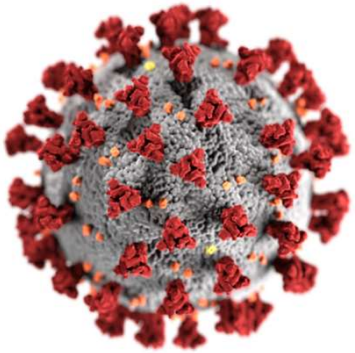
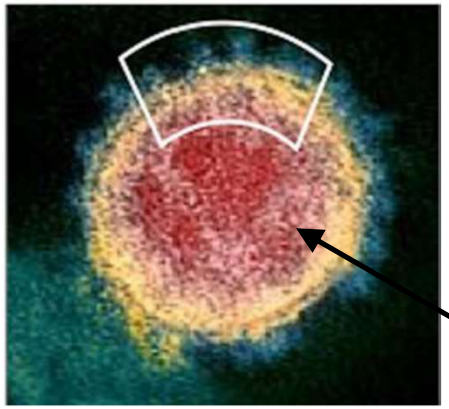
# Covid-19

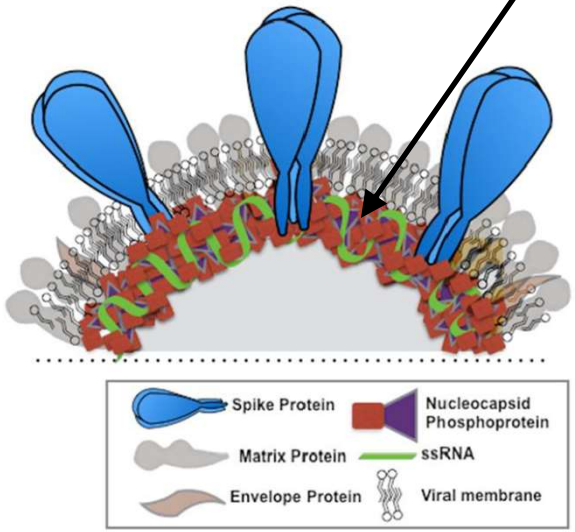
## Espline SARS-CoV-2

# SARS-CoV-2 structure and assay target



https://phil.cdc.gov/Details.aspx?pid=23311






**Assay target =**
**NP（Nucleocapsid protein)**

NP is released from membrane and RNA by pretreatment with detergent

NP is measured/detected by sandwich immunoassay

Dinesh et al. (2020). Structural basis of RNA recognition by the SARS-CoV-2 nucleocapsid phosphoprotein bioRxiv https://dx.doi.org/10.1101/2020.04.02.022194

# Different types of COVID-19 tests

| | Real-time PCR | Antigen test | | Antibody test |
|---|---|---|---|---|
| | | CLEIA (Lumipulse SARS-CoV-2 Ag) | Immuno-chromatography assay (Espline SARS-CoV-2) | |
| Purpose | Detecting the presence of antigen ( = current infection) | | | Determining past infection (= immunity rates in a community) |
| Sample type | Nasopharyngeal swab fluid or Saliva | Nasopharyngeal swab fluid or Saliva | Nasopharyngeal swab fluid | Blood (Serum) |
| Target | Viral RNA | Nucleocapsid protein | Nucleocapsid protein | IgM/IgG antibodies |
| Sensitivity | ★★★ | ★★★ | ★★ | Depends on kit |
| Specificity | ★★★ | ★★★ | ★★★ | Depends on kit |
| Test operation | Instrument-based | Instrument-based | Manual (available for PoC) | Manual (available for PoC) |
| Sample-to-answer time | ~ 2 days | <1hr | <1hr | <1hr |
| Others | | Capable of testing 60 or 120 samples per hour on Fujirebio instrument, Lumipulse G600I or G1200 | | |

# Espline SARS-CoV-2 antigen test kits

- Immuno-chromatography (ICA)
- Sample type: nasopharyngeal swab fluid
- Sample volume: 20uL
- Test result TAT: ~30 minutes
- Recommended in Japanese Guideline

