JICA COVID-19 Webinar Series
– Sharing of Japanese Experiences –

How antigen-detecting rapid diagnostic tests (Ag-RDTs) will change the course of COVID-19 pandemic

The role and effective use of Ag-RDTs for COVID-19 and experience in the use of Ag RDTs in Japan

Nagasaki University Graduate School of Biomedical Sciences
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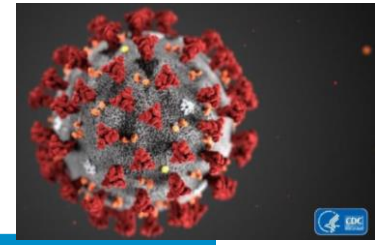
World Health Organization



Health Topics | Countries | Newsroom | Emergencies | Data

Emergencies preparedness, response

- Home
- Alert and response operations
- Diseases
- Biorisk reduction
- Disease outbreak news

Pneumonia of unknown cause – China

Disease outbreak news
5 January 2020

On 31 December 2019, the WHO China Country Office was informed of cases of pneumonia of unknown etiology (unknown cause) detected in Wuhan City, Hubei Province of China. As of 3 January 2020, a total of 44 patients with pneumonia of unknown etiology have been reported to WHO by the national authorities in China. Of the 44 cases reported, 11 are severely ill, while the remaining 33 patients are in stable condition. According to media reports, the concerned market in Wuhan was closed on 1 January 2020 for environmental sanitation and disinfection.

The causal agent has not yet been identified or confirmed. On 1 January 2020, WHO requested further information from national authorities to assess the risk.

COVID-19 suddenly appeared in Wuhan, China in end of 2019, and has spread all over the world.

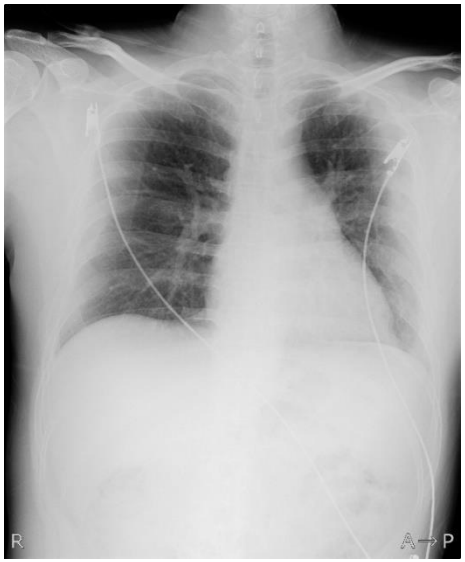
COVID-19 Case

Age/sex: 30s, male

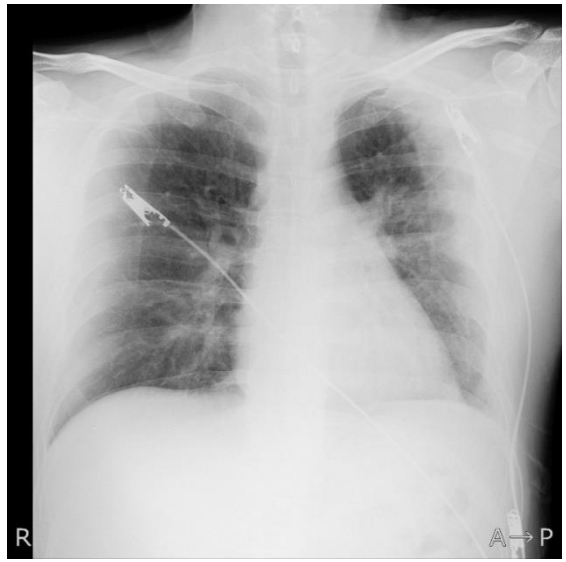
Pre-existing conditions: none noted

History of present illness: Family gathering on the 22nd of month X-1 (with visit from a COVID-19-infected area). He developed a cough on the 8th of month X, and with both a fever in the range of 38° C and diarrhea on the 9th of month X, he visited a local doctor. The fever of 37.5° C and diarrhea persisted, and government testing was requested on the 14th of month X. He was found to be SARS-CoV-2 PCR positive on the 17th of month X and was admitted to our hospital on the same day.

