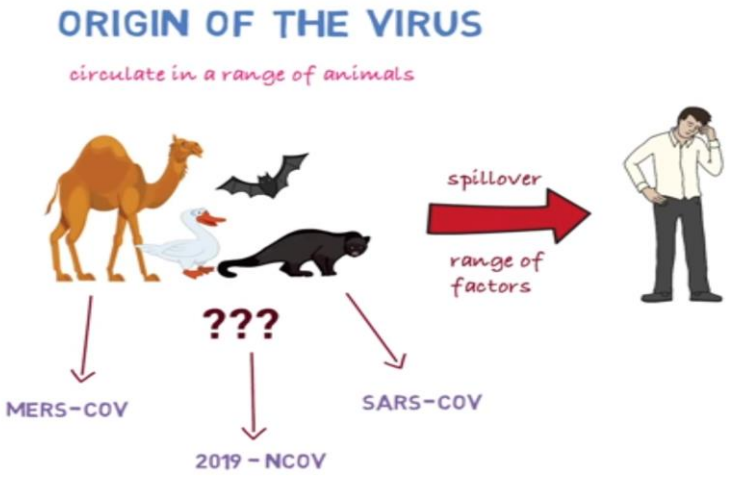
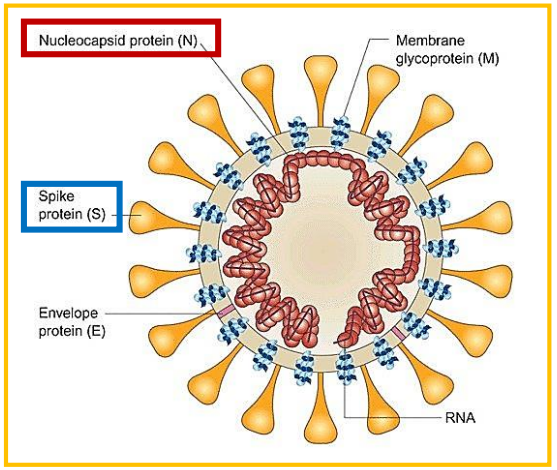
COVID-19 Antibody test

Boditech Med Inc.

Coronavirus

- **Coronavirus is RNA virus with 27-32 kb genome and can infect human, bat, birds, and other animals**
- **4 coronavirus subtypes (Alpha, Beta, Delta, Gamma)**
  - **Alpha, Beta: found in human, other animals**
  - **Delta, Gamma: found in animals**
- **A novel coronavirus (nCoV) is a new strain that has not been previously identified in humans and causes COVID-19 disease.**
- **Clinical symptoms including fever, coughing sneezing, and pneumonia.**





Human Coronavirus

There are seven strains of human coronaviruses

Common cold coronavirus

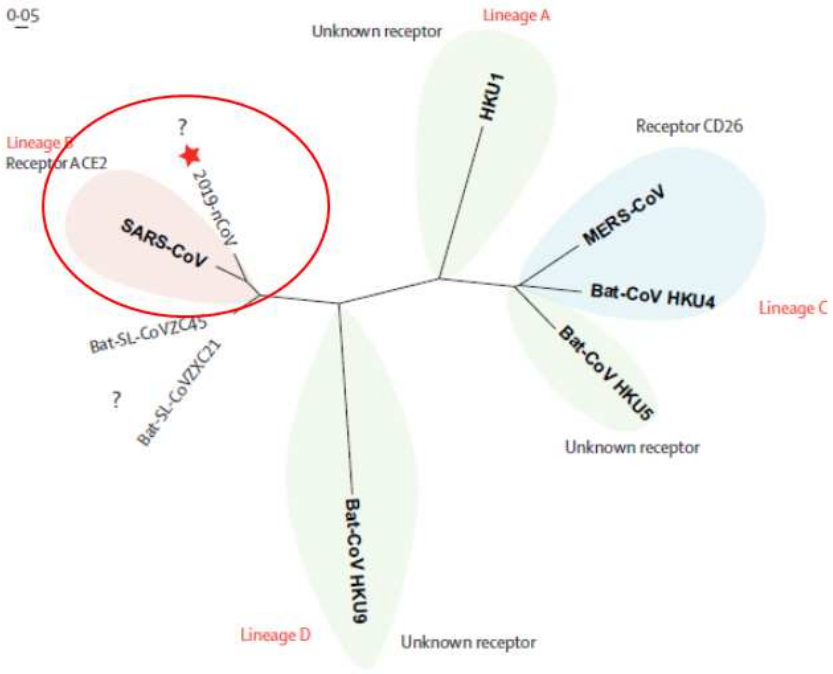
HCoV-229E | HCoV-OC43 | HCoV-OC43 | HKU1

