



DEPARTMENT OF LABORATORY MEDICINE AND PATHOLOGY

Update on Molecular Testing for COVID-19

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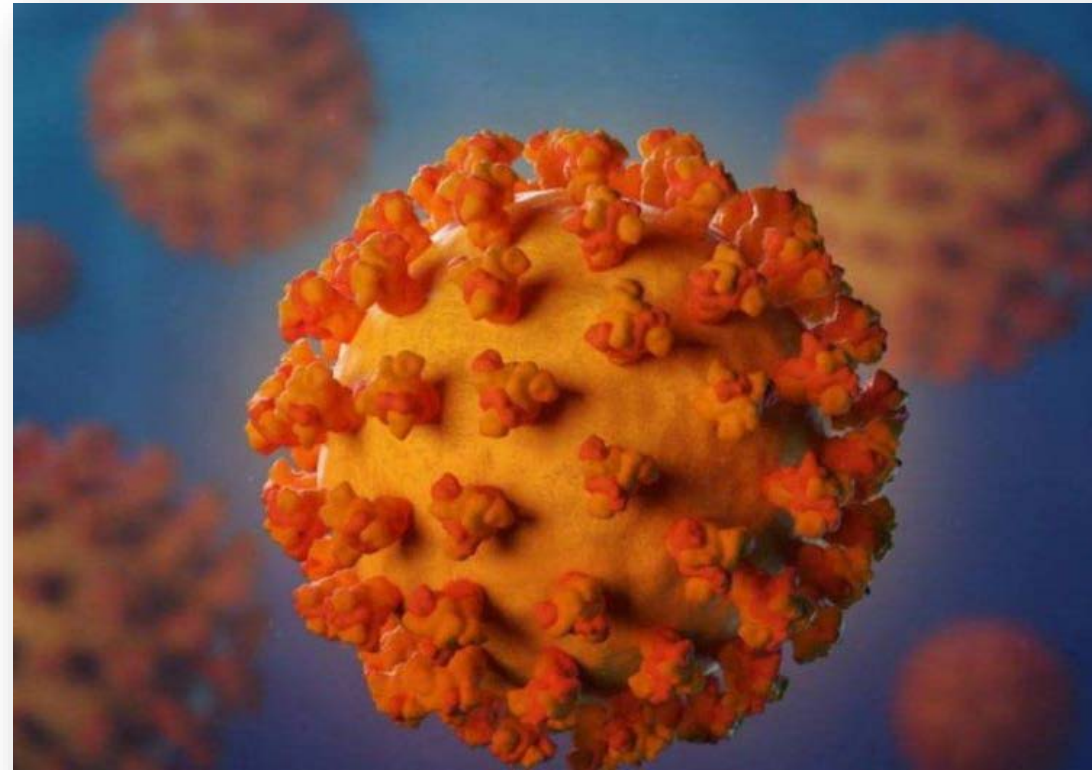
Objectives

- Discuss the intended use of molecular tests (e.g., real-time PCR) for the detection of SARS-CoV-2
- Highlight common questions related to molecular testing for COVID-19
- Update on latest advancements in molecular testing

Coronaviruses: From the Common Cold to Global Contagion

Common human coronaviruses:

