Scientific update on COVID-19

Updated on December 21\textsuperscript{th} 2020
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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
- 7 coronaviruses known to infect humans
  - 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
    - MERS-CoV = CFR of 37% (2012 - )
    - SARS-CoV-2 = CFR unknown (2019 - )

Coronaviridae Study Group Nat Microbiol. Apr 2020
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
Persistence of virus RNA

49 patients with 490 specimens → 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

Time to loss detection

• 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?

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Median (95% CI)</th>
<th>95th percentile (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat swab</td>
<td>15.6 (11.8–20.7)</td>
<td>32.8 (25.9–62.9)</td>
</tr>
<tr>
<td>Sputum</td>
<td>20.0 (14.1–27.0)</td>
<td>43.7 (33.6–60.4)</td>
</tr>
<tr>
<td>Nasopharyngeal swab</td>
<td>22.7 (18.8–27.5)</td>
<td>46.3 (39.0–55.2)</td>
</tr>
<tr>
<td>Feces</td>
<td>24.5 (21.2–29.3)</td>
<td>45.6 (40.0–52.6)</td>
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</table>

Data are presented in days after illness onset

Mild cases, n = 43

Severe cases, n = 6

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<tr>
<td>Throat swab</td>
<td>33.9 (24.2–47.3)</td>
<td>58.9 (39.4–81.7)</td>
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<tr>
<td>Sputum</td>
<td>30.9 (23.5–39.1)</td>
<td>44.7 (36.3–58.0)</td>
</tr>
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<td>Nasopharyngeal swab</td>
<td>33.5 (25.7–42.7)</td>
<td>49.4 (38.4–68.5)</td>
</tr>
<tr>
<td>Feces</td>
<td>32.5 (26.3–39.1)</td>
<td>48.9 (41.3–59.7)</td>
</tr>
</tbody>
</table>

Limits

• 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

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 on 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
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
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
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

Positive rates:

- 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

**Limit:** this differ according to the typology of patients and disease stages.
Dynamic in viral shedding

94 symptomatic patients → 414 throat swabs 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

**Dynamic in viral shedding**

- 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<sub>95%</sub> [30–57%]). Infectiousness decline quickly within 7 days

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

He X et al. Nat Med. May 2020
Oral & fecal viral shedding

401 patients → 1758 rectal swabs during 0 to 98 days after illness onset

- 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

517 pairs (respiratory + rectal samples) from the 80 patients positive in rectal swabs

- 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

Factors independently associated with the duration of fecal viral shedding:

- Neutrophil level OR: 1.55 IC₉₅%[1.05 – 2.40]
- Interval between antiviral treatment and illness onset OR: 1.17 IC₉₅%[1.01 – 2.34]

→ 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?
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.
SARS-CoV-2 detection

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

Viral load was equivalent between NPS and saliva samples (Kendall’s coefficient of concordance = 0,87)

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<th>Contact-tracing cohort (n=161)</th>
<th>Airport Quarantine cohort (n=1,763)</th>
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<tbody>
<tr>
<td></td>
<td>saliva</td>
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</tr>
<tr>
<td></td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>positive</td>
<td>38</td>
<td>3</td>
</tr>
<tr>
<td>negative</td>
<td>6</td>
<td>114</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tr>
<td>NPS</td>
<td>86%, CI$_{90}$% [77 – 93%]</td>
<td>99,93%, CI$_{90}$% [99,77 – 99,99%]</td>
</tr>
<tr>
<td>Saliva</td>
<td>92%, CI$_{90}$% [83 – 97%]</td>
<td>99,96%, CI$_{90}$% [99,85 – 100,00%]</td>
</tr>
</tbody>
</table>

→ 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

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

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

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

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

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.
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
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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