SARS-CoV, MERS-CoV

SARS-CoV | MERS-CoV

Novel coronavirus

2019-nCov



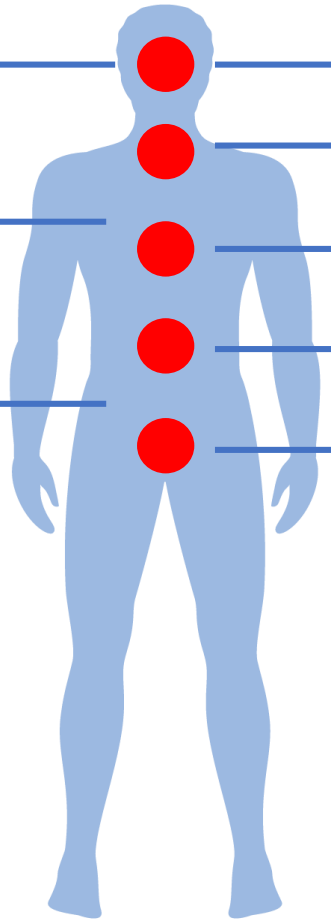
Comparisons

	Covid-19	MERS	SARS	Common cold coronavirus
Origin	First reported in December 2019 in Wuhan, China	First reported in 2012 in Saudi Arabia	First reported in 2002 in southern China	Four coronavirus strains are thought to be responsible for 15-30% of common cold
Trans-mission	Likely from touching or eating an infected, as yet unidentified animal. Human-to-human transmission occurs through close contact	Often from touching infected camels or consuming their milk or meat. Limited transmission between humans through close contact	Believed to have spread from bats, which infected civets. Transmitted mainly between humans through close contact	Close contact with infected humans or touching a surface that carries the virus
Cases	More than 70,000 confirmed cases at Feb 18	2,494 confirmed cases; 858 deaths(as Nov. 30, 2019) Mortality rate of 34%	8,098 cases; 774 deaths. Mortality rate of about 10%	Millions each year. Generally nonlethal with rare exceptions.
Current Status	Cases reported mainly in China and spreading over the world	All cased lined to Arabian Peninsula. Others in 12 countries and death have been declining since 2016	No new cases reported since 2004. 87% of previous cases in China and Hong Kong	Circulates year-round, but more common in fall/winter

Symptoms of Coronavirus infection

Severe symptoms

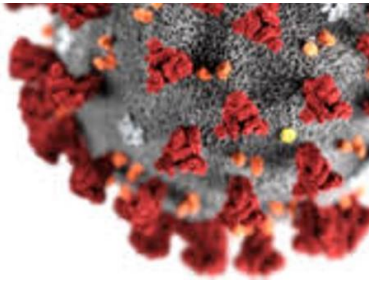
- High fever (40°C or more)
- Pnuemonia
- Kidney failure



Common symptoms

- Fever
- Dry cough
- Mild breathing difficulty
- Gastrointestinal issues
- Diarrhea
- General body aches

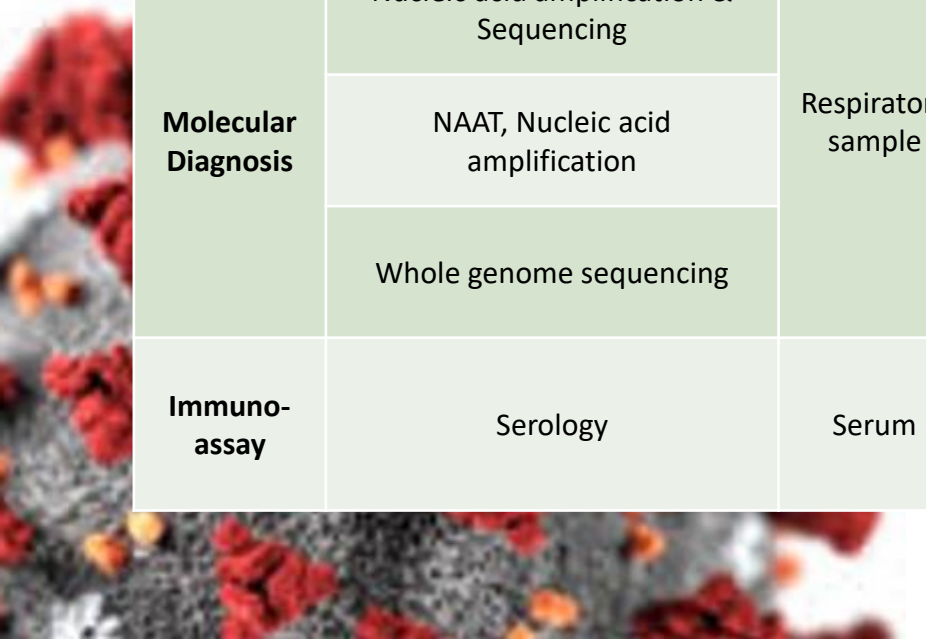
Diagnosis of Coronavirus infection



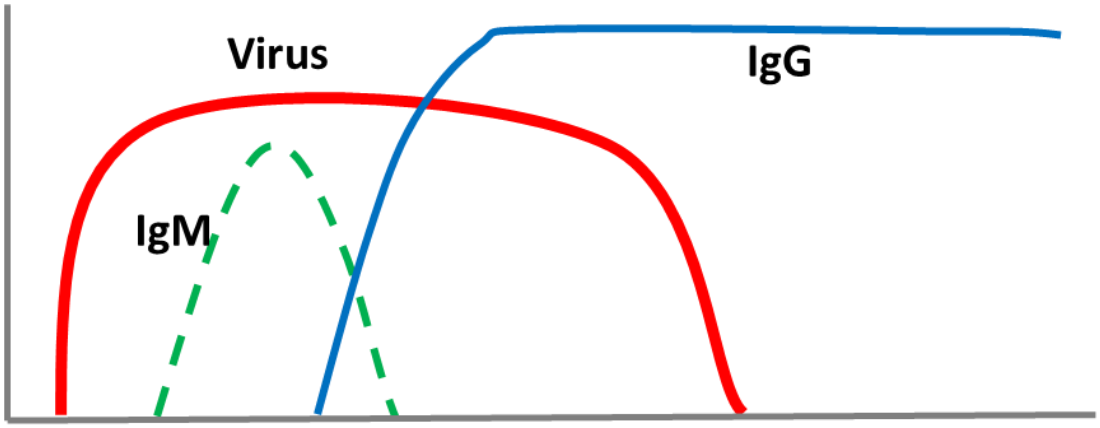
WHO Guideline for novel coronavirus diagnosis

Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases
Interim guidance
17 January 2020
World Health Organization

	Test	Type of sample	Comments
Molecular Diagnosis	Nucleic acid amplification & Sequencing	Respiratory sample	Collect on presentation. Done by an expert laboratory. RT-PCR followed by sequencing if positive.
	NAAT, Nucleic acid amplification		Collect on presentation. Done by an expert laboratory. Various PCR methods are under validation
	Whole genome sequencing		Collect on presentation. Done by an expert laboratory. Well quipped facility with long and costly testing
Immuno-assay	Serology	Serum	IgG/IgM test in blood sample. Paired samples necessary for confirmation, the first sample collected in week 1 of illness and the second collected 2-3 weeks late



Test windows



Molecular test PCR

Antibody test COVID-19 Ab

Cold Spring Harbor Laboratory
bioRxiv
THE PREPRINT SERVER FOR BIOLOGY
January 23, 2020.

Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin

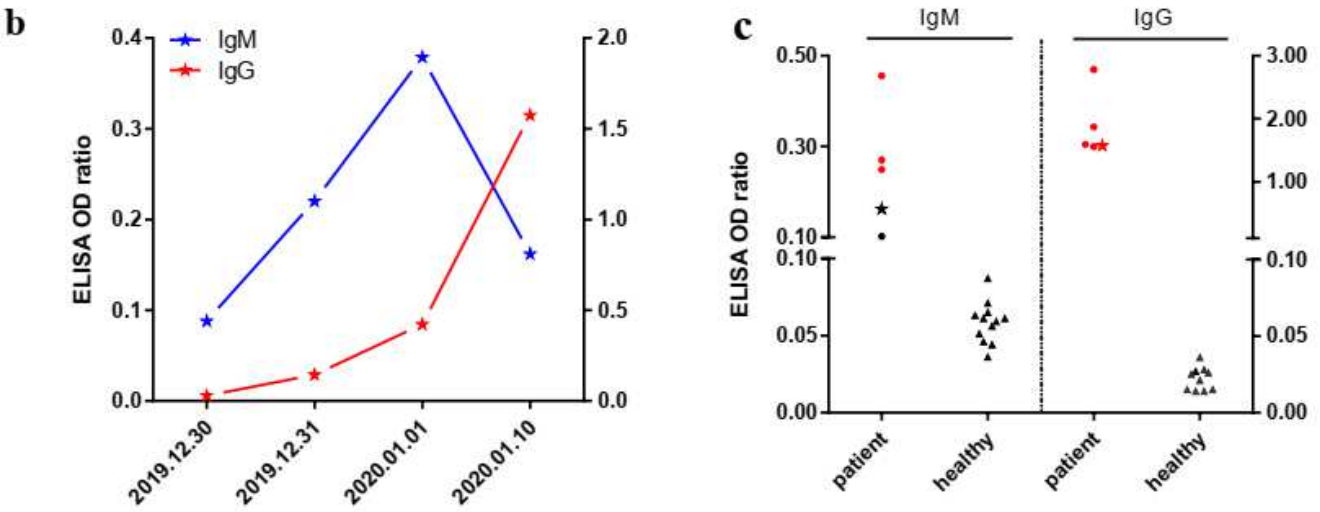


Fig. 2 Serological investigation of patient samples

b, dynamics of nCoV-2019 antibodies in one patient who showed sign of disease on 2019.12.23
c, serological test of nCoV-2019 antibodies in five patients.
* For b and c, cut-off was set up as 0.2 for IgM test and 0.3 for IgG test, according to healthy controls.

Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes

ABSTRACT

In December 2019, a novel coronavirus (2019-nCoV) caused an outbreak in Wuhan, China, and soon spread to other parts of the world. It was believed that 2019-nCoV was transmitted through respiratory tract and then induced pneumonia, thus molecular diagnosis based on oral swabs was used for confirmation of this disease. Likewise, patient will be released upon two times of negative detection from oral swabs. However, many coronaviruses can also be transmitted through oral–fecal route by infecting intestines. Whether 2019-nCoV infected patients also carry virus in other organs like intestine need to be tested. We conducted investigation on patients in a local hospital who were infected with this virus. We found the presence of 2019-nCoV in anal swabs and blood as well, and more anal swab positives than oral swab positives in a later stage of infection, suggesting shedding and thereby transmitted through oral–fecal route. We also showed serology test can improve detection positive rate thus should be used in future epidemiology. Our report provides a cautionary warning that 2019-nCoV may be shed through multiple routes.

