Welcome to HyTest Webinar Diagnostics of COVID-19: Achievements, Challenges and Perspectives

October 29, 2020

1. Introductions: Company and Speaker *by Dr. Netta Fatal*
2. Talk 40 min *by Dr. Alexander Semenov*
3. Q&A 20 min *by Dr. Alexander Semenov and Dr. Karina Seferian*

HyTest Ltd.

We develop and produce monoclonal antibodies and antigens that are mainly used as key components in laboratory tests.

- Established in 1994
- Headquarters in Finland, operations in China, Russia and North America
- Sales to over 50 countries
- Active participation in IFCC and AACC standardization committee work
- Operations ISO 9001:2015 compliant

HyTest

Comprehensive product line

PRODUCT CATEGORIES

- Monoclonal antibodies
- Polyclonal antibodies
- Antigens
- Plasma and serum
- Over 1,000 different reagents

KEY PRODUCT AREAS

Cardiac Markers | Metabolic Syndrome | Infectious Diseases | Inflammation | Veterinary

OTHER PRODUCT AREAS

Blood Coagulation and Anemia | Immunology and Serology | Fertility and Pregnancy | Hormones | Tumor Markers | Neuroscience

Gangliosides | Kidney diseases | Inflammation | Microbial and Plant Toxins | Biodefence | Molecular Biology

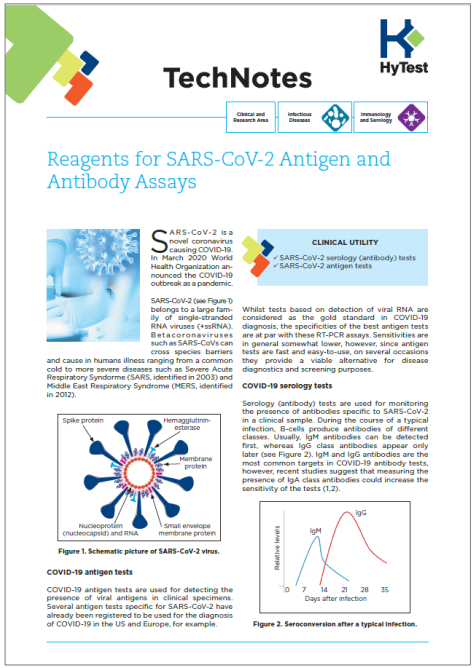
HyTest

SARS-CoV-2 reagents from HyTest

- SARS-CoV-2 **monoclonal antibodies** for antigen tests
  - Anti-NP
  - Anti-Spike (RBD)

- SARS-CoV-2 **recombinant antigens** for serology tests
  - Nucleoprotein
  - Spike RBD

- Secondary antibodies for serology tests
  - Anti-IgG
  - Anti-IgM
  - Anti-IgA



TechNotes

HyTest

Clinical and Research Area | Infectious Diseases | Immunology and Serology

Reagents for SARS-CoV-2 Antigen and Antibody Assays

SARS-CoV-2 is a novel coronavirus causing COVID-19. In March 2020 World Health Organization announced the COVID-19 outbreak as a pandemic.

SARS-CoV-2 (see Figure 1) belongs to a large family of single-stranded RNA viruses (+ssRNA). Betacoronaviruses such as SARS-CoVs can cross species barriers and cause in humans illness ranging from a common cold to more severe diseases such as Severe Acute Respiratory Syndrome (SARS, identified in 2003) and Middle East Respiratory Syndrome (MERS, identified in 2012).

CLINICAL UTILITY

- ✓ SARS-CoV-2 serology (antibody) tests
- ✓ SARS-CoV-2 antigen tests

Whilst tests based on detection of viral RNA are considered as the gold standard in COVID-19 diagnosis, the specificities of the best antigen tests are at par with these RT-PCR assays. Sensitivities are in general somewhat lower, however, since antigen tests are fast and easy-to-use, on several occasions they provide a viable alternative for disease diagnostics and screening purposes.

Spike protein
Hemagglutinin-esterase
Membrane protein
Nucleoprotein (nucleocapsid) and RNA
Small envelope membrane protein

Figure 1. Schematic picture of SARS-CoV-2 virus.

COVID-19 antigen tests

COVID-19 antigen tests are used for detecting the presence of viral antigens in clinical specimens. Several antigen tests specific for SARS-CoV-2 have already been registered to be used for the diagnosis of COVID-19 in the US and Europe, for example.

COVID-19 serology tests

Serology (antibody) tests are used for monitoring the presence of antibodies specific to SARS-CoV-2 in a clinical sample. During the course of a typical infection, B-cells produce antibodies of different classes. Usually, IgM antibodies can be detected first, whereas IgG class antibodies appear only later (see Figure 2). IgM and IgG antibodies are the most common targets in COVID-19 antibody tests, however, recent studies suggest that measuring the presence of IgA class antibodies could increase the sensitivity of the tests (1,2).

IgG
IgM
Relative levels
0 7 14 21 28 35
Days after infection

Figure 2. Seroconversion after a typical infection.

HyTest

Today’s speaker: Dr. Alexander Semenov

- MSc. and PhD degrees from the Moscow State University (MSU)
- Senior Scientist and Project Manager, joined HyTest R&D in 2005
- Involved in research projects focused on BNP and NT-proBNP, in charge of developing antibodies and immunoassays specific to these heart failure biomarkers
- Co-author of about 20 publications and patents
- Member of The Joint Committee for Traceability in Laboratory Medicine (JCTLM) Proteins Review Team

Panelist Dr. Karina Seferian

- MSc and PhD degrees from the Moscow State University (MSU)
- Senior Scientist and Project Manager, joined HyTest R&D in 2003
- Involved in research projects related to the heart failure biomarker Brain Natriuretic Peptide (BNP). Ongoing research is focused on the inflammatory biomarker serum amyloid A (SAA)
- Co-author of about 10 scientific publications and patents

HyTest

Diagnostics of COVID-19: achievements, challenges and perspectives

Alexander G. Semenov, PhD

Coronavirus disease 2019 (COVID-19) pandemic

- On 7 January 2020 the Chinese Center for Disease Control and Prevention (China CDC) officially announced the outbreak of a novel pneumonia caused by a pathogenic coronavirus in Wuhan, China

