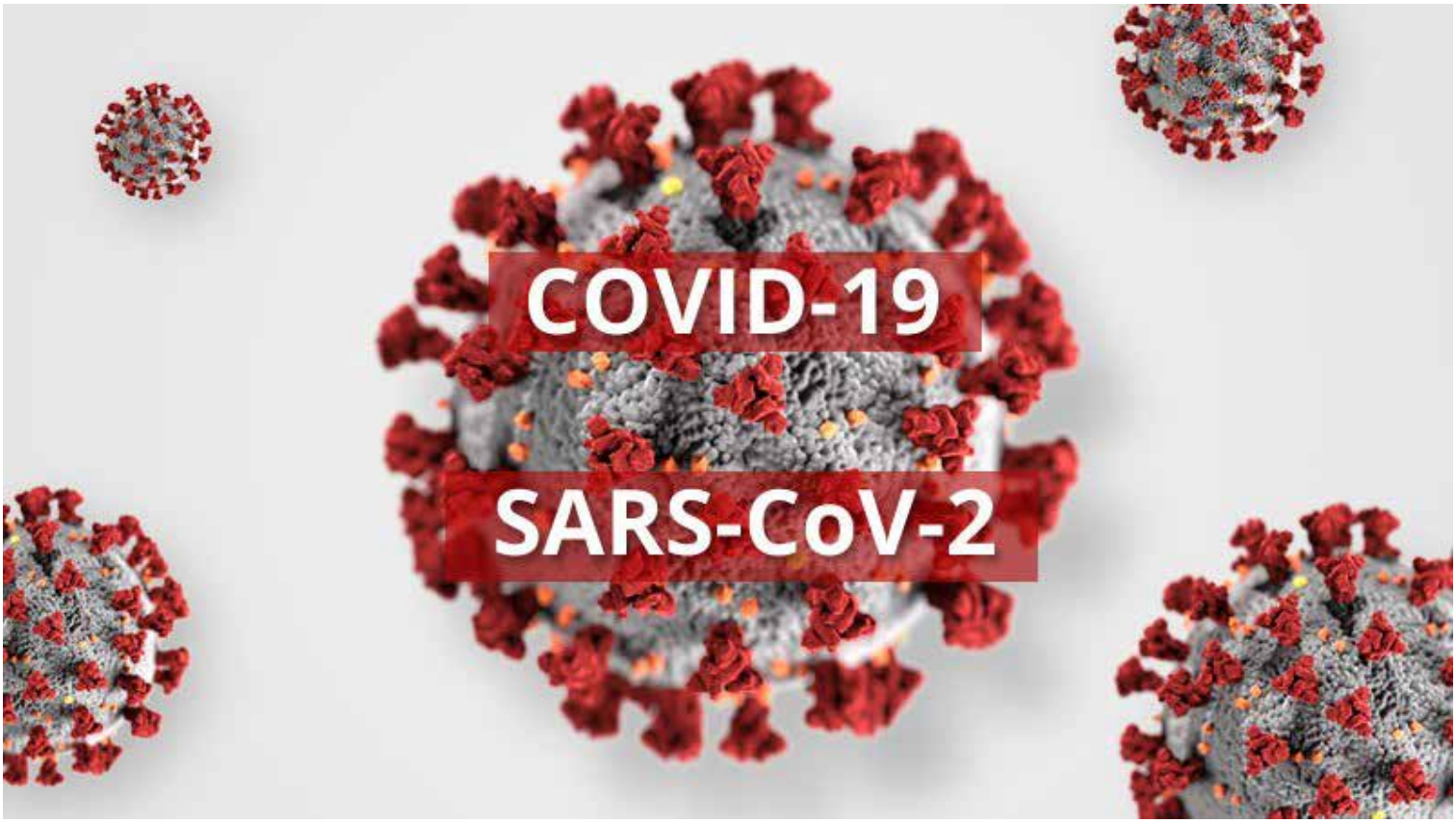


Saving the world one test at a time. The right Superhero for Covid19!


