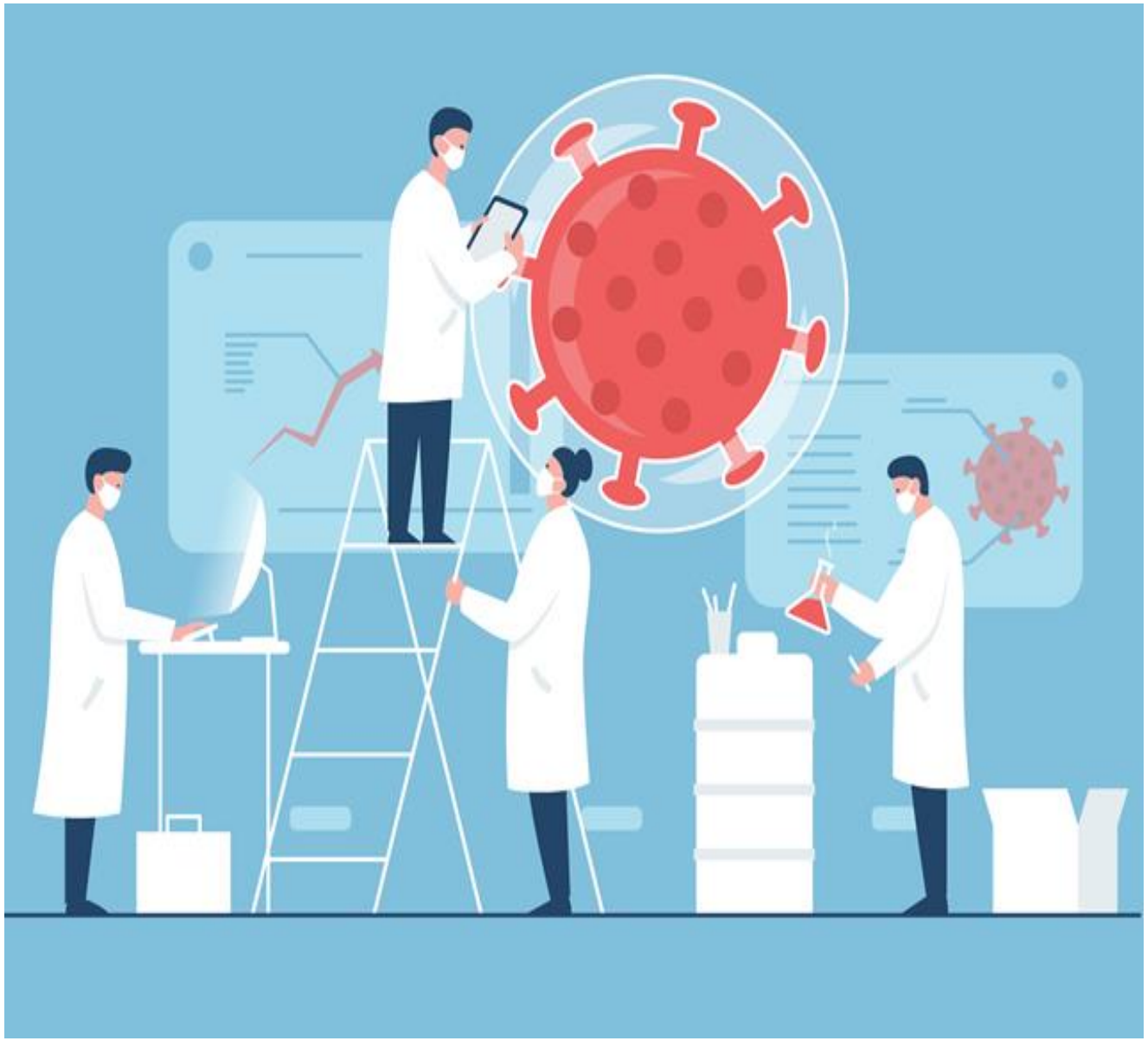


INTERPRETATION OF LABORATORY RESULTS AND LABORATORY ROLES IN COVID-19 DIAGNOSIS

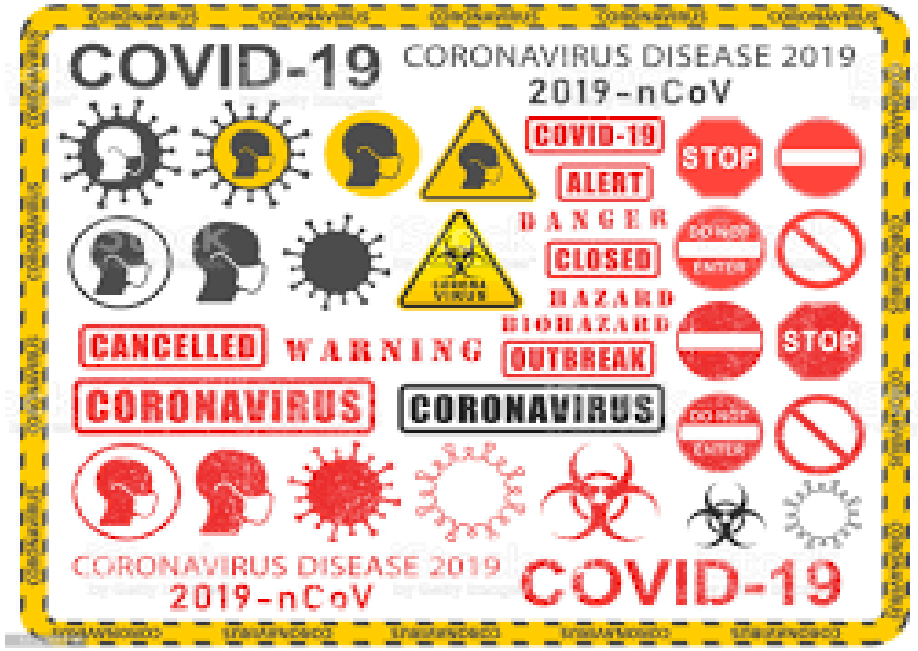
BY: MINA HEIDARI

CONTENTS:

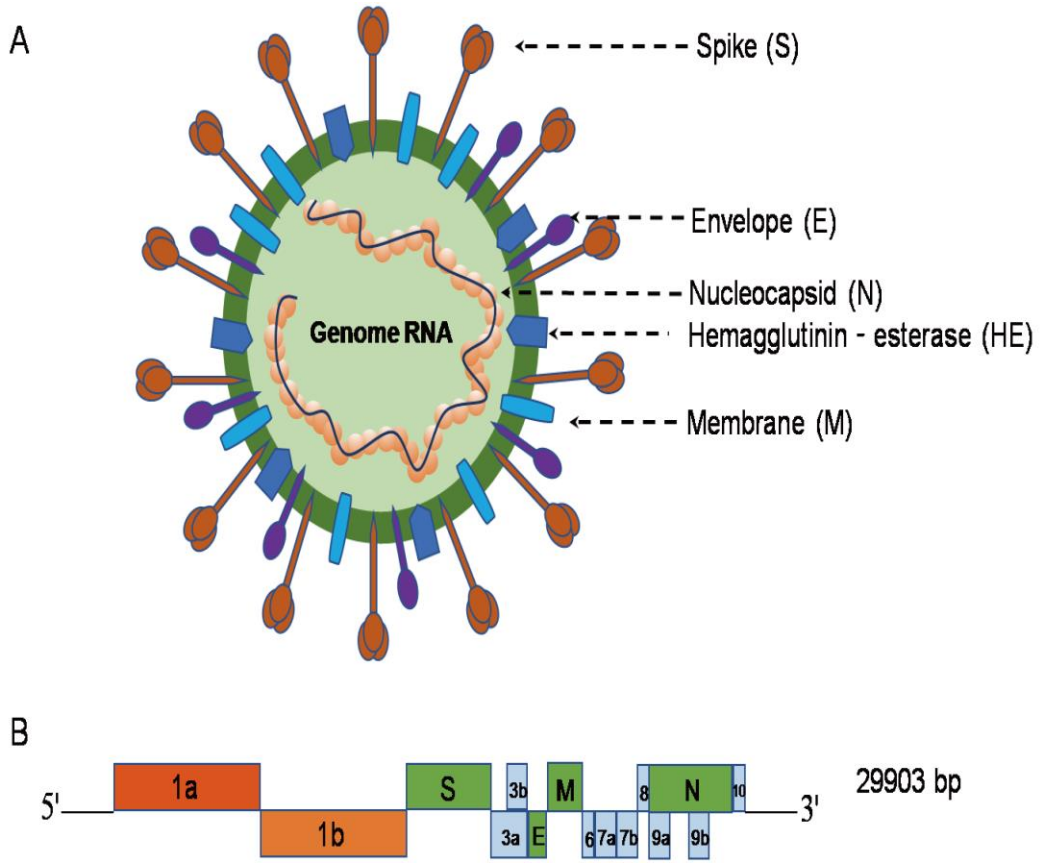
- Virology
- Definition of variants
- Laboratory roles
- Biochemical, serologic and molecular diagnosis and monitoring of Covid-19
- Pre-analytical and analytical issues regarding Covid-19 laboratory tests' interpretation

INTRODUCTION

- SARS-CoV-2, is a newly emerging virus belonging to the *Coronaviridae* family, presumably derived from a bat SARS-like coronavirus and transmitted to humans after the emergence of mutations in the spike glycoprotein (protein S) and nucleocapsid (N protein) .
- This zoonotic pathogen, called coronavirus disease 2019 (COVID-19) by the **World Health Organization** (WHO), is assumed to be the latest global biological human hazard.



VIROLOGY :



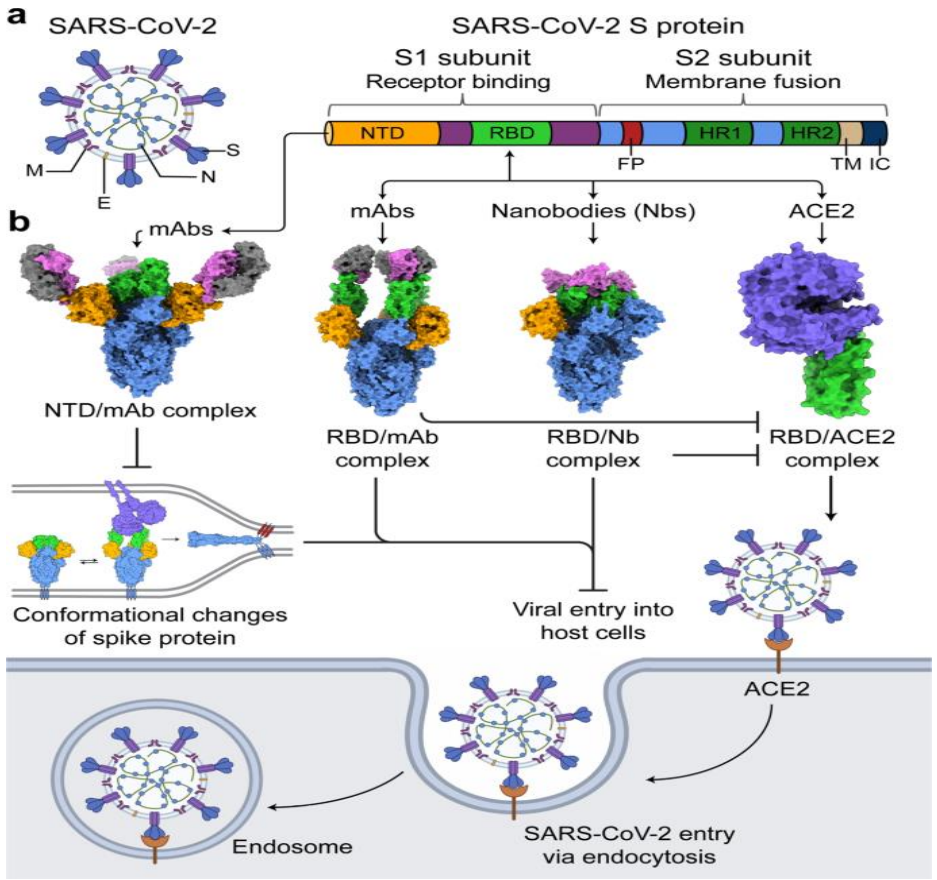
- (**A**) β-coronavirus is an enveloped, non-segmented, positive-sense single-stranded RNA virus genome in a size ranging from 29.9 kb. The virion has a nucleocapsid composed of genomic RNA and phosphorylated nucleocapsid (N) protein, which is buried inside phospholipid bilayers and covered by the spike glycoprotein trimmer (S). The membrane (M) protein hemagglutinin-esterase (HE) and the envelope (E) protein are located among the S proteins in the virus envelope.
- (**B**) 5′ and 3′ terminal sequences of the SARS-CoV-2 genome. The gene order is 5′-replicase ORF1ab-S-envelope(E)-membrane(M)-N-3′. ORF3ab, ORF6, ORF7ab, ORF8, ORF9ab, and ORF10 are located at the predicted positions shown in the picture. 1a, 1b, 3a, 3b, 6, 7a, 7b, 8, 9a, 9b, 10 in the picture represent different ORF genes.

PHYSIOCHEMICAL PROPERTIES

- The virus particle has a diameter of 60~100 nm and appears round or oval . Most of the knowledge about the physicochemical properties of CoVs comes from SARS-CoV and MERS-CoV. SARS-CoV-2 can be inactivated by UV or heated at 56 °C 30 min, and also sensitive to most disinfectants such as diethyl ether, 75% ethanol, chlorine, peracetic acid, and chloroform .
- It has been reported that SARS-CoV-2 was more stable on plastic and stainless steel than on copper and cardboard, and viable virus was detected up to 72 h after application to these surfaces. On cardboard, the half-life of SARS-CoV-2 was longer than that of SARS-CoV and the longest viability of both viruses was on stainless steel and plastic .

RECEPTOR INTERACTIONS AND CELL ENTRY

- Human angiotensin-converting enzyme 2 (ACE2) is a functional receptor hijacked by SARS-CoV-2 for cell entry . ACE2 is a membrane protein expressed in lung, heart, kidney, and intestine mainly associated with cardiovascular diseases .
- In addition to cleavage of angiotensin (Ang) I to produce Ang-(1-9), ACE2 also provides a direct binding site for the S proteins of CoVs . The S protein of CoVs exists in a metastable pre-fusion conformation that undergoes a dramatic structural rearrangement to fuse the viral membrane with the host cell membrane .
- This process is triggered by the **S1 subunit** and a host–cell receptor binding, which destabilizes the pre-fusion trimer, resulting in the S1 subunit shedding and the S2 subunit transition to a highly stable post-fusion conformation . To engage a host–cell receptor, the **receptor-binding domain (RBD) of S1** undergoes hinge-like conformational movements that transiently hide or expose the determinants of receptor binding .



