih.gov/index.php) assists scientists interested in quantitative high-throughput screening (qHTS) experiments. The Center established and now maintains an automated robotic facility capable of screening 40 plates of 1,536 wells per hour, which equals about 1.5 million compounds per day, all under BSL-2 conditions (Figure 2). To process the staggering amount of data generated from these screens, NCATS has developed custom bioinformatics pipelines and unique approaches to the statistical analysis of large, matrixed datasets. NCATS scientists also conduct cutting-edge research on compound synthesis, assay miniaturization, automation, and fundamental biological and chemical techniques in order to further advance large-scale drug screen technology. The powerful resources of NCATS are not restricted to the NIH research community, as NCATS regularly partners with universities and medical centers, other U.S. federal agencies, small businesses and industry, and patient groups and advocacy organizations.

The successful identification and clinical evaluation of remdesivir as a treatment for COVID-19 remind us to be optimistic about scientific research and its potential in any situation. Though there is still a need for better therapeutic options for COVID-19, the EUA approval of remdesivir is a significant accomplishment. We know that this will not be the last pandemic, but as long as we continue developing and utilizing research techniques like large-scale drug screens, we can remain hopeful that effective drugs will be identified faster and more frequently no matter the pathogen.

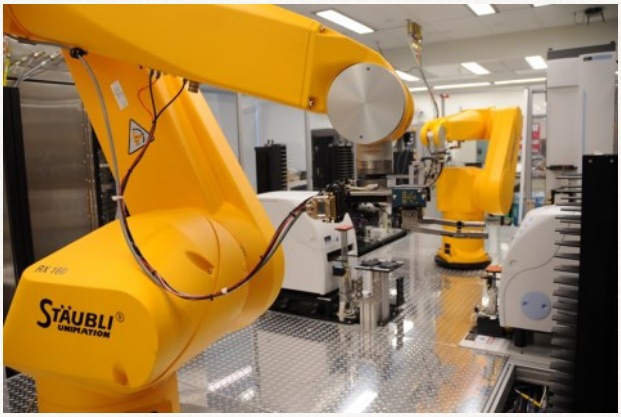

Figure 2. NCATS automated drug screening facility.

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INA-RESPOND Newsletter

DIAGNOSTIC ASPECT OF COVID-19 (PART 1)

By: Yan Mardian

SCIENCE CORNER

COVID-19 has become the whole world's attention since WHO decided to categorize it as Pandemic since last March 2020. Until now, only 12 countries/territories around the globe that spared by the disease or no official report through extensive global tracking. As we know, COVID-19 is caused by SARS-CoV -2, a large positive-sense single-stranded RNA, that comprised of around 30 kbp nucleotide and four structural proteins, i.e., nucleocapsid protein (NP) that holds the viral RNA, spike protein (SP), envelope protein (EP), and membrane protein (MP), that create the viral envelope. This new virus is also suspected of having 10-20 greater binding affinity with the human receptor ACE2 receptor than its precede, SARS-CoV, in 2003, which might explain the greater scope of disease throughout the globe.

In response to the rapidly evolving COVID-19 pandemic, countries have used different testing approaches depending on testing capacity, public health resources, and the spread of the virus in the community. The US-CDC recommends priority for testing 3 groups: hospitalized patients with presentations compatible with COVID-19, other symptomatic persons at risk for poor outcomes, and persons who had close contact with someone with suspected or confirmed COVID-19 within 14 days of illness onset or have a history of travel in an affected area. These patients should be evaluated with a molecular diagnostic test, as described later.

The spectrum of symptomatic infection ranges from mild to critical; most infections are not severe. Specifically, in a report from the Chinese Center for Disease Control and Prevention that included approximately 44,500 confirmed infections with an estimation of disease severity:

Mild (no or mild pneumonia) was reported in 81 percent.

Severe disease (eg, with dyspnea, hypoxia, or >50 percent lung involvement on imaging within 24 to 48 hours) was reported in 14 percent.

Critical disease (eg, respiratory failure, shock, or multiorgan dysfunction) was reported in 5 percent.

Pneumonia appears to be the most frequent serious manifestation of infection, with fever, dry cough, shortness of breath, and fatigue as the most frequent symptoms observed. However, recent data also suggest that gastrointestinal symptoms (diarrhea and nausea), new onset of losing smell and/or taste, conjunctivitis, and dermatologic findings (maculopapular, vesicular lesion) could also become presumptive clues in COVID-

