# COVID-19:
# Testing and Variants

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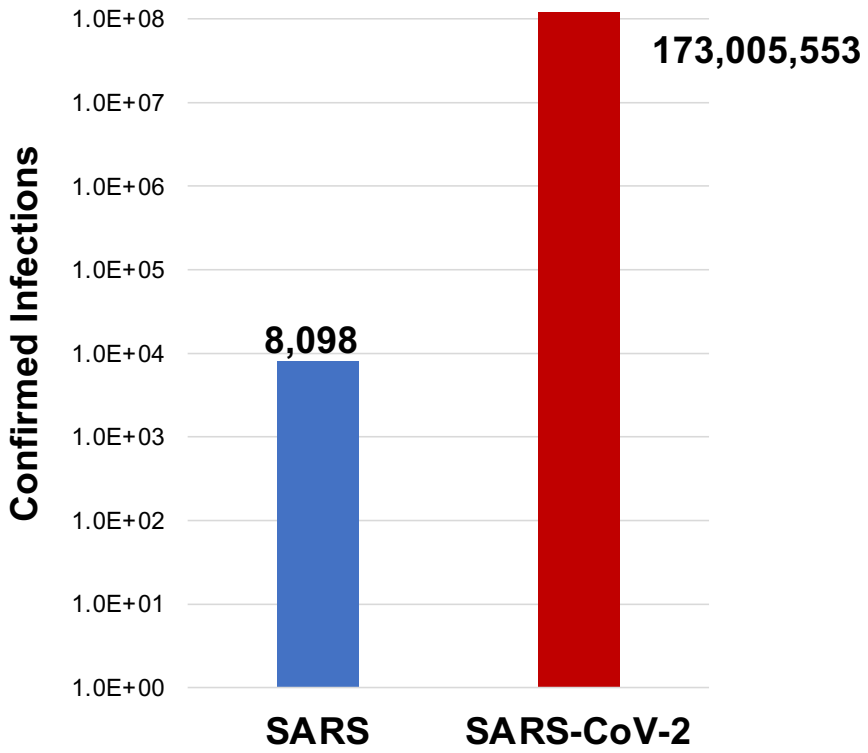
CHAN ZUCKERBERG BIOHUB

# The scale of the COVID-19 pandemic is far greater than that of SARS in 2003




World Health Organization 2021

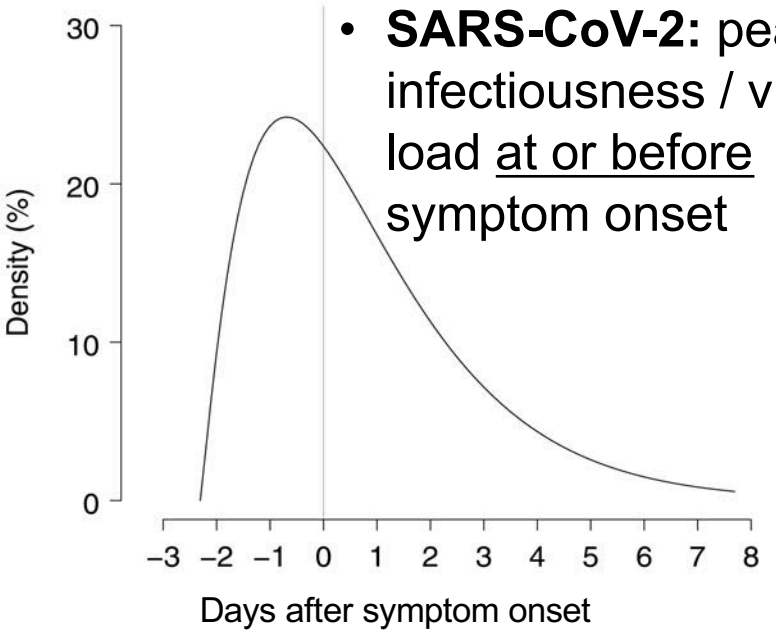
# Pre-symptomatic and asymptomatic transmission is a defining feature of COVID-19


**Diamond Princess 3/2020:**

- 712 (19.2%) of passengers tested positive
- **46.5%** were asymptomatic at time of testing

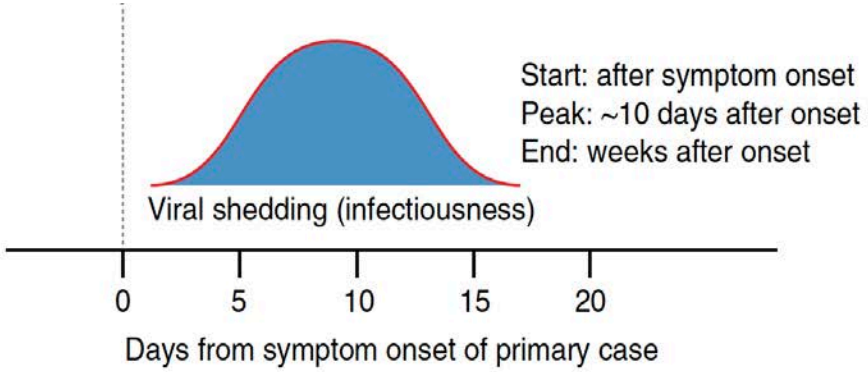
Moriarty et al. MMWR. 2020.

# Unlike SARS-2003, peak infectiousness for SARS-CoV-2 occurs at or before symptom onset




- **SARS-CoV-2:** peak infectiousness / viral load <u>at or before</u> symptom onset

- **SARS-2003:** peak infectiousness / viral load <u>10 days after</u> symptom onset




He et al. Nature Medicine. 2020.

# COVID-19 diagnostic testing

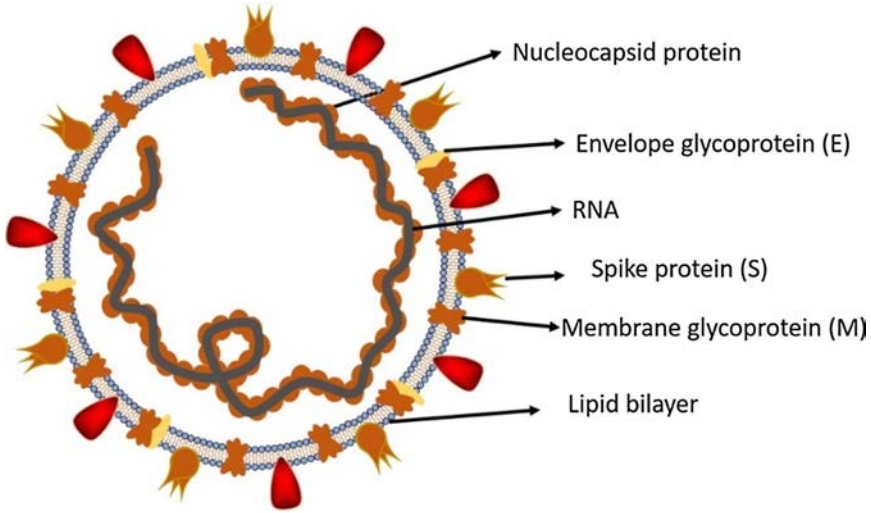
*Learning objectives*

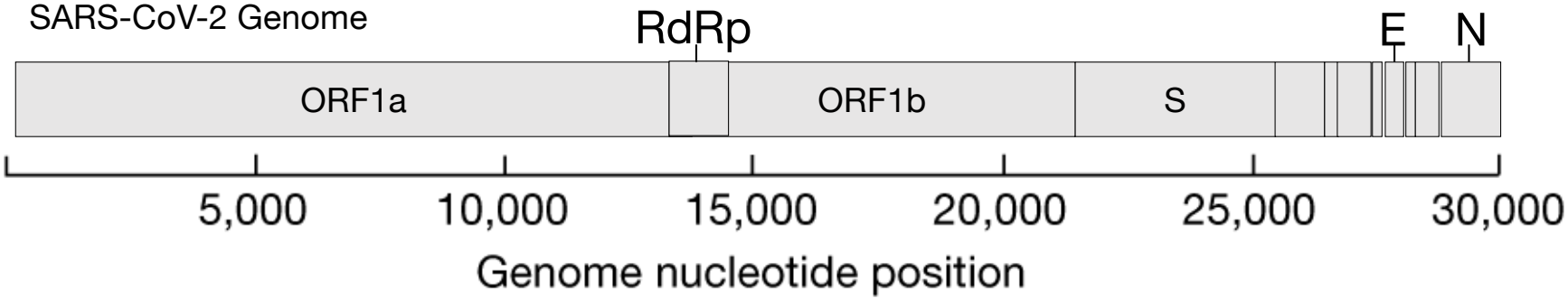
1. Types of COVID-19 testing
2. Dynamics of viral load and infectiousness
3. Sensitivity/specificity, and false negative, false positive tests
4. Use cases for highly sensitive PCR tests and less sensitive rapid antigen/nucleic acid tests

# Types of COVID-19 diagnostic testing

## 1. SARS-CoV-2 nucleic acid amplification tests

- PCR most common
- Detects viral RNA



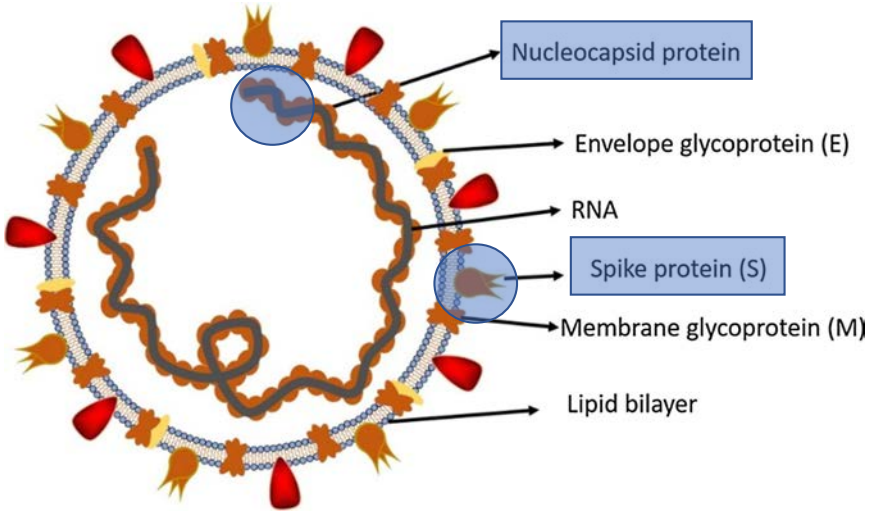



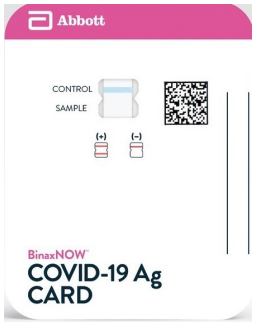

Chan et al. JCM. 2020. Shereen et al. J. Adv. Res. 2020.

# Types of COVID-19 diagnostic testing

## 2. SARS-CoV-2 antigen tests

- Detect viral proteins




Chan et al. JCM. 2020. Shereen et al. J. Adv. Res. 2020.