SARS-COV-2 VARIANT CLASSIFICATIONS AND DEFINITIONS

- **Viruses like SARS-CoV-2 continuously evolve as changes in the genetic code (genetic mutations) occur during replication of the genome**.
- Key Definitions:
- **Mutation:** A mutation refers to change in a virus's genome (genetic code). Mutations happen frequently, but only sometimes change the characteristics of the virus.
- **Lineage:** A lineage is a group of closely related viruses with a common ancestor. SARS-CoV-2 has many lineages; all cause COVID-19.

- **Variant:** A variant is a viral genome (genetic code) that may contain one or more mutations. In some cases, a group of variants with similar genetic changes, such as a lineage or group of lineages, may be designated by public health organizations as a Variant of Concern (VOC) or a Variant of Interest (VOI) due to shared attributes and characteristics that may require public health action.

VARIANT BEING MONITORED (VBM)

- Alpha (B.1.1.7 and Q lineages)
- Beta (B.1.351 and descendent lineages)
- Gamma (P.1 and descendent lineages)
- Epsilon (B.1.427 and B.1.429)
- Eta (B.1.525)
- Iota (B.1.526)
- Kappa (B.1.617.1)
- Mu (B.1.621, B.1.621.1)
- Zeta (P.21.617.3)

- Variants designated as VBM include those where data indicates there is a potential or clear impact on approved or authorized medical countermeasures or that have been associated with more severe disease or increased transmission but are no longer detected, or are circulating at very low levels, in the United States.

VARIANT OF INTEREST (VOI) / VARIANT OF CONCERN (VOC)

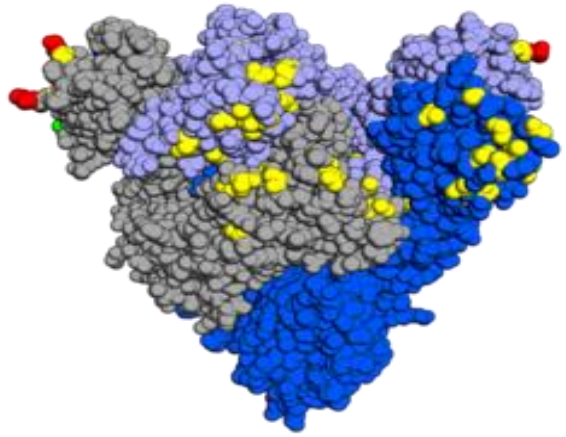
- **Delta** (B.1.617.2 and AY lineages)
- **Omicron** (B.1.1.529 and BA lineages)

- A variant for which there is evidence of an increase in transmissibility, more severe disease (for example, increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures

VARIANT OF HIGH CONSEQUENCE (VOHC)

- A VOHC has clear evidence that prevention measures or medical countermeasures (MCMs) have **significantly** reduced effectiveness relative to previously circulating variants.
- To date, no variants of high consequence have been identified in the United States

THE OMICRON



S gene dropout or S gene target failure :

- The Omicron variant has a total of 60 mutations compared to the original Wuhan variant: **Thirty-two mutations affect the spike protein**, the main antigenic target of antibodies generated by infections and of many vaccines widely administered. Many of those mutations had not been observed in other strains.The variant is characterised by 30 amino acid changes, three small deletions, and one small insertion in the spike protein compared with the original virus, of which 15 are located in the receptor-binding domain .
- It also carries a number of **changes and deletions in other genomic regions**. Additionally, the variant has three mutations at the furin cleavage site. The furin cleavage site increases SARS-CoV-2 **infectivity**.

LABORATORY ROLE

The global coronavirus disease 2019 (COVID-19) has presented **major challenges for clinical laboratories**, from initial diagnosis to patient monitoring and treatment .

Clinical laboratories are operating to **support the global fight** against this **ongoing pandemic .**

Initial response to this pandemic involved the **development**, **production**, and **distribution of diagnostic molecular assays** at an unprecedented rate .

In addition to molecular testing, **serological assays** to detect antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are now becoming available from numerous diagnostic manufacturers.

LABORATORY MEDICINE EFFICIENTLY CONTRIBUTE TO:

- **Etiological identification** of sars-cov2 infection
- Effectively involved in **epidemiologic surveillance**
- Determination of **prognosis**
- Patient **follow-up**
- **Therapeutic monitoring**

CRITICAL ROLES OF LABORATORY IN MANAGING COVID-19

Diagnostic tests :

- Molecular test :

RT-PCR (Gold standard)

OTHER NAAT

- Serology antibody testing

Anti-spike or Anti-RBD IgM, IgG, IgA

- Rapid antigen testing

Monitoring and prognostic tests:

- Hematology :

- CBC diff.

- Coagulation tests

PT, PTT, INR,D-Dimer, Fibrinogen level

- Inflammatory mediators :

CRP, ESR, Procalcitonin, Ferritin

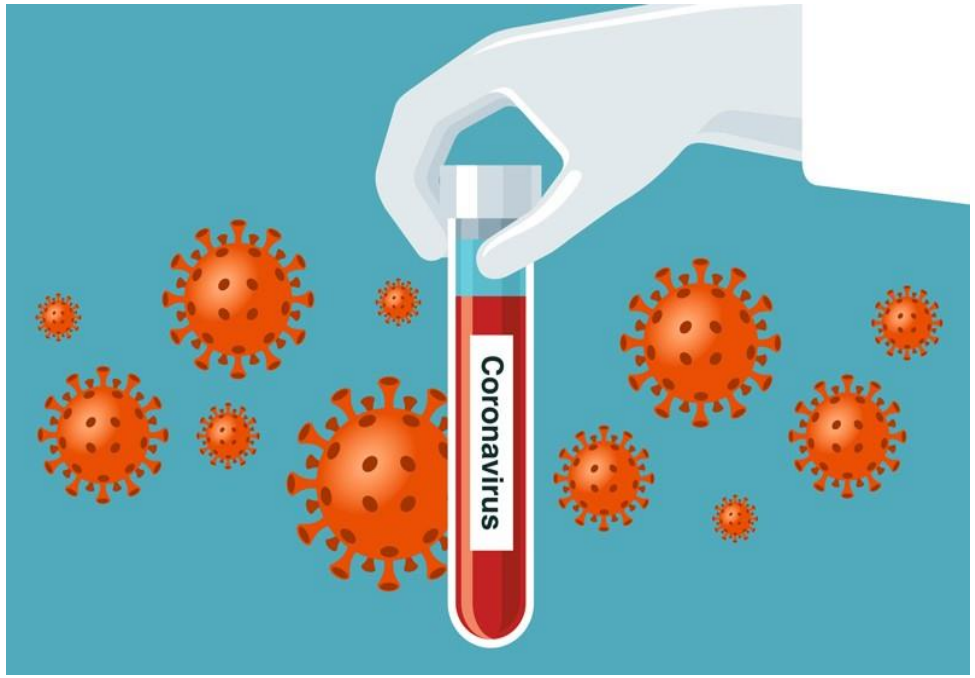
- Biochemical parameters :