Pawel Czech
Founder at Nex.D

RT-LAMP – hitting the gold standard.

If there's one phrase we have all heard time and time again since this pandemic started, it's that 'RT-PCR is the gold standard test for COVID-19.'

So what is a 'gold standard' in this regard?

In medicine, a gold standard test is usually the diagnostic test or benchmark test that is the best available for the task at hand. Molecular testing techniques have developed significantly over the last 30 years, but all of these techniques essentially began with a technique called PCR - polymerase chain reaction. This technique allowed us to amplify and detect small amounts of the genetic material DNA in an exponential manner. Over time, PCR gave rise to several modified and enhanced techniques, such as RT-PCR, qPCR, and others. If a scientific technique or test is well known to be the best, and over time it accrues a lot of scientific evidence to support its accuracy, then this naturally becomes the gold standard.

What is RT-PCR? Where do these terms come from and what do they actually mean?

RT-PCR stands for Reverse-Transcription Polymerase Chain Reaction.

PCR is a very common scientific technique that has been widely used in research and medicine for several decades, in order to detect genetic information. RT-PCR is a version of the PCR technique, used when the genetic material called RNA (rather than DNA that humans have) is being detected. RT-PCR is now being used as a test to detect SARS-CoV-2 (the virus that causes COVID-19), because the genetic material inside this virus is made of RNA. This type of test has frequently been used as a frontline test for COVID-19, as it directly tests for the presence of the viral RNA.

RT-PCR tests are sensitive and reliable tests, capable of producing results in 3-4 hours, although this usually takes longer as samples must first be sent to specialized external laboratories (6-8 hours on average).

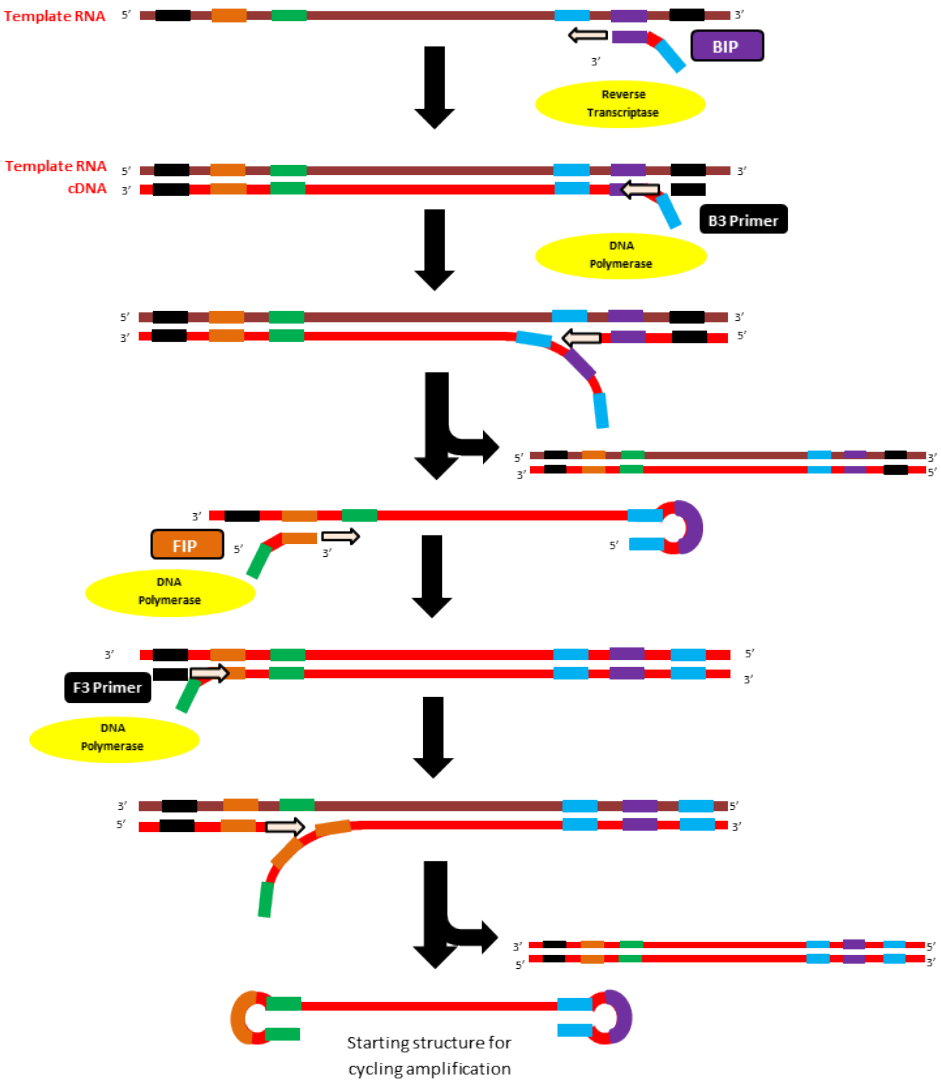
Many diagnostic and research companies produce RT-PCR products, tests, and machines, so the technology is widely available. Some RT-PCR tests are developed as an `all-in-one' kit, reducing laboratory handling and potential for contamination.