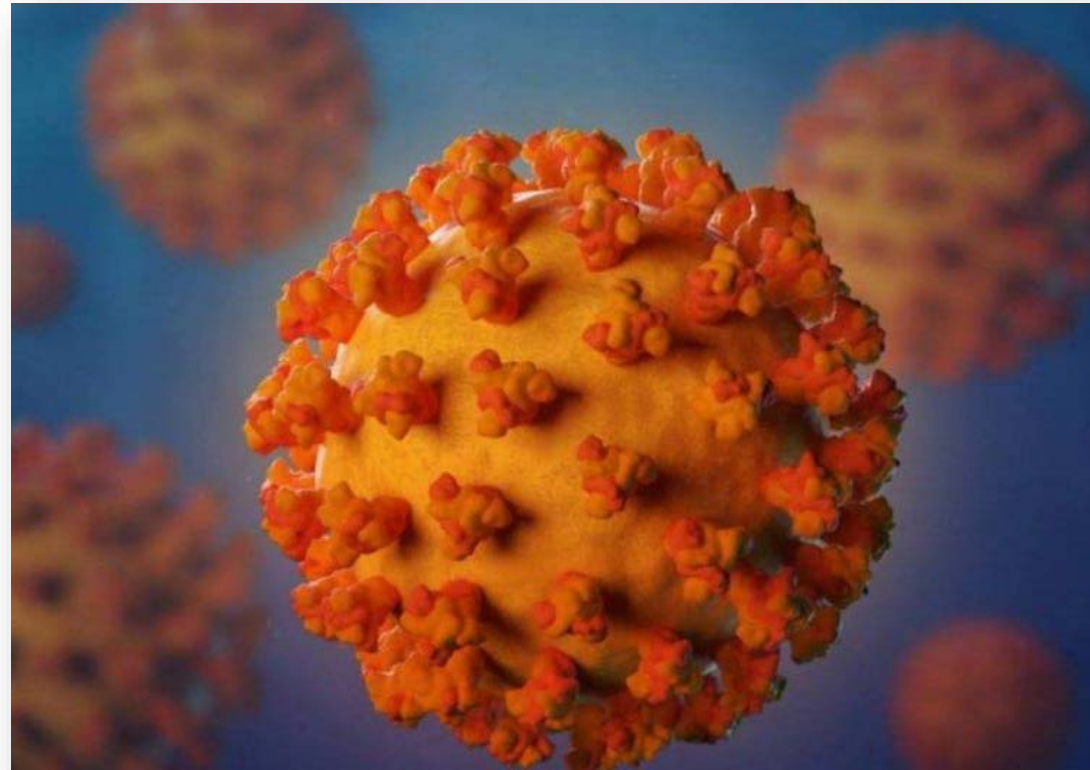
- HCoV-OC43
- HCoV-NL63
- HCoV-229E
- HCoV-HKU1



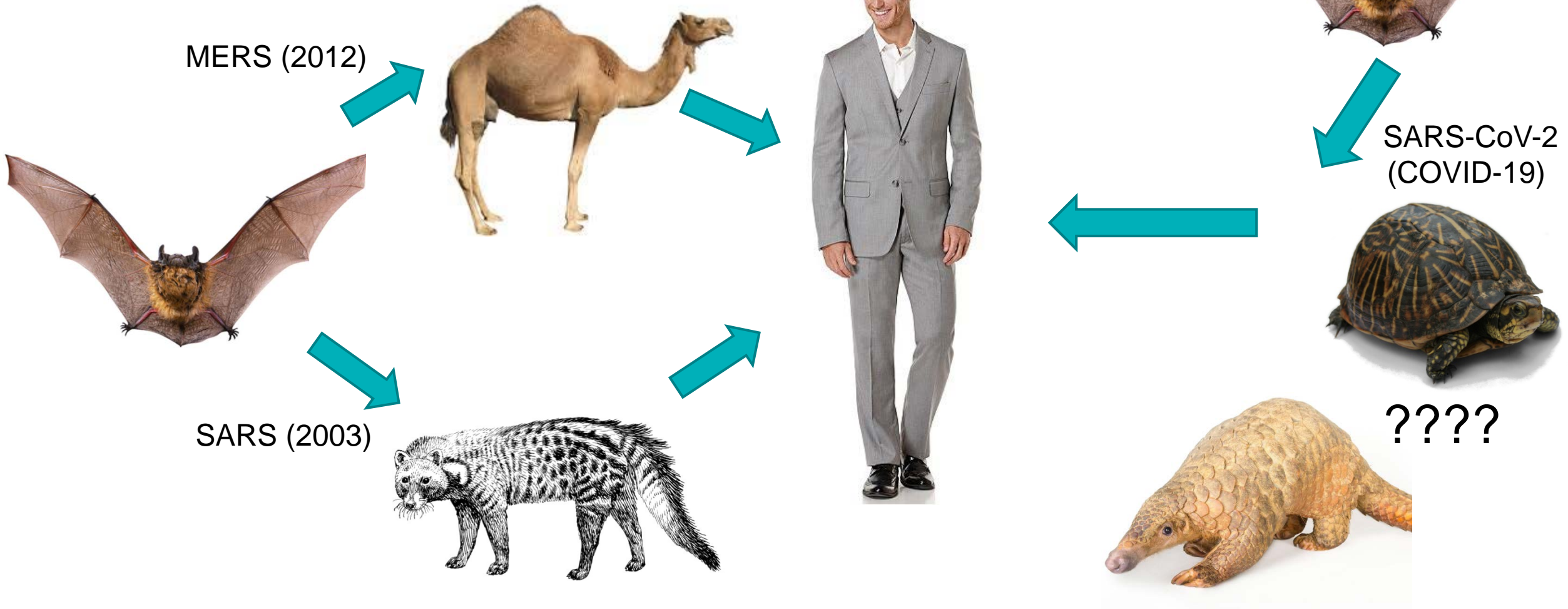
Coronaviruses: From the Common Cold to Global Contagion

