شاخص های شناسایی ویروس و روش PCR

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SARS-CoV-2: The Virus

- **Enveloped, with a ssRNA genome**
- **4 Coronavirus genera**
  - *Alphacoronavirus (Mammals)*
    - **229E and NL63**
  - *Betacoronavirus (Mammals)*
    - **OC43 and HKU1**
    - **SARS-CoV (2002-2003)**
    - **MERS-CoV (2012)**
    - **SARS-CoV-2 (2019-?)**
  - *Gammacoronavirus (Birds)*
  - *Deltacoronavirus (Birds)*
- **Bats are the natural reservoir for SARS-CoV-2**
  - **Pangolins and/or turtles as intermediate hosts?**

SARS-CoV-2: The Virus

- **Order *Nidovirales*,**
- **Family *Coronaviridae*,**
- **Subfamily *Orthocoronavirinae***
- **Genus *Betacoronavirus* (Alpha, Beta, Gamma and Delta)**

- **Similar to other coronaviruses, SARS-CoV-2 is an enveloped, positive sense, single stranded RNA virus with a genome of nearly 30,000 nucleotides**

- **Six kinds of human CoVs have been previously identified**
- **HCoV-NL63 and the HCoV-229E (Alphacoronavirus genus)**

- **HCoV-OC43**
- **HCoVHKU1**
- **SARS-CoV**
- **MERS-CoV which belong to the Betacoronavirus genus**

