COVID-19
ONLINE EDUCATIONAL PROGRAM

SARS-CoV-2 Virological Diagnostic

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academic medical education

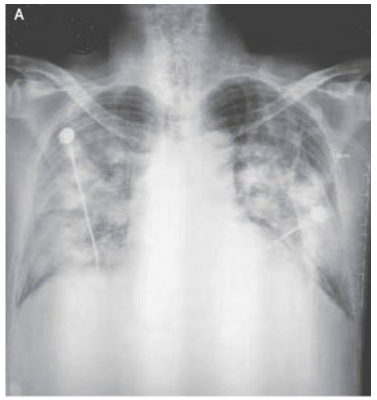
Disclosures

- **AG Marcelin** has no commercial interests.
- **AG Marcelin** has received travel grants, honoraria, and study grants from various pharmaceutical companies including Gilead Sciences, Merck-Sharp & Dohme-Chibret, Jansen and ViiV Healthcare.
- **AG Marcelin** prepared the content of this presentation using his own material with no commercial input.
- **AG Marcelin** may discuss cases and circumstance when drugs are used off label; this is his own personal clinical experience. For the proper use of medications, please review the Product Monographs.

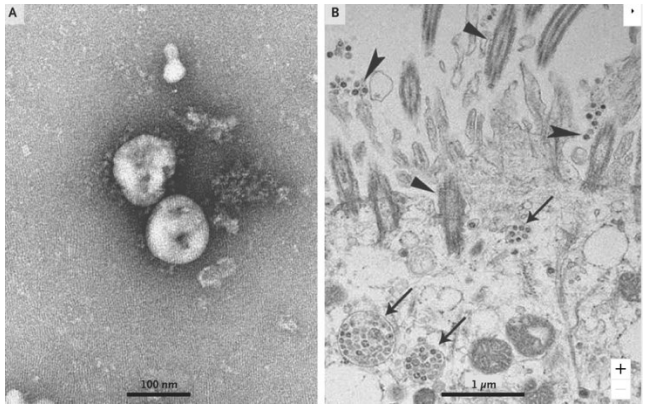
- Virus
- Viral diagnostics
  - RNA
    - Assays performance
    - Kinetic
    - Viral load
    - Body fluids/tissues
  - Antigen
- Conclusion

SARS-CoV-2

- In late December 2019, cases of severe pneumonia were reported
  - Epidemiologically associated with the Huanan seafood market in Wuhan, China
  - Unknown etiologic agent identified



