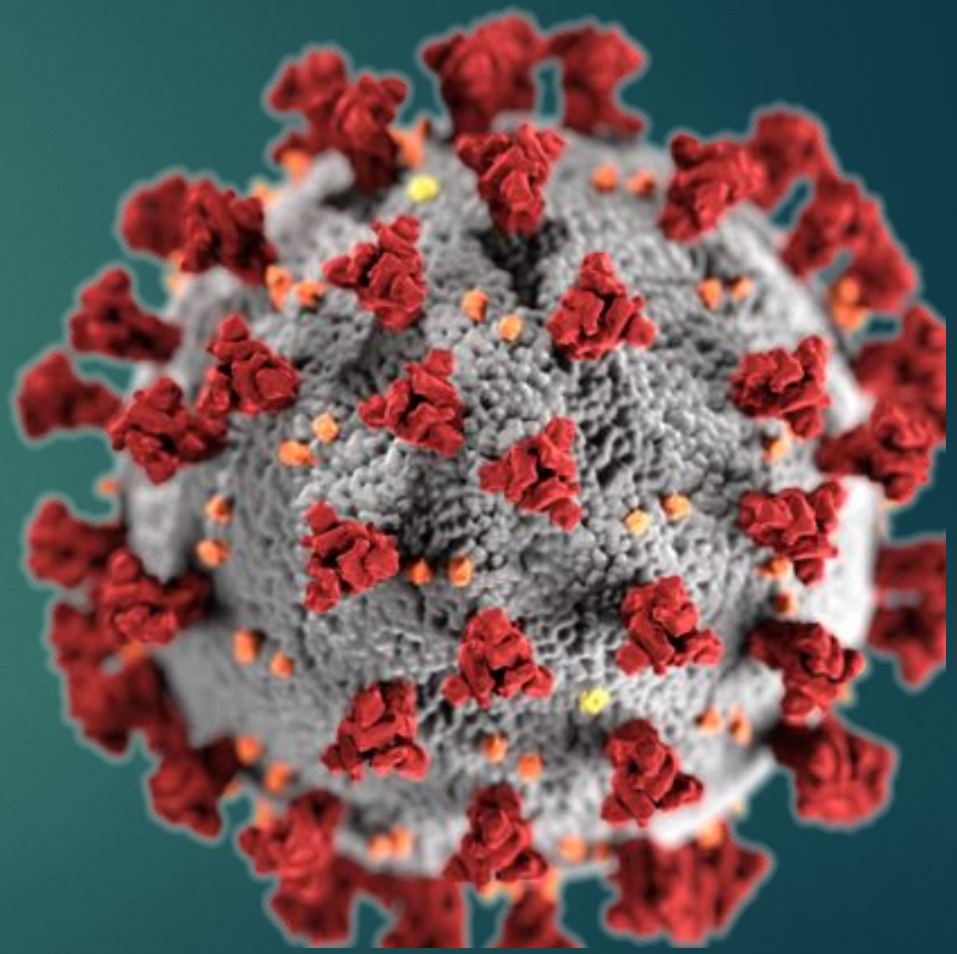
The Basic Science of SARS-CoV-2



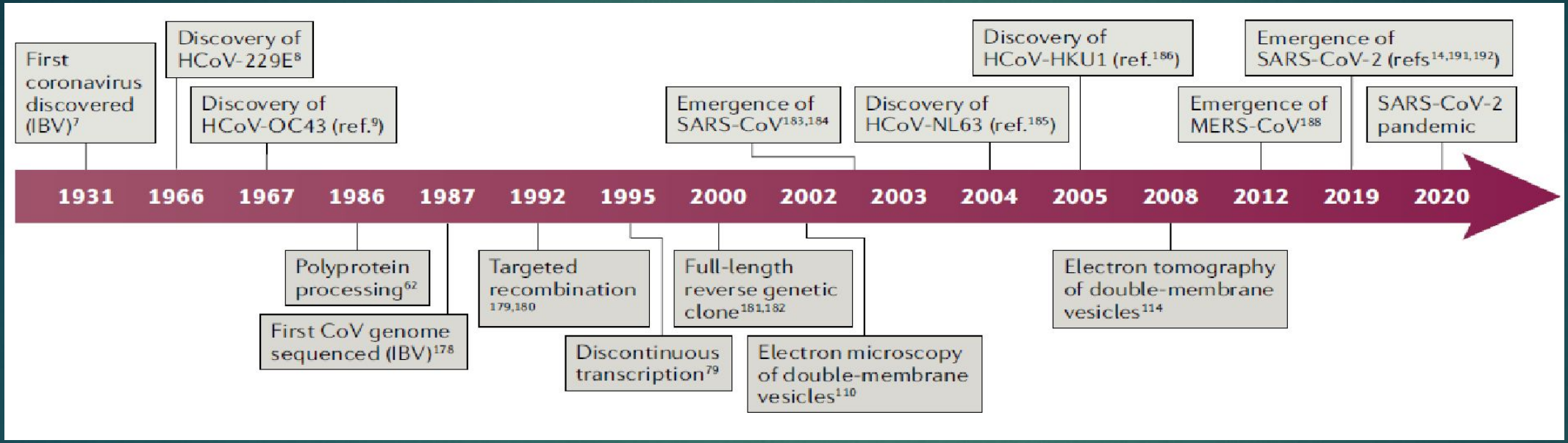
GLOBAL COMMUNITY LECTURE SERIES

DR S DUBE

Agenda

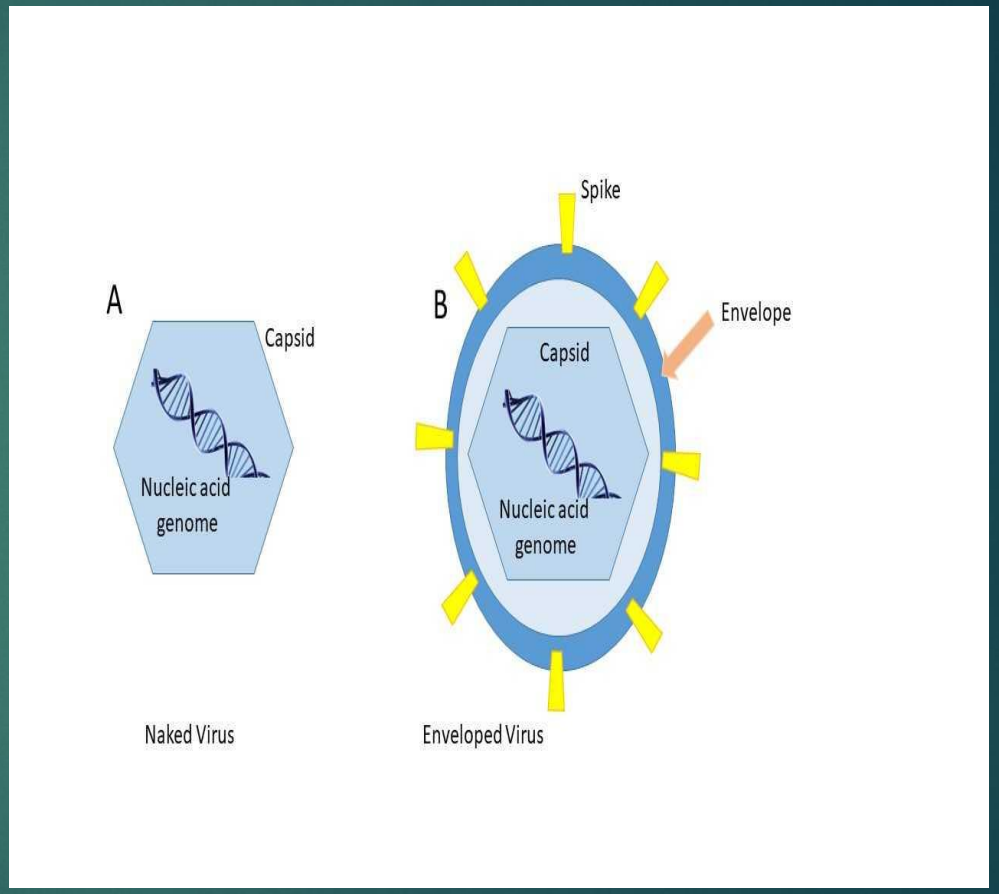
- Epidemiology
- Basics of virology
- Viral nomenclature
- Structure of the virus
- Testing
- Life cycle of the virus
- Natural history of the disease

