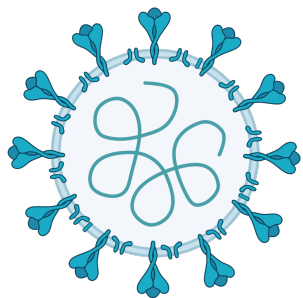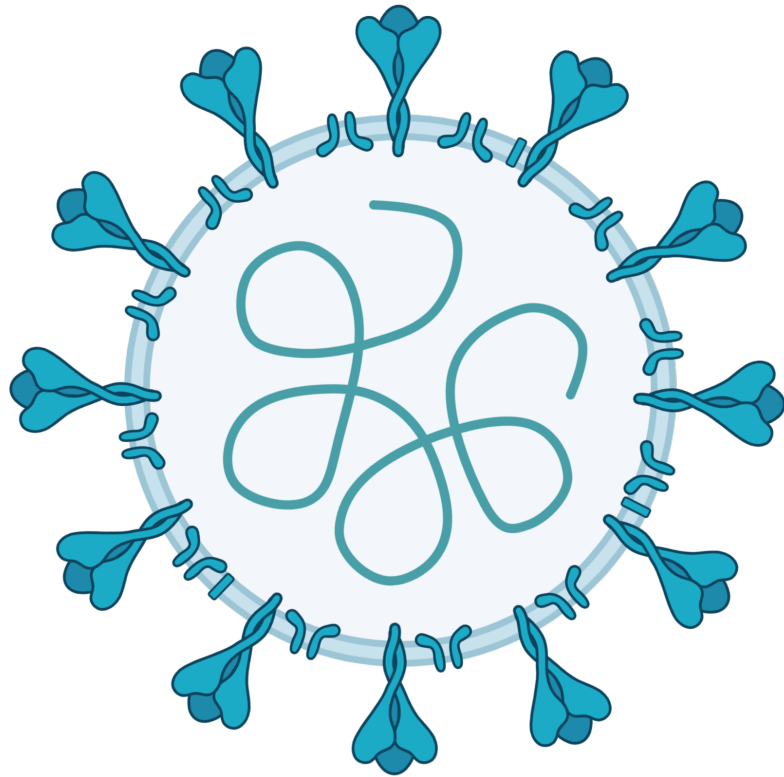# SARS-CoV-2: Ask an Immunologist

Olivia Hatton, PhD

GS222 @ Colorado College

January 5, 2021

Testing
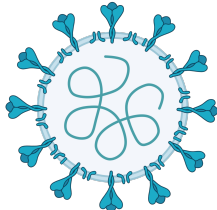
Herd Immunity & Vaccines

Disease & Treatments

How Do Molecular Tests Work?
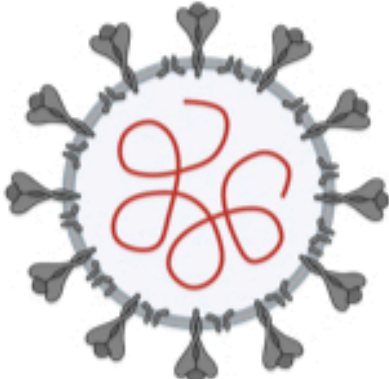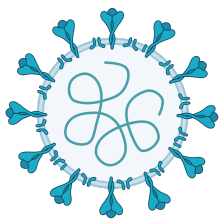## I - Types of SARS-CoV-2 Diagnostic Tests

	Molecular (a.k.a. PCR)	Antigen	Antibody (a.k.a. serology)
What It Detects	Instructions for making more virus (viral RNA genome)	Parts of the virus (viral proteins)	Your immune response to the virus (IgG or IgM)
Type of Sample	Swab or Savlia	Swab or Saliva	Blood
Turnaround Time	Days (Rapid = Minutes)	Minutes	Days
Accuracy	High	Moderate	Moderate
Best For	Diagnosis	Screening	Rates of Exposure
What It Tells You	Virus Present; *Active* Infection	Virus Present; *Active* Infection	Antibodies to Virus Present; *Previous* Infection

Table modified from Service, RF. (2020, August 3). Radical shift in COVID-19 testing needed to reopen schools and business, researchers say. *Science* doi:10.1126/science.abe1546. Table created with BioRender.com
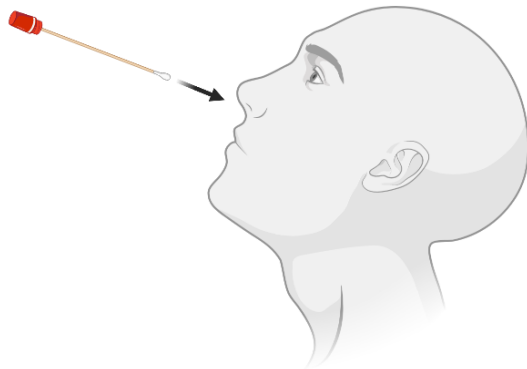
How Do Molecular Tests Work?
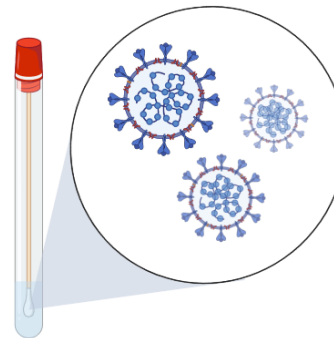## II - COVID-19 Diagnostics Testing through RT-qPCR

1 **Nasopharyngeal swab** <15 min
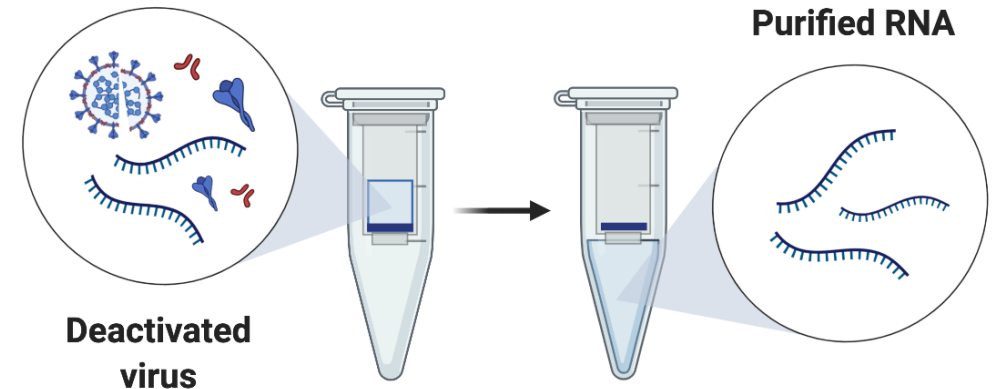
Cotton swab is inserted into nostril to absorb secretions.

2 **Collected specimen** 0-72 h

Specimen is stored at 2-8°C for up to 72 hours or proceed to RNA extraction.

3 **RNA extraction** ~45 min

Purified RNA is extracted from deactivated virus.

Purified RNA

Deactivated virus

4 **RT-qPCR** ~1 h per primer set

Purified RNA is reverse transcribed to cDNA and amplified by qPCR.

Retro transcription

266   13,468   21,563   29,674

5'   3'

ORF1a   ORF1b

RdRp

E   **N***
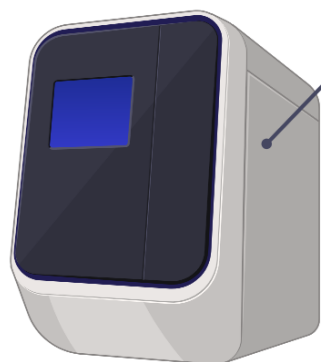
Primers and probes for screening

E_Forward: ACAGGTACGTTAATAGTTAATAGCGT
E_Probe1: FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
E_Reverse: ATATTGCAGCAGTACGCACACA

E gene
First-line screening tool

RdRp_Forward: GTGARATGGTCATGTGTGGCGG
RdRp_Probe1: FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ
RdRp_Probe2: FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
RdRp_Reverse: CARATGTTAAASACACTATTAGCATA

RdRp gene
Confirmatory testing

* N gene testing is not further used because it is slightly less sensitive.

5 **Test results** real time

Positive SARS-CoV-2 patients cross the threshold line within 40.00 cycles (< 40.00 Ct).