LDH, AST, ALT, BILLI, ALB, Cr

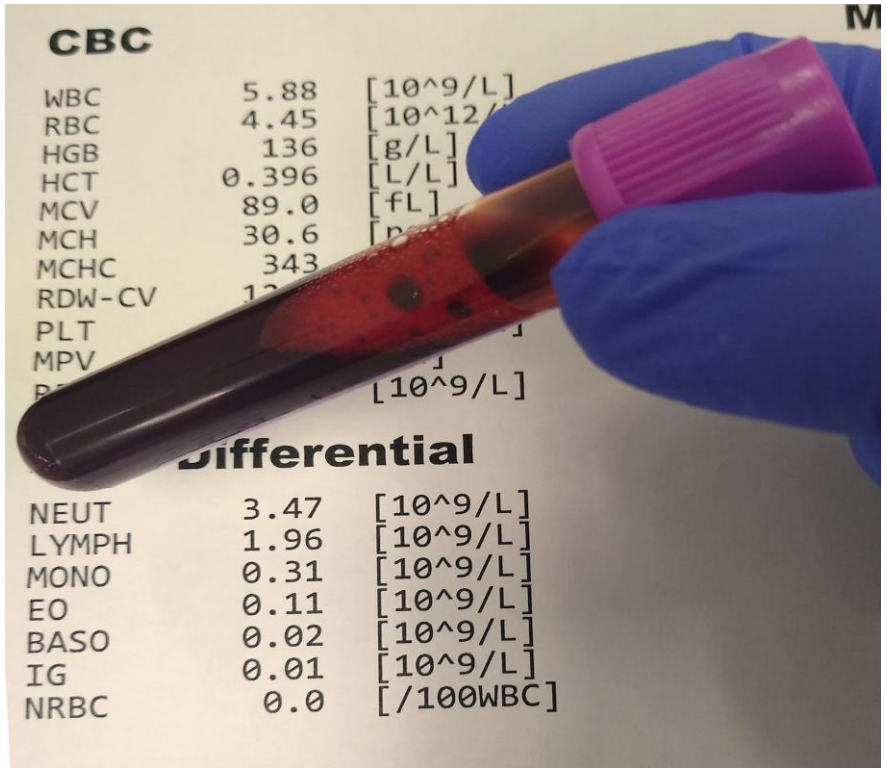
VALUE OF **CBC,COAGULATION TESTS**, AND **INFLAMMATION-RELATED** PARAMETERS

- To better represent how abnormal laboratory findings are important in COVID-19 diagnosis and prognosis, we searched the Scopus, PubMed, and Web of Science medical databases using the keywords “COVID-19,” “2019-nCoV,” or “coronavirus 2019.” We ultimately selected **19 articles** (**totaling 2988 patients**, 484 of whom (16.1%) were severely affected) that provided a panel of laboratory examinations in COVID-19 patients.

(Laboratory findings in COVID-19 diagnosis and prognosis, Clinica Chimica Acta Volume 510, November 2020, Pages 475-482)



CBC DIFF :



- **NORMAL LYMPHOCYE COUNT:**

ADULT: 1000-4800 per microliter of blood

CHILDREN: 3000-9500 per microliter of blood

- **LYMPHOPENIA :**

count less than 1000 per microliter of blood

- **THROMBOCYTOPENIA :**

Count less than 100,000 per microliter of blood

SOME REVIEW ARTICLE RESULTS:

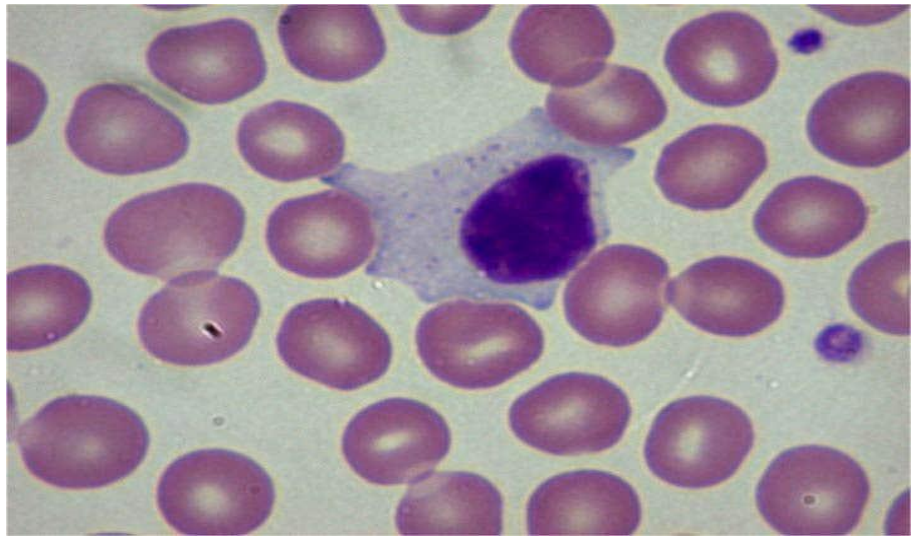
- Data from **15 published articles** reflecting the value of blood cell count and differential percentages of lymphocytes and neutrophils from patients with severe/non-severe COVID-19 showed:
- Lymphopenia is a prominent finding in most patients, some studies have reported an increased number of neutrophils.
- **Decreased lymphocytes accompanied by mild thrombocytopenia are among the most common abnormal findings** attracting attention in CBC of COVID-19 patients.
- Lymphocyte counts lower than 0.8×10^9/L may be associated with COVID-19 severity, number of neutrophils higher than 3.5×10^9/L may reflect a poor clinical outcome.
- Yang et al. reported that the **elevated** neutrophil-to-lymphocyte ratio (NLR) may predict COVID-19 prognosis.
- The results of a recent study revealed that the incidence of **critical illness** in COVID-19 patients aged more than 50 was 9.1% for patients having **NLR < 3.13**, while it was 50% for those with **NLR ≥ 3.13 .**

(J. Liu, Y. Liu, P. Xiang, L. Pu, H. Xiong, C. Li, M. Zhang, J. Tan, Y. Xu, R. Song: **Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage)**

(Ai-Ping Yang [1], Jian-Ping Liu [2], Wen-Qiang Tao [3], Hui-Ming Li · **The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients)**

POTENTIAL MECHANISM LEADING TO LYMPHOCYTE DEFICIENCY

- activation-induced **apoptosis** caused by inflammatory cytokine storm such as TNF-alpha and IL-6
- **Direct infection** of lymphocytes by the virus
- Virus may directly destroy **lymphatic organs** such as spleen and thymus
- **Suppresion of proliferation** due to metablic states caused by virus,such as hyperlactic acidemia



COAGULATION AND PRO-INFLAMMATORY FACTORS

- PT : 11-13.5 seconds
- PTT : 30-45 seconds
- INR : 0.8-1.1
- **D-DIMER** : 220- 500 ng/ml
- FIBRINOGEN : 200-400 mg/dl

