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VIROLOGY

REACTing
research & action
targeting emerging infectious diseases

https://reacting.inserm.fr/

Scientific update on COVID-19

Updated on December 21th 2020

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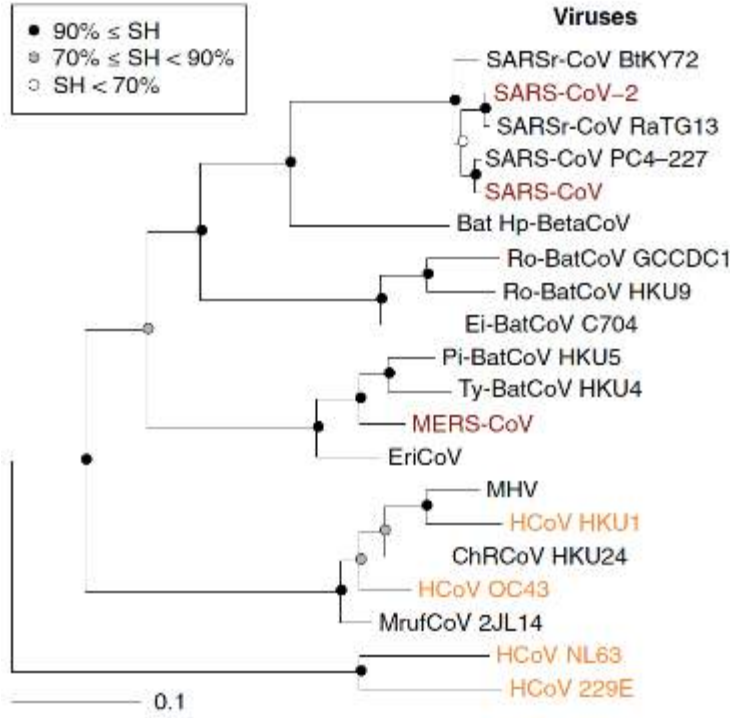
VIROLOGY

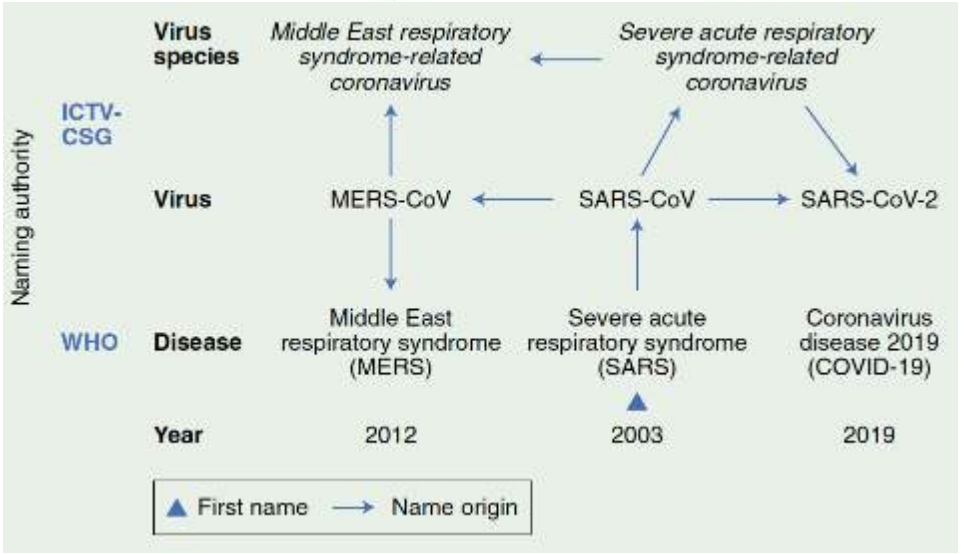
Questions:

- Which type of virus is SARS-CoV-2?
- What is the stability and viability of SARS-CoV-2?
- What is the impact of the mutation of SARS-CoV-2?
- What do we know about viral load and shedding according to different samples?
- What is the description of the immune responses in infected patients?
- Alternative to the nasopharyngeal swab for SARS-CoV-2 detection?

SARS-CoV-2

- Part of family of enveloped positive-strand RNA viruses (*coronaviridae*)
- Belongs to the *betacoronavirus genus*
  - 98% similarity with bat coronavirus RaTG13
  - 79% genetic similarity with SARS-CoV
- <u>7 coronaviruses known to infect humans</u>
  - 4 coronavirus infect mainly the upper respiratory tract
    - HCoV HKU1 – OC43 – NL63 – 229E
  - 3 coronavirus can replicated in lower respiratory tract and cause pneumonia with high case fatality rates
    - SARS-CoV = Case Fatality Rate (CFR) of 10% (2002 – 2003)
    - MERS-CoV = CFR of 37% (2012 -)
    - SARS-CoV-2 = CFR unknown (2019 -)





Stability of SARS-CoV-2

IN VITRO

Outcome: positive viral culture

Surface stability

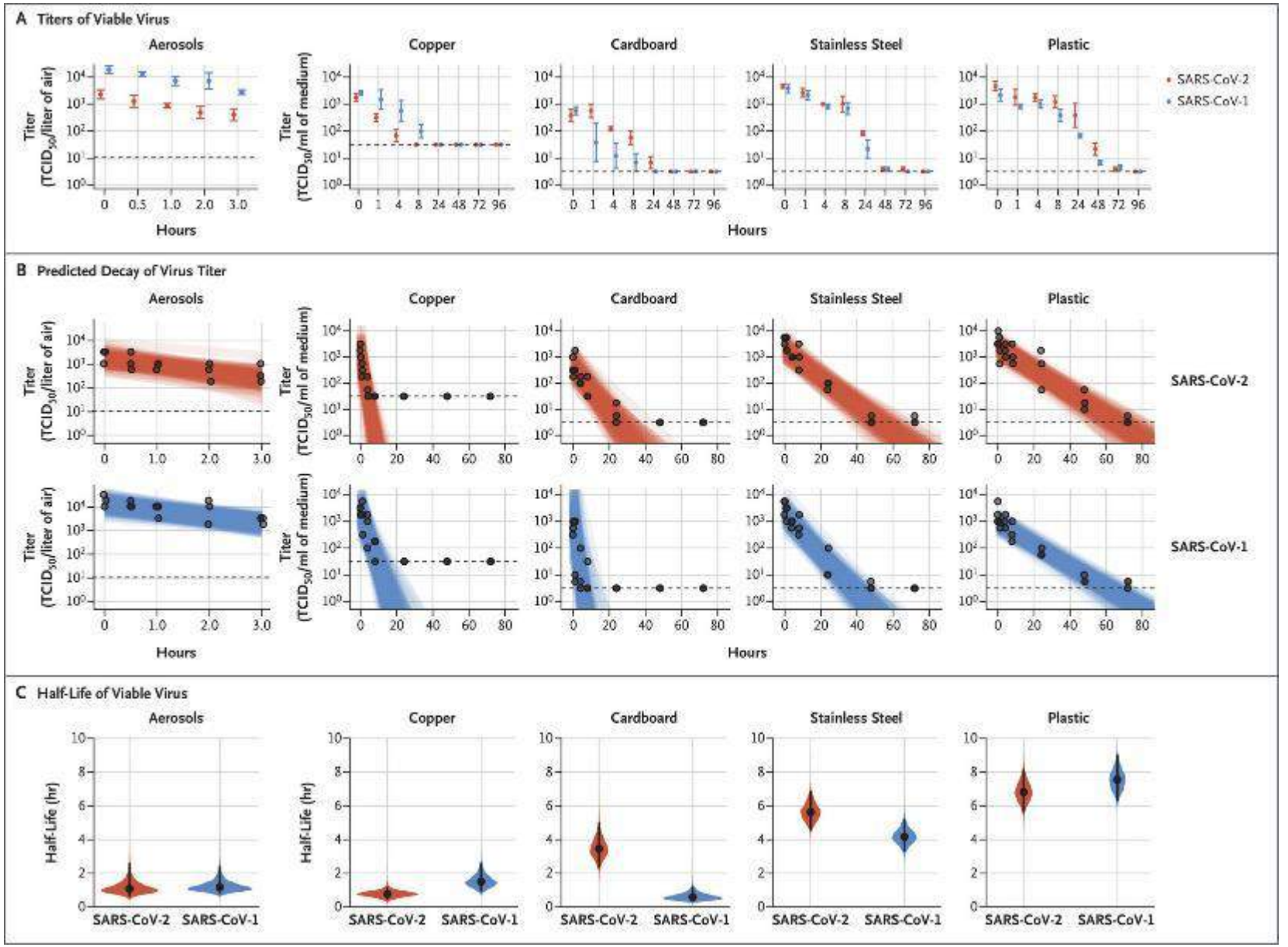
- Plastic and stainless steel: **72 hours**
- Cardboard: **24 h**
- Copper: **4 hours**