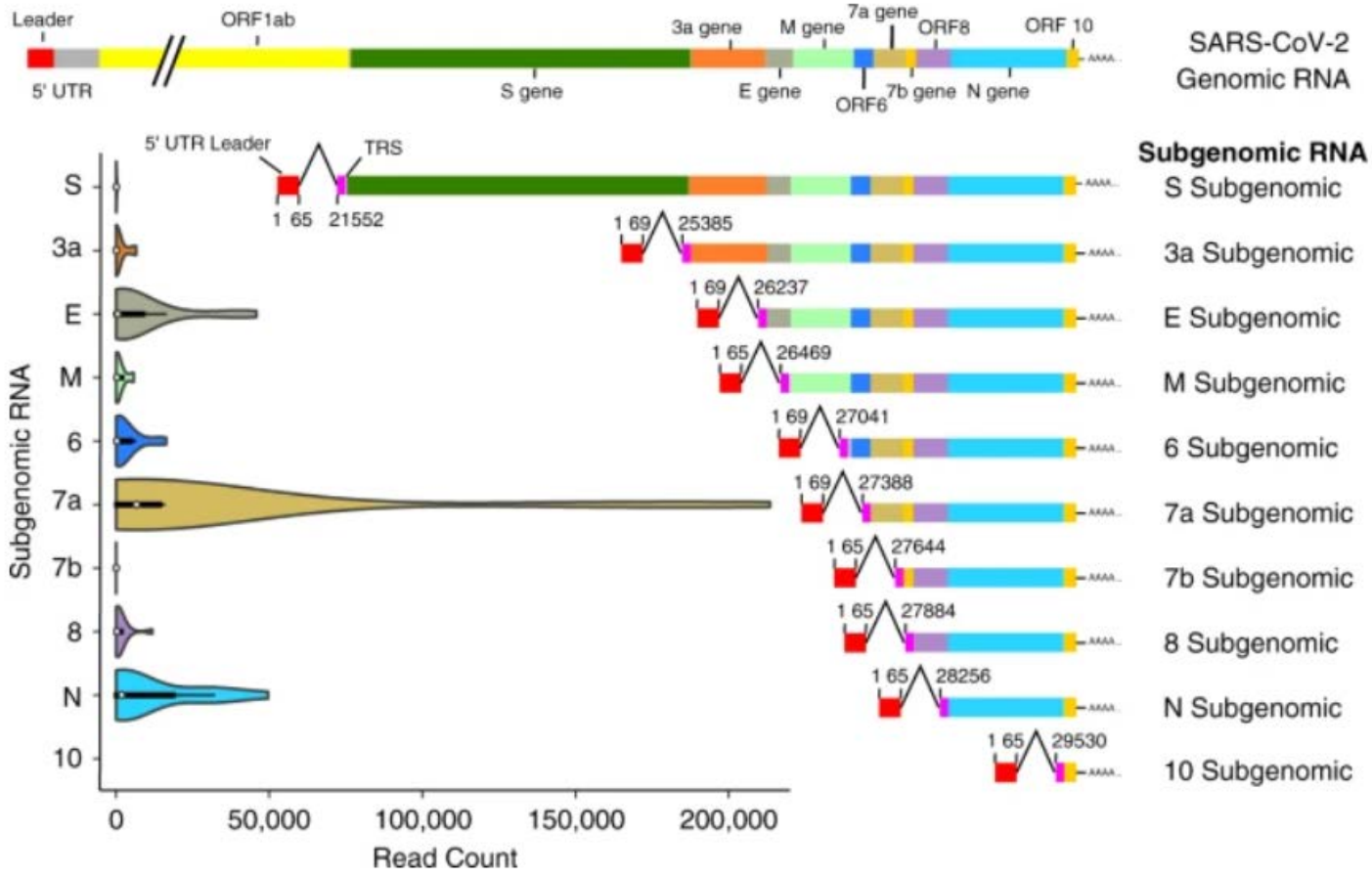
SARS-CoV-2: Replication & transcription

- After having entered the host cell, replication of coronaviruses initially involves generation of a complementary negative sense genome length RNA for amplification of plus strand virus genome RNA, as well as transcription of a series of plus strand subgenomic RNAs all with a common leader joined to gene sequences in the 3′-end of the virus genome.