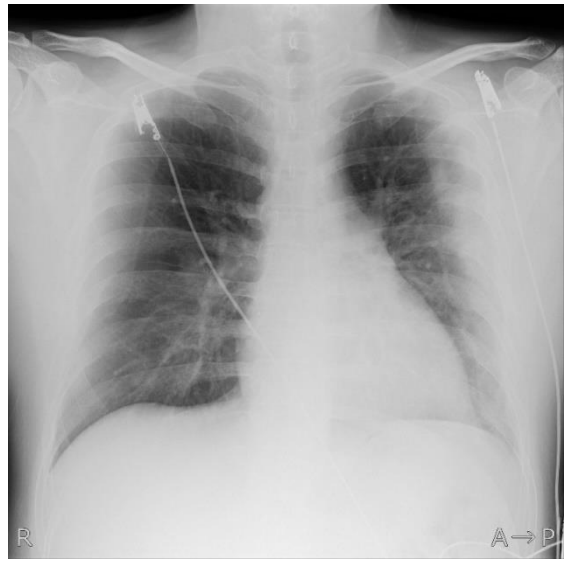
day 1 upon admission



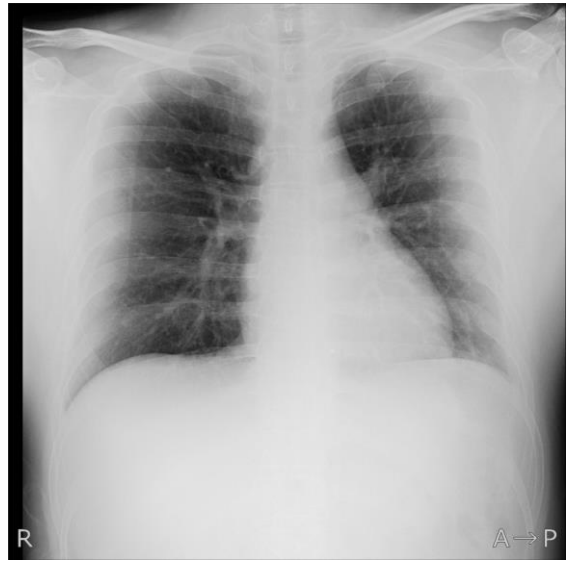
day 6



day 10



day 27



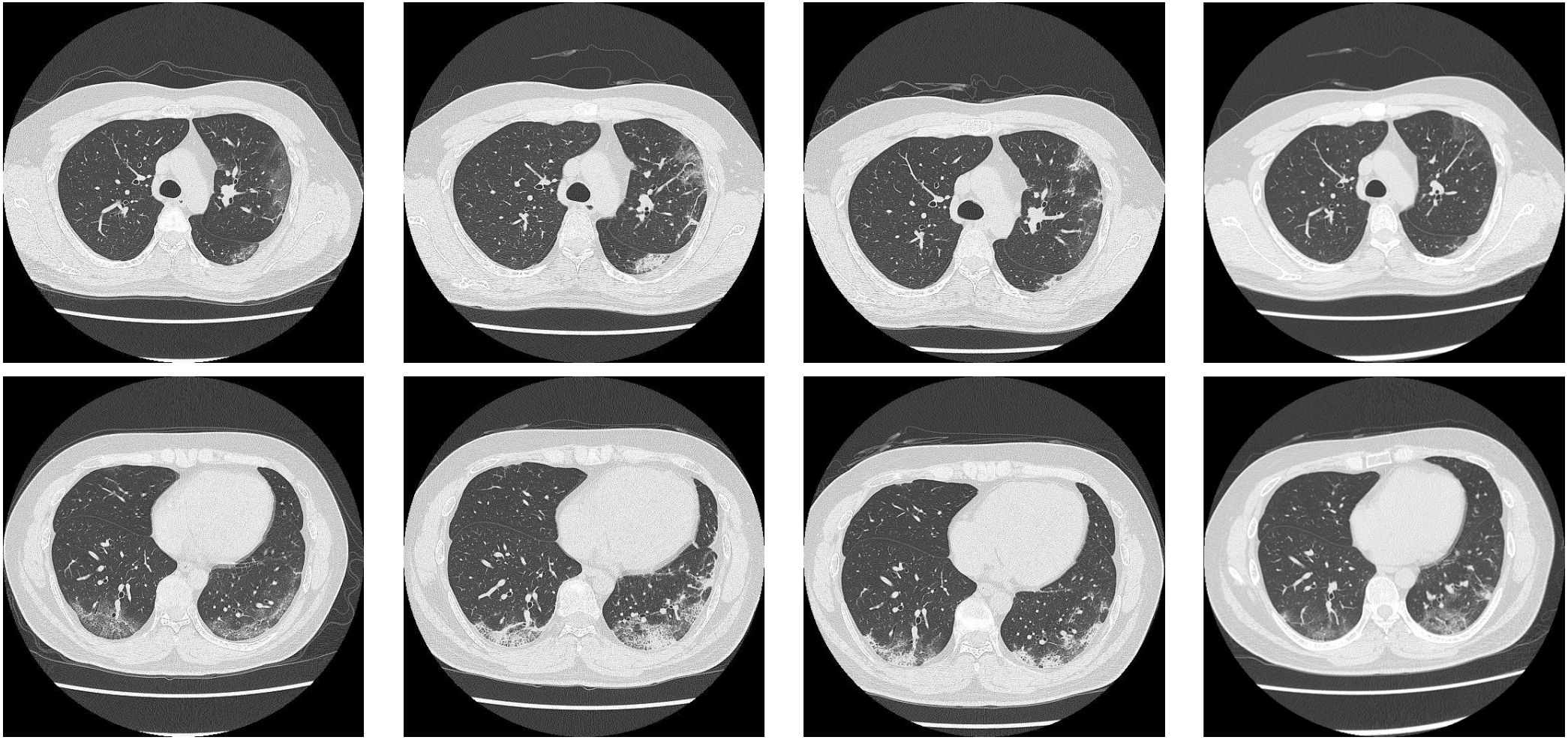
Designated Infectious Disease Medical Institution for Nagasaki Prefecture

