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INDONESIA RESEARCH PARTNERSHIP ON INFECTIOUS DISEASE

KEMENTERIAN KESEHATAN REPUBLIK INDONESIA

NEWSLETTER

January 2021

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Efficacy and Effectiveness of Covid-19 Vaccines

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NATIONAL INSTITUTE OF HEALTH RESEARCH AND DEVELOPMENT

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FEATURES

INA-RESPOND

Newsletter

TRIPOD & PROACTIVE Study Updates

By: Eka Windari R., Lois E. Bang, Venty Muliana Sari, Melinda Setiyaningrum

STUDY UPDATES

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PARTICIPANT STATUS

Per 02 January 2021, the total ongoing participants in the TRIPOD study are 15 out of 490 enrolled participants. From those 15 ongoing participants, one is still on TB treatment while 14 are waiting for their 6-month post-treatment visit. Two hundred and forty participants have completed the study, while 235 participants are terminated early (including death). Therefore, there are still 3.06 % participants from the total enrolled participants in the follow-up status. From the uploaded CRFs, all participant from site 520, 570, and 590 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, 9 participants from site 560 (RSUP dr. Kariadi Semarang), 4 participants from site 580 (RSUP dr. Sardjito Jogjakarta), and 1 participant from site 600 (RSUP dr. Adam Malik Medan).

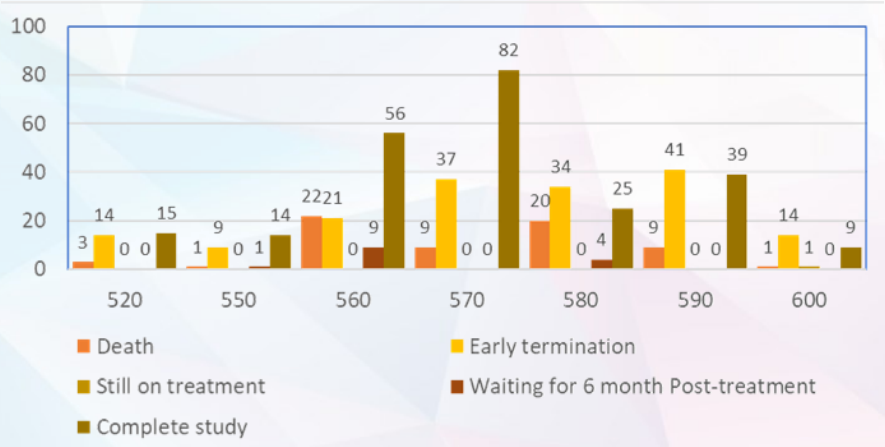

Figure 1. Participant status per site based on uploaded CRF per 2 January 2021

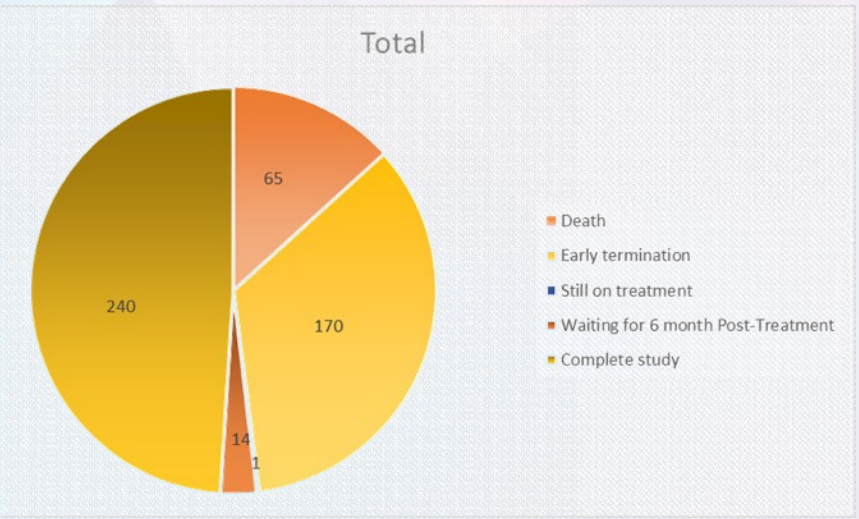

Figure 2. Total participant status based on uploaded CRF per 2 January 2021

The database Quality assurance (except for TB Treatment pages) has been conducted for site 520, 570, and 590 from 24 November – 22 December 2020.

The Site Close-out Visit (SCV) has been conducted for site 520 on 30 November – 1 December and site 570 on 15-16 December 2020. The upcoming SCV for site 590 will be conducted on 19-20 January 2021

AWAITING CULTURE AND DST RESULT

The result for baseline culture and DST results from all sites are complete.

INA104

PARTICIPANT STATUS

One pediatric subject from Site 610 (RSU Kab Tangerang) completed the study's last visit (Follow Up Month 36) on 20 Jan 2021. This is the first completed subject for this study. Other subjects from this site will follow since it is the first activated site, and other sites are starting to have their Follow Up month 30.

As of 12 Jan 2021, from the 4,336 subjects enrolled, 202 subjects have End of Study status due to the following reasons: 154 subjects are dead, 22 subjects move away to a city where site PROACTIVE is not available, 21 subjects withdraw, and five subjects have negative HIV test result. To date, there are 4,134 active subjects in this study. Forty -seven subjects are transfer IN/OUT between Proactive Sites.

Below is the table of Enrollment and Active Participants by Sites:

No	Site# / Name	1st Enrollment	Enrollments stop	# Screened			# Enrolled			Participants Transfer In	Participants Transfer Out	End of Study Participants	Active Participants
				Ped	Adult	Total	Ped	Adult	Total				
1	510 – Hasan Sadikin	7-Feb-19	31-Dec-19	12	226	238	10	198	208	3	2	4	205
2	520 – Sanglah	7-Nov-19	30-Jun-20	7	220	227	5	138	143	3	1	1	144
3	530 – Cipto M.	3-May-18	31-Aug-19	38	365	403	36	274	310	7	2	12	303
4	540 – Sulianti Saroso	25-Feb-19	31-Dec-19	26	225	251	20	162	182	0	1	4	177
5	550 – Wahidin	14-Mar-18	31-Aug-19	17	695	712	10	327	337	0	0	20	317
6	560 – Kariadi	14-Aug-18	31-Aug-19	21	285	306	12	218	230	3	2	11	220
7	570 – Soetomo	26-Apr-18	31-Aug-19	7	365	372	6	307	313	4	3	31	283
8	580 – Sardjito	14-Sep-18	30-Sep-19	5	290	295	4	216	220	3	3	4	216
9	590 – Persahabatan	19-Jul-18	31-Aug-19	12	324	336	10	239	249	2	2	29	220
10	600 – Adam Malik	12-Mar-18	31-Aug-19	17	778	795	2	336	338	4	5	21	316
11	610 – Tangerang	10-Jan-18	31-Aug-19	60	890	950	17	310	327	1	1	20	307
12	630 – Ansari Saleh	17-Jul-18	31-Aug-19	19	447	466	9	236	245	1	6	3	237
13	640 – St. Carolus	13-Aug-18	30-Sep-19	0	380	380	0	225	225	8	3	0	230
14	650 – Budi Kemuliaan	2-Aug-18	31-Aug-19	4	306	310	4	225	229	3	4	16	212
15	660 – AW Sjahranie	3-Oct-18	30-Sep-19	25	292	317	17	205	222	2	6	3	215
16	670 – Zainoel Abidin	9-Apr-19	31-Dec-19	17	384	401	5	121	126	0	3	7	116
17	680 – Soedarso	4-Jul-19	31-Dec-19	8	139	147	8	107	115	1	0	5	111
18	690 – Abepura	2-Jul-19	30-Jun-20	7	201	208	4	133	137	1	2	6	130
19	700 – TC Hilers	8-Jul-19	30-Jun-20	14	236	250	10	170	180	1	1	5	175
Total				316	7,048	7,364	188	4,148	4,336	47	47	202	4,134