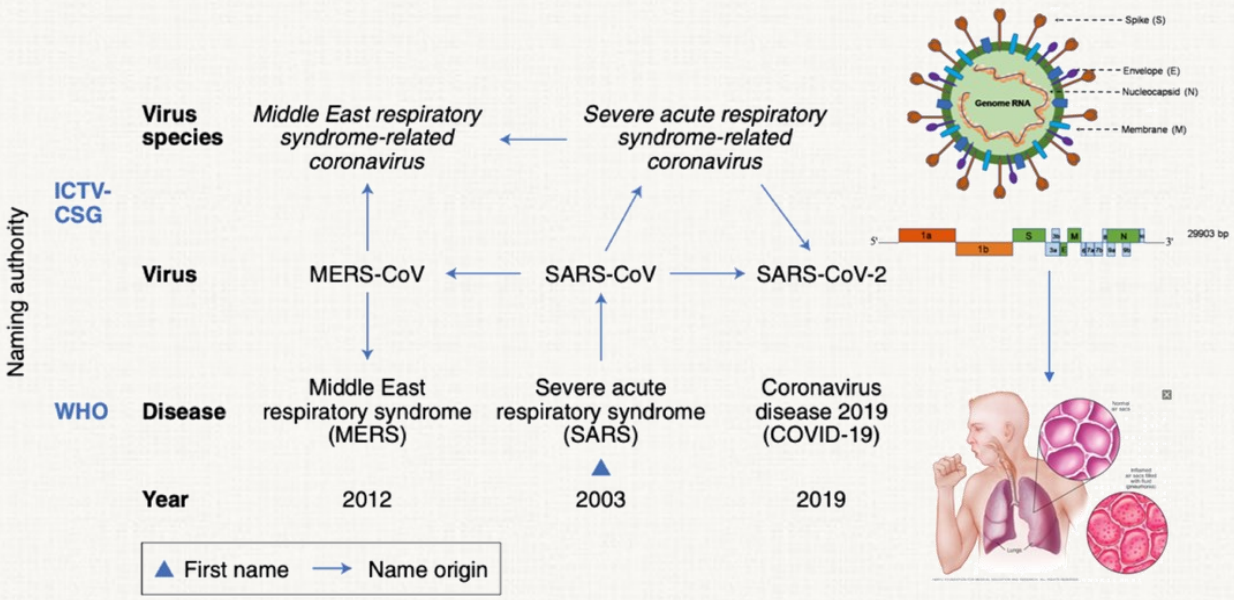

Fig. 1. SARS-CoV-2 causing COVID-19 pandemic

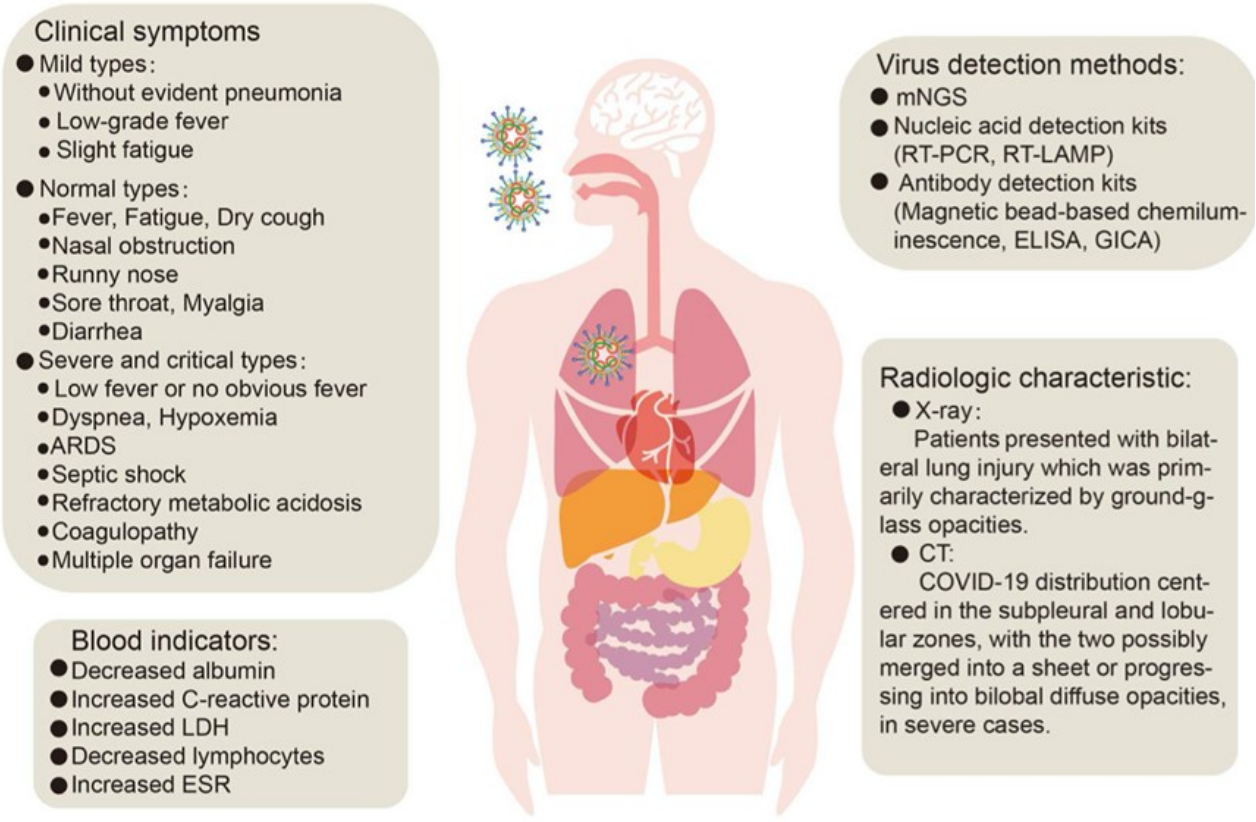

Fig. 2. The clinical manifestation, imaging and laboratory diagnostic of COVID-19

19 diagnosis, especially on people with close contact with confirmed cases.

Typical laboratory findings among hospitalized patients with COVID-19 include lymphopenia, elevated aminotransaminase levels, elevated lactate dehydrogenase levels, and elevated inflammatory markers (e.g., ferritin, C-reactive protein, and erythrocyte sedimentation rate). Several laboratory features, including high D-dimer levels and more severe lymphopenia, have been associated with critical illness or mortality. Chest radiographs may be normal in early or mild disease. Common abnormal radiograph findings were consolidation and ground-glass opacities, with bilateral, peripheral, and lower lung zone distributions; lung involvement increased over the course of illness, with a peak in severity at 10 to 12 days after symptom onset. In a study of 1014 patients in Wuhan who underwent both RT-PCR testing and chest CT for evaluation of COVID-19, a "positive" chest CT for COVID-19 (as determined by a consensus of two radiologists) had a sensitivity of 97 percent, using the PCR tests as a reference; however, specificity was only 25 percent. The low specificity may be related to other etiologies causing similar CT findings. However, chest CT abnormalities have also been identified in patients prior to the development of symptoms and even prior to the detection of viral RNA from upper respiratory specimens.