IgM to IgG conversion in 5 days

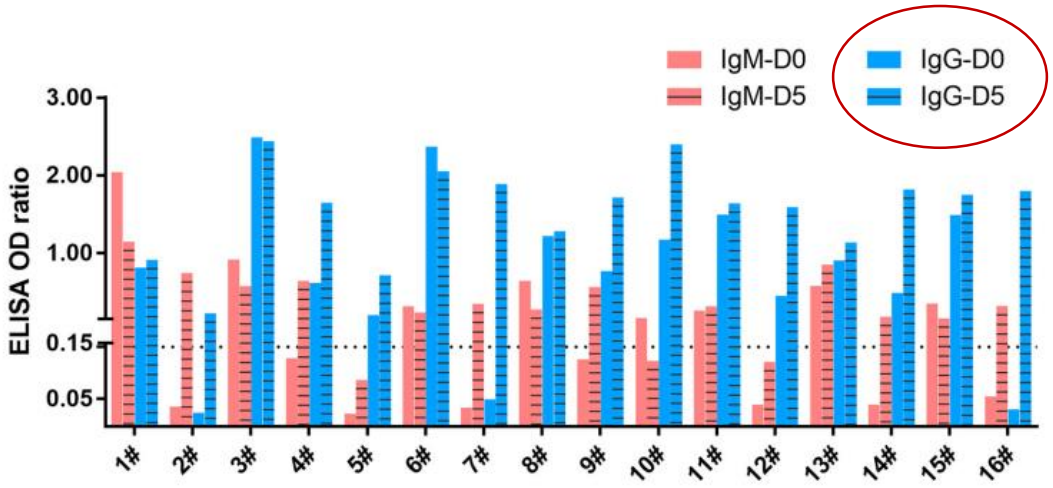
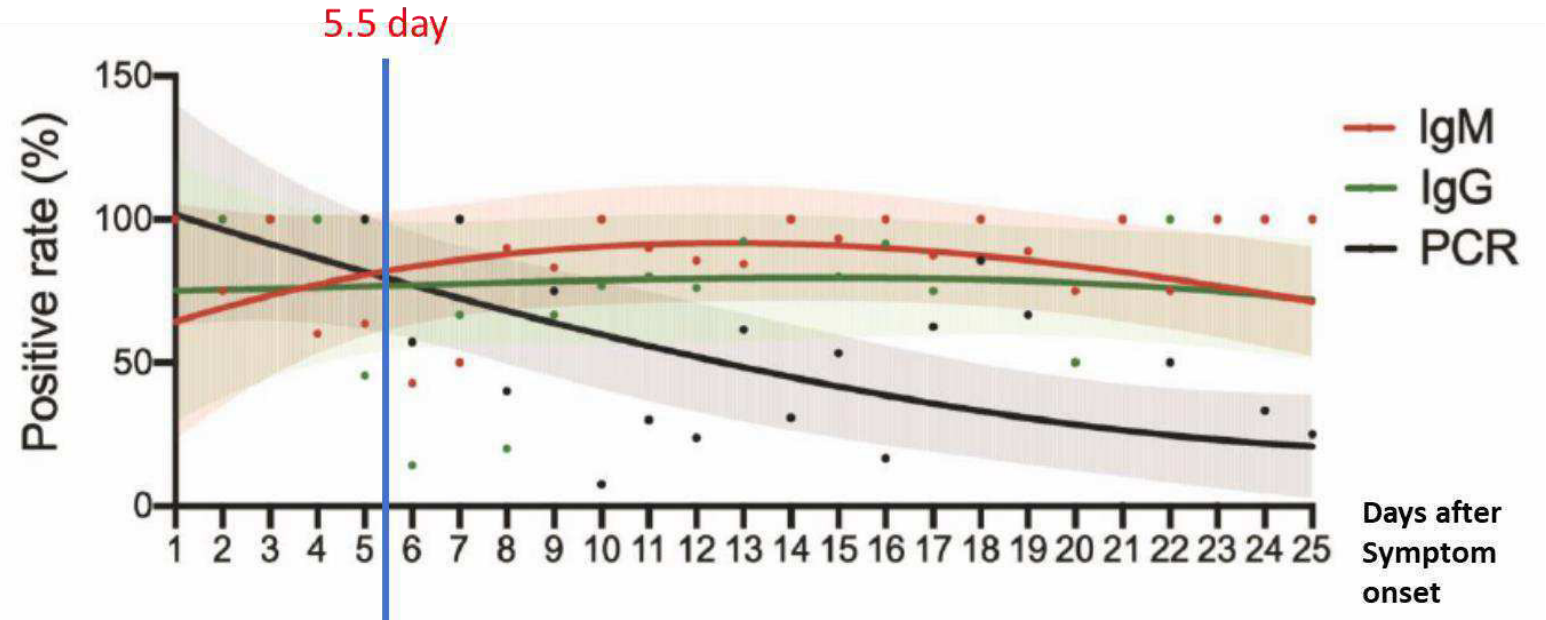


Figure 1. Serological detection of 2019-nCoV. Dashed line indicates cutoff, which was determined based on data from healthy controls.