Fluorescence

Positive

Threshold

Negative

Cycles
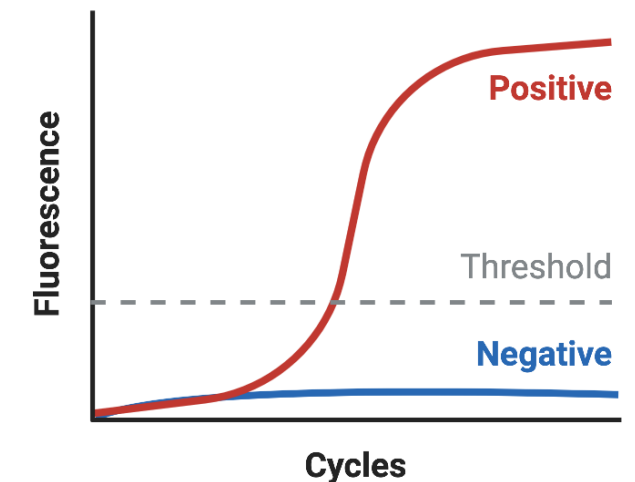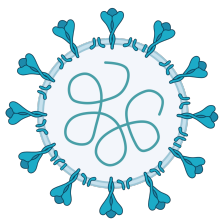
How Do Molecular Tests Work?
III - Fluorescent Probe-Based Quantitative PCR (qPCR)

40 Cycles

① Initiation ② Denaturation (95°C) ③ Primer annealing (60°C) ④ Extension (72°C)

Template DNA

5'
3'

Denaturation

5'
3'

5'

Reverse Primer

Forward Primer

Bound probe

3'

Polymerization — Polymerase

5'
3'

Fluorescent reporter released

F

5'
3'

Key concept

Fluorescent Reporter — Quencher

Ground state fluorophore

Fluorescent signal

F

Polymerase cleavage

Excited state fluorophore

Results

Fluorescence

Amplification (Positive)

Threshold

No amplification (Negative)

Cycles

How Do Molecular Tests Work?
III - Nicking Enzyme Amplification Reaction (NEAR, IDNow)
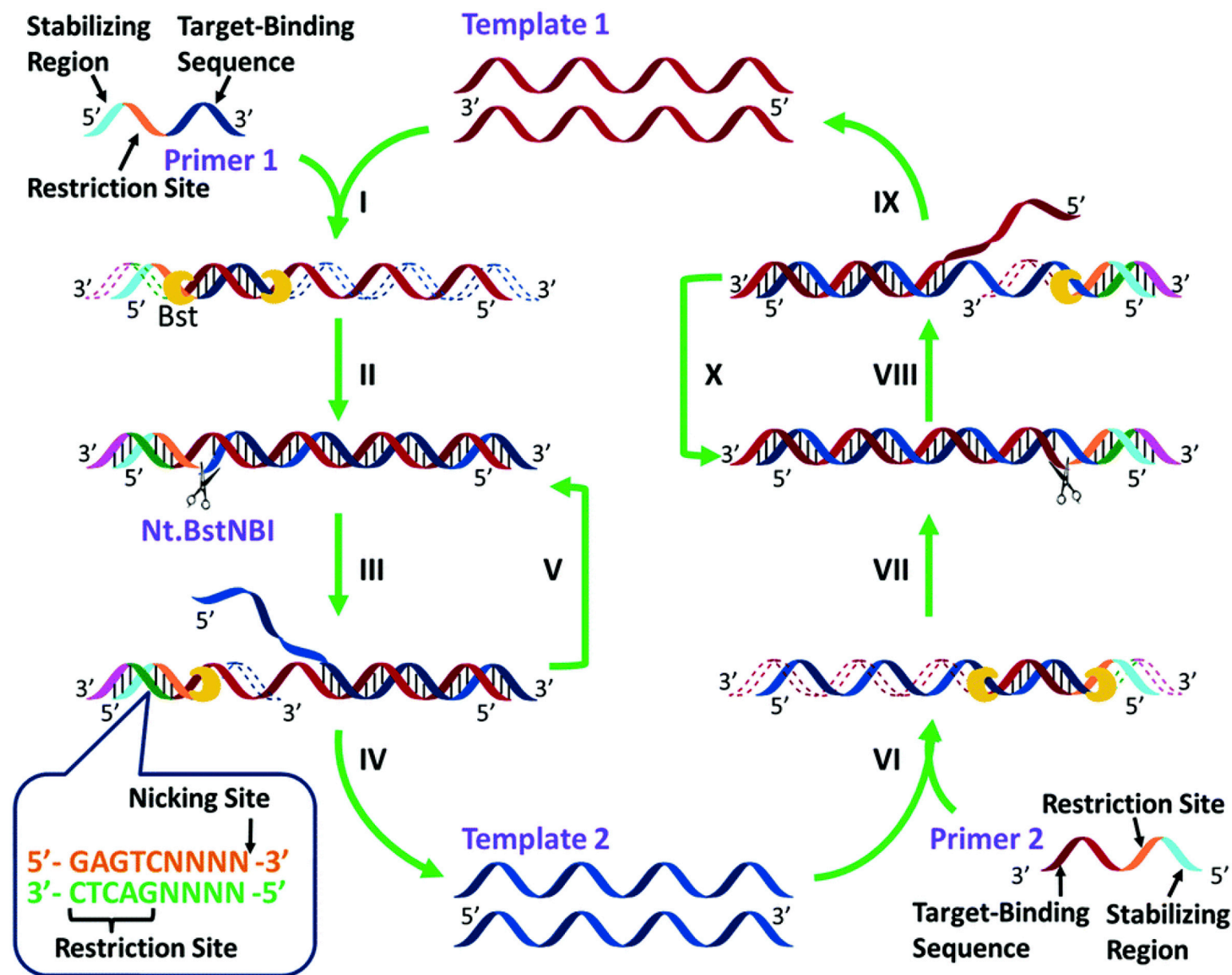
- **Key Differences from PCR**
  - Cutting site (restriction site) in primer
  - Presence of enzyme to make cuts (restriction enzyme - Nt.BstNBI)
  - Strand-displacing polymerase (Bst)

- **Advantages**
  - *Faster* - reaction can be run at a single temperature (isothermal)
  - *More sensitive* - >2 fold amplification per cycle (PCR)

- **Disadvantages**
  - *Specificity* - common to see non-specific products; high background
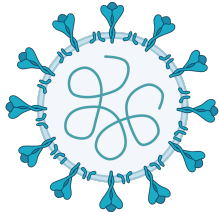
Why Are Antigen Tests Less Accurate?
I - How Lateral Flow Antigen Testing Works
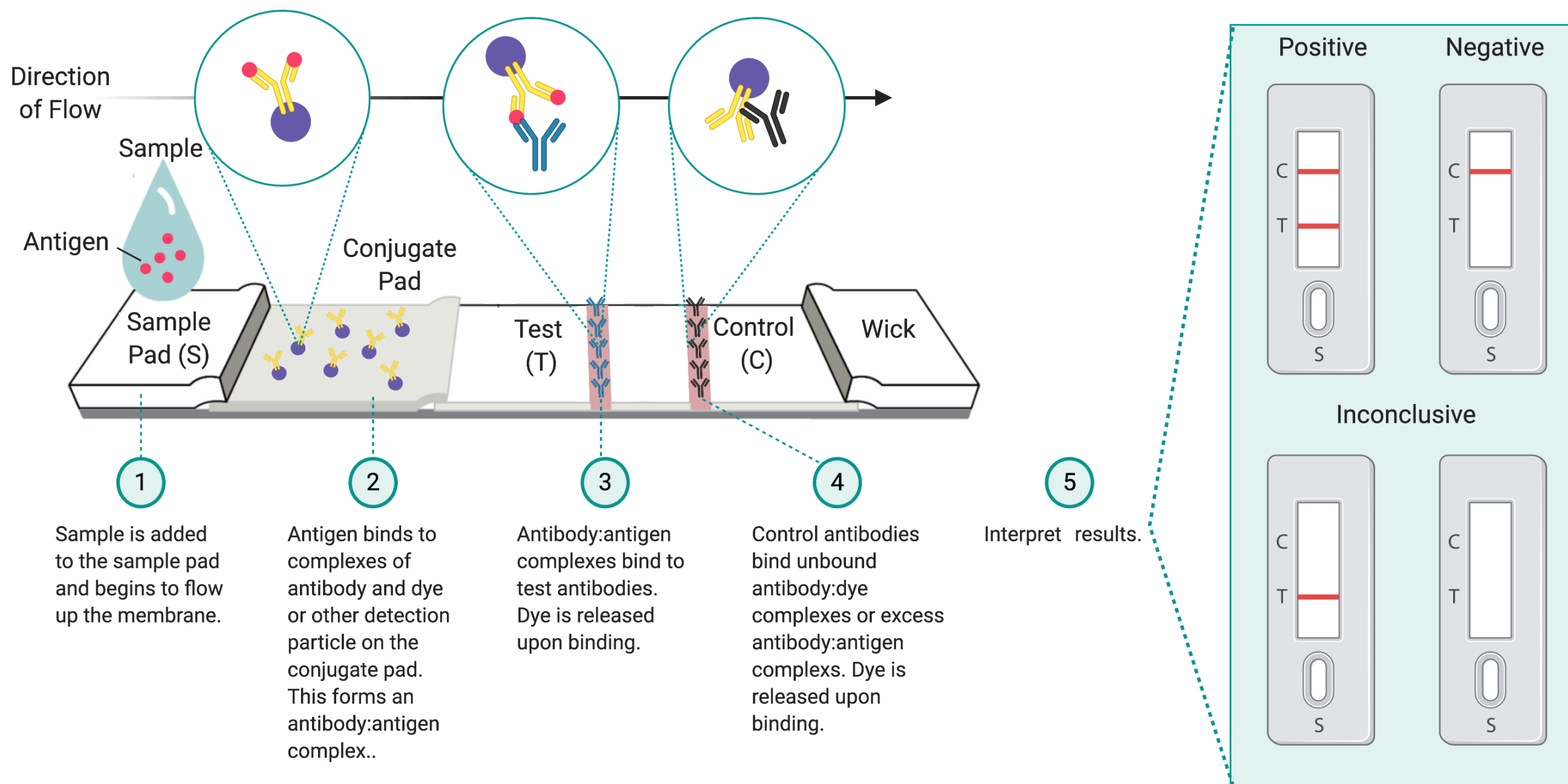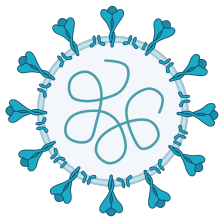
Direction of Flow