The confirmatory diagnosis of COVID-19 is made by direct detection of SARS-CoV-2 RNA by nucleic acid amplification tests (NAATs), primarily reverse transcription-polymerase chain reaction (RT-PCR), which is the most widely used of In-vitro Diagnostic (IVD) assays of COVID-19. Upper respiratory samples are the primary specimens for SARS-CoV-2 NAAT. However, false-negative tests from upper respiratory specimens have been well documented. If initial testing is negative but the suspicion for COVID-19 remains and determining the presence of infection is important for management or infection control, repeating the test should be performed

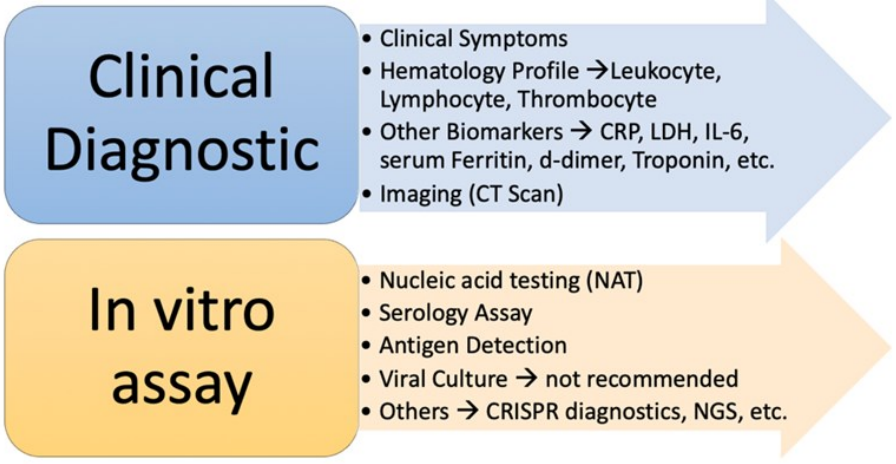

Fig.3. COVID-19 Diagnostic Approach

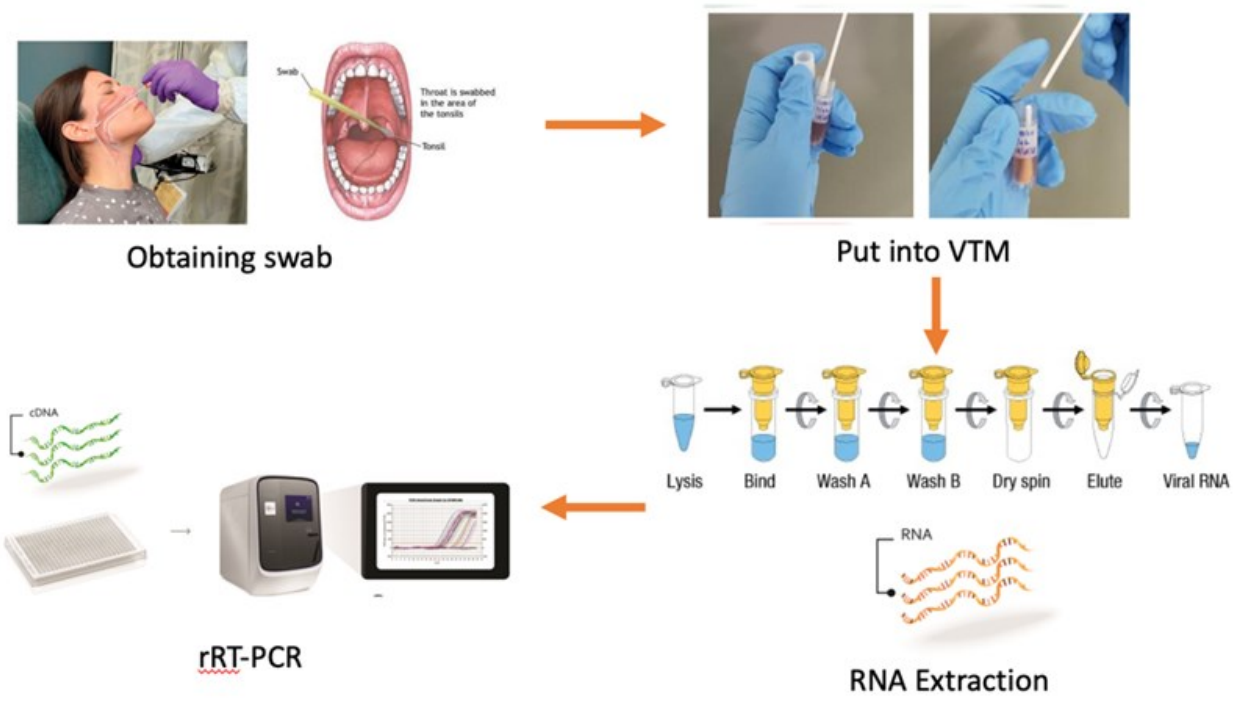

Fig.4. Standard real-time RT-PCR assay procedure

24 to 48 hours after the initial test. In such cases, the WHO and IDSA recommend testing a lower respiratory tract specimen if the patient has evidence of lower respiratory tract illness. Samples should be obtained by using a flocked swab, if available, to enhance the collection and release of cellular material. Swabs with an aluminum or plastic shaft are preferred. Swabs that contain calcium alginate, wood, or cotton should be avoided, because they may contain substances that inhibit PCR testing. Ideally, swabs should be transferred into universal transport medium immediately after sample collection to preserve viral nucleic acid. Inadequate sample collection may result in a false-negative test. After specimen collection, samples undergo RNA extraction followed by qualitative RT-PCR for target detection.