day 1 upon admission

day 6

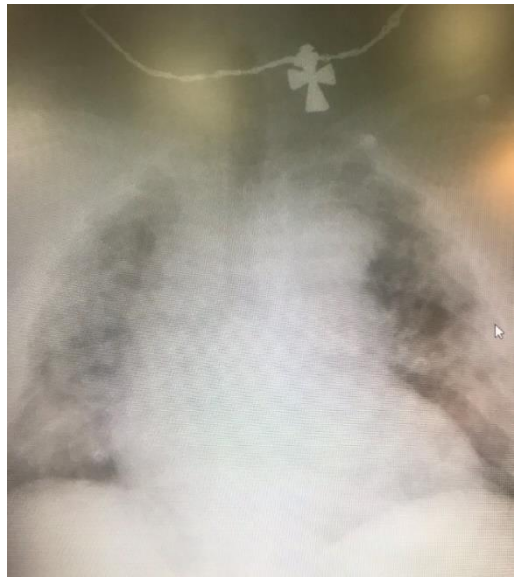
day 10

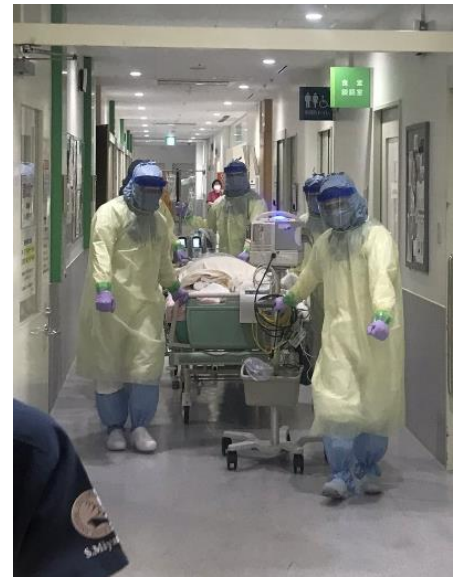
day 27

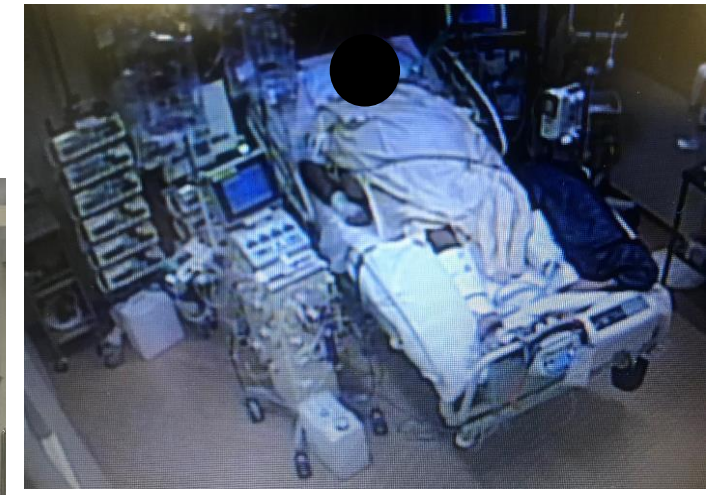


Designated Infectious Disease Medical Institution for Nagasaki Prefecture

A severe case of Nagasaki University Hospital







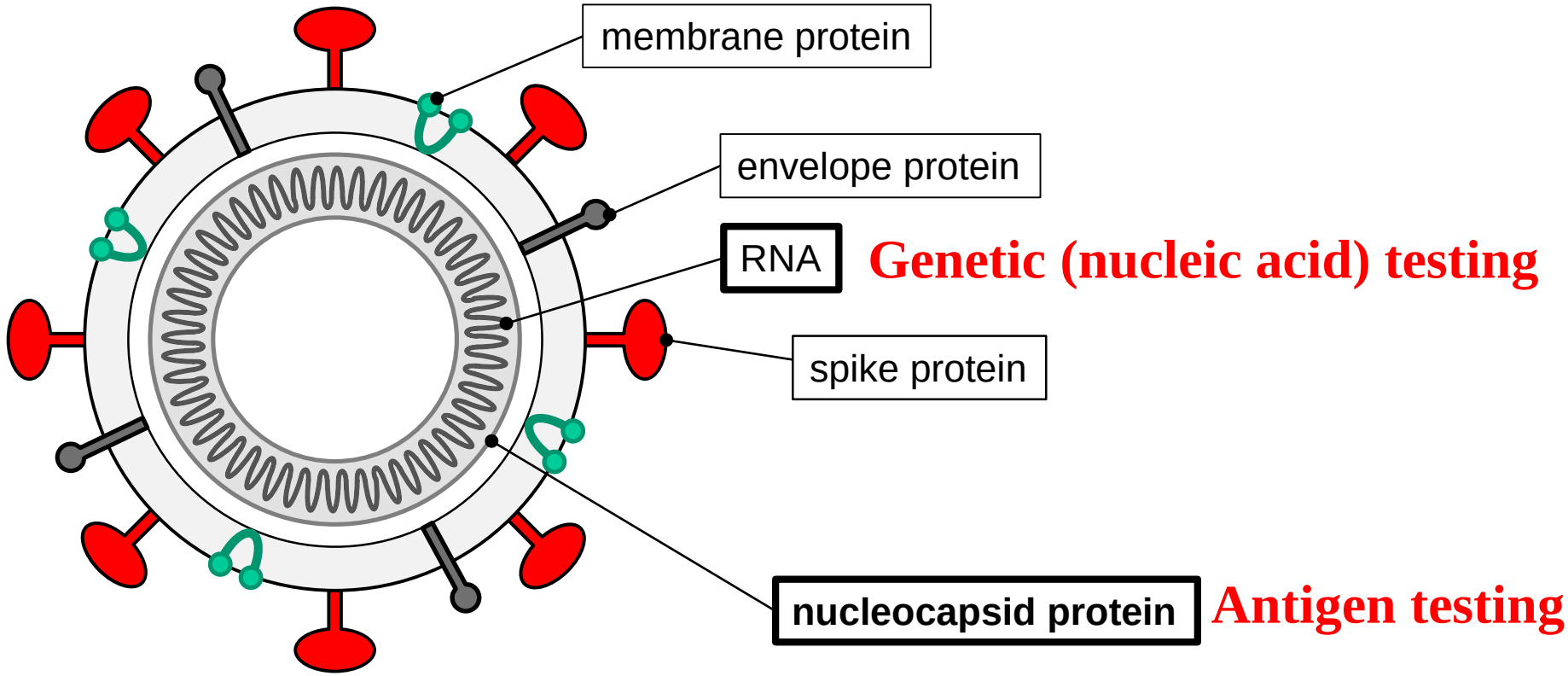
Extracorporeal membrane oxygenation (ECMO) is needed to save this patient.

Nagasaki University Hospital

Importance of laboratory diagnosis

- **Since therapeutic drug or vaccine has been developing for the novel coronavirus, it is most effective to diagnose and isolate patients by testing.**
- **There are three tests for detecting coronaviruses: genetic testing, antigen testing, and antibody testing.**

Structure of SARS-CoV-2 and laboratory testing



Japanese Society of Laboratory Medicine
Ad Hoc Committee on the Novel Coronavirus
(February 2020 –)

Chairman: YANAGIHARA Katsunori (Nagasaki University)
Committee member: IINUMA Yoshitsugu (Kanazawa Medical University)
Committee member: OTSUKA Yoshihito (Kameda Medical Center)
Committee member: OKAYAMA Akihiko (University of Miyazaki)
Committee member: KAYABA Hiroyuki (Hirosaki University)
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Committee member: TAKAHASHI Satoshi (Sapporo Medical University)
Committee member: NAGAO Miki (Kyoto University)
Committee member: MISAWA Shigeki (Juntendo University)
Committee member: MORINAGA Yoshitomo (University of Toyama)

7 medical doctors and 3 medical technologists

Genetic (nucleic acid) testing

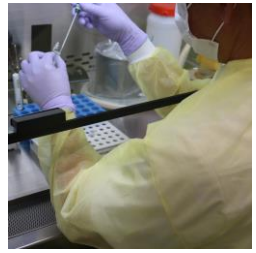
Method of increasing and detecting genes by a technique such as PCR

Advantages: It can detect even extremely small quantities with high sensitivity.

Disadvantages: It requires special equipment.
The procedure is complicated.
It is performed by a skilled clinical laboratory test technician.
It takes time.