# SARS-CoV-2 nucleic acid vs antigen tests

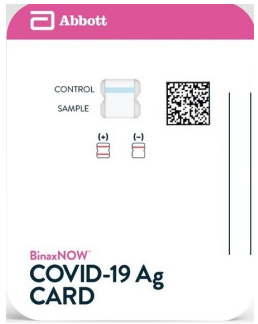
**Limit of Detection (genome copies/mL)**

$10^2$ → $10^5$

## RT-PCR
- 45-180 min
- Gold standard


## Rapid NAAT
- 15 min

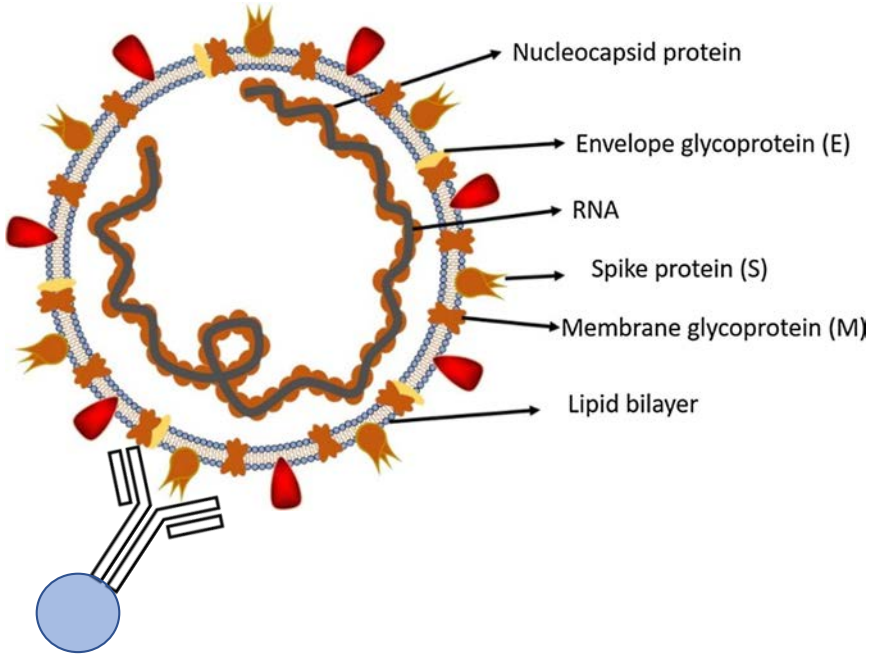

## Antigen
- 15 min



# Types of COVID-19 diagnostic testing

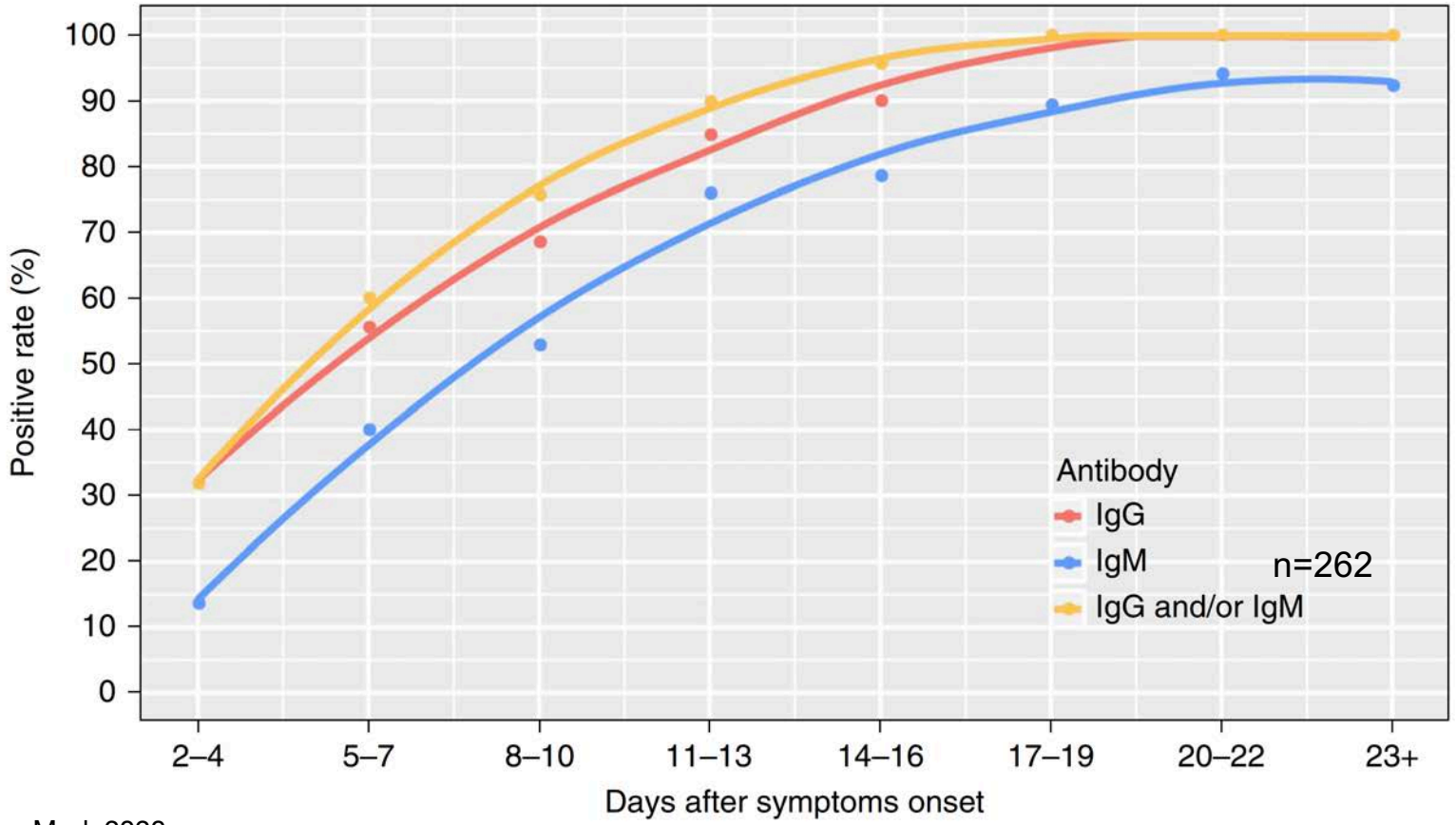
## 3. SARS-CoV-2 antibody tests

- Detect human antibodies against SARS-CoV-2 viral proteins




Chan et al. JCM. 2020. Shereen et al. J. Adv. Res. 2020.

# Antibody tests: not useful for diagnosing acute COVID-19




Long et al. Nature Med. 2020.

# SARS-CoV-2 nucleic acid/antigen tests

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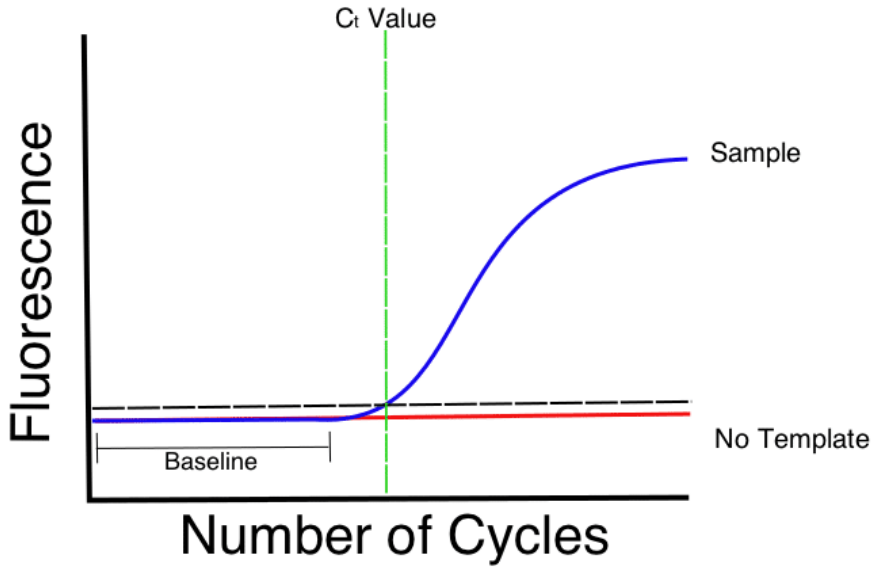
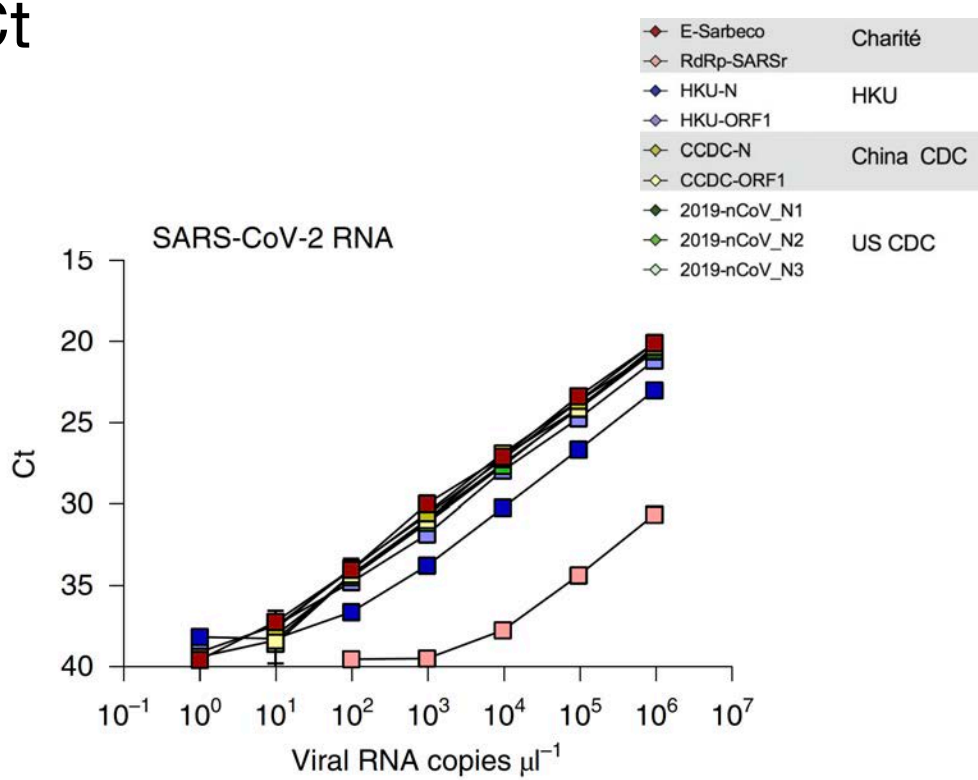
# SARS-CoV-2 RT-PCR

- Ct: cycle threshold –cycles needed for detectable signal
- Higher viral load = lower Ct




Vogels et al. Nature Microbiology. 2020. https://doi.org/10.1038/s41564-020-0761-6.