A novel and robust real-time RT-PCR assay were developed by Tib-Molbiol, Germany, in collaboration with various partners by the 2nd week of January 2020. It was highly specific for SARS-CoV-2 RNA and did not cross-react with other coronaviruses. The test detects the SARS-CoV-2 RNA via envelope (E) and RNA-dependent RNA polymerase (RdRp) gene assays. The E-gene assay was used for first-line screening, while the RdRp gene assay was employed for confirmatory testing. In another approach developed by US-CDC, the nucleocapsid (N) gene was used for specific detection of SARS-CoV-2. A cycle threshold value (Ct-value) less than 40 is defined as a positive test, while a Ct-value of 40 or more is defined as a negative test. A Ct-value <40 for only one of the two nucleocapsid protein [N1 and N2] is defined as indeterminant and requires confirmation by retesting.

Currently, there are now over 300 tests for SARS-CoV-2 listed in the diagnostic pipeline of molecular testing. FIND, which stands for Foundation for Innovative New Diagnostics, is working in partnership with WHO to accelerate development and access to diagnostics as part of the global response to the COVID-19 pandemic. This diagnostics resource center is designed to support policymakers and healthcare providers with up-to-date information on tests and testing for SARS-CoV-2. FIND conducted independent evaluations at the Hôpitaux Universitaires de Genève, to verify the limit of detection (LOD) and the clinical performance (as reported by the manufacturers) of the following molecular test kits. The LOD analysis was performed using cultured viral stocks from a clinical isolate from Switzerland and quantified using an E gene standard. The clinical performance analysis was conducted on extracted samples from individuals suspected to have COVID-19 that were tested using an in-house PCR protocol that was optimized based on the Tib Molbiol assay. Data for the first tests evaluated are summarized below. More results will be added as they become available. Tests were selected for evaluation according to the scoring criteria, but the order in which the assessments were conducted does not reflect any endorsement or prioritization.

However, despite being highly accurate, conventional real-time RT-PCR assay often requires hours to generate a result and

Company	Gene target	Verified LOD (copies / reaction)	Avg Ct (lowest dilution 10/10)	Clinical sensitivity (50 positives)	Clinical specificity* (100 negatives)	Product No.	Product name	Lot No.	PCR platform	Supplier recommended Ct cut-off
altona Diagnostics	E	1–10	35.45	92% (95%CI: 81, 97)	100% (95%CI: 96, 100)	821003/ 821005	RealStar® SARS-CoV-2 RT-PCR Kit 1.0	023567	BioRad CFX96 deep well	None; any signal can be considered positive
	S	1–10	35.99	92% (95%CI: 81, 97)	100% (95%CI: 96, 100)					
Atila BioSystems Inc.	ORF1ab	50–100	N/A	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	iAMP-COVID-100-RUO	Atila iAMP COVID-19 Detection (isothermal detection)	COVID20200320	BioRad CFX96 deep well	Any signal is considered positive (isothermal)
	N	1–10	N/A	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)					
BGI Health (HK) Co. Ltd	ORF1	1–10	32.43	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	MFG030010	Real-time Fluorescent RT-PCR kit for detection 2019-nCOV (CE-IVD)	6220200305	Roche LightCycler 480	≤38
Boditech Med. Inc	E	10–50	34.9	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	UFPK-4	ExAmplar COVID-19 real-time PCR kit (L)	WLQCB02L	BioRad CFX96 deep well	≤42
	RdRP	50–100	33.46	90% (95%CI: 79, 96)	100% (95%CI: 96, 100)					
CerTest Biotec	ORF1ab	10–50	35.16	98% (95%CI: 90, 100)	100% (95%CI, 96, 100)	VS-NC0112L VS-NC0212L	VIASURE SARS-CoV-2 Real Time PCR Detection Kit	NC0212L-023	BioRad CFX96 deep well	<40
	N	1–10	35.46	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)					
DAAN Gene Co. Ltd	ORF1	1–10	38.76	100% (95%CI: 93, 100)	96%* (95%CI: 90, 98)	DA0930-DA0932	Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing)	2020007	Roche LightCycler 480	≤40
	N	1–10	36.97	100% (95%CI: 93, 100)	98%* (95%CI: 93, 99)					
EUROIMMUN	ORF1ab/N	1–10	37.88	100% (95%CI: 93, 100)	98%* (95%CI: 93, 99)	MP 2606-0425	EURORealTime SARS-CoV-2	I200320AL	Light Cycler 480 II	Any signal considered positive
GeneFirst Limited	ORF1	1–10	35.45	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	MPA-COVID19	The Novel Coronavirus (2019-nCoV) Nucleic Acid Test Kit	00072	BioRad CFX96 deep well	≤37.0 positive; 37-40 indeterminate; >40 negative
	N	1–10	36.72	98% (95%CI: 90, 100)	100% (95%CI: 96, 100)					
KH Medical Co. Ltd	S	1–10	37.94	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	RV008	RADI COVID-19 Detection Kit	V008.200202	BioRad CFX96 deep well	≤40
	RdRP	10–50	36.74	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)					
Primerdesign Ltd	RdRP	1–10	36.7	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	Z-Path-COVID-19-CE	Coronavirus COVID-19 genesig® Real-Time PCR assay	JN-02780-0009	LightCycler 480	Any signal regarded as positive
R-Biopharm AG	E	1–10	37.99	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	PG6815RUO	RIDA®GENE SARS-CoV-2 RUO	21120N	BioRad CFX96 deep well	None; any signal can be considered positive
SD Biosensor Inc.	E	1–10	37.43	100% (95%CI: 93, 100)	97%* (95%CI: 92, 99)	M-NCOV-01	STANDARD M nCoV Real-Time Detection Kit	MNC00120005	Roche LightCycler 480	≤41
	ORF1	1–10	36.99	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)					
Seegene Inc.	E	1–10	33.3	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	RP10244Y RP10243X	Allplex™ 2019-nCoV Assay	RP4520C24	BioRad CFX96	≤40
	N	1–10	36.74	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)					
	RdRP	1–10	34.73	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)					
Tib Molbiol	E	1–10	33.34	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	53-0776-96 6754155001	ModularDx Kit SARS-CoV (COVID19) E-gene (Tib Molbiol) + LightCycler Multiplex RNA Virus Master (Roche)	48202019 48274100	Roche LightCycler 480	Define the cut-off 2–4 cycles higher than observed Cp value for 10 copies