Once a sample has been collected, chemicals are used to remove any proteins, fats, and other molecules, leaving only the genetic material (called nucleic acid) behind. This will be a mixture of the tested person's genetic material, as well as any viral RNA that might be present.

Enzymes in the testing kits convert the viral RNA into DNA, a step that is required in order to then allow this genetic material to be amplified, thus allowing virus detection by using a PCR machine. This machine cycles through heating and cooling temperature to help amplify the amount of viral genetic material, so that roughly 35 billion copies of viral DNA are made for each single viral RNA strand that was originally present.

Fluorescent markers are typically used to bind to the amplified DNA and produce light, which is detected by the machine to produce the test result. If the intensity of the light produced within the sample reaches a certain threshold, this is classed as a positive test. The more viral genetic material, the more fluorescent markers there are that bind to it, and the stronger the fluorescent signal is that the machine detects. The number of PCR temperature cycles that are required to reach the fluorescence threshold is recorded, and this gives an estimate of how much virus was present in the patient sample.

So, is RT-PCR the 'gold standard' then? - well, to give you that classic politicians' answer: "yes and no".



Let's step back for a second. The WHO doesn't actually state that RT PCR is the gold standard; it says to be clear that NAAT (Nucleic Acid Amplification Test) is the gold standard reference). NAATs enable sensitive detection of low-density viral infections.

Types of nucleic acid amplification tests:

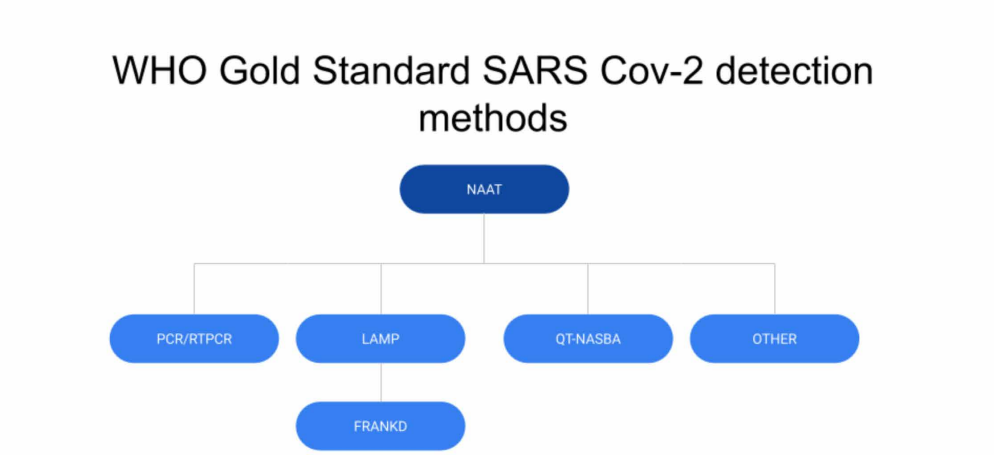
Polymerase chain reaction (PCR) – including nested (n), quantitative (q) or real-time reverse transcription (RT) PCR, Reverse transcription loop-mediated isothermal amplification (RT-LAMP), and quantitative nucleic acid sequence-based amplification (QT-NASBA) – are among the key NAATs developed.

