Laboratory Diagnosis of SARS-CoV-2: NAAT & Ag RDT

Seyed Alireza Nadji, PhD

Professor of Medical Virology

Head, Virology Research Center

National Research Institute of Tuberculosis and Lung Diseases

Shahid Beheshti University of Medical Sciences

21 Feb, 2021
Tehran-Iran

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Several Indications for SARS-CoV-2 Diagnostic Testing

- Surveillance – understanding disease epidemiology, monitoring impact
- Clinical Case Management and Contact Tracing
  - Discharge or release from isolation/quarantine
- Prior infection/exposure and immunity
- **Prognosis** - predicting who is likely to develop severe disease or who could benefit from Rx

Focus is primarily on humans but needs extend to animals and the environment

Several COVID-19 Diagnostic Approaches

Mechanism	• Virus detection • Immune response
Samples	• Respiratory tract, stool • Blood, serum, plasma
Biomarkers	• RNA • Proteins • Antibodies
Methods	• RT-PCR and isothermal amplification • Sequencing • Immunoassay for detection of antigens and antibodies and neutralization assays

Shaheed Beheshti Universityof Medical Sciences

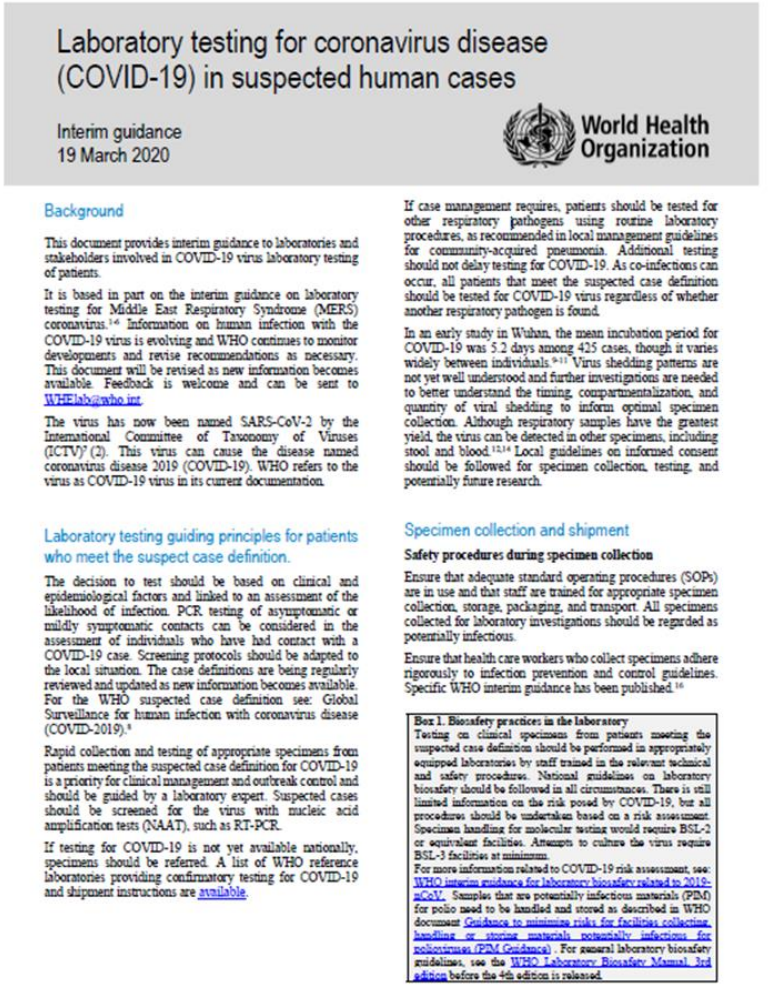
Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Interim WHO guidance:

Laboratory testing for COVID-19

https://apps.who.int/iris/bitstream/handle/10665/331501/WHO-COVID-19-laboratory-2020.5-eng.pdf?sequence=1&isAllowed=y



Laboratory testing for coronavirus disease (COVID-19) in suspected human cases

Interim guidance
19 March 2020

World Health Organization

Background

This document provides interim guidance to laboratories and stakeholders involved in COVID-19 virus laboratory testing of patients.

It is based in part on the interim guidance on laboratory testing for Middle East Respiratory Syndrome (MERS) coronavirus.[1-6] Information on human infection with the COVID-19 virus is evolving and WHO continues to monitor developments and revise recommendations as necessary. This document will be revised as new information becomes available. Feedback is welcome and can be sent to WHElab@who.int.

The virus has now been named SARS-CoV-2 by the International Committee of Taxonomy of Viruses (ICTV)[7] (2). This virus can cause the disease named coronavirus disease 2019 (COVID-19). WHO refers to the virus as COVID-19 virus in its current documentation.

Laboratory testing guiding principles for patients who meet the suspect case definition.

The decision to test should be based on clinical and epidemiological factors and linked to an assessment of the likelihood of infection. PCR testing of asymptomatic or mildly symptomatic contacts can be considered in the assessment of individuals who have had contact with a COVID-19 case. Screening protocols should be adapted to the local situation. The case definitions are being regularly reviewed and updated as new information becomes available. For the WHO suspected case definition see: Global Surveillance for human infection with coronavirus disease (COVID-2019).[8]

Rapid collection and testing of appropriate specimens from patients meeting the suspected case definition for COVID-19 is a priority for clinical management and outbreak control and should be guided by a laboratory expert. Suspected cases should be screened for the virus with nucleic acid amplification tests (NAAT), such as RT-PCR.

If testing for COVID-19 is not yet available nationally, specimens should be referred. A list of WHO reference laboratories providing confirmatory testing for COVID-19 and shipment instructions are available.

If case management requires, patients should be tested for other respiratory pathogens using routine laboratory procedures, as recommended in local management guidelines for community-acquired pneumonia. Additional testing should not delay testing for COVID-19. As co-infections can occur, all patients that meet the suspected case definition should be tested for COVID-19 virus regardless of whether another respiratory pathogen is found.

In an early study in Wuhan, the mean incubation period for COVID-19 was 5.2 days among 425 cases, though it varies widely between individuals.[9-11] Virus shedding patterns are not yet well understood and further investigations are needed to better understand the timing, compartmentalization, and quantity of viral shedding to inform optimal specimen collection. Although respiratory samples have the greatest yield, the virus can be detected in other specimens, including stool and blood.[12,14] Local guidelines on informed consent should be followed for specimen collection, testing, and potentially future research.

Specimen collection and shipment

Safety procedures during specimen collection

Ensure that adequate standard operating procedures (SOPs) are in use and that staff are trained for appropriate specimen collection, storage, packaging, and transport. All specimens collected for laboratory investigations should be regarded as potentially infectious.

Ensure that health care workers who collect specimens adhere rigorously to infection prevention and control guidelines. Specific WHO interim guidance has been published.[16]

Box 1. Biosafety practices in the laboratory

Testing on clinical specimens from patients meeting the suspected case definition should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on laboratory biosafety should be followed in all circumstances. There is still limited information on the risk posed by COVID-19, but all procedures should be undertaken based on a risk assessment. Specimen handling for molecular testing would require BSL-2 or equivalent facilities. Attempts to culture the virus require BSL-3 facilities at minimum.

For more information related to COVID-19 risk assessment, see: WHO interim guidance for laboratory biosafety related to 2019-nCoV. Samples that are potentially infectious materials (PIM) for polio need to be handled and stored as described in WHO document Guidance to minimize risks for facilities collecting, handling or storing materials potentially infectious for polioviruses (PIM Guidance) . For general laboratory biosafety guidelines, see the WHO Laboratory Biosafety Manual, 3rd edition before the 4th edition is released.

Laboratory testing for COVID-19
Virus detection

- Nucleic acid amplification tests (NAAT)
- Rapid Antigen detection
- Viral sequencing
- Viral culture

Specimens to be collected from symptomatic patients and contacts

Table 1. Specimens to be collected from symptomatic patients and contacts

	Test	Type of sample	Timing
Patient	NAAT	Lower respiratory tract - sputum - aspirate - lavage Upper respiratory tract - nasopharyngeal and - oropharyngeal swabs - nasopharyngeal wash/nasopharyngeal aspirate. Consider stools, whole blood, urine, and if diseased, material from autopsy.	Collect on presentation. Possibly repeated sampling to monitor clearance. Further research needed to determine effectiveness and reliability of repeated sampling.
Patient	Serology	Serum for serological testing once validated and available.	Paired samples are necessary for confirmation with the initial sample collected in the first week of illness and the second ideally collected 2-4 weeks later (optimal timing for convalescent sample needs to be established).
Contact in health-care centre associated outbreaks or other settings where contacts have symptoms, or where asymptomatic contacts have had high-intensity contact with a COVID-19 case.	NAAT	Nasopharyngeal and oropharyngeal swabs.	Within incubation period of last documented contact.
	Serology	Serum for serological testing once validated and available.	Baseline serum taken as early as possible within incubation period of contact and convalescent serum taken 2-4 weeks after last contact (optimal timing for convalescent sample needs to be established).