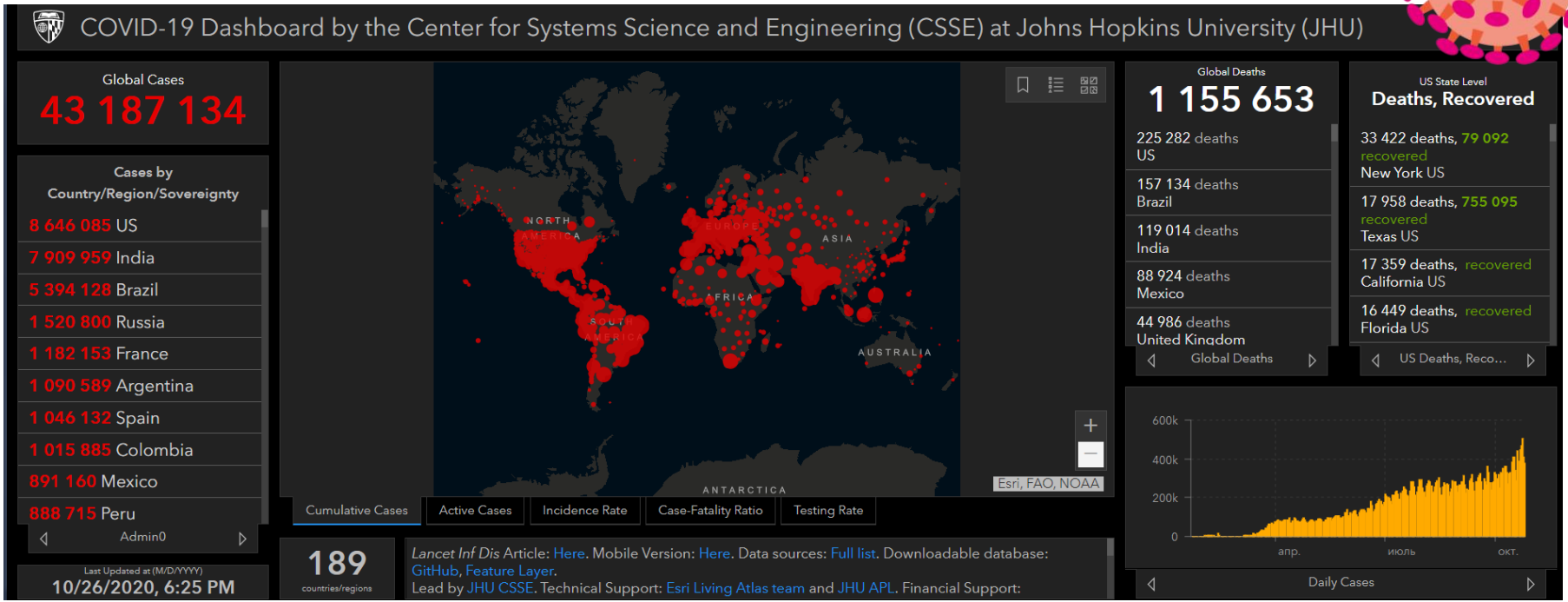
- This novel coronavirus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and its associated clinical syndrome was named corona virus disease 2019 (COVID-19)

- On 11 March 2020 the new infectious disease was recognized as a pandemic by WHO

World Health Organization

HyTest

COVID-19 pandemic



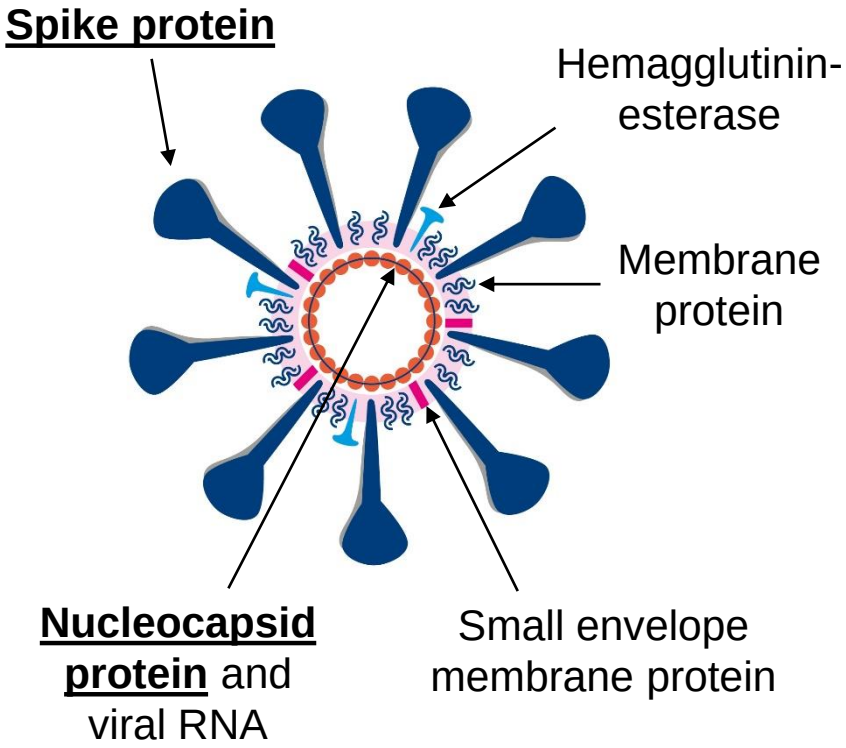
https://coronavirus.jhu.edu/map.html

HyTest

1. SARS-CoV-2 virus
2. Transmission and clinical characteristics of SARS-CoV-2
3. Nucleic acid testing for detection of SARS-CoV-2
4. SARS-CoV-2 antigen tests
5. Serological testing of SARS-CoV-2

1. SARS-CoV-2 virus
2. Transmission and clinical characteristics of SARS-CoV-2
3. Nucleic acid testing for detection of SARS-CoV-2
4. SARS-CoV-2 antigen tests
5. Serological testing of SARS-CoV-2