So what is a NAAT? A NAAT is a nucleic acid amplification technique.

The most well known of the NAAT is the RT-PCR test, but that is like saying the most well-known superhero is Spiderman therefore Spiderman is the only superhero worth knowing!

If we have learned anything from the world of Marvel comics, it is that there are a lot of worthy superheroes out there, and some may be, in their own way, even more, amazing than The Amazing Spiderman...

So is FRANKD a NAAT?



Yes. As a reminder, according to the World Health Organization (WHO), the types of Nucleic Acid Amplification Tests (NAATs) include: Polymerase chain reaction (PCR) – including nested (n), quantitative (q) or real-time reverse transcription (RT) PCR, Reverse Transcription loop-mediated isothermal amplification (RT-LAMP), and quantitative nucleic acid sequence-based amplification (QT-NASBA).

FRANKD is a NAAT, more specifically, FRANKD is a type of Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) test, which is often called RT-LAMP or Isothermal test.

RT-LAMP is a similar process to RT-PCR, but instead of using a series of time-consuming temperature cycles to help produce DNA copies of the viral RNA, RT-LAMP is conducted at a constant temperature of about 60°C, using additives to the testing sample (called primers and a special type of the enzyme called polymerase).

The amount of DNA produced in RT-LAMP is much higher than in RT-PCR, and a positive test result can even be seen visually with the naked eye, without requiring a machine to read the results.

Compared to RT-PCR, RT-LAMP is a more modern technology but is technically simpler and easier for technicians to perform, making it a potentially more widespread and useful technique for the detection of COVID-19 during this global pandemic.