Specimen collection and shipment

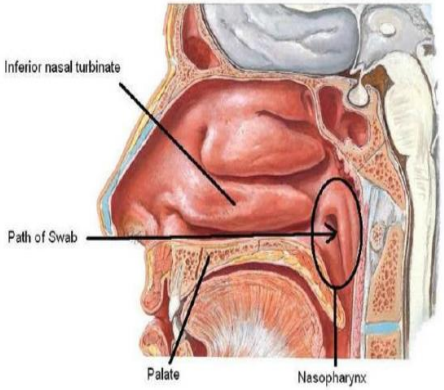
Table 2. Specimen collection and storage (adapted from[4, 27, 28])

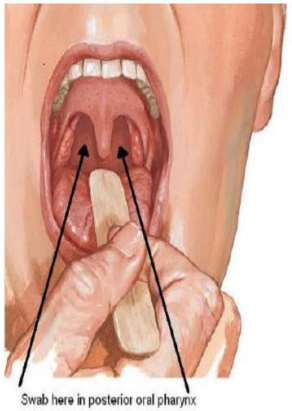
Specimen type	Collection materials	Storage temperature until testing in-country laboratory	Recommended temperature for shipment according to expected shipment time
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	2-8 °C	2-8 °C if ≤5 days –70 °C (dry ice) if >5 days
Bronchoalveolar lavage	Sterile container *	2-8 °C	2-8 °C if ≤2 days –70 °C (dry ice) if >2 days
(Endo)tracheal aspirate, nasopharyngeal or nasal wash/aspirate	Sterile container *	2-8 °C	2-8 °C if ≤2 days –70 °C (dry ice) if >2 days
Sputum	Sterile container	2-8 °C	2-8 °C if ≤2 days –70 °C (dry ice) if >2 days
Tissue from biopsy or autopsy including from lung.	Sterile container with saline or VTM.	2-8 °C	2-8 °C if ≤24 hours –70 °C (dry ice) if >24 hours
Serum	Serum separator tubes (adults: collect 3-5 ml whole blood).	2-8 °C	2-8 °C if ≤5 days –70 °C (dry ice) if >5 days
Whole blood	Collection tube	2-8 °C	2-8 °C if ≤5 days –70 °C (dry ice) if >5 days
Stool	Stool container	2-8 °C	2-8 °C if ≤5 days –70 °C (dry ice) if >5 days
Urine	Urine collection container	2-8 °C	2-8 °C if ≤5 days –70 °C (dry ice) if >5 days

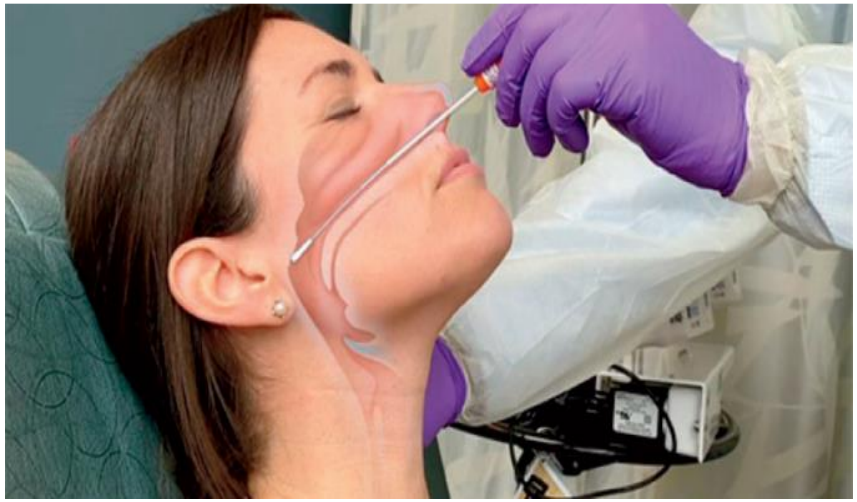
* For transport of samples for viral detection, use viral transport medium (VTM) containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens. If VTM is not available sterile saline may be used instead (in which case, duration of sample storage at 2-8 °C may be different from what is indicated above).

Aside from specific collection materials indicated in the table also assure other materials and equipment are available: e.g. transport containers and specimen collection bags and packaging, coolers, and cold packs or dry ice, sterile blood-drawing equipment (e.g. needles, syringes and tubes), labels and permanent markers, PPE, materials for decontamination of surfaces, etc.

Swab Specimen Collection







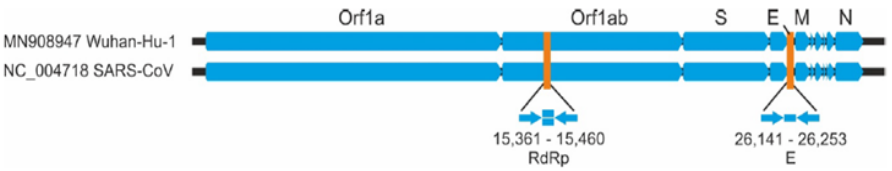
Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Nucleic acid amplification tests (NAAT) for COVID-19

- Routine confirmation of cases of COVID-19 is based on detection of unique sequences of virus RNA by NAAT
- real-time reverse-transcription polymerase chain reaction (rRT-PCR) with confirmation by nucleic acid sequencing when necessary.
- The viral genes targeted so far include the N, E, S and RdRP genes.
- RNA extraction should be done in a biosafety cabinet in a BSL-2 or equivalent facility.



Summary table of available protocols in this document

Institute	Gene targets
China CDC, China	ORF1ab and N
Institut Pasteur, Paris, France	Two targets in RdRP
US CDC, USA	Three targets in N gene
National Institute of Infectious Diseases, Japan	Pancorona and multiple targets, Spike protein
Charité, Germany	RdRP, E, N
HKU, Hong Kong SAR	ORF1b-nsp14, N
National Institute of Health, Thailand	N

https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf?sfvrsn=de3a76aa_2

The sensitivities of the tests to individual genes are comparable according to comparison studies except the RdRpSARSr (Charité) primer probe, which has a slightly lower sensitivity likely due to a mismatch in the reverse primer

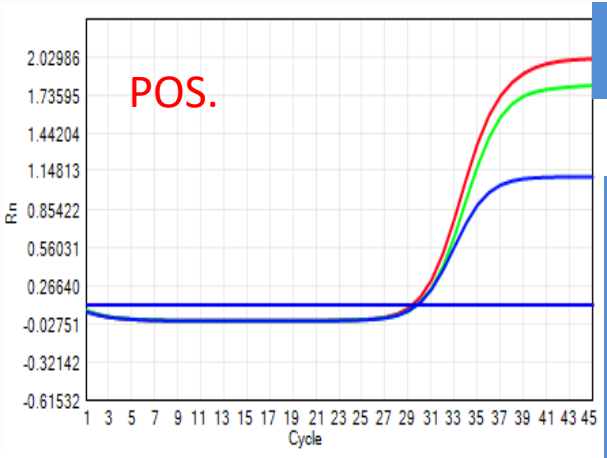
.J Clin Microbiol. 2020;JCM.00557-20. Published online April 8, 2020. doi:10.1128/JCM. 00557-20