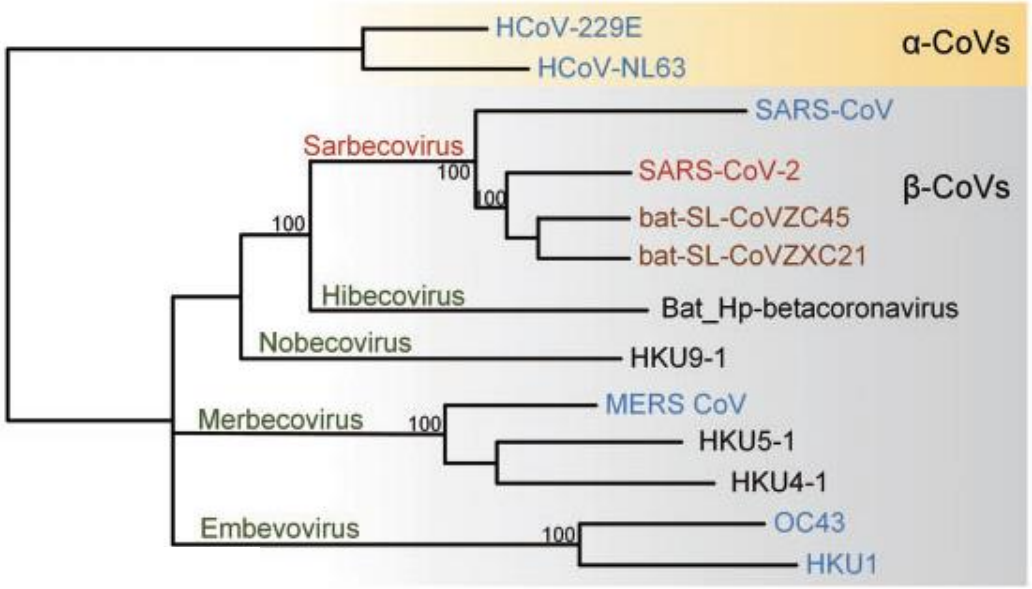
SARS-CoV-2 is a novel coronavirus of zoonotic origin



- A linear, single-stranded, positive-sense RNA virus (+ssRNA)
- The 3'-end of the genome encodes 4 structural proteins as well as accessory proteins
- The virus has an RNA proofreading mechanism keeping the mutation rate relatively low

HyTest

Phylogenetics of SARS-CoV-2



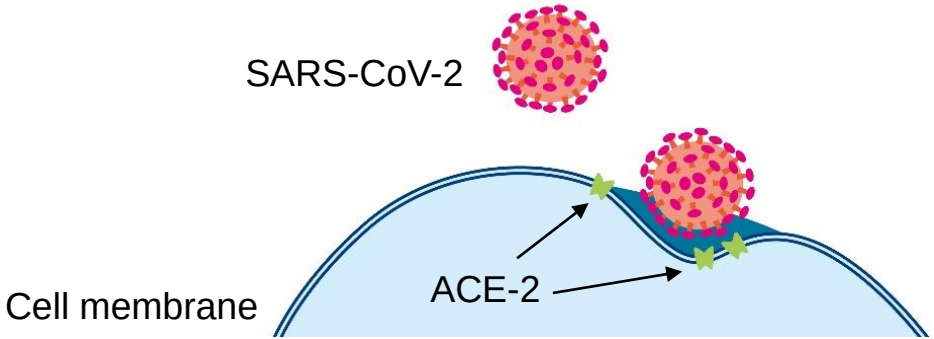
Shi J. et al. Clin Chem, 66:8, 1030-1046 (2020)

There are currently 7 CoVs that can cause human respiratory diseases, but to date, only SARS-CoV, MERS-CoV, and SARS-CoV-2 have caused a large outbreak with high mortality

HyTest

Mechanisms of entry of SARS-CoV-2

- SARS-CoV-2 enters the body through the nose and throat (e.g. when virus-laden droplets are inhaled)
- Receptor-binding domain (RBD) interacts with angiotensin-converting enzyme 2 (ACE-2)
- ACE2 receptors are abundantly expressed in the respiratory system (the highest expression in alveolar epithelial cells)



HyTest

1. SARS-CoV-2 virus
2. Transmission and clinical characteristics of SARS-CoV-2
3. Nucleic acid testing for detection of SARS-CoV-2
4. Serological testing of SARS-CoV-2
5. SARS-CoV-2 antigen tests

Transmission of SARS-CoV-2

- SARS-CoV-2 likely originated from the Chinese horseshoe bat

- The initial patients were infected mainly by wild animals
- Human-to-human transmission

- SARS-CoV-2 infects the respiratory system initially, and virus replication in alveoli causes alveolar vascular rupture as the disease progresses