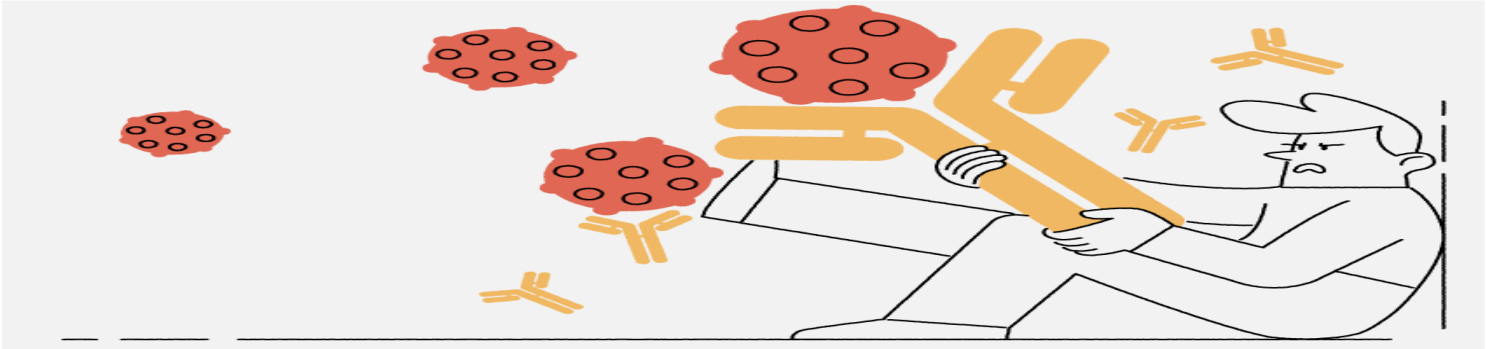
- CRP : Less than 3mg/l
- ESR : 1-13 and 1-20 mm/h
- FERRITIN : 24-336 and 11-307 micg/l
- PROCALCITONIN : Less than 0.05 nglml
- **IL-6** : Less than 3 or 6 pg/ml

BIOCHEMICAL PARAMETERS :

Hepatic, Renal and Cardiac biomarkers

- AST : 10-40 U/L
- ALT : 7-56 U/L
- LDH : 140-280 U/L
- CPK : 20- 200 TO 395 U/L
- ALBUMIN : 3.4-5.4 g/L
- CREATININ : 0.7-1.3 mg/dl
- TROPONIN : Less than 0.04 ng/L

SEROLOGY TESTS



- Serology testing for COVID-19 can be defined as analysis of **plasma, serum, or whole blood** for the detection of antibodies, especially immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA), that are specific for SARS-CoV-2 antigens, including the **spike glycoprotein** and **nucleocapsid** protein .
- Nearly all immunocompetent persons develop an adaptive immune response following SARS-CoV-2 infection, including B and T cell mediated immunity due to antiviral humoral and cellular immune responses, respectively.

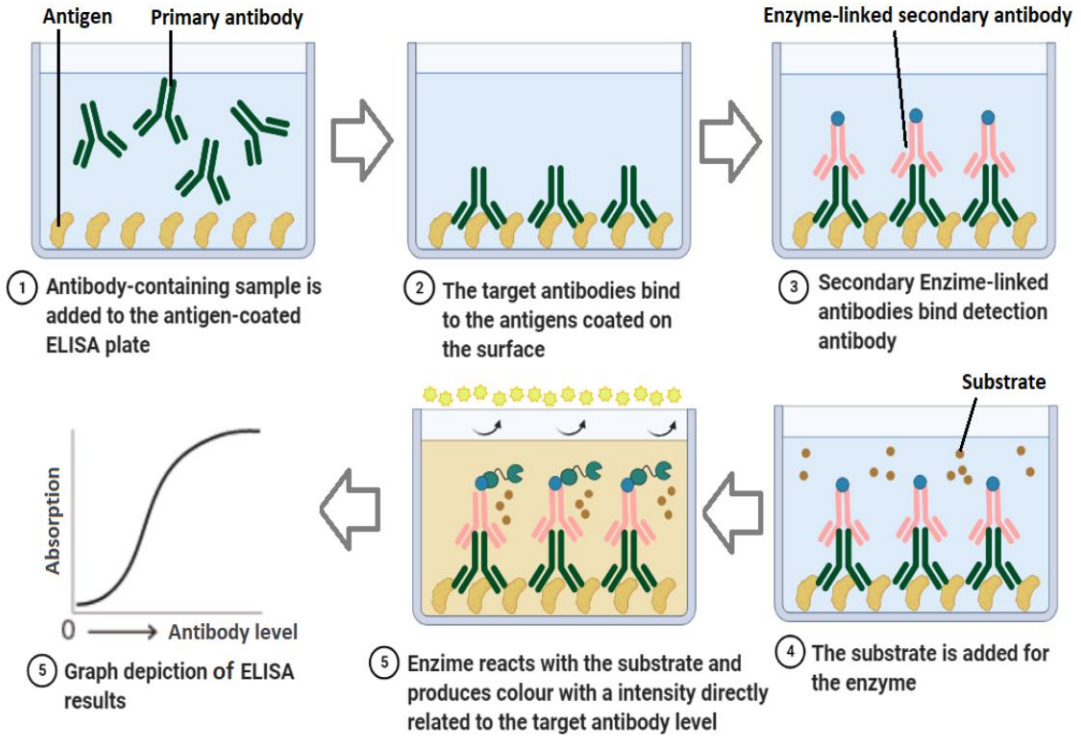
- In humans, the humoral response includes antibodies directed **against S and N proteins**. The S protein contains two subunits, S1 and S2. The S1 subunit contains the RBD that mediates binding of virus to susceptible cells. **RBD is the main target for neutralizing antibodies**. Antibodies—including IgM, IgG, and IgA—against S and its subunits can be detected in serum within 1-3 weeks after infection . **IgM and IgG antibodies can arise nearly simultaneously** ; however, IgM (and IgA) antibodies decay more rapidly than IgG .
- The clinical significance of measuring serum **IgA** in SARS-CoV-2 infection is **not known**; however secretory IgA plays an important role in protecting mucosal surfaces against pathogens by neutralizing respiratory viruses, including SARS-CoV-2

SEROLOGY TEST METHODS:

- Testing methodologies vary from rapid diagnostic tests used at the POC (disposable immunochromatographic lateral flow assays), to enzyme-linked immunosorbent assays (ELISA) or chemiluminescent immunoassays run on fully automated clinical laboratory instruments.

ELISA METHOD

- The **enzyme-linked immunosorbent assay (ELISA)** is an immunological assay commonly used to measure **antibodies, antigens, proteins** and **glycoproteins** in biological samples.
- In ELISA, various antigen-antibody combinations are used, always including an enzyme-labeled antigen or antibody, and enzyme activity is measured **colorimetrically.**
  The enzyme activity is measured using a substrate that changes color when modified by the enzyme. Light absorption of the product formed after substrate addition is measured and converted to numeric values.
  Depending on the antigen-antibody combination, the assay is called a direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA etc.



WHEN TO ORDER COVID-19 SEROLOGY TESTS ?

- Past COVID-19 infection
- To better assess the prevalence of infection at a population level
- **Confirming suspected cases**, especially in patients with either mild to moderate illness or tested in the late phase of COVID-19, not detected with molecular assays
- Identification of potential convalescent plasma donors
- Immunological/epidemiological surveillance and in monitoring immune responses of future COVID-19 illness

CDC COMMENT:

- Currently available antibody tests for SARS-CoV-2 assess IgM and/or IgG to one of two viral proteins: S or N. Because COVID-19 vaccines are constructed to encode the spike protein or a portion of the spike protein, **a positive test for S IgM and/or IgG could indicate prior infection and/or vaccination**. To evaluate for evidence of prior infection in a person with a history of COVID-19 vaccination, a test external icon that specifically evaluates **anti-N IgM/IgG** should be used.