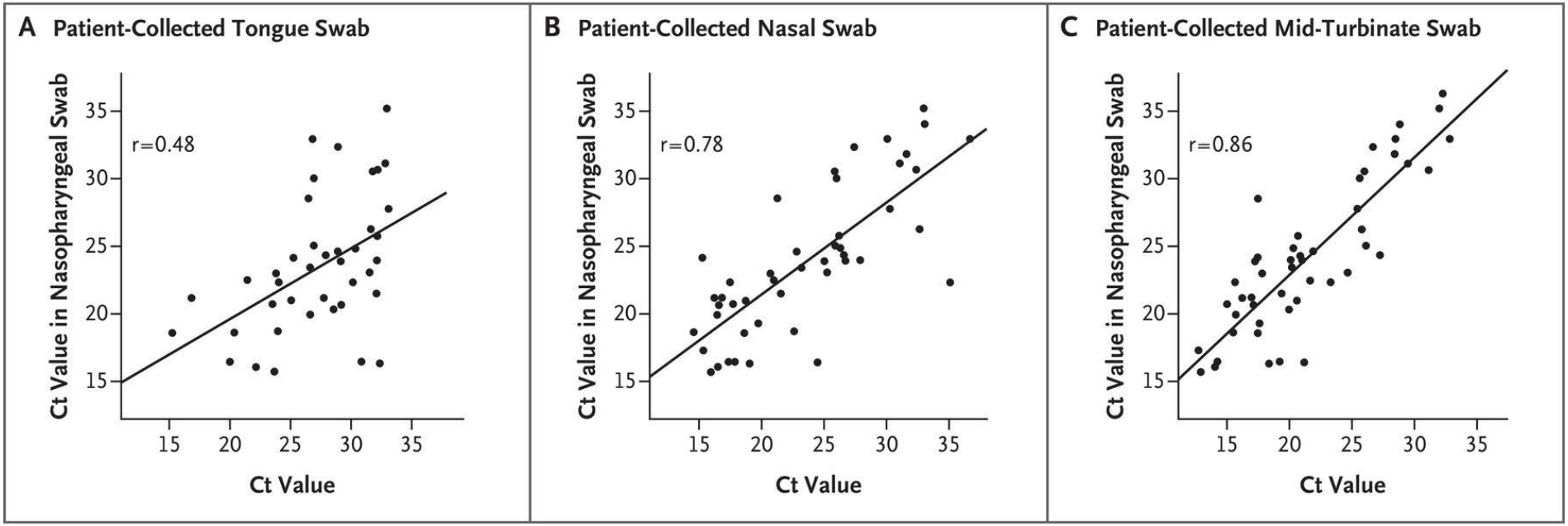
**Sensitivity:** true positive rate of a test
**Specificity:** true negative rate of a test

# SARS-CoV-2 PCR sensitivity varies by sample collection site

| | Sensitivity |
|---|---|
| Oropharyngeal (OP) swab | 32-61% |
| Nasopharyngeal (NP) swab | 54-89% |
| NP+OP | 80-89% |
| Sputum | 72-89% |
| Bronchoalveolar lavage | 93-100% |


. Hansen et al. CID. 2021. Wang et al. JAMA. 2020., Lee et al. CID. 2020.

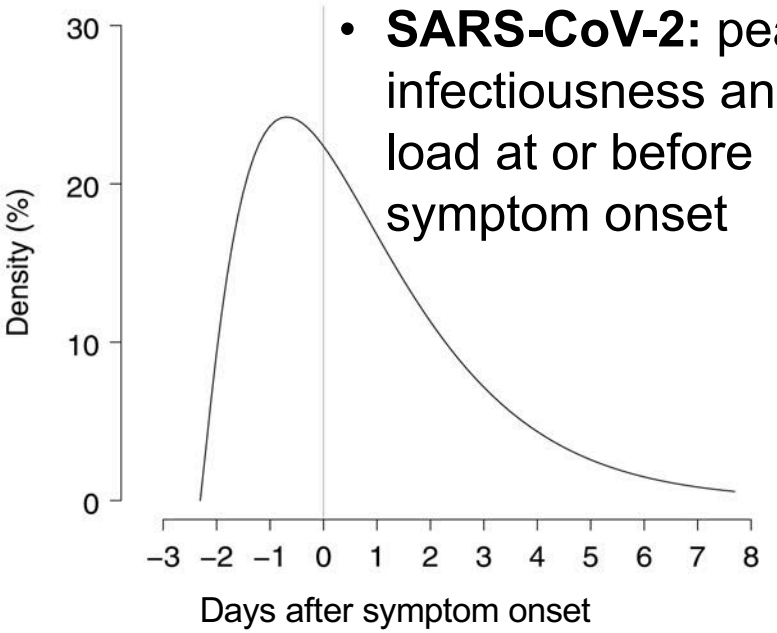
# Clinician-collected NP swab vs patient collected swabs




Tu et al. 2020. NEJM.

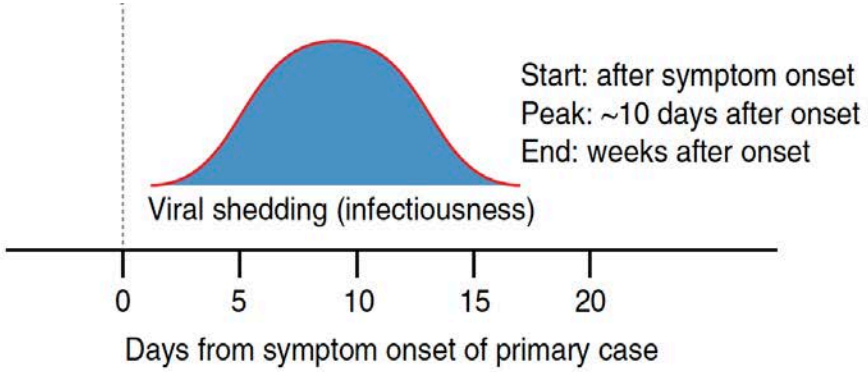
# Do we really need a highly sensitive test?

# SARS-CoV-2 peak viral load and infectiousness occurs at or before symptom onset



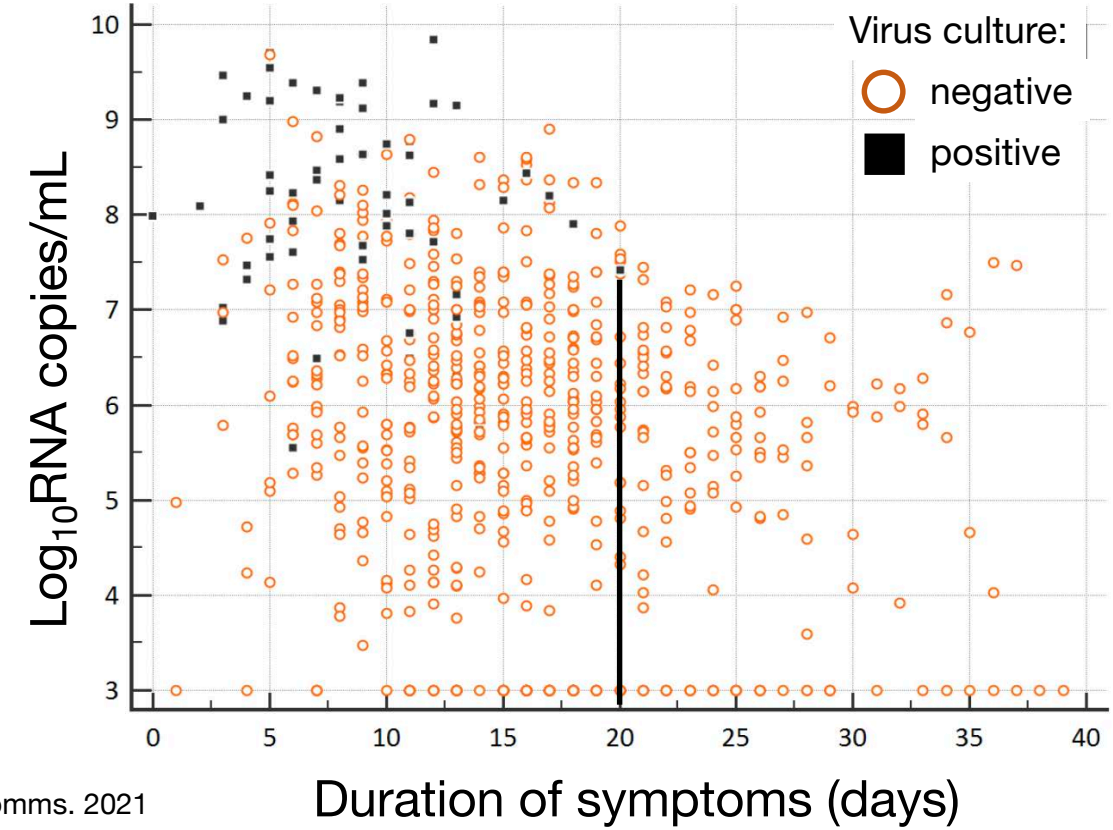

- **SARS-CoV-2:** peak infectiousness and viral load at or before symptom onset

- **SARS-2002:** peak infectiousness / viral load 10 days after symptom onset




He et al. Nature Medicine. 2020.

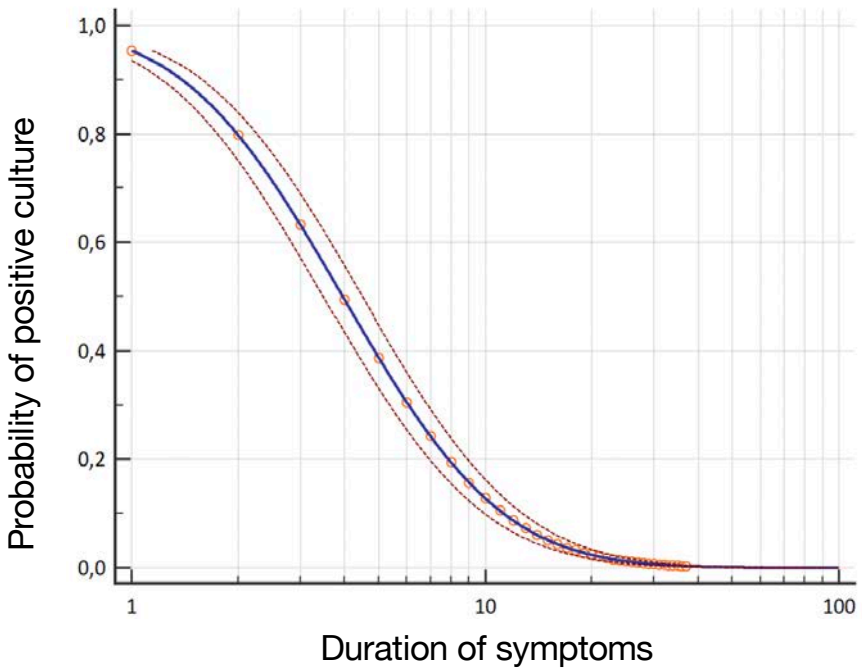
# SARS-CoV-2 RNA persists but infectiousness decreases over time