Sample

Antigen

Conjugate Pad

Sample Pad (S)

Test (T)

Control (C)

Wick

1
Sample is added to the sample pad and begins to flow up the membrane.

2
Antigen binds to complexes of antibody and dye or other detection particle on the conjugate pad. This forms an antibody:antigen complex..

3
Antibody:antigen complexes bind to test antibodies. Dye is released upon binding.

4
Control antibodies bind unbound antibody:dye complexes or excess antibody:antigen complexs. Dye is released upon binding.

5
Interpret results.

Positive

Negative

Inconclusive

C
T
S
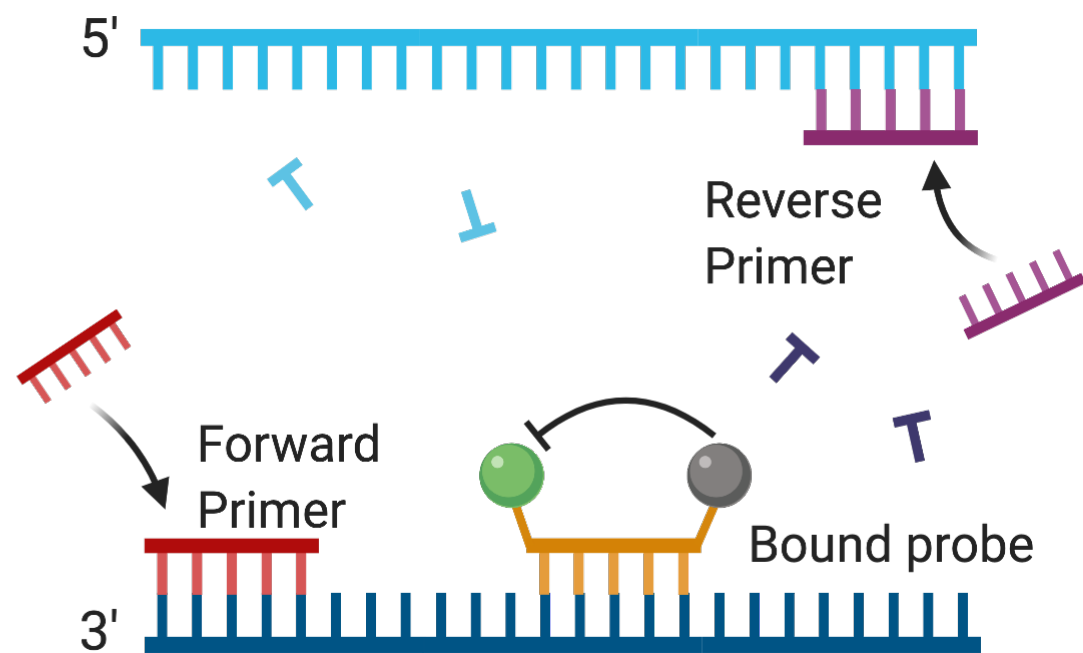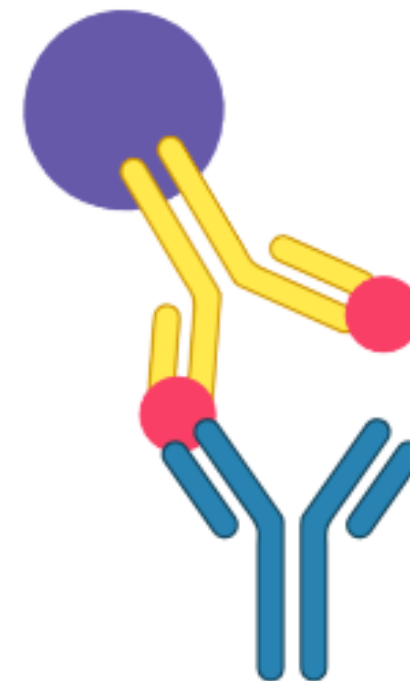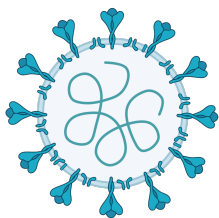
Why Are Antigen Tests Less Accurate?
II - All Tests Have Limits …

PCR-based tests can detect small amounts of viral genetic material, so a test can be positive long after a person stops being infectious.

Rapid antigen tests detect the presence of viral proteins and can return positive results when a person is most infectious.

Antibody tests detect the body's immune response to the virus and are not effective at the earliest phase of infection.

