SARS-CoV-2 Diagnostics

A brief overview

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Disclosures

- Editor for IDSA COVID-19 Real-time Learning Network
- Scientific advisor for PhAST Diagnostics
- Scientific advisory board for GlaxoSmithKline

It is risky to hold strong opinions on virtually any aspect of the COVID-19 pandemic

What is controversial one day is dogma the next

Best to view everything here as the opinion of the speaker, and judge the data for yourself

Case

- A 75 year old nursing home resident learned that she was in close contact with a fellow resident 3 days ago who was later diagnosed with COVID-19. She is asymptomatic.
- As part of the facility policy, she underwent antigen and PCR testing with an anterior nasal swab. Both results return negative.
- How would you interpret this result and how would you act on it?

  A. A true negative, no further testing needed

  B. A possible false negative, repeat antigen testing in 2 - 4 days

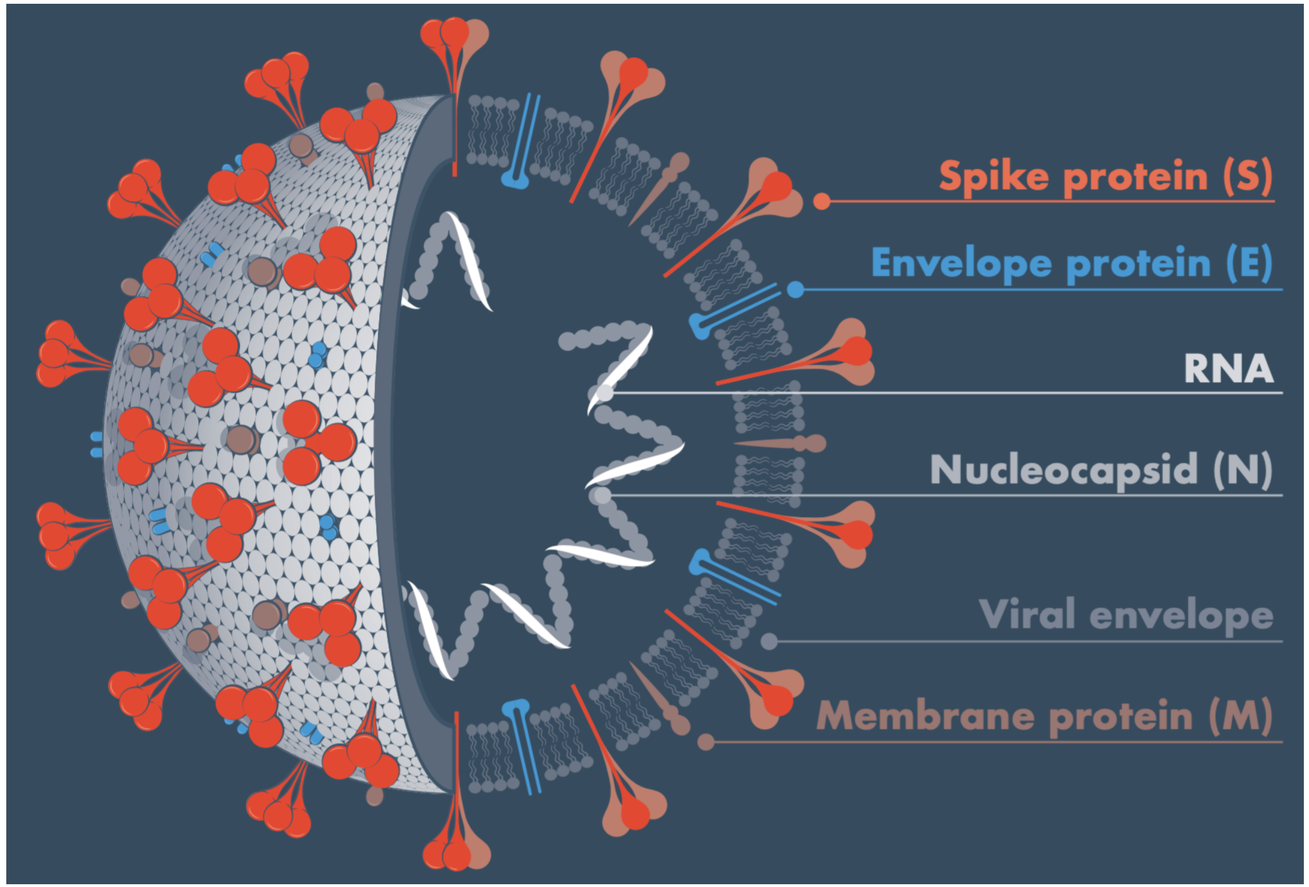
  C. A possible false negative, repeat PCR testing in 2 - 4 days