PREANALYTICAL CONSIDERATIONS

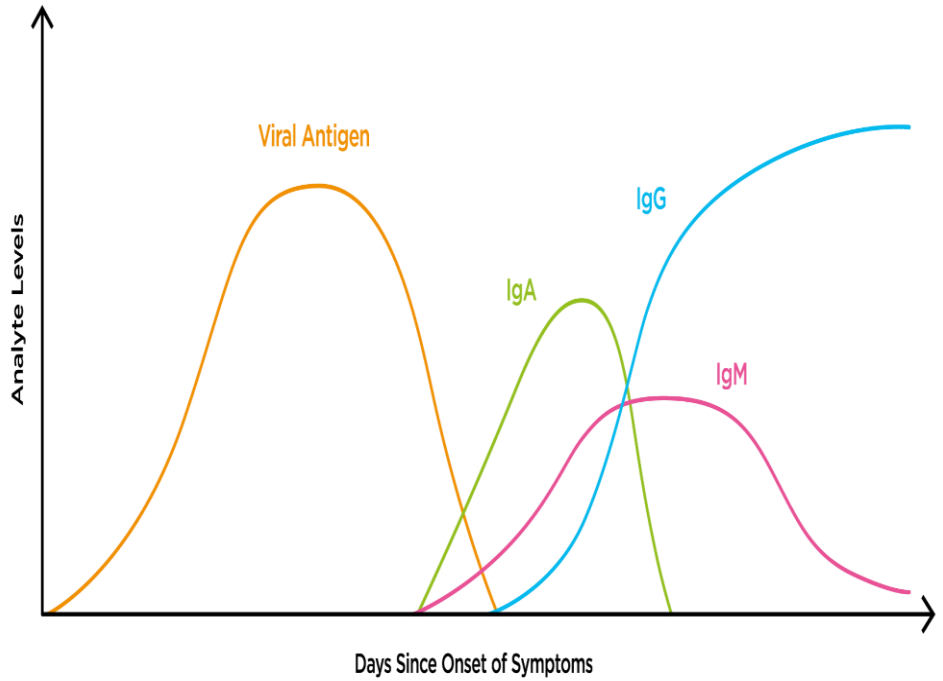
- **Time of testing**

The timeframe of serological testing and performance characteristics of antibody tests are closely linked. Accumulating data suggest that seroconversion occurs approximately 7–14 days after symptom onset .

Other studies using an internally developed ELISA report much earlier detection of IgM with one report indicating detectable IgM in 85% of COVID-19-confirmed patients 1–7 days after symptom onset .

- **Patient characteristics**

Another important consideration in serology testing is the patient population in which the test has been evaluated. Recent data suggest that patients with severe COVID-19 have higher IgG and IgM titers compared to those with non-severe COVID-19 .



ANALYTICAL CONSIDERATIONS

- **Cross-reactivity**

Preliminary claims in manufacturer package inserts are encouraging, with most companies indicating **no cross-reactivity**. However, the number of viral strains evaluated varies from manufacturer to manufacturer, and more peer-reviewed data are urgently needed.

- **Antibody target**

IgM is unlikely to play the primary role in COVID-19 antibody testing due to traditional specificity challenges associated with high false-positive rates

RAPID ANTIGEN TESTS

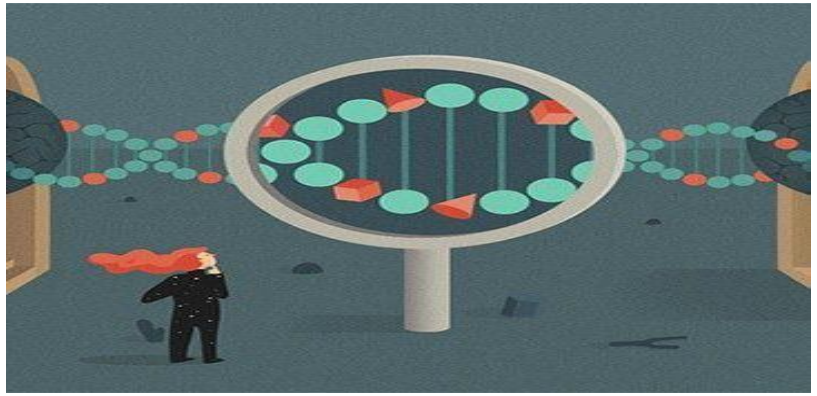
- Antigen tests are immunoassays that detect the presence of a specific viral antigen, which implies current viral infection. Antigen tests are currently authorized to be performed on **nasopharyngeal or nasal swab** specimens placed directly into the assay's extraction buffer or reagent.

- Antigen tests are relatively inexpensive, and most can be used at **the point-of-care**. Most of the currently authorized tests return results in approximately 15–30 minutes. Antigen tests for SARS-CoV-2 are generally less sensitive than RT-PCR and other nucleic acid amplification tests (NAATs) for detecting the presence of viral nucleic acid.
- However, NAATs can remain positive for weeks to months after initial infection and can detect levels of viral nucleic acid even when virus cannot be cultured, suggesting that the presence of viral nucleic acid may not always indicate contagiousness.
- Antigen tests have been used for screening testing for COVID-19 in congregate housing settings, such as nursing homes. This repeat testing has quickly identified people with COVID-19, informing infection prevention and control measures, thus preventing transmission. In this case, and where rapid test turnaround time is critical, there is value in providing immediate results with antigen tests, even though they may have lower sensitivity than NAATs.

INTERPRETATION CONSIDERATIONS

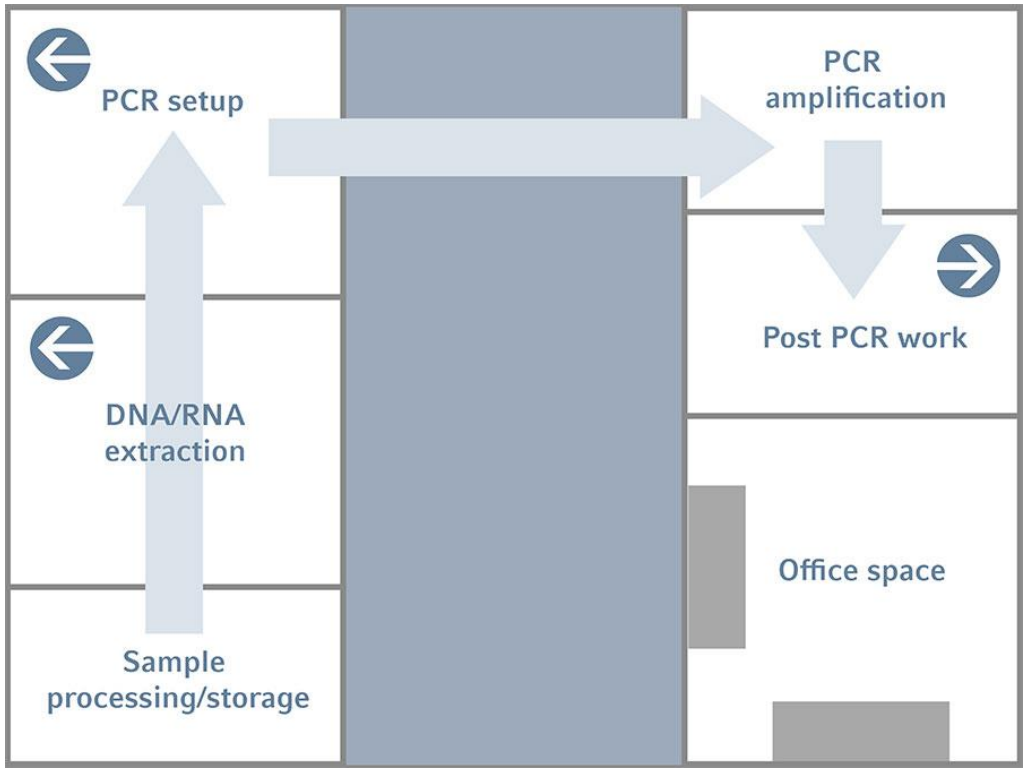
- The specificity of antigen tests is generally as high as most NAATs, which means **that false positive test results are unlikely** when an antigen test is used according to the manufacturer's instructions. Despite the high specificity of antigen tests, false positive results will occur, especially when used in communities where the prevalence of infection is low .
- In general, for all diagnostic tests, the lower the prevalence of infection in the community, the higher the proportion of false positive test results.

