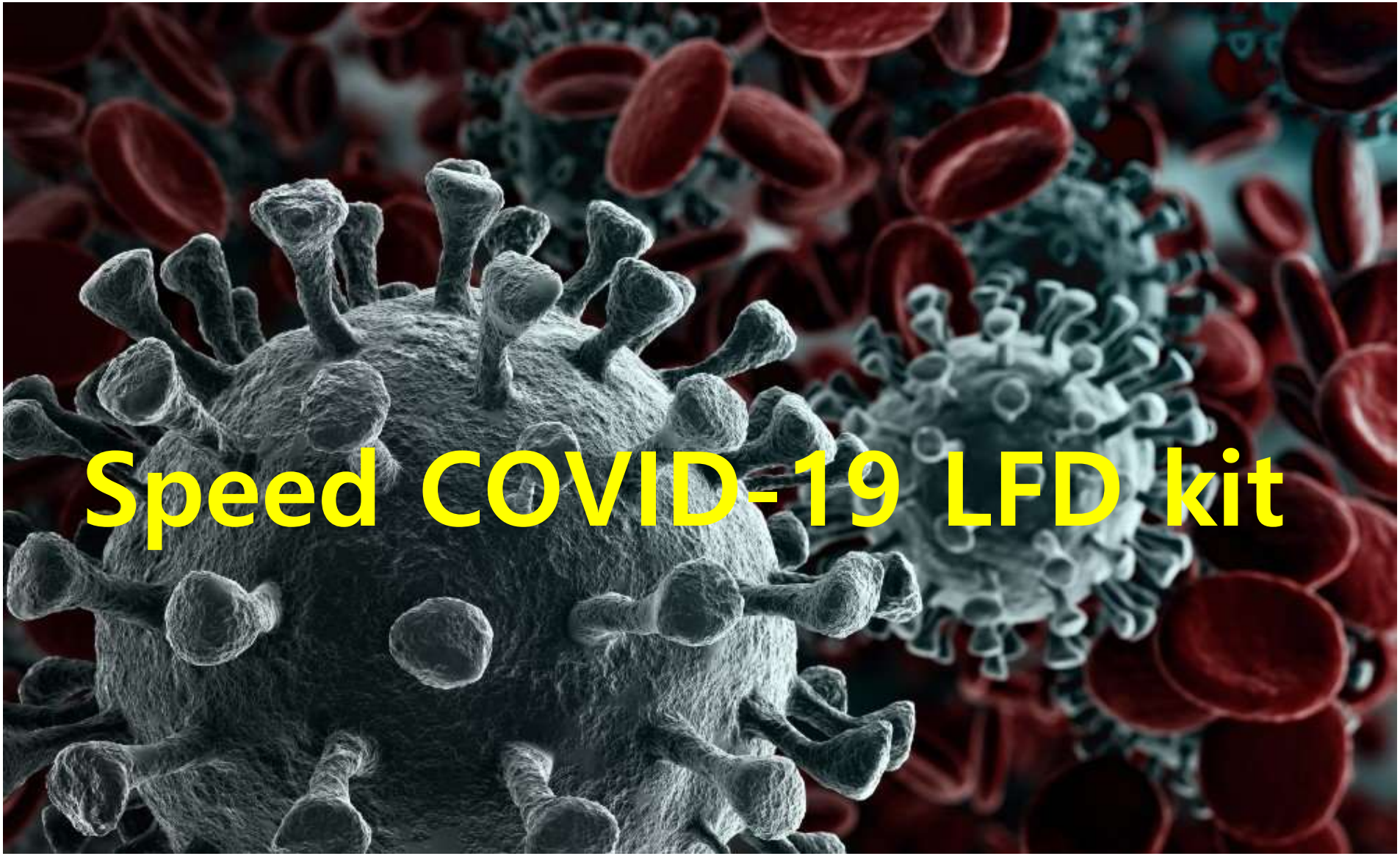

Speed COVID-19 LFD kit

Speed COVID-19 RPA-LFD kit (molecular diagnosis) Principal

Recombinase polymerase amplification (RPA) is a single tube, isothermal alternative to the polymerase chain reaction (PCR). By adding a reverse transcriptase enzyme to an RPA reaction it can detect RNA as well as DNA, without the need for a separate step to produce cDNA,. Because it is isothermal, RPA can use much simpler equipment than PCR, which requires a thermal cycler. Operating best at temperatures of 37–42 °C and still working, albeit more slowly, at room temperature means RPA reactions can in theory be run quickly simply by holding a tube. This makes RPA an excellent candidate for developing low-cost, rapid, point-of-care molecular tests. RPA was developed and launched by TwistDx Ltd. (formerly known as ASM Scientific Ltd), a biotechnology company based in Cambridge, UK.