  D. A possible false negative, repeat antigen and PCR testing in 2 - 4 days

  E. A possible false negative, repeat PCR testing in 7 days

Key aspects of the virus pertinent to diagnostics

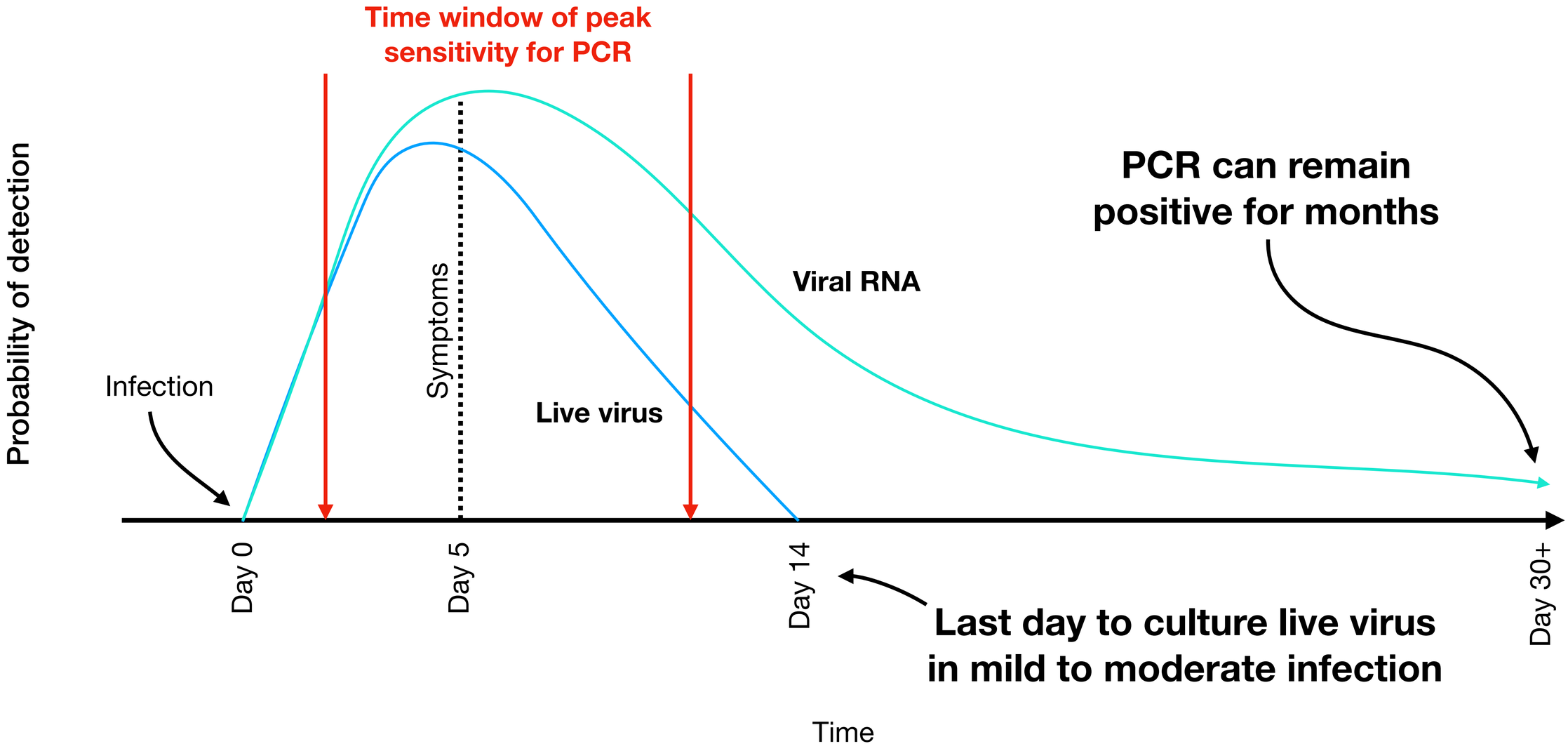
Virus structure and targets



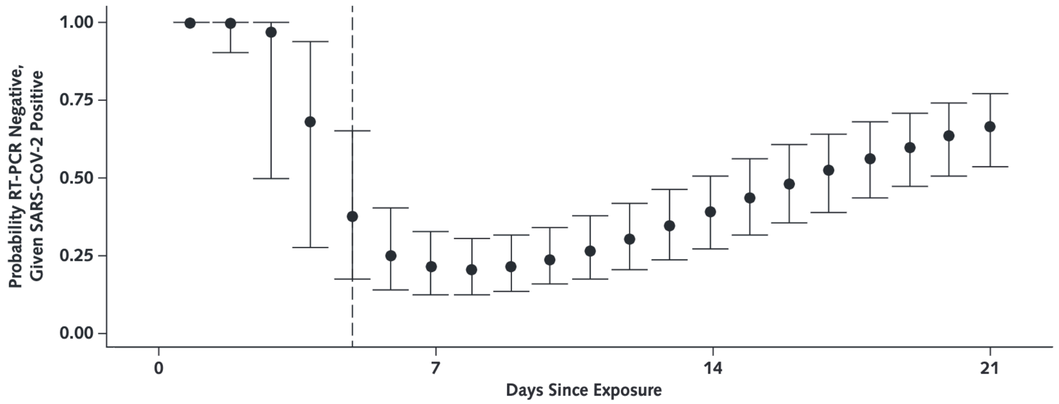
- Spike, nucleocapsid genes specific to SARS-CoV-2
- Envelope gene common to sarbecoviruses (SARS, SARS-CoV-2)
- Non-structural gene targets include ORF1ab
- Most assays target >1 gene and/or >1 section of a gene

Viral load kinetics

And its relationship to diagnostics



Clinical performance at the patient level



Kucirka, Ann Int Med, 2020

- Peak sensitivity around day ~5 from exposure
- Test sensitivity likely higher in those with symptoms since they are more likely to present for care
  - Asymptomatic people can be anywhere along this time axis
- Serial testing in 24 to 72 hours greatly increases sensitivity for detecting active infection

Asymptomatic/presymptomatic transmission

Paradigm shift in testing strategies

- Modeling and small epidemiologic studies suggest somewhere between 40% to 80% of new infections occur from asymptomatic or presymptomatic individuals
- Pandemic control requires repeated testing of both symptomatic and asymptomatic people

	SARS-CoV-2	Other respiratory viruses
Frequency of testing	>1 time	Usually once
Test results	Semi-quantitative	Qualitative
Target population	Symptomatic & asymptomatic	Symptomatic only

The SARS-CoV-2 testing paradigm

Active case detection in people with **high pretest probability** for infection (symptomatic or close contacts of known case)

Screening in people with **low pretest probability** of infection (asymptomatic)

Clinical management

Infection control

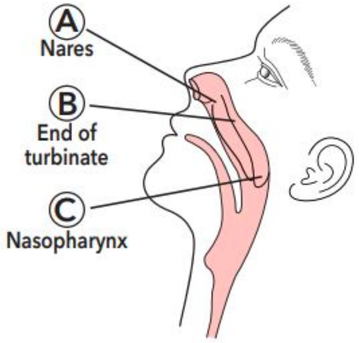
Nucleic acid amplification methods

Molecular diagnostics

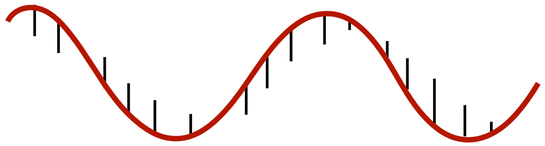
- The polymerase chain reaction (PCR) is the primary method of laboratory detection for SARS-CoV-2
- Assays detect the presence of viral nucleic acid in a sample (and by proxy imply presence in the human)
- Results are qualitative but store amplification data on the instrument