©nature
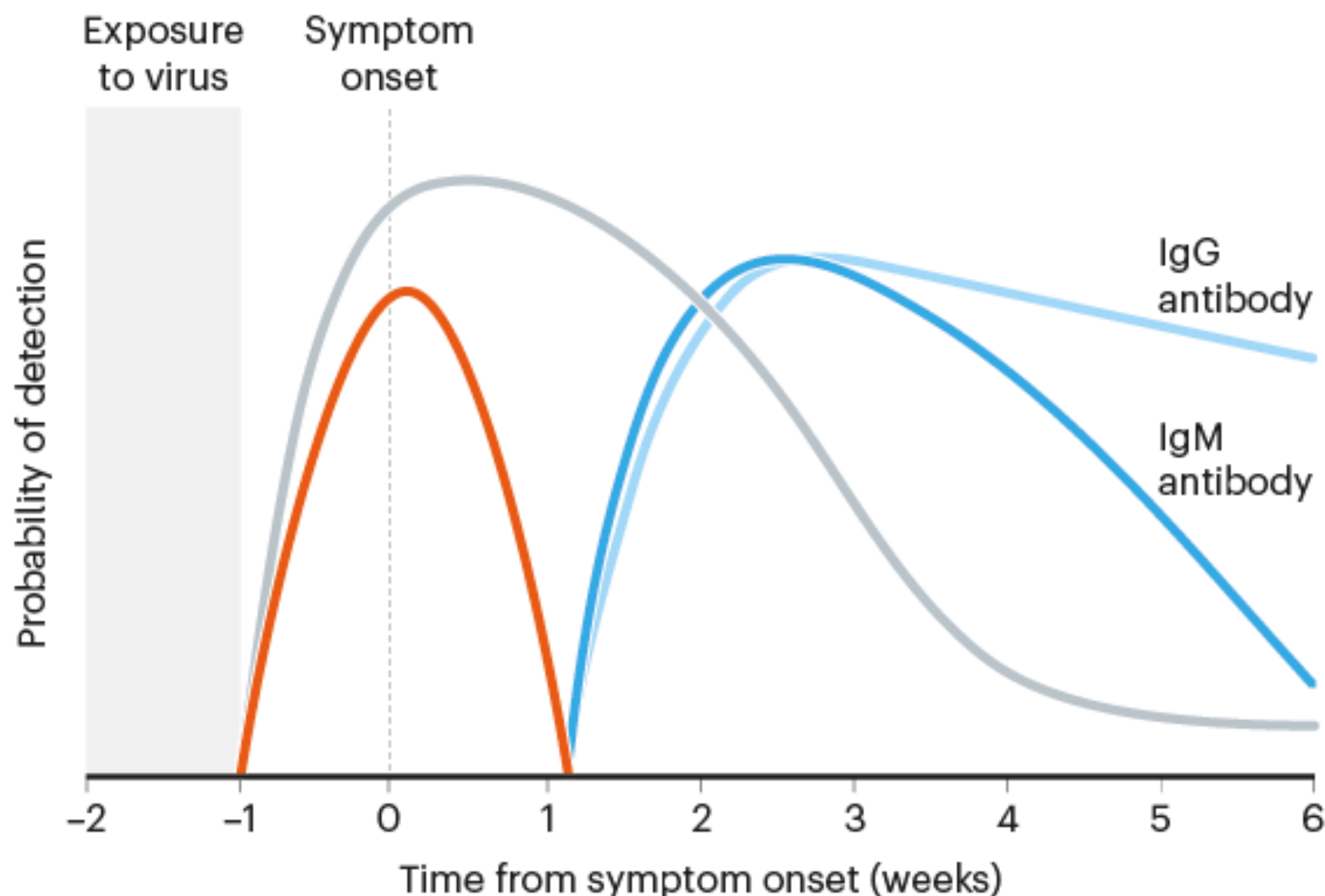
Why Are Antigen Tests Less Accurate?
IV - … But Each Test Has A Purpose

Types of SARS-CoV-2 Tests

	Molecular (a.k.a. PCR)	Antigen	Antibody (a.k.a. serology)
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Table modified from Service, RF. (2020, August 3). Radical shift in COVID-19 testing needed to reopen schools and business, researchers say. *Science* doi:10.1126/science.abe1546. Table created with BioRender.com

Testing
Miscellaneous Quick Answers

Why Were There Shortages in Testing?
- Production (materials, facilities, staff, etc)
- Testing (materials, instrumentation, CLIA certified labs & staff, etc)
- Allocation of Tests & Materials

Why Does It Take Over A Day To Get Results?
- **Point-of-Care / Rapid Tests**
  - Abbott IDNow (molecular: ~15 min/test, 1 test/run)
  - Cepheid Xpress (molecular: ~35 min/test, 2-80 tests/run)
  - Quidel Sofia2 (antigen: ~15 min/test, 1 test/run)
  - Ellume COVID-19 home test (antigen: ~15 min/test, 1 test/run)
- **RT-qPCR (molecular)**
  - Straight from collection,: ~ 120 min/test, 10-90 tests/run*
    - *depends on size of plate, pooling, # of primer sets*
  - Bottlenecks: transport to lab, certified staff, time from sample collection, sample back-log, etc
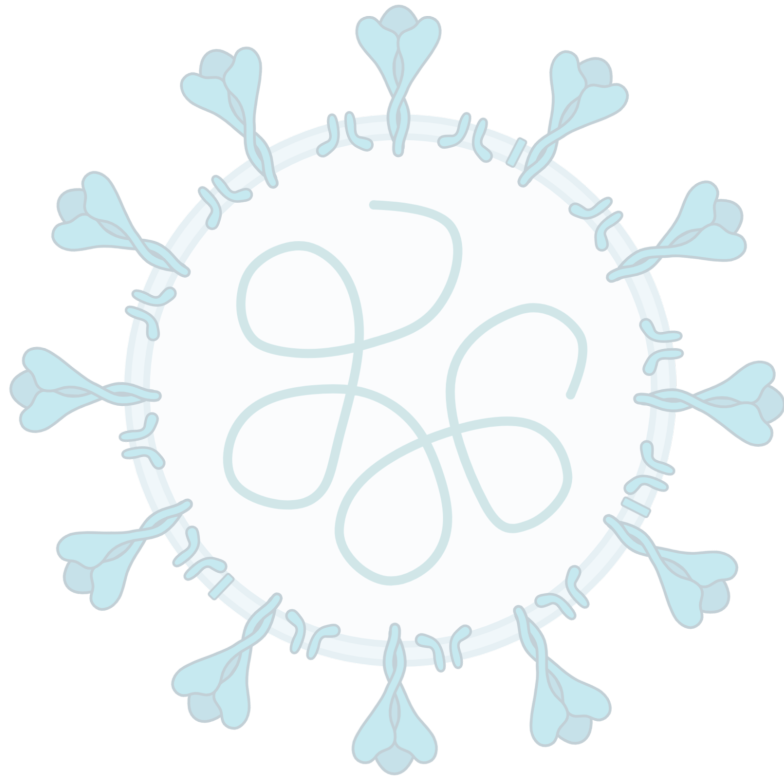
Testing

**Herd Immunity
& Vaccines**

Disease
& Treatments

What is Herd Immunity & How is it Achieved?

Principles of
HERD IMMUNITY & SOCIAL DISTANCING
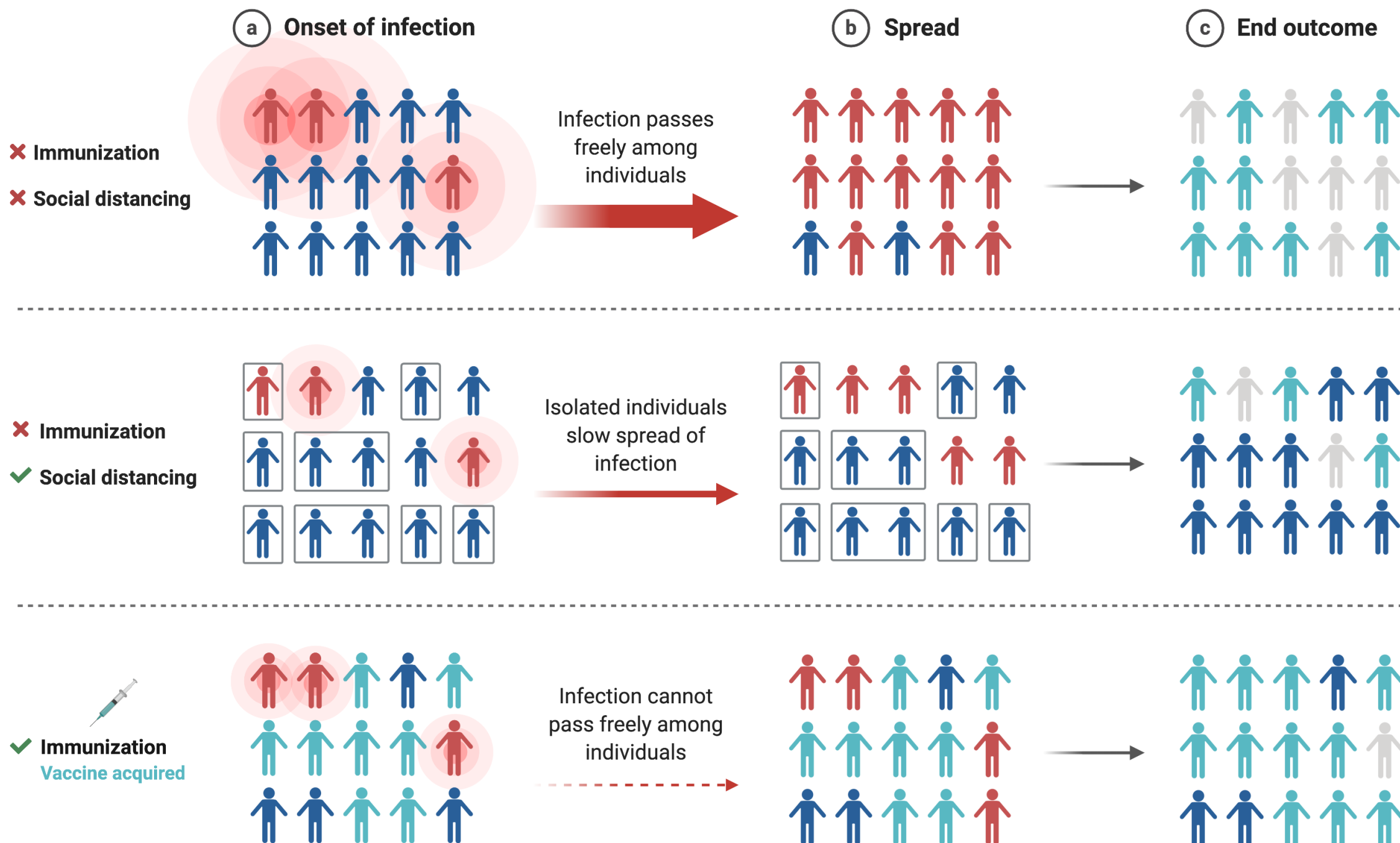
Healthy | Social distancing | Infected | Immunity acquired | Deceased