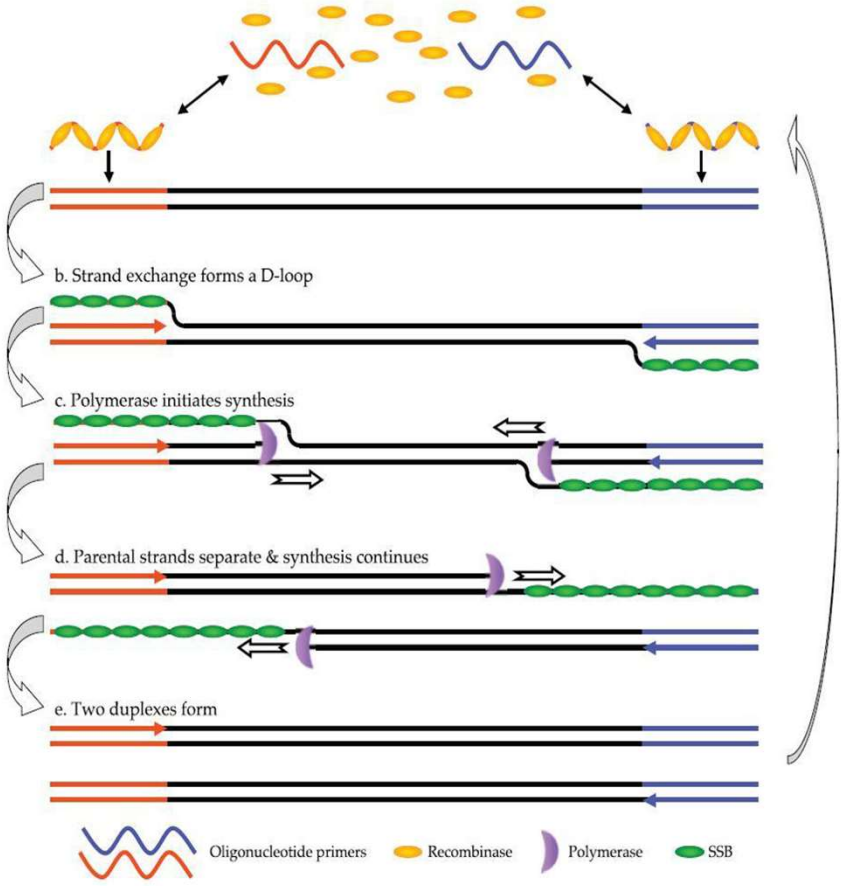


Fig. 1. RPA cycle.

The RPA process employs three core enzymes – a recombinase, a single-stranded DNA-binding protein (SSB) and strand-displacing polymerase. Recombinases are capable of pairing oligonucleotide primers with homologous sequence in duplex DNA. SSB bind to displaced strands of DNA and prevent the primers from being displaced. Finally, the strand displacing polymerase begins DNA synthesis where the primer has bound to the target DNA. By using two opposing primers, much like PCR, if the target sequence is indeed present, an exponential DNA amplification reaction is initiated. No other sample manipulation such as thermal or chemical melting is required to initiate amplification. At optimal temperatures (37–42 °C), the reaction progresses rapidly and results in specific DNA amplification from just a few target copies to detectable levels, typically within 10 minutes, for rapid detection of viral genomic DNA or RNA, pathogenic bacterial genomic DNA, as well as short length aptamer DNA.

The three core RPA enzymes can be supplemented by further enzymes to provide extra functionality. Addition of exonuclease III allows the use of an exo probe for real-time, fluorescence detection akin to real-time PCR. Addition of endonuclease IV means that a nfo probe can be used for lateral flow strip detection of successful amplification,. If a reverse transcriptase that works at 37–42 °C is added then RNA can be reverse transcribed and the cDNA produced amplified all in one step. Currently only the TwistAmp exo version of RPA is available with the reverse transcriptase included, although users can simply supplement other TwistAmp reactions with a reverse transcriptase to produce the same effect. As with PCR, all forms of RPA reactions can be multiplexed by the addition of further primer/probe pairs, allowing the detection of multiple analytes or an internal control in the same tube.

Recombinase Polymerase Amplification (RPA)

TwistDx Ltd

- Founded in 2002 by Dr Niall Armes
- Recombinase Polymerase Amplification (RPA) - the isothermal alternative to PCR
- Acquired by Alere Inc. in March 2010
- Based in Cambridge, UK
- 650+ customers in 56 countries
- 180+ peer-reviewed publications

TwistDx

Recombinase Polymerase Amplification (RPA)

RPA benefits

Amplification in 5-10 mins > speed
Tolerance to many inhibitors / crude sample > simple work flow
Ambient incubation, optimal at 37-45°C > low equipment burden
Ambient temperature shipping > cold-chain independence

> point of care / field use

Single molecule detection > sensitive
Proprietary probe systems > specific
Single pair of primers > simple design
Duplex/Triplex/IC assays > multiplex compatible
Fluorescence/lateral flow/gel > multiple detection formats
One step RT addition > RNA and DNA detection
real-time/digital/NGS/SNP/nesting > application versatile

> comparable with PCR

Speed + sensitivity + specificity + flexibility + simple work flow

> stand out isothermal

TwistDx

Recombinase Polymerase Amplification (RPA)