Real-time reverse-transcriptase (RT) PCR

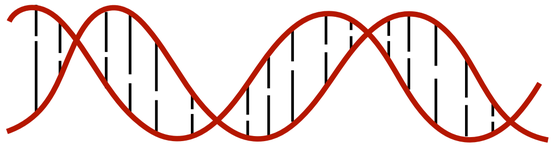
1. Sample collected



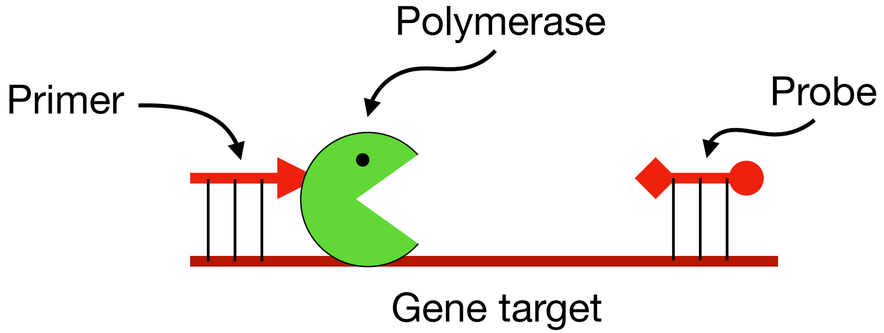
2. RNA extracted and purified



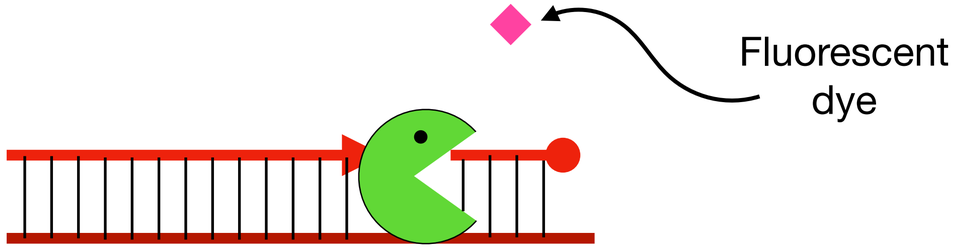
3. RNA reverse transcribed into DNA



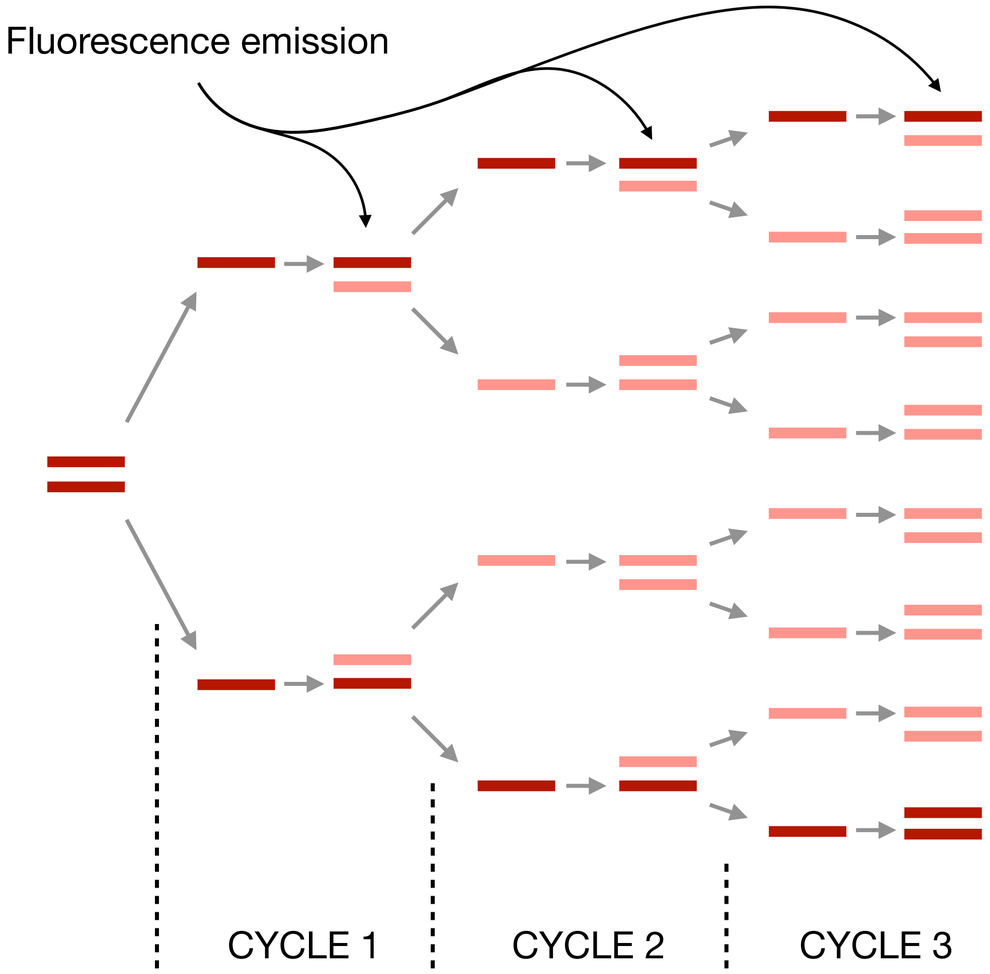
4. Gene targets amplified

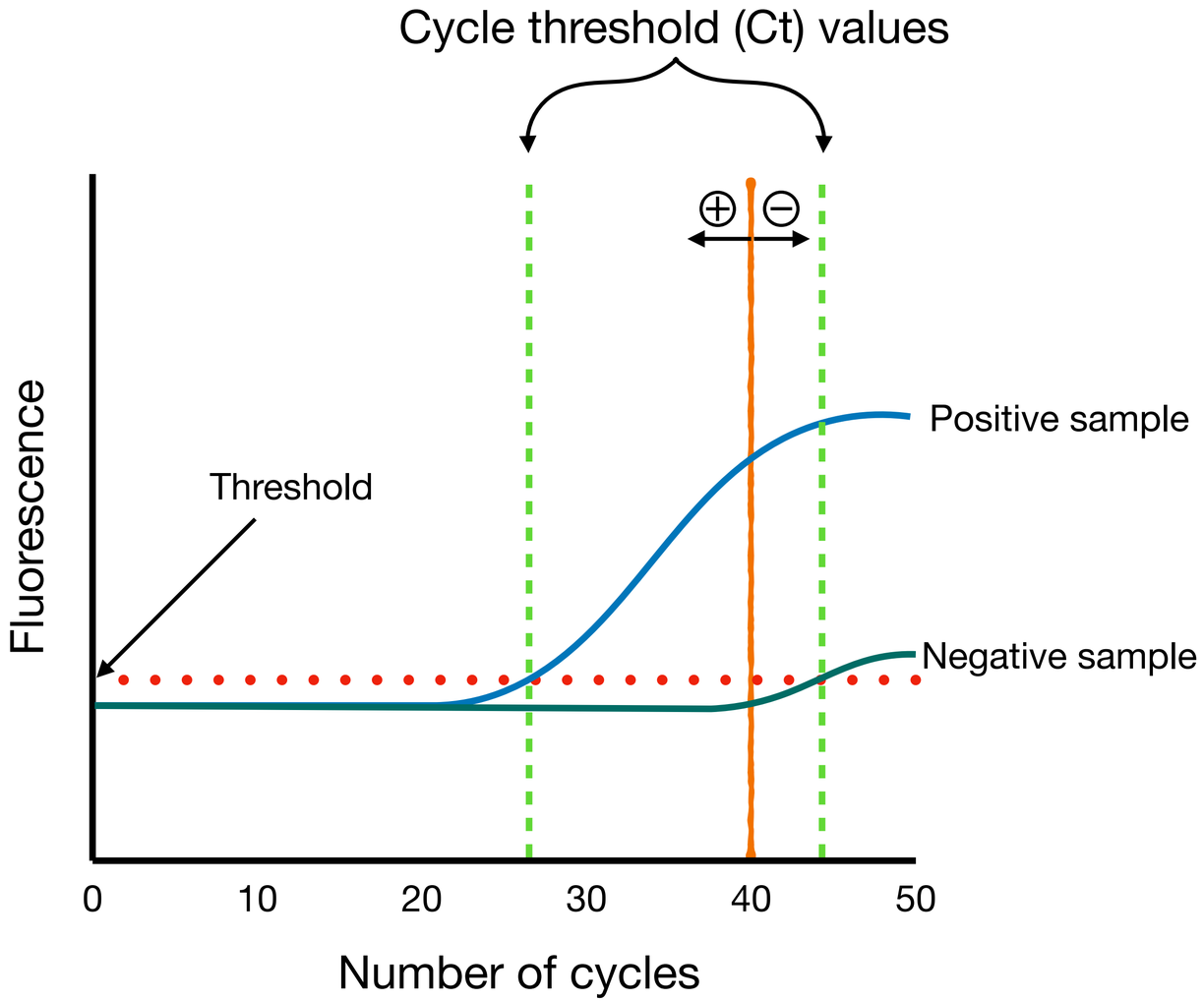


5. Fluorescence detected in real time



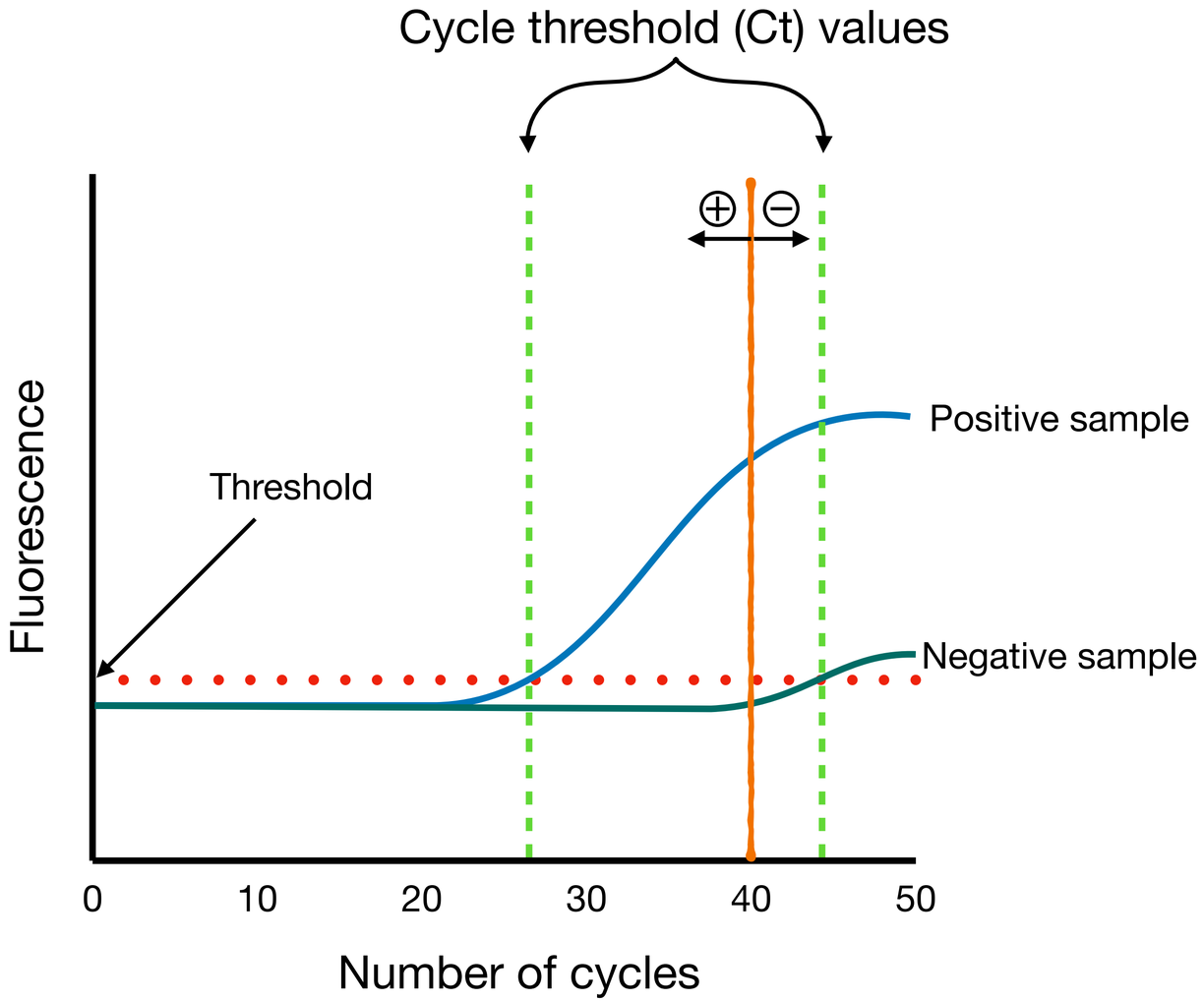
Real-time reverse-transcriptase (RT) PCR





Real-time reverse-transcriptase (RT) PCR