Fig.5. SARS-CoV-2 Molecular Assay Evaluation Results, Last Updated: May 15th 2020

another 24 to 48 hours if the patient's sample needs to be transported to a central lab for processing. Patients may continue spreading the virus during those time lags. There's also a possibility of contamination during manual laboratory procedure. In order to meet this issue, Switzerland-based Roche Group to begin selling its real-time RT-PCR test, in which patient samples are fed into Roche's fully automated cobas® SARS-CoV-2 6800 and 8800 systems, which provide reliable and high-quality results for clinical decision-making for patients with suspected COVID-19 (coronavirus) infection. The cobas® SARS-CoV-2 Test is a qualitative assay that allows the detection of nucleic acids in samples from patients who meet COVID-19 (coronavirus) clinical and/or epidemiological criteria. The tests are for use on the automated, high throughput cobas® 6800/8800 Systems under Emergency Use Authorization. The cobas® SARS-CoV-2 Test is a single-well dual-target assay, which includes both specific detection of SARS-CoV-2 (COVID-19 Coronavirus), along with a conserved region of the E-gene and pan-sarbecovirus detection for the sarbecovirus subgenus family that includes SARS-CoV-2. The assay has a full

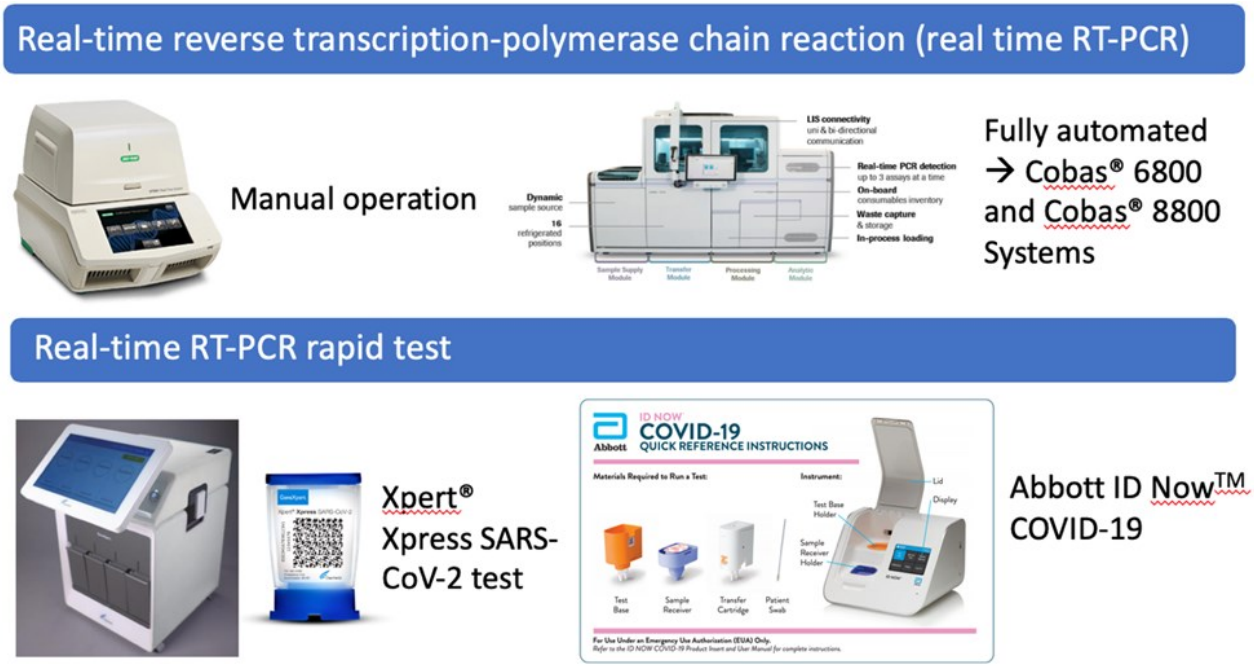

Fig. 6. Nucleic acid testing (NAT) /Molecular Testing of COVID-19

-process negative control, positive control, and internal control. The systems provide up to 96 results in about three hours and a total of 384 results for the cobas® 6800 System and 1056 results for the cobas® 8800 System in 8 hours.