RPA applications

Medical diagnostics

Biodefense

Veterinary

Water and food testing

Agriculture

TwistDx

Principle of PCRD

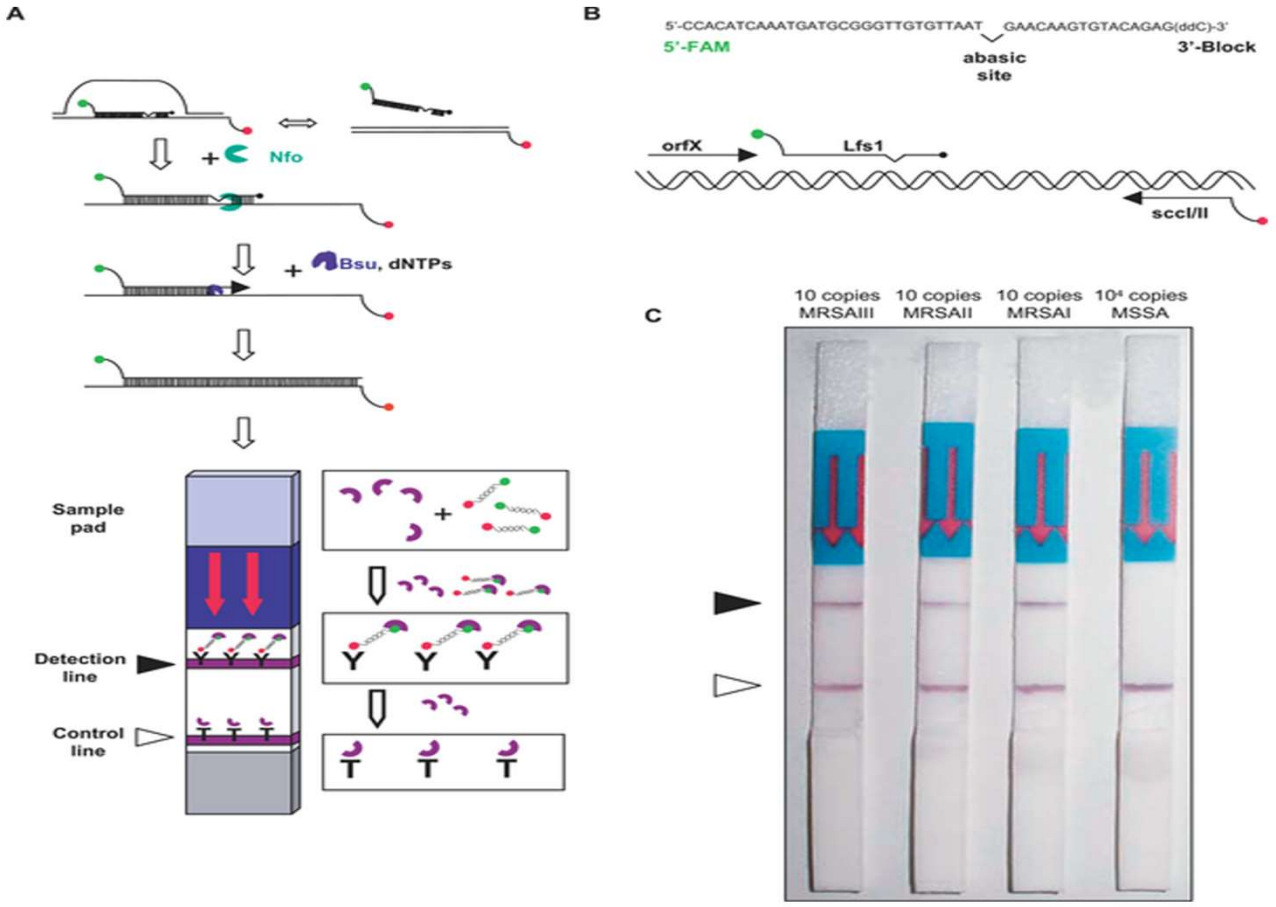


Fig. Schematic of RT-RPA result detection by dip stick method using FAM label (green), biotin label (red), α-biotin antibody (Y, detection line, filled arrowhead), and α-biotin), α-FAM gold (purple), species-specific α-[α-FAM-gold] antibody (T, control line, open arrowhead)

2019 nCoV RPA-LFD POCT model

2-3 min

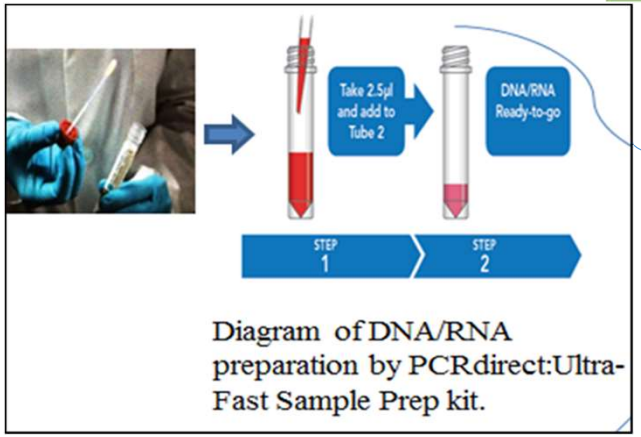
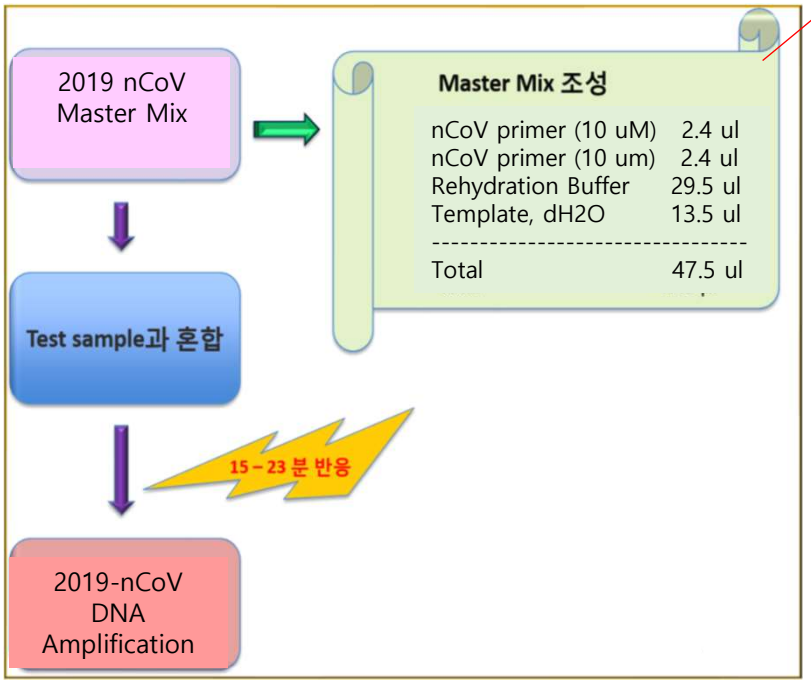
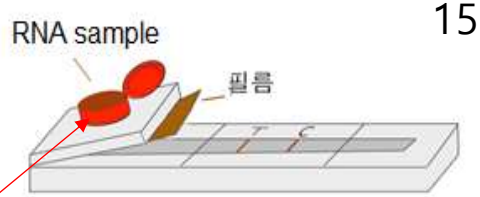
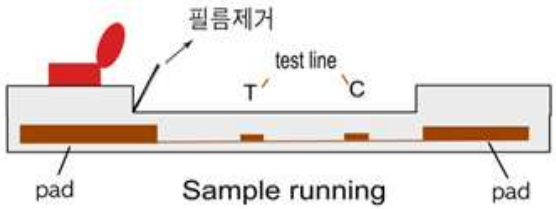


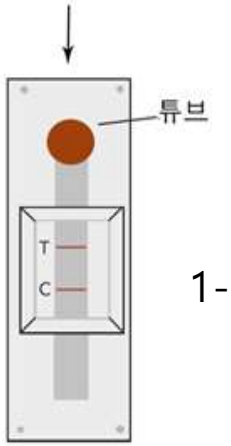
Diagram of DNA/RNA preparation by PCRdirect:Ultra-Fast Sample Prep kit.