Shaheed Beheshti Universityof Medical Sciences

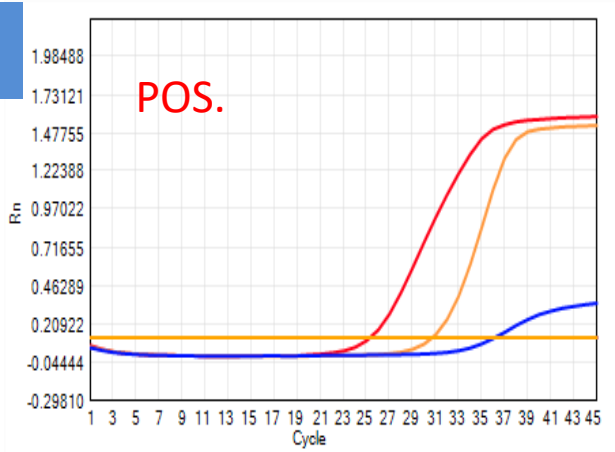
Virology Research Center

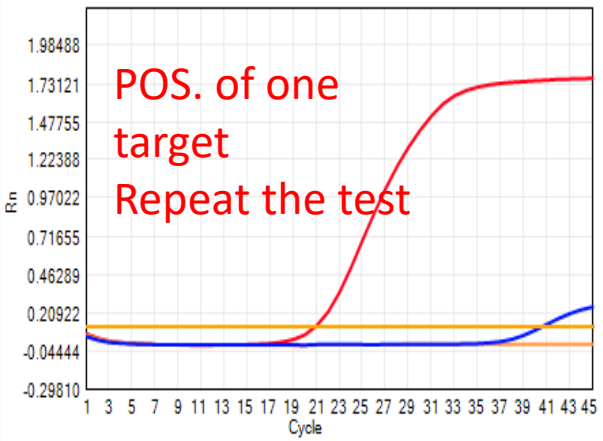
National Research Institute of Tuberculosis and Lung Disease (NRITLD)

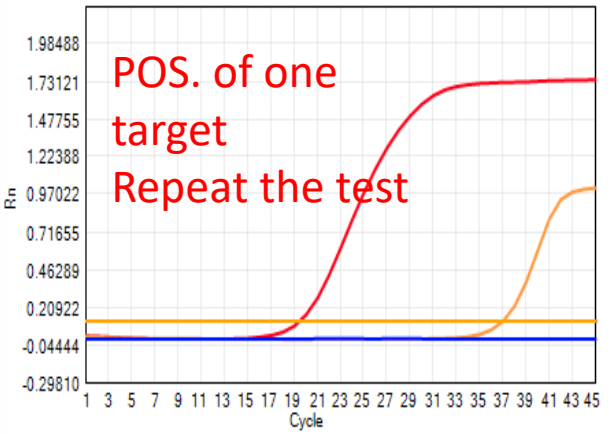
Interpreting rRT-PCR Tests

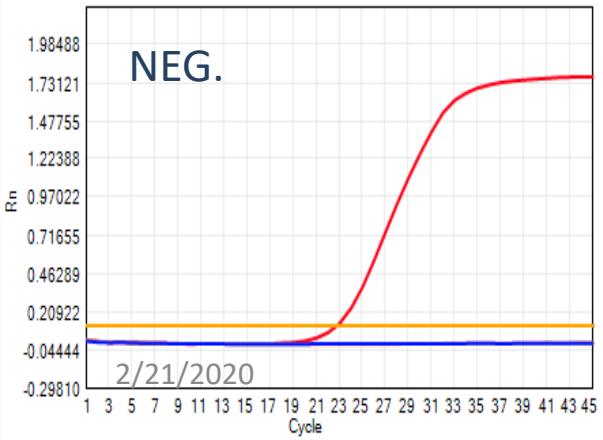


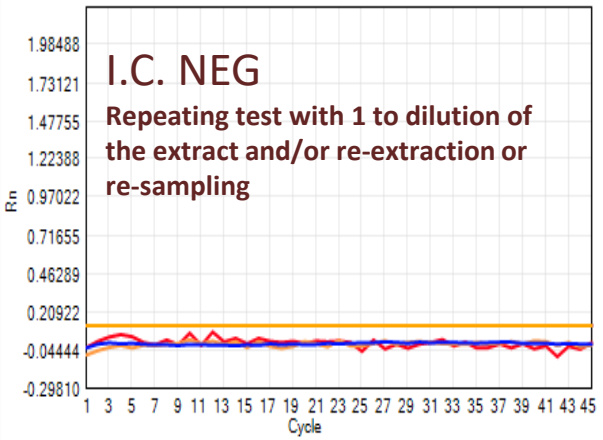
- Double gene: ≤35 C.T = Pos.
- Double gene: 35…38 C.T ; repeat the test
  - Same result; Pos.
- C.T≥ 39 = Neg.
- One target gene; repeat the test
  - Same results; re-sampling
- I.C. =Neg.; repeat the test with 1:10 dilution of extract
  - Same result; re-extraction or re-sampling

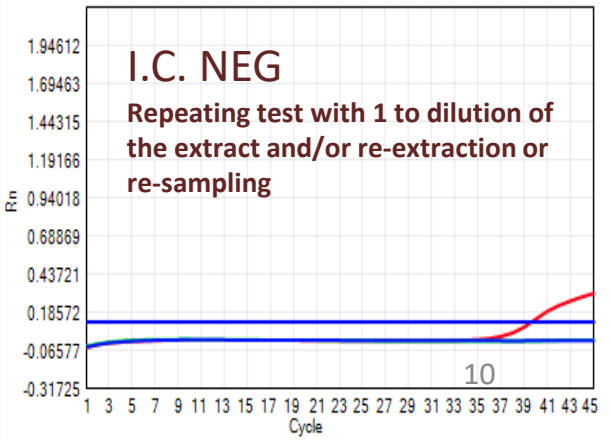












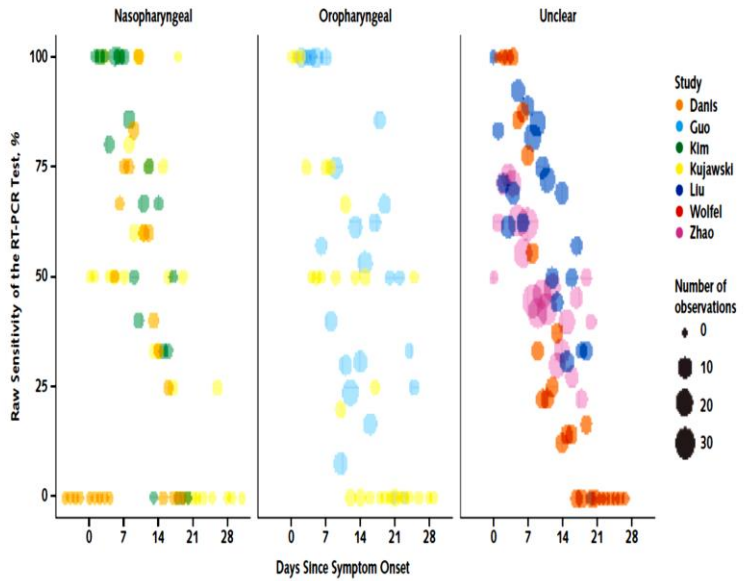
Detection of SARS-CoV-2 in different types of clinical specimens

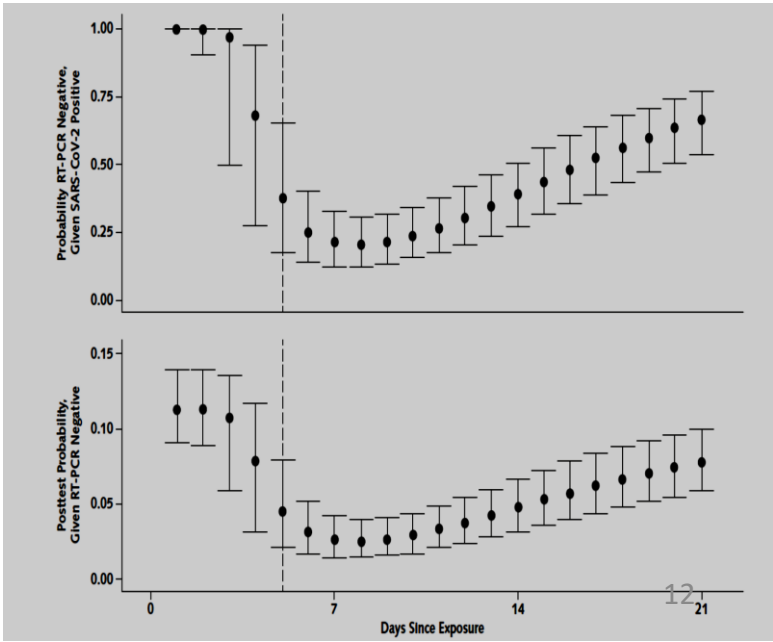
- data suggest the sensitivity of the COVID19 RT-PCR :
  - 32% for oropharyngeal,
  - 63% for nasopharyngeal (NP)
    - It was reported Pharyngeal washing has the same sensitivity ??
  - 73% for sputum samples
  - 93% for tracheal aspirates/ bronchoalveolar lavage (BAL)
  - Saliva ??
- False-negative results mainly occurred due to
  - inappropriate timing of sample collection in relation to illness onset
  - deficiency in sampling technique, especially of nasopharyngeal swabs.
- Specificity of most of the RT-PCR tests is 100%