- Ct value: “The number of cycles needed for an amplicon to become detectable above background”
- The greater the amount of starting material present in a reaction, the fewer the number of cycles necessary to cross the threshold
  - Lower Ct values indicate higher amounts of target RNA in a sample (and vice versa)

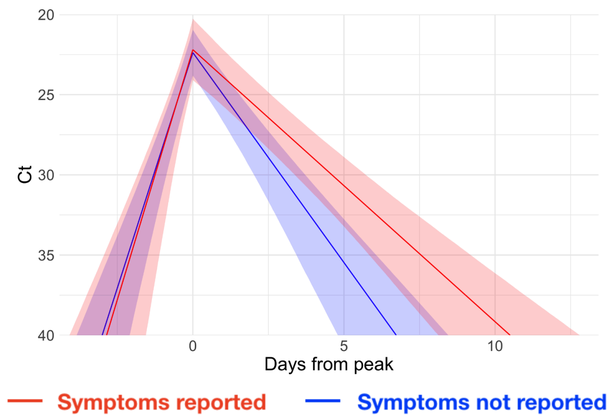


Cycle threshold values

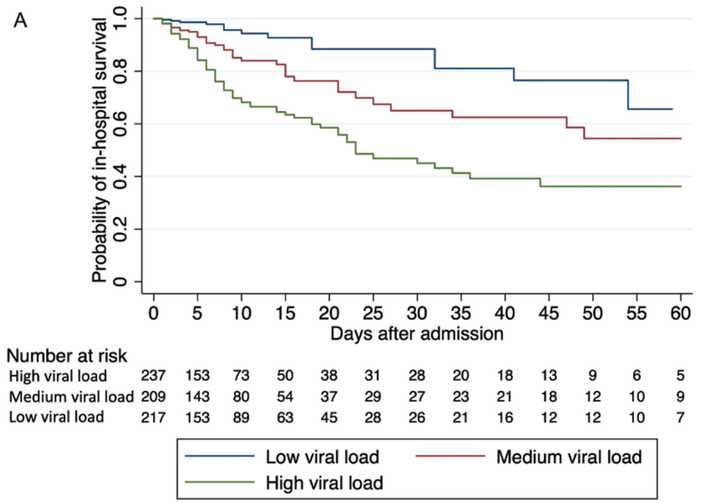
Patient level use cases

- Help determine time point in illness course (when trended)
- Prognostication
- Risk of transmission