Novel coronavirus RT (reverse transcription PCR) testing

0.5h to 1h for 12 samples
Manual operation



0.5h to 1h for 12 samples
Manual operation

12 samples/0.5h/Run

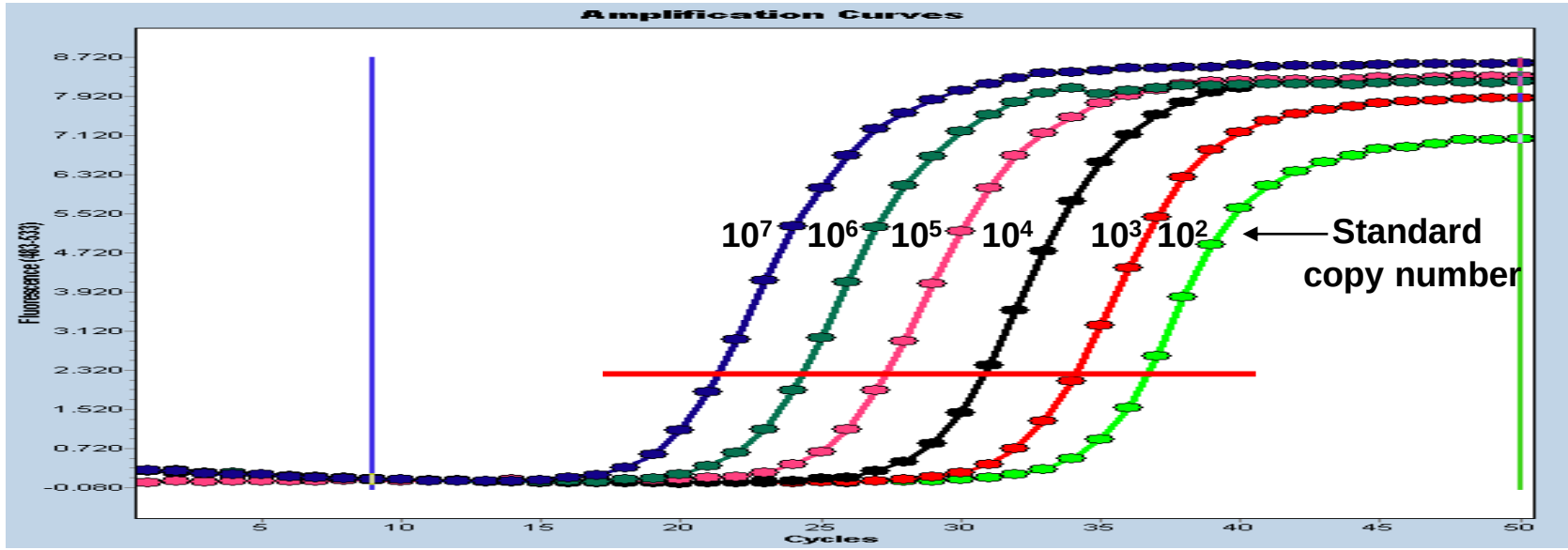
0.5h for 12 samples
Manual operation

1.5h / run

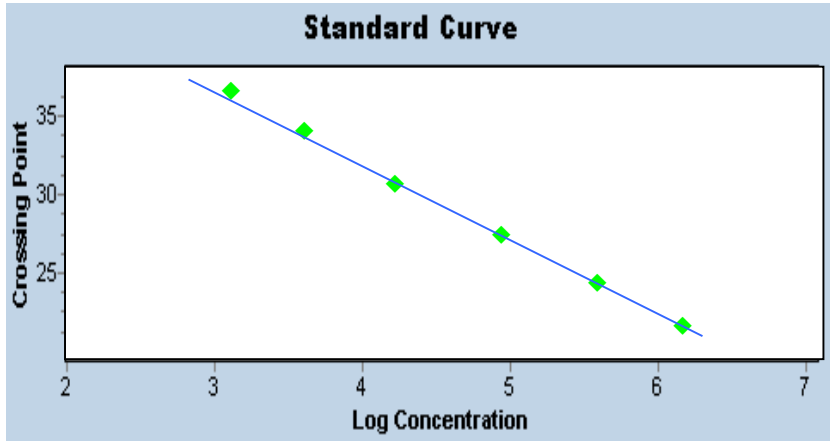
Specimen preprocessing → RNA extraction preparation → RNA extraction → PCR preparation → PCR

About 3-5 hours for the whole process

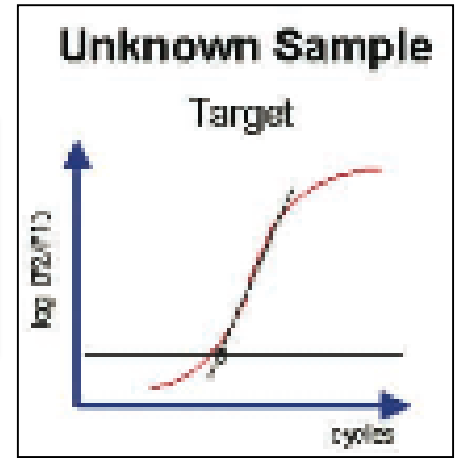
Absolute quantification of nucleic acid by real-



The logarithmic concentration is plotted on the horizontal axis,
the crossing point is plotted on the vertical axis,
and a calibration curve is created.



The number of copies of an unknown sample can be obtained.



Fully automated genetic testing equipment

Antigen testing

Advantages: It produces quick and easy results. (30 min)
No special equipment is required.
(there is also equipment usage testing)

It can be performed anywhere.

Disadvantages:
It cannot detect pathogens unless there are a lot to a certain extent.
It has low sensitivity.

Test results of antigen testing in clinical specimens

With the RT-PCR method	Positive match rate	Negative match rate	Overall match rate
All specimens (n=72)	37.0% (10/27 cases)	97.8% (44/45 cases)	75.0% (54/72 cases)
100 copies/test or more	83.3% (5/6 cases)	NA	NA
30-99 copies/test	16.7% (1/6 cases)	NA	NA
Less than 30 copies/test	33.3% (5/15 cases)	NA	NA

- Ministry of Health, Labor and Welfare Novel Coronavirus Response Headquarters. Guidelines for the Utilization of the SARS-CoV-2 Antigen Detection Kit
- Fujirebio Inc. Epsline SARS-CoV-2 Package Insert

Data from these sources was used during preparation

Evaluation of antigen test kits

Comparison of ESPLINE with SARS-CoV-2 PCR 62 positive cases

		qRT-PCR Ct value range				
		<20	20 to <25	25 to <30	30 to ≦40	Total
number of qRT-PCR positive specimens (n=62)		9	23	18	12	62
ESPLINE SARS-CoV-2	positive	9	23	16	2	50
	negative	0	0	2	10	12
concordance rate (%)		100.0	100.0	88.9	16.7	80.6

Takeda Y et al., *medRxiv.* 2020.