Room Temperature
15-20 min







1-5 min

Speed COVID-19 LFD kit

Speed COVID-19 LFD kit is a real-time RT-RPA test intended for the qualitative detection of RNA from the SARS-CoV-2 in nasopharyngeal, nasal, and oropharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider.

Test Principle

Speed COVID-19 LFD kit is a diagnostic kit with a method of amplifying a specific part of the Orf1a gene of the new coronavirus via RPA method for 15 minutes at 37°C. Results are confirmed through the PCRD nucleic acid detector (Abingdon health, Ltd), and positive test results are measurable within 10 minutes.

Product Description

Novel coronavirus, also known as 2019-nCoV Wuhan coronavirus and Wuhan seafood market pneumonia virus is a positive-sense, single-stranded RNA coronavirus. The first suspected cases were notified to the WHO on 31 December 2019, with the first instances of symptomatic illness appearing just over three weeks earlier on 8 December 2019. The virus was genomically sequenced from a sample from a person with pneumonia during the 2019–20 Wuhan coronavirus outbreak.

Reported symptoms have included fever, fatigue, dry cough, shortness of breath, respiratory distress, pneumonia, kidney failure and death in severe cases. Among the majority of those hospitalized, vital signs were stable on admission, and they had leukopenia and lymphopenia. However, a quarter among those infected have experienced severe symptoms. Most of these patients also presented underlying conditions such as hypertension, diabetes or cardiovascular disease.

This virus belongs to the family of coronaviruses. Coronaviruses form a large family of viruses, and the illnesses they cause can range from the common cold to more severe diseases such as the Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). Coronaviruses are a broad family of viruses, but only six (229E, NL63, OC43, HKU1, MERS-CoV, and SARS-CoV) were previously known to infect people; 2019-nCoV made it seven.