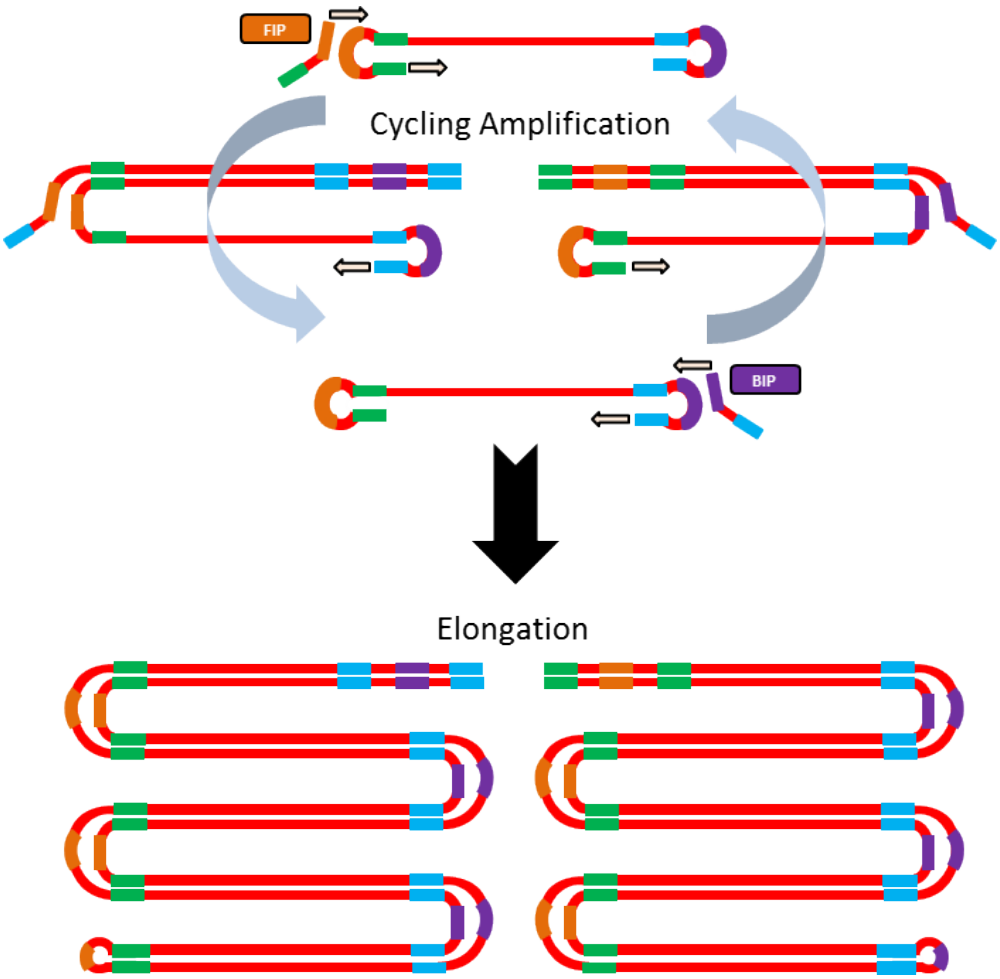
As it is a newer technology, there is understandably less historical scientific evidence published on its use in COVID-19, but diagnostic companies like GeneMe are currently performing clinical trials to help provide robust evidence to support its widespread use.

How it works:

RT-LAMP assays for COVID-19 start with the collection of samples from the nose or throat of an individual using a swab, but can also use samples collected using other less invasive methods, such as mucous/saliva produced from coughing/drooling, or from a cheek swab.

Like for RT-PCR, the viral RNA in the sample is converted to DNA, which allows it to be copied and amplified. The amplified viral copy DNA using RT-LAMP technology and

reagents can be detected in two ways. The first method is the analytical method, which uses a fluorescent dye intercalation analysis in the thermocycler (the PCR processing machine). As the dyes interact with the viral copy DNA, the intensity of the light detected on the thermocycler can be measured to give the approximate number of viral RNA molecules that were initially present in the sample. The second method is the end-point type analysis, which uses a specific dye that, in the presence of SARS-CoV-2, visibly changes the color of the reaction mixture. This color change can be detected by the naked eye, in order to determine if the reaction is positive or negative. However, this second analysis type is more prone to errors and is not suitable as an accurate diagnostic method.

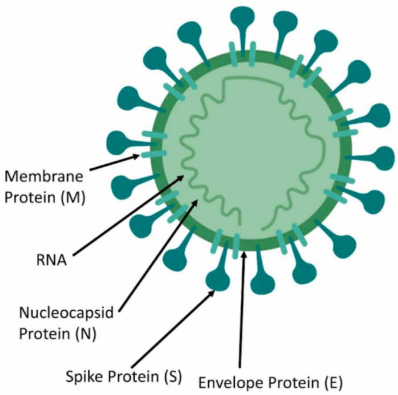


What does the test detect?

Like RT-PCR, RT-LAMP detects whether or not viral copy DNA (produced from viral RNA) is present in a sample from a patient. It does this by capturing and amplifying regions of the virus' genetic material, usually, genes encoding the Spike protein, N protein, or Envelope, or multiple regions at once (see virus diagram below).