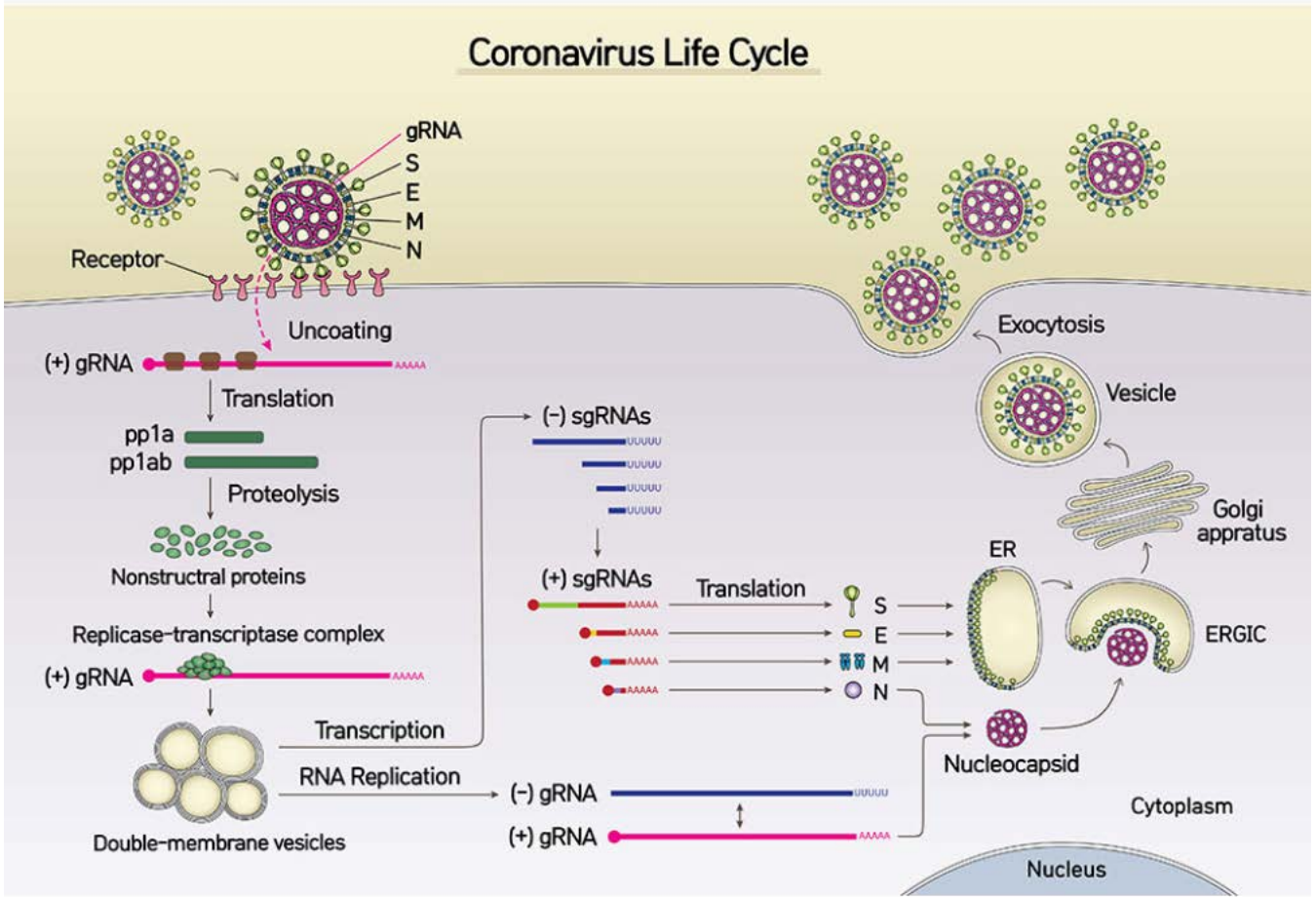
SARS-CoV-2: Replication & transcription

- When the spike protein of SARS-CoV-2 binds to the receptor of the host cell, the virus enters the cell, and then the envelope is peeled off, which lets genomic RNA be present in the cytoplasm.
- The ORF1a and ORF1b RNAs are made by genomic RNA, and then translated into pp1a and pp1ab proteins, respectively.
- Protein pp1a and ppa1b are cleaved by protease to make a total of 16 nonstructural proteins.
- Some nonstructural proteins form a replication/transcription complex RdRp, which use the (+) strand genomic RNA as a template.
- The (+) strand genomic RNA produced through the replication process becomes the genome of the new virus particle.
- Subgenomic RNAs produced through the transcription are translated into structural proteins (S: spike protein, E: envelope protein, M: membrane protein, and N: nucleocapsid protein), which form a viral particle.
- Spike, envelope, and membrane proteins enter the endoplasmic reticulum, and the nucleocapsid protein is combined with the (+) strand genomic RNA to become a nucleoprotein complex. They merge into the complete virus particle in the endoplasmic reticulum-Golgi apparatus compartment, and are excreted to extracellular region through the Golgi apparatus and the vesicle.