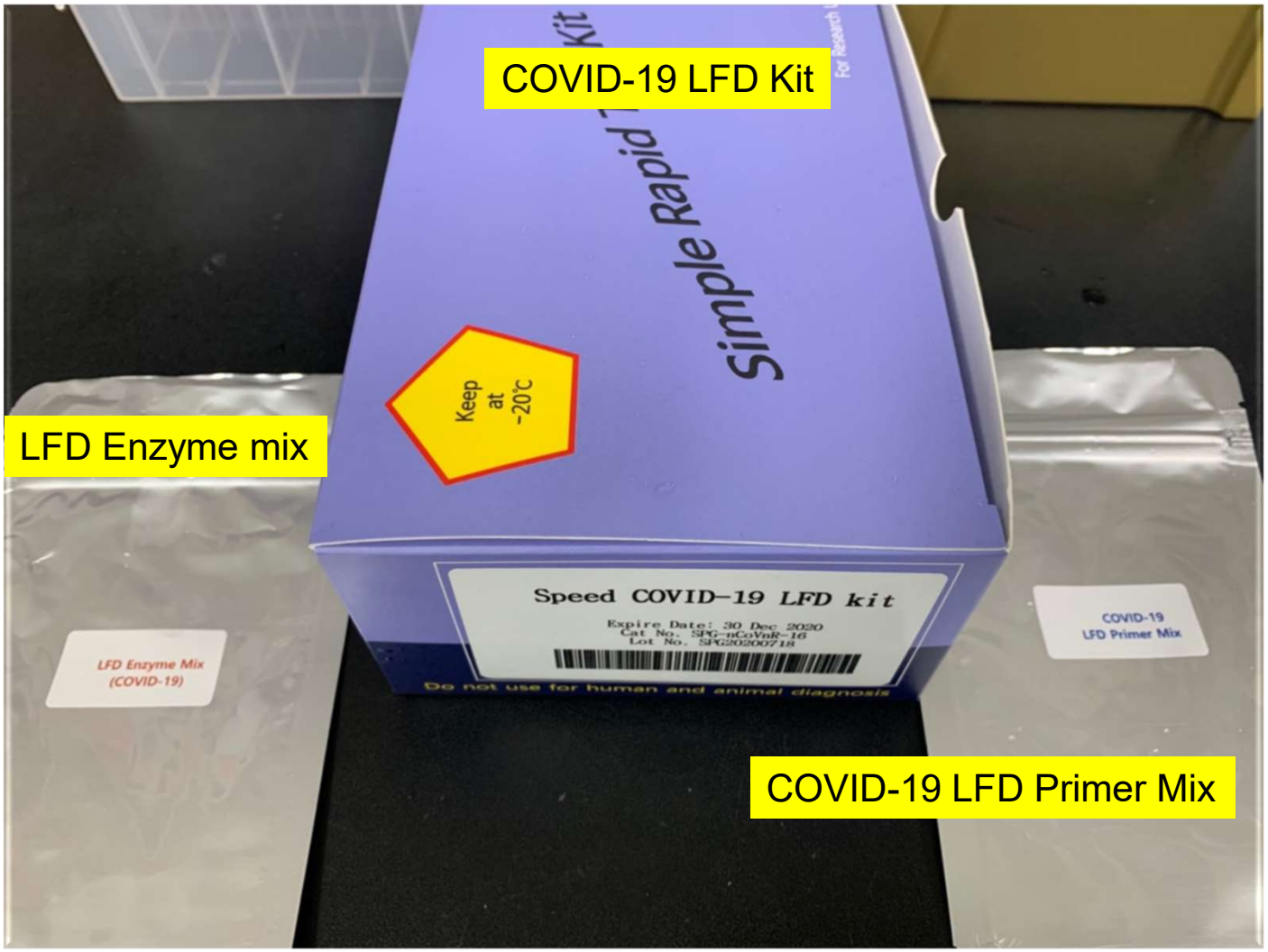
Product Description

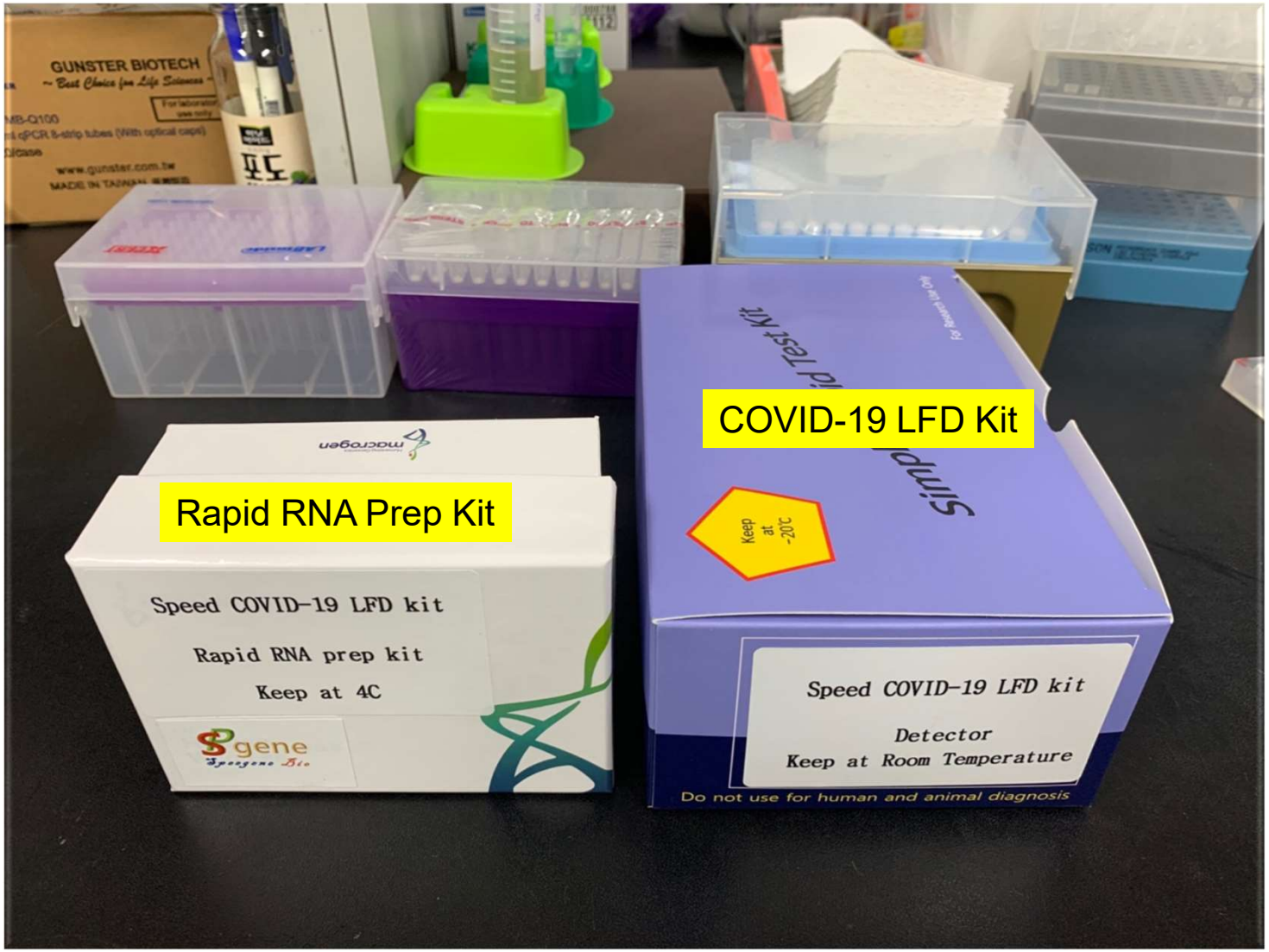
The Speed COVID-19 LFD kit contains three parts of a specific ready-to-use system for the detection of nCoV, RNA extraction, Gene amplification, and Amplicon detection on site manner. RNA extraction is supported by Arcis Biotechnology Ltd within a few minutes. Gene amplification is supported by newly developed isothermal gene amplification technology, called RPA method (TwistDx Ltd). The master contains enzymes and reagents including specific primer set which is specially designed to amplify the Orf1a gene for the unique amplification of nCoV within 20 min at room temperature. The labelled amplicon can be detected in a few minutes by PCRD Nucleic Acid Detector which is a single-use *in vitro* immunochromatographic test supported by Abingdon Health Ltd. Therefore, total reaction can be done within 30 minute without expensive equipment.