MOLECULAR TESTING : RT-PCR

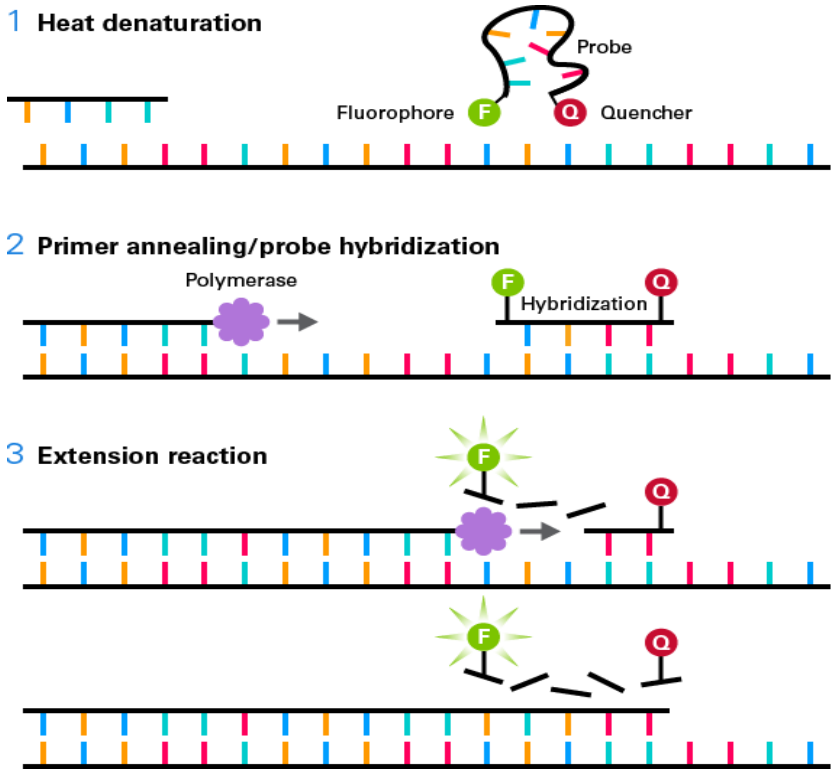


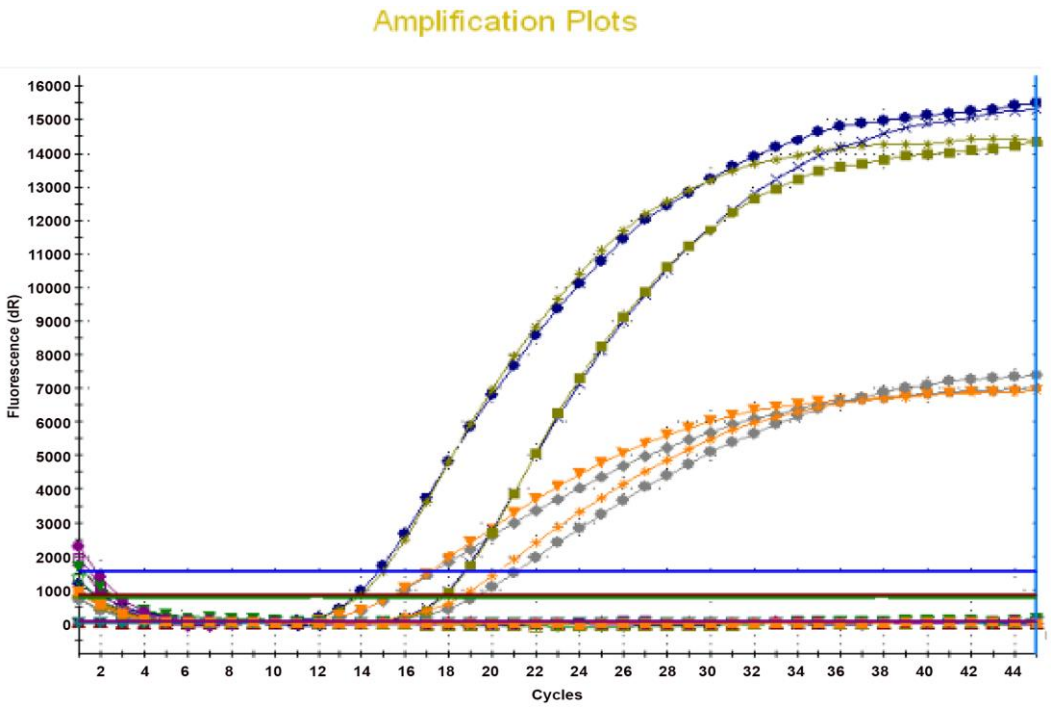
- The **reverse transcription polymerase chain reaction** (RT-PCR) is an enzymatic and chemical process by which short strands of ribonucleic acid (RNA) are converted to deoxyribonucleic acid (DNA) and **copied** in a doubling time reaction (amplification) to concentrations that can be detected and visualised by the human eye.
- This method has been in use for over two decades for the detection of viruses which have an RNA genome in a range of clinical samples, and most recently it is the primary method to confirm the presence of SARS-CoV-2. Following the discovery of the SARS-CoV-2 virus in China, the **full genome sequence** was released globally and this allowed for the development of RT-PCR tests to detect the virus.
- This was a **vital step**, as to specifically detect any virus using RT-PCR prior knowledge of the sequence is required, as it is short genome fragments that the test targets to amplify.

SAMPLE PROCCESSING



PCR TEST PRINCIPLE :

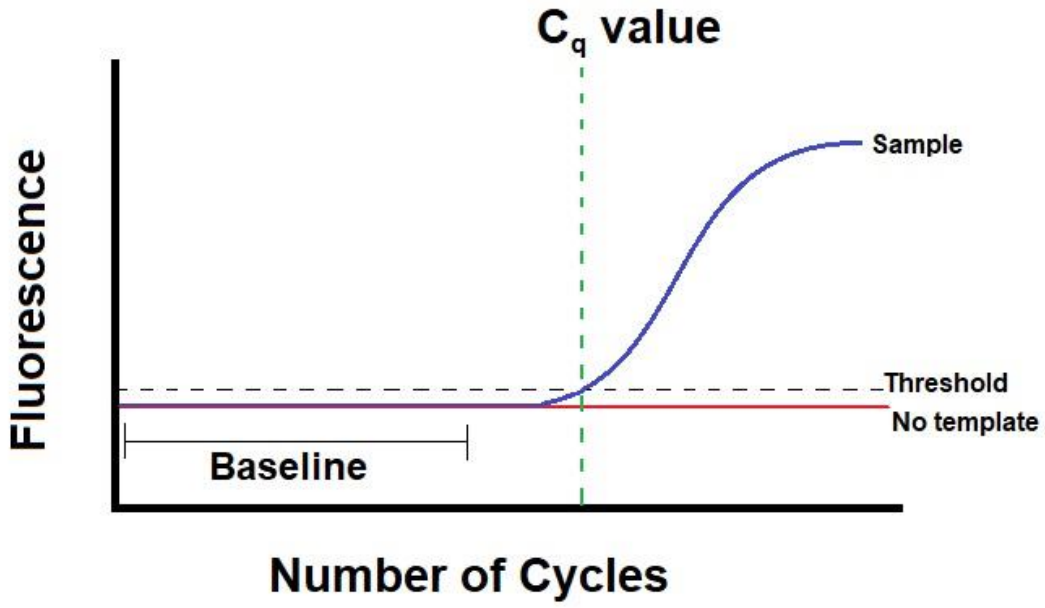




CYCLE THRESHOLD (CT) VALUE

What does CT mean?