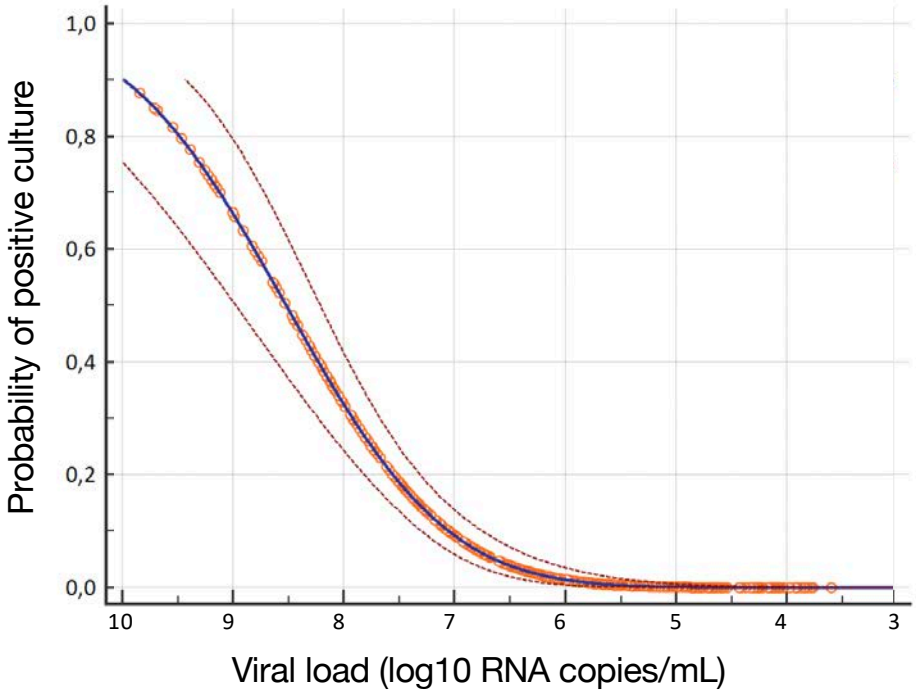



Van Kampen et al. Nature Comms. 2021

N = 129

# Infectiousness decreases over time and correlates with SARS-CoV-2 viral load



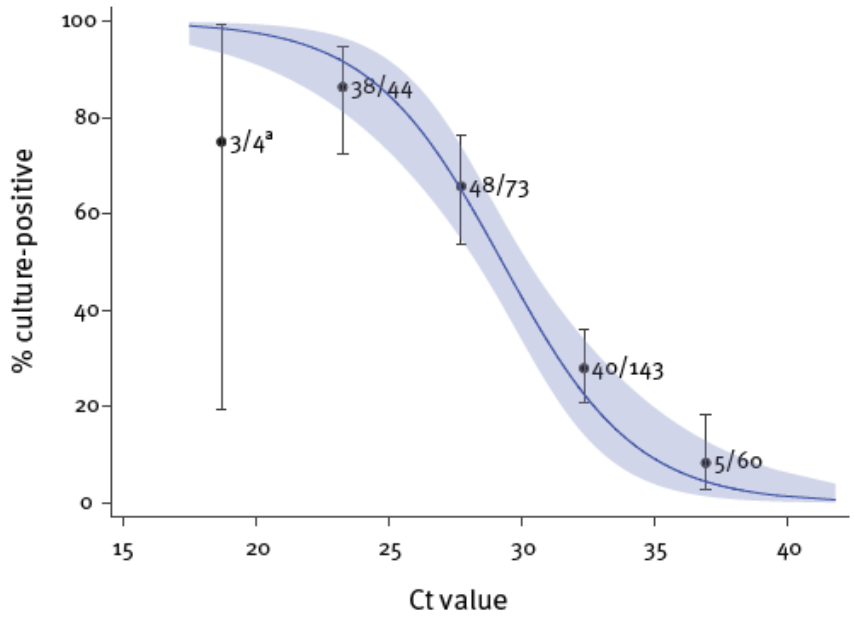




Van Kampen et al. Nature Communications. 2021.

# Ct value versus culture positivity

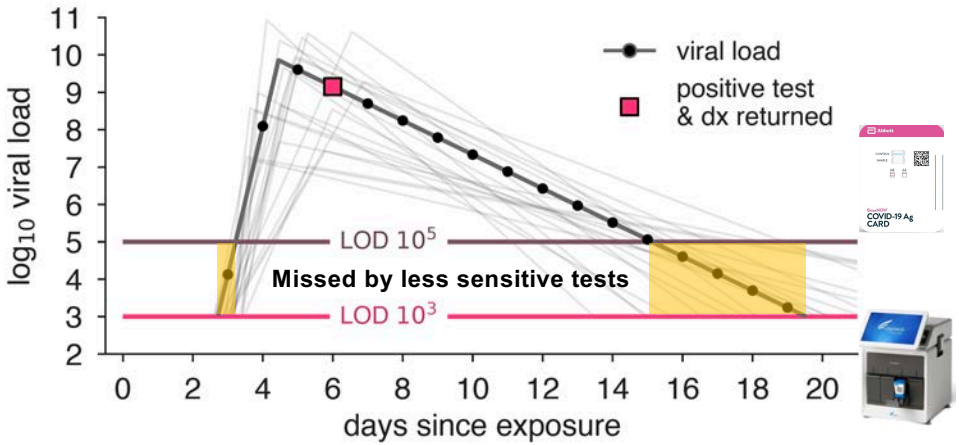
**FIGURE 2**

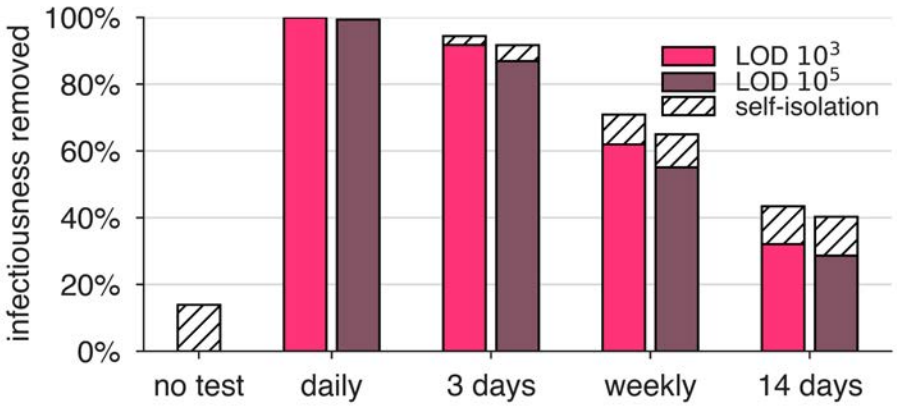
Relationship between RT-PCR Ct value and culture positivity in mixed effects logistic regression analysis, SARS-CoV-2, England, January–May 2020 (n = 324)




Singanayagam A et al. Eurosurveillance. 2020;25(32):2001483. 2020
https://www.cebm.net/study/duration-of-infectiousness-and-correlation-with-rt-pcr-cycle-threshold-values-in-cases-of-covid-19-in-england/

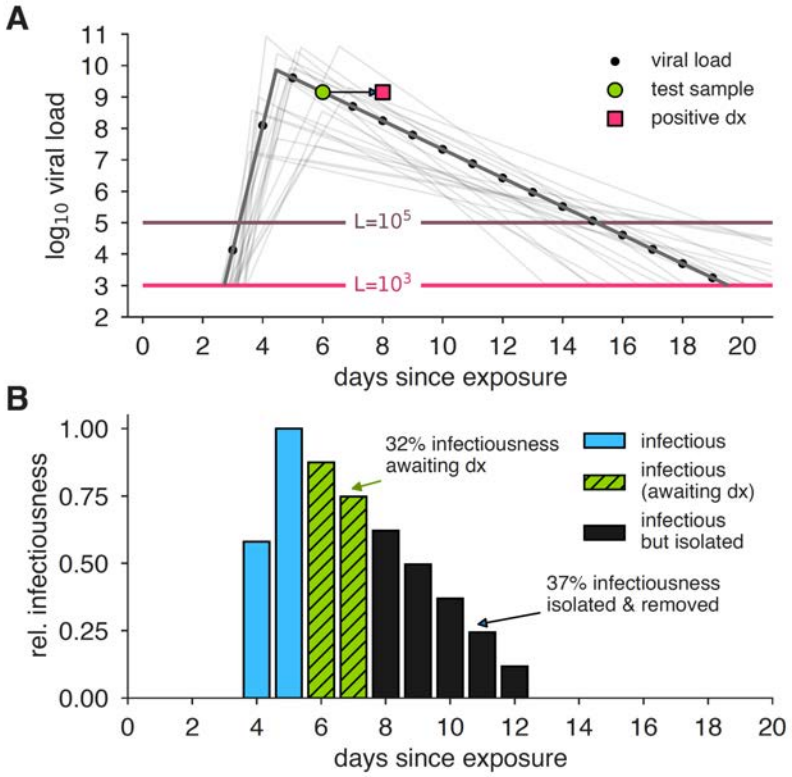
# Test sensitivity is secondary to <u>frequency</u> for effective COVID-19 surveillance




Minna et al. NEJM. 2020.
Larremore et al. Science Advances. 2020.

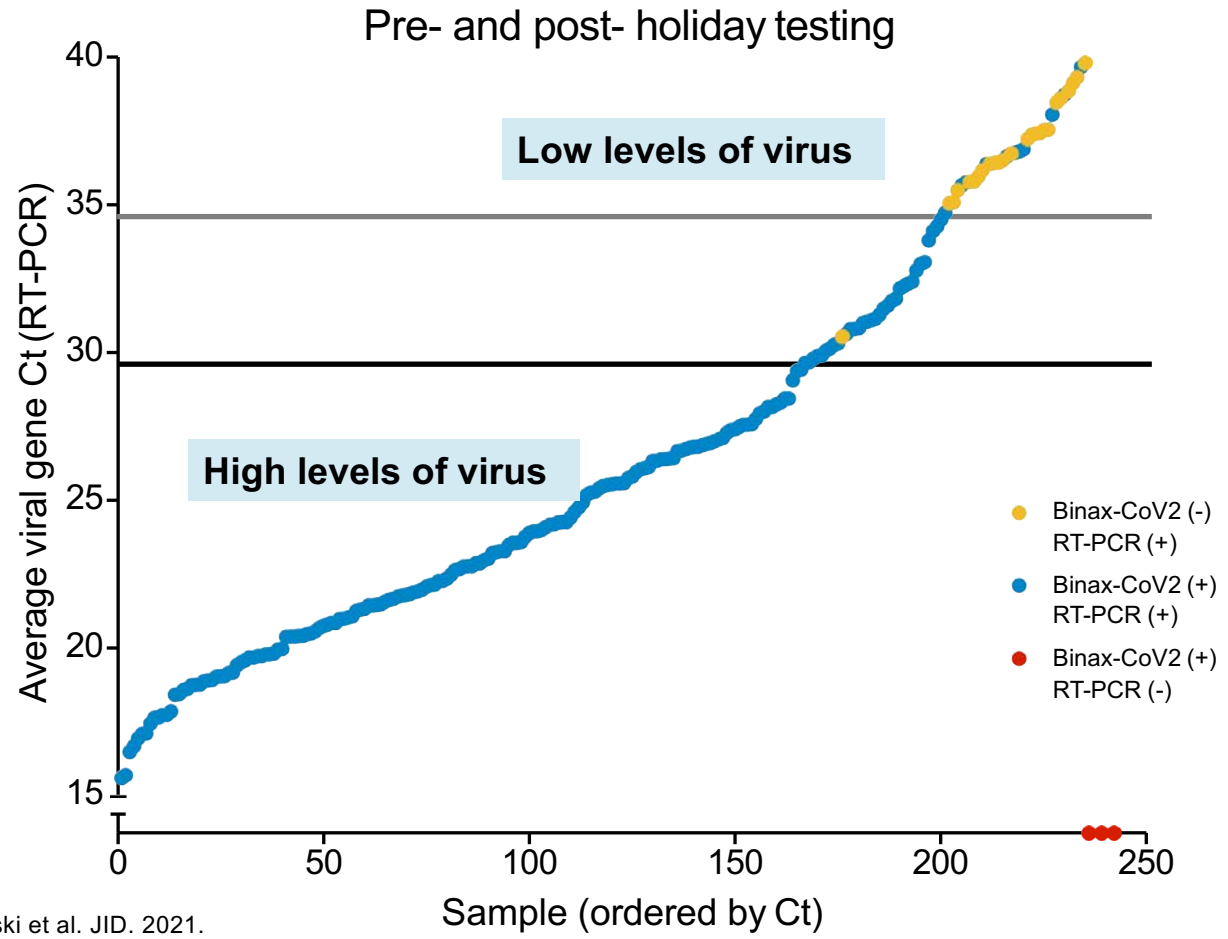
# Test sensitivity is secondary to frequency and turnaround time for COVID-19 surveillance




Larremore et al. Science Advances. 2020.