Detection rate by days

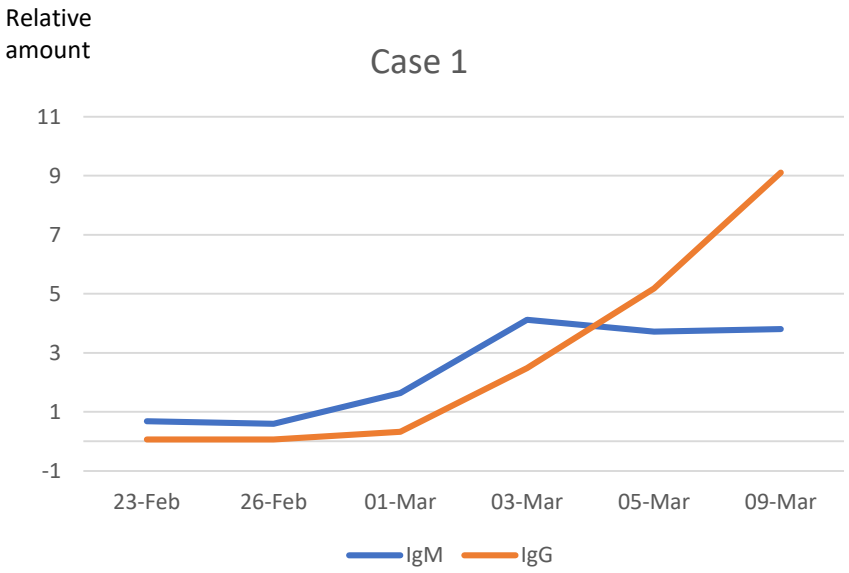


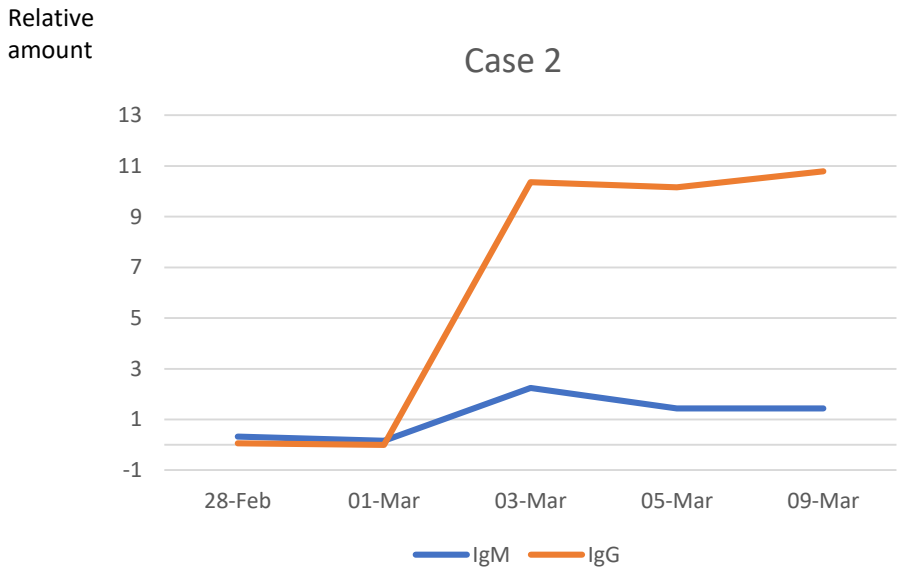
Detection rate	0-5.5 day	6-13 day	>14 day
PCR	> 90%	80	< 50 %
IgG/IGM	~ 75 %	75.6 ~ 93.1%	

5.5 days after symptom onset, IgM/IgG detection rate is better than PCR positive rate

Seroconversion of COVID-19 patients

By AFIAS COVID-19 Ab test



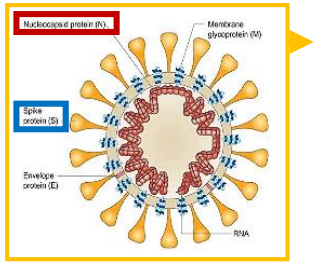


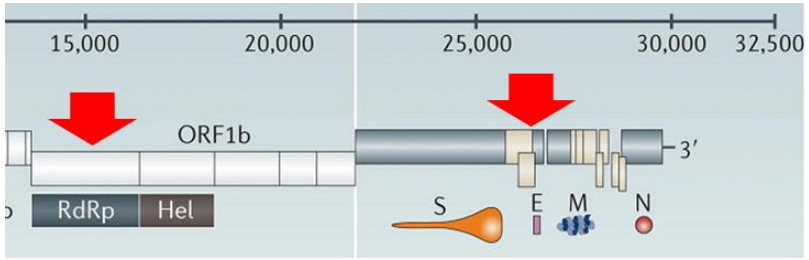
"G " University hospital at Korea

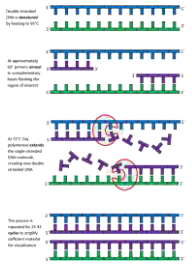
	RT-PCR	Boditech Antibody test	Rapid test (Antibody)
Target	Virus (RNA)	Antiviral IgM/IgG	Antiviral IgM/IgG
Sample	Nasopharyngeal/ oropharyngeal swab	Blood(WB/S/P)	Blood(WB/S/P)
Sample hazard level	Highly contagious	Low to non-contagious	Low to no-contagious
Time(TOT)	1-6 hrs	10 min	10-15 min
Sensitivity	High	High to medium	Low
Testing windows	Pre-symptom to onset of symptom	3-6 days after onset of symptom	1 week after onset of symptom
Result	Qualitative with Ct value	Qualitative with COI value	Qualitative
Clinical value	Confirmatory test	Screening and monitoring of COVID-19	Screening

Molecular diagnosis of Coronavirus

Confirmatory test with high precision and sensitivity







Disadvantage of MDx..

1. Long total turn around time including sample preparation(> 6 Hrs)
2. Require well equipped facility
3. Require highly trained technician/clinician
4. Chance of false negative
   (there is report that 30-50% of false negative in some cases

Needs for screening POC test !

Specification of Covid-19 Ab

Product name	AFIAS COVID-19 Ab	ichroma COVID-19 Ab
Assay type	TRF LFA*, IgM/IgG test	
Sample type	whole blood/ Serum/plasma	
Sample prep/ extraction	Fingertip blood(C-tip, 30 ul) / tube blood (100ul)	Fingertip blood(10 ul) / tube blood (10 ul)
Target	Anti-viral IgM/IgG	
Assay time	10 Min.	
Analytic sensitivity	Positive agreement 100% Negative agreement 96.7%	
Device (Reader)	AFIAS-1/6 (Automated)	ichroma II (Manual)
Package unit	24kit/box	25kit/box

* TRF LFA; Time resolved fluorescence lateral flow immunoassay

ichroma™

COVID-19 Ab

This is not a complete Instruction for use. For more detailed instructions, please refer to IFU.