INA-RESPOND Newsletter

SCIENCE CORNER

ANTIGEN-DETECTION RAPID DIAGNOSTIC TEST: A SIMPLE KIT THAT PLAYS A MEANINGFUL ROLE IN COVID-19 PANDEMIC COMPLEXITY

By: Adhella Menur

New year, new start. All the people worldwide embrace 2021 with the abundance of hopes that we will end the COVID-19 pandemic. Sadly, it won't come easy. The SARS-CoV-2 is still giving us many surprises as they want to survive and linger with us. At the end of 2020, the virus did not let us relax with the good news of the COVID-19 vaccines as they came with newly identified worrying variants from England, South Africa, and Brazil. Scientists are concerned about how the new variants appear, why they are more infectious, and how they affect disease severity or vaccine efficacy. Public health officials, infectious disease experts, and even Moderna CEO suggested a high likelihood that COVID-19 will become an endemic disease, and the public should prepare for that. However, there is always a blessing in disguise. Over 380,000 virus genomes were sequenced in just one year to help scientists get real-time data about the evolution and work together to solve the puzzle.1,2

In England, the new strain called B.1.1.7 now becomes the most common variant. It was first identified in September 2019 in Kent County in England and now representing more than 50% of new COVID-19 confirmed cases until December 2019. While a new particular mutation can rise in frequency by chance if it is carried by a super-spreader, moved to a new un-infected location, or introduced into a new segment of the population, for the case of B.1.1.7 variant that brings multiple spike protein mutations (deletion 69/70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H), it has a unique origin story. Some scientists believe that the virus may have mutated in a person who was immunocompromised. Unlike the flu virus, the novel coronavirus can correct mistakes when it replicates due to its proofreading enzyme. It tends to have a fairly stable genome (mutation rate estimated to be near 2.5 x 10-6 substitutions/ nucleotide/ cell infection). However, people who have weakened immune systems may contain the infectious virus for months. It can give the virus many chances to acquire mutations that help it replicates or evades the immune system. Another origin possibility is the increase of mutation odds that can happen in chronically ill patients treated with experimental therapies like COVID-19 convalescent plasma because of the illness's length time that gives the virus to replicate more. British scientists suggest that this variant is significantly more transmissible than previously circulating variants, with increased transmissibility of up to 70% attributed to the N501Y mutation that makes this variant binds more tightly to the ACE2 receptor and P681H mutation that sits next to the "furin cleavage site," which is where the spike protein must be cleaved for the virus to enter cells. According to another study, this variant is detected in a polymerase chain reaction (PCR) test with higher viral loads, making it easier for the virus to spread. Another concern about this variant is its ability to spread amongst children easier than before because of its stickiness to the host cell. This variant also impacts the diagnostic assay result. Its deletion 69/70 mutation leads to a conformational change in the spike protein, therefore causing a negative result of S-gene in the PCR test. Luckily, most commercial PCR tests have multiple targets to detect the virus, such that even if a mutation impacts one of the targets, the other PCR targets will still work.2-4

Multiple mutations in the spike protein were also independently found in the new South Africa variant called B.1.251. This variant was first identified in Nelson Mandela Bay, South Africa, in samples dating back to the beginning of October 2020. It now appears to be the predominant variant in the country. It contains nine changes in the spike protein that can be divided into two subsets: one cluster in N-Terminal Domain (NTD) that includes four substitutions and a deletion (L18F, D80A, D215G, Δ242-244, and R246I), and another cluster of three substitutions in Receptor Binding Domain (RBD) i.e., K417N, E484K, and N501Y. Unlike the B.1.1.7 lineage detected in the UK, this variant does not contain the deletion at 69/70 and E484K mutation. As we know, NTD and RBD are two immunodominant regions of the viral spike protein that frequently targeted by neutralizing antibodies and several potent monoclonal antibodies. The accumulation of mutations specifically within those two immunodominant regions of spike is highly suggestive of escape from neutralizing antibodies. Specifically, the E484K mutation has been shown to reduce antibody recognition and causing the virus to be more resistant to monoclonal antibody treatment, which has helped some people fight the virus when administered early on in their infection. E484K confers resistance to class 2 neutralizing antibodies. Moreover, K417N mutation would abolish key interactions with class 1 neutralizing antibodies and contribute to immune evasion. This variant's uniqueness answers why re-infection among people pre-

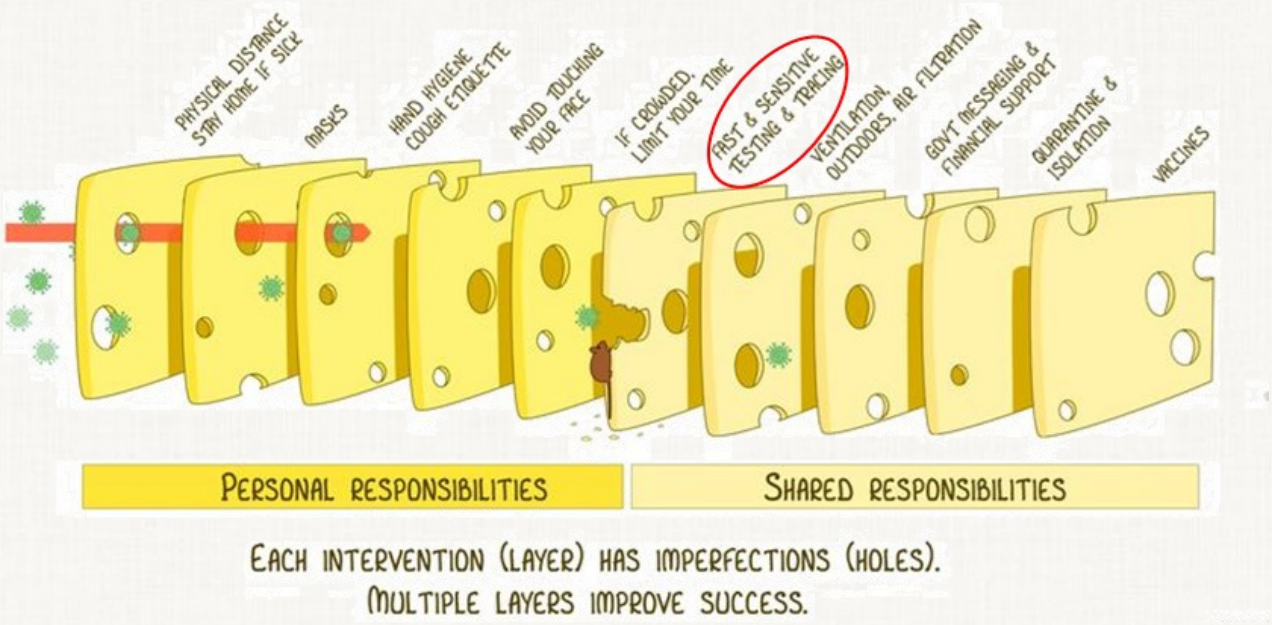

Figure 1. The Swiss Cheese respiratory virus pandemic defense. Recognizing that no single intervention is perfect at preventing spread.9

sumed to have acquired some degree of immunity due to previously having had SARS-CoV-2 could happen. This variant worries scientists because of their ability to infect more and escape the host immunity, which might reduce vaccine effectiveness. Elucidating the role of non-neutralizing antibodies and the efficacy of T cell responses to this strain offer an alternative solution.5,6,7