Effects of SARS-CoV-2 on different organs

Strokes, seizures, confusion, and brain inflammation

Inflamed and damaged alveolus

Infection of gastrointestinal tract causes diarrhea

Conjunctivitis/ inflammation

Blood clots, heart attacks, and cardiac inflammation

Kidney damage

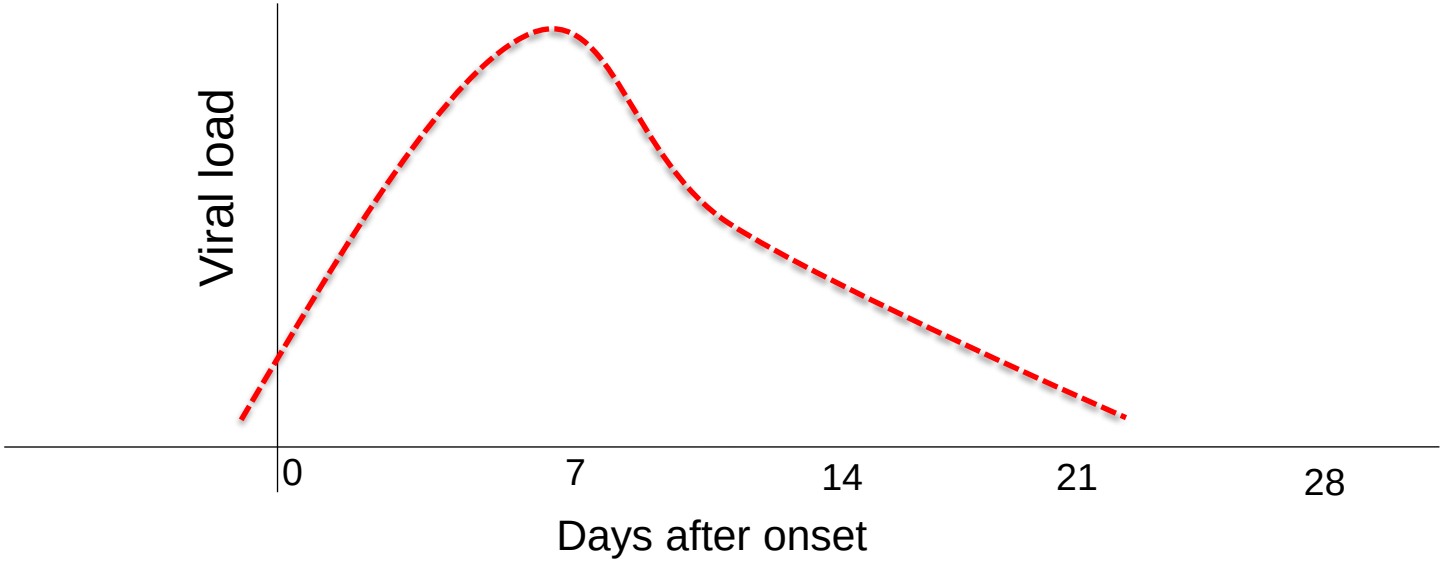
Liver disfunction

HyTest

Clinical characteristics of infected patients

- The general incubation period is 1 to 14 days (usually 3–7 days)
- Clinical symptoms of infected patients: fever, cough, expectoration, headache, myalgia or fatigue, dyspnea, diarrhea
- Some individuals infected with SARS-CoV-2 may never develop symptoms (asymptomatic cases)

Viral loads in different stages of SARS-CoV-2 infection



Incubation → Early stage → Progressive stage → Recovery stage

HyTest

Clinical specimens for SARS-CoV-2 testing

- Viral RNA has been detected in nasopharyngeal swabs, oropharyngeal swabs, throat swabs, sputum, bronchoalveolar lavage fluid, whole blood, serum, stool, urine, saliva, rectal swabs and conjunctival swabs

- The viral load is considerably higher in respiratory tract specimens than in nonrespiratory specimens

- Collection and detection of lower respiratory tract specimens are recommended even if the upper respiratory tract specimens are negative

Biosafety precaution and protection

- It is recommended that all specimens collected for laboratory investigation should be considered **potentially infectious**

- RNA extraction, nucleic acid amplification assays and sequencing should be conducted in BSL-2 laboratory or facility using procedures equivalent to BSL-2

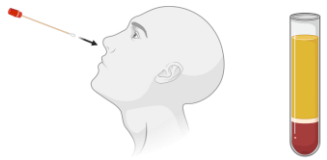
- Point of care (POC) or near-POC assays can be performed on a bench without employing a BSC, when the local risk assessment so dictates and proper precautions are in place

https://www.who.int/publications/i/item/laboratory-biosafety-guidance-related-to-coronavirus-disease-2019-(covid-19)

The WHO message:

“Test, test, test!”

Sensitivity and specificity of a diagnostic test



		Disease status	
		Subjects with disease	Subjects without disease
Test	Positive	True Positive (TP)	False Positive (FP)
	Negative	False Negative (FN)	True Negative (TN)

No test gives a 100% accurate result

$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

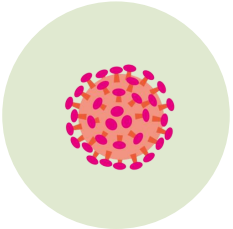
$$\text{Specificity} = \frac{TN}{FP + TN}$$

HyTest

1. SARS-CoV-2 virus
2. Transmission and clinical characteristics of SARS-CoV-2
3. Nucleic acid testing for detection of SARS-CoV-2
4. SARS-CoV-2 antigen tests
5. Serological testing of SARS-CoV-2

WHO interim guidance for SARS-CoV-2 diagnostic testing

“Wherever possible, suspected active SARS-CoV-2 infections should be tested with NAAT, such as rRT-PCR.”



“SARS-CoV-2 Ag-RDTs <…> can be used to diagnose SARS-CoV-2 infection in a range of settings where NAAT is unavailable or where prolonged turnaround times preclude clinical utility.”

“Serology should not be used as a standalone diagnostic to identify acute cases in clinical care or for contact tracing purposes.” “Serological assays <…> can be useful in various settings.”

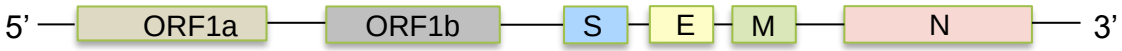
https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2

HyTest

Nucleic acid testing for detection of SARS-CoV-2

- The diagnosis of SARS-CoV-2 infection in clinical laboratories worldwide mainly depends on the detection of the viral RNA by RT-PCR

- Gene regions including ORF1b, N, and S genes are commonly targeted regions for RT-PCR



- Increasing the number of specific targets in the RT-PCR system may increase the testing sensitivity, as SARS-CoV-2 is prone to mutation

HyTest

Nucleic acid testing for detection of SARS-CoV-2

- Diagnosis of the infection mainly depends on positive viral nucleic acid test results and not on clinical symptoms
- The selection of specimens for molecular assays is crucial
- Viral loads of respiratory tract specimens are highest in bronchoalveolar lavage fluid, followed by the sputum, nasal swabs, and pharyngeal swabs
- A negative result from an oral nasopharyngeal swab is not sufficient for a hospital discharge

Quality assurance of nucleic acid test for SARS-CoV-2

- False positive results are mainly caused by cross-contamination between specimens/residual contamination, and the cross-reaction of other viruses due to nonspecific primers

False Positive

- False negative results are mainly due to unreliable detection reagents and non-standardized testing protocols

- Insufficient virus in a specimen is caused mainly by incorrect sampling sites or sampling techniques and improper sampling time (the site and quality of sampling matters!)