In advancement with global widespread COVID-19, the demand for low-complexity, rapid (results within 1 hour) molecular diagnostic is getting increased. Nowadays, this need was developed as cartridge-based assays on platforms that can be used as Point-of-Care Molecular Diagnostics. The most prominent real-time RT-PCR rapid test is the Xpert® Xpress SARS-CoV-2 test by Cepheid, USA, which provides results in just 45 min using the GenXpert benchtop system. It is a rapid and automated point-of-care (POC) molecular test that enables the qualitative detection of SARS-CoV-2 in the nasopharyngeal swab, nasal wash, or aspirate specimens from suspects. The test requires only a minute for sample preparation, employs Cepheid's Xpert® Xpress cartridge technology, and targets multiple regions of the viral genome. It has also received the Food and Drug Administration (FDA) emergency use authorization (EUA).

Another Rapid Molecular testing, Abbott ID NowTM COVID-19 test, is the most recent breakthrough IVD assay that detects SARS-CoV-2 in just 5 min. It is a molecular POC test that utilizes the isothermal nucleic acid amplification technology for the qualitative detection of viral RNA from SARS-CoV-2. The test can be used in any location, such as hospitals, clinics, physicians' offices, or in outbreak hotspots of COVID-19. It requires just a portable touchscreen-operated instrument, i.e., ID Now, which is lightweight (6.6 pounds) and compact (the size of a small toaster). It employs a molecular test for the RdRp gene and can take throat, nasal, nasopharyngeal and oropharyngeal swabs as samples. The kit contains 24 tests, positive and negative controls, swabs for sample collection, and pipettes. It has just received the FDA EUA and is being seen as a remarkable achievement worldwide. However, study raises questions about false negatives of the Abbott ID NOW COVID-19 test. Researchers at the Cleveland Clinic tested 239 specimens known to contain the coronavirus using Abbott ID NOW. However, the ID NOW only detected the virus in 85.2% of the samples, meaning it had a false-negative rate of 14.8 percent. Abbott said any problems with the test could stem from samples being stored in viral transport media before being tested, instead of being inserted directly into the ID NOW testing machine. As a result, the company recently instructed all users to only test samples put directly into the machines.

Improving the nucleic acid extraction and amplification process and shortening the overall testing times are urgent problems to be solved. In addition, problems such as false negatives are difficult to avoid, and it may require multiple tests to determine the status of infection. To this end, Gootenberg et al. developed a new method based on CRISPR/Cas13-based SHERLOCK technology for SARS- CoV-2 testing. Based on the SHERLOCK method, which stands for Specific High-sensitivity Enzymatic Reporter unLOCKing, the kit works by programming a CRISPR molecule to detect the presence of a specific genetic signature – in this case, the genetic signature for SARS-CoV-2 – in a nasal swab, nasopharyngeal swab, oropharyngeal swab or bronchoalveolar lavage (BAL) specimen. When the signature is found, the CRISPR enzyme is activated and releases a detectable signal. Recently on May 7th, 2020, the company has received Emergency Use Authorization (EUA) from the U.S. Food

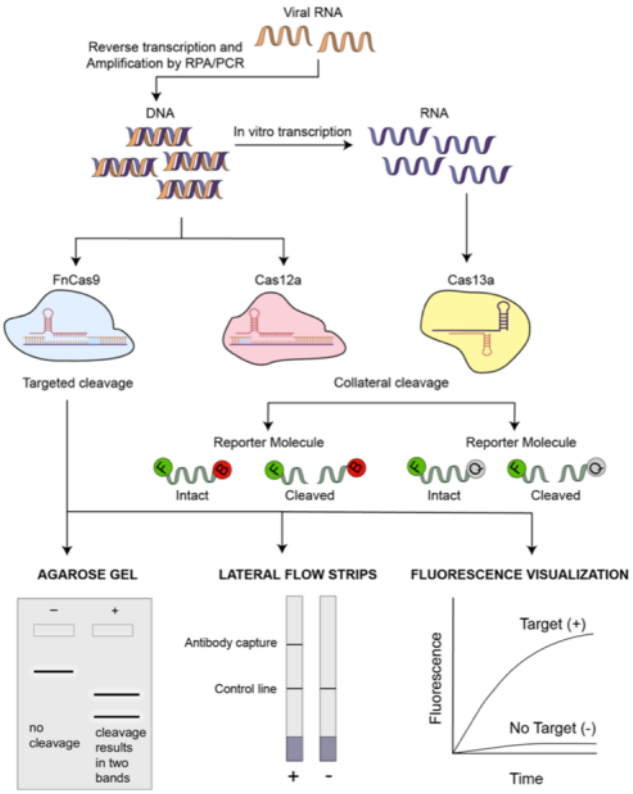

Fig. 7. An overview of the general schematic of CRISPR/Cas based COVID-19 testing methods.

and Drug Administration (FDA) for its Sherlock™ CRISPR SARS -CoV-2 kit for the detection of the virus that causes COVID-19, providing results in approximately one hour.

In the research setting, the authoritative identification method for SARS-CoV-2 is virus culture and high-throughput sequencing of the whole genome. However, the application of high-throughput sequencing technology in clinical diagnosis is limited because of its equipment dependency and high cost. Next-generation sequencing (NGS) provides scientists a high-throughput method to quickly analyze the virus's genetic code and trace its origin. While isolation of the live virus followed by growing the virus in cell culture is usually done for pathogenesis research or assessment of probable therapeutic compound in vitro. However, virus culture must be conducted in a biosafety Level-3 facility to ensure safety. Therefore, those two approaches are not recommended for clinical use of COVID-19 diagnosis.

Apart from molecular testing, the recent development of COVID-19 testing also includes antibody and antigen assay based-test, which can act as a complementary diagnosis method and are useful in the point-of-care setting. Furthermore, the non-standard collection method of pharynx swab, for molecular testing, can easily lead to misdiagnosis. And the samples collected from different parts of individuals will also affect the test result. Moreover, the collection process is extremely risky for medical staff. Therefore, serological detection can make up for the deficiency of nucleic acid detection. We will explain the principles of antibody and antigen-based assays in the next month's Newsletter. Please stay healthy, everyone! Cheers.