Coronaviruses associated with severe disease:

- SARS (2002-2003)
- MERS (2012)
- SARS-CoV-2 (2019-2020)



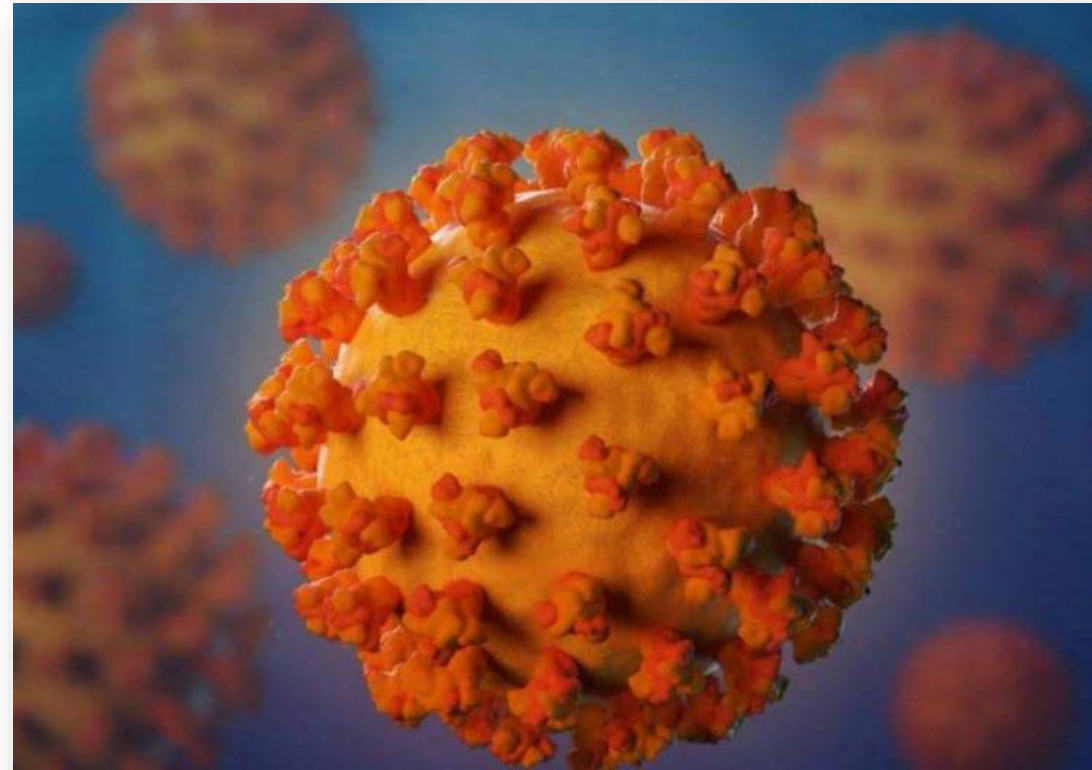
Why do certain coronaviruses cause more severe disease?