Another newly identified variant that threads us is found in Brazil called the P.1. The P.1 variant is a branch of the B.1.1.28 lineage that was first reported by the National Institute of Infectious Diseases (NIID) in Japan in four travelers from Brazil on 9 January 2021. The P.1 variant was identified in 42% of the specimens sequenced from late December 2020 in Manaus, Amazon. In fact, it is estimated that approximately 75% of the Manaus's population had been infected with SARS-CoV2 as of October 2020. However, since mid-December 2020, the region has observed an extreme surge in COVID-19 cases, and many patients had to be transported out of their hospitals due to severe oxygen shortage. This variant's emergence raises concerns of a potential increase in transmissibility or propensity for SARS-CoV-2 re-infection of individuals. The P.1 variant contains 17 unique amino acid changes and three deletions. Its mutations include the N501Y mutation, which it has in common with the variants reported in the UK and South Africa, E484K, and K417N/T. There is evidence to suggest that some of the mutations in the P.1 variant may affect its transmissibility and antigenic profile, which may affect the ability of antibodies generated through previous natural infection or through vaccination to recognize and neutralize the virus. Scientists thought that recent vaccines would still prevent serious illness from the variants but might be less effective at preventing a mild or asymptomatic infection from them; more studies are needed.5,8

Although those three SARS-Cov-2 variants appear to be more infectious, they have been observed not to affect disease severity until now. So, can we take a break? Absolutely not! High transmission means an increment of cases that have many awful impacts. More patients who need to be cared for will overwhelm medical facilities and reduce the quality of care, leading to higher death rates than expected. The high transmission also gives the virus many chances to develop further mutations. It is like we provide a playground for the virus. Quickly suppressing the pandemic is an undeniably urgent responsibility for us. The core principles of action are to avoid importing new variants, prevent their spread, and improve molecular surveillance; concordance with the enforcement of strict health protocol, vaccination, and medical care.

One of the Swiss Cheese layers is to enhance fast and sensitive testing and tracking that leads to rapid detection and isolation of new cases. Our effort for strengthening the layer is wisely using an effective and efficient testing tool. COVID-19 antigen rapid detection test (Ag-RDT) is one of the testing tools that can fulfill the needs, considering Nucleic Acid Amplification Tests (NAAT) as the gold standard for COVID-19 diagnostic is still difficult to access despite government effort to enhance molecular laboratory facility and has a delay time-to-result (1-3 days). Ag-RDT measures the presence or absence of the viral proteins (antigens), most commonly the abundant nucleocapsid protein.

Figure 2. Illustration of Ag-RDT testing12

In terms of new variant viruses that hold many mutations, particularly in spike protein, there has not been any report that it would negatively impact rapid antigen detection tests. Thanks to the use of nucleocapsid protein in most of the commercially available Ag-RDTs. A few rapid antigen detection tests are based on detection of the spike protein, and therefore it cannot be ruled out that the identified mutations will not have an effect on them.10,11

The preferred sample type is nasopharyngeal (NP) swab, there is hope that saliva or mouthwash will provide a viable alternative, but at present, this appears less accurate. If the target antigen is present in sufficient concentrations in the sample, it will bind to specific antibodies fixed to a paper strip enclosed in a plastic casing and generate a visually detectable signal, typically within 15-30 minutes. The antigen(s) detected is expressed only when the virus is actively replicating; therefore, such tests are best used to identify acute or early infection. The sensitivity compared to NAAT in samples from the upper respiratory tract (nasal or NP swabs) appears to be highly variable, ranging from 30-94%, but specificity is consistently reported to be high (>97%). Ag-RDT is most likely to perform well in patients with high viral loads (Ct values ≤25 or >106 genomic virus copies/mL), which usually appear in the early symptomatic phases of the illness (within the first seven days of illness). This offers the opportunity for early diagnosis and interruption of transmission through targeted isolation and cohorting of the most infectious cases and their close contacts. A study revealed that a routine application of Ag-RDTs would increase the proportion of suspect cases who receive their test results the same day from 33 to 97%. It is important to remember that patients who present too early after contact with the case, pre-symptoms stage, or more than seven days after the onset of symptoms are more likely to have lower viral loads, and the likelihood of false-negative results with Ag-RDT is higher. Hence, the success of testing depends on several factors, including

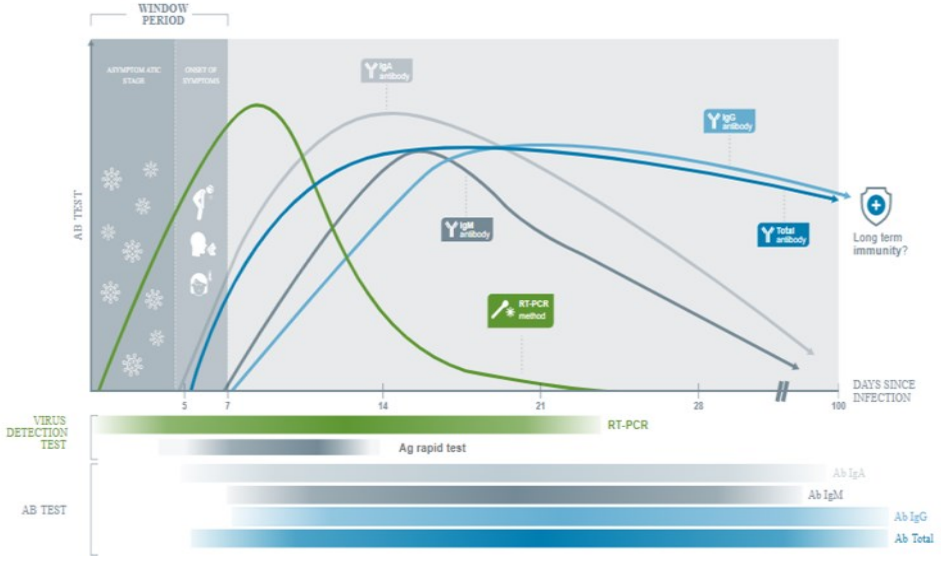

Figure 3. The time-point to choose suitable testing10

the correct time from onset of illness, the concentration of virus in the specimen, the quality of the specimen collected from a person and how it is processed, and the precise formulation of the reagents in the test kits. 10,13,14

In testing a symptomatic person, we should consider the prevalence setting. Ag-RDT testing should be performed in a high prevalence setting. It could be performed in a low prevalence setting if only PCR capacity is limited. The best timing for Ag-RDT testing is within 5-7 days after symptoms onset. Any results are suggested to be confirmed with PCR. Confirmatory testing should take place as soon as possible after the antigen test and not longer than 48 hours after the initial antigen testing. When a symptomatic person receives a negative antigen test result followed by a negative confirmatory PCR, the healthcare provider should consider whether the person has had exposure to a person with COVID-19 within the past 14 days. If the person has had exposure, that person should follow infection control measures for 14 days after their most recent exposure to a person with COVID-19. If PCR testing capacity is limited, serial Ag-RDT testing after 2-4 days from the first negative result could be done. In testing an asymptomatic person, the best timing is within seven days following exposure or as soon as possible. Serial Ag-RDT testing can also be done and repeated every 2-3 days until the end of possible incubation time, with the first positive result confirmed with PCR.13,15, 16