# Binax-CoV-2 rapid antigen test

# SARS-CoV-2 Binax antigen assay vs PCR Ct value




Total Binax-CoV2 tests: 3298

- Binax-CoV2(+) RT-PCR(+): 210 (6.4%)
- Binax-CoV2(+) RT-PCR(-): 3 (0.1%)
- Binax-CoV2(-) RT-PCR(+): 23 (0.7%)
- Binax-CoV2(-) RT-PCR(-): 3062 (92.8%)

Pilarowski et al. JID. 2021.

# When do we need a sensitive test?

- ***Hospitalized patients***
- ***High risk settings***

- *Surveillance*

Limit of Detection (genome copies/mL)

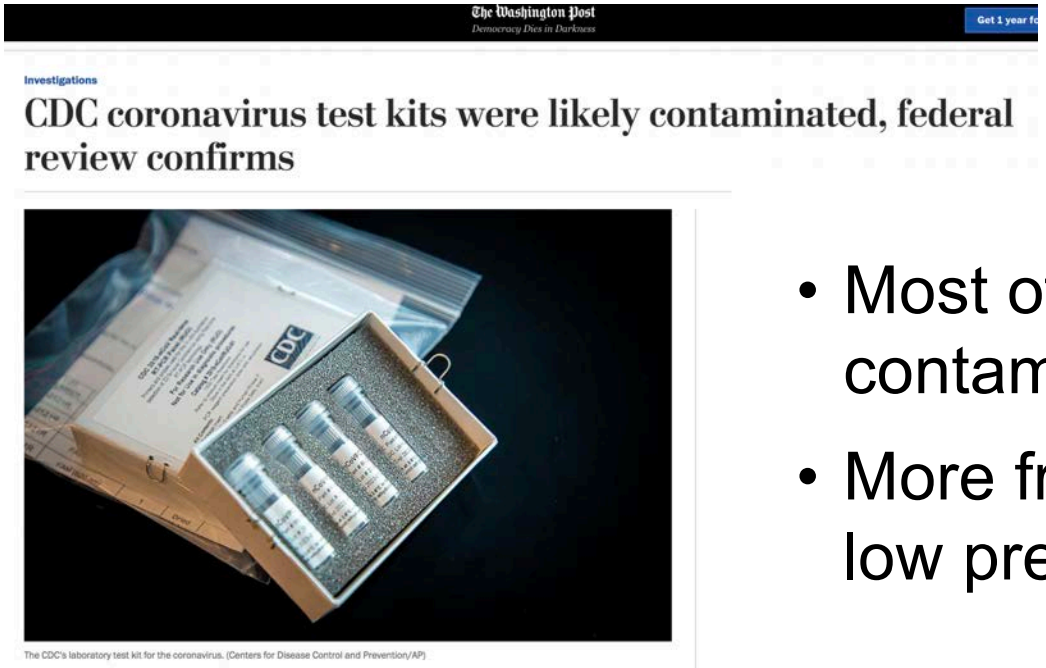
$10^2$ → $10^5$

**RT-PCR**


**Rapid-NAAT**

**Antigen**

# False positive SARS-CoV-2 tests can also be a problem




- Most often due to laboratory contamination.
- More frequent in the context of low prevalence screening testing.

# SARS-CoV-2 variants

*Learning objectives*

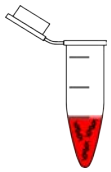
1. How we detect SARS-CoV-2 variants
2. The most widely recognized SARS-CoV-2 variants of concern
3. Reasons why we should be concerned about variants
4. Protection offered by vaccines against variants

# How do we detect variants?

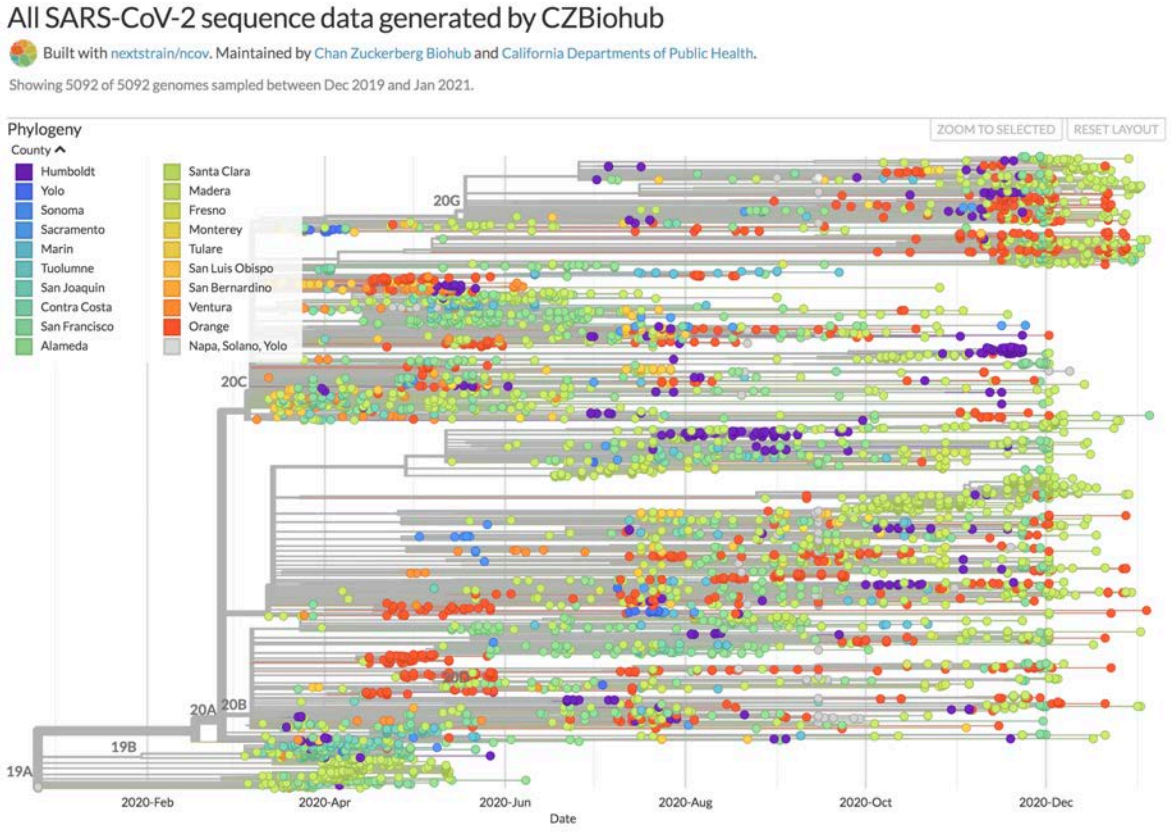
*Standard tests can't distinguish variants of concern*



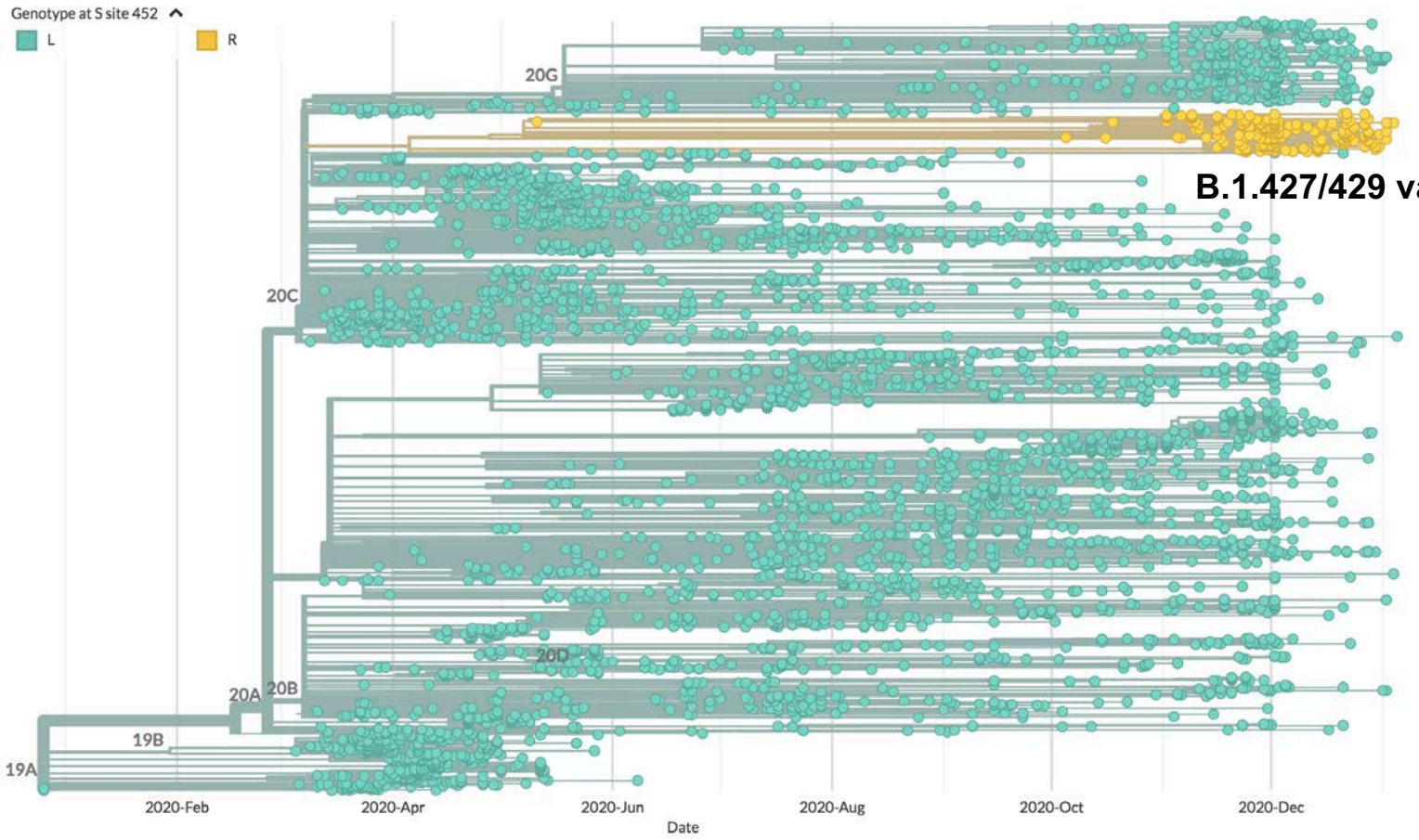
# How do we detect variants? *sequencing*