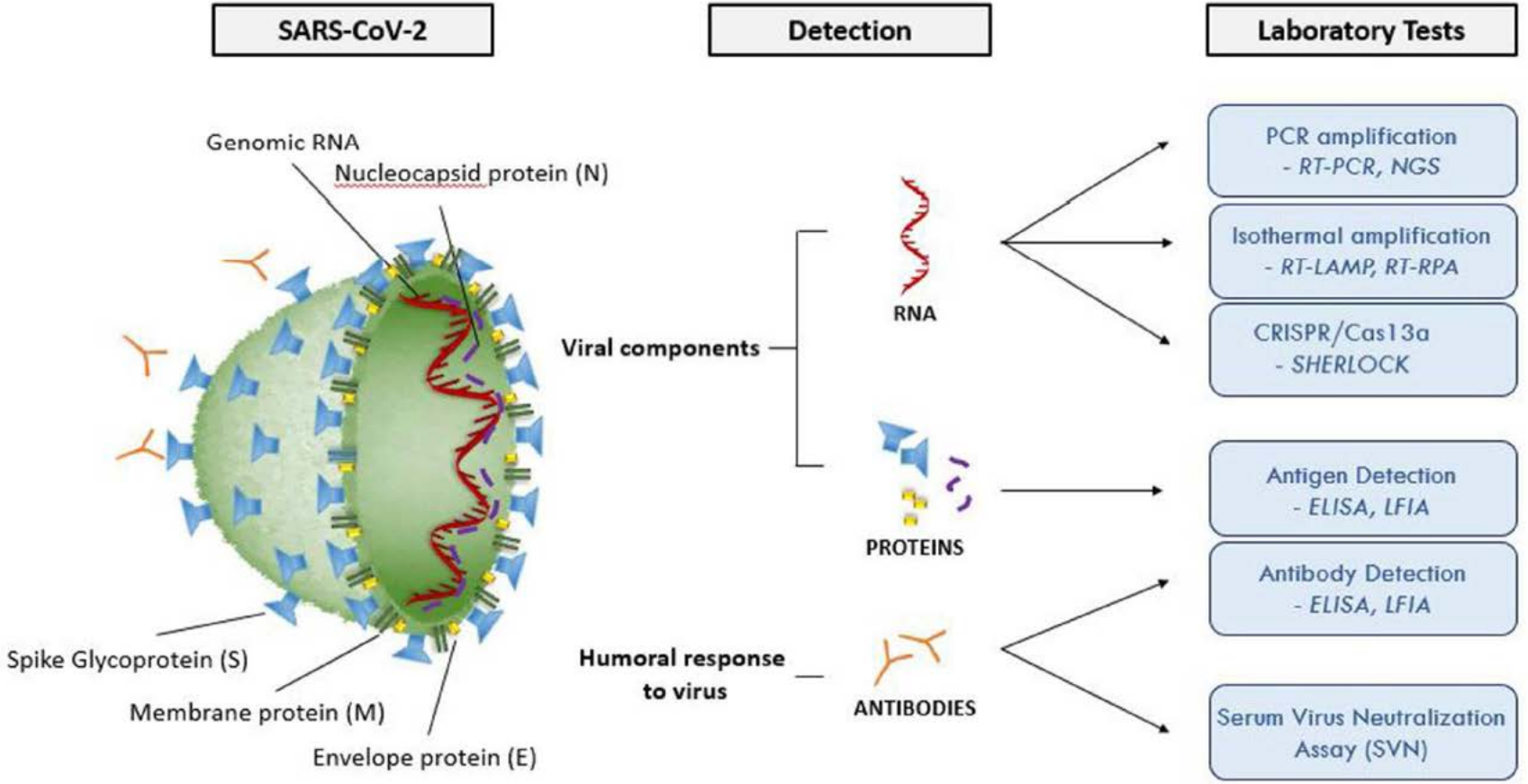
SARS-CoV-2 Genomic & Subgenomic RNA



SARS-CoV-2: Replication & transcription



Molecular structure of SARS-CoV-2 and summary of the available laboratory tests and their target molecules.



Laboratory Testing of COVID-19

Virusisolation: dangerous, slow, impractical.

- Molecular assay
- –Diagnosis of acute infection, very sensitive and specific
- –Rapid development is possible, based on sequence information
- –Expensive, devices

Immunoassay

- –Cheap, less sensitive, less specific, slow development for reliable kits
- –Antigen: no amplification → low sensitivity, only for limited-resource setting.
- –Antibody: diagnosis of asymptomatic/past infection, sero-surveillance
- •High levels occur in the second and third week of illness.

Reverse-transcription real-time PCR (rRT-PCR)

- Target genes: *E*, *RdRp*, *N,S*
- Diagnosis
- –Areas with no known COVID-19 virus circulation
- At least two different targets
- One positive NAAT results and sequencing
- –Areas with established COVID-19 virus circulation
- Screening by RT-PCR of a single discriminatory target is possible
- –One or more negative results do not rule out the possibility of COVID-19
- Poor quality of specimen or transport problem
- Very early and late stage of COVID-19
- Technical problem: virus mutation or PCR inhibition (Internal control)

Current Diagnostic Tests for COVID-19

- The symptoms expressed by COVID-19 patients are nonspecific and cannot be used for an accurate diagnosis:

  Fever , Cough, fatigue, sputum production and shortness of breath

- The first genome sequence of SARS-CoV-2 was added to the GenBank sequence repository on January 10, 2020.