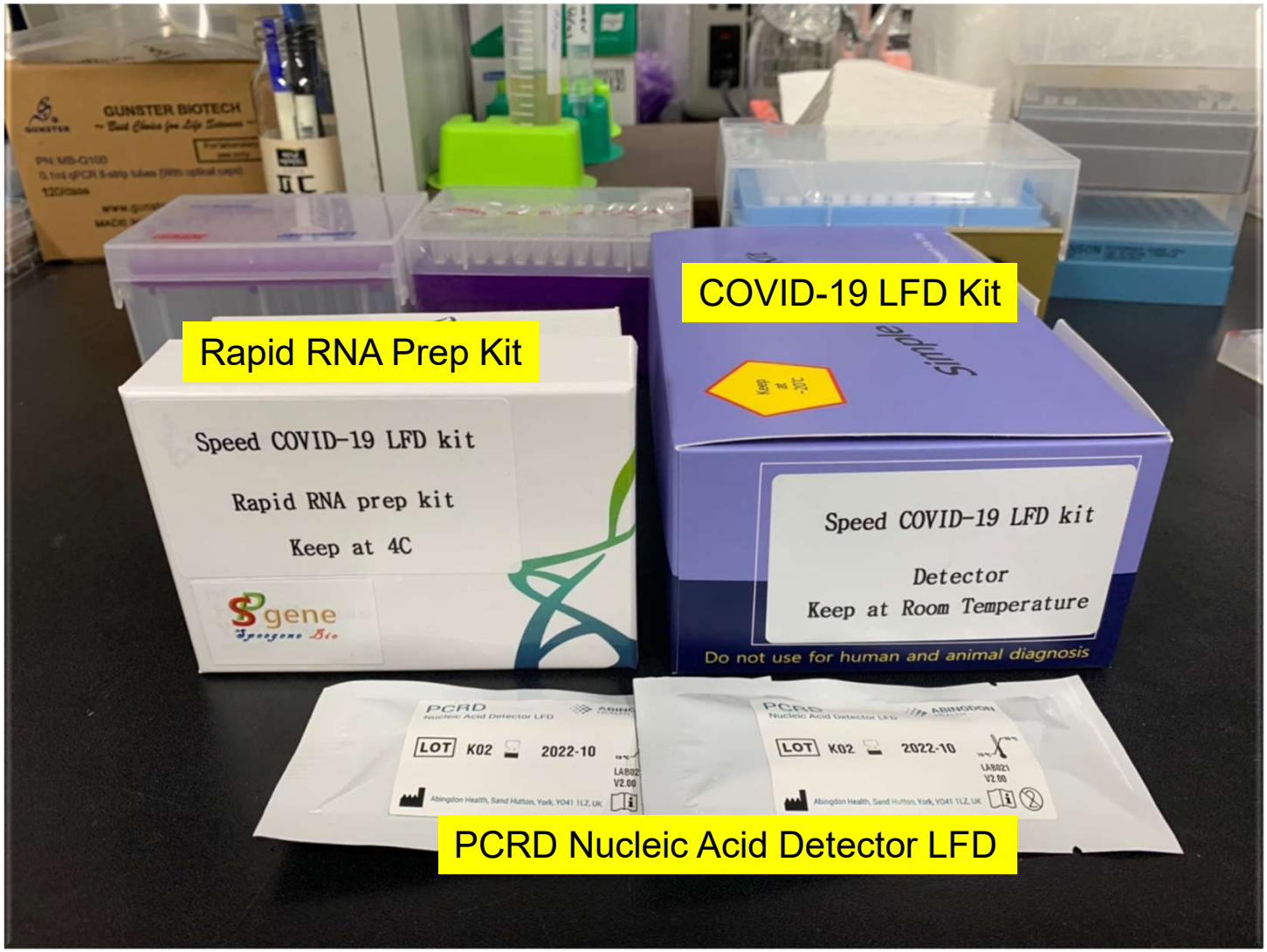
Component of Kit

No		Name	Q'ty / 16 rxn	Storage condition
Rapid RNA Prep Kit	1	Lysis Buffer (White tube)	150 ㎕, 16 ea	4℃
	2	Wash Buffer (Orange tube)	20 ㎕, 16 ea	4℃
COVID-19 LFD Kit	3	COVID-19 LFD Primer Mix	50 ㎕, 16 ea	-20℃
	4	LFD Enzyme mix	Lyophilized tube, 16 ea	-20℃
	5	Positive control (Orf1 gene-DNA)	40 ㎕, 1 ea	-20℃
PCRD Detector	6	Extraction Buffer	5 ml, 1 ea	*RT
	7	PCRD Nucleic Acid Detector LFD	30 ㎕, 1 ea	RT

Component of Kit




GUNSTER BIOTECH
~ Best Choice for Life Sciences ~
www.gunster.com.tw
MADE IN TAIWAN
Rapid RNA Prep Kit
Speed COVID-19 LFD kit
Rapid RNA prep kit
Keep at 4C
COVID-19 LFD Kit
Keep at -20C
Speed COVID-19 LFD kit
Detector
Keep at Room Temperature
Do not use for human and animal diagnosis


Rapid RNA Prep Kit
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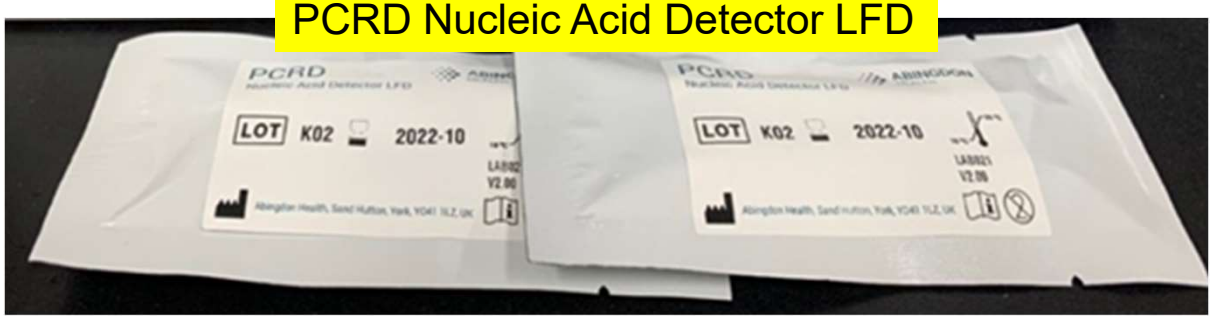
PCRD Nucleic Acid Detector LFD

PCRD Nucleic Acid Detector LFD



RNA extraction

*** Corona virus RNA can be extracted easily in a few minutes with Arcis DNA Sample Prep kit made by Arcis Biotechnology Ltd.**

1) Put less than 90 μℓ of clinical samples from sputum, nasopharyngeal swab, oropharyngeal swab, stool, urine etc into white tube (lysis buffer) of Rapid RNA prep kit.
2) Mix it well and incubate 1 minute at room temperature.
3) Transfer 5 μℓ of it into orange tube (wash buffer).
4) Use 5 μℓ in RPA reaction in next step.

RPA reaction

Transfer 1-5 μℓ of RNA extract into COVID-19 LFD Primer Mix and mix well.

1) **And then drop down the mixture into the lid of tube.**
2) **Switch the lid with that of LFD Enzyme mix.**
3) **Mix it well and incubate 15-20 min at RT.**

Amplicon Detection

Prepare the PCRD Nucleic Acid Detector.

1) **Mount 20 μℓ of RPA reaction on the rim of device.**
2) **Add 90 μℓ of PCRD extraction buffer for dropping down into the sample hole of PCRD device.**
4) **Leave the PCRD device to develop for 10 minutes while the result develops.**
5) **Read the results visually at 10 minutes.**
6) **Ignore any changes which occur after 10 minutes.**

Performance Characteristics

1) **Assay range: For guidance, in lab studies PCRD device has been shown to detect between 0.02 ug/ml and 0.001 ug/ml ds DNA.**
2) **However, levels of sensitivity will depend on the outcome of your own RPA reaction. The detection level for your assay should therefore be determined empirically.**
3) **Specificity: PCRD device is specific for the detection of DIG and FITC.**
   It does not detect Dinitophenol (DNP) of Sulforhodamine 101 Acid Chloride (Texas Red).