Fig. 1

From: Revisiting the dangers of the coronavirus in the ophthalmology practice



Structural Protein	Function of Protein
Nucleocapsid Protein (N)	• Bound to RNA genome to make up nucleocapsid
Spike Protein (S)	• Critical for binding of host cell receptors to facilitate entry of host cell
Envelope Protein (E)	• Interacts with M to form viral envelope
Membrane Protein (M)	• Central organiser of CoV assembly • Determines shape of viral envelope

- It has been noted that some CoVs do not need to have the full ensemble of structural proteins to make virions, highlighting that certain proteins may be dispensable or compensated by the function of non-structural proteins.

Main structure of coronaviruses.

Samples can be collected in the same way as for RT-PCR, usually from the nose or throat using a swab stick, or through mucus produced when a person coughs strongly. In order to amplify and measure the viral RNA, it must first be converted to DNA using an enzyme and copied many times. The results of the test are determined by the fluorescence signal measured in the thermocycler machine, or on the cloudiness/color change of the reaction mixture.

What does the result mean?

Like RT-PCR, RT-LAMP tests are highly sensitive and reliable at detecting the virus, if performed on a sample taken from an infected part of the body whilst an active infection is occurring.

A Positive test result: – A positive RT-LAMP result means that the person the sample was taken from is currently infected by the virus.

A Negative test result: – A negative RT-LAMP result could mean that the person is not currently infected by this virus, the virus is not present at the site the sample was taken from, or that it is too early, or too late in the infection to detect replicating virus. This is why, when test results are negative, new patient samples are taken a few days later to reduce the chance of incorrectly missing an infected person.

RT-LAMP tests cannot detect if a person has had the virus and then cleared it after the end of the COVID-19 disease (i.e. RT-LAMP tests do not detect antibodies), they only detect when the virus is actually present.

Advantages and limitations of RT-LAMP tests:

Advantages of RT-LAMP:

- RT-LAMP is a significantly quicker technique than RT-PCR, and can produce results in 30 minutes from a sample being tested (as opposed to a number of hours for RT-PCR).

- Technically, RT-LAMP is a simpler and cheaper method compared to RT-PCR, and can be done inside or outside of hospital laboratories, reducing the time between taking a

sample and issuing a diagnosis (in general, RT-PCR has to be done inside a specialist laboratory).

- RT-LAMP can detect current infections of disease, allowing medical staff to determine who is currently infected and who is not.

Disadvantages of RT-LAMP:

- The technology is newer than RT-PCR, and so naturally does not have such an established background of research behind it. Tests using RT-LAMP technology for COVID-19 are still being assessed in clinical settings.
- The science behind building these tests is more difficult than RT-PCR.
- The distribution of virus varies across the respiratory tract between patients, so even if a person is infected with the virus, it may only be detectable in sputum or a nasopharyngeal swab, but not necessarily at both locations at the same time.
- RT-LAMP tests for COVID-19 can only tell if a person is currently infected with this particular coronavirus. It can't provide information on other diseases or symptoms and does not tell staff if a patient has been previously infected with the virus or if a patient has any immunity to the virus.

So if FRANKD is a type of RT-LAMP (or isothermal test) how does it differ from those described above?

FRANKD differs from most RT-LAMP tests in that it has very high specificity and consequently a decreased risk of false-positive results, which are common technological problems when working with RT-LAMP. Thanks to an innovative patented polymerase enzyme, combined with a special protein (NeqSSB - which binds to single-stranded DNA and RNA) FRANKD is a uniquely global solution for rapid COVID-19 diagnosis.

The patented polymerase enzyme provides high specificity and speed in producing DNA in the reaction, which allows it to produce massive amounts of DNA in a very short time. Practically, this means that FRANKD gives fast and reliable results by detecting nucleic acid of the virus in a NAAT method. FRANKD also uses special ingredients (called oligonucleotides) that are crucial for the accurate and efficient detection of the virus.

The method of developing these components is GeneMe Ltd intellectual property, which has been implemented in the FRANKD test. We have compared our testing components to other published isothermal testing methods for SARS CoV-2 detection, and none of them give similar levels of reaction performance (e.g. specificity, sensitivity, or speed of testing).