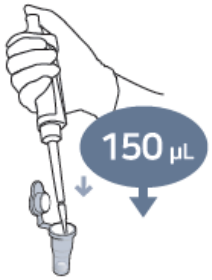
Test Components

Test Cartridge | Detector tube (Granule) | Detector diluent vial | ID Chip

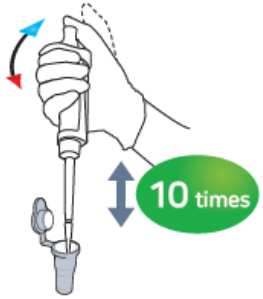
Test Procedure

1 Draw 150 µL (Detector diluent)

2 Add it into Detector tube

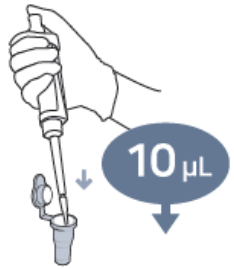


3 Pipetting the mixture 10 times.



4 Draw 10 µL (Whole blood / Serum / Plasma)

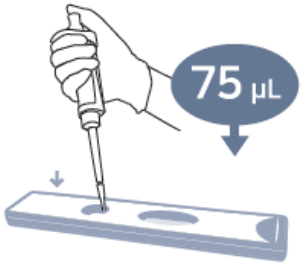
5 Add it into Detector tube



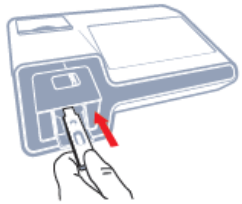
6 Shake 10 times

7 Draw 75 µL

8 Load the sample mixture

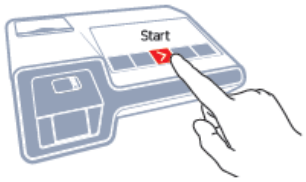


9 Insert the test cartridge



10 Wait 10 minutes

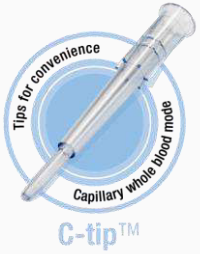
11 Press 'Start'



12 Read the test result

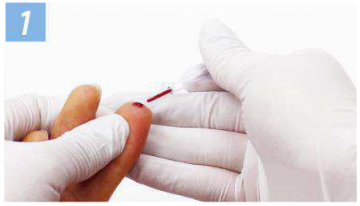
Test procedure

1. Fingertip blood
2. Simple operation
3. Data collection by network
4. 10 min result on site



3 simple steps

1



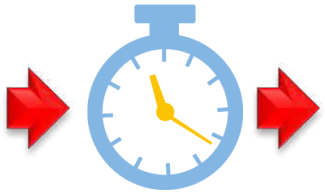
Collect capillary blood

2

Place the C-tip™ in the cartridge tip hole

3

Press START to run the test.



10 min

Reader Specification

iCHROMA II

Interface
7" touch screen
Built-in thermal printer
USB port / Ethernet / SD card slot
WiFi and bluetooth dongle (optional)
LIS / HIS compatible

Memory
1,000 patient results
1,000 QC results
100 User IDs

Analyzer
276 x 220 x 91 mm
1.3 kgs
100 – 240V AC, 50-60Hz
Battery (AA×4)

Up to 30 test/hours

AFIAS-1

Interface
5" touch screen
Built-in thermal printer
RS 232 / USB port / Ethernet / SD card slot
LIS / HIS compatible

Memory
5,000 patient results
5,000 QC results
500 ID chips
100 User IDs

Throughput
Up to 10 tests / hour

Analyzer
320 x 204 x 180 mm
3.9 kgs
100 – 240V AC, 50-60Hz
Internal temperature control

Up to 6 test/hours

AFIAS-6

Interface
7" touch screen
Built-in thermal printer
RS 232 / USB port / Ethernet / SD card slot
LIS / HIS compatible

Memory
5,000 patient results
5,000 QC results
500 ID chips
100 User IDs

Throughput
Up to 36 tests / hour

Analyzer
420 x 336 x 293 mm
15.1 kgs
100 – 240V AC, 50-60Hz
Internal temperature control

Up to 36 test/hours

Thank you!

PCR practical issues

- Sampling issues

- CDC recommend nasopharyngeal AND oropharyngeal swab. nasopharyngeal sampling requires some training and common cause of false negative
- It is reported that during nasopharyngeal swab sampling, candidates often sneezing or coughing, which is potential hazard for the health worker.
- Sample is biosafety level 2 but SARS-Cov-2 virus is biosafety level 3. Thus, during transport and extraction (until it completed and collect RNA of virus). It need to be treated as biosafety level 3 and extreme caution.
- Virus is liable in even in protective solution(VTM). 3-4 hours at RT and 24 hours at refrigerated. Sample transfer from collection site to Lab for PCR causes the false negative

PCR practical issues

- Performing analysis (virus RNA extraction and PCR amplification)

- As previously mentioned, extraction should be performed biosafety level 3 with high level protection equipment. It requires well trained person. Also prolonged working at BL3 environment gives burden and easily slow down performance.(It is one of the bottleneck of PCR procedure)
- In case different lab uses different PCR device, it is hard to compare directly each result
- PCR requires well trained person for the operation and result analysis.
  → In Korea, at least 2 independent PCR positive is required for COVID-19 Confirmation. Due to inconsistency of result, it is reported that 10 times of independent PCR had been performed in one patient
- Contamination issues: lab test is performed a lot test, positive result tube contains amplified (hundreds millions of copy) RNA and it is potential contaminant of PCR device and Lab.