Espline

# Product overview

FUJIREBIO

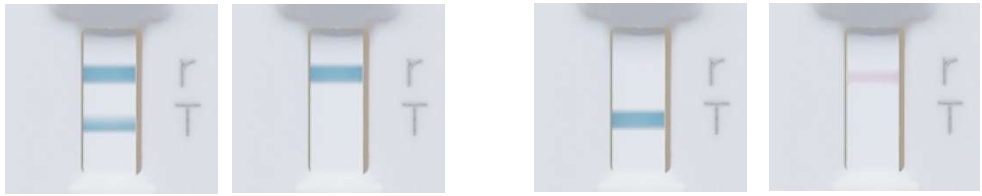
## Simple test procedure

Prepare sample processing solution → Extract samples into a squeeze tube → Leave to stand 5 min → Apply 2 drops of sample onto the sample window → Press the convex button → Reaction time

検体処理液の分注 → 検体抽出 → 滴下チップをはめて静置 → 滴下 → 押す → 反応時間


## Easy result interpretation

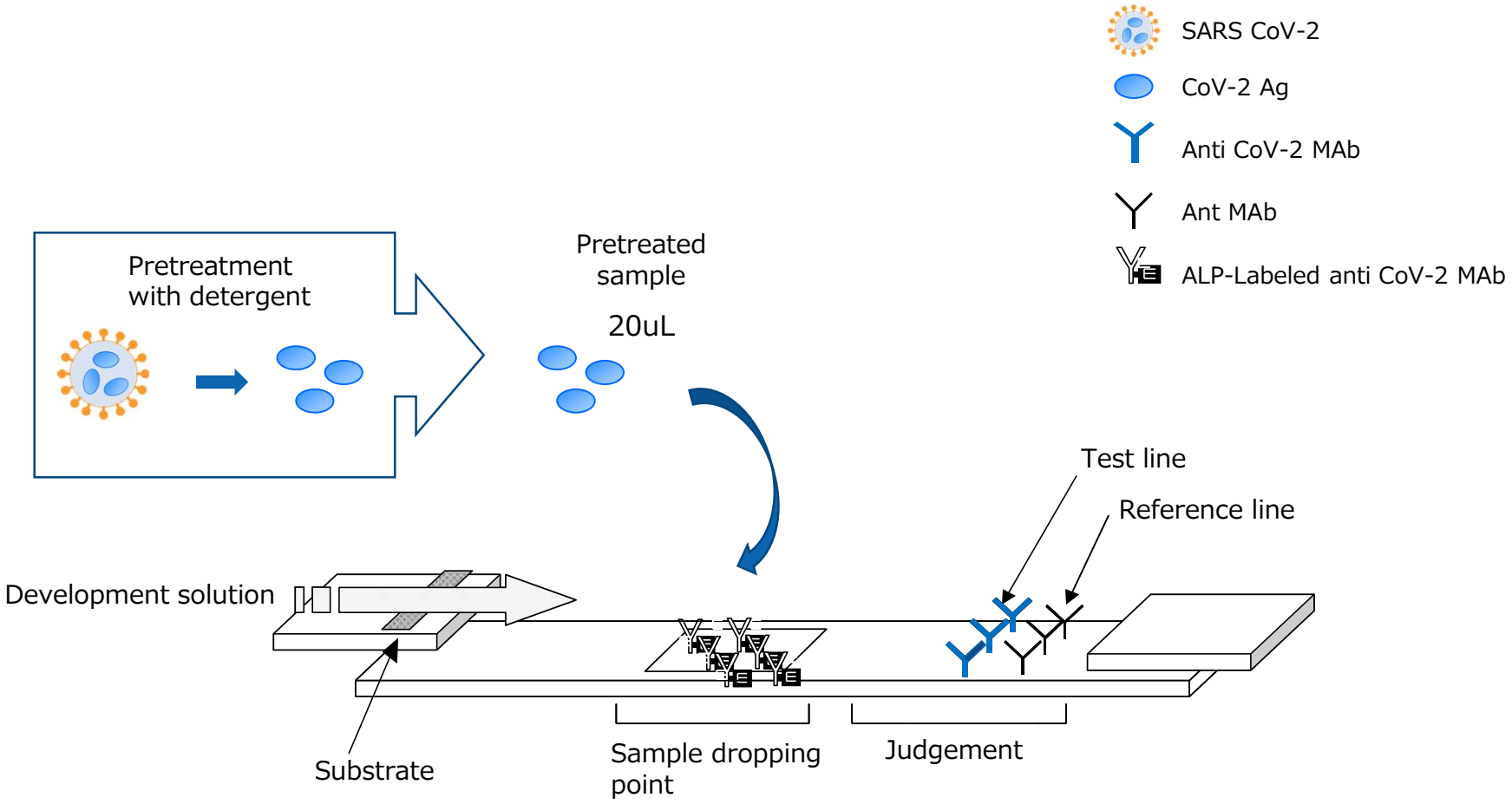
Positive　Negative　Retest necessary



H.U.GROUP

Espline

# Assay principle



# Positioning of Antigen tests

- Surveillance testing versus clinical testing
  - Clinical testing:
    - Symptomatic patients
    - Need for high accuracy and sensitivity
    - Not limited by costs
    - Diagnose delay but you can isolate such a person
  - Surveillance testing
    - Asymptomatic individuals
    - Need for speed
    - Speed of reporting is more important than sensitivity

- Disadvantage of sensitive tests
  - Unnecessary quarantine days due to identification in the recovery period who still have the virus but below the infectious threshold

- NEED FOR:
  - Need for Speed, frequency of testing, speed of result reporting, and as sensitive as possible (but marginally important)
  - Identifying super spreaders

**Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance**
Daniel B Larremore, Bryan Wilder, Evan Lester, Soraya Shehata, James M Burke, James A Hay, Milind Tambe, Michael J Mina, Roy Parker, **doi:** https://doi.org/10.1101/2020.06.22.20136309