SARS-CoV-2 (COVID-19): Molecular Testing

Molecular tests (real-time PCR) have generally targeted a combination of the following genes:

- Nucleocapsid (N)
- Open reading frame 1ab (Orf)
- Envelope (E)
- RNA dependent RNA polymerase (RdRp)



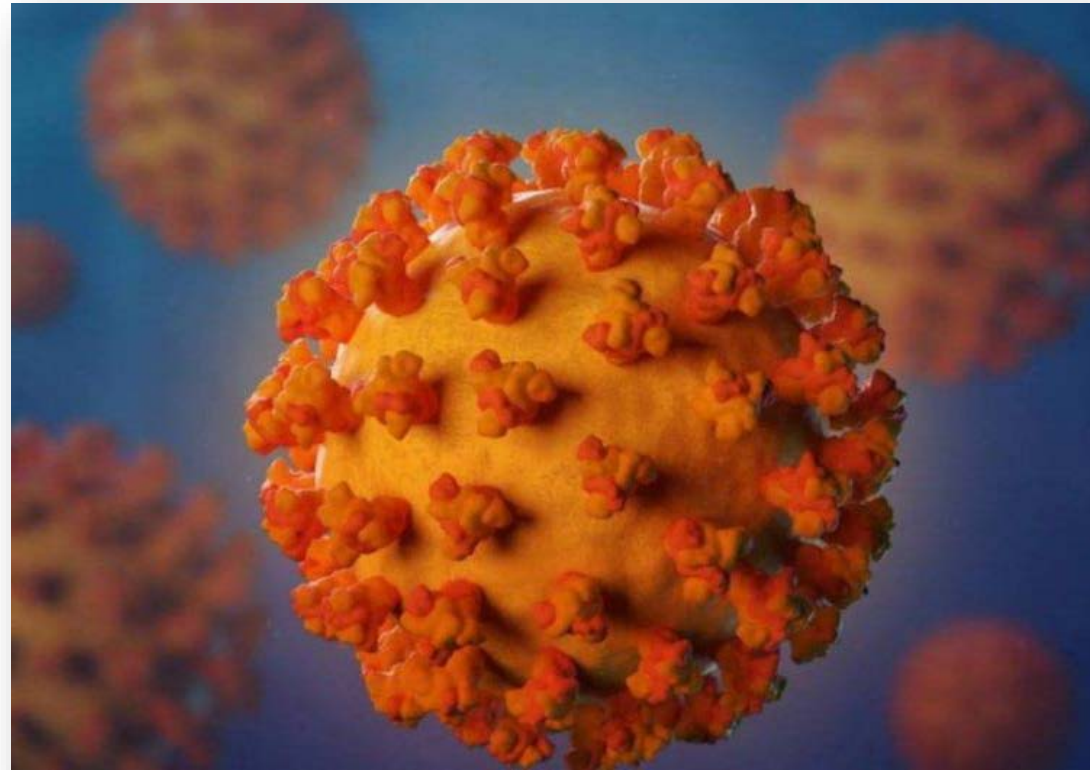
SARS-CoV-2 (COVID-19): Molecular Testing

Appropriate sample types:

- Nasopharyngeal swab (**preferred**)
- Oropharyngeal (throat) swab
- Saliva (?)

If evidence of LRTI:

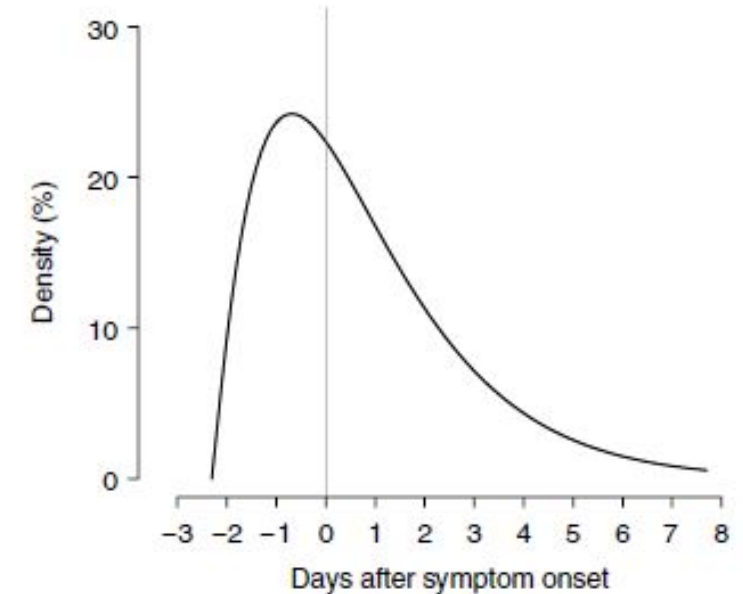
- Sputum
- BAL fluid
- Tracheal secretions



SARS-CoV-2 (COVID-19): Common Questions

When is SARS-CoV-2 shed at the highest amount?