- In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal. The CT (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (exceeds background level).
- CT levels are inversely proportional to the amount of target nucleic acid in the sample : the lower the CT level the greater the amount of target nucleic acid in the sample.



PCR TEST INTERPRETATION

Ct Value	Indication	Interpretation
<25	High levels of SARS-CoV-2 genomic load	Patients with higher SARS-CoV-2 genomic loads are more likely to develop severe outcomes and require intubation and severe outcomes. Patient needs to be monitored.
25-30	Moderate levels of SARS-CoV-2 genomic load	
>30	Low levels of SARS-CoV-2 genomic load	Low SARS-CoV-2 genomic load can be found early in infection when viral replication has just begun. Additionally, it can indicate the later phases of infection after the virus has been cleared and has left behind remnants of its genomic content. Interpretation requires clinical context.

MATERIALS INCLUDED IN THE 2019-NCOV REAL-TIME RT-PCR DIAGNOSTIC PANEL

- The 2019-nCoV Real-Time RT-PCR Diagnostic Panel contains four reagents: **(CDC 's laboratory kit)**
- Three primer-probe mixes for:
  - 2019-nCoV_**N1**: targets virus nucleocapsid (N) gene for specific detection of SARS-CoV-2
  - 2019-nCoV_**N2:** targets virus nucleocapsid (N) gene for specific detection of SARS-CoV-2
  - RP: targets human **RNase P gene** for detection of human nucleic acids; control for sample integrity
- nCoVPC: noninfectious positive control material; yields a positive result in each assay included in the panel
- OTHER KITS: **N, RdRp**

  N, S, RdRp

CDC **INFLUENZA SARS-COV-2** (FLU SC2) MULTIPLEX ASSAY

- The CDC Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay is a (RT-PCR) test that detects and differentiates RNA from SARS-CoV-2, influenza A virus, and influenza B virus in upper or lower respiratory specimens.
- The assay provides a sensitive, nucleic-acid-based diagnostic tool for evaluation of specimens from patients in the **acute phase of infection.**
- The CDC Flu SC2 Multiplex Assay is a four-in-one assay that includes:
- One primer mix and one probe mix. Primers and probes target:
  - Virus nucleocapsid (**N)** gene for specific detection of **SARS-CoV-2**
  - Matrix (**M1)** gene for specific detection of **influenza A** virus
  - Nonstructural 2 **(NS2**) gene for specific detection of **influenza B** virus
  - **RNase P** gene (RP) for specific detection of human nucleic acid that serves as an internal control
- Positive controls: SC2PC and Seasonal Influenza Positive Control (SIPC), that together confirm all four targets in the assay are working correctly

PREANALYTICAL CONSIDERATIONS

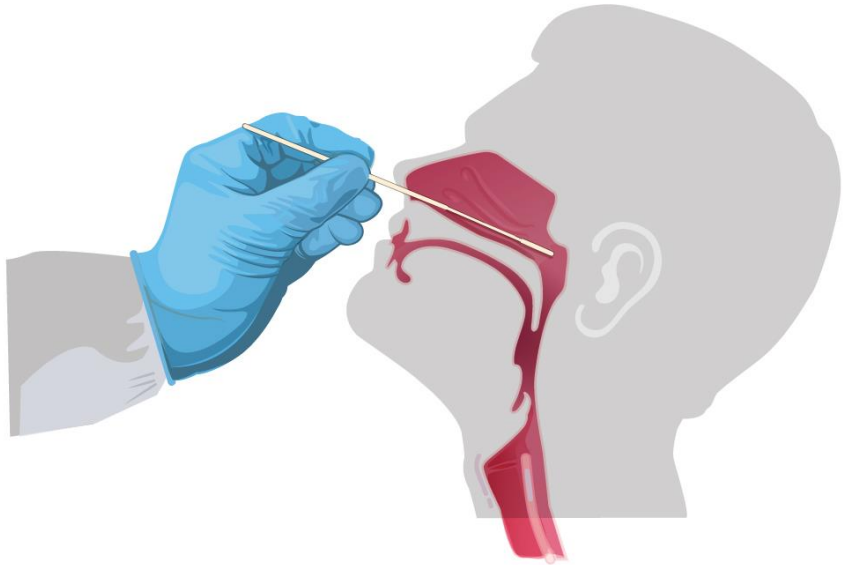
Specimen types :

- Upper respiratory specimens include nasopharyngeal (NP), oropharyngeal (OP, i.e. throat), nasal mid-turbinate (NMT), and anterior nares swabs, along with NP wash/aspirate or nasal wash/aspirate.
- Lower respiratory tract specimens include sputum, bronchoalveolar lavage (BAL), tracheal aspirate, pleural fluid, and lung biopsy.
- Salivary
- FECES
- CSF

SPECIMEN COLLECTION

Instructions for collecting NP specimen (performed by a trained healthcare provider):

- Tilt patient's head back 70 degrees.
- Gently and slowly insert a minitip swab with a flexible shaft (wire or plastic) through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx.
- Gently rub and roll the swab.
- Leave swab in place for several seconds to absorb secretions.
- Slowly remove swab while rotating it. Specimens can be collected from both sides using the same swab, but it is not necessary to collect specimens from both sides if the minitip is saturated with fluid from the first collection.
- If a deviated septum or blockage create difficulty in obtaining the specimen from one nostril, use the same swab to obtain the specimen from the other nostril.
- Place swab, tip first, into the transport tube provided



TIME OF TESTING

- The diagnostic testing window is perhaps one of the most important factors impacting test sensitivity. False negatives may be caused by **low viral loads in the early and late stages of infection.**
- 1-3 days **after onset of symptoms**
- 5-7 days for **asymptomatic** ones who were in close contact with covid-19 patients
- Afternoon hours (**around 2 pm**)

OTHER PREANALYTICAL ISSUES

- preanalytical issues include inadequate procedures for collection, handling, **transport and storage** of the specimens (especially OP and NP swabs), as well as **inadequate sample material in** terms of **poor quality or volume** .
- **Interfering substances** present in the specimens, **sample contamination.**

ANALYTICAL CONSIDERATIONS

- Pippeting errors
- Thermal cycler adjustments
- Trained molecular staffs and **EXPERTISE** in data analysis
- Gene target :

mismatches between the RNA sequence of the specimen and the primer and probes of the assay, which can lead to false negatives


MANY THANKS
FOR YOUR
ATTENTION!