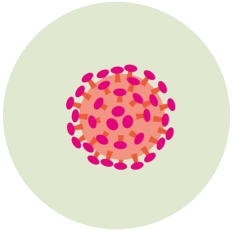
False Negative

SARS-CoV-2 whole-genome sequencing

- Whole-genome sequencing is a powerful tool to understand the transmission dynamics of outbreaks
- Immediate sharing and analysis of data during outbreaks is recommended as an integral part of outbreak response
- Sequencing methods include: metatranscriptomics, hybrid capture-based, amplicon, and nanopore targeted sequencing
- Hundreds of SARS-CoV-2 genomes have been released on public databases

1. SARS-CoV-2 virus
2. Transmission and clinical characteristics of SARS-CoV-2
3. Nucleic acid testing for detection of SARS-CoV-2
4. SARS-CoV-2 antigen tests
5. Serological testing of SARS-CoV-2

SARS-CoV-2 antigen tests vs PCR and antibody tests



Antigen test is an alternative diagnostic method that relies on the immunodetection of the viral antigens in biological samples

PCR tests are expensive and require special equipment and highly skilled personnel. Results are not fast!

Antibody tests are easy to use and affordable but not very effective in the initial phase of the infection

HyTest

SARS-CoV-2 antigen tests with EUAs

Date EUA Issued or Last Updated	Entity	Diagnostic (Most Recent Letter of Authorization) and Date EUA Originally Issued	Attributes	Authorized Setting(s)[1]	Authorization Documents[2]
07/17/2020	Quidel Corporation	Sofia SARS Antigen FIA 05/08/2020	Lateral Flow, Fluorescence, Instrument Read	H, M, W	HCP, Patients, IFU
07/23/2020	Becton, Dickinson and Company (BD)	BD Veritor System for Rapid Detection of SARS-CoV-2 07/02/2020	Chromatographic Digital Immunoassay, Instrument Read	H, M, W	HCP, Patients, IFU
08/18/2020	LumiraDx UK Ltd.	LumiraDx SARS-CoV-2 Ag Test 08/18/2020	Microfluidic Immunofluorescence Assay, Instrument Read	H, M, W	HCP, Patients, IFU
08/26/2020	Abbott Diagnostics Scarborough, Inc.	BinaxNOW COVID-19 Ag Card 08/26/2020	Lateral Flow, Visual Read	H, M, W	HCP, Patients, IFU
10/02/2020	Quidel Corporation	Sofia 2 Flu + SARS Antigen FIA 10/02/2020	Lateral Flow, Fluorescence, Instrument Read, Multi-Analyte	H, M, W	HCP, Patients, IFU
10/13/2020	Access Bio, Inc.	CareStart COVID-19 Antigen test 10/08/2020	Lateral Flow, Visual Read	H, M, W	HCP, Patients, IFU
10/23/2020	Celltrion USA, Inc.	Sampinute COVID-19 Antigen MIA 10/23/2020	Magnetic Force-assisted Electrochemical Sandwich Immunoassay (MESIA)	H, M	HCP, Patients, IFU

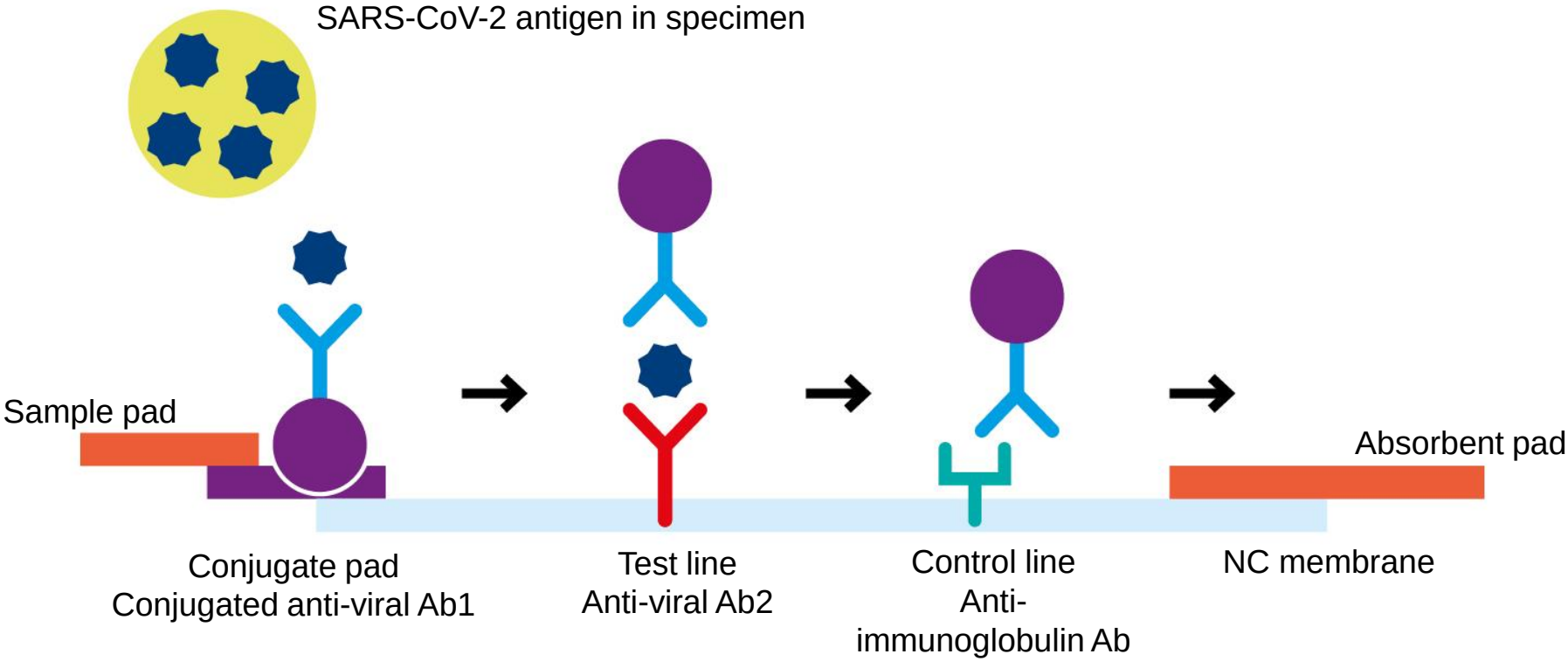
https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

HyTest

SARS-CoV-2 antigen tests

- Antigen tests have performance limitations. However, the circumstances of the pandemic call for the availability of more tests
- Rapid antigen tests may provide the advantage of fast time to results and low-cost detection but require **a relatively high viral count**
- Nucleocapsid and Spike proteins are used as antigens for detection
- Immunochromatographic assay is the most commonly used method for the detection of SARS-CoV-2 antigens