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INA-RESPOND Newsletter

EFFICACY VS. EFFECTIVENESS ~ EXPLANATORY VS. PRAGMATIC: THINKING FOR THE BEST DESIGN TO FIT THE OBJECTIVE

By: Aly Diana

Randomized trials are hard work, and this toil is only worth it because of the prospect of a substantial reward, namely the results. Stakeholders use these results to directly support decisions on delivering an intervention that will improve health outcomes for patients and the public. In other words, for every intervention, the ultimate goal is to gain benefits for the place we call the real-life world.

However, generally, randomized trials are broadly characterized as an effectiveness or efficacy trial. People have been using the corresponding terms "pragmatic" vs. "explanatory" more often lately. Put merely, pragmatic trials help providers decide between options for care, and explanatory trials test research hypotheses under ideal study circumstances. Pragmatic trials seek answers to whether an intervention will work under usual conditions. In contrast, explanatory trials are best to test causal hypotheses in a setting where confounders can be minimized to the greatest degree possible. Typically, the findings from pragmatic trials have high generalizability (i.e., they are likely to hold in most clinical circumstances). In contrast, explanatory trials are the most likely laboratory experiments where confidence is high that any differences found between study conditions (e.g., treatment vs. control) were the result of scientific testing under rigorously controlled conditions. Generally, the control condition for purely pragmatic trials is usual care, whereas, for explanatory trials, it is a placebo or an active comparator condition.

These two approaches represent different attitudes to decision making on the usefulness of interventions. Results from pragmatic trials can be directly adopted as a new policy, while results from explanatory trials should commonly be further tested before translated into policy. Regardless of the widespread concern that explanatory randomized trials can be poor predictors of the real-world Effectiveness of the intervention and seem to take us further away from our ultimate goal, there always be merit in the explanatory trials, given the objectives and particular situations. Only in an idealized setting, an intervention has the best chance to demonstrate a beneficial effect. In some cases, it would be very challenging to show a beneficial effect in a real-world situation. Can we imagine conducting the first trial of HIV or Tuberculosis drugs using a pragmatic approach?

"It was more of a '*triple-blind*' test. The patients didn't know which ones were getting the real drug, the doctors didn't know, and, I'm afraid nobody knew."

It feels that we are standing at two opposite sides. However, we should recognize that there is no simple threshold, and most trials are neither entirely pragmatic (effectiveness) or explanatory (efficacy) and lie instead on a continuum between these poles. Therefore, the most important thing is to design a study that will fit the objective. The objective of a highly pragmatic trial would be to maximize the applicability of the intervention to usual care across a range of local and distant settings. The objective of a highly explanatory trial would be to maximize the intervention's chance of demonstrating an effect through the expected mechanism, with little attention paid to the issue of whether this outcome would be achieved under real-world conditions.

Therefore, we need to evaluate whether our design fits the objective. There is one tool, called PRECIS-2, that may help us with the evaluation process. PRECIS-2 has nine domains—

Continue to page 18

COMIC CORNER

INA-RESPOND Newsletter

A BETTER HEART IS JUST A STRETCH AWAY

By: Edrick Purnomo Putra

LIFESTYLE & SPORT

Aging is inevitable. As we age, the degenerative process occurs in our bodies. Age-related arterial stiffness or arteriosclerosis is described as an independent risk factor of cardiovascular disorders and mortality. Arterial stiffness leads to a decreased buffer capacity of the arteries and an increase in pulse pressure and pulse wave velocity. This will cause an early return of the reflected waves, thereby augmenting late systolic pressure. As a result, the left ventricle needs to generate extra workload to compensate for this augmented pressure, which also increases the oxygen demand, and in the long run, left ventricular hypertrophy will develop as a risk of heart failure. Incompliant arteries also increase the pulsatile pressure deeper into the periphery and damage microvasculature on end-organ systems, such as the brain and the kidneys.[1] Arterial stiffness is also determined by the intrinsic elastic properties of smooth muscle and/or connective tissues (e.g., elastin-collagen composition) in the arteries.[2]

Some other chronic conditions may also contribute to arterial stiffness. Atherosclerosis is considered an inflammatory disease characterized by endothelial dysfunction and the formation of atherosclerotic plaque, which is also a risk factor of cardiac events. This inflammatory response will also increase arterial stiffness, so the connection between atherosclerosis and arteriosclerosis could be established. Both markers of inflammation and arterial stiffness may predict cardiovascular events in the future.[1] Therefore, preventing these things from happening is an important issue.

Flexibility is one of the components of physical fitness, specifically the health-related components together with cardiorespiratory endurance, body composition, muscle strength, and endurance. Even though flexibility is traditionally linked to performance on daily activities and sports, as the name suggests, flexibility is believed to have an impact on human health. Recent studies indicated that flexibility is associated

with cardiovascular health risk. Therefore, flexibility may no longer be simply viewed as a supportive measure in functional movements in daily activity or musculoskeletal injury risk management in sports.[2]

A simple way to measure body flexibility is by using the sit-and-reach test, which is used in many studies. This test measures the flexibility of the hamstring, hip, and lower back, which is usually called 'trunk flexibility.' Trunk flexibility can be easily evaluated over all ages and in any practical field.[3] Present studies show that poor trunk flexibility could be a predictor of age-related arterial stiffening, independent of other fitness components. Flexibility is determined by skeletal muscle and/or connective tissue in the tendon, ligaments, and fascia. Age-related alterations in the muscles or connective tissues in the arteries may correspond to similar age-related alterations in the whole body. A 5-year longitudinal study shows that poor trunk flexibility accelerates the progression of age-related arterial stiffness. In this study, arterial stiffness was measured by carotid-femoral pulse wave velocity (cfPWV), and it showed a greater annual rate of change in poor flexibility group.[4]