a Onset of infection   **b** Spread   **c** End outcome

✖ Immunization
✖ Social distancing

Infection passes freely among individuals

✖ Immunization
✔ Social distancing

Isolated individuals slow spread of infection

✔ Immunization
Vaccine acquired

Infection cannot pass freely among individuals

When is Herd Immunity Achieved?
The Relationship Between R_0 & p_I

- Herd immunity is achieved when, on average, one infected person in a generates less than one secondary case ($R<1$)

$$R = (1-p_C)(1-p_I)R_0$$

R = effective reproduction number

p_C = reduction of transmission due to non-pharmaceutical interventions (NPI)

p_I = proportion of immune individuals

R_0 = basic reproduction number; varies

When is Herd Immunity Achieved?
The Relationship Between R_0 & p_I

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$$R = (1-p_C)(1-p_I)R_0$$

R = effective reproduction number

p_C = reduction of transmission due to non-pharmaceutical interventions (NPI)

p_I = proportion of immune individuals
R_0 = basic reproduction number; varies

- Without NPI ($p_C = 0$), herd immunity ($R<1$) is achieved when:

$$p_I = 1-1/R_0$$

	R_0	p_I (as a%)
Seasonal Influenza	1.28 (median)	22% (median)
Measles	12 - 18	92 - 94%
SARS-CoV-2	2.5 - 4	60 - 75%

What Types of Vaccines Are In Development?

I - Types of Vaccines

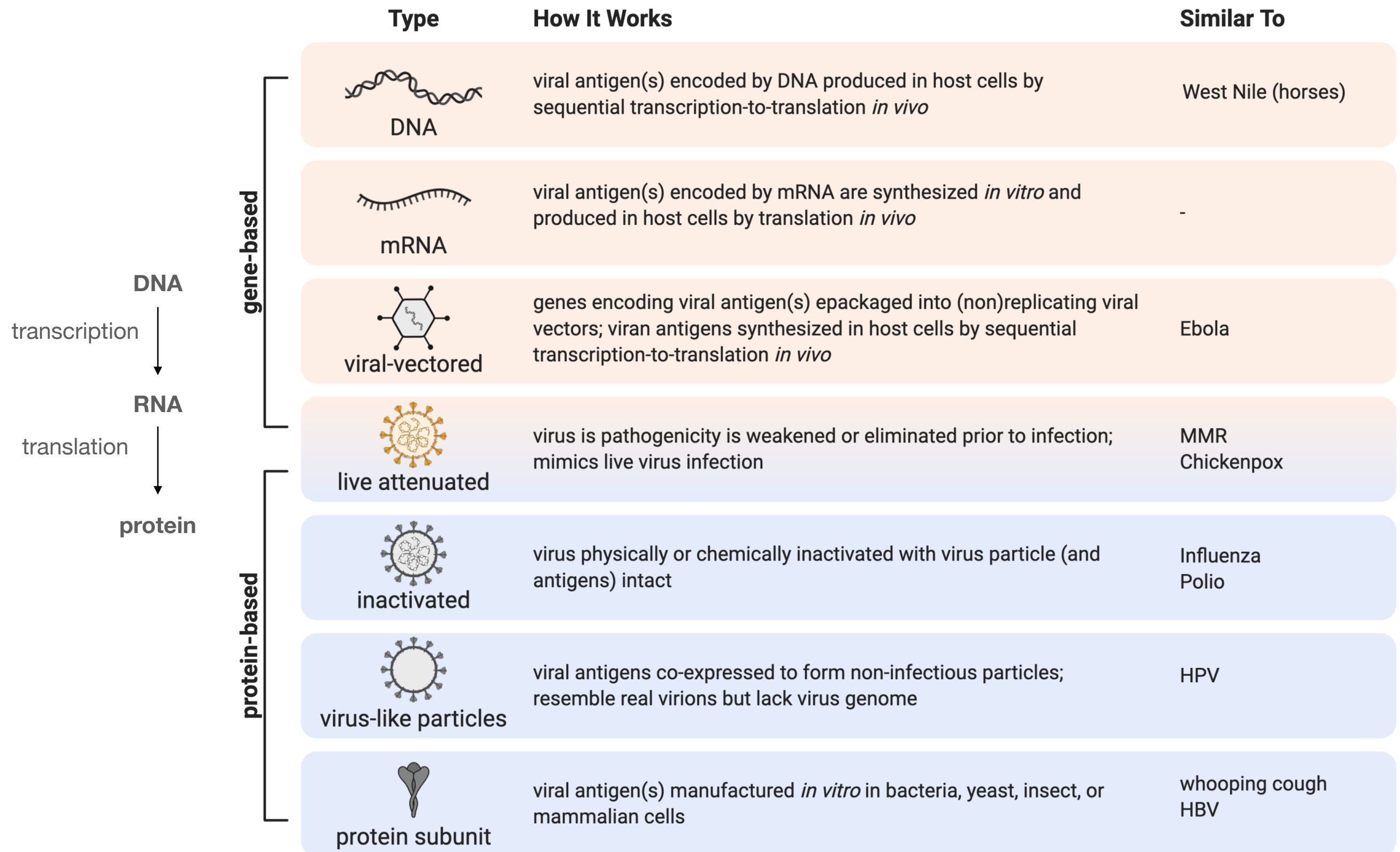|	Type	How It Works	Similar To
gene-based	DNA	viral antigen(s) encoded by DNA produced in host cells by sequential transcription-to-translation *in vivo*	West Nile (horses)
	mRNA	viral antigen(s) encoded by mRNA are synthesized *in vitro* and produced in host cells by translation *in vivo*	-
	viral-vectored	genes encoding viral antigen(s) epackaged into (non)replicating viral vectors; viran antigens synthesized in host cells by sequential transcription-to-translation *in vivo*	Ebola
protein-based	live attenuated	virus is pathogenicity is weakened or eliminated prior to infection; mimics live virus infection	MMR Chickenpox
	inactivated	virus physically or chemically inactivated with virus particle (and antigens) intact	Influenza Polio
	virus-like particles	viral antigens co-expressed to form non-infectious particles; resemble real virions but lack virus genome	HPV
	protein subunit	viral antigen(s) manufactured *in vitro* in bacteria, yeast, insect, or mammalian cells	whooping cough HBV

DNA

transcription

RNA

translation

protein

What Types of Vaccines Are In Development?
II - The Mechanism of SARS-CoV-2 Entry as a Vaccine Target