FRANKD also offers a complete COVID-19 testing solution, providing a Sample Collection Kit that includes the FRANKD buffer which helps inactivate the virus, making the work with our test safer for the operator. FRANKD buffer is compatible with freeze-dried reagents in the testing tubes provided, so the swab sample can be directly added to the testing tube without the need for a purification step. This means that FRANKD can offer not only the reagents for accurate isothermal detection of the COVID-19 virus but the whole rapid diagnostic testing solution, from sample collection to securely issuing the test resu¹t.

So how does RT-LAMP compare to RT-PCR in terms of accuracy?

Well let's break down the two measures we always talk about:

A clinical trial of the FRANKD test has shown the following reliability measures:

1. Sensitivity 97%
2. Specificity 100%

What is Sensitivity? – This is the ability of a diagnostic test to give a positive result when the person being tested has the disease and is supposed to be tested as positive (i.e. the true positive rate)

What is Specificity? – This is the ability of a diagnostic test to indicate a negative result when the person being tested does not have the disease and is supposed to be negative (i.e. the true negative rate).

So FRANKD has a Sensitivity of 97% and a Specificity of 100% when compared to the RT-PCR technique, which is currently the most commonly used.

Remember, not all RT PCR tests are equal, just like not all superheroes are equal. They all have different strengths, different approaches, and different weaknesses.

Advantages of RT-PCR:

- RT-PCR is accepted by scientists and medical staff as a robust and well-documented technique.
- With RT-PCR being so common in research and medicine, the technology is already in place in some laboratories to test for COVID-19.
- RT-PCR can detect current infections of disease, allowing medical staff to determine who is currently infected and who is not.

Disadvantages of RT-PCR:

- RT-PCR relies on time-consuming heating/cooling cycles to help amplify any viral genetic material present in the sample so that it can be detected. This process takes time and so delays the issuing of results. This means RT-PCR takes longer to generate a test result than an RT-LAMP test such as FRANKD (hours vs. <30 minutes)
- RT-PCR is more technically challenging to perform than RT-LAMP tests such as the FRANKD test and requires more specialized and expensive equipment and staff to perform the test.
- The distribution of virus varies across the respiratory tract between patients, so even if a person is infected with the virus, it may only be detectable in sputum or a nasopharyngeal swab, but not necessarily at both locations at the same time.

- RT-PCR for COVID-19 can only tell if a person is currently infected with this particular coronavirus. It can't provide information on other diseases or symptoms and does not tell staff if a patient has been previously infected with the virus or if a patient has any immunity to the virus.

Ok - so I get that FRANKD is a gold standard test, but why haven't we heard of this sort of test before, and why has everyone been talking about RT PCR?

There is no doubt that RT-PCR is very accurate and trusted by the medical community, and when lives are at stake you need to be super accurate and super reliable. However, RT-PCR is also (comparatively) super slow! Because of the fundamental science of the technique, it is unlikely to be going to speed up over time - it can't go to training camp and suddenly get faster or learn to fly. Also, the technical procedures in RT-PCR are complex, which limits its use to the hands of highly skilled professionals.

So when governments talk about RT-PCR being the gold standard they are correct. But unfortunately, just like gold, the reagents and ingredients required to carry out RT-PCR have become a scarce commodity during the COVID-19 pandemic, and a commodity that everyone is trying to get hold of at the same time. It is worth remembering that although gold is certainly a marvelous and precious metal, there are other marvelous metals that are as good, or even better in their own way- we only need to look at Wolverine's adamantium claws to be assured of that.

So RT-PCR may well be like Spiderman - well-known and wonderful no doubt - but we need as many superheroes as we can get to help us through this global emergency, and we need them quickly.

The FRANKD test uses remarkable and innovative modern technology to diagnose COVID-19 at speed and with accuracy. Which superhero is FRANKD then? Perhaps one in particular springs to mind from another (DC comics) universe. The superhero Flash has been described as 'possessing super speed, including the ability to run, move, and think extremely fast, use superhuman reflexes, and seemingly violate certain laws of physics.' It seems like a good match for us at least.