Trained healthcare or laboratory staff, or trained operators with protective equipment, are still needed to carry out NP-swab, Ag-RDT testing, test analysis, and reporting the results to clinical staff and public health authorities. Those requirements are flaws for Ag-RDT if we want to recommend it as a self-testing at home. NP swab samples are frequently perceived as uncomfortable by patients and difficult to do it independently. A study, based on evidence that supports the use of anterior nasal (AN) swabs collected by patients themselves for PCR, was conducted to assess the use of AN swabs for Ag-RDT self-testing. Ag-RDT (STANDARD Q) with AN sampling showed a sensitivity of 74.4% and specificity of 99.2% compared to PCR. The sensitivity with NP sampling was 79.5%, and specificity was 99.6%. In patients with high viral load (>7.0 log10 RNA SARS-CoV2/swab), the sensitivity of the Ag-RDT with AN sampling was 95.7% and 100% with NP sampling. The Ag-RDT frequently did not detect patients with lower viral load or with symptoms >7 days. If such testing could be repeated frequently and immediately ahead of situations when transmissions are likely to occur, self-testing with Ag-RDT may have a significant impact on the pandemic. Further study is needed to improve the testing method. One that we should not forget is to always practice safe handling and proper waste management. In short, Ag-RDT is like a weapon, and how to optimize it to the fullest depends on the user.17

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INA-RESPOND Newsletter

FROM OUR PARTNER

SARS-COV-2 GENOMIC VARIANTS

By: Katy Shaw—Saliba

Introduction

Reports of new SARS-CoV-2 variants in the United Kingdom (UK) and South Africa before the December holidays set off alarm bells globally, resulting in boarder closures, lockdowns, and travel bans (cite). What are these genomic variants? Why are they of concern? Does this mean that the vaccines won't work? 11 months into the SARS-CoV-2 pandemic, have there been other SARS-CoV-2 variants of impact?

Why do new SARS-CoV-2 genomic variants emerge?

SARS-CoV-2 has one of the largest genomes of RNA viruses (Figure 1). The positive sense, single stranded genome encodes: nonstructural proteins (functions: genome transcription and replication), structural proteins (functions: virion structure, entry/replication, and immune evasion), and accessory proteins (functions: immune evasion) (1, 2).

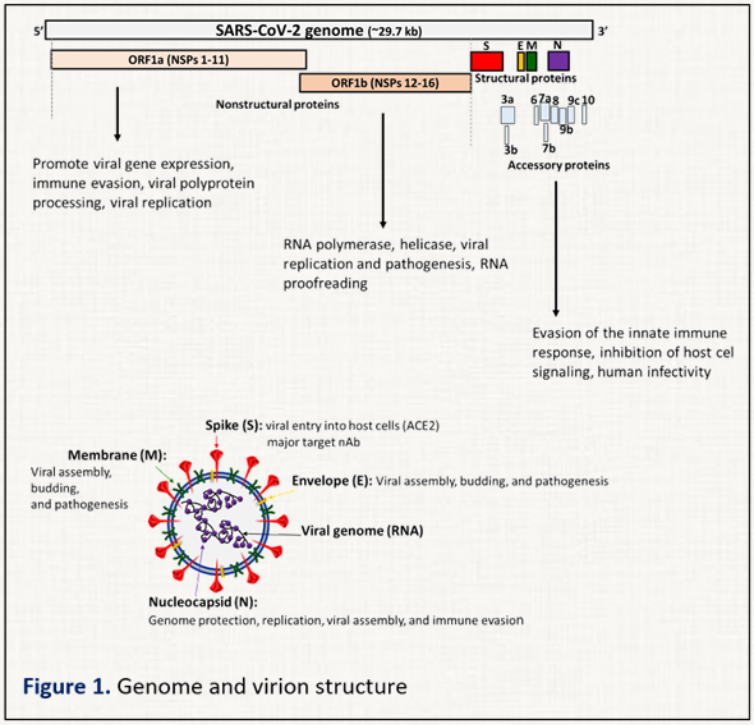


Figure 1. Genome and virion structure

In addition to having the largest genomes of the RNA viruses, coronaviruses also have a unique feature: their genome has a proof-reading capability. This is not to say their replication is perfect; mutations still arise, the SARS-CoV-2 genome just mutates at a much slower rate than other RNA viruses such as influenza or HIV (3).

As the virus replicates, a single infected individual contains copies of the original virus they were infected with and copies of the virus with mutation(s) (termed "variants", see box on terminology) (4). Thus, if someone is persistently infected, many different variants can arise. (5, 6). What is the fate of these variants?

Figure 2. Fates of mutations

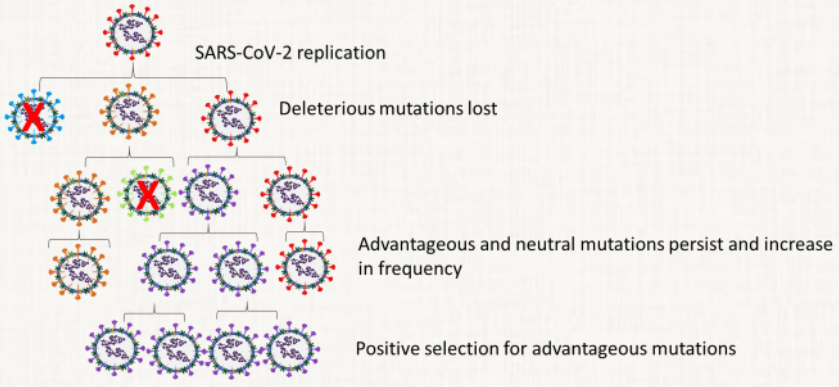


Figure 2. If the variant contains mutation(s) are deleterious, they will be lost. Variants that have mutation(s) that are neutral or advantageous will be retained and may increase in frequency in the human population. When the prevalence is low in a population, the frequency of variants can be more pronounced and it can be difficult to distinguish neutral mutations from advantageous mutations (7). Advantageous mutations can be identified is if they have emerged

repeatedly or independently, if they replace other previous strains, and/or they provide a measurable advantage to the virus (8).

Why are variants a concern?

Advantageous mutations can have a number of consequences that impact the virus and host both at the individual level and the population level. Figure 3A and B.

Increased viral load

Mutations increase viral load by increasing the efficiency in which the virus infects cells, increasing the replication efficiently, and/or helping evade immune responses (more below). Viral load has been tied to disease severity (9) and increased viral load in the upper respiratory tract can increase transmission potential (10).

Enhanced transmission

Similar to viral load, mutations can enhance transmission by increasing viral load in the upper respiratory tract, enhancing viral replication, and/or increasing the environmental stability of the virus.

Differences in disease severity

Mutations may change disease severity by altering the immune response, increasing viral load, or other mechanisms. Increased disease severity and death is a major concern for viral variants. However, decreased disease severity could also be a concern. For instance, if the virus resulted in more mild or asymptomatic infections, that could increase transmission because people might not be aware that they are ill.

Diagnostic evasion

Mutations can occur in genes (or proteins) that are targeted by diagnostic tests. For instance, if a mutation affects the ability of a primer to bind in an RT-PCR diagnostic assay, the assay may not be able to detect the variant.

Therapeutic evasion

Mutations can assist the virus in evading therapeutic agents such as monoclonal antibodies and antiviral drugs if they alter the therapeutic target.