SARS-CoV-2

S2

S1

ACE2

① Cleavage of SARS-CoV-2 S protein

S2' site

S1/S2 site

TMPRSS2 protease

② Activation of S2 domain

Activated S2

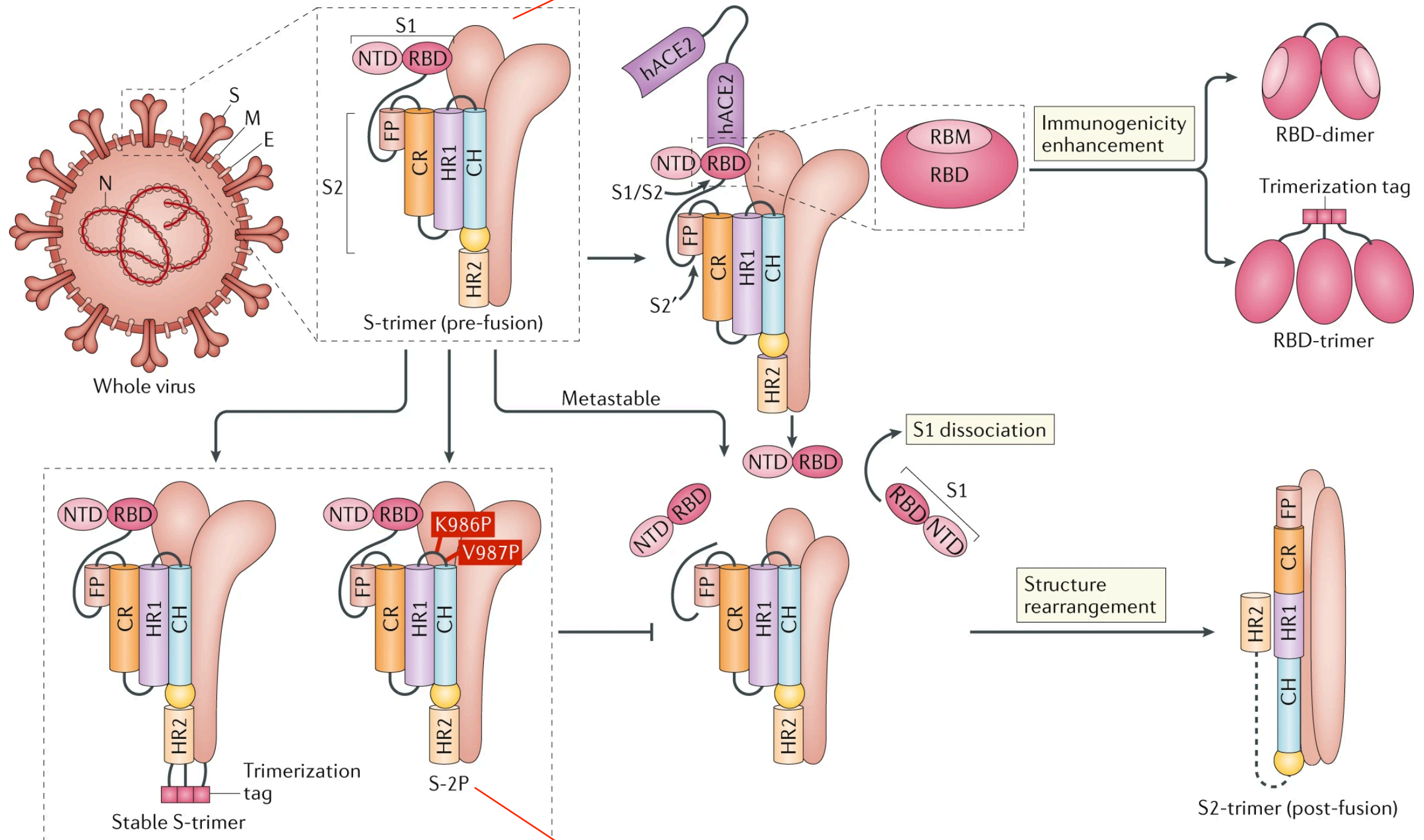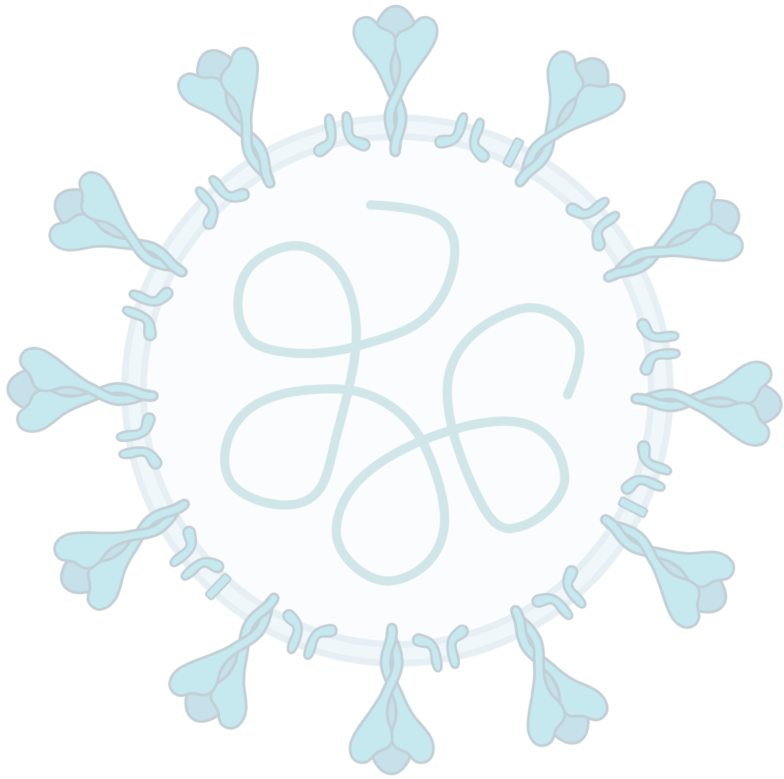③ Fusion of viral and host membranes

Target cell
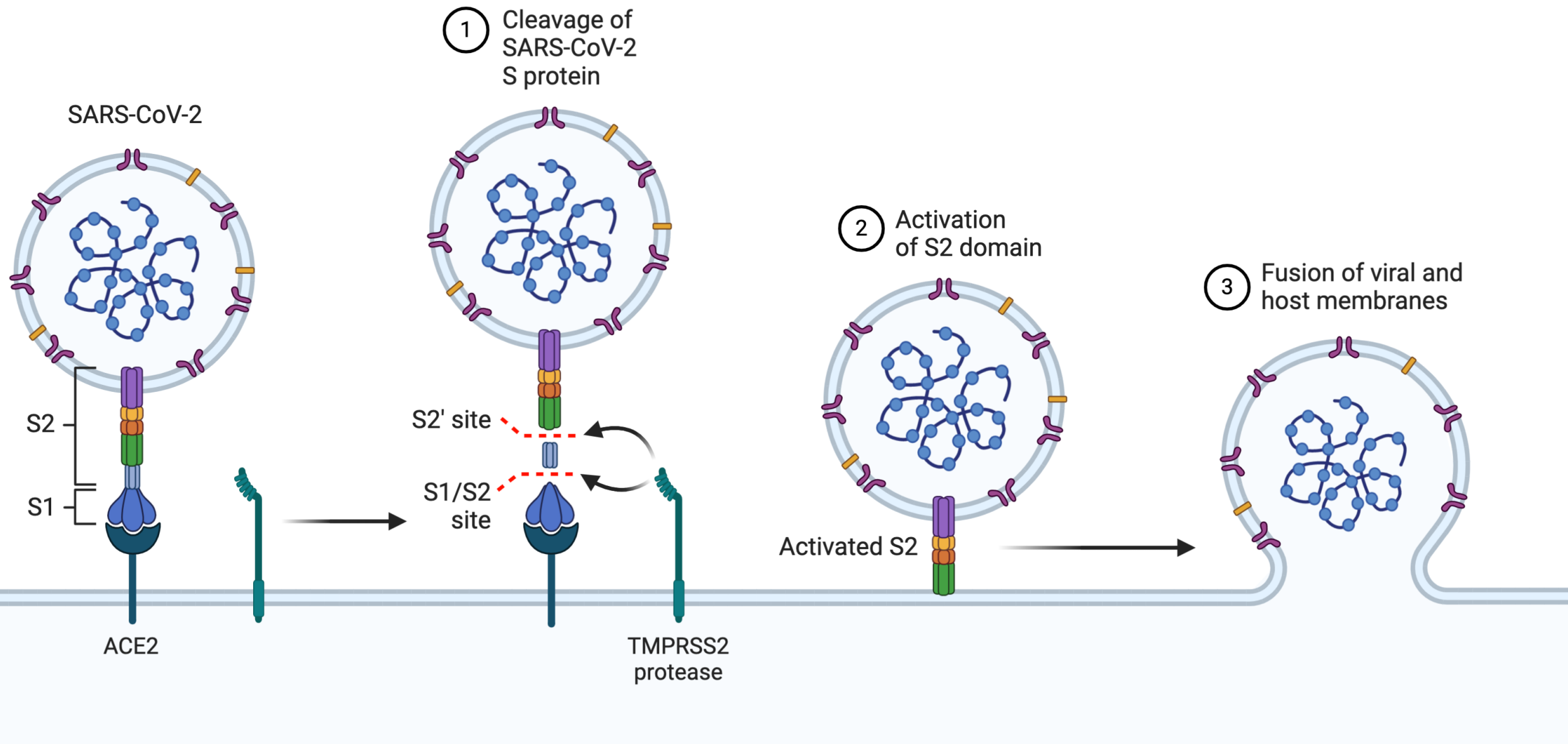
What Types of Vaccines Are In Development?
III - Major Targets Used in COVID-19 Vaccine Candidates