Chest radiograph

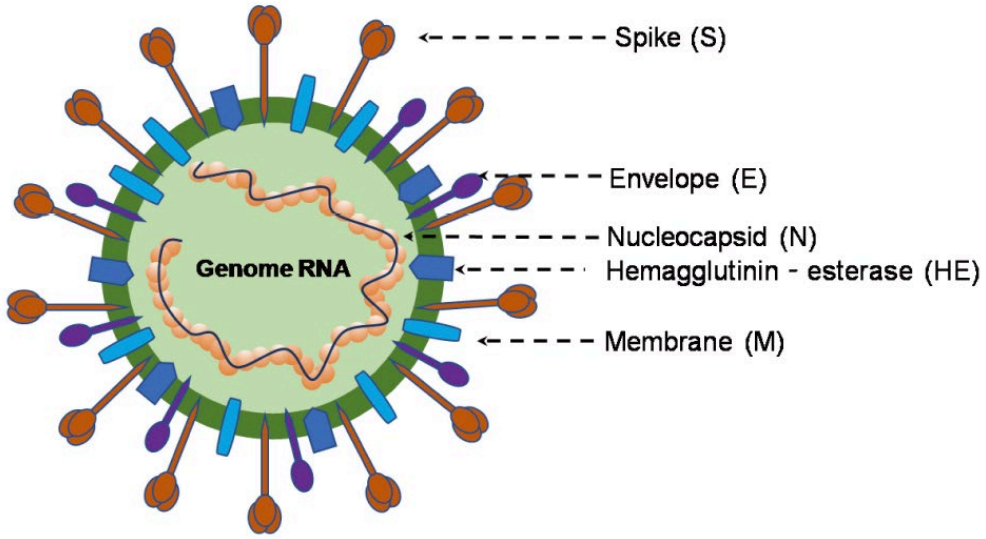


Visualization of 2019-nCoV with Transmission Electron Microscopy

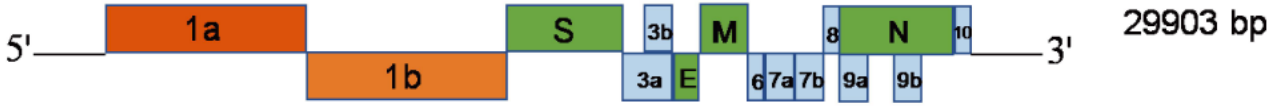
Zhu et al, N. Eng. J. Med, 2020; A novel coronavirus genome identified in a cluster of pneumonia cases—Wuhan, China 2019–2020 China CDC Weekly; Report of clustering pneumonia of unknown etiology in Wuhan City. Wuhan Municipal Health Commission, 2019.

SARS-CoV-2

- β coronavirus
- Viral particle
  - Diameter 100nm
  - Enveloped
  - Spike protein: mediated attachment to host cell



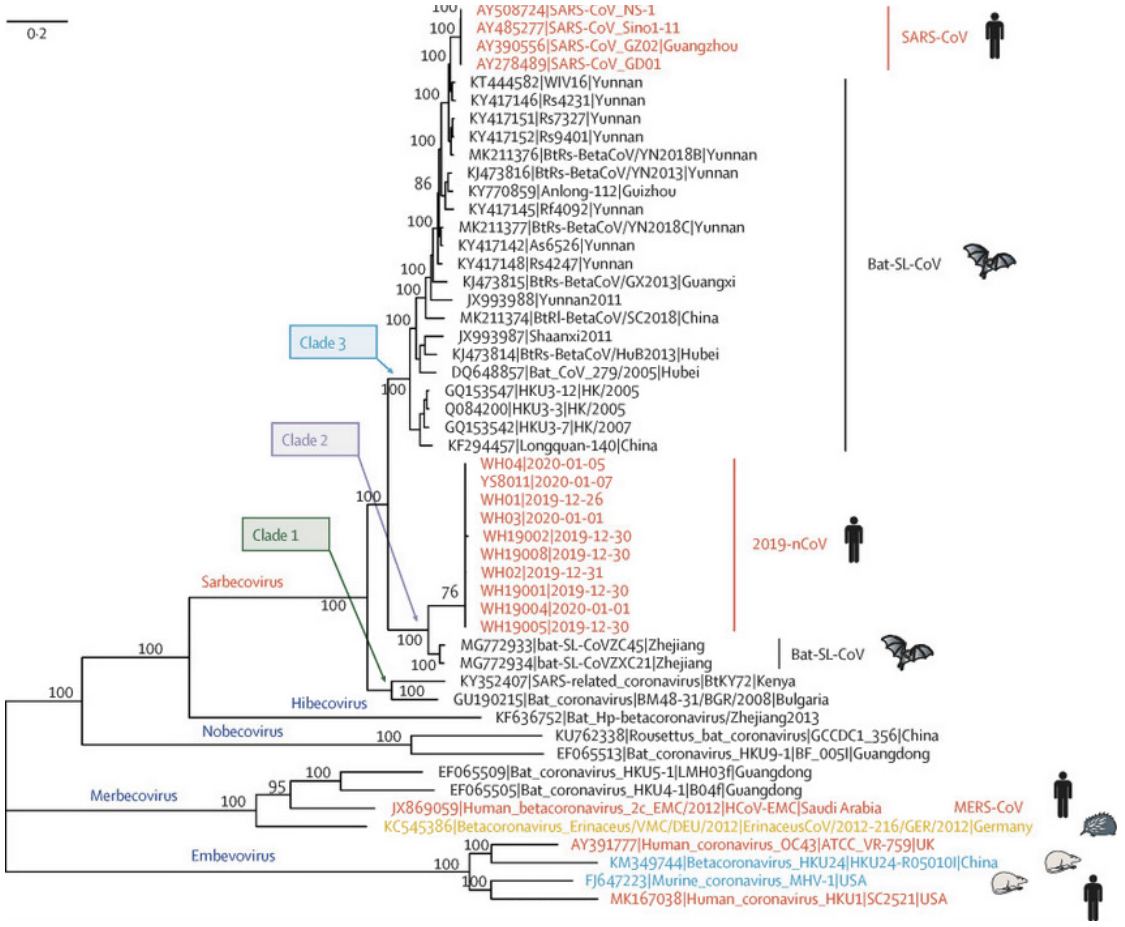
  - Genome: positive-sense, single-stranded, RNA (30 kb)