COVID-19 rapid antigen test kit approved in Japan (As of May 12, 2021)

Only Japanese products are listed (in order of approval date)

https://www.mhlw.go.jp/stf/newpage_11332.html

Product Name	Applicant Company	Approval Date	Clinical Performance (Comparison with RT-PCR method using domestic test samples)		Time to Result	Specimen Type	Storage Temperature	Product Information Website/ Contact Information
			Positive match rate	Negative match rate				
ESPLINE SARS-CoV-2	Fujirebio Inc.	May 13, 2020	66.7%	100%	30 minutes	Nasopharyngeal swab, Nasal swab	1-30℃	https://www.fujirebio.com/en/products-solutions/espline-sarscov2
QuickNavi-COVID19 Ag	Denka Co., Ltd.	August 11, 2020	53.4%	96.4%	15 minutes	Nasopharyngeal swab, Nasal swab	2-30℃	https://www.denka.co.jp/eng/contact/
ImmunoAce SARS-CoV-2	TAUNS LABORATORIES, INC.	October 13, 2020	76.2%	100%	15 minutes	Nasopharyngeal swab, Nasal swab	2-30℃	https://www.tauns.co.jp/en/contact-e/
PRORAST SARS-CoV-2 Ag ADTest SARS-CoV-2	ADTEC Corporation/LSI Medience Corporation	January 29, 2021	73.8%	100%	15 minutes	Nasopharyngeal swab, Nasal swab	1-30℃	https://www.adtec-inc.co.jp/
FUJI DRI-CHEM IMMUNO AG HANDY COVID-19 Ag	FUJIFILM Corporation	February 15, 2021	75.6%	100%	15 minutes	Nasopharyngeal swab, Nasal swab	1-30℃	https://www.fujifilm.com/contact/
ALSONIC COVID-19 Ag	Alfresa Pharma Corporation	March 12, 2021	66.7%	95.0%	5 minutes	Nasopharyngeal swab, Nasal swab	2-30℃	https://www.alfresa-pharma.co.jp/inquiry/form/
KBM LineCheck nCoV (Stick Type)	KOHJIN BIO CO., LTD.	March 17, 2021	78.6%	100%	1-10 minutes	Nasopharyngeal swab	2-30℃	https://kohjin-bio.jp/e-mail-inquiries/
ImmunoArrow SARS-CoV-2	TOYOBO CO., LTD.	May 12, 2021	74.1%	100%	15 minutes	Nasopharyngeal swab, Nasal swab	–	https://www.toyobo-global.com/gl/support/

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Journal of Infection and Chemotherapy

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Original Article

Evaluating a novel, highly sensitive, and quantitative reagent for detecting SARS-CoV-2 antigen

Ryo Kobayashi [a, c], Ryosei Murai [a], Koichi Asanuma [a], Yoshihiro Fujiya [b, c], Satoshi Takahashi [a, b, c, *]

[a] *Division of Laboratory Medicine, Sapporo Medical University Hospital, Sapporo, Japan*
[b] *Division of Infection Control, Sapporo Medical University Hospital, Sapporo, Japan*
[c] *Department of Infection Control and Laboratory Medicine, Sapporo Medical University School of Medicine, Sapporo, Japan*

The concordance rate between the chemiluminescent enzyme immunoassay (CLEIA) and immunochromatographic assay (ICA)

Table 1
Concordance between Espline SARS-CoV-2 and Lumipulse Presto SARS-CoV-2 Ag in the positive group.

		Lumipulse Presto SARS-CoV-2 Ag		Total
		(+)	(−)	
Espline SARS-CoV-2	(+)	24	0	24
	(−)	48	28	76
Total		72	28	100

The concordance rate between the CLEIA and ICAwas 52% .
The CLEIA judged 72 of the 100 patient samples as positive.

The concordance rate between the chemiluminescent enzyme immunoassay (CLEIA) and Nucleic acid Amplification Test (NAT)

Table 2
Concordance between the SARS-CoV-2 antigen and nucleic acid tests.

a

		2019 Novel Coronavirus Detection Kit		Total
		(+)	(-)	
Lumipulse Presto SARS-CoV-2 Ag ((+)≥1.00 pg/mL)	(+)	56	16	72
	(-)	18	10	28
Total		74	26	100
Sensitivity: 75.7% (95% confidence interval: 65.0%–86.5%)				
Concordance rate: 66.0%				

b

		2019 Novel Coronavirus Detection Kit		Total
		(+)	(-)	
Lumipulse Presto SARS-CoV-2 Ag ((+)≥1.00 pg/mL)	(+)	0	8	8
	(-)	0	192	192
Total		0	200	200
Specificity: 96.0% (95% confidence interval: 93.0%–99.0%)				
Concordance rate: 96.0%				
Concordance in the positive group (n = 100) (a), and in the negative group (n=200) (b)				

The SARS-CoV-2 nucleic acid test judged 74 of the 100 samples as positive at the same time as antigen measurement.
Therefore, the sensitivity of the CLEIA was 75.7%.
The specificity when testing the 200 negative samples was 96%.

How antigen-detecting rapid diagnostic tests (Ag-RDTs) will change the course of COVID-19 pandemic ?

Rethinking Covid-19 Test Sensitivity — A Strategy for Containment

Michael J. Mina, M.D., Ph.D., Roy Parker, Ph.D., and Daniel B. Larremore, Ph.D.

The NEW ENGLAND JOURNAL of MEDICINE

Michael J. Mina, MD, PhD

- Department of Epidemiology, Department of Immunology and Infectious Diseases,
- **Harvard** T.H. Chan School of Public Health

Daniel B. Larremore, PhD

- Department of Computer Science, **University of Colorado Boulder**
- **Harvard** John A Paulson School of Engineering and Applied Sciences

There are 3 major challenges with Covid-19:

1. A significant number of asymptomatic and infectious "silent spreaders" (ie. estimated 30.8% in Japan[2]);
2. With increased cases, we need to identify and isolate infectious individuals to create a safe community or safe bubble;
3. Rapid, de-centralized, and large-scale screening cannot be achieved with RT-PCR.