- Peak viral shedding ~24 h **prior** to symptom onset
- Detection in upper airway (i.e., NP swab) likely drops after 7 days post onset



He, X. Nature Med, 15 Apr 2020

SARS-CoV-2 (COVID-19): Common Questions

What is the sensitivity of the COVID-19 PCR test? Is it really 70%?

- Likely depends on several factors:
 - Timing of collection
 - During first week of disease: Upper respiratory sample
 - During later stages of disease: Lower respiratory sample
 - Sample type
 - NP swab > OP swab during early disease
 - Sputum, BAL fluid recommended during later stages of disease
 - Quality of sample collected
 - Specific test performance characteristics (often sacrifice sensitivity for speed)

SARS-CoV-2 (COVID-19): Common Questions

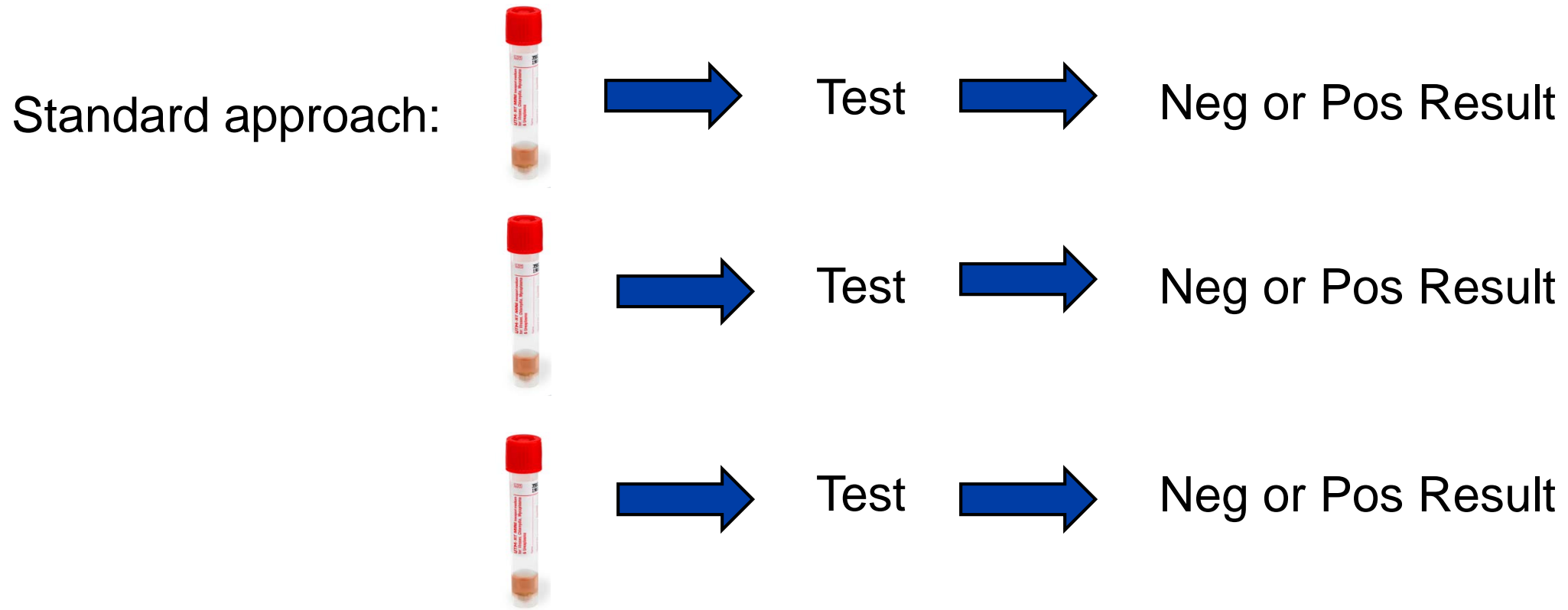
Why do some patients test positive for weeks, or even months?