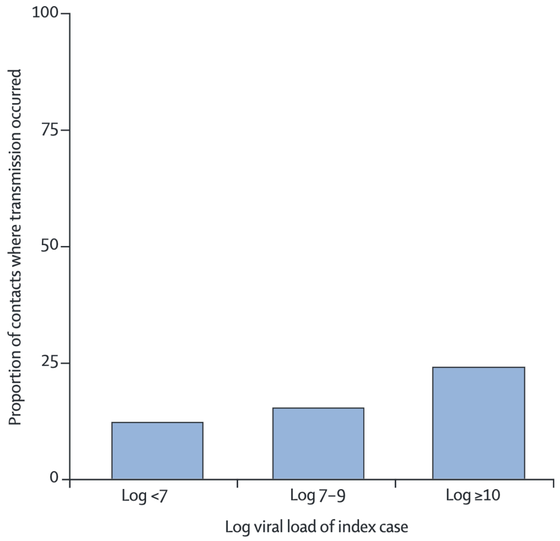
All are areas of controversy



Kissler, medRxiv, 2021



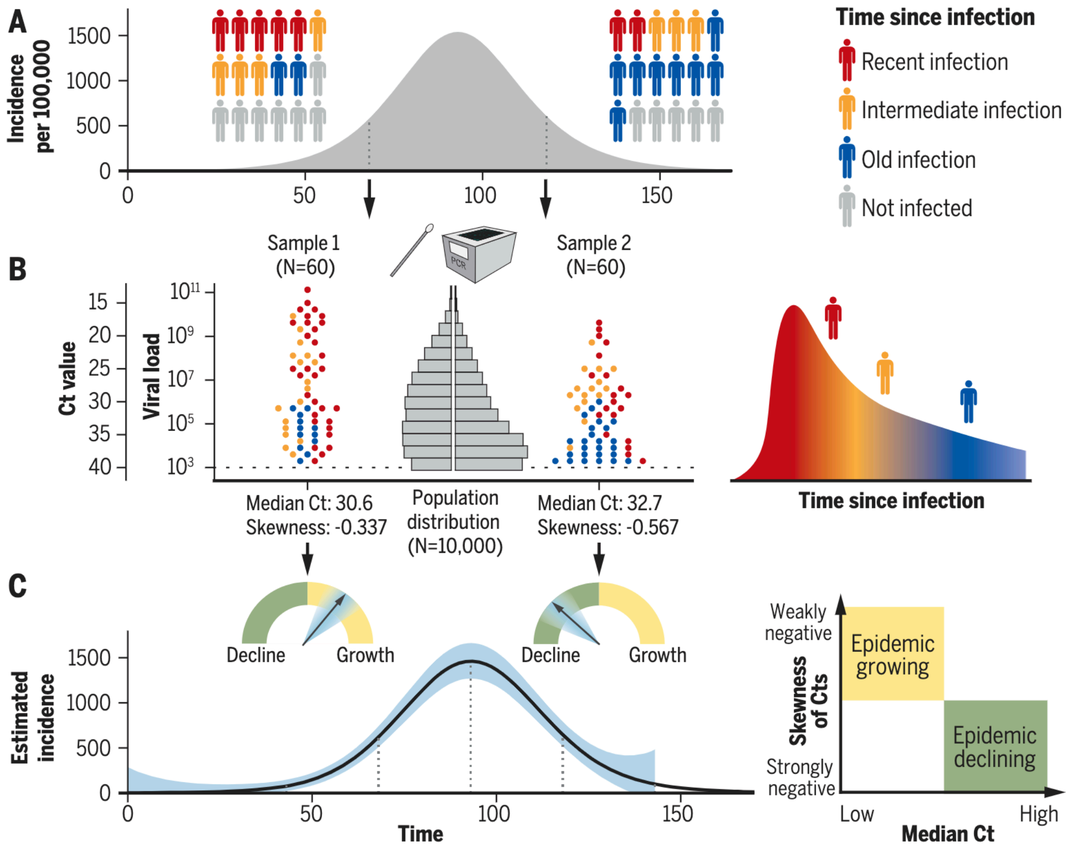
Magleby, Clin Inf Dis, 2020



Marks, Lancet Inf Dis, 2021

Cycle threshold values

Population level use case



Hay, Science, 2021

Median and skewness of cross-sectional distributions of Ct values can model dynamics of a local epidemic

Cycle threshold values

Impacted by multiple factors

Assay-specific variables

- Method by which instrument sets threshold
- Conditions present in an individual reaction

Host-specific variables

- Body site of sampling
- Time point in illness
- Immunity

Virus-specific variables

- Propensity for replication and rate of decay by variant

Pre-examination variables

- Quality of sample collection
- Time from collection to analysis

Cycle threshold value

Bottom line/s

- No method for standardizing Ct values across assays and labs
  - Cannot compare values from different platforms (though automated platforms have systematic biases that can be modeled)
- Can vary substantially even within a single platform due to pre-analytic factors
- Does not equate to a viral load as values not normalized
- Can have utility when ‘low’ and when used serially in a given patient and with a good understanding of nuances

Should be interpreted with caution and with consultation

Unexpected SARS-CoV-2 PCR test results

Approaches to interpretation

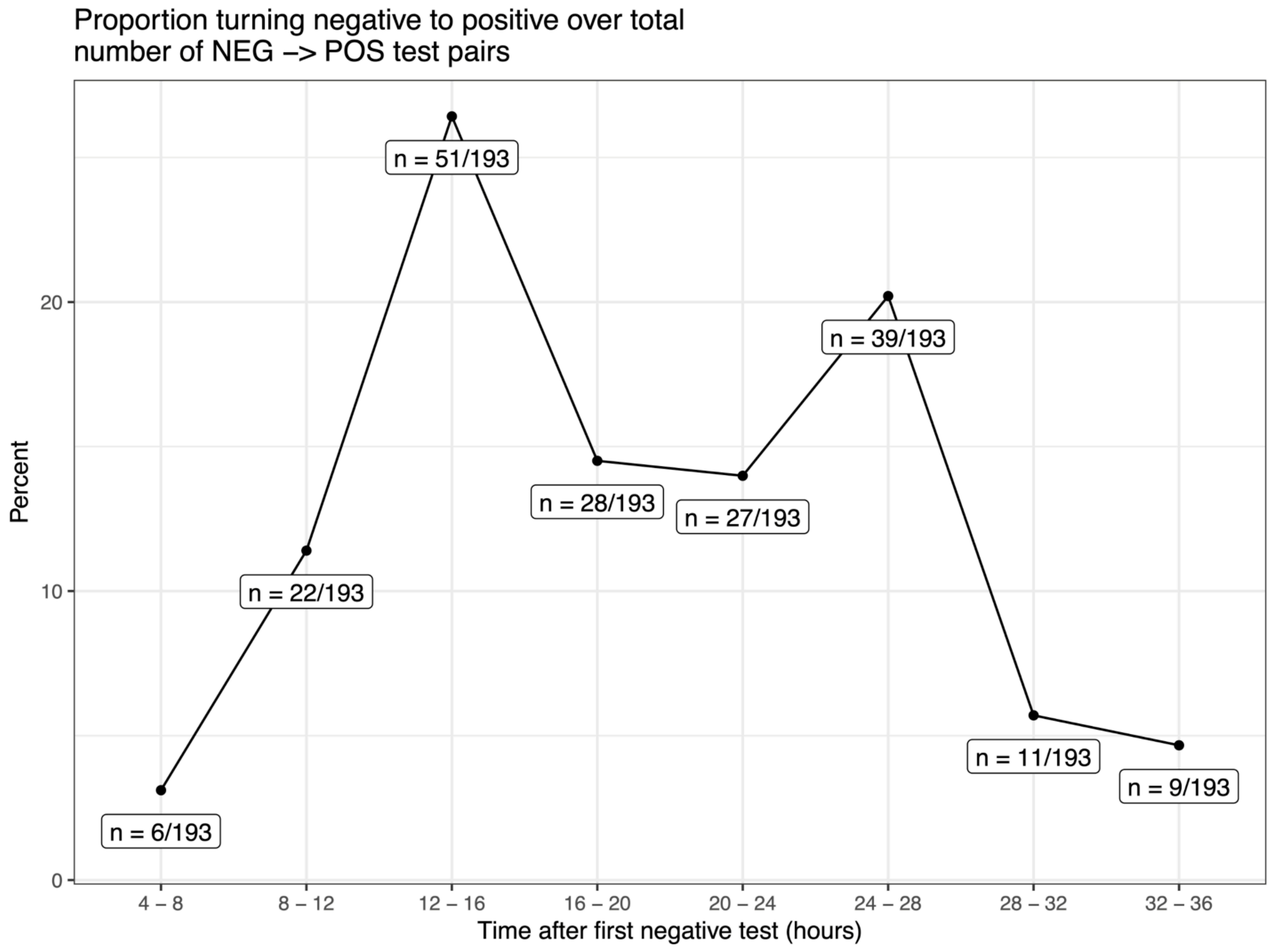
- **Negative** tests in patients with **high pretest probability** should be treated as false negatives and should be repeated in 24-72 hours
- **Positive** tests in patients with **low pretest probability** have multiple interpretations
  - What is the Ct value?
    - If ‘low’, then true asymptomatic infection
    - If ‘high’, and repeat tests low, then represents early infection
    - If ‘high’ and repeat tests are also ‘high’, may represent residual RNA
    - If ‘high' and repeat tests are all negative, may represent a false positive