Mina and Larremore have modelled for surveillance effectiveness considering test sensitivities, frequency, and sample-to-answer reporting time. [3]

Mina MJ, Parker R, Larremore DB. Rethinking Covid-19 Test Sensitivity - A Strategy for Containment. *N Engl J Med.* 2020;383(22):e120.
Larremore DB, Wilder B, Lester E, et al. Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance. medRxiv. 2020:2020.2006.2022.20136309.

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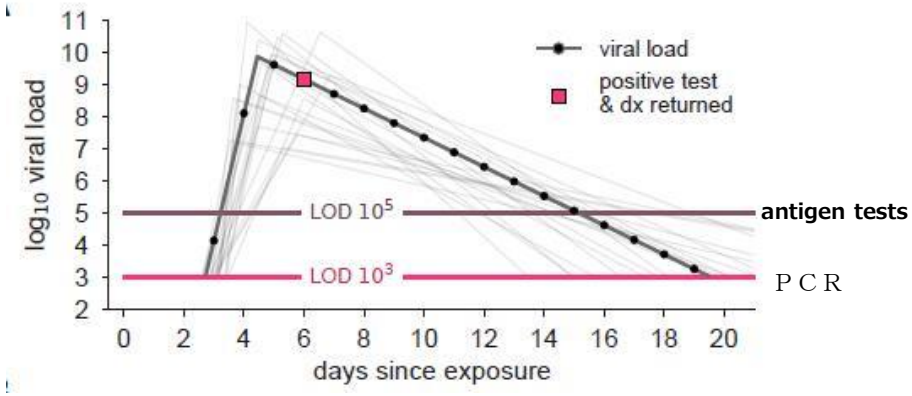

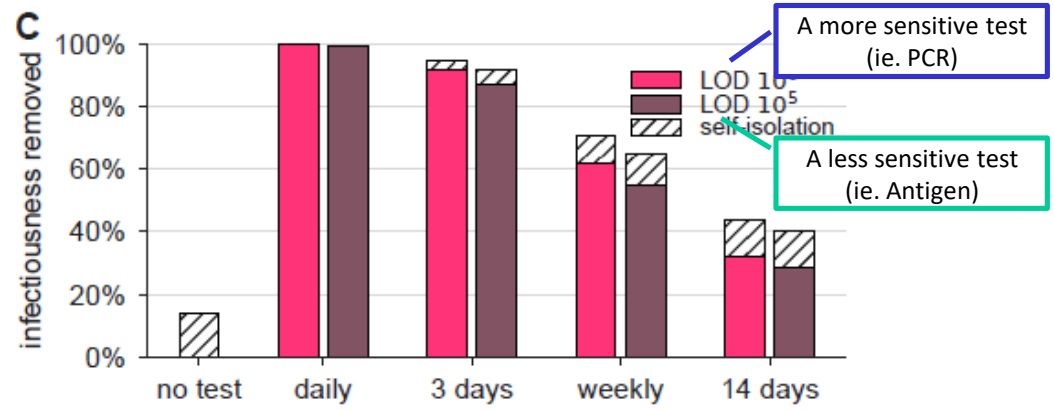

The model simulated 10,000 individuals using viral load trajectories identified from literature. It considered 20% of patients would undergo isolation based on symptoms.[1]

The model then looked at the effectiveness of different testing frequencies on removing infectiousness from the cohort. It has found that more frequent testing (daily or every 3 days) can remove 85%-100% of infectious individuals. Self-isolation based on symptoms can only remove <15% infectiousness.[1]

Larremore DB, Wilder B, Lester E, et al. Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance. medRxiv. 2020:2020.2006.2022.20136309.

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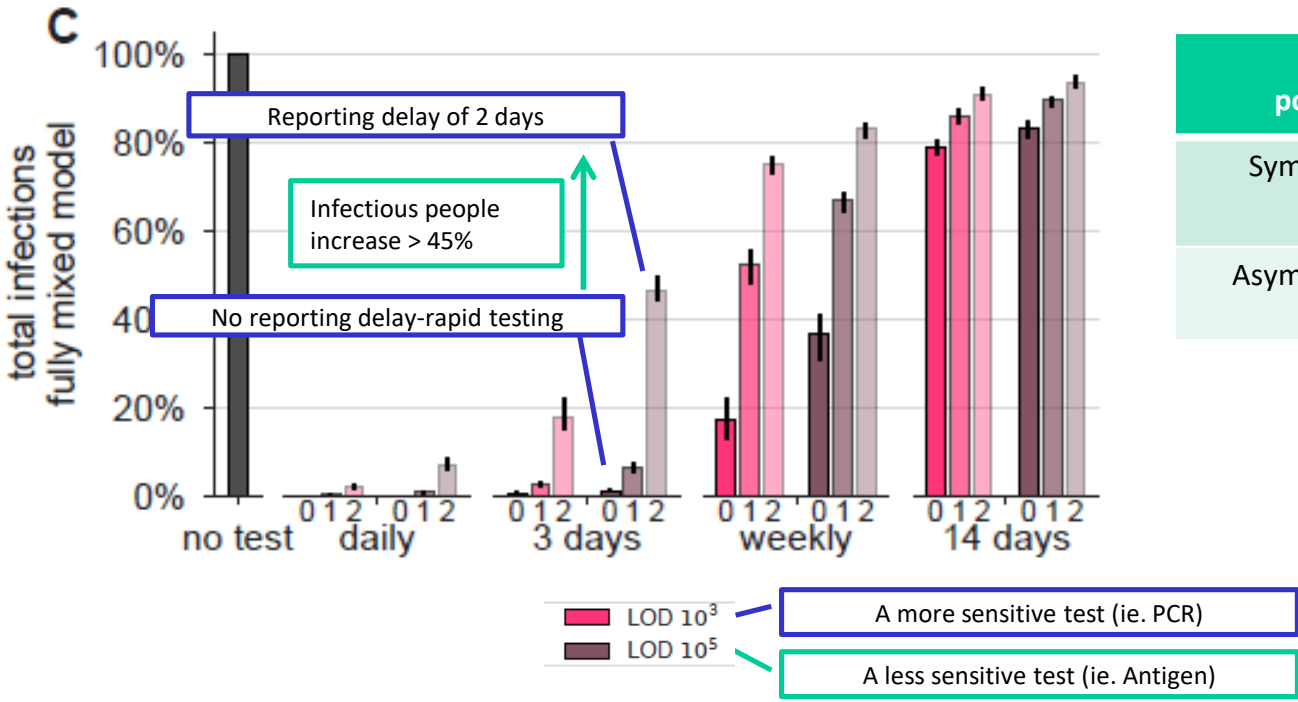
The model also found out that diagnostic reporting delay can lead to less effective control in viral spread.[1]