Why do some patients go from positive, to negative, to positive?

- Persistent infection? Reinfection? Remnant viral RNA?
- Several studies have been published that address these questions (South Korean CDC, Singapore, Canada)
 - Still learning, but common themes include:
 - PCR cycle threshold values increase over time
 - Viral infectivity (as measured by viral culture) appears to drop off by days 8-11 after onset of symptoms
 - CDC has updated guidance from test-based to symptom- or time-based strategy for discontinuing isolation

SARS-CoV-2 (COVID-19): Common Questions

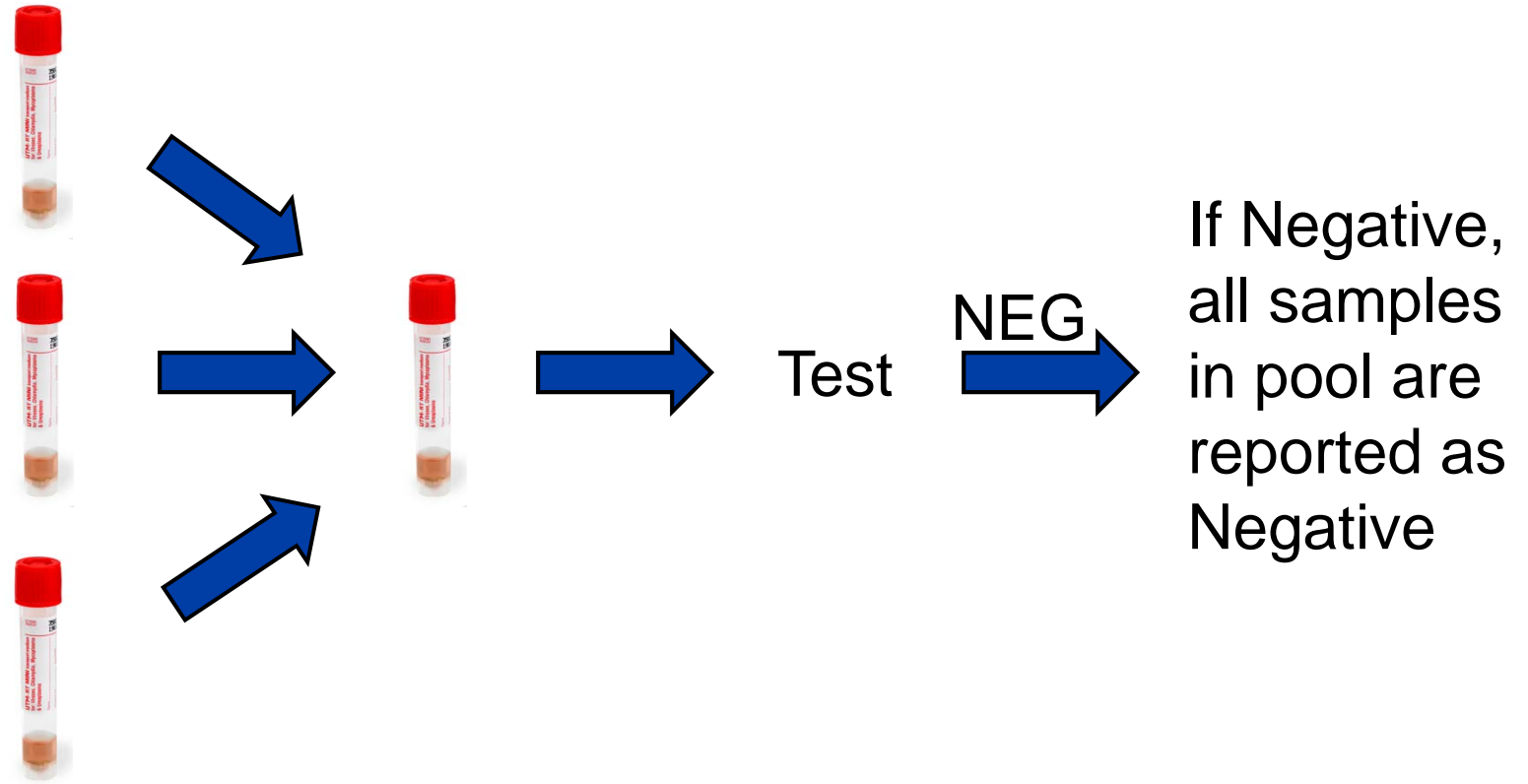
What about pooled testing? What is it, and should we use it?