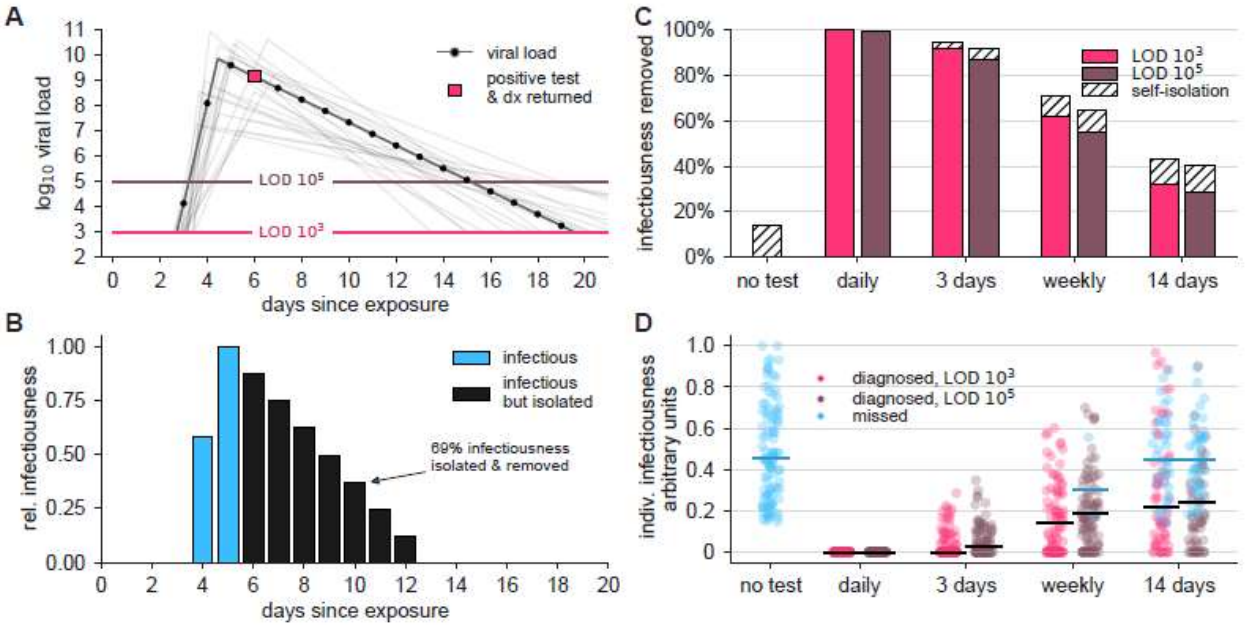



Figure 1: **Surveillance testing effectiveness depends on frequency.** (A) An example viral load trajectory is shown with LOD thresholds of two tests, and a hypothetical positive test on day 6, two days after peak viral load. 20 other stochastically generated viral loads are shown to highlight trajectory diversity (light grey; see Methods). (B) Relative infectiousness for the viral load shown in panel A pre-test, totaling 31% (blue) and post-isolation, totaling 69% (black). (C) Surveillance programs using tests at LODs of $10^3$ and $10^5$ at frequencies indicated were applied to 10,000 individuals trajectories of whom 20% would undergo symptomatic isolation near their peak viral load if they had not been tested and isolated first. Total infectiousness removed during surveillance (colors) and self isolation (hatch) are shown for surveillance as indicated, relative to total infectiousness with no surveillance or self-isolation. (D) The impact of surveillance on the infectiousness of 100 individuals is shown for each surveillance program and no testing, as indicated, with each individual colored by test if their infection was detected during infectiousness (medians, black lines) or colored blue if their infection was missed by surveillance or detected positive *after* their infectious period (medians, blue lines). Units are arbitrary and scaled to the maximum infectiousness of sampled individuals.

Espline

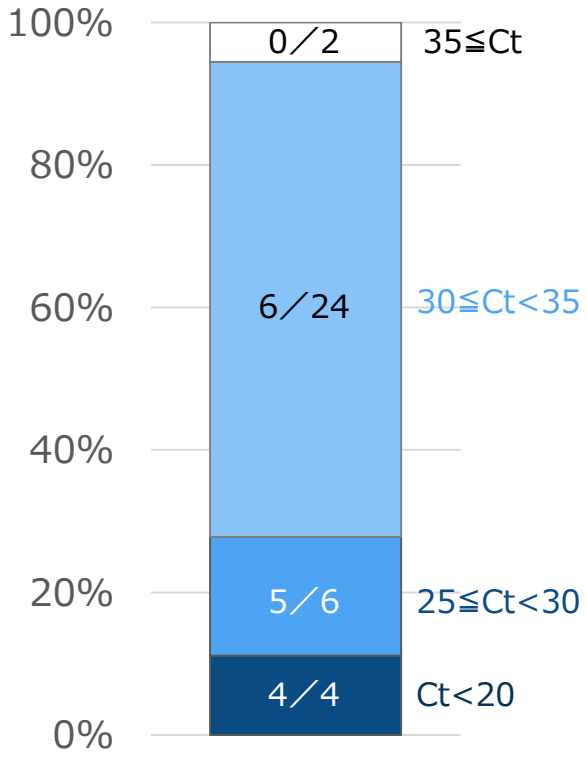
# Comparison with qRT-PCR (1/2)

Espline (EL) shows as good a detection rate as qRT-PCR for clinical samples with Ct30 or less.

| | | qRT-PCR | | Total | PPV |
|---|---|---|---|---|---|
| | | Positive | Negative | | NPV |
| EL | Positive | 15 | 2 | 17 | 88% |
| | Negative | 21 | 68 | 89 | 76% |
| | Total | 36 | 70 | 106 | |
| | Concordance | 42% | 97% | | |