ACTGCATAGC
GGTAACTGCAT
CATAGCAATC



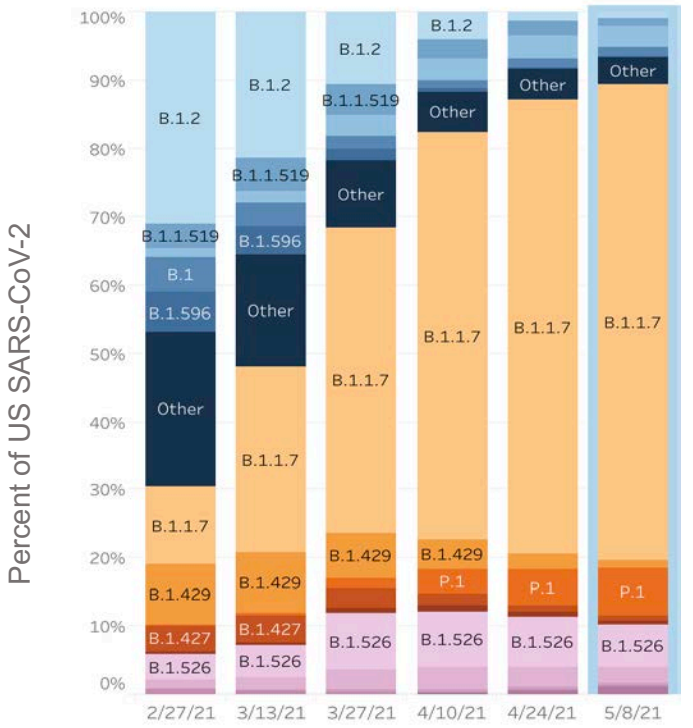
# Emergence of B.1427/B1.429 variants in California




Data: CZ Biohub/GSAID/Nextrain

# Several SARS-CoV-2 variants of concern have recently been identified

- **B.1.1.7** (UK)
- **B.1.351** (South Africa)
- **P.1.** (Brazil)
- **B.1.427/B.1.429** (CA)
- **B.1.617.2** (India)




https://covid.cdc.gov/covid-data-tracker/#variant-proportions

# SARS-CoV-2 variants have mutations leading to amino acid changes in the the spike protein

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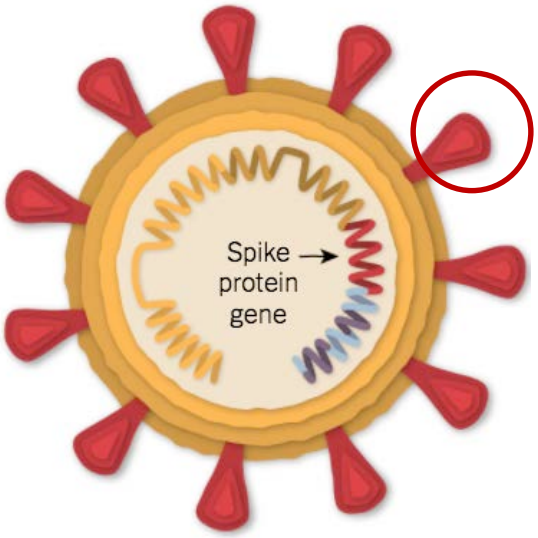
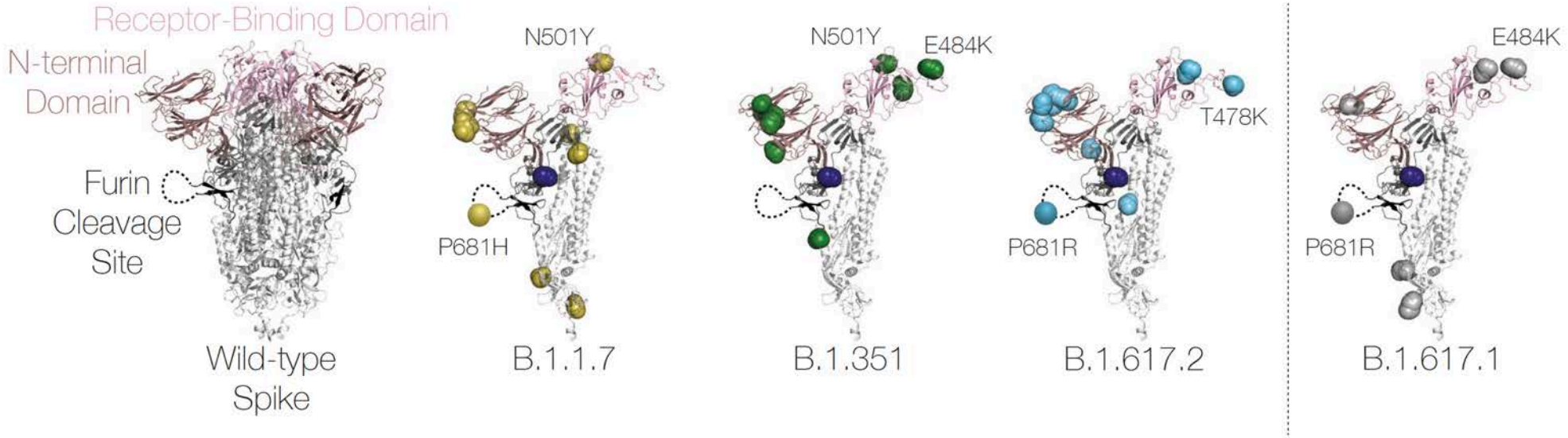



**SARS-CoV-2**

Rambaut et al. https://virological.org , New York Times

# SARS-CoV-2 variants have mutations leading to amino acid changes in the the spike protein




Wall et al. Lancet 2021.

## Why are we concerned about variants?

- Potential for increased transmissibility
- Potential for vaccine/immune escape

# Potential for increased transmissibility

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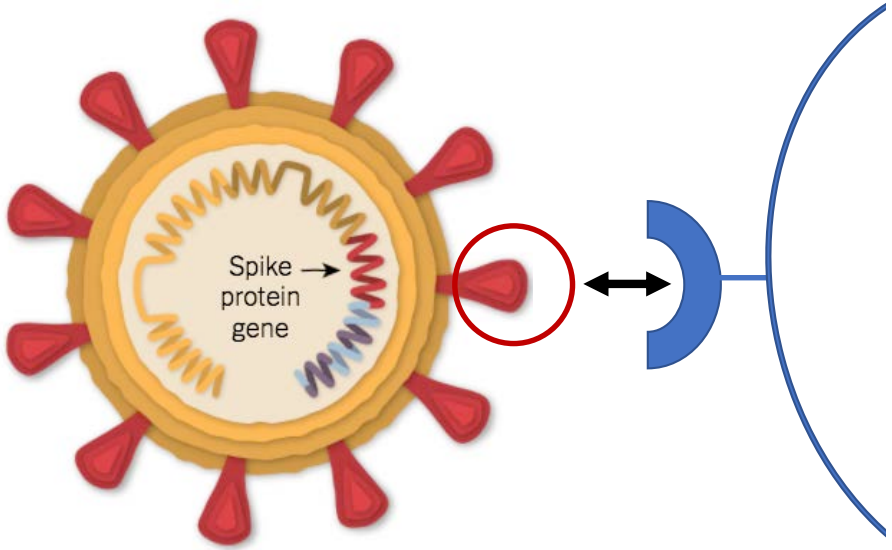
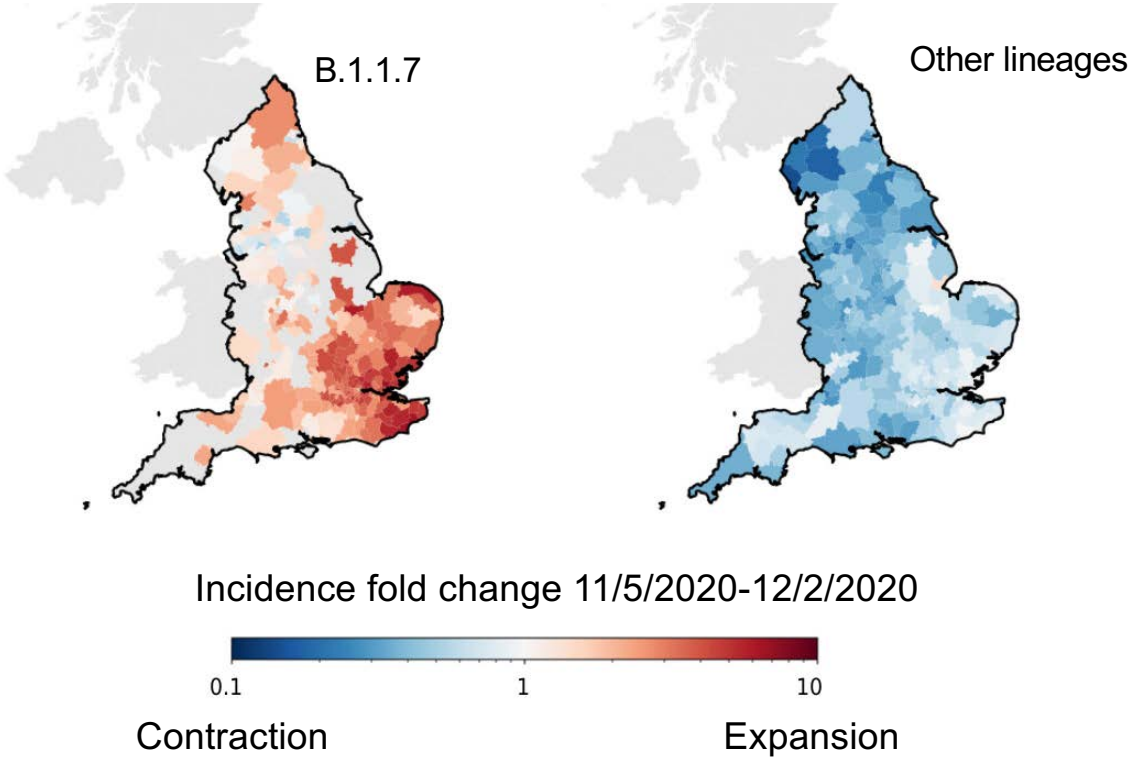