JAMA. doi.org/10.1001/jama.2020.3786

False-Negative Rate of RT-PCR–Based SARS-CoV-2 Tests by Time Since Exposure

- Over the 4 days of infection before the typical time of symptom onset (day 5), the probability of a false-negative result in an infected person decreases from
  - 100% (95% CI, 100% to 100%) on day 1
  - 67% (CI, 27% to 94%) on day 4
- On the day of symptom onset, the median false-negative rate
  - 38% (CI, 18% to 65%) on day 5
  - decreased to 20% (CI, 12% to 30%) on day 8
  - then began to increase again, from 21% (CI, 13% to 31%) on day 9 to 66% (CI, 54% to 77%) on day 21.

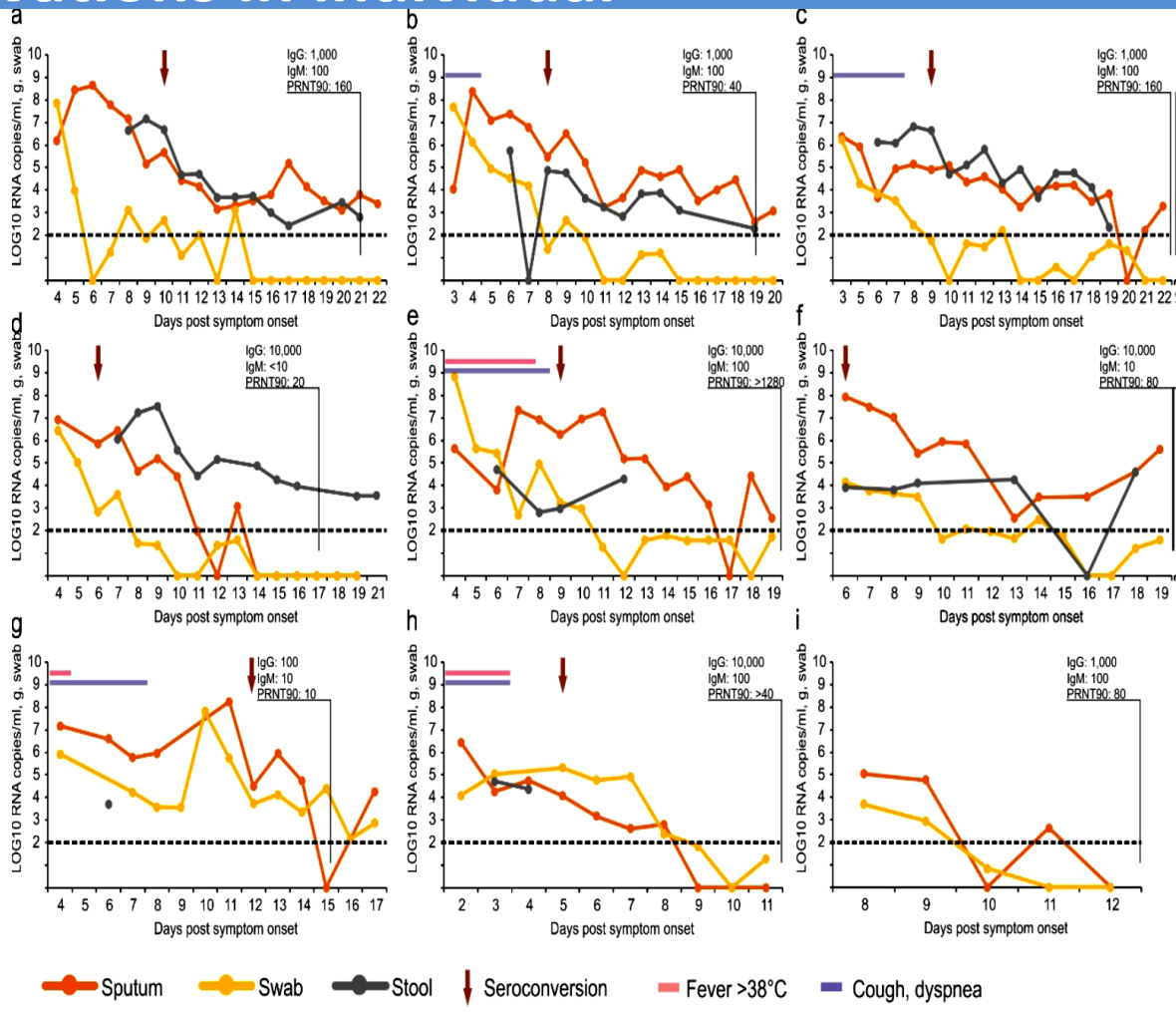




Shaheed Beheshti Universityof Medical Sciences
Virology Research Center
National Research Institute of Tuberculosis and Lung Disease

Viral load kinetics, seroconversion and clinical observations in individual

- Pharyngeal virus shedding was very high during the first week of symptoms
  - peak at 7.11 X 10e+8 RNA copies / throat swab, day 4.
- Infectious virus was readily isolated from throat- and lung-derived samples, but not from stool samples in spite of high virus RNA concentration.
- Blood and urine never yielded virus.
- Shedding of viral RNA from sputum outlasted the end of symptoms.
- Seroconversion occurred after 6-12 days, but was not followed by a rapid decline of viral loads.
- Asymptomatic persons seem to shed virus longer than symptomatic ones and show weak immunologic reaction than to symptomatic one.



Nature 581, 465–469 (2020)

Complexity	Manufacturer	Product/information	Virus Detection Method	Platform/Instrument	Influenza Viruses Detected	Influenza A Virus Subtypes Differentiated	Other Respiratory Viruses Differentiated	Approved Specimens[3]	Test Time
High, Moderate	BioFire Diagnostics, LLC (Commercially Available)	BioFire Respiratory Panel 2.1 (RP2.1)	Nucleic Acid Detection	FILMARRAY® 2.0 and FILMARRAY® TORCH systems	Influenza A, Influenza B	A(H1), A(H1)pdm09, A(H3)	SARS-CoV-2, Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus	NPS	1 hour
High, Moderate, Waived	BioFire Diagnostics, LLC (Commercially Available)	BioFire Respiratory Panel 2.1-EZ (RP2.1-EZ)	Nucleic Acid Detection	FILMARRAY® 2.0 EZ Configuration System	Influenza A and B	A(H1), A(H1)pdm09, A(H3)	SARS-CoV-2, Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus	NPS	Approximately 45 minutes
High, Moderate	GenMark Diagnostics, Inc	ePlex Respiratory Pathogen Panel 2	Nucleic Acid Detection	ePlex System	Influenza A and B	A(H1), A(H1)pdm09, A(H3)	SARS-CoV-2, Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B	NPS in viral transport media	<2 hours
High, Moderate	QIAGEN (Commercially Available)	QIAstat-Dx Respiratory SARS-CoV-2 Panel	Nucleic Acid Detection	QIAstat Dx Analyzer System 1.0	Influenza A, Influenza B	A(H1), A(H1)pdm09, A(H3)	SARS-CoV-2, Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, SARS-CoV-2, Human Metapneumovirus A+B, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Rhinovirus/Enterovirus, Respiratory Syncytial Virus A+B	NPS in universal transport media	1 hour
High, Moderate	Roche Molecular Systems, Inc. (Commercially Available)	cobas SARS-CoV-2 & Influenza A/B	Nucleic Acid Detection	Cobas 6800/8800 Systems	Influenza A, Influenza B	Not Differentiated	SARS-CoV-2	Healthcare provider-collected NPS and NS, and self-collected NS (collected in a healthcare setting with instruction by a healthcare provider)	3-8 hours
High, Moderate, Waived	Roche Molecular Systems, Inc. (Commercially Available)	cobas SARS-CoV-2 & Influenza A/B Nucleic Acid Test	Nucleic Acid Detection	Cobas Liat Systems	Influenza A, Influenza B	Not Differentiated	SARS-CoV-2	Healthcare provider-collected NPS and NS, and self-collected NS (collected in a healthcare setting with instruction by a healthcare provider)	20 minutes
High, Moderate	Cepheid (Commercially Available)	Xpert Xpress SARS-CoV-2/ Flu/RSV	Nucleic Acid Detection	GeneXpert Dx and GeneXpert Infinity systems	Influenza A and B	Not Differentiated	SARS-CoV-2, RSV	NPS, NS, NW/NA	<40 minutes
Waived	Cepheid (Commercially Available)	Xpert Xpress SARS-CoV-2/ Flu/RSV	Nucleic Acid Detection	GeneXpert Xpress System (Tablet and Hub Configurations)	Influenza A and B	Not Differentiated	SARS-CoV-2, RSV	NPS	<40 minutes
High, Moderate, Waived	Quidel	Sofia 2 Flu + SARS Antigen FIA	Antigen Detection	Sofia FIA Analyzer	Influenza A, Influenza B	Not Differentiated	SARS-CoV-2	NPS, NS within first 5 days of onset of symptoms	15 minutes
High	Quest Diagnostics	Quest Diagnostics RC COVID-19 +Flu RT-PCR	Nucleic acid detection	Roche cobas SARS-CoV-2 & Influenza A/B	Influenza A and B	Not Differentiated	SARS-CoV-2	When ordered by a healthcare provider: NS specimen is self-collected at home using the Quest Diagnostics Self-Collection Kit for COVID-19 +Flu	Patient ships the self-collected specimen to Quest Diagnostics overnight via FedEx. Test results are provided electronically to the healthcare provider and the patient.
High	CDC (Public Health Use Only, Not Commercially Available)	Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay*	Nucleic Acid Detection	Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument	Influenza A, Influenza B	Not Differentiated	SARS-CoV-2	NPS, NPW, NPA, NS, NA, TS, sputum, TA, BAL	4 hours