Should use same assay and specimen type when trending

Serology can help

When to repeat?

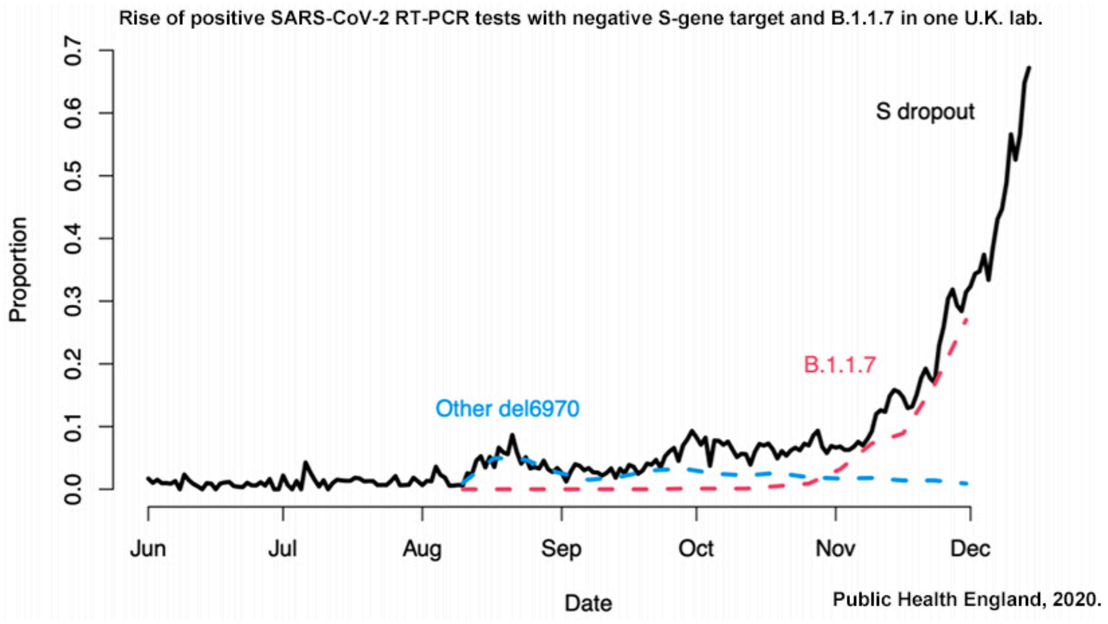
In 12 to 24 hours



Kanjilal, IDWeek 2021

- Analysis of 193 test pairs performed within 36 hours of each other where 1st test was negative and 2nd test positive
- Highest proportion lay in the 12-24 hours after the first test
- Lower respiratory tract specimens associated with higher likelihood of 2nd test positivity

Impact of variants on test performance

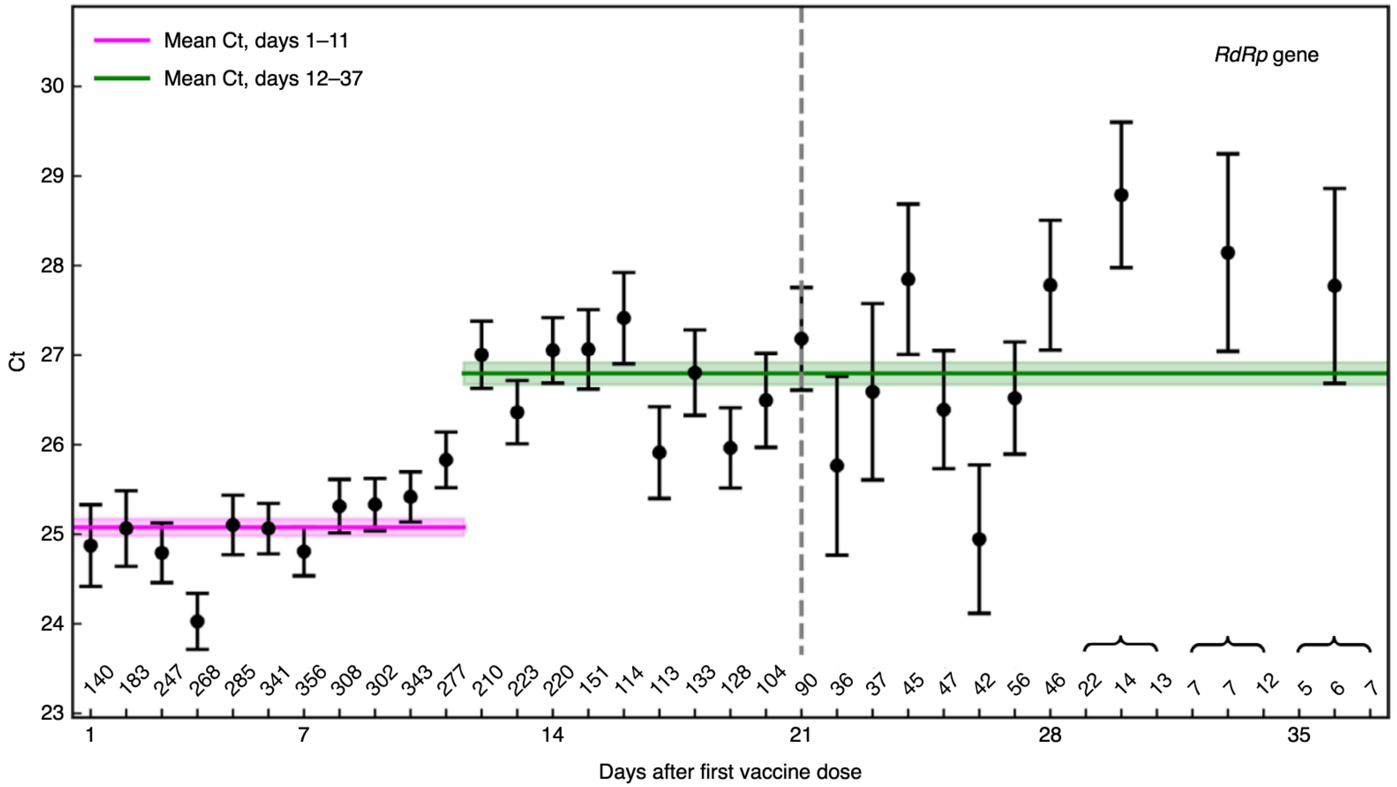


- Rise of Alpha variant (B.1.1.7) resulted in spike gene target failure (‘dropout’) for certain RT-PCR assays (i.e., TaqPath COVID-19 Combo Kit, ThermoFisher)
  - Helpful for surveillance as other targets in the assay remain positive
- FDA maintains a website that is (supposedly) kept up to date on the impact of new variants on assay sensitivity