Increased cell infectivity

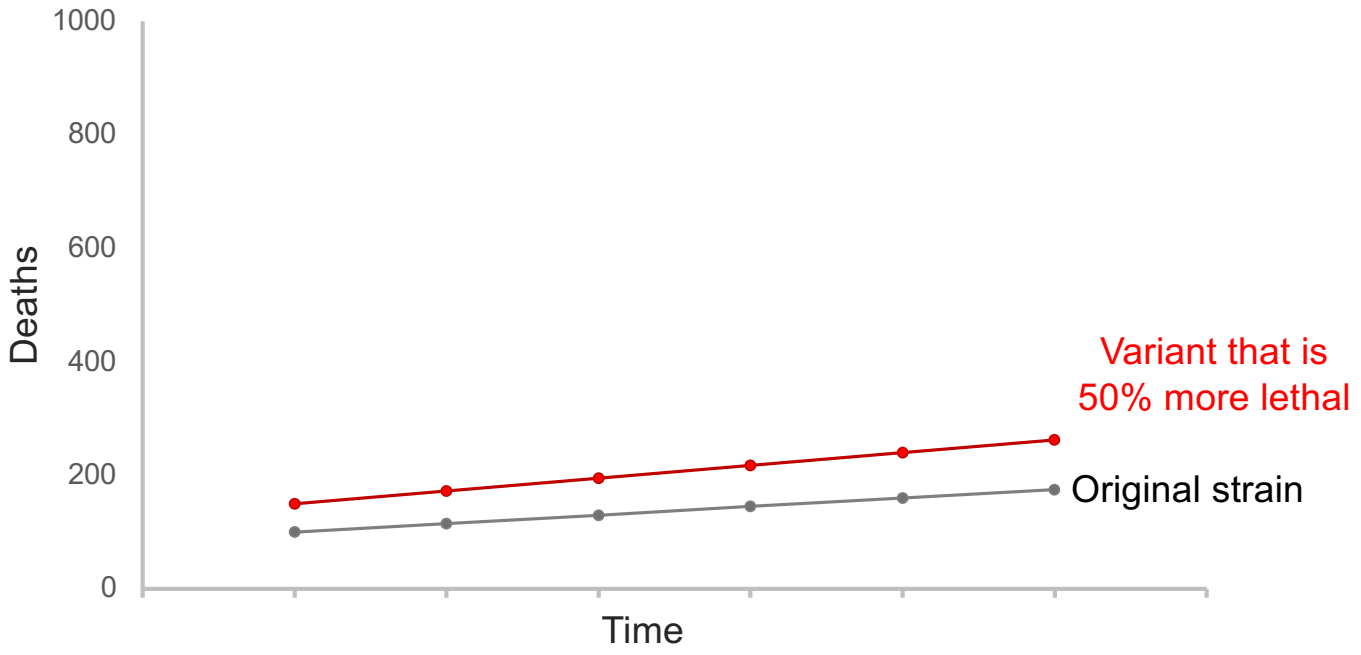
Rambaut et al. https://virological.org , New York Times

# Transmissibility case study: B.1.1.7 (UK variant)




Vöhringer et al. https://virological.org

# Even if a variant is not more lethal, greater transmissibility can increase deaths




Kucharski. London School of Hygiene and Tropical Medicine.

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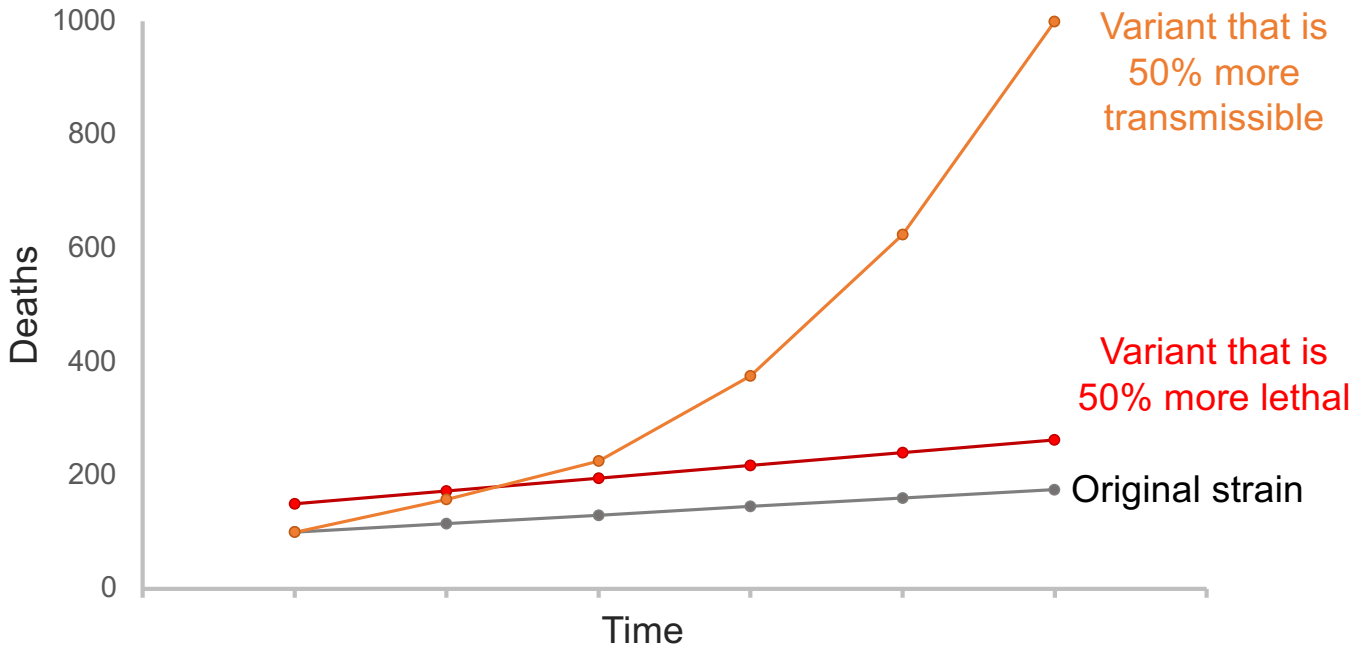


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# Potential for vaccine/immune escape

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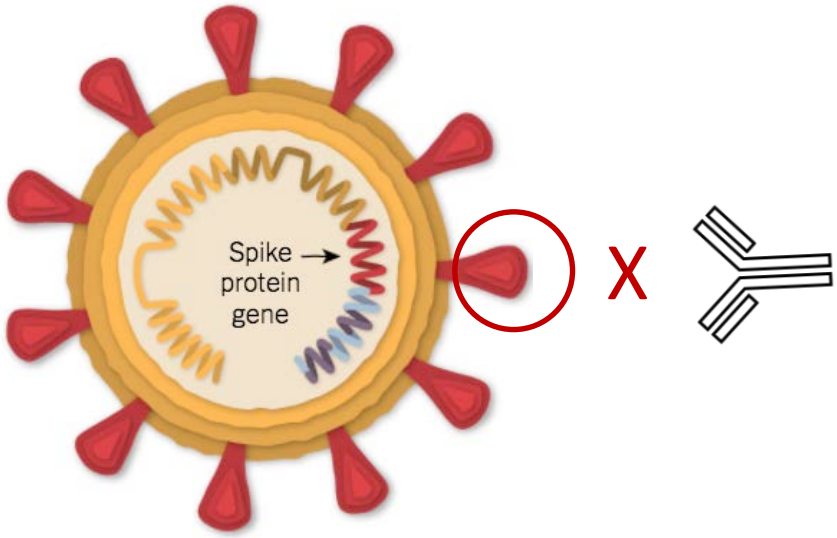
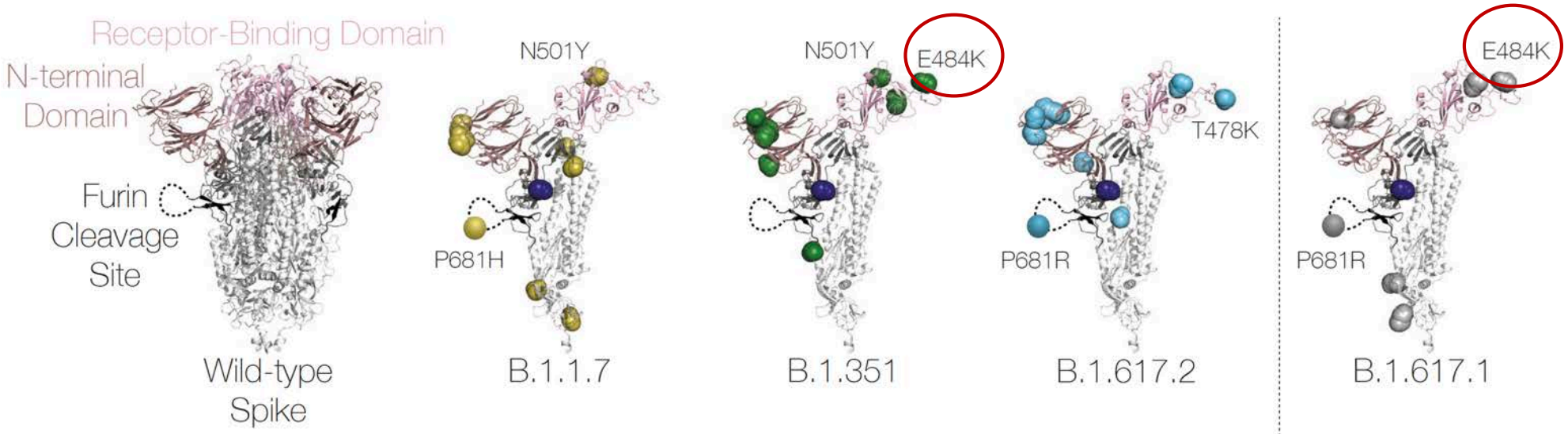



Resistance to antibody neutralization

Rambaut et al. https://virological.org , New York Times

# E484K reduces antibody neutralization




Wall et al. Lancet 2021.