- Since then, more than 1000 sequences have been made available on the Global Initiative on Sharing All Influenza Data (GISAID) and GenBank

A number of RT-PCR kits for detection of SARS-CoV-2

Institute	Targets
CDC, US	N gene (Three set)+Rnase P
CDC, China	ORF1ab+N gene
Charite,Gemany	RdRp+E
Hong kong University	ORF1b (nsp14)+N
Nationa Institute of Infectious Diseases, Japan	N gene
Nation Institute of Health, Thailand	N gene
Pasteur Institut , France	RdRp, E

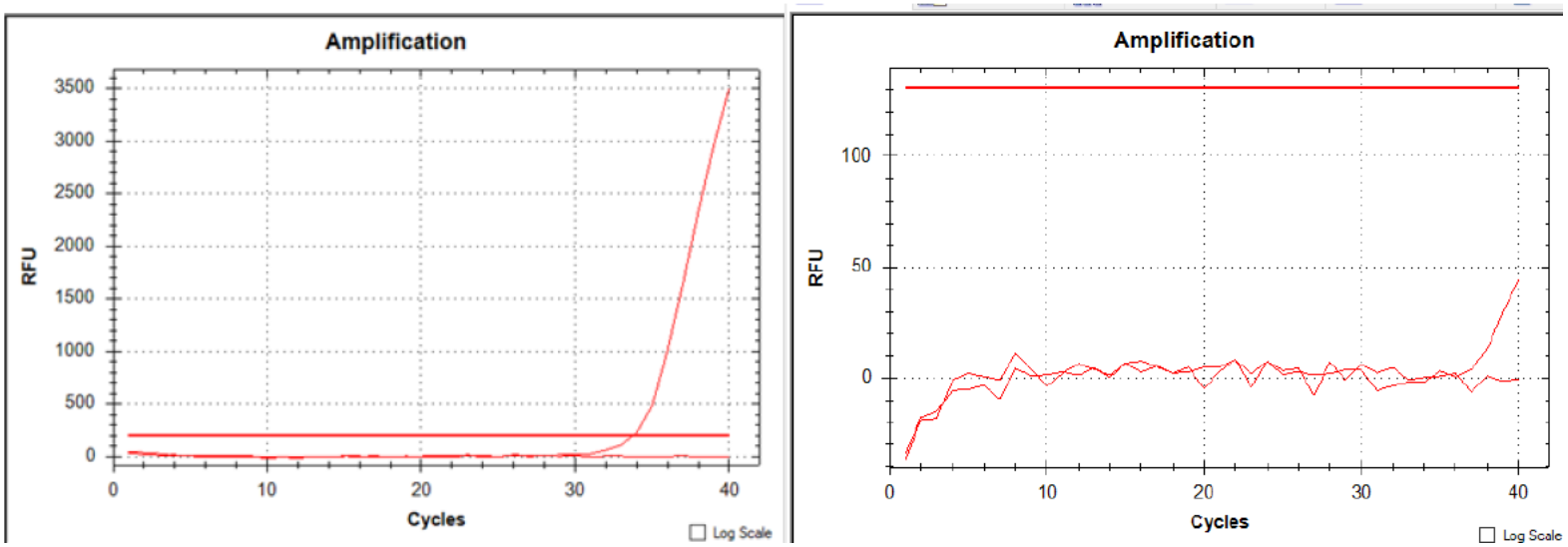
Corman et al., 2020

- Conserved sequences:
- (1) the RdRP gene in the open reading frame ORF1ab region
- (2) the E gene
- (3) the N gene
- Both the RdRP and E genes had high analytical sensitivity for detection (technical limit of detection of 3.6 and 3.9 copies per reaction),
- whereas the N gene provided poorer analytical sensitivity (8.3 copies per
- reaction).

- One-Step Assay (reproducible, lower target amplicon generation)
- Two-Step Assay (more sensitive, more timeconsuming)

Retesting is always important

- Values close to the cut-off values in specimens with low viral loads may indicate false-negative or false-positive results. Thus, a laboratory physician should interpret the results and if necessary, retest using residual or new specimens.



Advantages of RT-PCR

- RT-PCR is the frontline diagnostic test for COVID-19 that is capable of analyzing thousands of specimens in a single day and shows a testing sensitivity of 95% (Corman et al., 2020).