Drill-down of qRT-PCR positive cases

Espline results for aRT-PCR positive samples



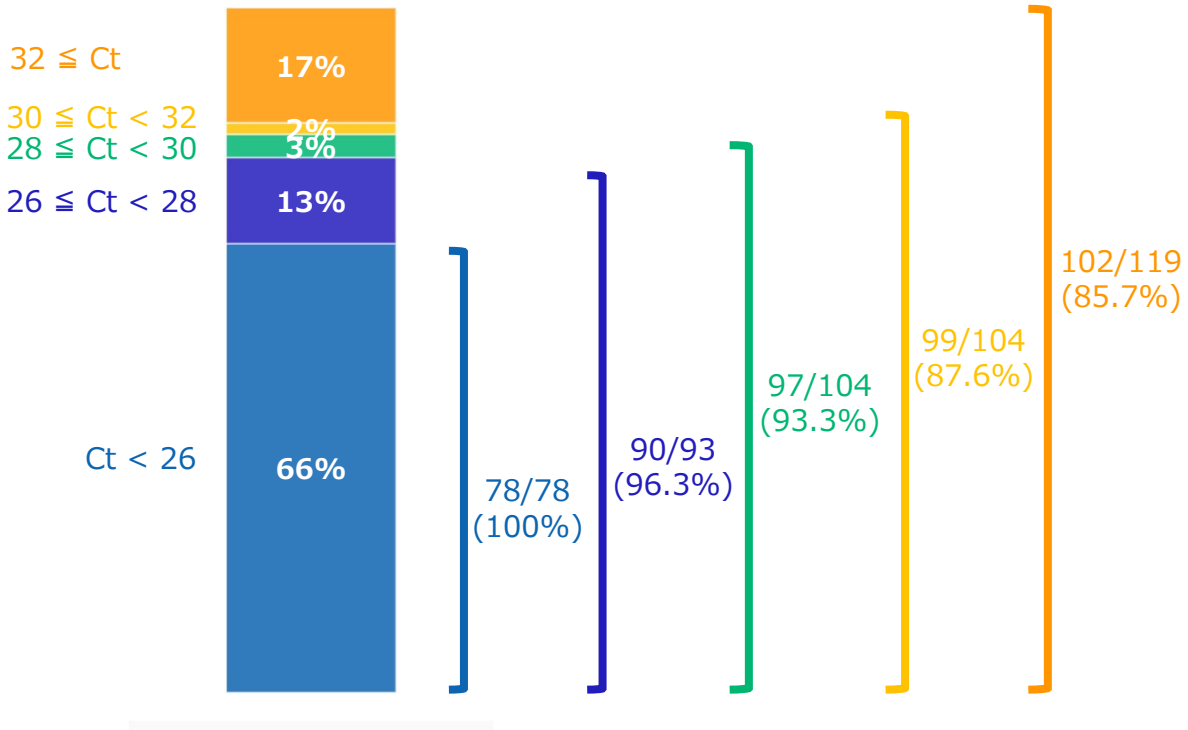

Studied by Toho university
Under article submission（Ishii et al.）

Espline

# Comparison with qRT-PCR (2/2)

Espline shows as good a detection rate as qRT-PCR for clinical samples with Ct30 or less.

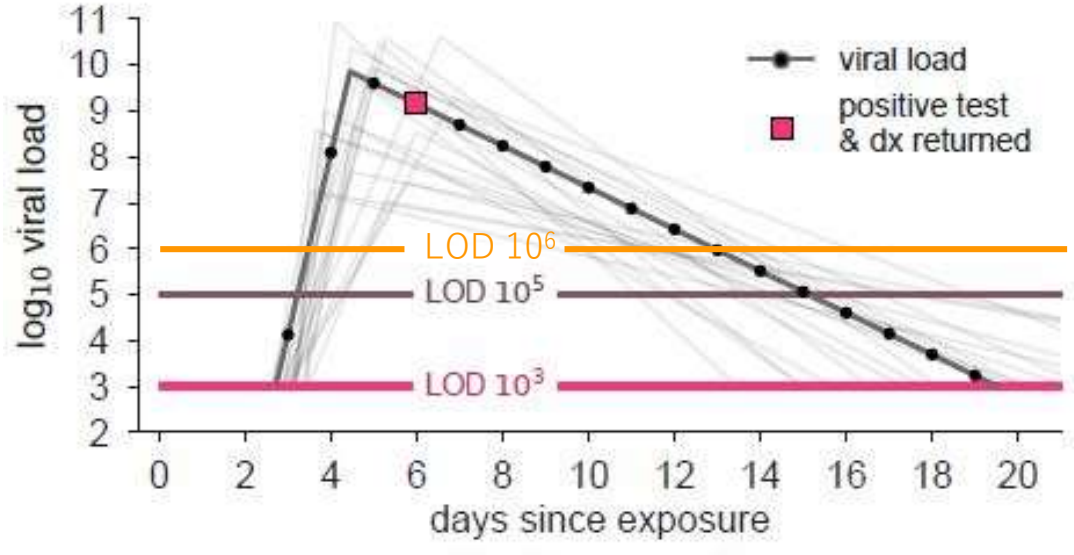
Positive rate of Espline for the nasopharyngeal swab samples in UVT (Universal Viral Transport) that were tested positive by qRT-PCR