Oxford/AstraZeneca (viral vector)

Pfizer/BioNTech/Fosun Pharma (mRNA)
Moderna/NIAID (mRNA)

Dai & Cai *Nat. Rev. Immuno.* (2020)
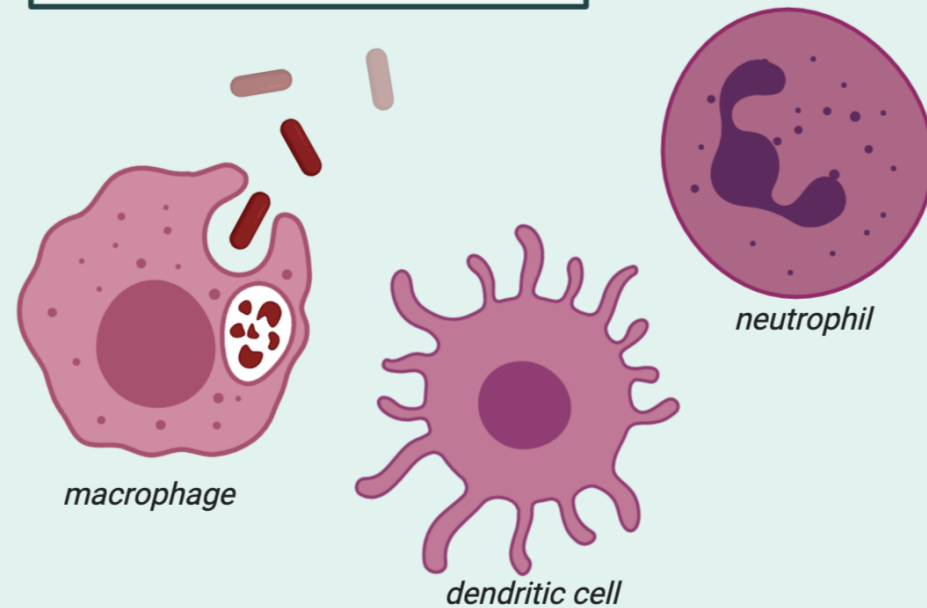
Testing

Herd Immunity
& Vaccines

Disease
& Treatments

Why Does COVID-19 Have Varying Severity?
I - A Very Brief & Simplified Overview of Immunity

INNATE IMMUNITY

macrophage

neutrophil

dendritic cell

sensitivity: virus vs. bacteria vs. fungi vs. parasite
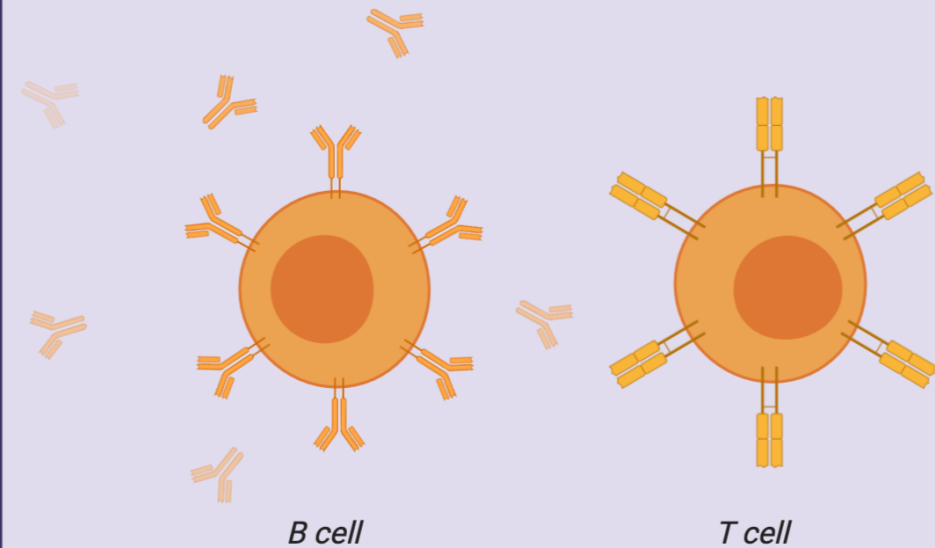
sensors: pattern-recognition receptors

functions: basic pathogen removal, activates proper adaptive response for type of pathogen

strengths: fast-acting first line of defesnse

weaknesses: non-specific, amnesia

ADAPTIVE IMMUNITY

B cell

T cell

sensitivity: SARS-CoV-1 vs. MERS vs. SARS-CoV-2

sensors: antigen receptors

functions: neutralize threat (antibodies from B cells), kill infected cells (T cells), learn from and remember best methods of attack
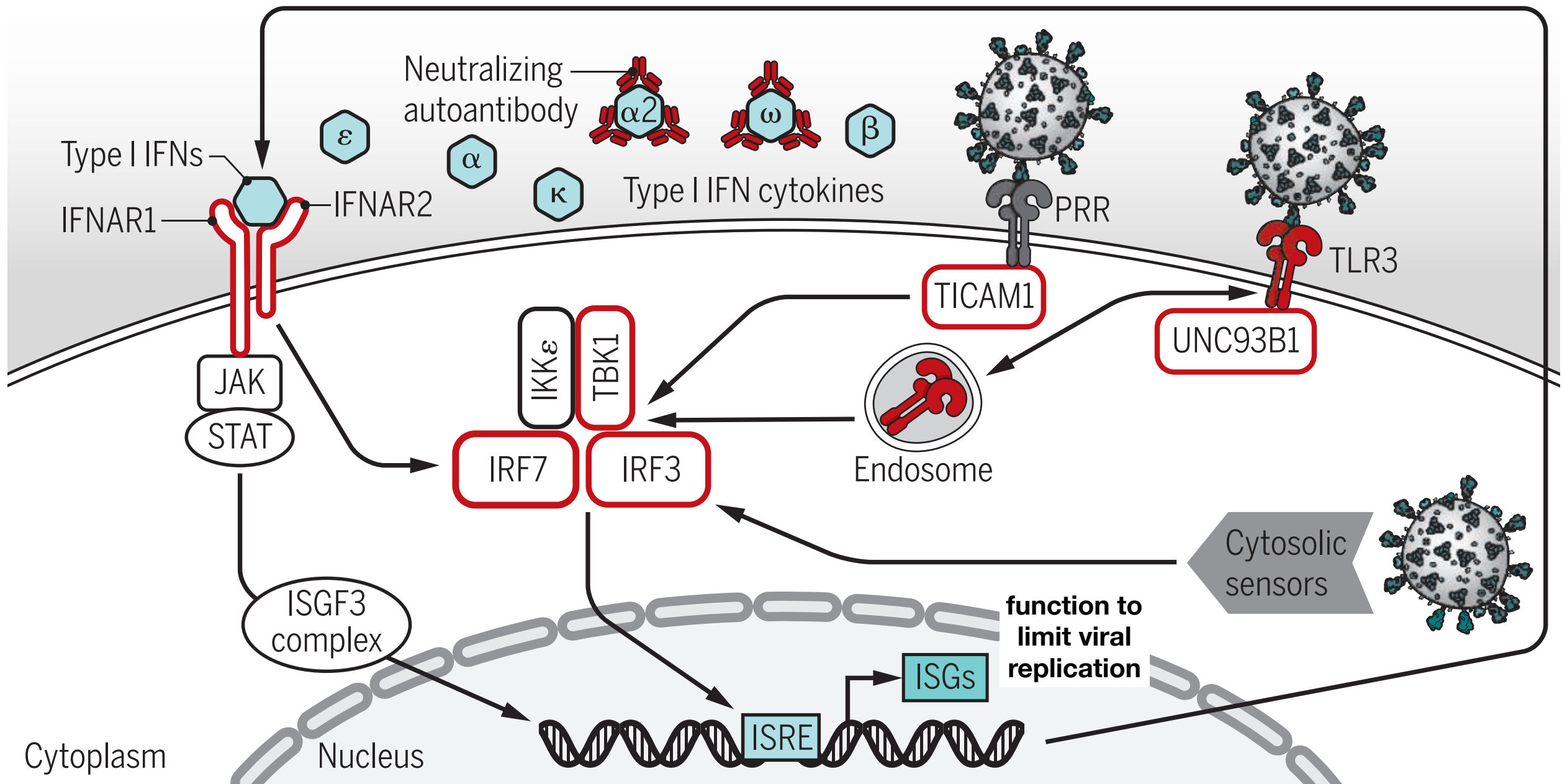
strengths: memory, specificty

weaknesses: takes a while to get into gear

Why Does COVID-19 Have Varying Severity?
II - COVID-19 Susceptibility & the Type I Interferon Pathway

red = neutralizing autoantibodies or loss-of-function genetic variants in subsets of patients with severe COVID-19