Principle of rapid antigen tests



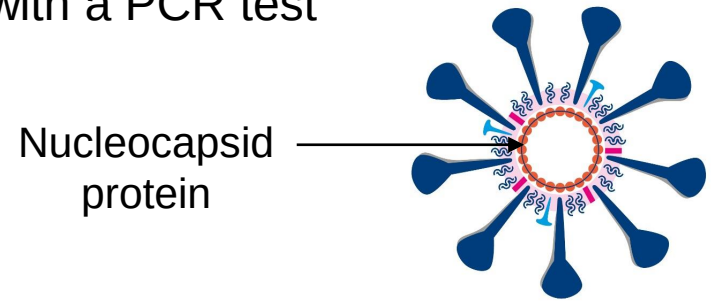
HyTest

Nucleocapsid-based antigen tests

- The nucleocapsid protein has emerged as a better target for diagnostic antigen tests because it's more conserved, and it's more abundant

- Currently there are several FDA authorized SARS-CoV-2 antigen tests that detect nucleoprotein in nasopharyngeal swabs (visual/instrument read)

- A positive result most likely indicates an active SARS-CoV-2 infection. A negative result may require to be confirmed with a PCR test



HyTest

Spike protein-based antigen tests

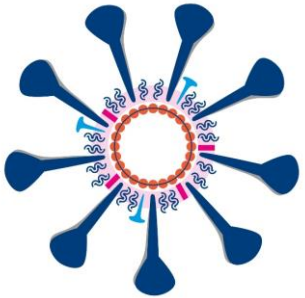
- Spike protein is involved in receptor recognition, viral attachment, and entry into host cells. It's less abundant than nucleocapsid.

- There a few commercially available (CE-IVD) rapid antigen assays to the SARS-CoV-2 spike protein and one FDA authorized test

- A correlation between high S1 concentrations in plasma and time between hospital admission and intubation was shown

(Ultra-sensitive Single Molecule Array assay, Ogata A. et al. Clin Chem 2020)

Spike protein →



HyTest

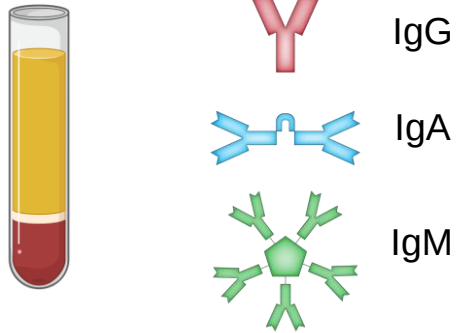
The role of antigen tests in COVID-19 diagnostics

- SARS-CoV-2 antigen assays can complement PCR and serological tests by identifying active infection or monitoring disease progression
- Antigen tests may be more practical to use for large numbers of people
- To assist healthcare providers and laboratories to quickly and reliably determine whether a patient is actively infected with the COVID-19 virus
- To assess if it is safe for people to return to work, return to school, travel and go about their daily lives

1. SARS-CoV-2 virus
2. Transmission and clinical characteristics of SARS-CoV-2
3. Nucleic acid testing for detection of SARS-CoV-2
4. SARS-CoV-2 antigen tests
5. Serological testing of SARS-CoV-2

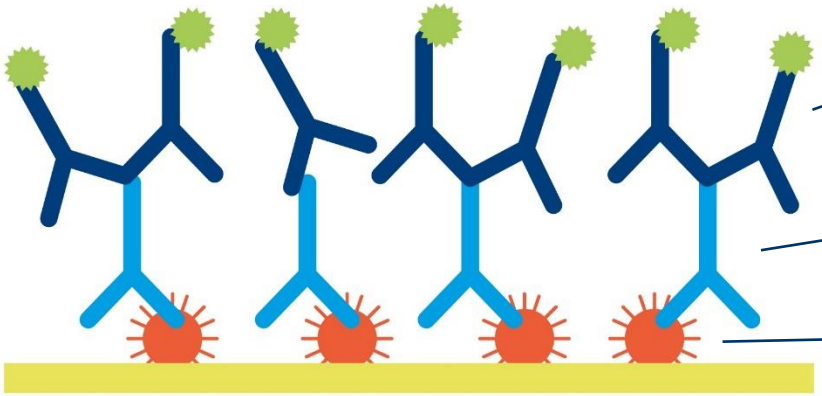
Serological testing of SARS-CoV-2

- Serology tests are used for the detection of antibodies in the blood in order to diagnose an active or previous infection
- Antibodies can be detected only several days after the exposure to the virus
- IgA has been reported less often than IgM and IgG



HyTest

Principle of serological testing of SARS-CoV-2



Secondary antibody (labelled)