Article under submission

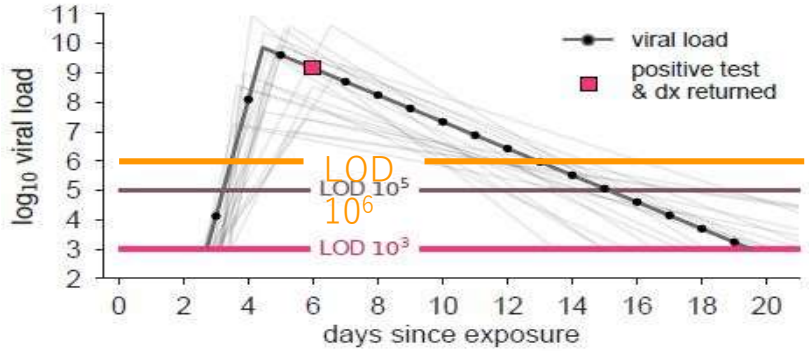
# ESPLINE SARS-CoV-2 Ag rapid test

- Correlation between ESPLINE SARS-CoV-2 and RT-PCR using 72 Japanese clinical samples:
  - The **concordance rate** between assays was **98%** (44/45 cases) for **negative samples** and **37%** (10/27 cases) for **positive samples**.
  - When **comparison** was **based** on the **number of viral RNA copy numbers** as determined by the one-step RT-PCR method, the **concordance rates** between the two assays was:
    - **83%** (5/6 cases) for **samples** with **100 copies or more / test**
    - **50%** (6/12 cases) for **samples** with **30 copies or more / test**.



# ESPLINE SARS-CoV-2 Ag rapid test

- Correlation between ESPLINE SARS-CoV-2 and RT-PCR using 124 Japanese administrative samples:
  - The **concordance rate** between assays was **100%** (100/100 cases) for **negative samples** and **67%** (16/24 cases) for **positive samples**. Overall concordance was 94% (116/124)
  - When **comparison** was **based** on the **number of viral RNA copy numbers** as determined by the one-step RT-PCR method, the **concordance rates** between the two assays was:
    - **100%** (12/12 cases) for **samples** with **1600 copies or more / test**
    - **93%** (14/15 cases) for **samples** with **400 copies or more / test**
    - **83%** (16/18 cases) for **samples** with **100 copies or more / test**.
  - Same samples solution was used for both and swab has been submerged in the viral transport media



Espline

# Cross reactivity

Espline showed no cross reaction with MERAS-CoV N protein, Influenza virus, or Human corona virus.

Cross reaction study using recombinant N protein of SARS, MARS, HCoV, and Influenza virus

| | Antigen | Cross reaction |
|---|---|---|
| 1 | SARS-CoV-2 | Yes |
| 2 | SARS-CoV | Yes |
| 3 | MERAS-CoV | No |
| 4 | HCoV-229E | No |
| 5 | HCoV-DC43 | No |
| 6 | HCoV-NL63 | No |
| 7 | HCoV-HKU1 | No |
| 8 | Influenza Type A H1N1 | No |
| 9 | Influenza Type A H3N2 | No |
| 10 | Influenza Type B | No |

Cross reaction study using cultured human corona virus

| | Cross reaction | |
|---|---|---|
| | Run 1 | Run 2 |
| 229E (ATCC VR-740) | No | No |
| OC43 (Amsterdam I) | No | No |
| OC43 (ATCC-VR1558) | No | No |
| NL63 (Tokyo/SGH-36) | No | No |
| HKU1 (Tokyo/SGH-15) | No | No |