- The anticipated limit of detection of the SARS-CoV-2 RT-PCR test is <10 copies/reaction (Chu et al., 2020) which allows early detection of low viral titers. Gene amplification indicates a positive result for the presence of SARS-CoV-2 RNA and should correlate with clinical observations, patient history, and epidemiological information.

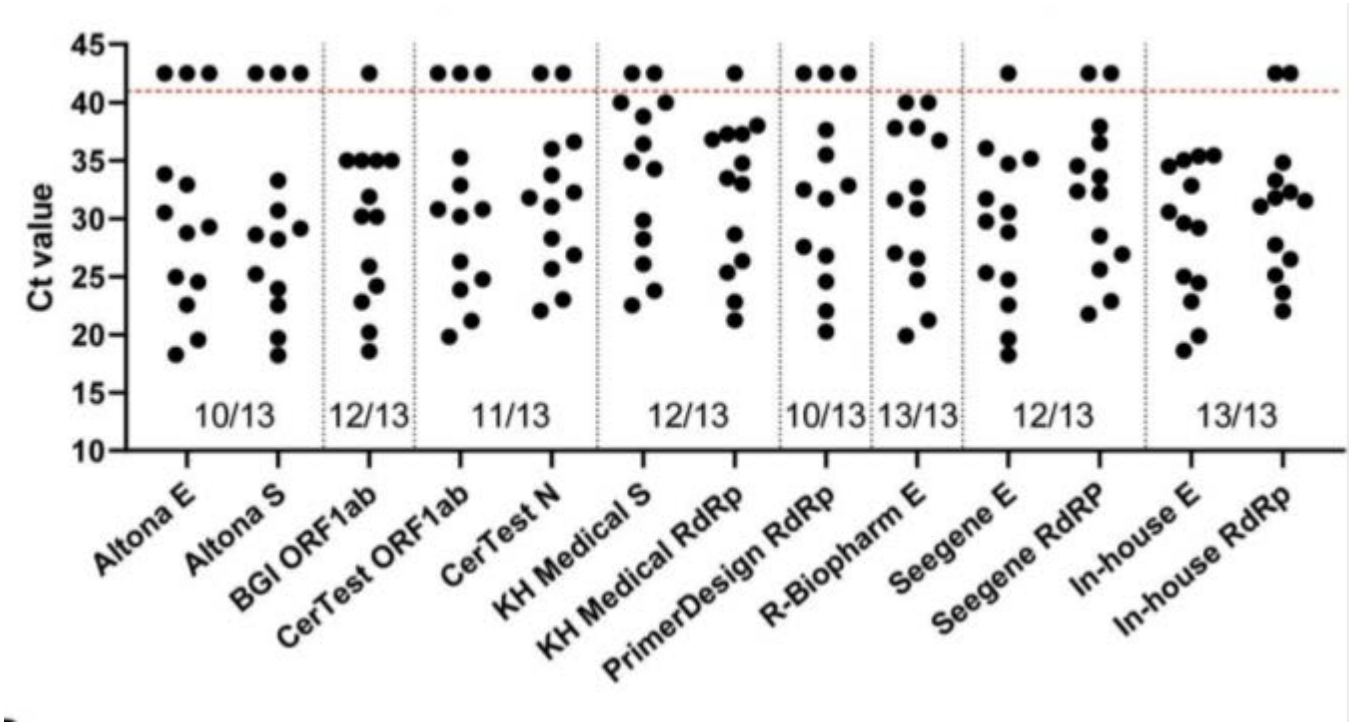
Disadvantages of RT-PCR

- False-negative results could potentially arise from mutations occurring in the primer and probe target regions in the SARSCoV-2 genome. Negative results do not preclude SARS-CoV-2 infection, and results should be validated with different primer
- sets against the same gene target and combined with patient history and other clinical data to accurately determine patient infection status.