No reports yet of difficulty identifying Delta variant

Impact of vaccination on test performance

- Early reports showed higher Ct values in infections occurring among the vaccinated

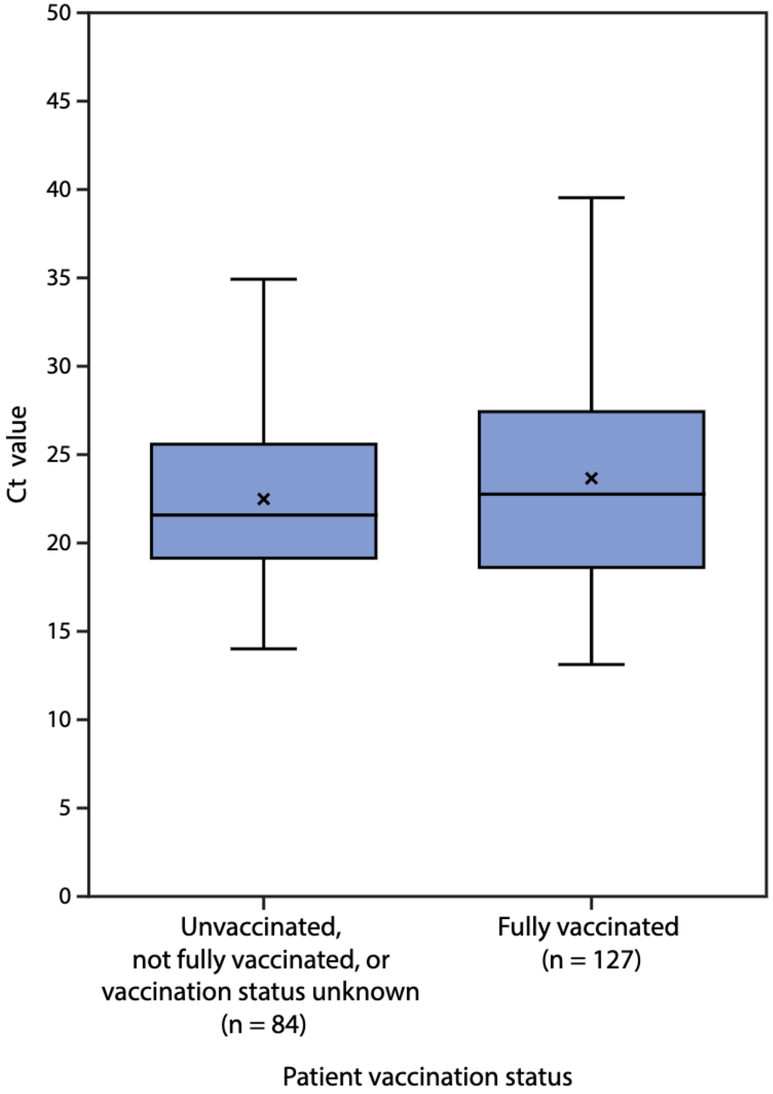


Levine-Tiefunbrun, Nat Med, 2021

Impact of vaccination on test performance

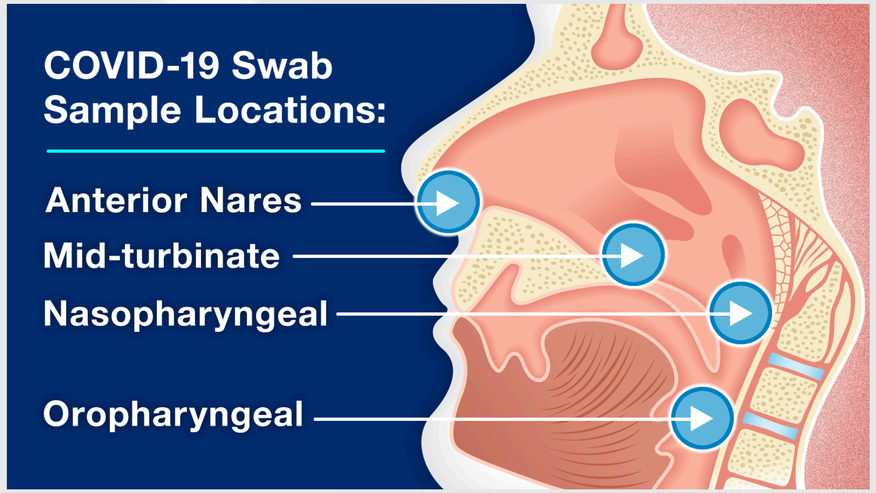
- During Delta wave, early reports suggest median Ct values appear similar to those seen among unvaccinated individuals
  - Impetus for renewed masking for all
- Impact on transmission not fully defined
- Does not provide insight into the kinetics of viral loads over time, which may differ between those with protective immunity and those who are immunologically naive

Brown, MMWR, 2021



Impact of alternative specimen types

- Nasopharyngeal swabbing has been the preferred body site of sampling for respiratory virus sampling
- The need for serial swabbing and PPE requirements has garnered intense interest in validating other body sites such as
  - Anterior nares
  - Mid-turbinate
  - Oropharyngeal
  - Saliva



FDA

Impact of alternative specimen types

Comparison of sensitivity

- Saliva equivalent to NP swab (83% vs 85%)*
- Oropharyngeal swabs equivalent to NP swab (84% vs 88%)**
- Anterior nasal / mid-turbinate lower sensitivity to NP swab (84% vs 98%)**
- Studies focus on patients with high pretest probability for infection
- When used for screening
  - Saliva 24% to 90%***
  - Anterior nasal / mid-turbinate 42% to 89%***

*Butler-LaPorte, JAMA Int Med, 2021

**Lee, J Clin Micro, 2021

***Kusumakar / Weiss, under review

Impact of alternative specimen types

Bottom line

- Great deal of heterogeneity and variation in study quality
  - Difficult to draw generalizable conclusions
- Differences in sensitivity most important for capturing people early in infectious period
  - Represents a small fraction of those tested
  - May be outweighed by operational benefits

Other common molecular methods

- Loop-mediated isothermal amplification (LAMP)
  - Abbot ID Now
- Transcription-mediated amplification (TMA)
  - Hologic Panther
- Multiplex nested PCR
  - Biofire RP2.1

These methods / assays do not provide Ct values

Rapid antigen tests

Antigen tests

- Immunochromatographic assays (ie lateral flow assays) that target the nucleocapsid protein
- Advantages
  - Rapid (~15 minutes)
  - Point-of-care
  - Can be self-administered
  - Compatible with nasal swabs
  - Decreased sensitivity relative to PCR