Viable in aerosol: **3 hours**

Half-life in aerosol:

- **1.1 to 1.2-h [0.64 – 2.24]**

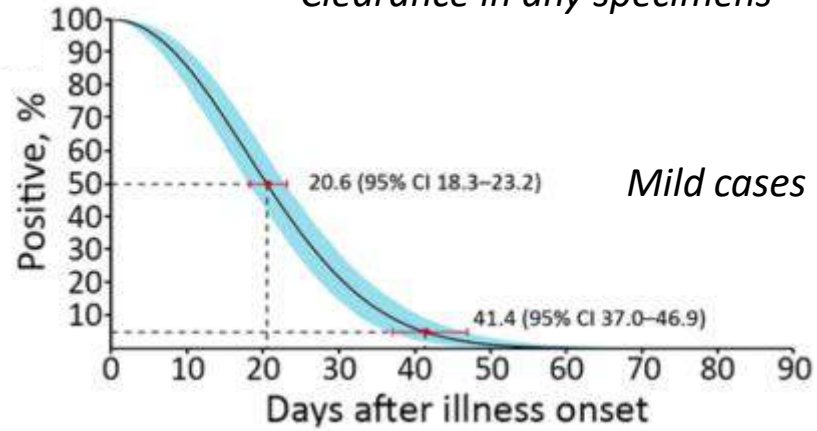
Aerosol transmission is possible in experimental conditions



Van Doremalen N *et al. NEJM.* Apr 2020

Persistence of virus RNA

Clearance in any specimens



Mild cases

<u>49 patients with 490 specimens</u> → 171 specimens positive for SARS-CoV-2 RNA

Frequency and duration of detectable SARS-CoV-2 RNA in body fluids?

Weibull model → time loss of SARS-CoV-2 RNA detection

<u>Time to loss detection</u>

- Time to loss detection was longer for NP swabs and feces
- Significant differences for mild cases among specimens

Prolonged persistence of SARS-CoV-2 RNA detection in hospitalized patient

→ Does not imply the existence of infectious virus particles

→ Still a need for preventive measures?

<u>Limits</u>

- Existence of infectious particles?
- Virus isolation and tests of specimen's infectivity
- not conducted
- Unspecified concentration of SARS-CoV-2 RNA
- May not be generalized to all population

Data are presented in days after illness onset

	Mild cases, n = 43		Severe cases, n = 6	
Specimens	Median (95% CI)	95th percentile (95% CI)	Median (95% CI)	95th percentile (95% CI)
Throat swab	15.6 (11.8–20.7)	32.8 (25.9–42.3)	33.9 (24.2–47.3)	53.9 (39.4–81.7)
Sputum	20.0 (14.1–27.0)	43.7 (33.6–60.4)	30.9 (23.5–39.1)	44.7 (36.3–58.0)
Nasopharyngeal swab	22.7 (18.8–27.5)	46.3 (39.0–55.2)	33.5 (25.7–42.7)	49.4 (38.4–68.5)
Feces	24.5 (21.2–28.3)	45.6 (40.0–52.8)	32.5 (26.3–39.1)	48.9 (41.3–59.7)

Jiufeng S *et al. Emerg Infect Dis*. May 2020

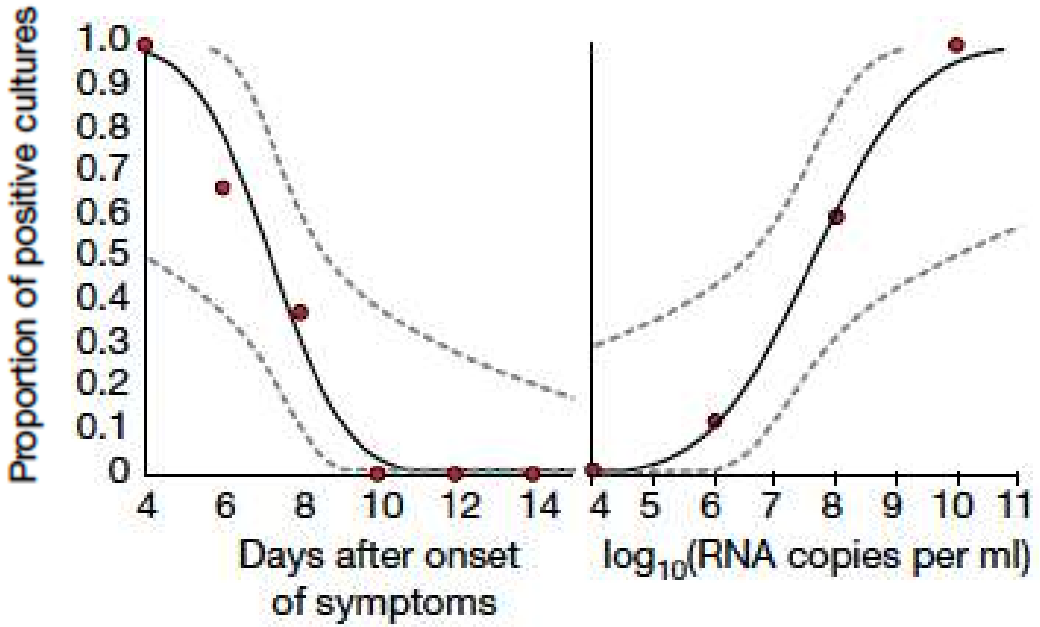
Viability

9 patients (Munich) – Virological analysis & information on virus infectivity

- Active virus replication in tissues of the upper respiratory tract
- No indications of replication in the digestive system
- Infectious virus on swab or sputum samples but not from stool samples
- None of urine and serum samples tested positive for RNA for SARS-CoV-2
- The success of virus isolation also depend of viral load

- **No isolates of the virus were obtained from samples taken after day 8 in spite of ongoing high viral loads.**

Virus isolation success based on probit distributions



Wölfel R *et al. Nature.* May 2020

Viral load

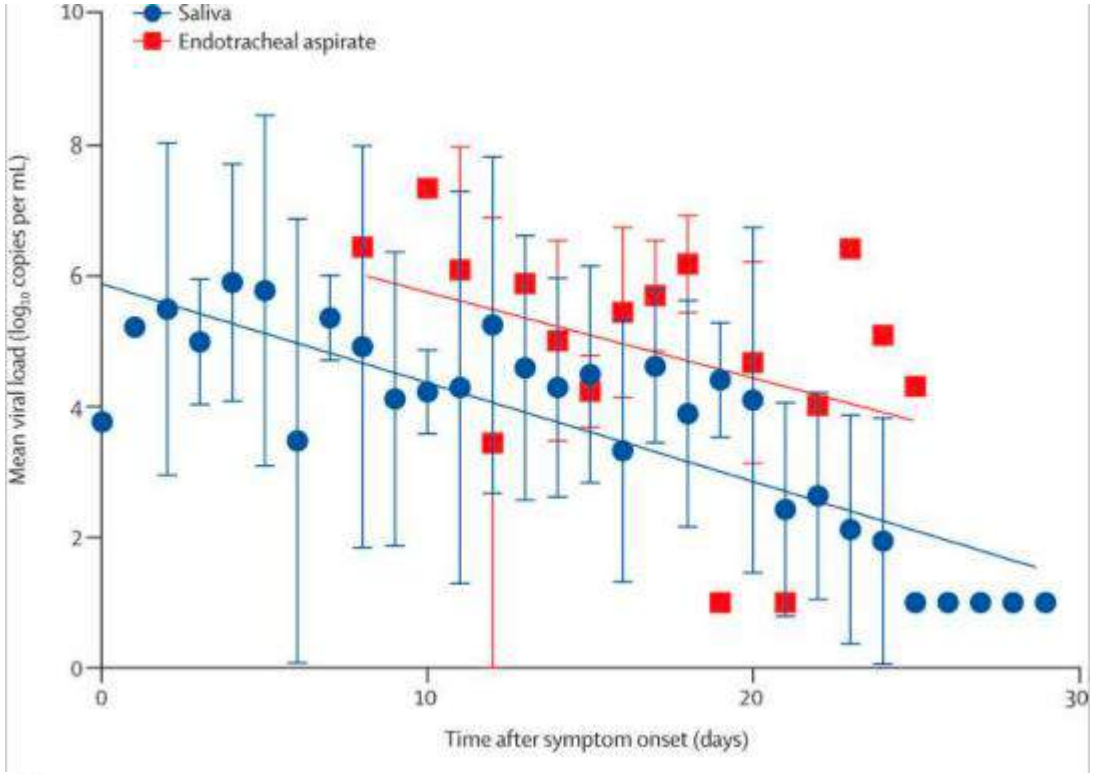
23 patients (median age: 62y) in Hong Kong → 173 respiratory specimens