With these findings, the authors concluded:[1,2]

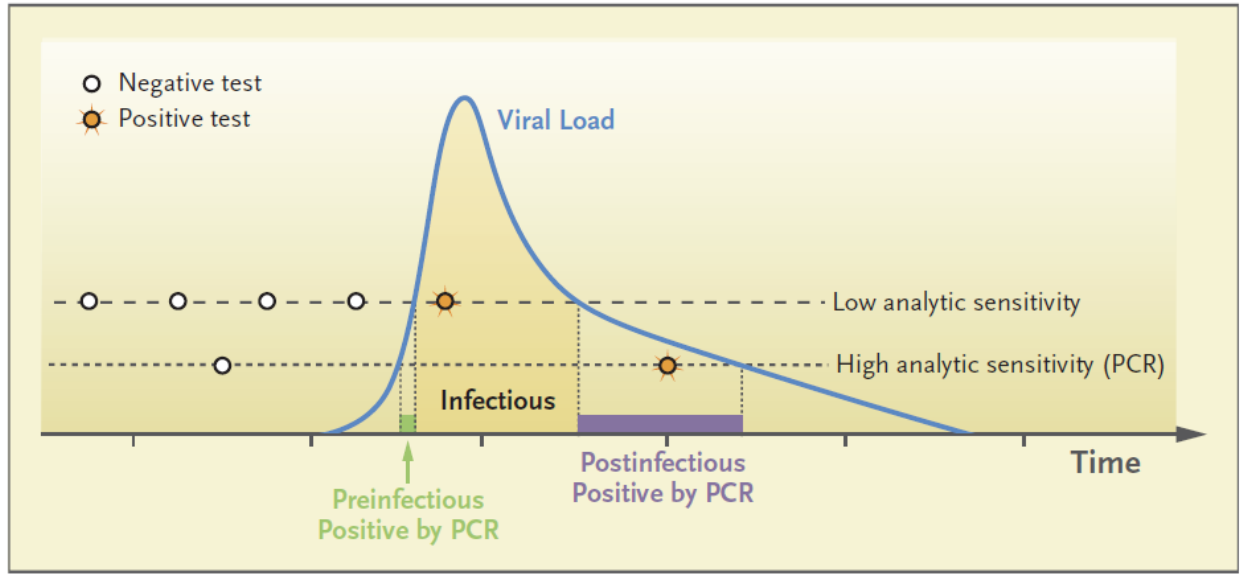
Testing population	Priority	Suitable test
Symptomatic	• Self isolation • Sensitivity	High sensitivity clinical diagnosis
Asymptomatic	• Time to result • Frequent testing	Rapid test (ie. Antigen)

Larremore DB, Wilder B, Lester E, et al. Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance. medRxiv. 2020:2020.2006.2022.20136309.

Rethinking Covid-19 Test Sensitivity — A Strategy for Containment

Michael J. Mina, M.D., Ph.D., Roy Parker, Ph.D., and Daniel B. Larremore, Ph.D.

- Frequency is more important than sensitivity in COVID-19 screening
- Frequent testing (daily or once every 3 days) can eliminate 85% to 100% of infected people



High-Frequency Testing with Low Analytic Sensitivity versus Low-Frequency Testing with High Analytic Sensitivity.

Mina MJ, Parker R, Larremore DB. Rethinking Covid-19 Test Sensitivity - A Strategy for Containment. *N Engl J Med.* 2020;383(22):e120.

Antigen-based testing but not real-time PCR correlates with SARS-CoV-2 virus culture

Andrew Pekosz, PhD,[1, 2] Charles K. Cooper, MD,[3] Valentin Parvu, PhD,[3] Maggie Li, MS,[1]
Jeffrey C. Andrews, MD,[3] Yukari C. Manabe, MD,[1, 4] Salma Kodsi, MS,[3] Jeffry Leitch, PhD
Devin S. Gary, PhD,[3] Celine Roger-Dalbert, MS[3]

Clinical Infectious Diseases
MAJOR ARTICLE
IDSA Infectious Diseases Society of America
hivma hiv medicine association
OXFORD
Impact factor 9.117 (2017)

Andrew S. Pekosz, PhD

- Department of Molecular Microbiology and Immunology,
- **Johns Hopkins** Bloomberg School of Public Health

Upper respiratory specimens are obtained from 251 individuals with one or more Covid-19 like symptoms at 21 geographically diverse mobile sites.
Patients are within 7 days post symptom onset.
Specimens from all 251 individuals went through PCR and antigen (Veritor) tests.
In 38 PCR positive specimens, viral culture was conducted to detect live virus.

-Infectious Diseases Society of America: Antigen-based testing but not real-time PCR correlates with SARS-CoV-2 virus culture /Andrew Pekosz, PhD

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Performance Values	Antigen Test Performance	rt-PCR Performance
PPA	96.4 (82.3–99.4)	100 (87.7–100)
NPA	98.7 (96.1–99.7)	95.5 (91.1–97.8)
PPV	90.0 (76.3–97.6)	73.7 (60.8–85.3)
NPV	99.5 (97.7–100)	100 (98.4–100)
OPA	98.4 (96.0–99.4)	96.0 (92.8–97.8)
Culture (+)/test (+)	27	28
Culture (–)/test (+)	3	10
Culture (+)/test (–)	1	0
Culture (–)/test (–)[a]	220	213

Veritor was highly sensitive in detecting infectious specimens

While rt-PCR is more sensitive in detecting the virus, it is less specific in identifying live virus.