- False positive results could be generated by cross-reactivity of primers with nucleic acids arising from co-infection with other viruses or bacteria. In these cases, the agent detected may not be the definite cause of disease. False positives can also occur if reagents in a laboratory become contaminated, which is a major concern, particularly with the high volume of testing encountered during a pandemic. A negative patient sample is useful to identify this error in testing

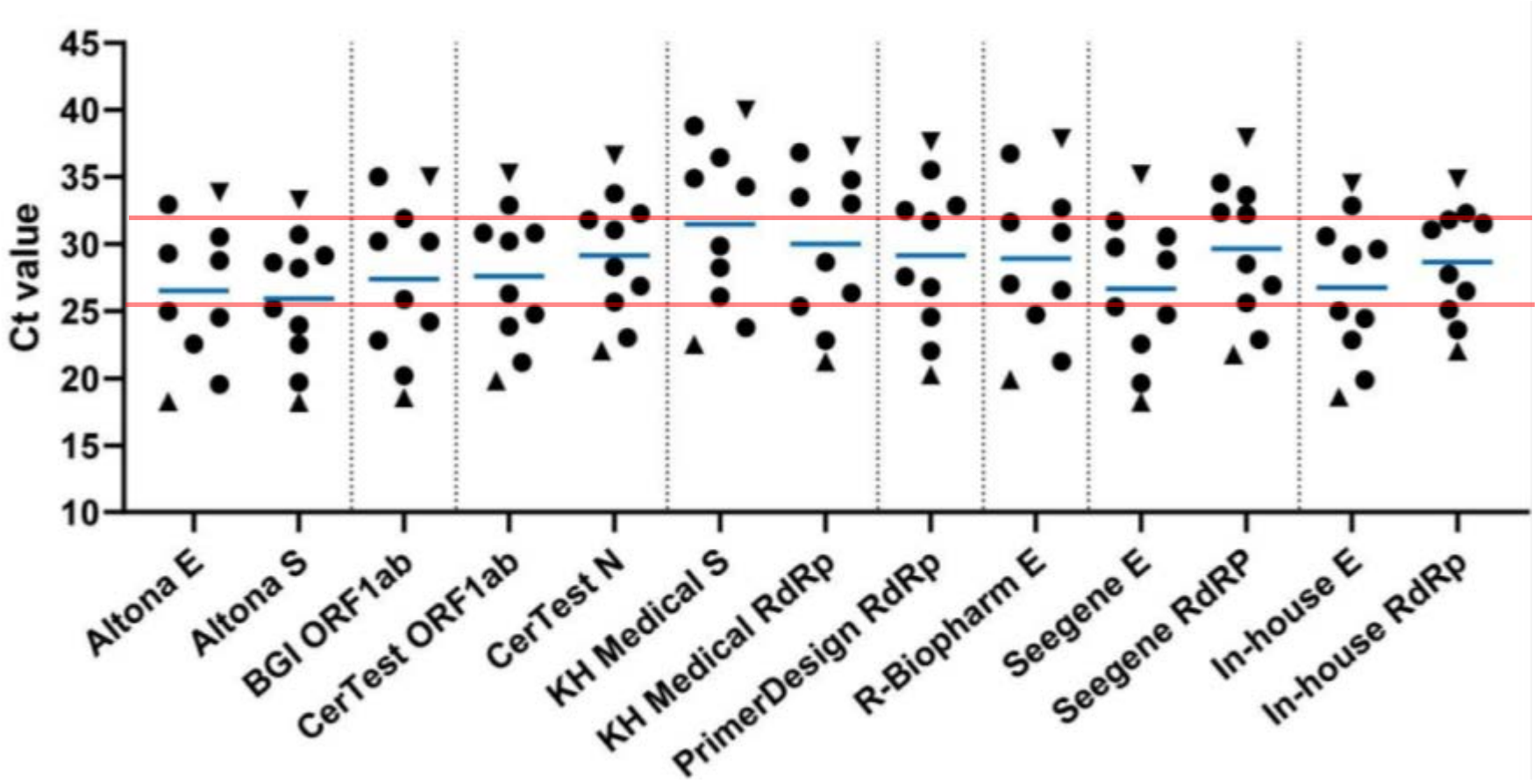
Comparison of seven commercial RT-PCR diagnostic kits for COVID-19