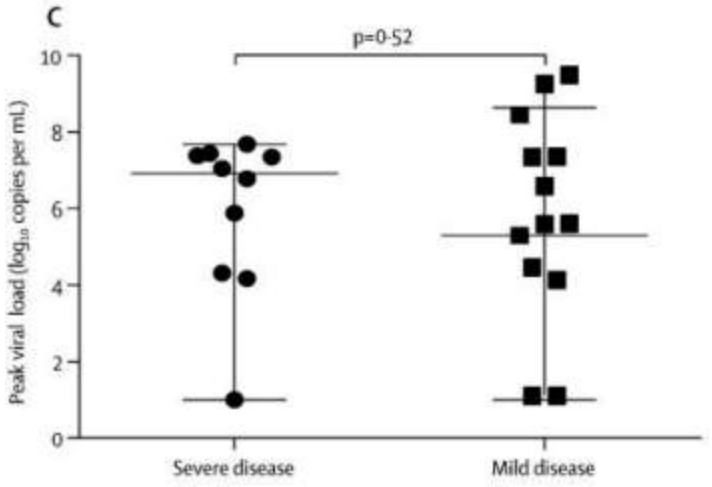
- Morning saliva samples
- Endotracheal aspirate (intubated patients)

Viral load:

- Median: 5,2 log_{10} copies per mL (IQR 4,1–7,0)
- Saliva viral load: higher during first week and declining after this point
- Endotracheal aspirate viral load: non-significant decline during the first weeks
- 7 patients had viral RNA detected 20 days after symptoms
- No association between prolonged detection and severity
- Older age was correlated with higher viral load
- No difference between mild and severe cases

Limit: low number of cases





To KK et *al. Lancet Infec Dis*. May 2020

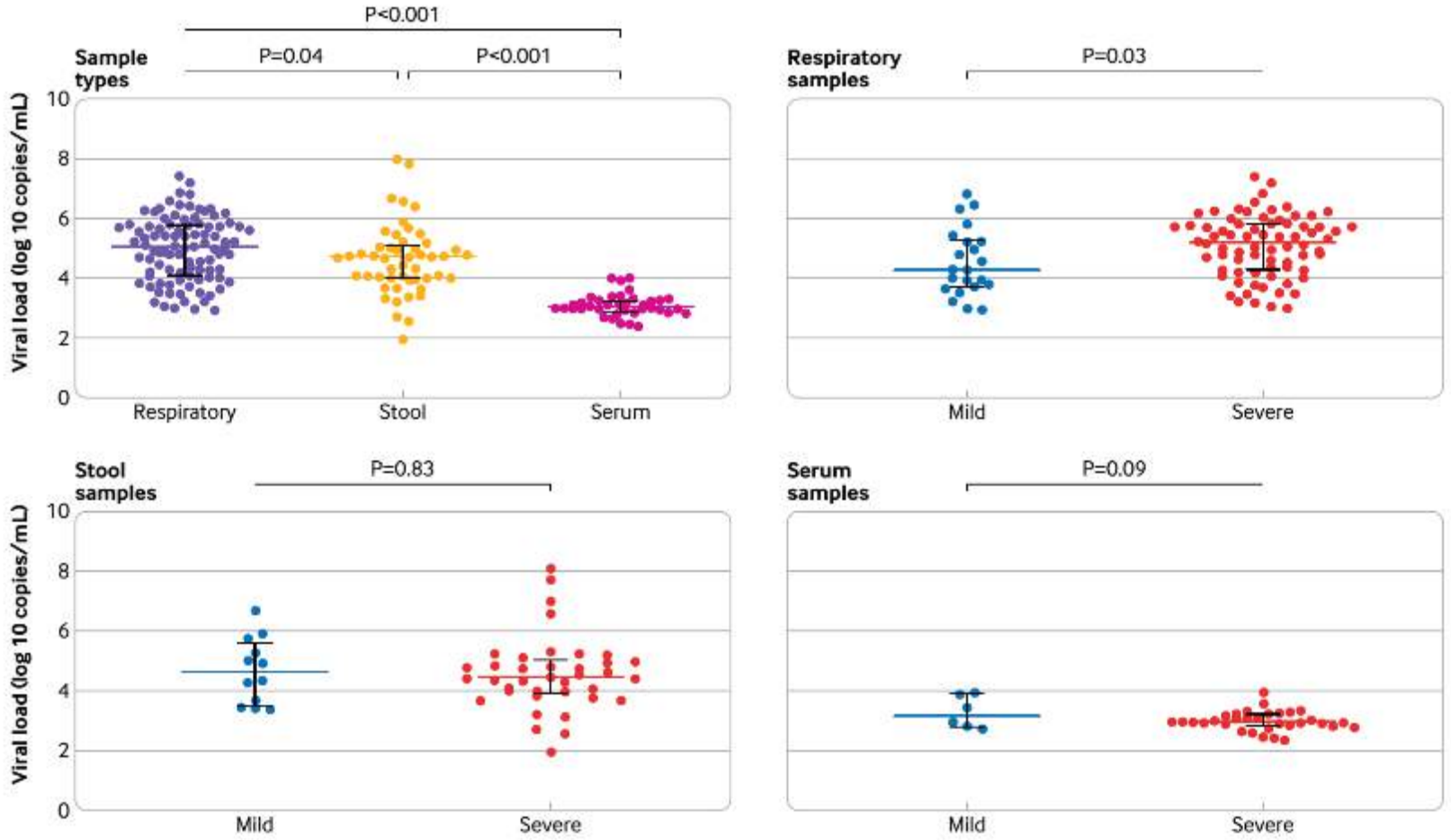
Viral load

96 patients (22 with mild disease and 74 with severe diseases) in China

Viral load:

- Duration of virus shedding in respiratory samples longer among severe patients (21 vs 14 days), also longer in patients >60 years old and male.
- 59% of patients with positive stool samples and presenting a longer viral shedding in stool than respiratory sample (22 vs 18 days).
- Viral load were slightly higher among severe cases.