Return to Work Criteria for HCP with SARS-CoV-2 Infection

Symptom-based strategy for determining when HCP can return to work

- *HCP with mild to moderate illness who are not severely immunocompromised:*
  - At least 10 days have passed *since symptoms first appeared* **and**
  - At least 24 hours have passed *since last* fever without the use of fever-reducing medications **and**
  - Symptoms (e.g., cough, shortness of breath) have improved

Note: HCP who are **not severely immunocompromised** and were **asymptomatic** throughout their infection may return to work when at least 10 days have passed since the date of their first positive viral diagnostic test.

- *HCP with severe to critical illness or who are severely immunocompromised[1]:*
  - At least 10 days and up to 20 days have passed *since symptoms first appeared*
  - At least 24 hours have passed *since last* fever without the use of fever-reducing medications **and**
  - Symptoms (e.g., cough, shortness of breath) have improved
  - Consider consultation with infection control experts

Note: HCP who are **severely immunocompromised** but who were **asymptomatic** throughout their infection may return to work when at least 10 days and up to 20 days have passed since the date of their first positive viral diagnostic test.

Test-Based Strategy for Determining when HCP Can Return to Work

- *HCP who are symptomatic:*
  - Resolution of fever without the use of fever-reducing medications **and**
  - Improvement in symptoms (e.g., cough, shortness of breath), **and**
  - Results are negative from at least two consecutive respiratory specimens collected ≥24 hours apart (total of two negative specimens) tested using an FDA-authorized molecular viral assay to detect SARS-CoV-2 RNA. See Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for 2019 Novel Coronavirus (2019-nCoV).
- *HCP who are not symptomatic:*
  - Results are negative from at least two consecutive respiratory specimens collected ≥24 hours apart (total of two negative specimens) tested using an FDA-authorized molecular viral assay to detect SARS-CoV-2 RNA. See Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for 2019 Novel Coronavirus (2019-nCoV).

In some instances, a test-based strategy could be considered to allow HCP to return to work earlier than if the symptom-based strategy were used.

many individuals will have prolonged viral shedding, limiting the utility of this approach.

A test-based strategy could also be considered for some HCP (e.g., those who are severely immunocompromised) in consultation with local infectious diseases experts if concerns exist for the HCP being infectious for more than 20 days.

Antigen-detecting COVID-19 Rapid Diagnostic Tests (RDTs)

Seyed Alireza Nadji, PhD

Professor of Medical Virology

Head, Virology Research Center

National Research Institute of Tuberculosis and Lung Diseases

Shahid Beheshti University of Medical Sciences

18 Dec, 2020
Tehran-Iran

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

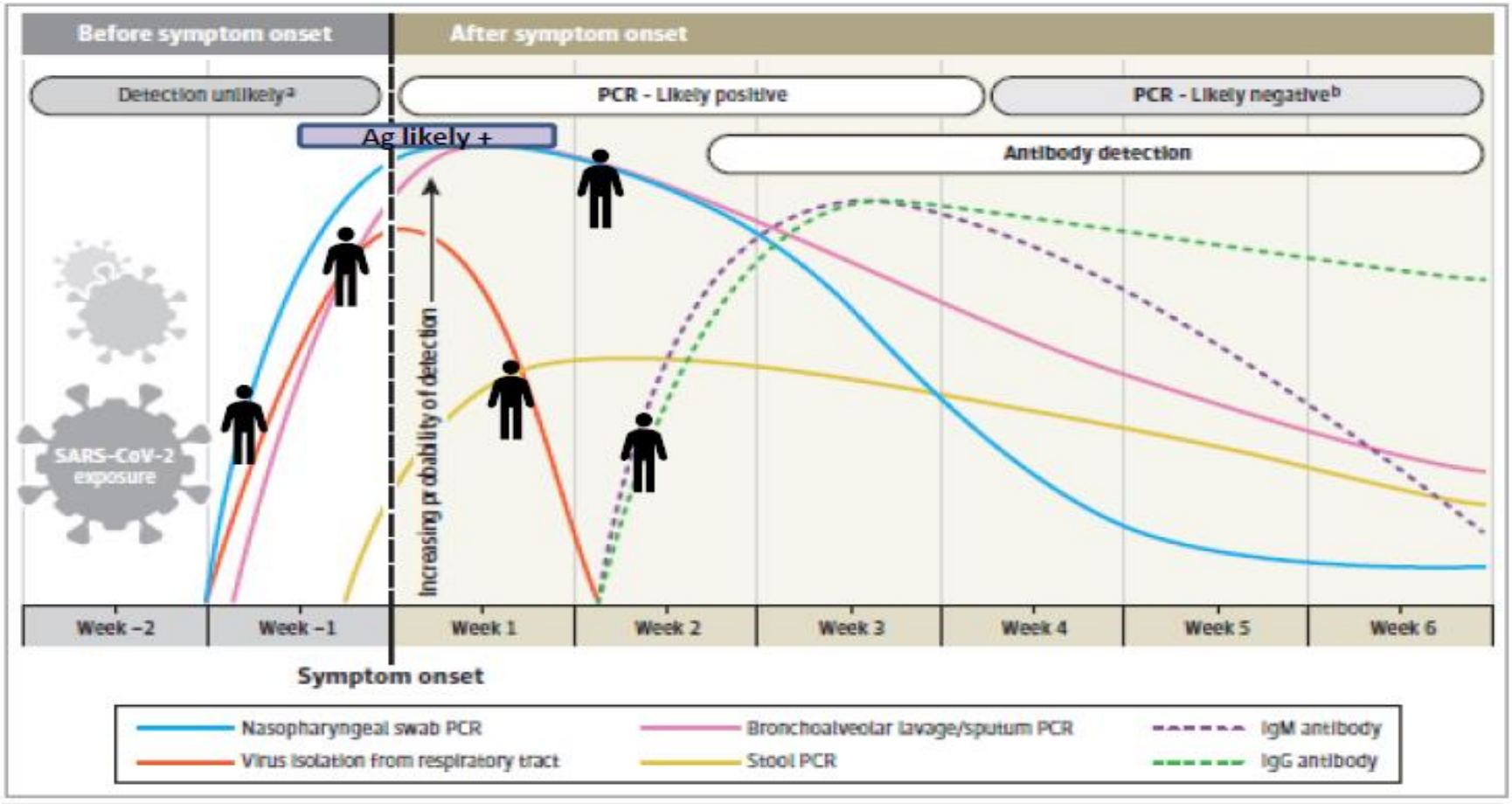
Outline

- Background
- Landscape and test characteristics
- Performance
  - Principles
  - Scientific evidence
- Target product profile