Altona Diagnostics,
BGI,
CerTest Biotec,
KH Medical,
PrimerDesign
R-Biopharm AG
Seegene

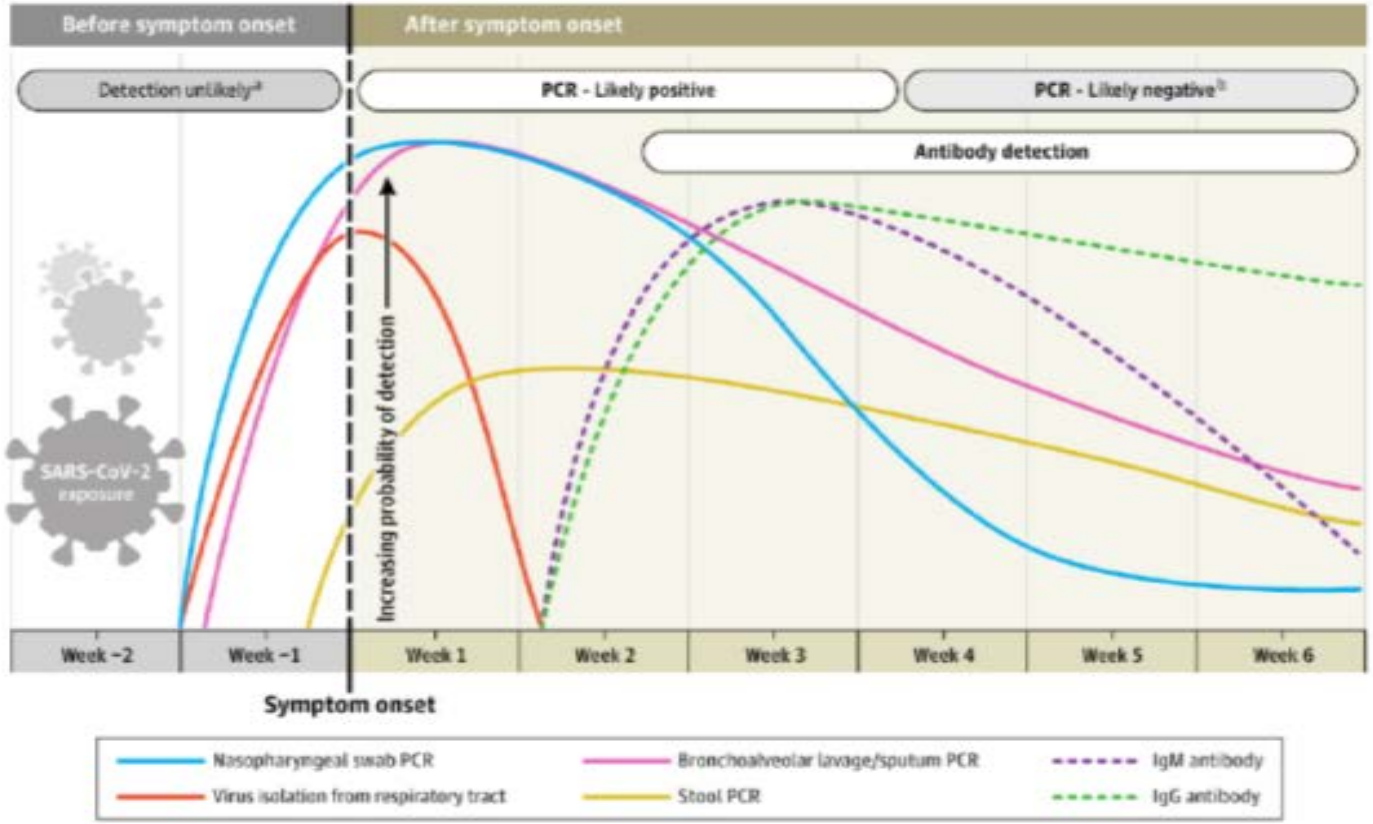


van Kasteren et al., 2020,

Comparison of seven commercial RT-PCR diagnostic kits for COVID-19



Estimated variation over time in COVID-19 testing



Sethuraman et al. JAMA. May 6, 2020

Thank You