Limit: a relatively low number of cases



To Zheng et *al. BMJ*. Apr 2020

Viral load

205 patients (mean age: 44y) → 1070 respiratory specimens:

- Pharyngeal swabs, urine, sputum, blood, feces
- Bronchoalveolar lavage fluid & fibro bronchoscopy brush biopsy

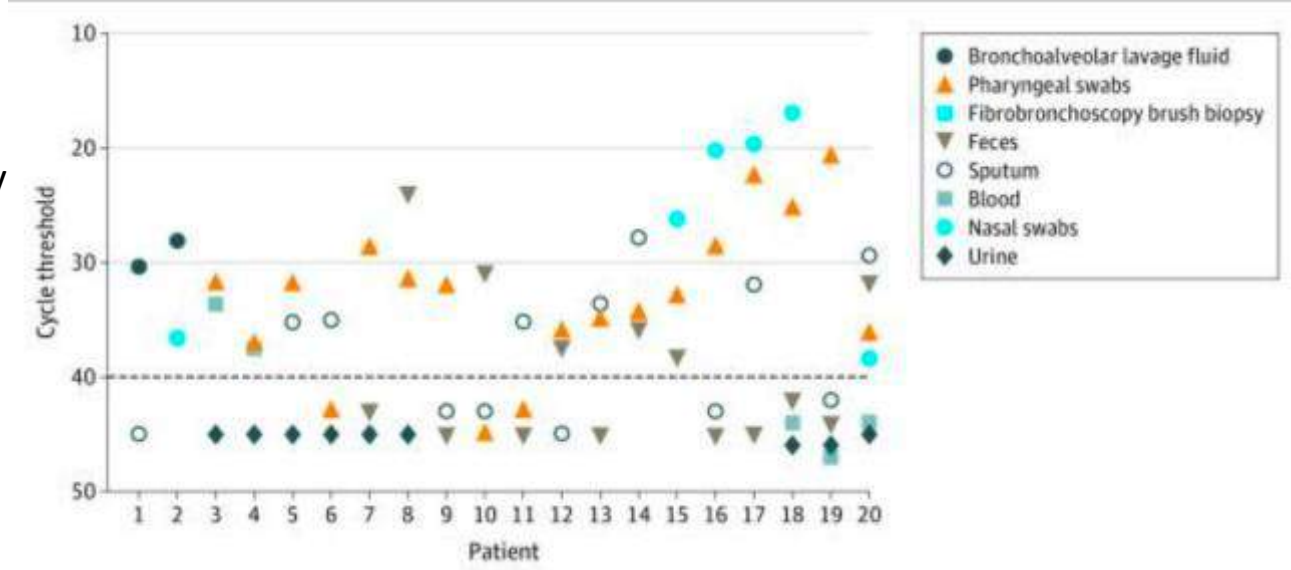
Cycle threshold: indicator of the copy number of SARS-CoV-2 RNA

Cycle threshold < 40 → positive for SARS-CoV-2 RNA

<u>Positive rates:</u>

- Highest positive rates → bronchoalveolar fluid (93%)
- Sputum (72%) – pharyngeal swabs (32%)
- Blood showed only 1% and urine 0%

- Mean cycle threshold for nasal swabs = 24,3 → higher viral load



→Testing of specimen from multiple sites
↑ sensitivity & ↓ false negative

<u>Limit</u>: this differ according to the typology of patients and disease stages.

Dynamic in viral shedding

94 symptomatic patients → <u>414 throat swabs</u> from symptoms onset up to 32 days after

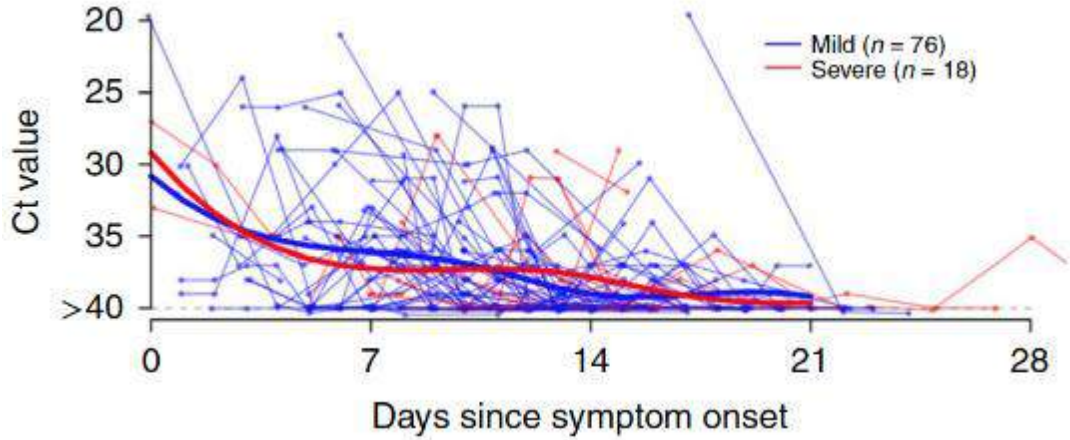
- Detection limit was Ct=40 (used to indicate negative samples)
- 50% were male
- Median age: 47 years
- No severe or critical patients

<u>Dynamic in viral shedding</u>

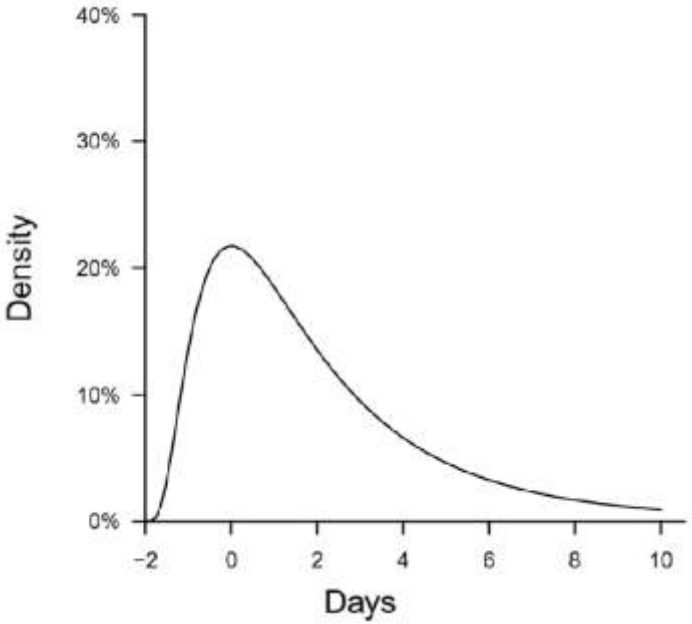
- Highest viral load soon after symptom onset
- Decreasing gradually after symptom onset
- **No difference in viral loads across sex, age groups, disease severity**

Viral shedding may begin 2 to 3 days before first symptoms

The estimated proportion **of presymptomatic transmission was 44%** ($CI_{95\%}$ [30–57%]). Infectiousness decline quickly within 7 days



Viral load detected by RT–PCR in throat swabs from patients infected with SARS-CoV-2



Simulated serial intervals assuming infectiousness started 2 days before symptom onset

He X et *al. Nat Med.* May 2020

Oral & fecal viral shedding

401 patients → <u>1758 rectal swabs during 0 to 98 days after illness onset</u>

- 80 patients positive for SARS-CoV-2 in the rectal swabs
  - Pediatrics: positive rate of 56,7%
  - Adults: positive rate of 16,9%
- Positive rate decreases over time

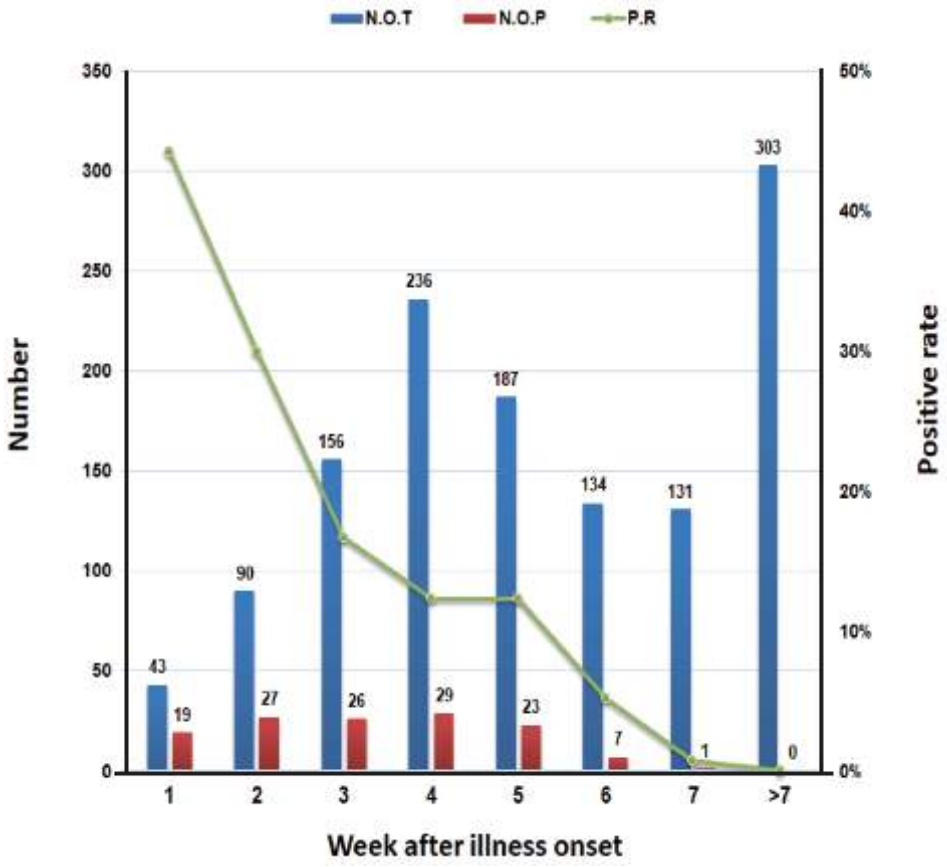
<u>517 pairs (respiratory + rectal samples) from the 80 patients positive in rectal swabs</u>