Viral particles are sensed by various PRRs, including cytosolic sensors. Type I IFNs are potent antiviral cytokines produced by innate immune cells. They bind a specific cell-surface receptor and signal through the JAK-STAT pathway to induce expression of ISGs that encode other antiviral proteins and various transcription factors. Subsets of patients with severe COVID-19 have loss-of-function genetic variants in several members of the type 1 IFN pathway (red) or neutralizing autoantibodies against type I IFNs, specifically IFN-α2 and IFN-ω.

modified from Beck & Aksentijevich *Science* (2020)

Why Are There So Many Long-Haulers?
## Data Below Not Yet Peer-Reviewed; Take with a Grain of Salt

- COVID-19 as a vascular disease (Chioh et al *medRxiv* 2020)
  - persistent immune activation —> endothelial cell dysfunction
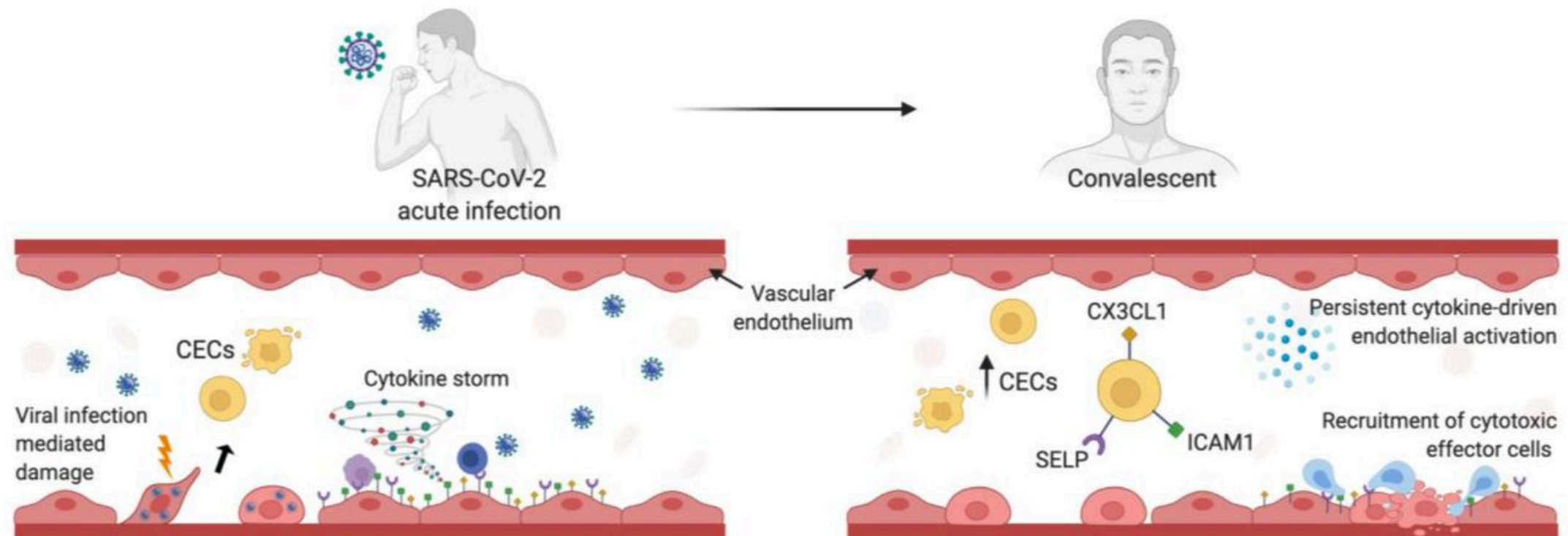
- Cytokine profile suggests long-haulers have the capability to activate T cells, but inability to recruit them (Patterson et al *bioRxiv* 2020)
  - additional support for COVID-19 as a vascular disease
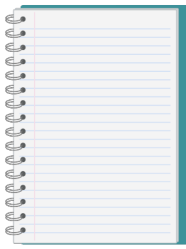
References
Information Up-To-Date as of December 28, 2020

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- Service RF. Radical Shift in COVID-19 Testing Needed To Reopen Schools And Businesses, Researchers Say. https://www.sciencemag.org/news/2020/08/radical-shift-testing-strategy-needed-reopen-schools-and-businesses-researchers-say (2020).

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- Dai L & Gao GF. Viral Targets for Vaccines Against COVID-19. *Nat. Rev. Immuno.* (2020) https://doi.org/10.1038/s41577-020-00480-0.
- Fontanet A & Cauchemez S. COVID-19 Herd Immunity: Where Are We? *Nat. Rev. Immuno.* **20**, 583-4 (2020).
- Metcalf CJE et al. Understanding Herd Immunity. *Trends In Immunology* **36**, 753-5 (2015).
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- Beck DB & Aksentijevich I. Susceptibility to Severe COVID-19. *Science* **370**, 404-5 (2020).
- Chioh FWJ et al. Convalescent COVID-19 Patients Are Susceptible to Endothelial Dysfunction Due to Persistent Immune Activation. *medRxiv* (2020) https://doi.org/10.1101/2020.11.16.20232835. ***NOT YET PEER REVIEWED***
- Patterson BK et al. Immune-Based Prediction of COVID-19 Severity and Chronicity Decoded Using Machine Learning *bioRxiv* (2020) https://doi.org/10.1101/2020.12.16.423122. ***NOT YET PEER REVIEWED***
- Zhang et al. Inborn Errors of Type I IFN Immunity in Patients with Life-Threatening COVID-19. *Science* **370**, eabd4570 (2020).

Graphics
- Created with BioRender.com

Additional Resources
To Quench Your Thirst For Knowledge

Testing
- [How Coronavirus Antibody, Genetic and Antigen Tests Work](#)
- [Coronavirus Test: Real Time RT-PCR - Animation](#)
- [Coronavirus Antibody Test - Animation](#)

Herd Immunity & Vaccines
- [Virology Lectures 2020 #19 - Vaccines](#)
- [COVID-19 Vaccine & Therapeutics Tracker](#)
- [What Does 95% Effective Mean? Teaching the Math of Vaccine Efficacy](#)
- [There Are Four Types of COVID-19 Vaccines: Here's How They Work](#)
- **Moderna/NIAID Pre-Clinical:** Corbett KS et al. Evaluation of the mRNA-1273 Vaccine Against SARS-CoV-2 in Nonhuman Primates. *N. Engl. J. Med.* **383**, 1544-55 (2020).
- **Oxford/AstraZeneca Pre-Clinical:** van Doremalen N et al. ChAdOx1 nCoV-19 Vaccine Prevents SARS-CoV-2 Pneumonia in Rhesus Macaques. *Nature* **586**, 578-82 (2020).
- **Pfizer/BioNTech Phase I:** Walsh EE et al. Safety and Immunogenicity of Two RNA-based COVID-19 Vaccine Candidates. *N. Engl. J. Med.* **383**, 2439-50 (2020).

Disease & Treatments
- [COVID-19 Vaccine & Therapeutics Tracker](#)
- [Immunology of COVID-19: Current State of the Science](#)

Other / General
- [This Week in Virology](#)
- New Mutant Strain
  - [Preliminary Genomic Characterization of an Emergent SARS-CoV-2 Lineage in the UK Defined By a Novel Set of Spike Mutations](#)
  - [Mutant Coronavirus in the United Kingdom Sets Off Alarms, But Its Importance Remains Unclear](#)
  - [Why the New COVID-19 Mutations Might Not Be As Scary As You Think](#)