SARS-CoV-2 (COVID-19): Common Questions

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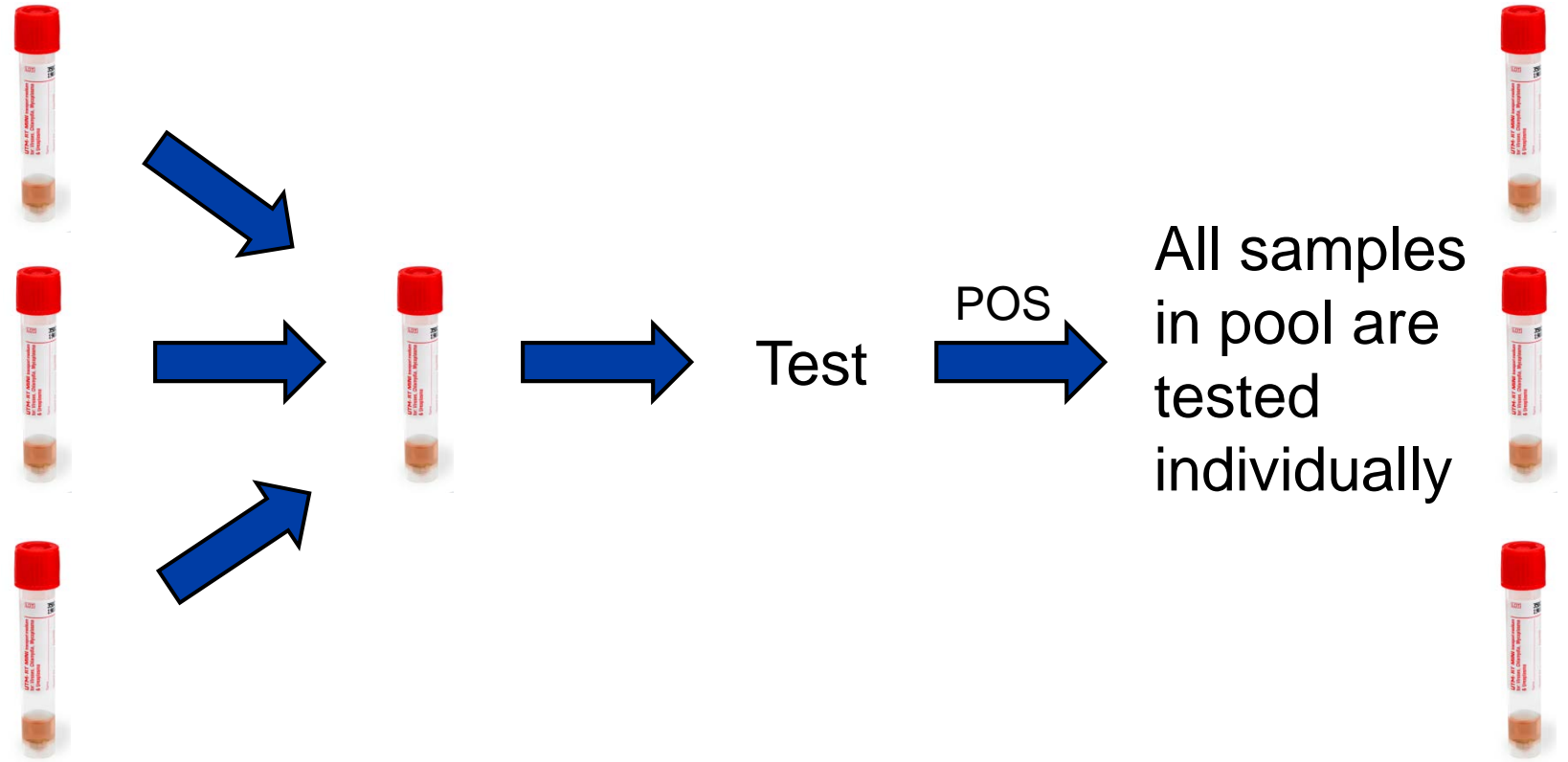
Pooling approach:



SARS-CoV-2 (COVID-19): Common Questions

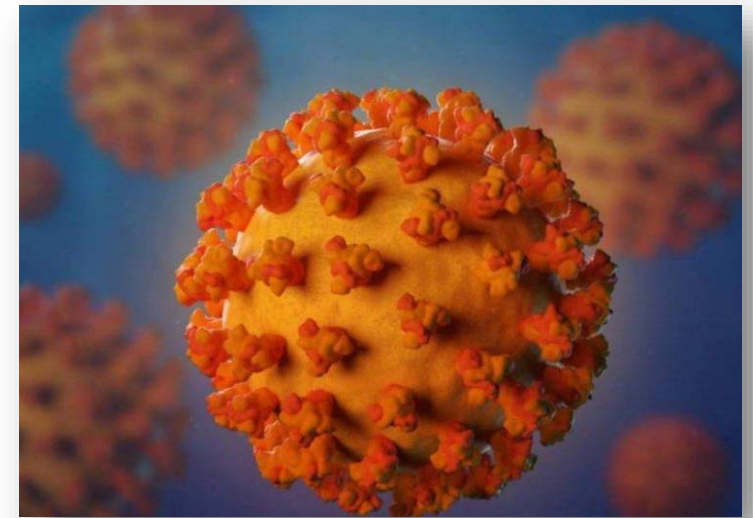
What about pooled testing? What is it, and should we use it?

Pooling approach:



SARS-CoV-2 (COVID-19): Pooled testing

- Advantages:
 - Increase testing capacity (and efficiency?)
 - Reduce reagent use
 - Decrease overall testing expenses
- Disadvantages/Considerations:
 - Decrease sensitivity, especially for detection of “low-positive” samples (dilution effect)
 - Requires low-prevalence setting (<10%)
 - Concern for lab contamination
 - Concern for reporting errors
 - Billing?



SARS-CoV-2 (COVID-19): Common Questions

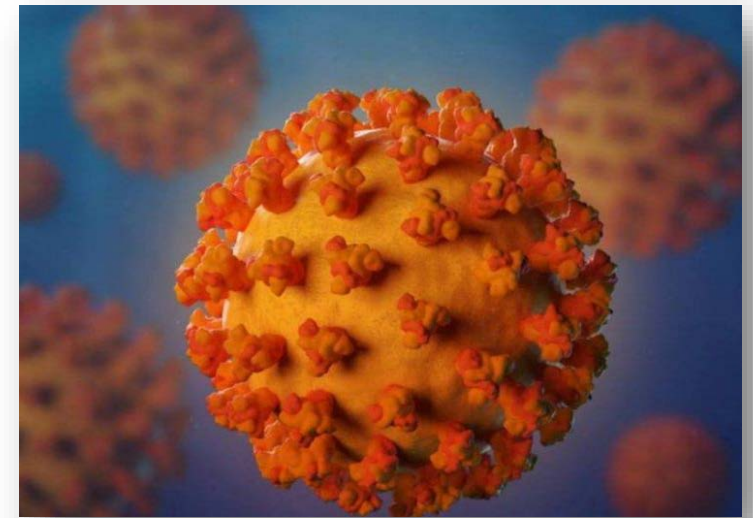
Is saliva testing a game-changer?

- Yale SalivaDirect assay received FDA EUA on August 15, 2020
- Saliva is a more convenient sample for most patients compared to an NP swab
- SalivaDirect requires samples be sent to a testing lab
- Sensitivity is likely lower (83-94%) than NP-based PCR tests
- Cost must factor in transport of sample to lab, staff, equipment, overhead



SARS-CoV-2 (COVID-19): Summary

- Acute COVID-19 is primarily diagnosed using molecular tests, such as real-time PCR
- Sensitivity of SARS-CoV-2 molecular tests is influenced by timing of sample collection, the specimen type, the quality of the sample, and the test itself
- Some patients will test positive for weeks, or even months after diagnosis
- Pooling represents an option for labs, but must be carefully considered
- Saliva is a potential specimen type, but may show lower sensitivity



Questions?