History of Coronaviruses



What is a virus

- Sub-microscopic particle consisting of nucleic acid encased in some type of protective shell (the viral capsid) to form the viral genome
- Naked or enveloped
- The genomes of viruses can be RNA or DNA and can be single or double-stranded and, in some cases, the virus can have several genome segments.
- Proteins on the surface of the viral capsid, or that extend through the viral envelope (e.g., spike proteins), are generally the proteins used for attachment to host cells



Viral classification

SARS-CoV-2 is a coronavirus of the beta-coronavirus subfamily. It is an enveloped virus with an RNA genome. Animal coronavirus infections mainly result in respiratory and enteric diseases

- Viruses are classified by
  - Morphology (shape)
  - Genome material (RNA or DNA)
  - Type of replication
  - Host
  - Type of disease

Coronaviruses (CoVs)

- Order Nidovirales
  - Suborder of Coronavirineae
  - Family Coronaviridae.
  - Subfamily of Orthocoronavirinae,
  - Genera: alphacoronavirus, betacoronavirus, gammacoronavirus and deltacoronavirus.
    - Human and aWhereas alphacoronaviruses and betacoronaviruses exclusively infect mammalian species,
    - gammacoronaviruses and deltacoronaviruses also infect avian species.

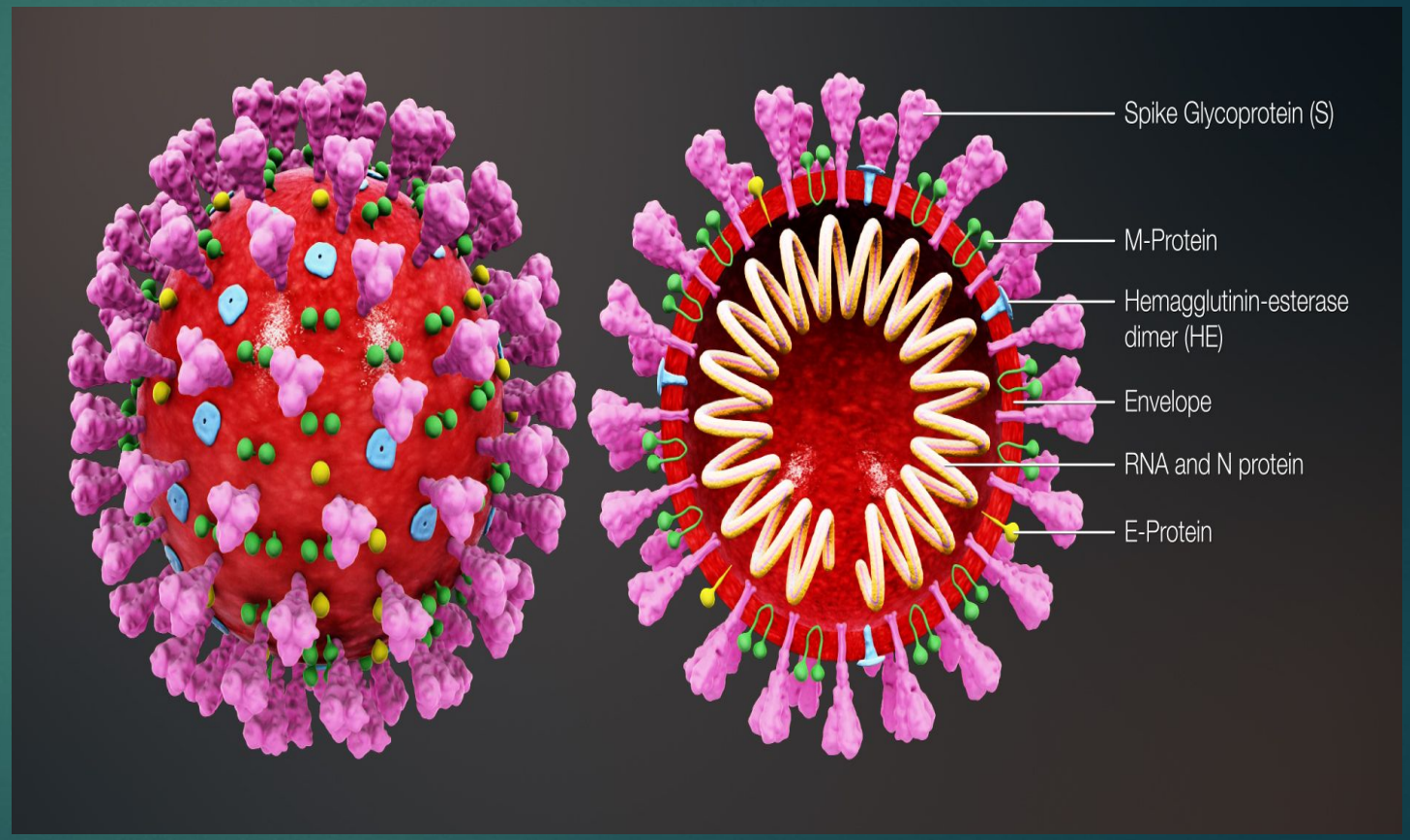
What's in a name

corona – latin for crown

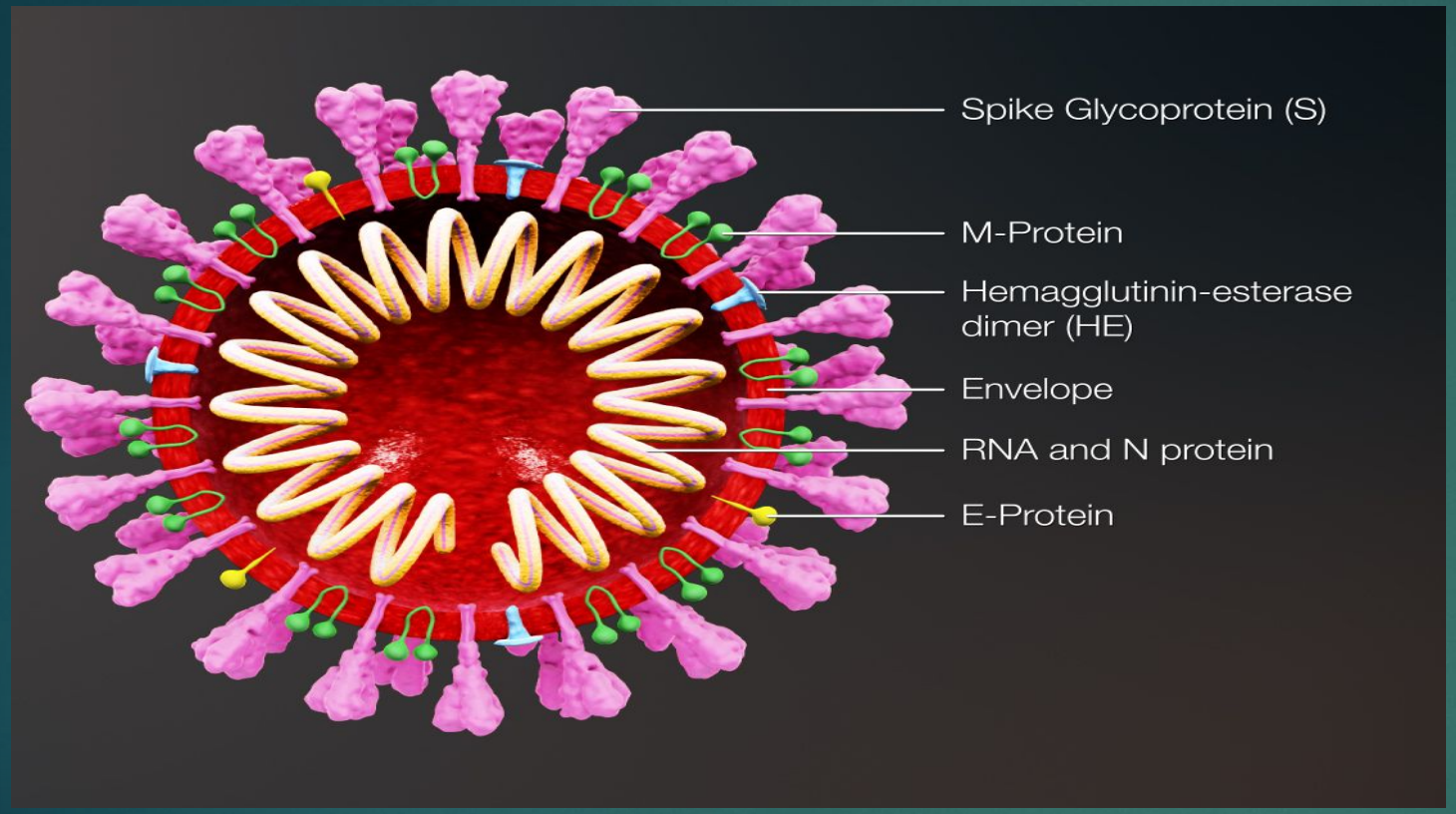
Coronavirus (CoV) — a large family of viruses that can cause disease in humans and animals

SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus-2) — the strain of virus causing the current pandemic

COVID-19 (Coronavirus Disease 2019) — the set of symptoms caused by SARS-CoV-2



Structure of the virus



Spike protein (S)
- ~150 kDa
- Attaches to ACE-2 receptor

Membrane protein (M)
- ~25–30 kDa
- Provides shape

Envelope protein (E)
- ~8–12 kDa
- Guides assembly and release

Nucleocapsid protein (N)
- ~50 kDa
- Protects RNA

RNA viral genome
- ~30,000 nucleotides (huge!)
- Encodes 29 proteins

Lipid envelope
- Acquired from host cell

- ***The coronavirus virion consists of structural proteins, namely spike (S), envelope (E), membrane (M), nucleocapsid (N) and, for some betacoronaviruses, haemagglutinin-esterase (not shown).***

More on structure

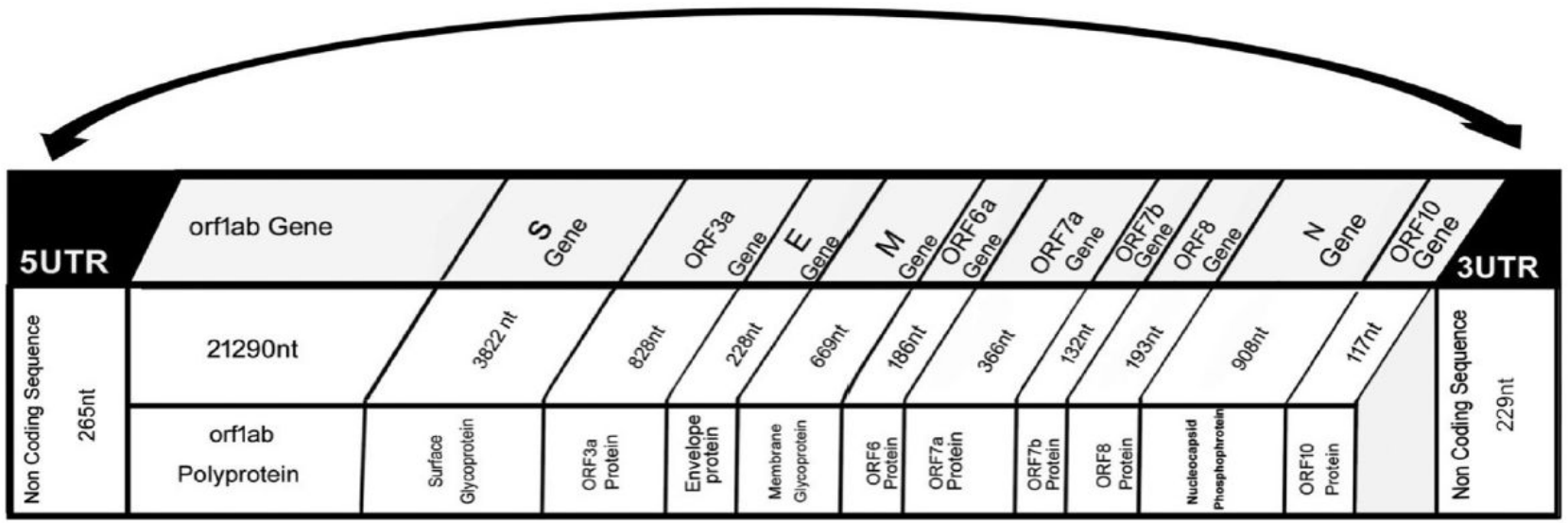


Fig. 1. Structure of the SARS-CoV-2 genome.