- Conclusions

Timing and targets

Figure. Estimated Variation Over Time in Diagnostic Tests for Detection of SARS-CoV-2 Infection Relative to Symptom Onset



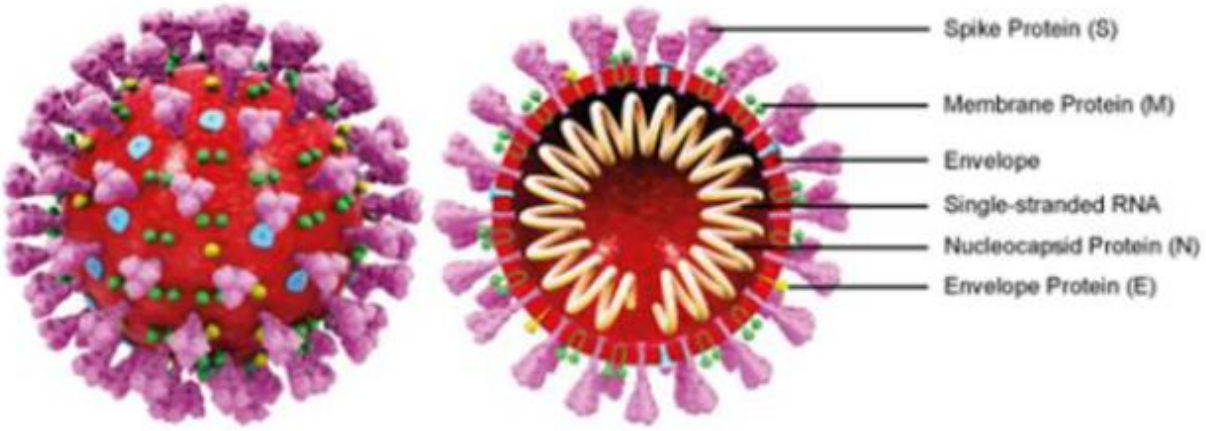
Estimated time intervals and rates of viral detection are based on data from several published reports. Because of variability in values among studies, estimated time intervals should be considered approximations and the probability of detection of SARS-CoV-2 infection is presented qualitatively.

[a] Detection only occurs if patients are followed up proactively from the time of exposure.

[b] More likely to register a negative than a positive result by PCR of a nasopharyngeal swab.

SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2; PCR, polymerase chain reaction. *Source: Sethuraman N et al. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA. 2020;323(22):2249–2251. doi:10.1001/jama.2020.8*

Antigen detecting tests



- (N) SARS-CoV-2 Nucleocapsid protein/Nucleoprotein
- (S) SARS-CoV-2 Spike protein
- (M) SARS-CoV-2 Membrane protein
- (E) SARS-CoV-2 Envelope protein.

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Landscape of 'registered and RUO' antigen detecting tests

Lateral Flow ICT +/- reader = RDT

- 24 manufacturers
- 31 products

- *No info on web*

- 14 manufacturers
- 17 products

Enzyme immunoassays (EIA)

- 8 manufacturers
- 10 products

No info on web

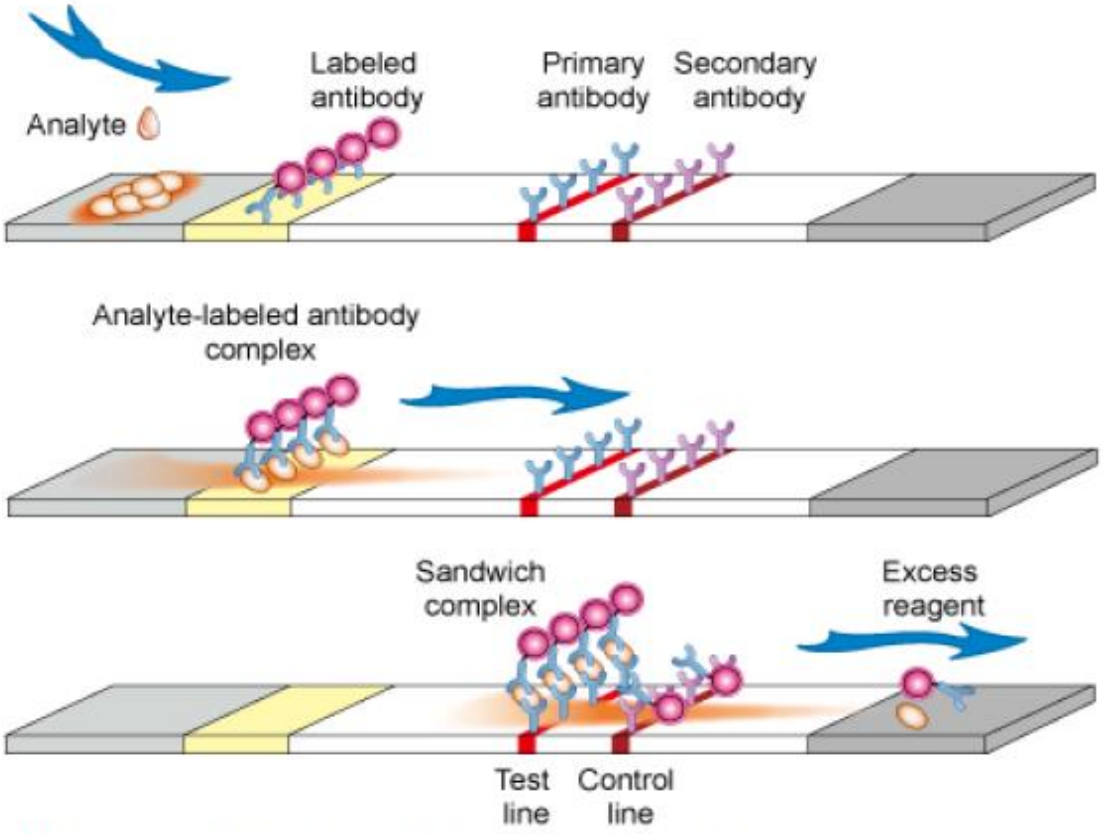
- 8 manufacturers
- 10 products

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Rapid diagnostic test – LFI



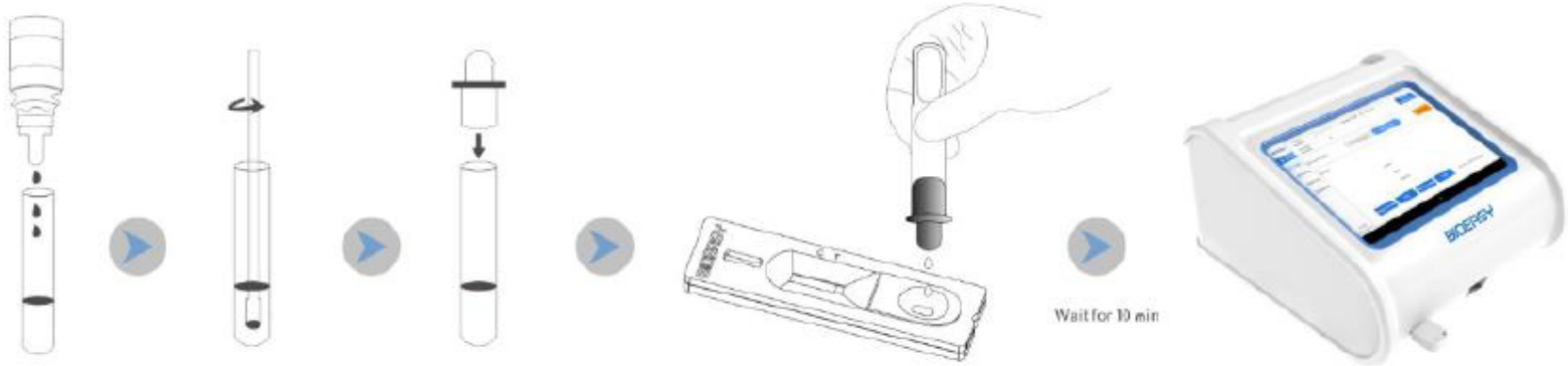
Source:https://www.cd-diatest.com/common-formats-of-lateral-flow-tests_d27