Antigen tests

Comparison with PCR

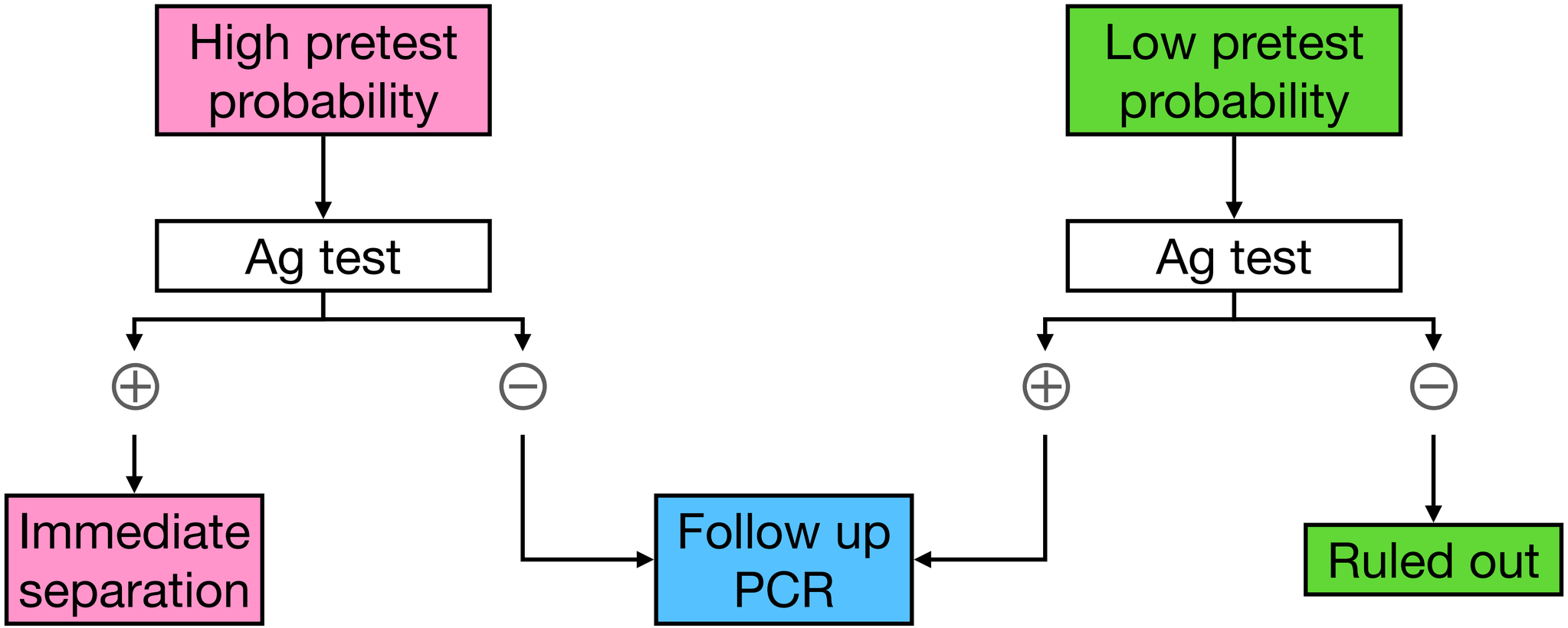
- Primary difference is a lower sensitivity and possibly a higher specificity for active illness
  - Will be falsely negative in a significant proportion of cases in early infection
  - Will be truly negative in cases with residual RNA
- Performance varies by symptomatology
  - Sensitivity ~80% in those with symptoms, ~44% in asymptomatic people*
- Performance varies by age
  - Sensitivity 45% in symptomatic children**

* Ford, Clin Infect Dis, 2021

** Villaverde, J Pediatrics, 2021

Antigen tests

Potential use cases to combine with PCR



Performance improves with serial testing

Case
Revisited

- A 75 year old nursing home resident learned that she was in close contact with a fellow resident 3 days ago who was later diagnosed with COVID-19. She is asymptomatic.
- As part of the facility policy, she underwent antigen and PCR testing with an anterior nasal swab. Both results return negative.
- How would you interpret this result and how would you act on it?

    A. A true negative, no further testing needed

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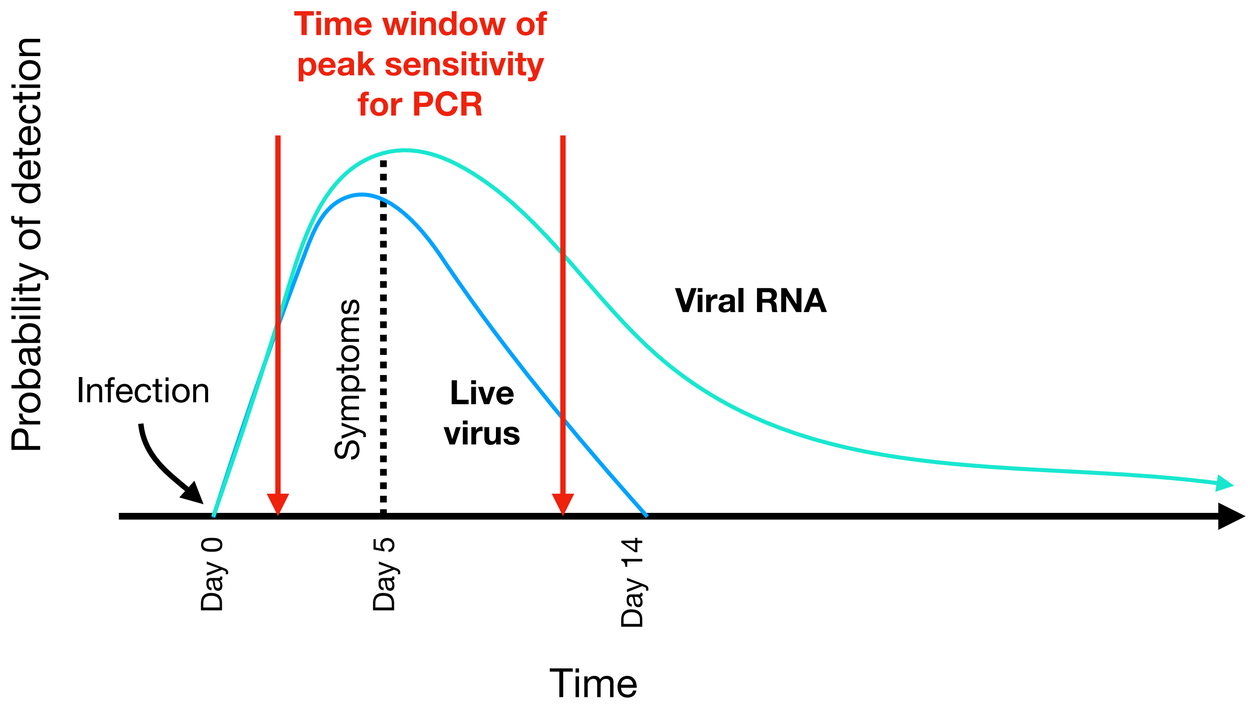
Case

SK interpretation



- False negative tests may arise if performed early or late in infection
- In cases where suspicion is high, best to repeat testing at a time when the viral load should peak (~day 5 to 7 after exposure)
  - A positive antigen test in a person with high pretest probability gives you an immediate answer
  - A negative antigen test would require further isolation until PCR results return
  - Ct value of PCR provides a ‘lower bound’ for how much virus may be present and can help provide insight into person-to-person spread

Thank you!