Genomic conclusion

Detecting the virus: Understanding immunology

Innate immunity

- relies on mechanisms already existing before microbe infects host
- is the first line of defense
- has no memory for subsequent exposure
- relies on non specific mechanisms

- Adaptive immunity
- Cell-mediated immune response (CMIR)
  - T-lymphocytes
  - eliminate intracellular microbes that survive within phagocytes or other infected cells
- Humoral immune response (HIR)
  - B-lymphocytes
  - mediated by antibodies
  - eliminate extra-cellular microbes and their toxins

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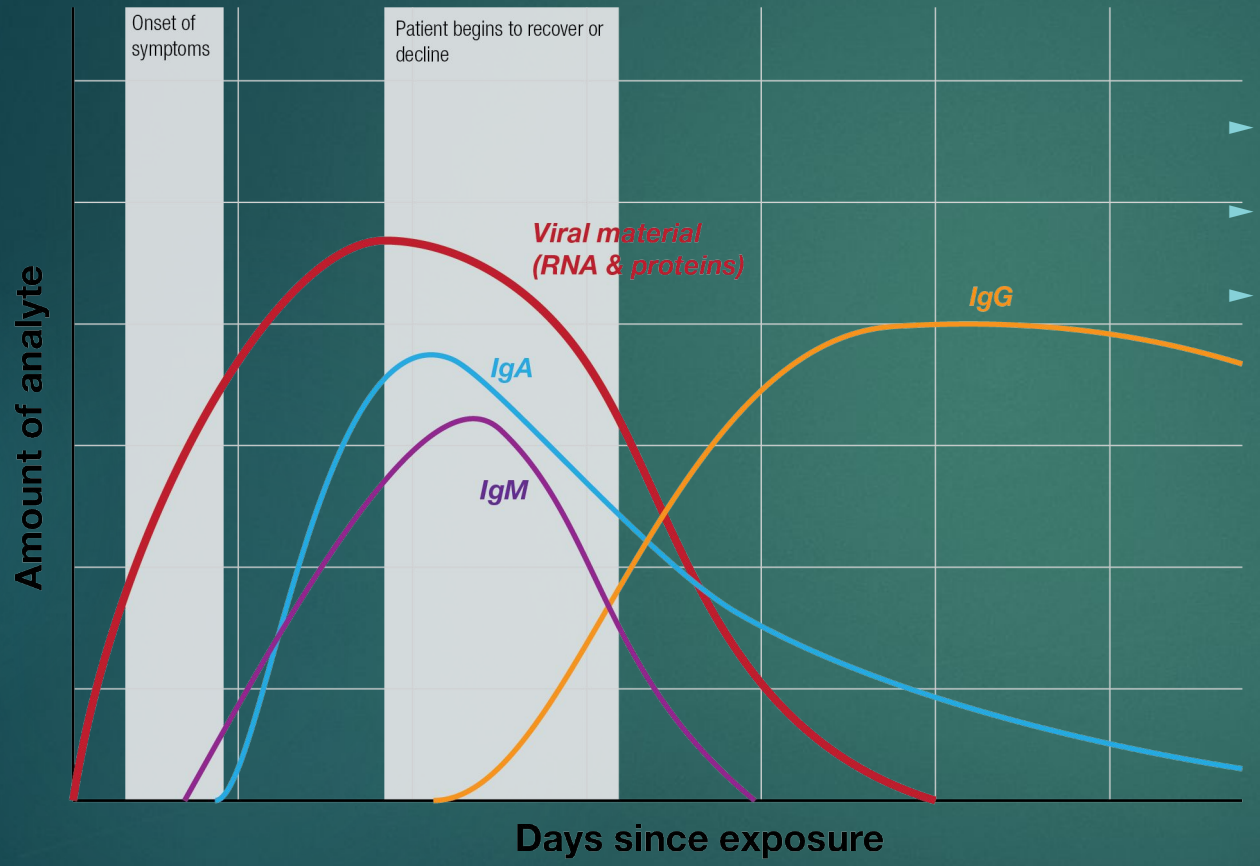
Detecting the virus



This graph is for illustrative purposes only - each type of infection has its own specific timeline.

- Which analytes are from the virus?
- Which are from the patient?
- Which analyte(s) would be the most useful for detection:
  - during the onset of symptoms?
  - after recovery?

Detecting the virus

Recommended diagnostic method for COVID-19

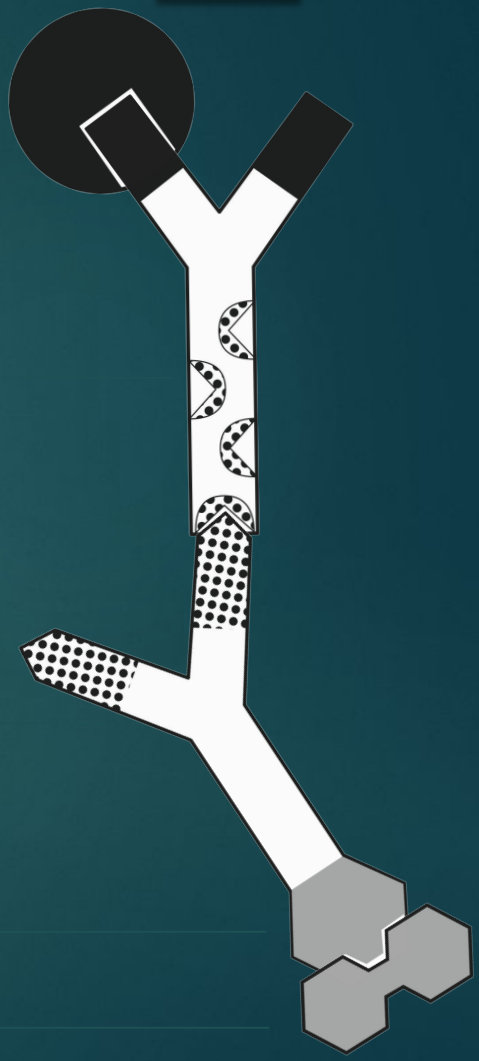
Real-time PCR method is recommended by WHO for COVID-19 diagnosis.