- 58 were double positive → coincidence rate increased during the disease progression
- 112 positive in rectal & negative in respiratory sample
- Higher viral load in rectal than respiratory samples

<u>Factors independently associated with the duration of fecal viral shedding</u>:

- Neutrophil level OR:1,55 $IC_{95\%}$[1,05 – 2,40]
- Interval between antiviral treatment and illness onset OR:1,17 $IC_{95\%}$[1,01 – 2,34]

NOT: number of tested - NOP: number of positive - PR: positive rate



→ Intestine = reservoir of SARS-CoV-2 RNA

The gastrointestinal viral reservoir is potentially a long-lasting fomite for SARS-CoV-2 transmission even for asymptomatic patients

→ **Still viable virus?**

Zhao F et *al. Gastroenterology.* May 2020

Positivity of viral culture

Viral culture is only rarely positive for low viral load (Ct values above 25 to 30) and after 8 to 10 days after symptom onset

Viral culture is not positive for feces sample

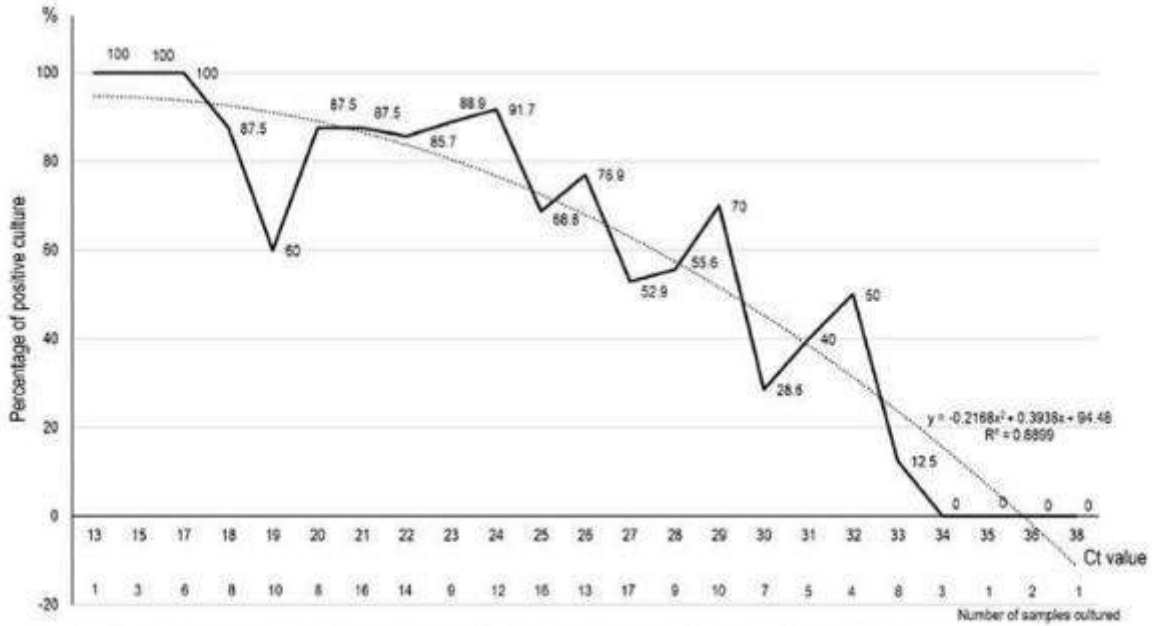
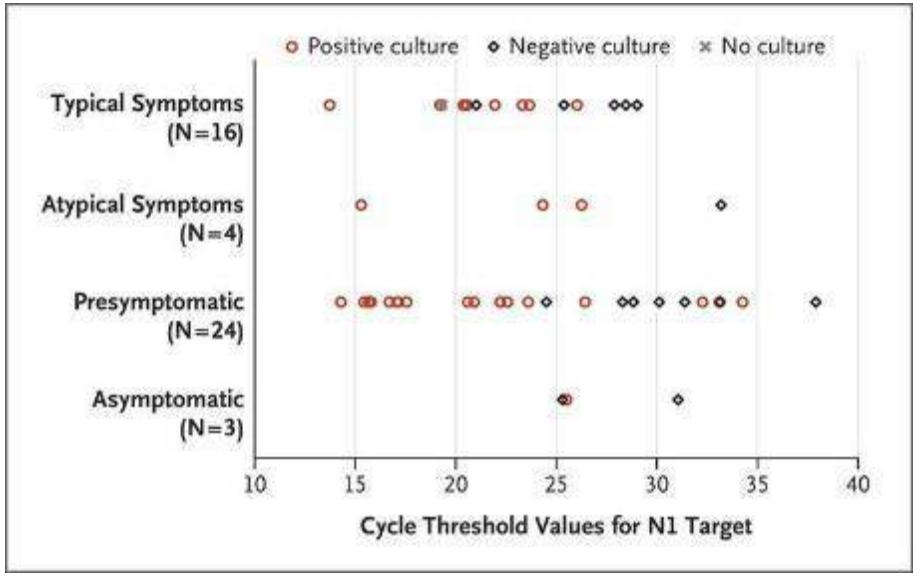
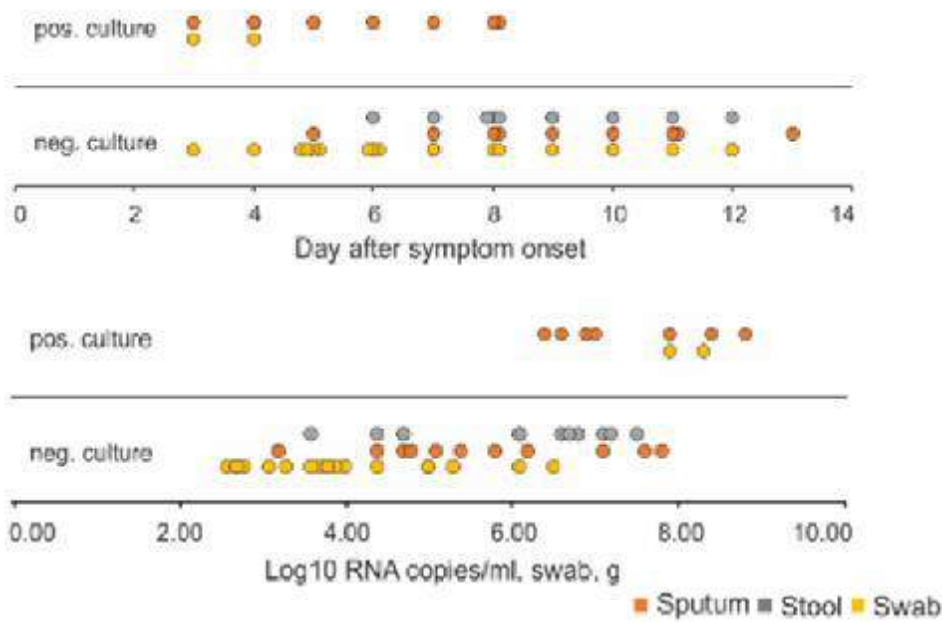