  It is reported that several case of device contamination, which resulted false positive (Pure water can produce positive PCR result)

OXFORD ACADEMIC

Impact factor=9.1

Clinical Infectious Diseases

ACCEPTED MANUSCRIPT

Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19) FREE

Li Guo, Ph.D, Lili Ren, Ph.D, Siyuan Yang, Ph.D, Meng Xiao, Ph.D, De Chang, MD, Ph.D, Fan Yang, Ph.D, Charles S Dela Cruz, MD, PhD, Yingying Wang, BS, Chao Wu, BS, Yan Xiao, MS ... Show more

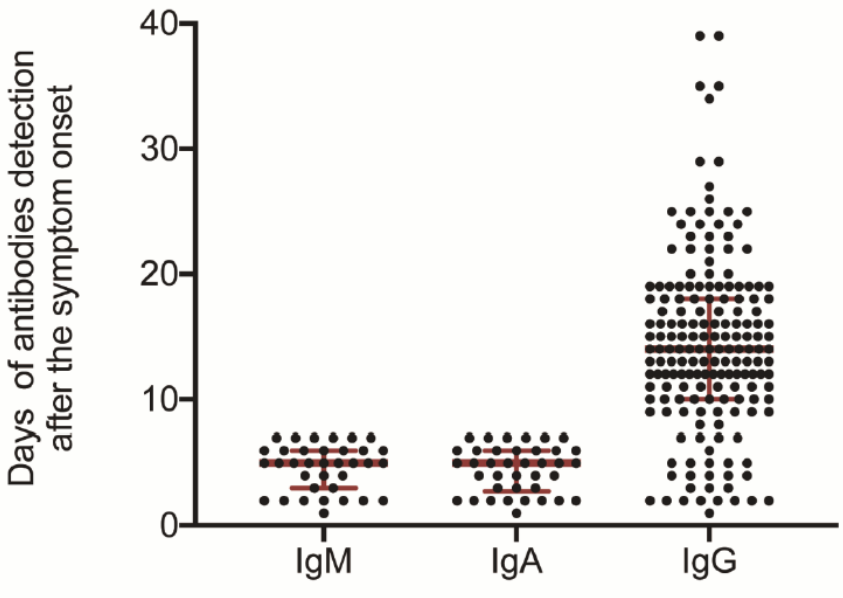
Author Notes

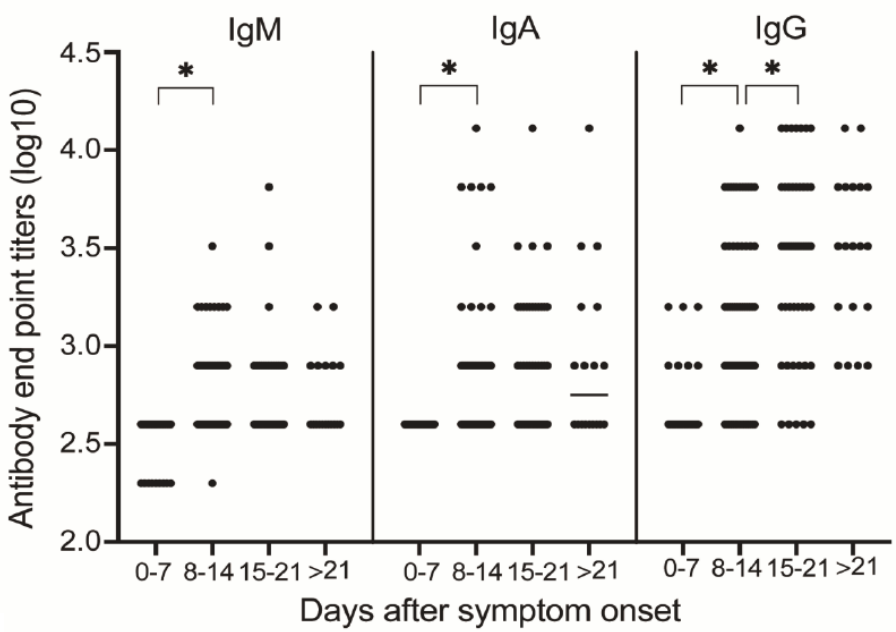
Clinical Infectious Diseases, ciaa310, https://doi.org/10.1093/cid/ciaa310

Published: 21 March 2020 **Article history** ▾

Results The median duration of ***IgM and IgA*** antibody ***detection were 5 days*** (IQR 3-6), while ***IgG*** was detected on 14 days (IQR 10-18) after symptom onset, with a ***positive rate of 85.4%, 92.7% and 77.9%*** respectively. ***In confirmed and probable cases, the positive rates of IgM antibodies were 75.6% and 93.1%, respectively.*** ***The detection efficiency by IgM ELISA is higher than that of qPCR method after 5.5 days of symptom onset***. The positive detection rate is significantly increased (98.6%) when combined IgM ELISA assay with PCR for each patient compare with a single qPCR test (51.9%).