COVID-19 TEST	Antigen-based immunoassy	Antibody-based immunoassay	Real-time PCR
Analyte	Antigen	Antibody	Gene
Detectable period	From a few days after onset of symptoms	From 7-28 days after onset of symptoms	All of stages
Sensitivity	50-70% (expected)*	More than 95%	More than 95%
Specificity	50-70%*	Not clear*	More than 95%
Detection of asymptomatic infection	Depending on the amount of viral antigen	At the later stage of infection	From the early stage of infection
Status of use	Only some regions	Only some regions	All of countries (recommended by WHO & CDC)

*Based on conventional antigen-based immunoassay and affected by seasonal coronaviruses
Reference: Oral presentation from online forum of KOFST(The Korean Federation of Science and Technology Societies)

Detecting the virus: Ag

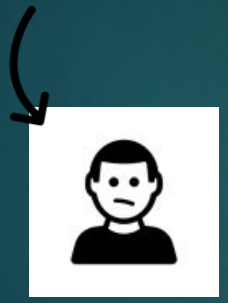
- **Antigen** — in an **antigen detection ELISA**, the patient sample is tested for the presence of antigens from viruses, bacteria, etc.
- **Primary antibody** — binds to the antigen
  - can be produced in a lab by injecting the target antigen into an animal and then harvesting the serum
- **Secondary antibody** — binds to the constant region of the primary antibody
  - made by injecting the primary antibodies from one animal into a different animal
  - secondary antibodies are attached to an **enzyme** which catalyzes a color change when substrate is added
- **Substrate** — changes color in the presence of the enzyme, indicating a positive result



Detecting the virus : Ab

Coronavirus Infection

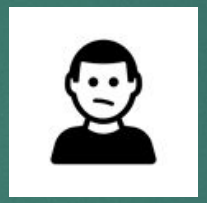
Coronavirus infects person



Person makes antibodies against coronavirus

Coronavirus Antibody Detection ELISA

Antigen
(lab-purified coronavirus S protein)

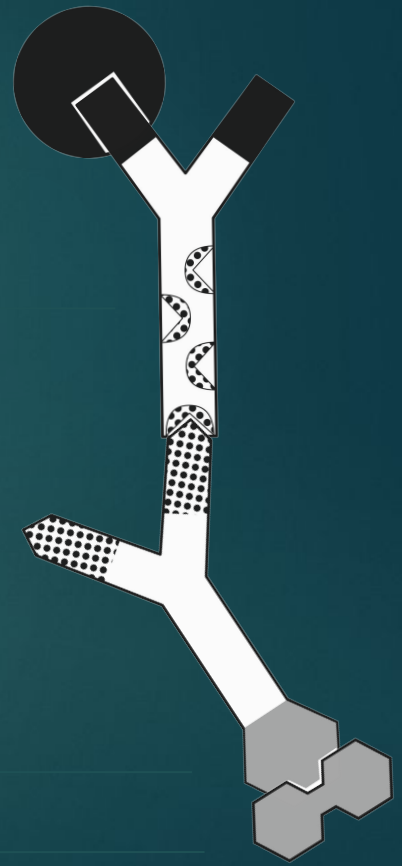


Patient Sample
(anti-coronavirus antibodies will be present in sample if patient was infected)

Secondary Antibody, goat anti-human
(binds if anti-coronavirus human antibodies are present in the sample)

Enzyme

Substrate



Detection the virus : PCR

- PCR (end point) — amplification of DNA template; analysis occurs after PCR, for example by gel electrophoresis
- Reverse transcription PCR (RT-PCR) — RNA is transcribed into DNA which is amplified by PCR
- Real-time PCR — PCR amplification is monitored in real time; can be quantitative (qPCR) or combined with RT-PCR (Real-Time RT-PCR)
- Droplet digital PCR (ddPCR) — PCR in which single a sample is fractionated into thousands of droplets prior to amplification with theoretically only one template per droplet; amplification either occurs or it doesn't (1 or 0, "digital"), which enables absolute quantitation
- Isothermal recombinase polymerase amplification (RPA) — a modification of PCR using different enzymes that allows amplification from either RNA or DNA at a single temperature (relevant to CRISPR-based diagnostics)

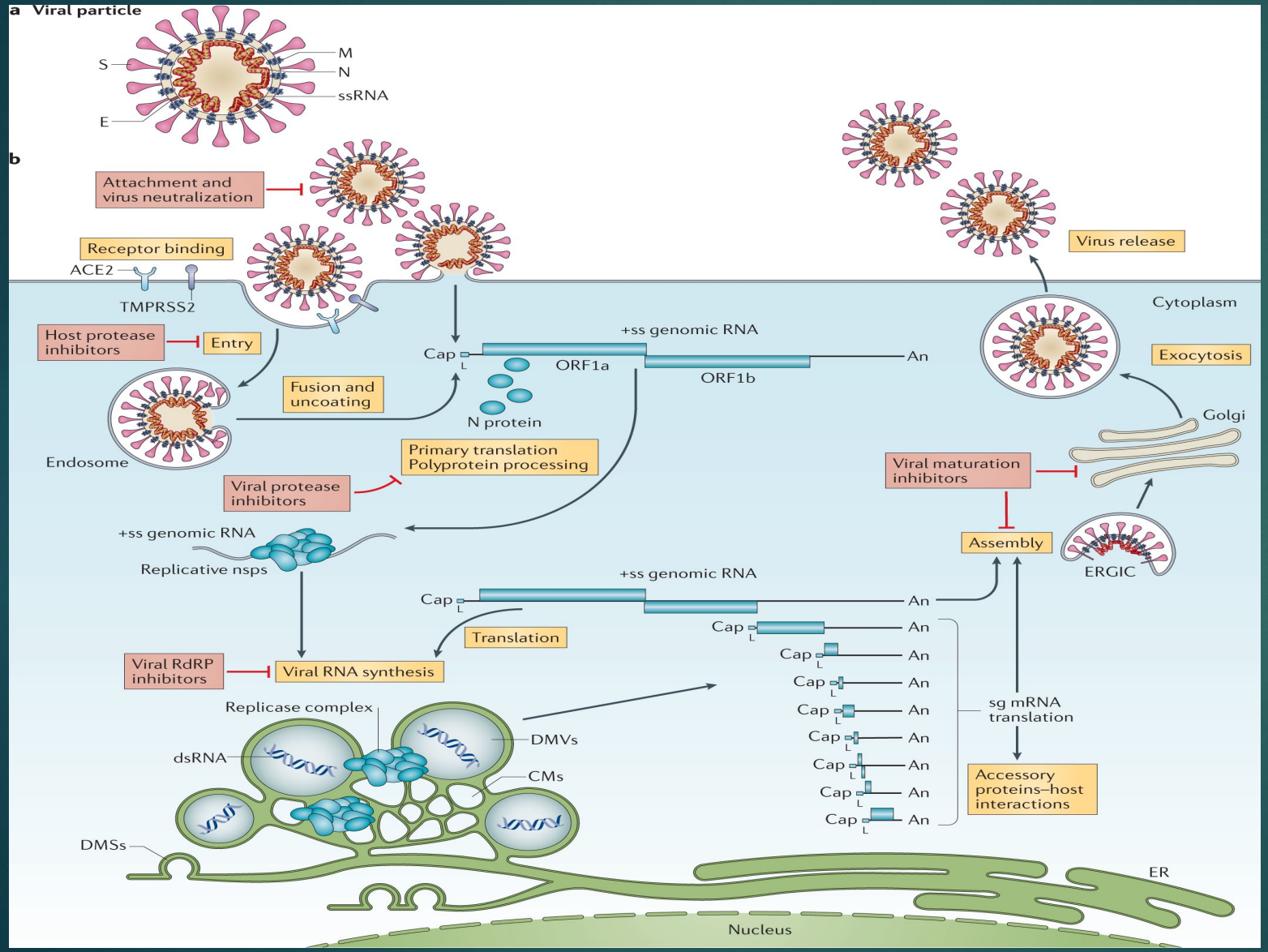
Application of the knowledge

- False positive and False negative results can be caused by:

I. Improper sample collection and transportation
II. Poor specimen quality
III. Improper laboratory processing
IV. Mutations
V. Concentrations near or below the LOD
VI. Limitation of the testing technology

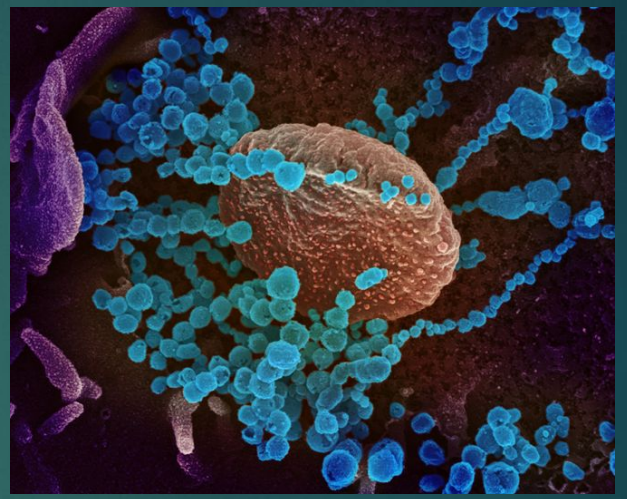
- One should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes
- Negative results do not exclude infection and must not be the sole basis of a patients managent
- Positive results indicates the detection of nucleic acid (RNA) and this may be present even after the virus is no longer viable

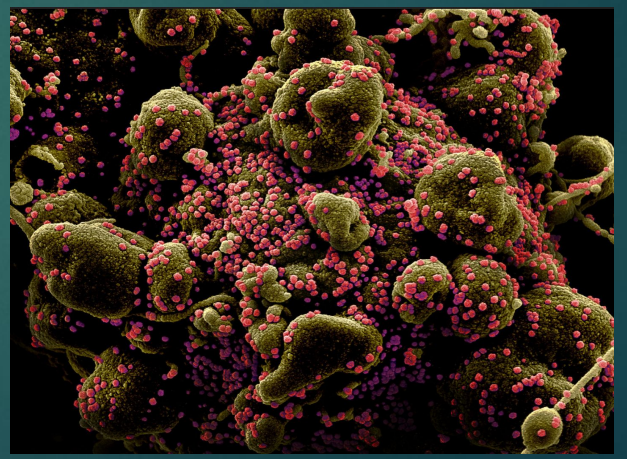
Life cycle of SARS- Cov-2



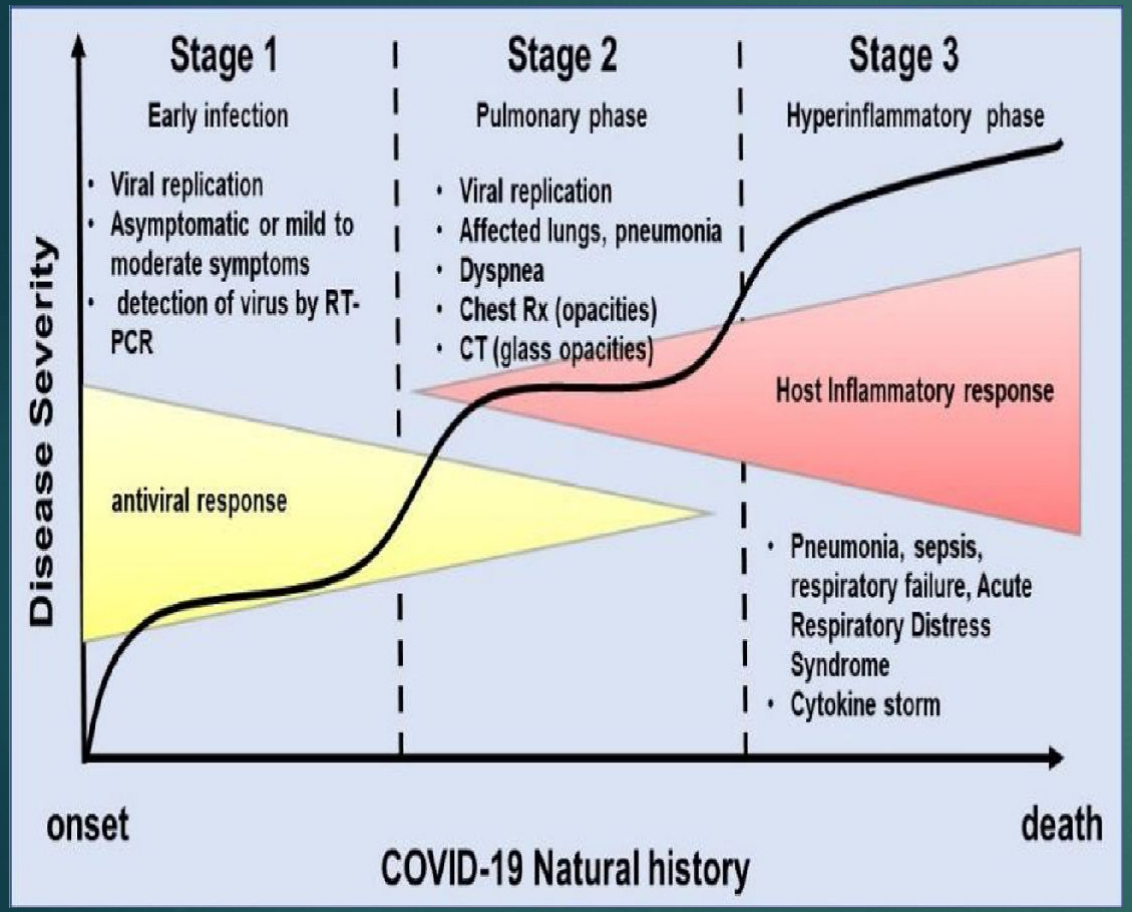
Summary of steps in the life cycle

- Binding
- Cleavage to permit fusion
- Fusion at the cellular or endosomal membrane
- Uncoating of the incoming genomic RNA
- Translation of two large open reading frames, ORF1a and ORF1b
- The polyproteins pp1a and pp1ab translationally processed into the individual non-structural proteins (nsps)
- Transcription of subgenomic mRNAs (sg mRNAs) comprising the characteristic nested set of coronavirus mRNAs
- Translocation into endoplasmic reticulum (ER) membranes and transit through the ER-to-Golgi
- Interaction with N-encapsidated, newly produced genomic RNA results in budding into the lumen of secretory vesicular compartments
- Virions are secreted from the infected cell by exocytosis





Natural history of the disease



1. The inhaled virus SARS-CoV-2 likely binds to epithelial cells in the nasal cavity and starts replicating.
   - *local propagation of the virus but a limited innate immune response.*
   - *virus can be detected by nasal swabs.*
   - *viral burden may be low, these individuals are infectious.*

2. Virus propagates and migrates down the respiratory tract along the conducting airways, and a more robust innate immune response is triggered. At this time, the disease COVID-19 is clinically manifest.