Whichever superhero this test or any other test is most like, one thing is for sure; the more superheroes we have in the world right now, literally the more lives will be saved.

So how does FRANKD compare to so-called "rapid" tests:

Well, first of all, a rapid diagnostic test (RDT) is designed for use where a preliminary screening test result is required rapidly and is especially useful in resource-limited settings. Lateral flow tests are probably the most well-known type of rapid diagnostic tests, similar to pregnancy tests, but there exist other systems such as dipstick tests, vertical flow test, etc.

RDT is different from FRANKD in their technology (antigen detection vs. NAAT) and their accuracy generally comes from the ability to detect viral antigens (such as viral proteins) in the swabs. There are many commercially available RDT but the ones for COVID-19 generally detect antibodies (which the body produces as part of an immune response to

infection by the virus, and which may last long after the virus has been cleared from the body), not the virus itself. Such tests, therefore, don't tell us about the presence of the virus.

Another question - why does the WHO insist on nose swabs rather than throat, mouth, or spit?

Well, it's quite simply a question of something called ‘viral load.’ This is the amount of virus present in a person when they are infected, and it may vary at different sites in the body.

In COVID-19, the viral loads in throat swab and sputum samples peaked at around 5–6 days after symptom onset, ranging from around 104 to 107 copies per mL during this time. Furthermore, 1,69×105 copies per mL are usually detected in the nasal swabs just after 3 days from onset.

So how does FRANKD scale when others can’t?

Firstly, we aren’t using the same reagents that most RT-PCR tests around the world because we produce FRANKD with the use of biotechnological methods, not chemical synthesis (like RT-PCR tests). Biotech production is usually done in engineered bacteria. These bacteria were designed to produce something specific in our case FRANKD enzymes. To scale up, we just need to grow more bacteria… our primary ingredient on which the bacteria create our enzymes is sugar! And if the world runs out of sugar… well, that doesn’t bear thinking about.

Secondly, we have taken an “Android” as opposed to the “Apple” strategy so the FRANKD test can be run on as many PCR machines as possible, thus maximizing the availability of the test to as many people as possible.

Thirdly, we bring the test closer to the people that need it. We use an inactivating buffer so that after the swab has been taken we reduce the biohazard to those processing the test so it can be conducted quickly and without requiring certain laboratory biosafety conditions.

Finally, we are working with manufacturers to make sure that we don't need to use a laboratory-based 'Rolls Royce' PCR machine to process the FRANKD test. Due to the more advanced scientific technology of RT-LAMP tests such as FRANKD (compared to RT-PCR), we don't need the PCR machine to be as expensive or complex. To stretch the analogy, we only need a simple car that does its one job to a high level of accuracy over and over again at an affordable price.

So how does this all play out… first and foremost, the virus will continue to set the timetable unless we get ahead of it. There is so much noise in this space at present that it can be difficult to cut through it all and hear the voices we need to hear. If you were to listen to one person, perhaps Bill Gates is as good as any other. If there is one person on the planet who has helped us to progress technologically and has a clear understanding of where we are and where we are going, then the founder of Microsoft might be a good candidate. Bill Gates has been preparing in his own well-thought-out and methodical response to this anticipated (by him) pandemic for the last 20 years. He is investing in the research initiative after a research initiative to help find a vaccine and improve testing. He has some very insightful words to say about testing for COVID-19:

"World War II was the defining moment of my parents' generation. Similarly, the coronavirus pandemic — the first in a century — will define this era. But there is one big difference between a world war and a pandemic: All of humanity can work together to learn about the disease and develop the capacity to fight it. With the right tools in hand, and smart implementation, we will eventually be able to declare an end to this pandemic — and turn our attention to how to prevent and contain the next one".

Bill Gates, April 2020

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