Primary antibody

Antigen

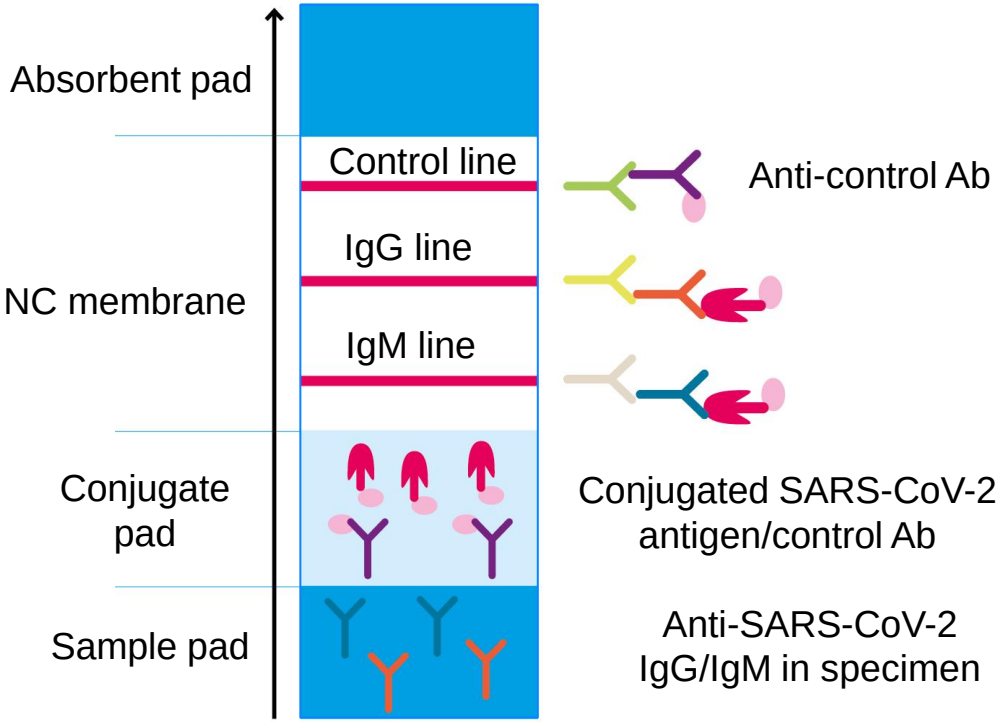
Anti-IgA/IgM or Anti-IgG

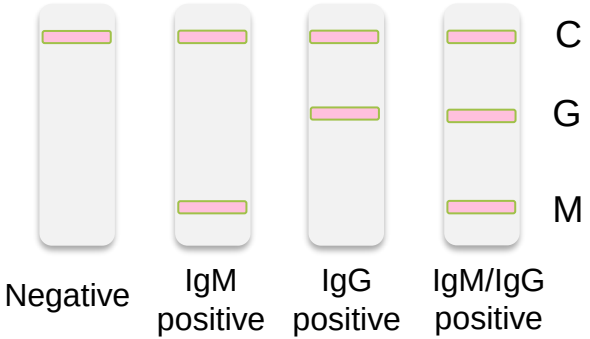
Antibody in serum (anti-SARS-Cov-2)

Rec. antigen (e.g. RBD/Nucleoprotein

HyTest

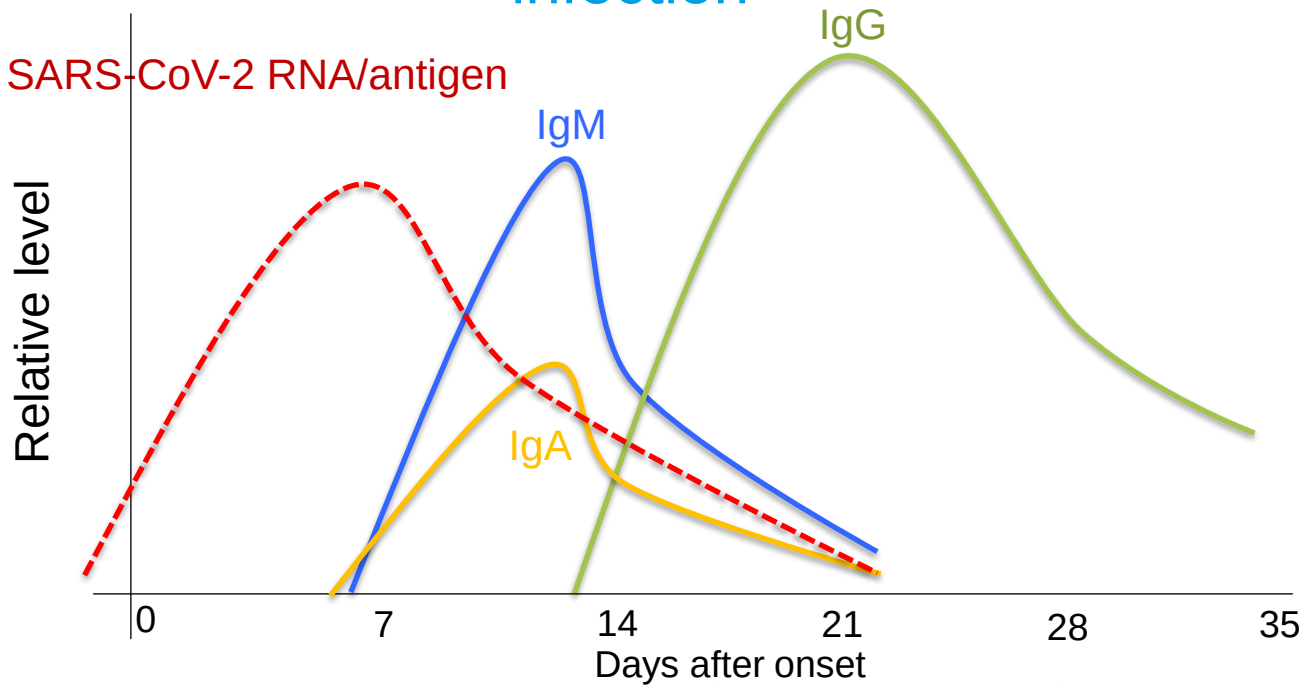
Principle of rapid antibody tests for SARS-CoV-2





HyTest

Levels of SARS-CoV-2 RNA/antigen, IgM and IgG after infection



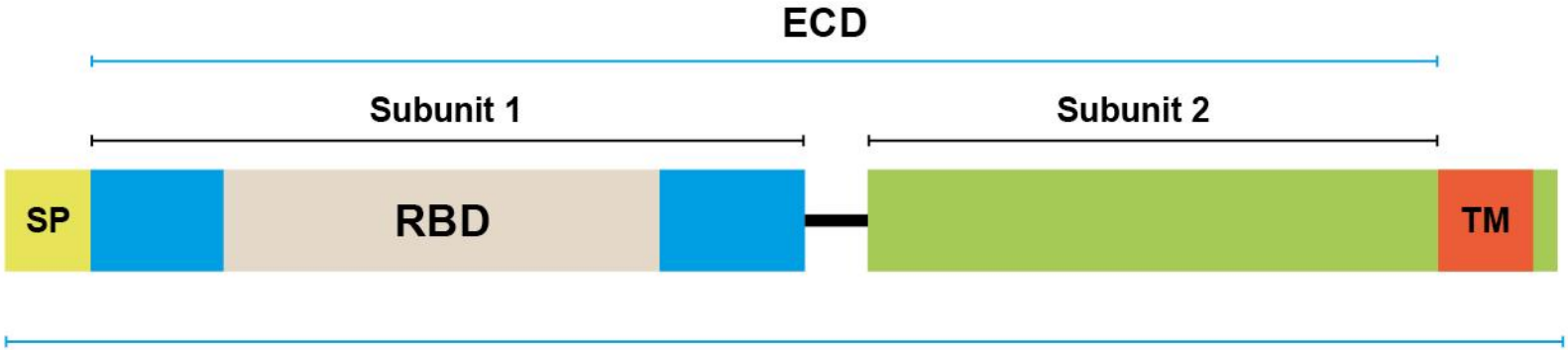
Incubation → Early stage → Progressive stage → Recovery stage