3. 20% of the infected patients will progress to stage 3 disease : pulmonary infiltrates and very severe disease. Initial estimates of the fatality rate are around 2%, but this varies markedly with age

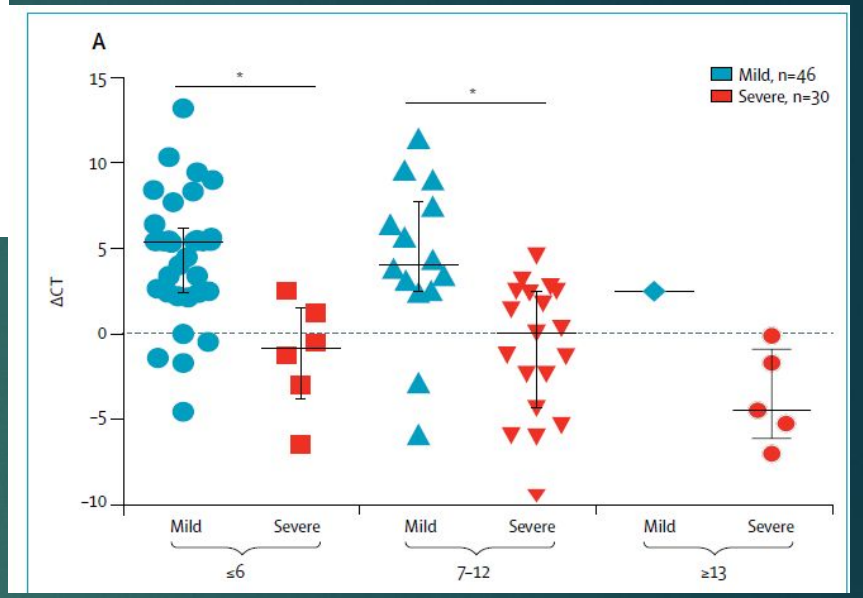
Elderly individuals are particularly at risk because of their diminished immune response and reduced ability to repair the damaged epithelium

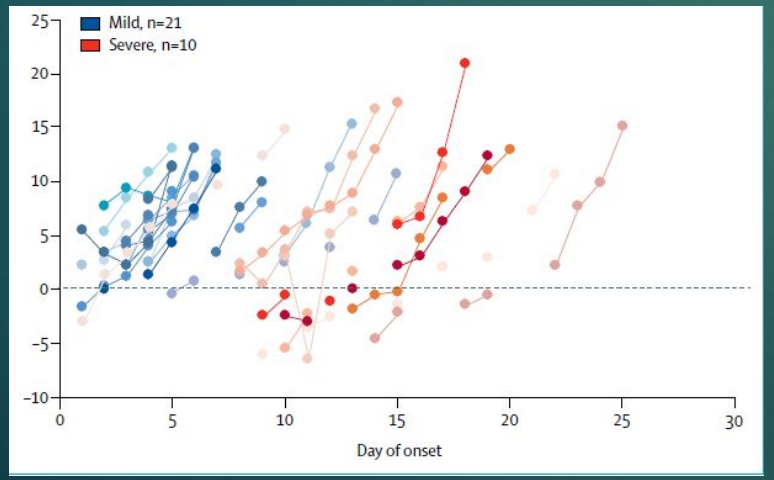
Relationship between viral load and disease severity

Prospective study- 76 patients
(Liu et al, The lancet inf dis 2020)

- NPS
- 30(39%) severe cases and 46 (61%) mild cases
- The Ct is the number of replication cycles to produce a fluorescent signal
- Lower Ct= Higher viral load
- Mean VL of severe disease was >60x that of mild disease

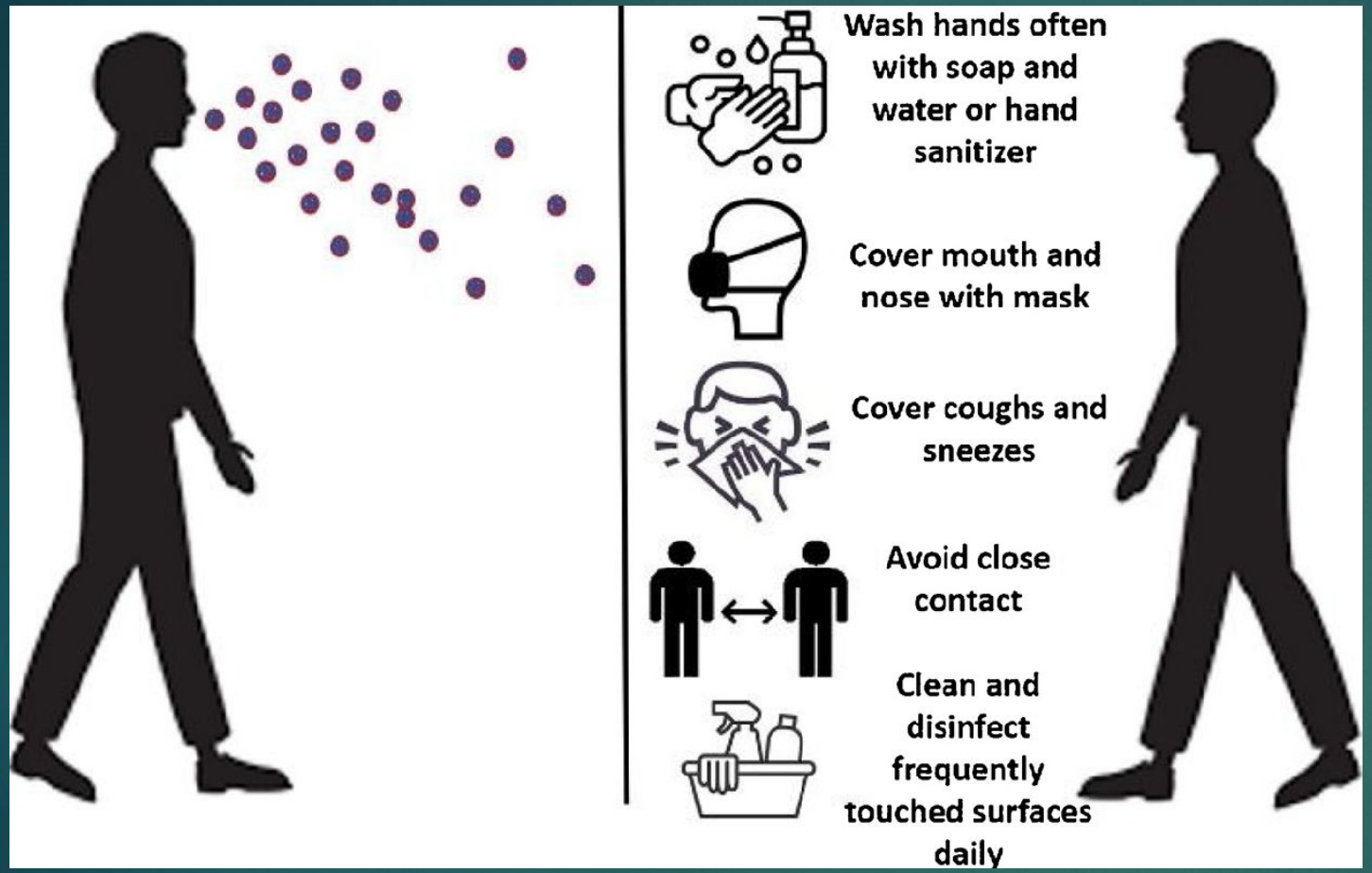
Viral dynamics in mild and severe cases of COVID-19