-Infectious Diseases Society of America: Antigen-based testing but not real-time PCR correlates with SARS-CoV-2 virus culture /Andrew Pekosz, PhD

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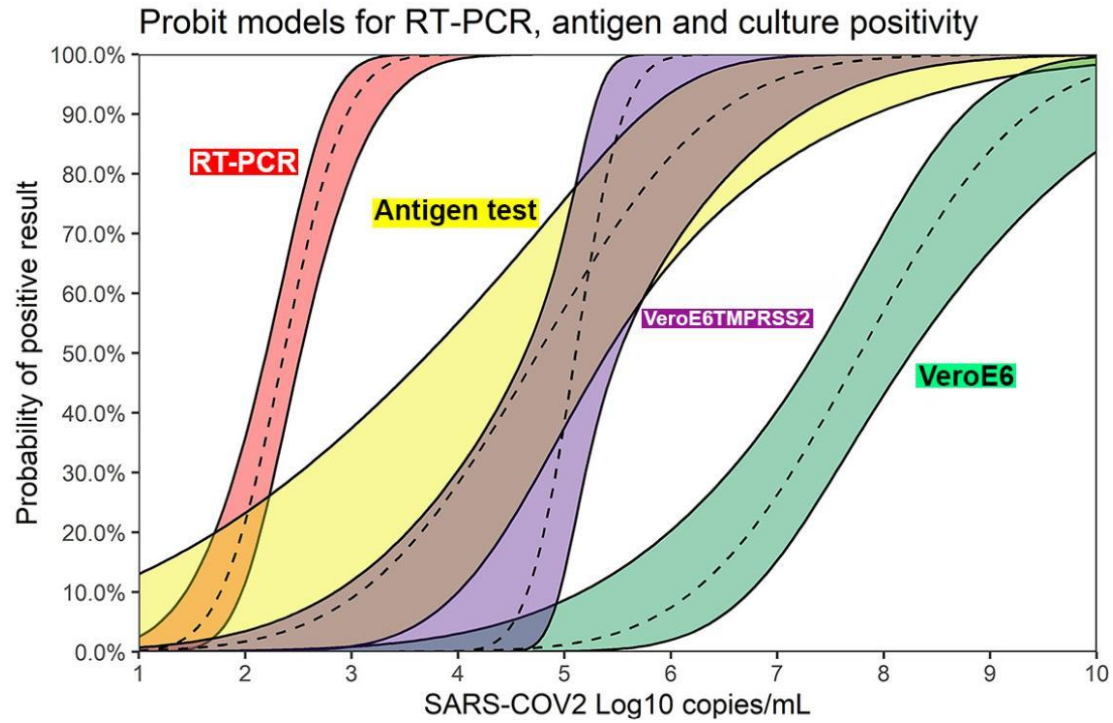
Using a probit model, the authors found stronger correlation between Veritor and viral culture (TMPRSS2) positivity, instead of rt-PCR and culture.



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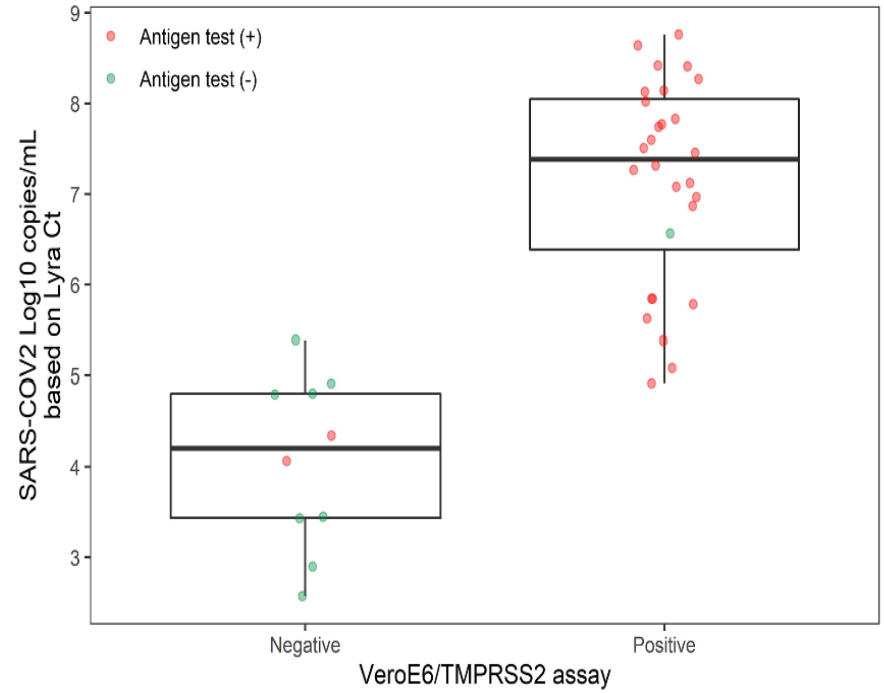
- Upper respiratory specimens are obtained from 251 individuals with one or more Covid-19 like symptoms at 21 geographically diverse mobile sites.（Patients are within 7 days post symptom onset.）
- Virus culture was performed in 38 PCR-positive samples to detect live virus
- They found a correlation between antigen testing (BD Veritor) and virus culture (TMPRSS2) positivity

Figure 1A.



-Infectious Diseases Society of America: Antigen-based testing but not real-time PCR correlates with SARS-CoV-2 virus culture /Andrew Pekosz, PhD

Possible problems in COVID-19 Testing

PCR or CLEIA assay

- Shortage of medical equipment, reagents, and testing laboratories
- Human resources for operating test devices
- Supply shortage of medical equipment for specimen collection
- Delays in transporting specimens to reference labs and feedback of test results
- Shortage of equipment for processing and analysis of test results
- Unstable electricity supply, causing negative effects on equipment (devises, air conditioning in laboratory areas)
- Quality control of laboratory tests

Storage and Transport Temperature

- Temperature control in warehouse
- Influence from other products in consolidation warehouse
- Traffic congestion during truck transportation
- Difficulty in cold transportation for the last mile delivery
- Lack of cold storage equipment for testing laboratories, health facilities, and transportation networks
- Unstable electricity supply

Tests at Border Points

- Standardization of test systems and strategies within a region
- Registration and management of a large number of people to be tested
- Delays in feedback of test results
- Quality control of test results
- - Border points (customs, quarantine) and surrounding areas, where many people come and go, may become hot spots for spreading infection
- - Since land routes are important for logistics in many countries, rapid diagnostic test is necessary

Rapid antigen tests can contribute to COVID-19 screening test

	Sensitivity	Rapidity	Cost
PCR	◎	×	△
Rapid antigen tests	△	◎	○