Overview of Test Characteristics

Target	• Nucleocapsid most commonly reported
Format	• Lateral flow dipstick or cassette • Visual read out or requires reader (walk- away or read-only)
Sample type	•Primarily naopharyngeal swabs but some are compatible with nasal swabs and sputum •Test asap; alternatives: swab at 2-8C 4-24 hours or in VTM for 3 d but not all products compatible with VTM and all list VTM as potentially compromising performance
Test kit	• some include swabs and controls
Reading time	• 5 mins, but most 30 mins
Storage conditions	• 2-30°C ; one exception: 40°C

Some examples

Test Procedure



Add 500μL(10 drops) of sample extraction buffer to the tube

Immerse the sample swab into the buffer and fully elute the the sample

Cover with the dripper

Add 100μL (2 drops) of the processed sample into the well

Read the result with Fluorescence Immunoassay Analyzer

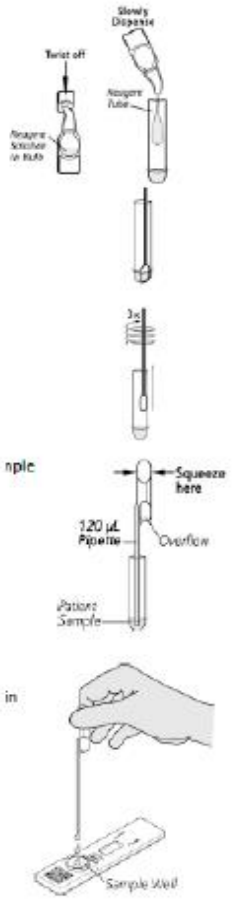
Specification

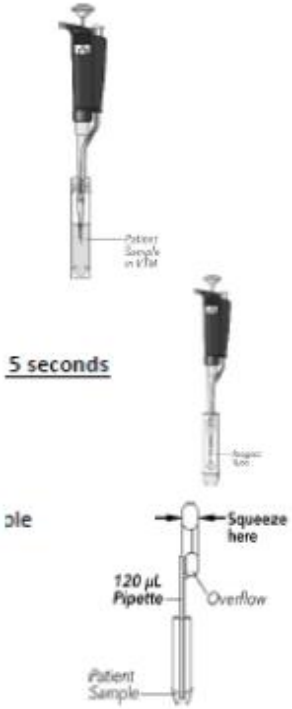
Cat. No.	Product	Format	Specimen	Pack	Qualification
YRLF04401025	Diagnostic Kit for 2019-Novel Coronavirus (2019-nCoV) Ag Test (Fluorescence Immunochromatographic Assay)	Cassette	Nasal swab/Deep sputum	25T	CE

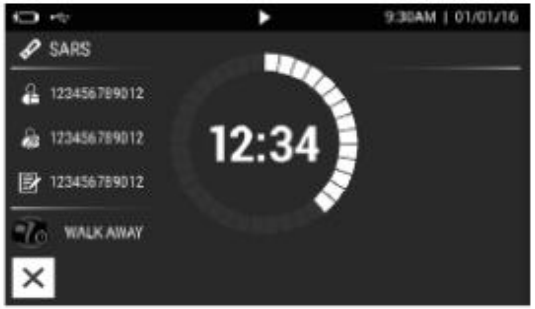
Shaheed Beheshti Universityof Medical Sciences

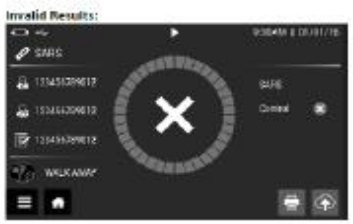
Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)





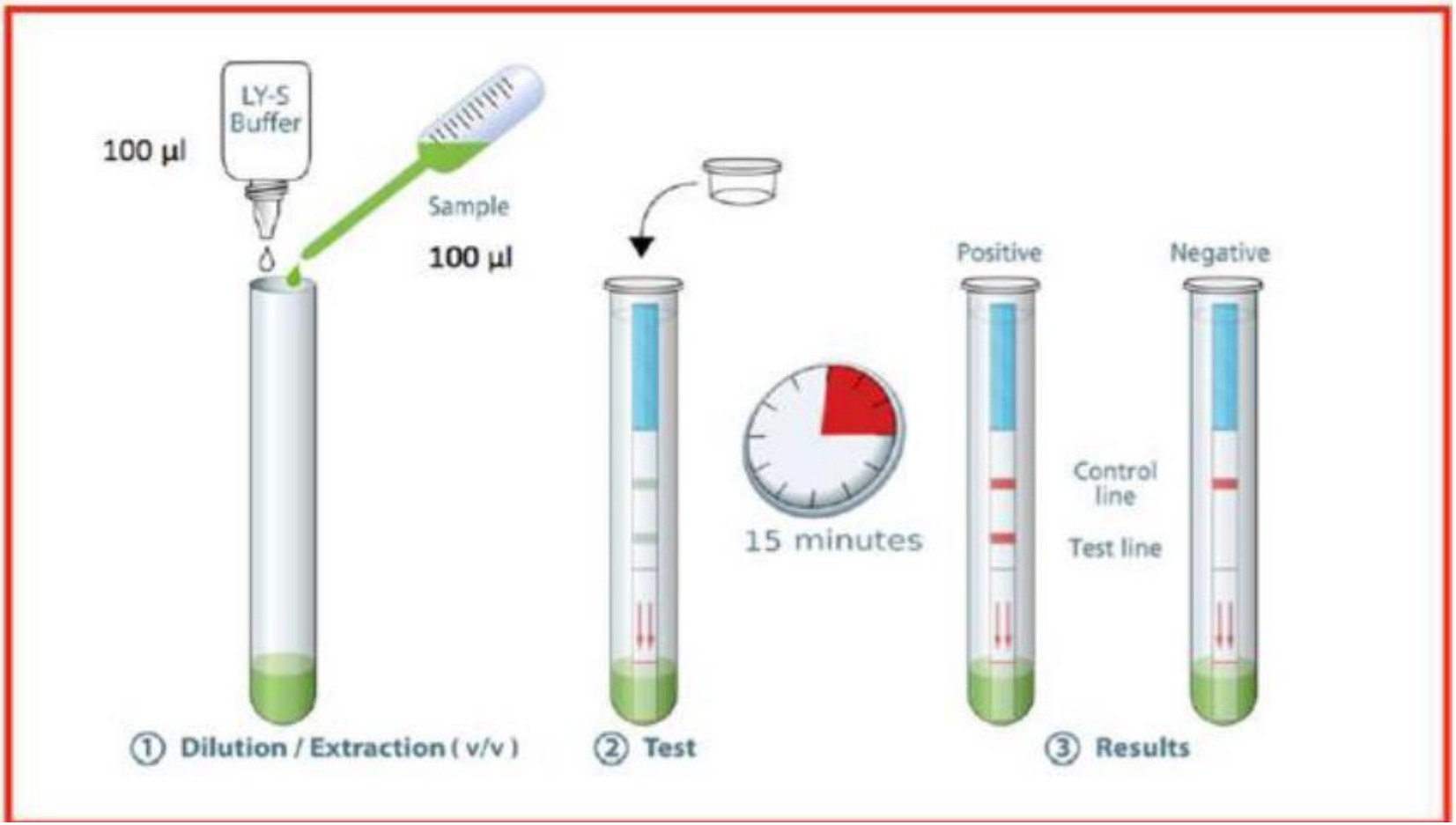




Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)


LY-S Buffer
100 µl
Sample
100 µl
15 minutes
Positive
Negative
Control line
Test line
① Dilution / Extraction (v/v)
② Test
③ Results

1 Insert the swab specimen and swirl the swab 5~10 times.

2 Remove the swab while gently squeezing the head of the swab.

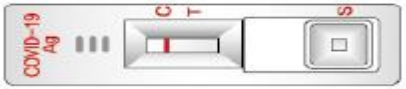
3 Close the assay diluent tube with a filter cap securely.

4 Invert the assay diluent tube and gently squeeze it to draw 3~4 drops (90~150μℓ) into a sample well on the device.

5 Read the result within 5~8 minutes.

■ Interpretation of Results

Negative

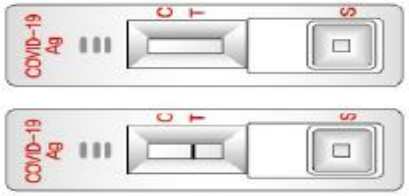


One **red** line "C" within the result window.