Exercise, as a part of physical activity, contributes to the prevention of many diseases, including cardiovascular diseases, when done regularly. It is clear from the studies that aerobic and resistance training affects endothelial function and arterial stiffness, but studies about stretching as flexibility training related to vascular health are still limited yet produce promising results.[5] A 4-week study of regular static stretching suggests that short term regular static stretching intervention induces a significant reduction in arterial stiffness in middle-aged men.[6] Another study with a longer period of intervention shows similar results. A 6-month regular whole-body static stretching intervention on pre-menopausal women shows that vascular endothelial function improves after three months, and arterial stiffness decreases after six months. However, these effects were reversed back to pre-intervention state after six months of detraining.[5]

When the shape of the muscle changes due to stretching, the blood vessels that run parallel to the muscle also stretch. Stretching causes mechanical stress to the arteries in the form of tension in vascular wall & vascular smooth muscle. Stretching also decreases the diameter of the arteries, which acts as a temporary restriction of the blood flow. When the restriction is released, reactive hyperemia occurs, and the blood flow increases. This will cause shear stress to endothelial cells. A synergistic effect of mechanical and shear stress lead to the secretion of vasodilatory factors such as nitric oxide (NO) and endothelium-derived hyperpolarizing factor from the vascular endothelial cells, which improves endothelial function. This will simultaneously change the property of blood vessels and consequently improve arterial stiffness.[5] A study in rats suggests that daily muscle stretching induces enhanced endothelium-dependent vasodilatation and angiogenesis, enhancing exercise-induced hyperemia in the skeletal muscles of aged rats. Local ischemia and/or mechanical stretching of intramuscular blood vessels are probable triggers of these vascular adaptations in chronically stretched skeletal muscle.[7]

Arterial stiffness is functionally determined by the vascular tone of the artery. Vascular tone is partially regulated by sympathetic nerve activity. Stretching of skeletal muscle causes an increase in sympathetic nerve activity via the central nervous system. Repetitive stimulation of transient sympathetic excitation induced by habitual stretching exercises, which improve flexibility, may chronically reduce resting sympathetic nerve activity. This reduction in sympathetic nerve activity may result in a decrease in arterial stiffness.[2] Structure of connective tissues may also play a role. Cross-links of collagen and elastin in the connective tissue determine tensile strength and elasticity of vascular wall and tendon or ligaments, which are related to both stiffnesses of arterial wall and flexibility of joints. Thus, the alternation of connective tissue by aging may be similar in the arteries and the trunk joint.[8]

Heart rate variability, the oscillation in the interval between consecutive R waves on the electrocardiogram (R-R intervals), has been established as a reliable and non-invasive tool for the assessment of cardiac autonomic function. Heart rate variability parameters can be used to derive the cardiac sympathovagal balance, the magnitude of sympathetic to parasympathetic nerve activity. A low heart rate variability has been associated with increased risk of cardiovascular events and mortality. Stretching exercise seems to be a useful therapeutic intervention to enhance heart rate variability in different populations. Although the mechanisms by which stretching exercise improves cardiac autonomic function are not yet well understood, increases in baroreflex sensitivity, relaxation, and NO bioavailability seem to play an important role.[9]

Hypertension is acknowledged as one of the greatest and most established risk factors for cardiovascular disease.[2] Another study shows that poor trunk flexibility is also associated with central blood pressure. While the previous explanation shows that stiff arteries cause augmented systolic pressure, this study shows that higher flexibility attenuates the age-related increase in central blood pressure. In this study, the correlation between flexibility and central blood pressure is significant after adjusting for age and sex. However, the significant correlation disappears after adjusting for cfPWV as an index of arterial stiffness, implying that arterial stiffness is an important mediator of the relationship between flexibility and central blood pressure. The age-related increase in central blood pressure may be counterbalanced by flexibility due to sympathetic nerve activity and/or endothelial function.

Stretching exercise reduces sympathetic nerve activity and increases serum NO bioavailability in healthy older adults.[8] These findings suggest that flexibility exercises could be used as a treatment of hypertension.[2,3,8]

As discussed above, it is evident that poor flexibility is an indicator of arterial stiffness[2–4], and regular stretching may improve vascular endothelial function and arterial stiffness itself.[5,6] Stretching exercise also enhances heart rate variability[9] and reduce blood pressure[8]. However, we need to remember that all this benefit will only happen if we do it regularly. One of the studies above shows that these effects are reversible if we stop doing exercise.[5] stretching may be a viable nonpharmacological intervention for the potential improvement of cardiovascular health in a variety of individuals, including those who are unable to perform traditional aerobic or resistance exercise.[9] American College of Sports Medicine (ACSM) recommends doing regular flexibility training at least 2-3 times per week with most days being active. Stretch on major muscles until the point of tightness or slight discomfort and hold it for 10-30 seconds for most adults. In older individuals, holding a stretch for 30-60 seconds may confer greater benefit. Also remember to stretch when the muscles are warm to make stretching more effective and safer.[10] So, don't forget to do your daily stretches!

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Continued from page 15

eligibility criteria, recruitment, setting, organization, flexibility (delivery), flexibility (adherence), follow-up, primary outcome, and primary analysis. We should score every domain from 1 (very explanatory) to 5 (very pragmatic). This assessment would be useful to facilitate discussion and consensus about our study. In my opinion, it's a good practice to follow.

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