Immune evasion (innate or adaptive)

Mutations can decrease the ability of the immune response to target the virus. Mutations can affect the innate response by interfering with the interferon response, disrupting cell signaling, disrupting innate immune cells (natural killer cells, dendritic cells, and mast cells), or decreasing specific elements of the viral genome (such as CpG dinucleotides) that are the target of the innate response (11, 12). Mutations can also occur in the targets of neutralizing antibodies that arise either from natural infection or vaccines leading to decreased population level immunity and decreased vaccine efficacy.

Figure 3A.

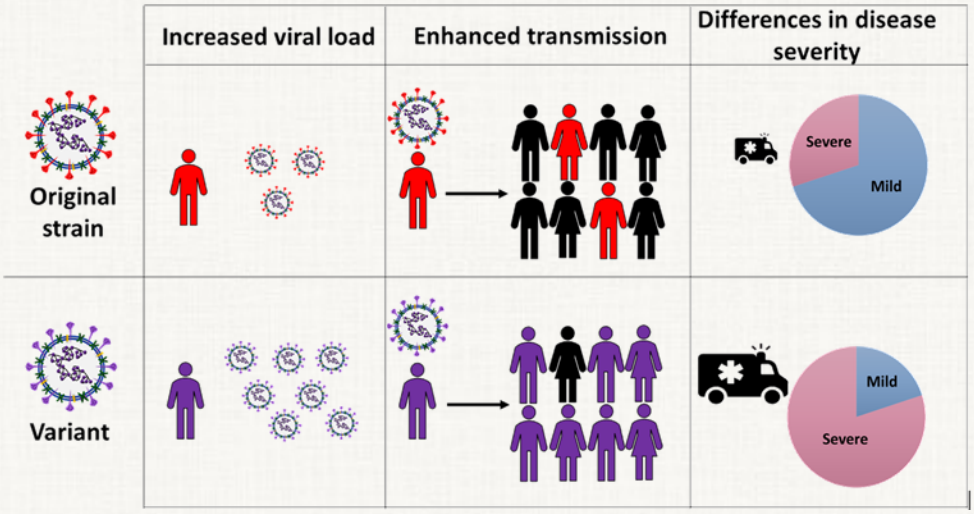
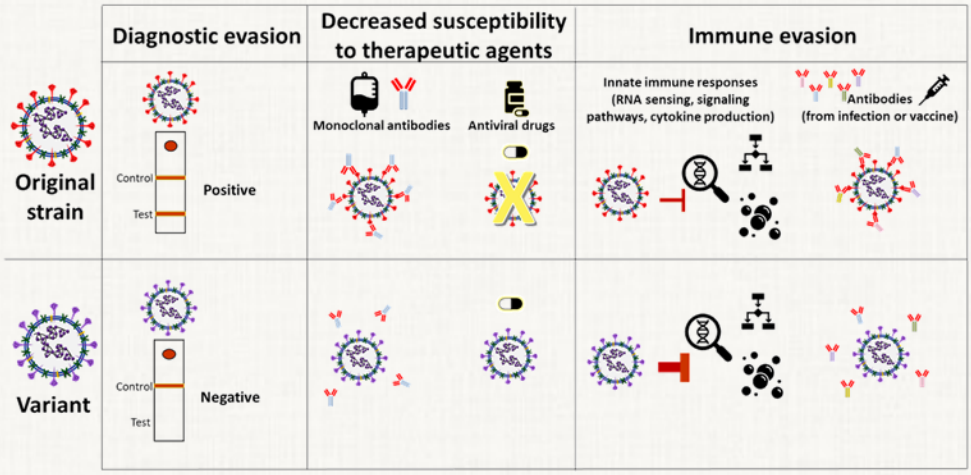


Figure 3B.



Reports on variants and their consequences

Founder effect

Often in outbreaks, as viruses spread to different geographical regions, certain mutations may being to dominate (founder mutations) (13). This was observed with the early global spread of SARS-CoV-2 (14) and reports of specific geographical variants (15-21).

D614G

To look for variants that may be increasing in frequency due to positive selection, a group at the Los Alamos National Laboratory looked for common variants that became more prevalent in distinct geographical locations (meaning the same variant was found independently and repeatedly in different locations without an obvious connection). Observing this phenomenon repeatedly indicates that a particular variant may be a candidate for conferring a selective advantage (advantageous mutation) (22).

The group focused specifically on the Spike protein because of its role for viral entry and since it is the major target of neutralizing antibodies. Comparing 28,576 sequences that had been uploaded to the GISAID SARS-CoV-2 database (https://www.gisaid.org/) by 29. May 2020 to the original reference strain from Wuhan revealed an aspartic acid (D) to glycine (G) change at position 614 in the Spike (herein referred to as D614G). In addition, there were 3 other mutations that almost always accompanied the D614G.

Mapping this haplotype over time revealed that prior to 1. March 2020, it only comprised 10% of the sequences deposited. However, it increased drastically to 67% from 1. March to 31. March 2020 and then 78% of sequences between 1. April to 18. May 2020. By June 2020, D614G was found in all viruses circulating globally (23). This indicates the G614 provides a selective advantage to the virus. What are the consequences of the D614G?

Increased viral load? Yes

Analysis of nasal specimens from patients infected with G614 had higher viral load than those with D614 (22, 24). In vitro studies using primary human respiratory tissue and lung epithelial cell lines demonstrated increased infectivity, stability, and replication (10, 25). This was also observed in nasal washes from the hamster model (25) .

Enhanced transmission? Yes

Structural analyses revealed that the D614G shifts the Spike protein into a conformation that would be better able to bind to the ACE2 receptor, which indicates that it can more efficiently bind to host cells and could lead to increased infectivity/transmission (23). In competition assays in primary human respiratory tissue, the G614 always dominated even when the infection was set up in a 9:1 D614:G614 ratio (25). Finally, using a hamster model, demonstrated enhanced transmission between hamsters (10).

Differences in disease severity? No

Despite increased viral load, examining clinical outcomes reveals the G614 does not increase or decrease disease severity (22, 24).

Diagnostic evasion? No

There is no evidence that D614G impacts the ability of diagnostic tests to detect SARS-CoV-2.

Therapeutic evasion? No

Neutralization assays revealed that convalescent serum from patients infected with D614 efficiently neutralized G614 viruses and binding assays with monoclonal antibodies including the Regeneron mAbs demonstrated that they were still effective at neutralizing the G614 virus (10, 25).

Immune evasion (innate or adaptive)? No

See above. In addition, the D614G variant emerged at a time when population level immunity was low, indicating it was unlikely the result of immune pressure.

Conclusion

Taken together, the D614G variant has enhanced replication and transmission but did not diminished neutralizing antibody binding.

New variants

All variants discussed below have diverged from the D614G (clade 20A Nextstrain nomenclature. B.1 Pangolin nomenclature).

In addition to the D614G, all of the variants discussed in this section also contain a mutation in the Spike protein Receptor Binding Domain (RBD) at position 501, where a tyrosine (Y) replaced the asparagine (N). Residue 501 forms a critical interaction with the host cell, a hydrogen bond with the Y41 of the host angiotensin-converting enzyme-2 (ACE2) receptor (26). The 501Y form has a higher affinity for this interaction (27, 28). This means that the viruses with the 501Y are better able to "stick" to the ACE2 receptor. Thankfully, it does not appear N501Y impacts neutralizing antibody binding (29) and does not appear to impact the current Pfizer or Moderna mRNA vaccines (30).