Positive

Two bands ; **black** "T" test line and **red** "C" control line within the result window.

Invalid



No "C" line within the result window.
It is recommended that the specimen be retested.

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Factors influencing test performance

- Host factors such as the time from illness onset and immune status – high viral load/antigen = better performance
- Sample type (upper, lower respiratory tract), quality and processing, including storage conditions and dilution in viral transport medium
- Viral factors including the concentration and duration of viral antigen shedding and structural variation in the target antigen
- Product design or quality. Antibodies may have poor affinity or be of insufficient quantity and poor packaging and exposure to heat and humidity can degrade antibodies. Unclear or incorrect instructions for use may lead to problems performing the test. These issues can all affect test performance
- Training and competency of the test operator; operator error in preparing the RDT, performing the test or interpreting the result can lead to erroneous conclusions. Because of the differing protocols of rapid tests, education of laboratory personnel about methods and limitations prior to their use is essential.

Measures of test performance

- **Analytical sensitivity** - is the ability of a test to detect a target analyte (e.g. antigen), in a sample usually expressed as the minimum detectable concentration of the analyte.
- **Analytical specificity** - the ability of an assay to measure on particular organism or substance, rather than others, in a sample.

This is done in the laboratory using well characterized and often 'spiked' samples reported in the instructions for use

Measures of test performance

- **Clinical sensitivity** - is the ability of a test to detect a target analyte (e.g. Antigen) in a patient/population

$$\text{sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

- **Clinical specificity** - the ability of an assay to measure on particular organism or substance, rather than others, in a patient/population

$$\text{specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

What is the effect of disease prevalence ?

- **Positive predictive value (PPV)**
  - probability that subjects with a **positive** test truly have the disease.

$$\text{PPV} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false positives}}$$

- **Negative predictive value (NPV)**
  - probability that subjects with a negative test truly do not have the disease

$$\text{NPV} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false negatives}}$$

Example – Population 10,000

Single step test	Sensitivity 70%; specificity 97%											
								Missed +ve cases				
Prevalence (%)	Sensitivity	Specificity	NPV	PPV	TP	FP	TN	FN	No. with disease	No. + tests	Total	% +ve tests
0.5	70.0	97.0	99.8	10.5	35.0	298.5	9651.5	15.0	50.0	333.5	10000.0	3.3
2.0	70.0	97.0	99.4	32.3	140.0	294.0	9506.0	60.0	200.0	434.0	10000.0	4.3
5.0	70.0	97.0	98.4	55.1	350.0	285.0	9215.0	150.0	500.0	635.0	10000.0	6.4
20.0	70.0	97.0	92.8	85.4	1400.0	240.0	7760.0	600.0	2000.0	1640.0	10000.0	16.4
50.0	70.0	97.0	76.4	95.9	3500.0	150.0	4850.0	1500.0	5000.0	3650.0	10000.0	36.5

Two step scenario												
Test 1:	Sensitivity 70%; specificity 97%											
Test 2:	Sensitivity 95%; specificity 99%											
								Missed +ve cases				
Prevalence (%)	Sensitivity	Specificity	NPV	PPV	TP	FP	TN	FN	No. with disease	No. + tests	Total	% +ve tests
0.5	66.5	100.0	99.8	91.8	33.3	3.0	9947.0	16.8	50.0	Test 1: 333.5 / Test 2: 36.2	10000.0	Test 1: 3.3% / Test 2: 10.9%
2.0	66.5	100.0	99.3	97.8	133.0	2.9	9797.1	67.0	200.0	Test 1: 434.0 / Test 2: 135.9	10000.0	Test 1: 4.3% / Test 2: 31.3%
5.0	66.5	100.0	98.3	99.2	332.5	2.9	9497.2	167.5	500.0	Test 1: 635.0 / Test 2: 335.4	10000.0	Test 1: 6.4% / Test 2: 52.8%
20.0	66.5	100.0	92.3	99.8	1330.0	2.4	7997.6	670.0	2000.0	Test 1: 1640.0 / Test 2: 1332.4	10000.0	Test 1: 16.4% / Test 2: 81.2%
50.0	66.5	100.0	74.9	100.0	3325.0	1.5	4998.5	1675.0	5000.0	Test 1: 3650.0 / Test 2: 3326.5	10000.0	Test 1: 36.5% / Test 2: 91.1%

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Current policy on use of COVID-19 Ag RDTs

Advice on the use of point-of-care immunodiagnostic tests for COVID-19

Scientific Brief

8 April 2020

In response to the growing COVID-19 pandemic and shortages of laboratory-based molecular testing capacity and reagents, multiple diagnostic test manufacturers have developed and begun selling rapid and easy-to-use devices to facilitate testing outside of laboratory settings. These simple test kits are based either on detection of proteins from the COVID-19 virus in respiratory samples (e.g. sputum, throat swab) or detection, in blood or serum, of human antibodies generated in response to infection.

WHO applauds the efforts of test developers to innovate and respond to the needs of the

- **WHO does not currently recommend the use of antigen-detecting rapid diagnostic tests for patient care, although research into their performance and potential diagnostic utility is highly encouraged**

- *Do we have sufficient evidence to base recommendations ?*
- *Are we ready to accept poorer performance if we can capture the most infectious cases faster and at least partially reduce the number people requiring RT-PCR ?*

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Target product profile – POCT where RT-PCR is not available or timely

- **Intended Use**
  - **In areas with confirmed SARS-CoV-2 community wide transmission or confirmed outbreaks in closed or semi-closed communities and in high risk groups**: Early detection of SARS-CoV-2 cases where molecular/reference assays are not available or services are overloaded leading to turn around times that are not useful for guiding clinical case management and infection control measures.
  - **In Suspected SARS-CoV-2 outbreak situations**: multiple positive cases highly suggestive of SARS-CoV-2
  - **Monitor trends in disease incidence**

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Target use setting

- The tests can be performed outside laboratories including routine and triage/screening point of healthcare facilities including emergency units, mobile units and in the community (contact tracing) by health care workers or laboratory technicians with appropriate training in sample collection, biosafety and the use of the test.

TPP: Performance requirements

- **Analytical sensitivity/Limit of detection**
  acceptable: equivalent to 10^6 genomic copies/ml or CT ≈ 25

  desirable: equivalent to 10^4 genomic copies/ml or CT≈35

- **Sensitivity** ≥ 70% desirable:≥80%
- **Specificity** ≥ 97% desirable: >99%

When prevalence is 10-20% : acceptable criteria PPV increases to >75-98% and NPV still high (>95%). At lower prevalence, PPV is low and positives would need to be confirmed

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

What if no baseline is available ?

- The importance of disease prevalence in interpreting the probability that positive results are true positives and negative test results are true negatives has been repeatedly stressed
- If surveillance data is not available then pilot introduction of testing with reference testing available is highly advisable before going to scale.

Do not use RDTs if .....

Lack of appropriate biosafety and infection prevention and control measures (IPC)	To safeguard health workers, sample collection for RDTs from patients with suspected COVID-19 requires that operators wear gloves, gown, mask and googles".
Management of the patient does not change based on the result of the test;	If test positive and test negative patients will be treated the same way then there is no benefit to testing.
Airport or border screening (confirmatory testing not immediately available)	Prevalence of COVID-19 will be highly variable amongst travellers, therefore it is not possible to determine PPV and NPV of test results. At a minimum test positives would require confirmatory testing to increase PPV and negative results would not rule out infection (only high viral loads/antigen will be detected) and the probability that a negative result means the patient does not have the disease is unknown.
Screening prior to blood transfusion	A positive RDT result would not necessarily correlate with presence of viremia and similarly a negative RDT result would not exclude possibility of viremia.

Conclusions

- Point of care COVID-19 antigen detecting RDTs are commercially available
- Independent data on test performance is limited and many factors can affect results
- Data suggests some tests can perform well in samples with high viral loads/antigen concentrations; cannot rule out infection and impact of missed cases unknown
- If RDTs can meet TPP requirements - potential use scenarios for case management, surveillance and outbreak investigations in areas where RT-PCR is unavailable or not timely
- WHO interim guidance will follow in near future informed by pending systematic reviews and independent evaluations

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Shaheed Beheshti Universityof Medical Sciences

Virology Research Center

National Research Institute of Tuberculosis and Lung Disease (NRITLD)

Questions?

Prof. S.A. Nadji

Email: s.a.nadji@sbmu.ac.ir