Fig. 1 Percentage of positive viral culture of SARS-CoV-2 PCR-positive nasopharyngeal samples from Covid-19 patients, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve

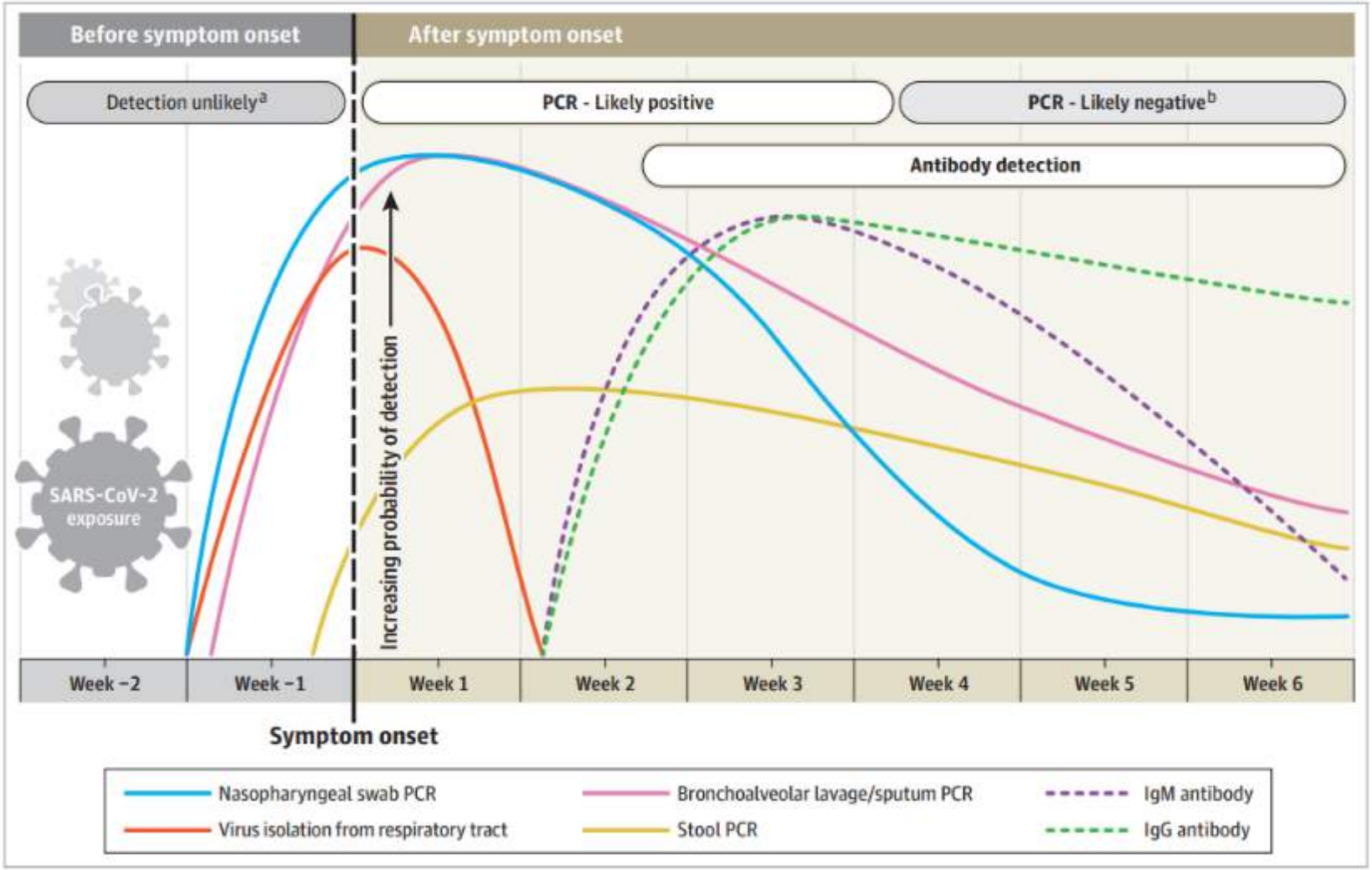




Arons MM *et al NEJM* May 2020

La Scola B *et al Eur J Clin Microbiol Infect Dis.* Jun 2020

SARS-CoV-2 detection



Limit: antibody response yet to be characterized among the various patients' populations

Estimated time intervals and rates of viral detection are based on data from several published reports. Because of variability in values among studies, estimated time intervals should be considered approximations and the probability of detection of SARS-CoV-2 infection is presented qualitatively. SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2; PCR, polymerase chain reaction.

[a] Detection only occurs if patients are followed up proactively from the time of exposure.

[b] More likely to register a negative than a positive result by PCR of a nasopharyngeal swab.

SARS-CoV-2 salivary detection

Rapid and accurate diagnostic tests are essential for controlling the ongoing Covid-19 pandemic

70 patients hospitalized with COVID-19 (nasopharyngeal swabs).

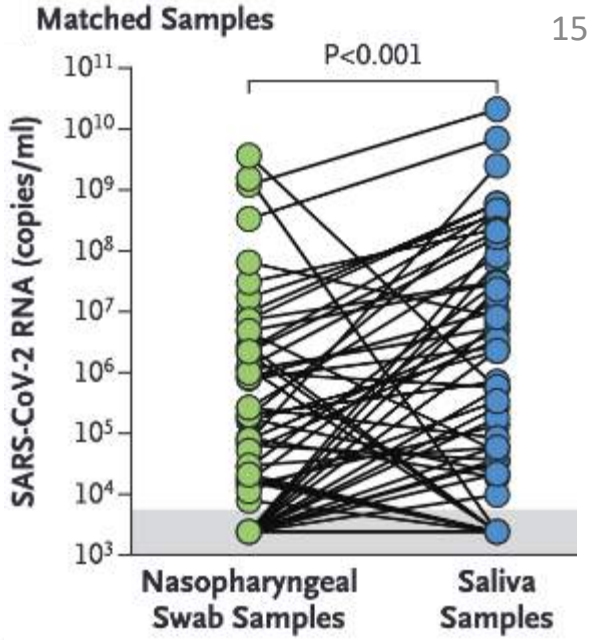
Additional samples (saliva specimens collected by the patients themselves + nasopharyngeal swabs collected by health care workers)

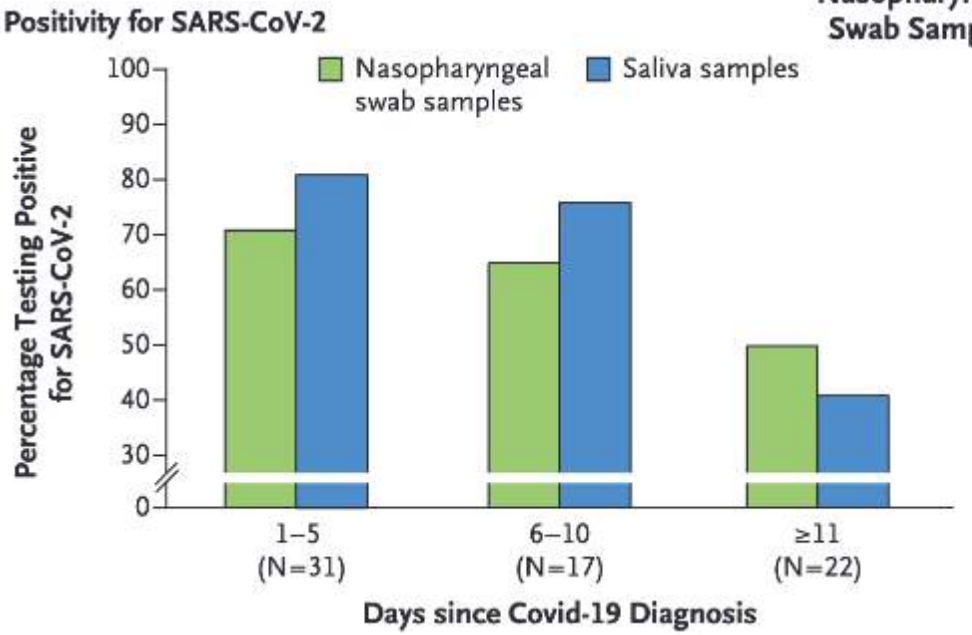
Detected more RNA copies in the saliva specimens than nasopharyngeal swabs (mean log copies per millilitre, 5.58 versus 4,93)