Two of the variants, B.1.315 and P.1, both contain additional mutations in the Spike RBD at E484 and K417. The E484K mutation changes the charge from negative to positive, which impact the shape of the RBD as it binds to the ACE2 receptor and

Variant nomenclature	Location and date first identified	Characteristic mutations
B.1.1.7 (20I/501Y.V1)	United Kingdom (UK) September 2020	ORF1ab: T1001I, A1708D, I2230T, Δ3675–3677 SGF Spike: Δ69–70 HV, Δ144 Y, **N501Y**, A570D, D614G, P681H, T761I, S982A, D1118H ORF8: Q27stop, R52I, Y73C Nucleocapsid: D3L, S235F
B.1.351 (20H/501Y.V2)	South Africa October 2020	ORF1ab: K1655N Spike: K417N, E484K, **N501Y**, D614G, A701V Envelope: P71L Nucleocapsid: T205I
P.1 (20J/501Y.V3) B.1.1.28 subclade (renamed "P.1")	Japan and Brazil January 2021	ORF1ab: F681L, I760T, S1188L, K1795Q, Δ3675–3677 SGF, E5662D Spike: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, **N501Y**, H655Y, T1027I ORF3a: C174G ORF8: E92K Nucleocapsid: P80R ORF9: Q77E ORF14: V49L

Table 1. New variants that have emerged and spread in late 2020.

it is thought that the E484K mutation may work synergistically with the N501Y to further increase binding (31). Mutational analyses with binding of polyclonal convalescent serum revealed that the E484K site has the most dramatic impact on neutralizing activity (32) and the K417 site also negatively impacts neutralization by both polyclonal sera and monoclonal antibodies (33, 34).

B.1.1.7 (20I/501Y.V1)

In December, headlines circulated regarding a Variant of Concern (VOC) that was identified in the United Kingdom (UK). This variant is referred to as B.1.1.7 (Pangolin lineage) or VOC 202012/01 or 20I/501Y.V1 (Nextstrain nomenclature).

While the variant was first detected in September (35), it wasn't until 14. December that England's Health Secretary reported a large increase in cases in south east England that was likely due to the B.1.1.7 variant (36). Analysis of available data by the advisory group to the United Kingdom Government's Chief Medical Advisor, New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG), lead to the conclusion that B.1.1.7 "demonstrates substantial increase in transmissibility compared to other variants" (37). This led to widespread lockdowns in the UK and headlines that read "Christmas is canceled."

As of 18. January 2021, B.1.1.7 was reported in 58 countries (38, 39). While many cases are imported, local transmission (meaning a case from someone without travel history) has been reported in: Europe (UK, Denmark, Netherlands, Italy, Ireland, Portugal, Spain, France, Switzerland, Belgium, Germany), the Middle East (Israel), Asia (China), South America (Brazil, Chile, Peru), and North America (US and Canada). Imported cases have been found in Malaysia. As of yet, B.1.1.7 has not been reported in Indonesia.

The B.1.1.7 variant has a total of 29 mutations that differ it from the original Wuhan strain and there are very few intermediary strains, indicating that the virus did not emerge due to gradual accumulation of mutations and therefore, likely emerged due to positive selection.

It could have emerged in an area with low sequencing coverage, however, given the large number of mutations in the Spike and global travel patterns, this is unlikely (40). It potentially could have emerged due to passage between an animal host and humans as was observed in mink farms in the Netherlands (41); however, the UK has reported that there's not a clear epidemiological link with the variant (40). The most likely explanation is that it emerged due to prolonged shedding in an immunocompromised host, which has been observed to previously result in variants (6).

Increased viral load? Yes

Analysis of RT-PCR results revealed decreased Ct values; indi-

cating increased viral load (37, 42). This could be partially driven by the N501Y, which increases the interaction force of the Spike and the ACE 2 receptor (27, 28) as discussed above.

Enhanced transmission? Yes

Initial analysis by the NERVTAG revealed increased growth rate from genomic data (70% faster) and increased R value (or the number of people an infected person transmits to) (37). Further pre-print analyses with more genomic and epidemiological evidence has revealed B1.1.7 has a roughly two-fold replication advantage at the population level (43); and this is due to increased R-value (number of people an infected person transmits to) rather than changes in the viral generation time (how fast the virus replicates) (24). Importantly, a preliminary report from the UK Genomics Consortium showed that following lockdown, while other strains and lineages of the virus decreased (R value 0.85), the B.1.1.7 variant continued to increase (R value of 1.25), indicating even with strict measures, the B.1.1.7 is efficiently transmitted (44).

Differences in disease severity? Potentially

Early examination of matched case-controls indicated there is no evidence that the B.1.1.7 variant impacts disease severity (45). However, on 22. January 2021, NERVTAG released more information indicating that three independent analyses indicate that there is a "realistic probability" of an increased risk of death with B.1.1.7 (46). Caveats to the report are that the deaths tend to lag and there also has been considerable pressure on the health system which may decrease level of care available. However, this confounder highlights the importance for good public health measures to mitigate the spread of B.1.1.7

Diagnostic evasion? Yes (for some assays)

The B.1.1.7 variant has a deletion in the Spike protein of residues 69 and 70 (Δ69–70). This deletion impacts some molecular diagnostic tests that target the Spike (24, 42, 47). This phenomenon has been termed "S gene drop out" or "S gene target failure" (SGTF) (48). The US Food and Drug Administration (FDA) has issued a letter to health care providers regarding three molecular tests (47). For the Accula SARS-Cov-2 Test, non-significant impact has been observed but out of an abundance of caution, the FDA is working with the company. The Linea COVID-19 Assay Kit detects multiple targets and sensitivity is not impacted. Similarly, TaqPath COVID-19 Combo Kit also detects multiple targets and therefore retains overall sensitivity.

The TaqPath assay has been used to screen for the B.1.1.7 mutation as each target can be viewed independently. Therefore, when SGTF is observed, it is assumed that the specimen is positive for B.1.1.7 (24, 42, 48). Screening for the B.1.1.7 variant using SGTF has been employed in the UK (24, 42) and Portugal (49). However, a preliminary report recently described a case in Wisconsin that the Δ69–70 mutation can occur in other variants and therefore may not be completely reliable for identifying only the B.1.1.7 variant (48, 50).

Therapeutic evasion? No

As yet, there's no evidence that B.1.1.7 evades therapeutics (antiviral, convalescent serum or hyperimmune IVIG, or monoclonal antibodies) (45).

Immune evasion (innate or adaptive)? Potentially (innate). No (vaccine or infection elicited)

In addition to the Spike mutations, B.1.1.7 contains mutations in the accessory protein 8 that are hypothesized to potentially impact interferon (IFN) signaling, an important innate immune response (26). As mentioned above, tests with sera from the Pfizer and Moderna mRNA vaccine trials demonstrated that the 501Y had very little impact on neutralization (30). Further, both Modern and Pfizer have stated that the Spike mutations in the B.1.1.7 variant "represent less than a 1% difference from the spike protein encoded."

B.1.351 (20H/501Y.V2)

A second variant of concern, B.1.351 or 20H/501Y.V2, was reported in South Africa on 18. December 2020. It was first identified in Nelson Mandela Bay at the beginning of October. This area was one of the hardest hit during the first pandemic wave for South Africa and B.1.351 rapidly spread to became the dominant strain within weeks throughout the Eastern and Western Cape Provinces (51).