# Are vaccines less effective against variants?

**Novavax NVX-CoV2373:**

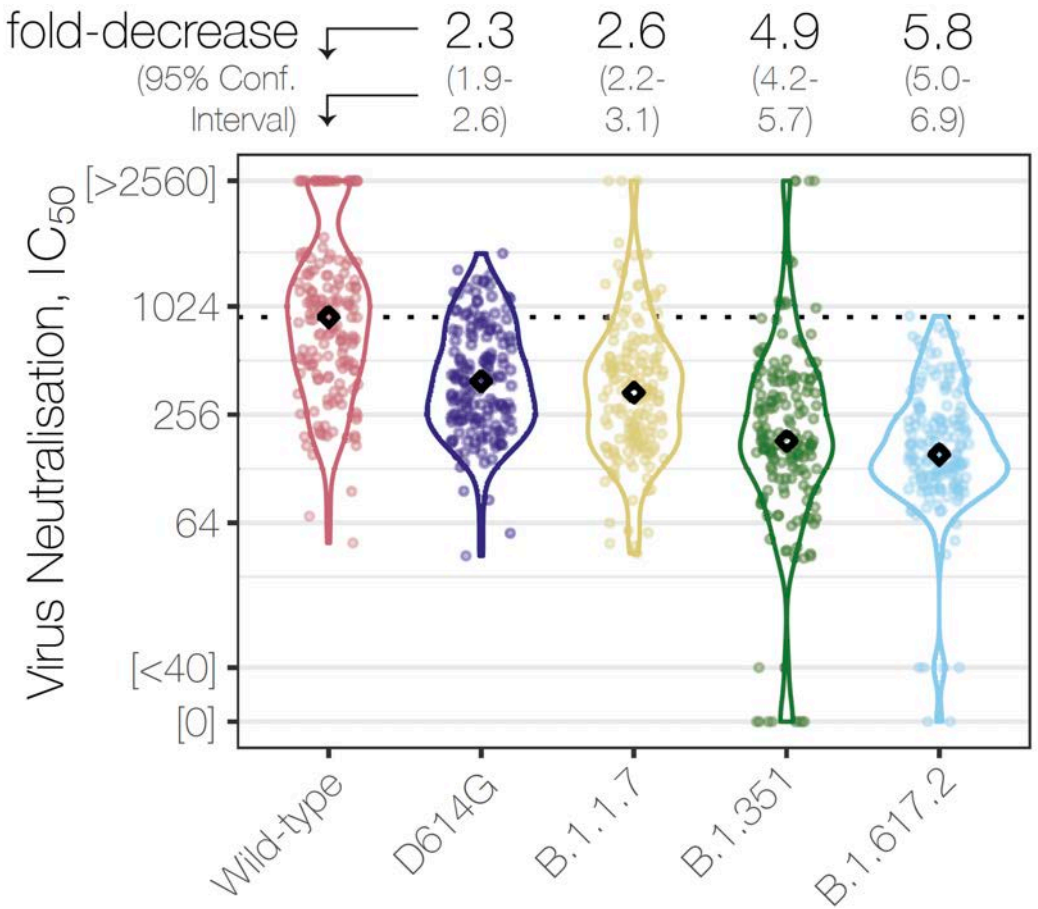
- B.1.3.5.1 (SA): **50.1% efficacy** against mild/moderate disease

**Efficacy of ChAdOx1 nCoV-19**

- B.1.3.5.1: **10.4% efficacy** against mild/moderate disease

N Engl J Med. 2021; 384:1899-1909. doi: 10.1056/NEJMoa2103055
N Engl J Med. 2021; 384:1885-1898. doi: 10.1056/NEJMoa2102214.

# Reduced antibody neutralization after Pfizer vaccine




Wall et al. Lancet 2021.

# Are vaccines less effective against variants?

**Novavax NVX-CoV2373:**

- B.1.3.5.1 (SA): **50.1% efficacy** against mild/moderate disease
- **No cases of severe disease, only 1 case in placebo group**

**Efficacy of ChAdOx1 nCoV-19**

- B.1.3.5.1: **10.4% efficacy** against mild/moderate disease
- **No cases of severe disease**

N Engl J Med. 2021; 384:1899-1909. doi: 10.1056/NEJMoa2103055
N Engl J Med. 2021; 384:1885-1898. doi: 10.1056/NEJMoa2102214.

# Pfizer/Moderna mRNA vaccines remain effective at preventing COVID-19 from variants

## Effectiveness of COVID-19 vaccines against the B.1.617.2 variant

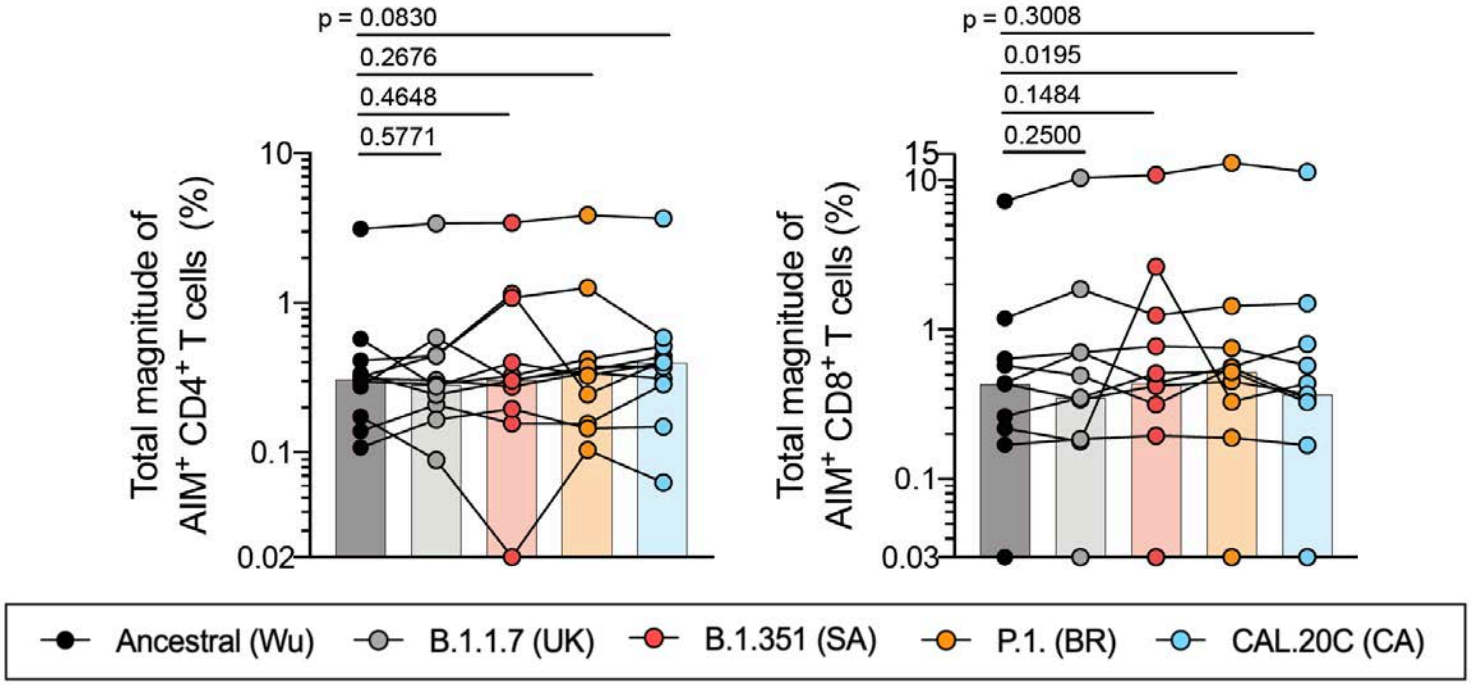
Jamie Lopez Bernal[1,2,3], Nick Andrews[1,2], Charlotte Gower[1], Eileen Gallagher[1], Ruth Simmons[1], Simon Thelwall[1], Julia Stowe[1], Elise Tessier[1], Natalie Groves[1], Gavin Dabrera[1], Richard Myers[1], Colin Campbell[1,2], Gayatri Amirthalingam[1,2], Matt Edmunds[1], Maria Zambon[1,3], Kevin Brown[1,2], Susan Hopkins[1,4], Meera Chand[1,5], Mary Ramsay[1,2]

Pfizer vaccine:

- 93% effective against B.1.1.7
- 88% effective against B.1.617.2

https://www.medrxiv.org/content/10.1101/2021.05.22.21257658v1

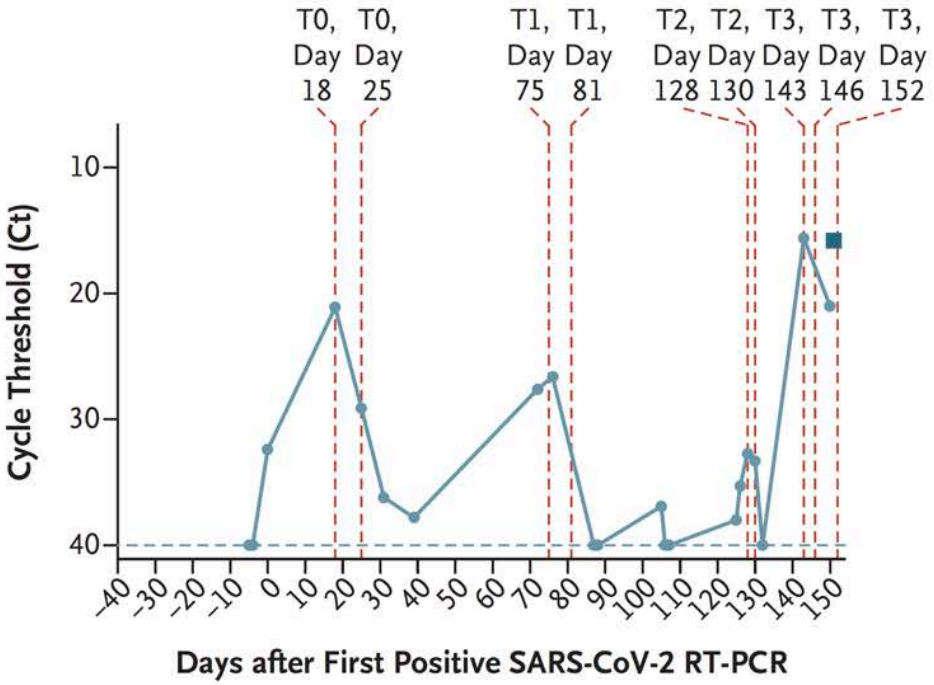
# T-cell responses to variants remain intact

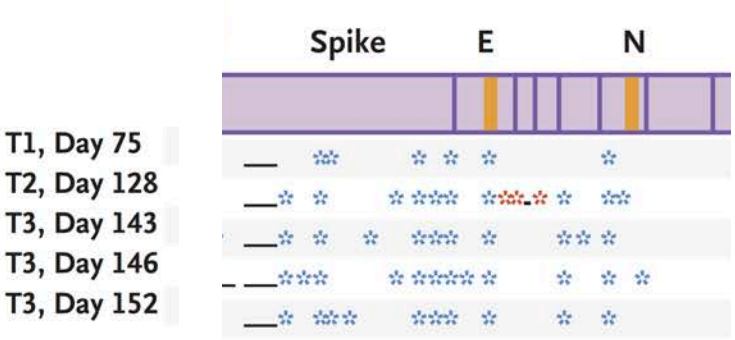



Tarke et al. BioRxiv. 2021.
Redd et al. OFID. 2021.

# Vaccines, masks and distancing are still effective

# How do variants arise? Accelerated viral evolution in immunocompromised patients may play a role




Choi et al. 2020. NEJM.

## **Key points:** Diagnostic testing:

1. Viral load and infectiousness is highest early during infection, at or before the time of symptom onset
2. PCR is the most sensitive type of test
3. Antigen tests perform very well at identifying active infections
4. Frequency and turnaround time are in most cases more important than test sensitivity.
5. Antibody tests do not detect new infections, only past infections

## **Key points:** SARS-CoV-2 Variants of Concern:

1. Variants of concern have mutations in the viral spike protein, which is recognized by the immune system and is essential for infecting cells
2. Sequencing is used to detect and track variants.
3. Some variants are more transmissible, and evade neutralizing antibodies
4. mRNA vaccines appear to protect against most variants, likely because of the T-cell mediated immunity