Considerations

1) **An initial quality control check on your primer pair and DNA is advised.**
2) **PCRD device is highly sensitive and may detect the presence of primer dimers and non-specific product as a “positive”.**
3) **Keep the LFD in protective container for as long as possible to reduce the risk of cross contamination.**

Reading and Result

<Example of RT-RPA LFD reaction with Positive and Negative control (Distilled water)>

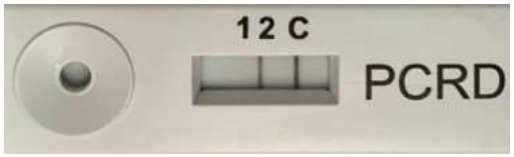
1) The Positive reaction shows dark band in line 2 and line C (control line)
2) The Negative control and distilled water show only one band in line C.
3) If the negative control or distilled water show the positive reaction, please repeat again the experiment.
4) If the false positive reaction shows again even in the repeated experiments, clean the experimental environment with RNase away or 70% alcohol including pipets, also. And use new cotton pipet tips.

INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Speed COVID-19 LFD kit Controls – Positive and Negative

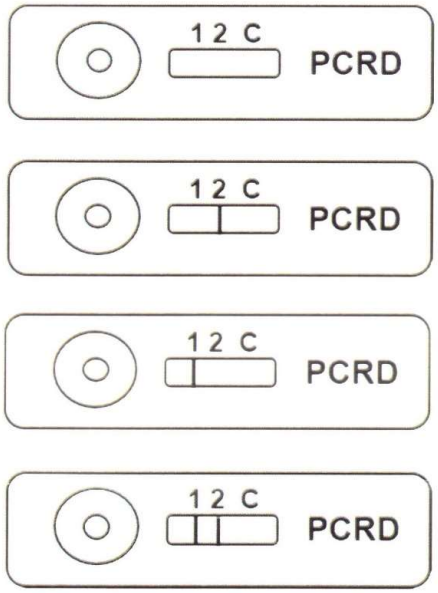
- Positive control: positive control is recognized by the presence of black lines on the strip at the T-line positions.



- Negative control: negative control is indicated by the presence of a single black line on the rest strip (C-line).

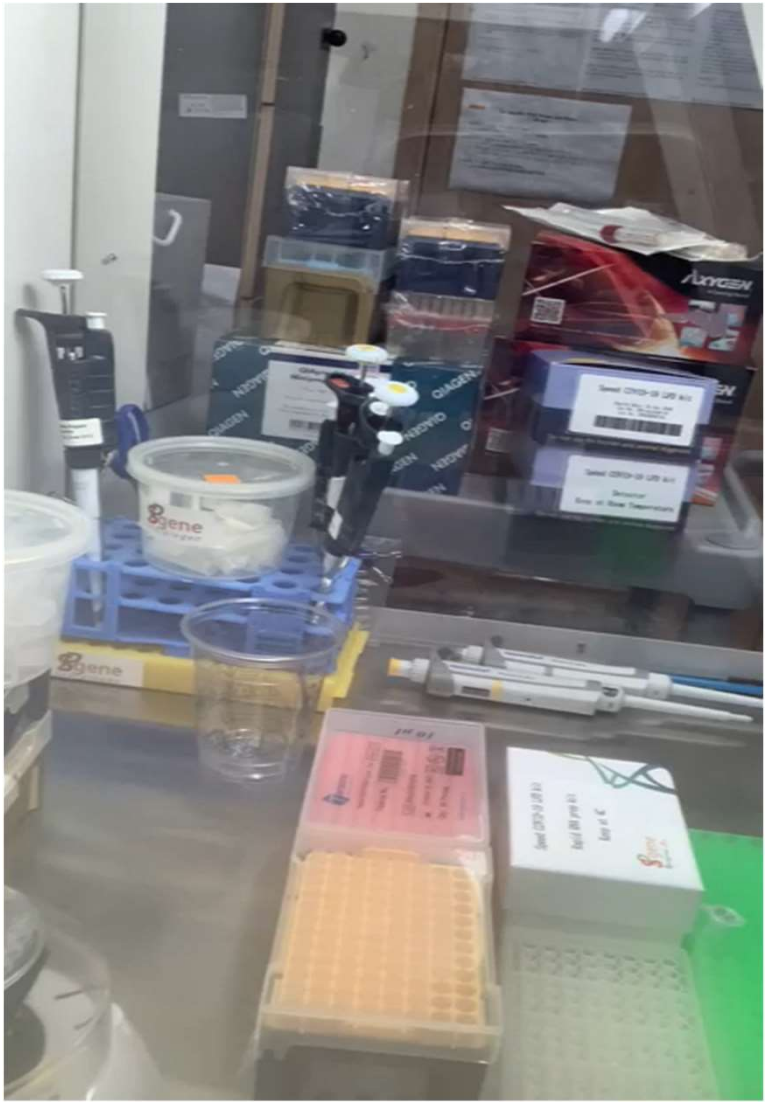
Invalid control results

- Repeat extraction, and RT-PPA. If additional clinical sample is unavailable, report Invalid or inconclusive results, which will request a new specimen be collected, if clinically indicated.