As of 18. January 2021, B.1.351 was reported in 24 countries with local transmission in South Africa, Botswana, Zambia, and the UK (38, 39). There have been imported cases to Australia but no local transmission. It has not been reported in the US or Indonesia.

Increased viral load? Yes

Preliminary evidence suggests that B.1.351 has higher viral load than previous strains circulating in South Africa (52).

Enhanced transmission? Yes

B.1.351 has the N501Y mutation which enhances the binding to the ACE2 receptor. In addition, the rapid displacement of other SARS-CoV-2 strains also indicates that B.1.351 likely has increased transmissibility (51, 53).

Differences in disease severity? Unknown

So far, there has been no evidence that B.1.351 impacts disease severity (52, 53). However, it will take time to fully gather data needed to make conclusions about mortality.

Diagnostic evasion? No

The B.1.351 variant does not have the same deletion in the Spike as B.1.1.7 and the other mutations do not impact diagnostic assays.

Therapeutic evasion? Yes

A pre-print examining structure-function and molecular modeling of the B.1.351 mutations on monoclonal antibody binding, predicts that the K417N and E484K abolish the salt bridges that are formed between the Spike RBD and some monoclonal antibodies; thus reducing their efficacy (54). This was demonstrated in a pre-print looking using a B.1.351 pseudo-virus where a significant decrease in the ability of monoclonal antibodies to neutralize the B.1.351 variant was observed (55). This was largely due both to the E484K and K417T mutations, but also with contribution from the other Spike protein mutations, particularly those in the N-terminal domain, which likely influence the structure of the Spike (Table 1) (55).

In the same pre-print, decreased neutralization with convalescent plasma was observed. When the pseudo-virus contained both the N-terminal domain and RBD mutations, there was no neutralizing activity in 21/44 of the donor sera and a substantial decrease in the rest (55). It is important to note that the convalescent plasma was collected from donors between May-September 2020. During this time, clades 19A, 20A, 20B were dominant, which contain the D614G but not the N501Y or other RBD/N-terminal mutations found in the variant (Nextstrain filtered for Africa) (56). B.1.351 is Pangolin lineage equivalent to the 20H/501Y.V2 clade.

Another pre-print with microneutralization assays with live B.1.351 virus and convalescent plasma from donors who were infected with the D614G virus but none of the other RBD/N-terminal domain mutations revealed a 6-200-fold reduction in neutralization. There was large variance among the donors in terms of degree of reduction (57).

Immune evasion (innate or adaptive)? Unknown (innate). Potentially (adaptive)

Given the findings with the convalescent plasma and decreased neutralization of the B.1.351 variant, there is concern about vaccine efficacy. While the 501Y showed a modest drop in neutralization with serum from participants in the Pfizer or Moderna mRNA vaccine trials (30), it will be important to test the effect of the other RBD and N-terminal mutations from the B.1.351.

P.1 (20J/501Y.V3)

The final variant of concern is the P.1 or 20J/501Y.V3 variant. P.1 is a branch off the B.1.1.28 lineage. In December in Manaus, Brazil, despite having an estimated 76% COVID-19 attack rate previously (58), cases and hospitalization began to increase again (59). Sequencing revealed the emergence and dominance of another variant of concern, P.1 (60). P.1 was also identified in travelers in Japan that had come from Brazil (45).

As of 18. January 2021, local transmission of P.1 has only been observed in Brazil (mainly in Manaus) and imported cases have been found in Italy and Japan (38, 39). No cases have been detected in the US or Indonesia.

The P.1 variant shares 3 mutation with the B.1.351 variant in the RBD of spike discussed above (N501Y, E484K, K417T) and has other distinct mutations in the Spike and other proteins (Table 1).

Increased viral load? Potentially

No reports have been released on viral load, however, P.1 has the N501Y mutation which enhances the binding to the ACE2 receptor.

Enhanced transmission? Potentially

Given the N501Y mutation and the rapid increase in cases in Manaus, it is likely that P.1 has enhanced transmissibility.

Differences in disease severity? Unknown

No reports have been released to indicate differences in disease severity.

Diagnostic evasion? No

The P.1 does not have the deletion that B.1.1.7 has in the Spike and there have not been any reports on decreased diagnostic accuracy with the P.1 variant.

Therapeutic evasion? Potentially

It is unknown if P.1 can evade current therapeutics. Given the RBD mutations that are shared with B.1.351, presumably the efficacy of monoclonal antibodies and convalescent plasma therapy could be reduced.

Immune evasion (innate or adaptive)? Potentially

There have been some reports of reinfections and the rise in infections in an area with high immunity is a major concern (59), but as with the B.1.351 variant, analysis of sera from vaccine recipients will provide critical insights.

Summary Table

	B.1.1.7 (20I/501Y.V1)	B.1.351 (20H/501Y.V2)	P.1 (20J/501Y.V3)
Increased viral load?	Yes	Yes	Potentially
Enhanced transmission?	Yes	Yes	Yes
Differences in disease severity?	Yes	Unknown	Unknown
Diagnostic evasion?	Yes	No	No
Therapeutic evasion?	No	Yes	Potentially
Immune evasion?	No	Potentially	Potentially

Conclusions

The emergence of SARS-CoV-2 variants reinforces the principle: viruses mutate. It also highlights the importance of genomic surveillance combined with epidemiology and basic laboratory experiments. While variants are a concern given their potential impacts on case numbers, disease severity, therapeutics, and vaccines; basic public health measures such as masks, hand-washing, and social distancing are still effective and should be double-down on. Finally, while the worry of decreased vaccine efficacy is important; given how effective the current SARS-CoV-2 vaccines are and the platforms on which they were developed; decreases in efficacy may still help curb the spread of SARS-CoV -2 and the vaccines will be able to be updated.

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INA-RESPOND Newsletter

HOW TO PREVENT AN ANKLE SPRAIN: AN UPDATE

By: Septi Mandala Putra

People tend to be more active and take part in sports and other physical exercises nowadays. Physically active individuals who often participate in activities that require jumping, changing direction, and pivoting are at increased risk for ankle sprain1.

An ankle sprain is one of the most common musculoskeletal injuries. Ankle sprains and the repetitive trauma often associated with the condition can lead to long-term disability, time lost from activity, and economic burdens for patients. Although the cost of treatment after a single ankle sprain is low, compounding expenses for extended care to address repetitive sprains in patients with conditions such as chronic ankle instability can increase the economic burden2.

An ankle sprain is characterized by the tearing of ankle ligaments. More than 70% of ankle sprains is a lateral ankle sprain, and 73% is the anterior talofibular ligament (ATFL)3. 2 million ankle sprains occur annually in the United States, 2 to 7 incidence rate of ankle sprain / 1000 person-years4. A research of 181 prospective epidemiology studies of ankle sprains among various populations shows that ankle sprains' incidence was higher in females than males (13,6 vs. 6,6 per 1000 exposures)3.

There are predisposing factors that can increase the risk of sustaining an ankle sprain. It can be classified as intrinsic (patient-related factors) or extrinsic (environmental characteristics). Intrinsic factors can be limited dorsiflexion of the ankle, reduced proprioception, deficiencies in postural control/balance (single leg balance test), reduced strength, foot posture index, anatomical abnormalities in the ankle and knee alignment, and cardiorespiratory endurance. Extrinsic risk factor includes the types of exercise or sports with a high incidence of ankle sprains like basketball, soccer, indoor volleyball, and climbing. In volleyball, landing after a jump is the most significant risk factor. Playing soccer on artificial turf and being a defender can increase ankle sprain incidence (42,3%)5.