IgM and IgG characteristics



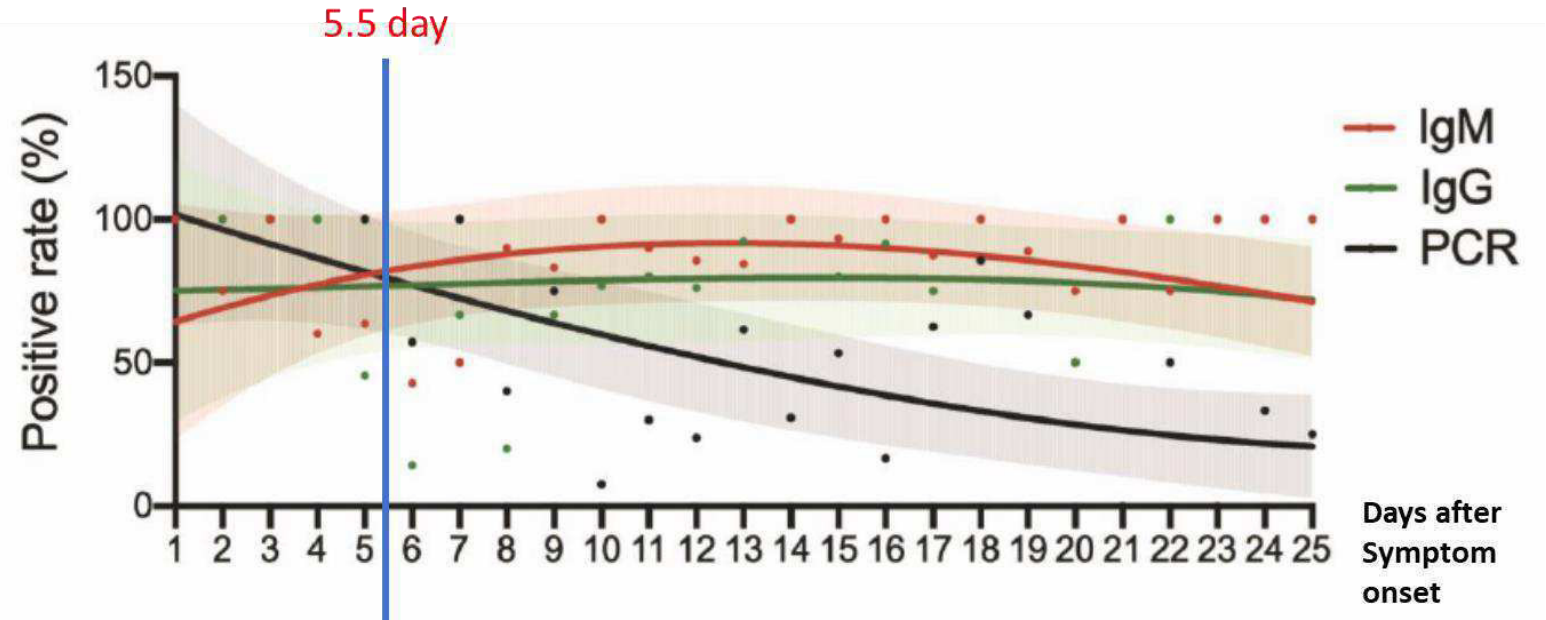


	IgG/A	IgG
Detection rate	85.4	77.9%
Detectable Day*	Day 5 IQR(3-6)	Day 14 IQR(10-18)

*Day after symptom onset
Post symptom onset(POS)

Day	IgG change	IgG change
0-7	Base level	Base level
8-14	Increased	Increased
15-21	plateaued	Increased
>21	plateaued	plateaued

Detection rate by days



Detection rate	0-5.5 day	6-13 day	>14 day
PCR	> 90%	80	< 50 %
IgG/IGM	~ 75 %	75.6 ~ 93.1%	

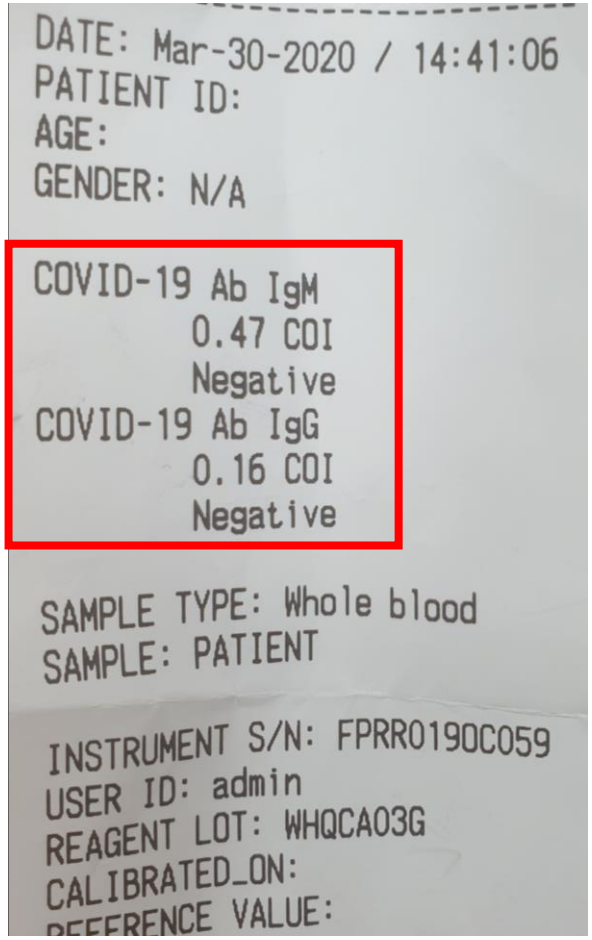
5.5 days after symptom onset, IgM/IgG detection rate is better than PCR positive rate

AFIAS COVID-19 Ab test result displayed by AFIAS-6
COVID-19 Ab IgM *COI Negative or Indeterminate or Positive COVID-19 Ab IgG *COI Negative or Indeterminate or Positive
*Test result is negative if COI is < 0.9, indeterminate if COI is 0.9-1.1 and positive if COI is >1.1-200.

		2019-nCOV RT-PCR assay		
		Positive	Negative	Total
AFIAS COVID-19 Ab	Positive	12	0	12
	Negative	0	145	145
	Indeterminate	0	5	5
	Total	12	150	162

- Positive Percent Agreement: 100%
- Negative Percent Agreement: 96.7%

Example of test result



SOFTWARE

	Application ver.	Firmware ver.
AFIAS-1	AA1.WW.02.27.30	FA1.WW.02.08.06
AFIAS-6	AA6.WW.02.34.00	FA6.WW.02.34.08
iCHROMA II	IR2.1.5.3	FI2.WW.02.30