Higher percentage of saliva samples than nasopharyngeal swab samples were positive

Saliva specimens and nasopharyngeal swab specimens have at least similar sensitivity in the detection of SARS-CoV-2 during the course of hospitalization

Limits: hospitalized patients, nasopharyngeal samples presented an unusually low sensitivity (≈70% for earlier samples) in this study





Saliva specimens could be effective in COVID-19 diagnosis, but needs to be confirmed for outpatients

Wyllies AL *et al. NEJM.* Aug 2020

Salivary detection of SARS-CoV-2 in asymptomatic subjects

Mass screening study – 1924 asymptomatic subjects:

- Close contact white clinically confirmed COVID-19 patients (CT cohort, *n= 161*)
- Asymptomatic travelers arriving at Tokyo & Kansai (AQ cohort, *n= 1763*)

Saliva sample (self-collected) & NPS sample (medical officers)

Comparison between paired samples

Estimated prevalence:

- CT cohort: 29,6%, $CI_{90\%}$[23,8 – 35,8%]
- AQ cohort: 0,3%, $CI_{90\%}$[0,1 – 0,6%]
- The true concordance probability was:
  0,998, $CI_{90\%}$[0,996 – 0,999%] in AQ cohort
- Viral load was equivalent between NPS and saliva samples (Kendall's coefficient of concordance = 0,87)

Diagnostic results of nasopharyngeal swab (NPS) and saliva test

Contact-tracing cohort (n=161)

NPS	saliva positive	saliva negative
positive	38	3
negative	6	114

Airport Quarantine cohort (n=1,763)

NPS	saliva positive	saliva negative
positive	4	1
negative	0	1758

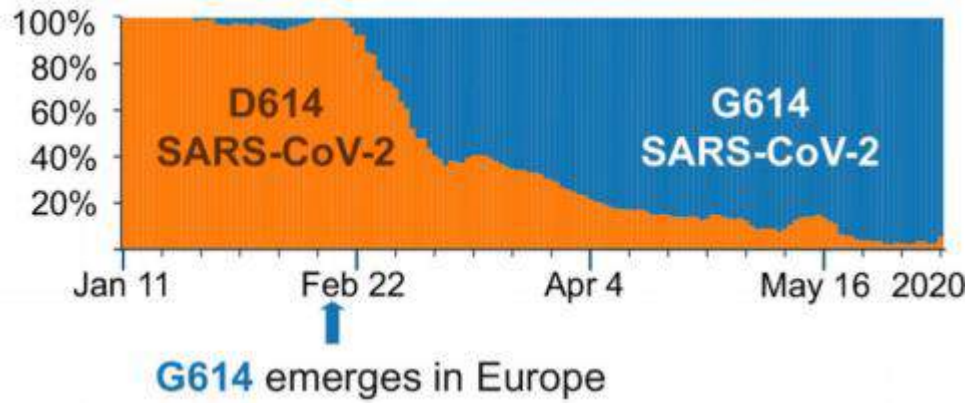
	Sensitivity	Specificity
NPS	86% , $CI_{90\%}$[77 – 93%]	99,93%, $CI_{90\%}$[99,77 – 99,99%]
Saliva	92% , $CI_{90\%}$[83 – 97%]	99,96%, $CI_{90\%}$[99,85 – 100,00%]

→ **Equivalent utility with similar sensitivity and specificity,**
→ **Self-collected saliva has significant advantages over NPS sampling,**
→ **Saliva may be a reliable alternative in detecting SARS-CoV-2 in asymptomatic**
→ **Limit:** the number of positive patients in the QC does not provide a strong evaluation of the saliva sensitivity in this population

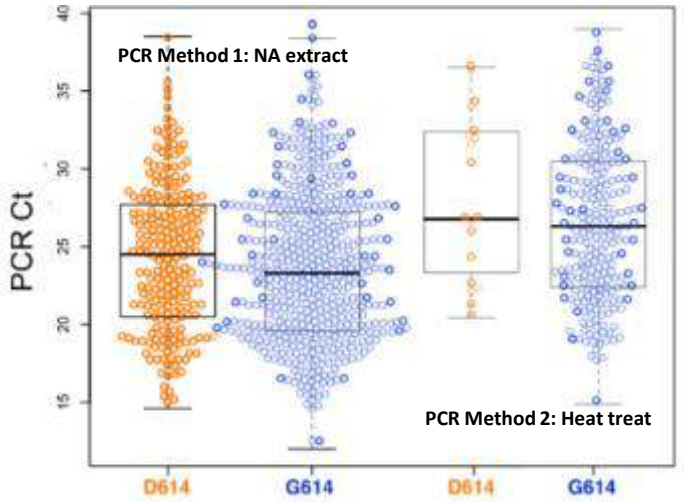
Changes in SARS-CoV-2 Spike

SARS-CoV-2 variant with Spike G614 has replaced D614 as the dominant pandemic form:

- Spike D614G amino acid change is caused by an A-to-G nucleotide mutation at position 23,403 in the Wuhan reference strain

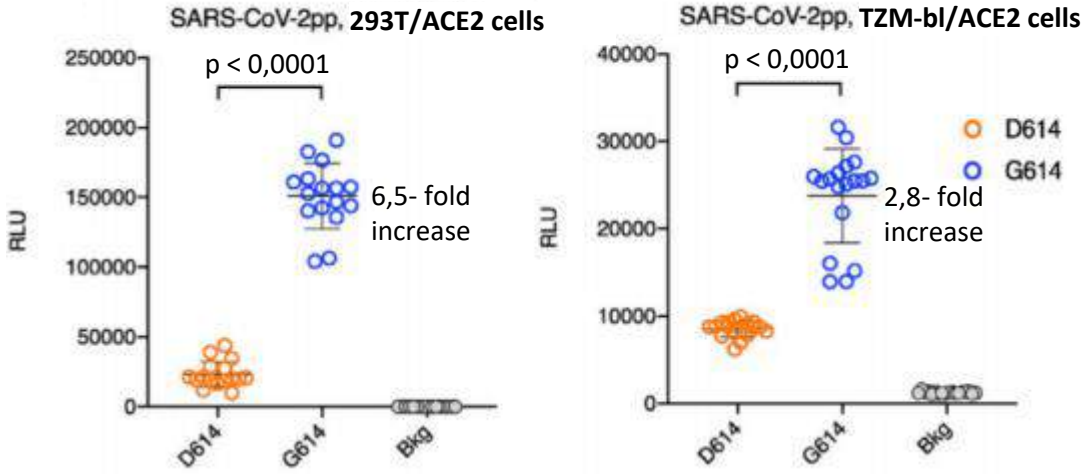


<u>G614 Is Associated with Potentially Higher Viral Loads in COVID-19 Patients but not with disease severity:</u>



- G614 is associated with a lower cycle threshold (Ct) required for detection (higher viral loads)

<u>Recombinant lentiviruses pseudo typed with the G614 Spike more infectious than corresponding D614 S-pseudo typed viruses</u>



Limits: this mutation is not single (*e.g.* associated to P314L in ORF1b) and represents the vast majority of cases in France among non-travelers since the very beginning of the outbreak

Korbert B *et al. Cell.* Aug 2020

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Spike mutation D614G & SARS-CoV-2 fitness

What is the impact on viral spread and vaccine efficacy of the spike protein mutation D614G ?

D614G amino acid substitution reached over 74% of all published sequences by June 2020.