Conducted by Suzuki T., National Institute of Infectious Diseases

Yamakawa K. Jpn J Med Pharm Sci Jun. 2020 Vol.77 No.6

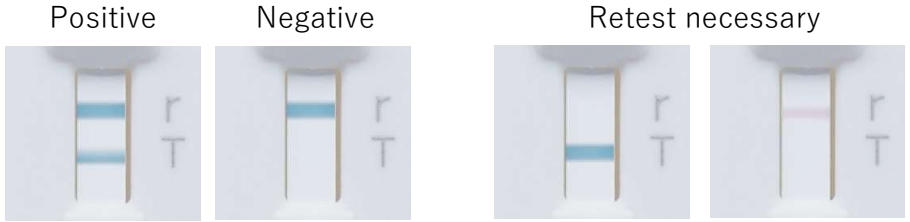
# ESPLINE SARS-CoV-2 Ag rapid test

## Espline SARS-CoV-2

- Immuno-chromatography (ICA)
- Sample type: nasopharyngeal swab fluid
- Sample volume: 20uL
- Test result TAT: ~30 minutes
- Japan PMDA cleared on May 13, 2020
- Reimbursement eligible on May 13, 2020
- Recommended in Japanese Guideline on May 13, and June 16, 2020

- Espline is a qualitative assay

Easy result interpretation




- Espline shows a detection rate as good as qRT-PCR for clinical samples with Ct30 or less.

Espline

# Japan guideline

https://www.mhlw.go.jp/content/000640554.pdf

<Unofficial translation by Fujirebio>

Guideline for the use of SARS-CoV-2 Antigen detection kit

Initial version: May 13, 2020
Updated: June 16

Ministry of Health, Novel Coronavirus Response Headquarters

This guideline describes the approach and important notes to promote the appropriate use of the rapid antigen detection kit (Product name: Espline SARS-CoV-2, manufacturer: Fujirebio, Inc.) for the diagnosis of Novel Coronavirus infection (COVID-19). At this point the purpose of the use of this kit is to detect positive patients quickly.

When tested positive by this kit, it can be considered as a definitive diagnosis. For a patient with suspected infection and **in between the 2nd to 9th day after symptom onset, negative result of this test kit does not require additional PCR testing.** On the other hand, this kit requires a higher amount of virus in sample than PCR, at this point **it is not suited to use this kit to a patient who has not presented symptom, because of the detectability limitation of this kit.**

Espline

# SD BIOSCIENCE vs ESPLINE

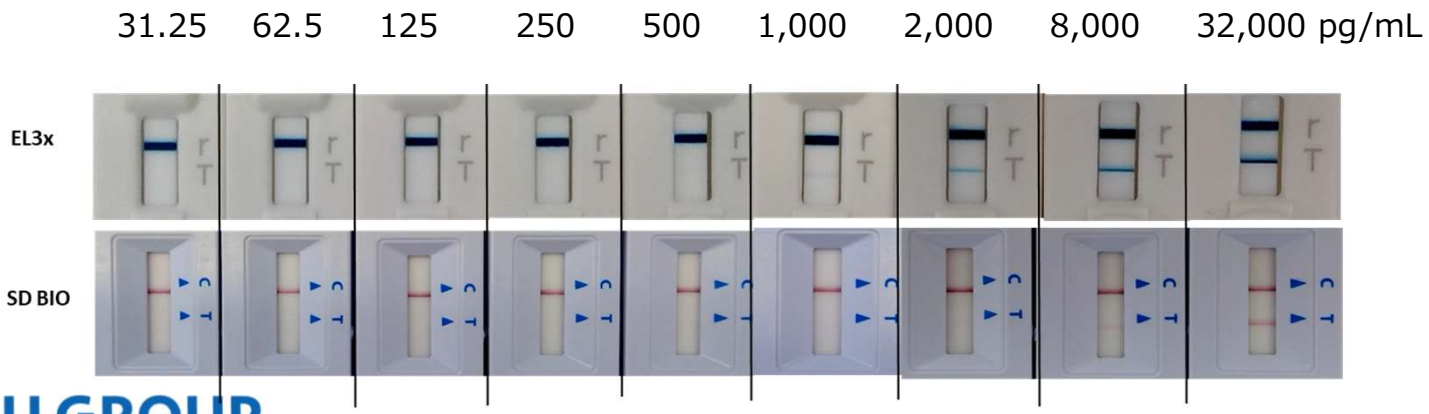
FUJIREBIO

## ■ Sensitivity with recombinant antigen

| Ag Concentration (pg/mL) | EL 3× | SD BIO |
|---|---|---|
| 32,000 | 4.0 | + |
| 8,000 | 3.0 | + |
| 2,000 | 1.5 | +w |
| 1,000 | 1.0 | - |
| 500 | 1.0 | - |
| 250 | 1w | - |
| 125 | - | - |
| 62.5 | - | - |
| 31.25 | - | - |