HyTest

Viral proteins used in serological tests

- Nucleoprotein, full-length spike, S1 domain, S2 domain or receptor-binding domain (RBD) — are used as targets in serological tests
- RBD domain and S1 subunit are more specific than full-length S or S2 subunit as antigens for COVID-19 serologic detection
- Some tests utilize both Spike and Nucleocapsid proteins
- Positive results are not necessarily indicators of presence of neutralizing antibodies

SARS-CoV-2 spike protein domain structure



Full-size Spike protein (1273 aar)

Spike protein fragments used in diagnostic assays:

- Subunit1 = S1 (14-685 aar)
- Subunit2 = S2 (686-1273 aar)
- ECD = Spike ectodomain = S1+S2
- RBD = Receptor-binding domain (part of S1 (319-541 aar))

HyTest

Homology of nucleocapsid and spike protein

SARS-CoV-2 vs	Nucleocapsid	Spike (RBD region)
SARS-CoV-1	**90.5%**	**75.7% (73.1%)**
MERS-CoV	45.1%	25.2% (15.3%)
HcoV_229E	23.1%	21.8% (11.7%)
HcoV_OC43	31.1%	26.4% (16.6%)
HcoV_NL63	23.3%	20.5% (12.6%)
HcoV_HKU1	29.1%	23.7% (14.4%)

Quality assurance of serological assays

- Cross-reactivity with other subtypes of coronaviruses should be considered
- Antibody assays are susceptible to the influence of endogenous interferents, including rheumatoid factors, heterophilic antibodies, and complements
- Preanalytical artefacts, such as specimen hemolysis, may yield false positive results

False Positive

Quality assurance of serological assays

- Sampling at unsuitable stages may lead to false negatives. IgA and IgM usually last a short time, and IgG may be produced in the later period

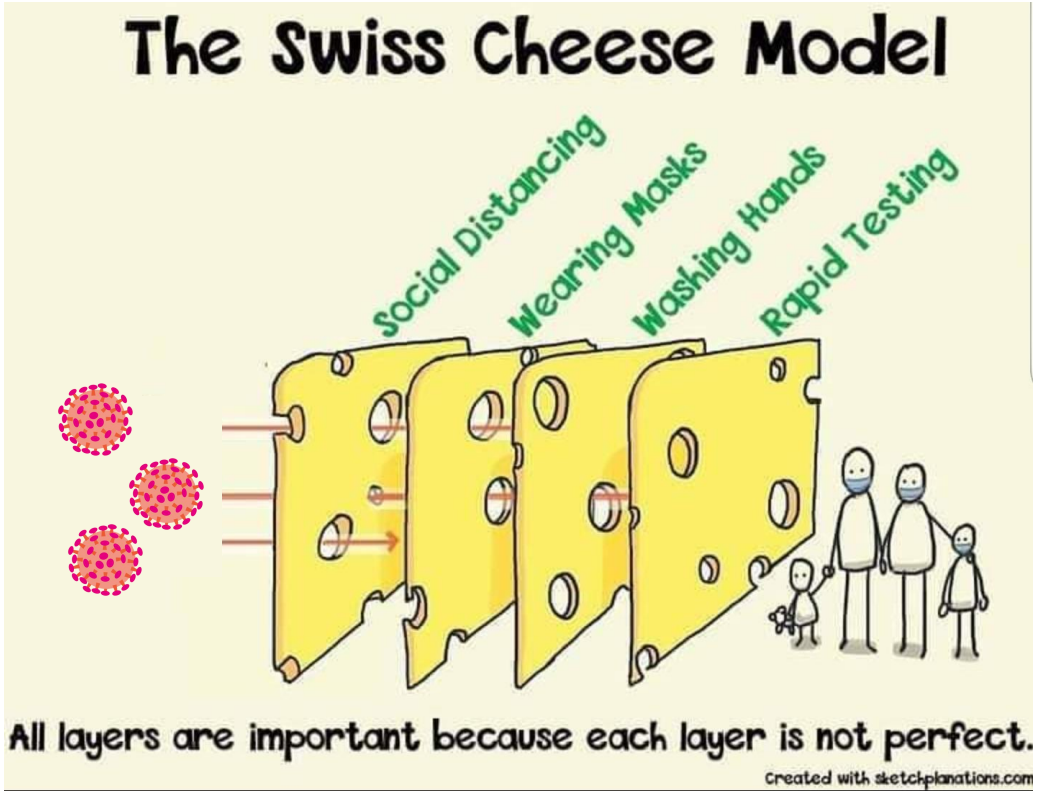
- Differences in individual immune response and antibody production also may lead to false negative results.

False Negative

- The combined IgM–IgG test was reported to have better practicality and sensitivity than tests for only IgM or IgG

What is the role of serological testing in COVID-19 diagnostics?

- If the results from serological assays can be used to infer if a patient is protected from future infections is not currently known
- A potential utility of serological assays could be after the emergence of vaccines to SARS-CoV-2
- Serology is possibly helpful in patients with signs and symptoms of COVID-19 that are persistently negative by PCR testing
- To distinguish COVID-19 infections from other potential causes of disease



Modifed from @sketchplanations/J. Reason

Concluding remarks

- The SARS-CoV-2 pandemic posed a serious threat to international healthcare system
- The rapid and early laboratory diagnosis is critical to control transmission
- Combination of molecular, serological and antigen tests is needed to improve the diagnostic accuracy of COVID-19
- Full understanding of the genomic characteristics, transmission and clinical features of SARS-CoV-2 will improve the accuracy of SARS-CoV-2 testing

Thank you for your participation!

www.hytest.fi

hytest@hytest.fi

HyTest