About half of recurrent ankle sprains result in disability and chronic pain. It is an important patient-oriented treatment goal if we prevent the repeat of ankle sprains incidence. Various modalities, including bracing, taping, and warm-up and strengthening exercises, have been used to avoid an ankle sprain's recurrence. Proprioceptive training has also been suggested6.

External prophylactic support

Ankle taping and bracing have been used to protect the ankle ligament from excessive sprain and remain popular at all levels. Although there are many variations of ankle taping applications and brace design, Zweirs et al.7 conclude 3 points:

- Mechanical supports: the primary benefit of ankle taping and bracing is to prevent and restricted all directions of ankle motion (inversion, eversion, plantar flexion, and dorsiflexion)
- Neuromuscular effects: taping and bracing can increase the stimulation of the cutaneous mechanoreceptors, enhancing proprioception by modifying the sensitivity of the surrounding joint.
- Psychological benefits8: some reports tell that enhanced perceptions of stability, confidence, and reassurance during activity and comfort level can make the participants feel that they don't have an ankle injury.

Ankle tape

Ankle brace

Exercise programs

Exercise programs such as stretching, strengthening, balancing, and sport-specific hopping and agility motions often give a positive effect to prevent an ankle sprain.

Stretching the gastrocnemius and soleus muscle can improve dorsiflexion range of motion in ankle ligaments and allow the joint to function in a more stable position.

Strengthening exercise like calf raise has a positive impact on reducing the risk of an ankle sprain if you are doing it right (the movement and control).

Balancing and proprioceptive exercises are the core components to prevent an ankle sprain. Examples of proprioceptive training for the ankle joint include balancing on a single leg with the eyes closed, balancing on a wobble board or ankle disk, and balancing on a single leg while completing a task such as catching or throwing a ball.

This can enhance both static and dynamic postural control and optimizing the body's ability to sense and correct the deviation in joint motion9.

Single limb balance in stable or/and unstable surface

Conclusions

External prophylactic support and exercise program has its strength and weakness. External prophylactic support appears to be more effective in preventing ankle sprain. A reusable brace is a more cost-effective method that can be used if there is no personal trainer, sports physician, or other practitioners who understand the exercise program. It can be used in a shorter time and can be applied easily (vs. taping). An exercise program is the most effective way to prevent ankle sprain if you have a coach/practitioner that can give the program. It can also potentially improve the performance10.

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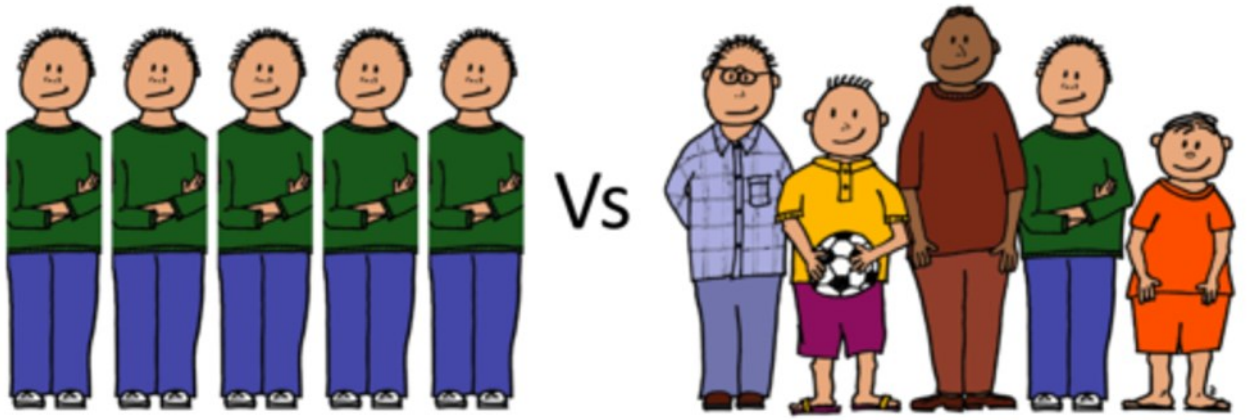
EFFICACY AND EFFECTIVENESS OF COVID-19 VACCINES

By: Aly Diana



Source: ISAPP Science Blog (https://isappscience.org/efficacy-and-effectiveness/)

COMIC CORNER

I am sure that we all know about the differences between efficacy and effectiveness. However, as these terms are almost used interchangeably in the layman world, I think it will not do any harm to re-state the definition of efficacy and effectiveness. **Efficacy** refers to the benefits and harms of an intervention under highly controlled conditions. **Effectiveness** examines interventions under circumstances that more closely approach real-world practice, with more heterogeneous patient populations, less-standardized treatment protocols, and delivery in routine clinical settings.

Why is it important?

All of the current phase 3 trials are designed as **efficacy studies** using individually randomized, placebo-controlled clinical trials (RCTs). This type of randomization also serves to isolate estimates of vaccine protection from herd protective effects and confines estimates of efficacy to direct vaccine protection of an individual, not a population. Moreover, the current phase 3 trials are powered **to detect protection against symptomatic infections**. Protection against severe COVID-19 disease and mortality is a key goal of implementing a COVID-19 vaccine in practice.

Recently, Pfizer/BioNTech has announced efficacy of 95%5; Gamaleya has announced efficacy of 92%; Moderna has announced efficacy of 94.5%; and Astra-Zeneca has announced efficacy of 70%. Sinopharm has now announced efficacy of 79%, and several countries participating in the Sinovac efficacy trials have announced efficacies (for the same product) of 50%, 65%, 78%, and 91%.

In these RCT studies, close attention is paid to timely attendance of study visits, cold-chain requirements, and study product administration. These variables might be difficult to control as vaccination of the general population is implemented. RCT designs (efficacy studies) might therefore **overestimate** the level of vaccine protection compared to real-world settings. The factors that may contribute include ways of transportation/ distribution and storage and how patients are vaccinated. In the 'real world,' a person might arrive three weeks late for the second vaccine dose, or the vaccine might have been in a refrigerator or freezer that had been

unmonitored and had a significant excursion of temperature or even not available when the time for second dose due. Vaccine effectiveness can also be affected by differences in the underlying medical conditions of people vaccinated in the real-world compared to those in the clinical trials. Vaccine effectiveness assessments can also provide important information about how well a vaccine works in groups of people not included or not well represented in clinical trials.

For these reasons, even when COVID-19 vaccines have achieved licensure via current phase 3 trials, **there will be substantial uncertainties about how useful the vaccines will be in practice**, and studies done after licensure, addressing vaccine effectiveness, including the level of protection of both vaccinated and non-vaccinated individuals in entire targeted populations, will be needed. Finding the design that would be both valid, reliable, and ethical is another challenge, especially during the pandemic. There are many things to consider, from quality, regulatory pathways of every country, vaccination access and equity, optimization of dose, schedule, and boosters, safety, genetic drift in evaluation of SARS-CoV-2, herd immunity, and many other factors.

However, we have to remember that "**vaccines do not save lives; vaccination does.**" Vaccination should be seen as one part of a comprehensive **mandatory** package of COVID-19 prevention, which will include masks, distancing, hygiene, and preparedness of healthcare facilities. Yes, we don't know what is the effectiveness of the vaccines right now. Understanding effectiveness will require the systematic implementation of post-licensure studies to understand the key parameters around herd immunity and policies derived from that knowledge. Nevertheless, when the effectiveness is lower than the efficacy, higher coverage is needed to reach herd immunity. So, let's take part and get vaccinated!

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