90% of mild cases were negative after day 10 post onset of symptoms
Severe cases still tested positive after day 10

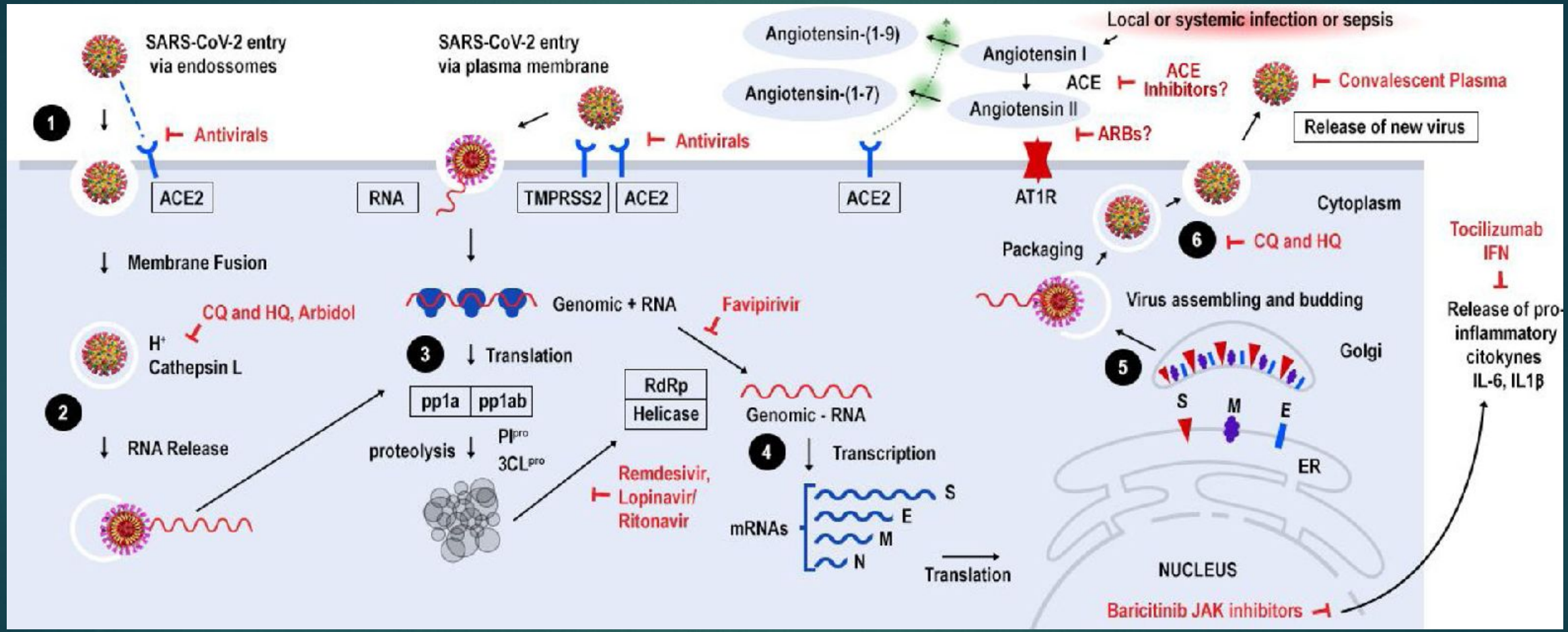
Natural history of the disease



PREVENTION
VACCINE
USEFUL THERAPIES

Effective intervention strategies relies on the knowledge of molecular and cellular

Treatment considerations



- ACE - Angiotensin- Converting Enzyme, ARB – Angiotensin Receptor Blocker, CQ - Chloroquine, HQ - Hydroxychloroquine, TMPRSS2–Transmembrane serine protease 2, IL-interleukin, JAK- Janus kinase.

Registered clinical trials

Drug/Treatment	[a]Number of registered clinical trials	Not yet recruiting	Recruiting	Active not recruiting	Terminated	Enrolling by invitation	Withdrawn	Suspended	completed	Unknown status
Anakinra	18	8	10	0	0	0	0	0	0	0
Anticoagulants	41	19	17	0	0	2	1	0	2	0
ARB (Angiotensin Receptor Blockers)	42	14	22	3	0	2	0	0	1	0
Azithromycin	93	35	44	5	1	1	1	5	1	0
Baricitinib	15	5	9	0	0	0	0	0	1	0
Chloroquine	73	28	35	2	0	3	1	2	2	0
Convalescent plasma	102	27	60	2	0	3	1	0	2	7
Dexamethasone	12	2	9	1	0	0	0	0	0	0
Favipiravir	24	13	8	2	0	1	0	0	0	0
Heparin	35	13	19	0	0	1	1	0	1	0
Hydroxychloroquine	218	71	102	16	2	8	3	8	8	0
Interferon alpha	17	7	8	0	0	1	0	0	1	0
Interferon beta	14	3	6	1	0	2	0	0	2	0
Interleukin 17A (IL-17A	3	1	2	0	0	0	0	0	0	0
Ivermectin	23	10	11	1	0	0	0	0	1	0
Lopinavir /Ritonavir	75	25	37	3	1	3	0	0	6	0
Losartan	14	5	8	0	0	1	0	0	0	0
Methylprednisolone	25	8	12	0	0	1	0	0	4	0
Nitazoxanide	12	7	5	0	0	0	0	0	0	0
Remdesivir	33	10	15	2	1	1	0	1	1	2
Tocilizumab	55	12	36	5	0	1	0	0	1	0
Umifenovir (Arbidol)	8	3	2	1	0	1	0	0	1	0
Vaccine	119	44	63	9	0	1	0	0	2	0
Vitamin C	25	12	11	0	0	1	0	1	0	0
Vitamin D	26	14	9	2	0	1	0	0	0	0
Zinc	15	10	3	1	0	1	0	0	0	0

Thankyou