Movies

How are you everyone!
I will show you how “Speed COVID-19 LFD kit” works shortly

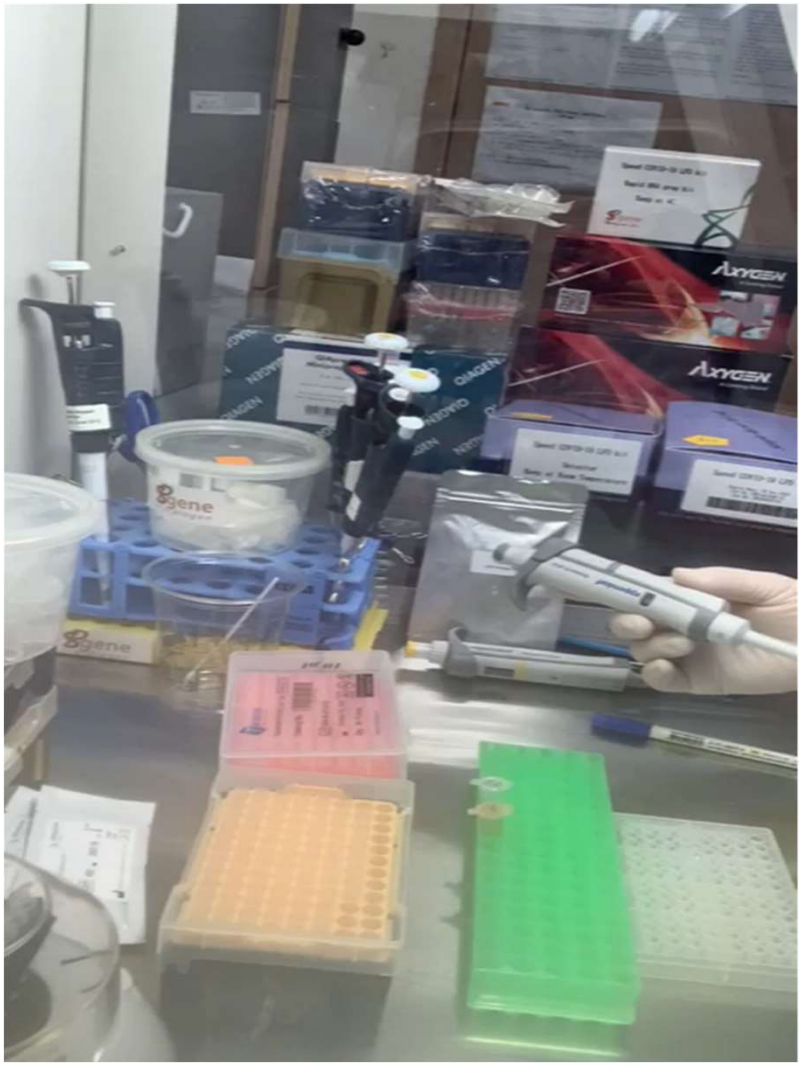


This kit consists of three part.
One is RNA extraction kit.
It takes only three minutes for RNA extraction from patient's samples.
The other one is RNA Amplification part.
It consists of Primer probe mix and LFD Enzyme mix.
Last one is Amplification detection kit.

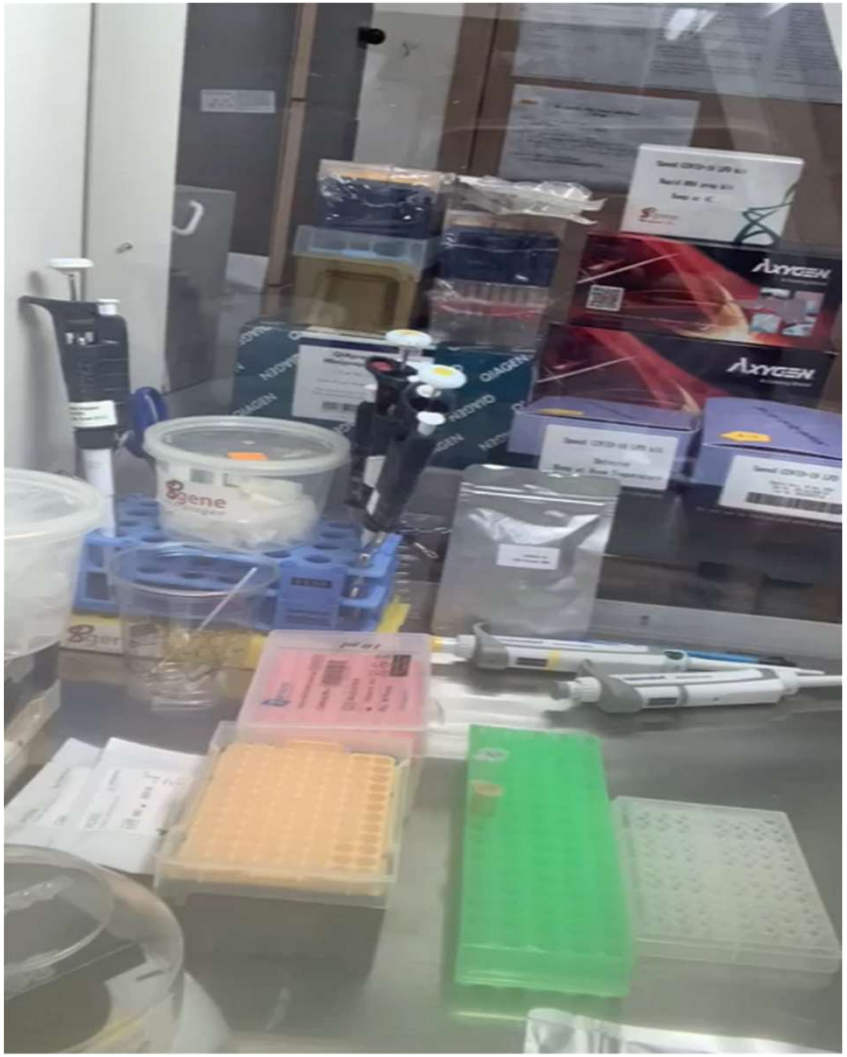
First of all, when you take the sample from patients with this kind of things.
You just put this one in the Lysis buffer for one minutes.

After that transfer 5 ul of lysed virus sample to wash buffer.
And then mix it well.

Next, transfer 5 ul of sample to "Primer Mix tube", and mix it well.



And then pull down it with centrifuge or manually like this.



Transfer all the sample of “Primer Mix tube” to LFD enzyme mix and mix it well.

And then pull down it with centrifuge or manually like this.

Let it react for 20 min at room temperature.

Before the reaction done, you should prepare the LFD kit, and sample name on it.

Transfer 20 ul of reaction sample on the rim of PCRD kit

Squeeze the two drop of Extraction buffer.

And wait for 10 min until finish the reaction.

And then read the result.

Positive reaction shows two line.
Negative reaction shows one line.

Thank you for watch video.