Protocol
Sample: SARS-CoV-2 NP Recombinant Ag
Sample processing :
EL 3×: Sample processing solution 2 10μL + sample 20μL
⇒5min⇒ drop 20μL
SD BIO: Sample processing solution 75μL + sample 15μL
⇒ drop 80μL

- Espline + Sample processing solution 2 showed approx. 10 times higher than SD Bioscience POCT.




H.U.GROUP

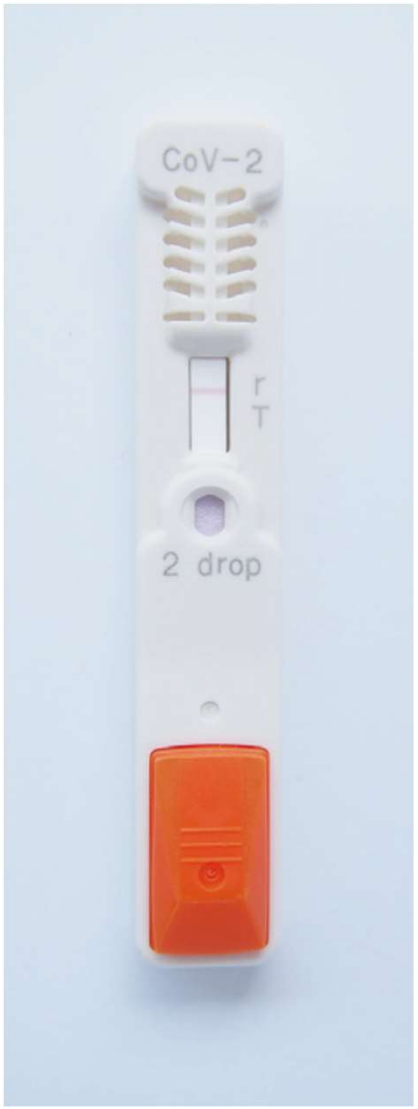
# Where is the test being used

- Rapid test demand in following areas;
  - Emergency test when PCR is not available (quick results, anywhere)
  - Screening test to identify patients for further test (to save PCR capacity)
  - Test to healthcare professionals
  - Test to broad public; back to work/school/weekly test etc.

- Competitor kits in market
  - Korean (SD Biocensor), Chinese and Japanese (Denka)
  - However, based on internal evaluation, Espline performs x10 higher sensitivity compared to other kits

  ➔ Espline has highest performance for rapid test, not using instrument

# Japanese kit




**European kit will not contain a Swab**

# Process to CE-marking of ESPLINE SARS-CoV-2

# timeline

| Due date | Task | responsibility |
|---|---|---|
| 31 july | Technical file ready (IFU,…) | FRI |
| 11 August | CE-Marking | FRE |
| 11 August | Press release | FRI - FRE |
| 15 September | Kits in warehouse | FRE |
| | | |

# ESPLINE® SARS-CoV-2

| P/N | Product name | test/box | Content | | Store |
|---|---|---|---|---|---|
| 260326 | ESPLINE® SARS-CoV-2 | 100 tests | Reaction cassette | 10 tests/Box x 10 | 2～30℃ |
| | | | Sample Extraction Solution (Squeeze tube) | 5 tubes/Bag x 20 | |
| | | | Applicator Tip | 10 tips/Bag x 10 | |

# FRE support

Victor.ruiz@fujirebio.com

Marketing Manager

Veronica.olmedo@fujirebio.com

sales.support@fujirebio.com

Rikkert.maertens@fujirebio.com

Marketing director