Effect on viral replication in cell culture:

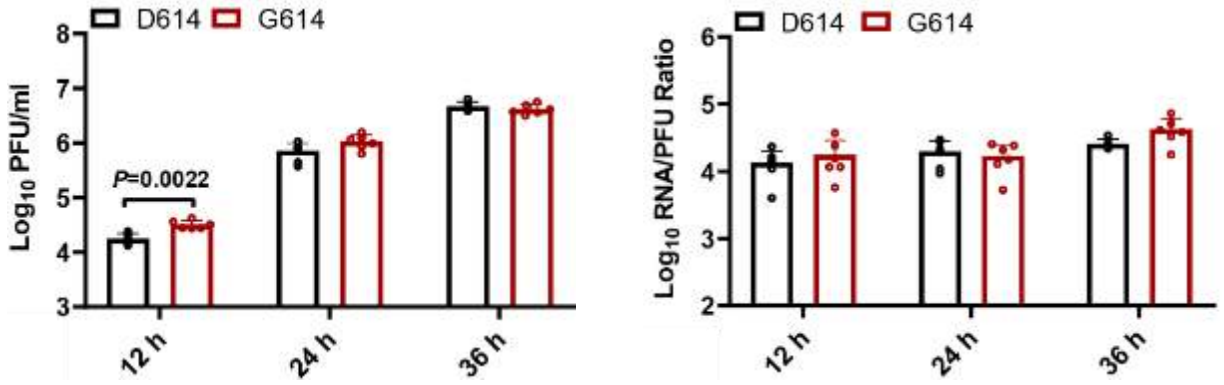
- Use of Vero E6 cells to test a pair of recombinant isogeneic viruses presenting a D614 or G614
- Two viruses replicated to comparable levels
- No difference was found on calculated the genomic RNA/PFU ratios.

→ **D614G mutation does not affect viral replication or virion infectivity in Vero E6 cells**

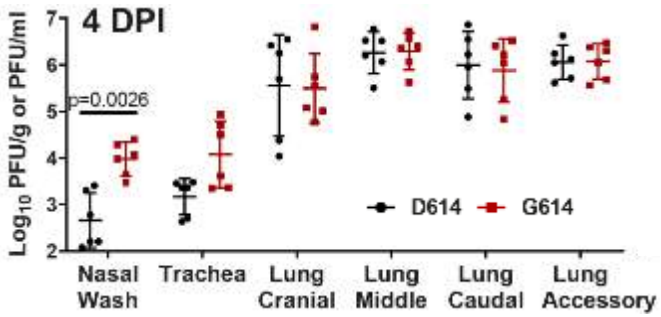
In vivo relevance of D614G mutation:

- Hamster model: intranasally infecting with D614 or G614
- Hamster infected with G614 produced higher infectious viral titers in the upper airway but not on lungs
- The RNA/PFU ratios of G614 virus were lower than D614 in upper airway but differences are negligible in lungs.

*Viral replication and genomic RNA/PFU ratios of D614 and G614 viruses produced from **Vero E6 cells***



*D614G substitution increases SARS-CoV-2 replication in the upper airway, but not the lungs, of **hamsters***



Plante JA *et al. Nature. Oct* 2020

Spike mutation D614G & SARS-CoV-2 fitness

In primary human airways tissue model:

- Infectious viral titers of G614 were higher than those of D614
- RNA/PFU ratios of D614 virus were 1.4- to 5.3-fold higher than those of G614 virus

→ G614 enhances viral replication through increased virion infectivity in primary human upper airway tissues

→ **Suggest the role of D614G mutation in viral transmissibility**

Effect on neutralization susceptibility:

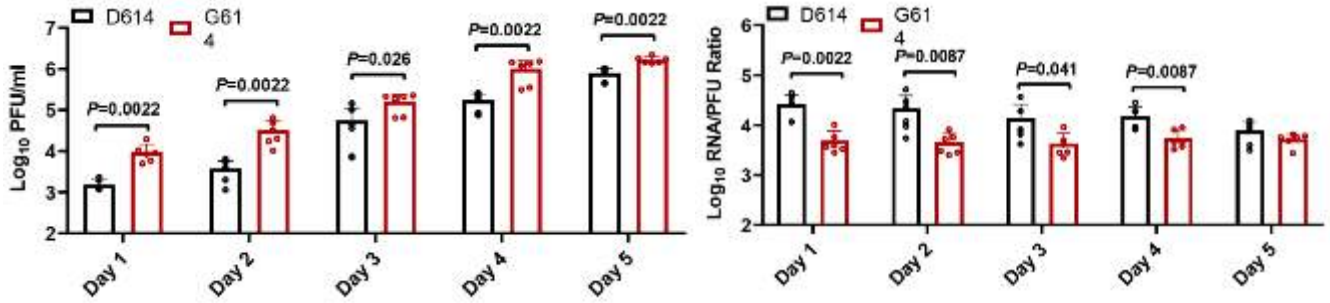
- D614G may confer higher susceptibility to serum neutralization
- D614G may modulate spike protein conformation to affect mAb neutralization

→ **Mutation may not reduce the ability of vaccine to protect against COVID-19**

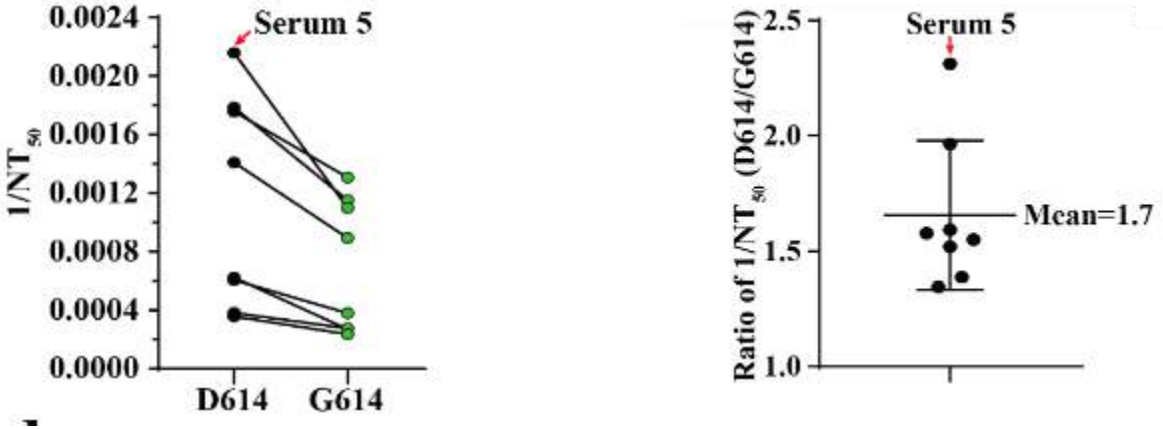
→ **Importance to test therapeutic mAbs against G614**

→ **Importance to monitor the impact of future mutations emergence with the introduction and use of vaccines**

*D614G substitution increases SARS-CoV-2 replication **in primary human airway tissues***



*Neutralizing activities of **hamster** sera against D614 and G614*



Plante JA *et al. Nature. Oct* 2020

VIROLOGY (December 21th 2020)

1. Which type of virus is SARS-CoV-2?

- RNA viruses that belong to the *betacoronavirus* genus

2. What is the stability and viability of SARS-CoV-2?

- Stability is similar to that of SARS-CoV-1 under experimental circumstances tested
- Aerosol and fomite transmission of SARS-CoV-2 is plausible

3. What is the impact of the mutation D614G for SARS-CoV-2?

- May increase transmission by increasing viral load in the upper airways without clinical impact
- Higher susceptibility to serum neutralization --> may not reduce the ability of vaccine to protect against COVID-19

4. What do we know about viral load and shedding according to different samples?

- Highest positive rates of SARS-CoV-2 in bronchoalveolar fluid among severe patients
- No influence of sex, age and disease severity on viral loads, has been observed
- Viral shedding may begin 2 to 3 days before first symptoms
- Detection of viral RNA does not necessarily mean that infectious virus is present, especially for low viral loads and >8 days from symptoms onset

5. What is the description of the immune responses in infected patients?

- IgG levels and neutralizing antibodies start to decrease within 2-3 months after infection

6. Alternative to the nasopharyngeal swab for SARS-CoV-2 detection?

- Saliva sample might be a good alternative to the NPS with several advantages, but asymptomatic populations are poorly characterized

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