Fehr et al, Methods Mol. Bio, 2015; Chan et al, Emerg. Microbes and Infections, 2020; Lu et al, The Lancet, 2020

SARS-CoV-2

- Whole genome sequencing
  - 88% identity with bat-derived Coronavirus
  - 79% with SARS-CoV
  - About 50% with MERS-CoV



Lu et al, The Lancet, 2020;

Tests to detect the COVID-19 can be divided in two main categories

- Molecular diagnostic tests, i.e. tests that will detect the presence of the virus
  - To help identify people who are infected

- Serologic tests, i.e. tests that will detect the immune response to the virus
  - To detect those who have already had the infection & developed antibodies

Detection of viral RNA by RT-PCR

- RT-PCR is the most commonly used and reliable test for diagnosis of COVID-19

  - Using nasopharyngeal swabs or other upper respiratory tract specimens, including throat swab

  - A variety of RNA gene targets are used by different manufacturers, with most tests targeting 1 or more of the envelope (*env*), nucleocapsid (*N*), spike (*S*), RNA-dependent RNA polymerase (*RdRp*), and *ORF1* genes.

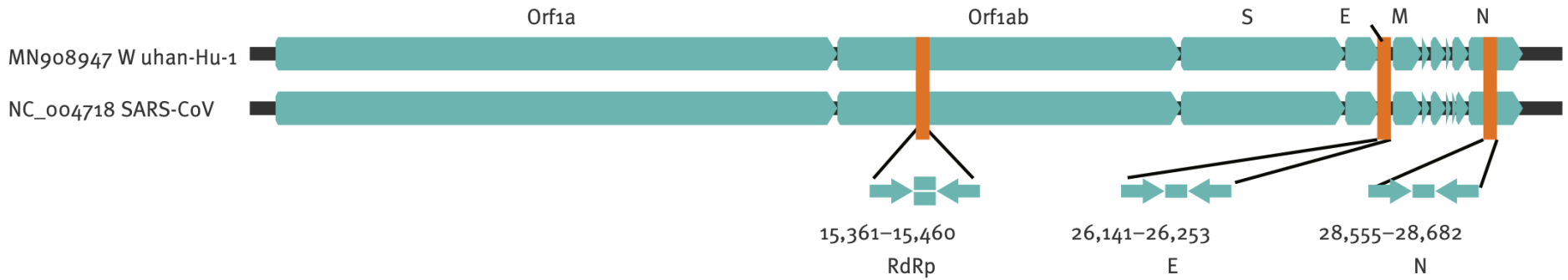
Nalla AK , et al. Comparative performance of SARS-CoV-2 detection assays using seven different primer/probe sets and one assay kit. *J Clin Microbiol*. 2020

RESEARCH

Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR

Victor M Corman[1], Olfert Landt[2], Marco Kaiser[2], Richard Molenkamp[3], Adam Meijer[4], Daniel KW Chu[5], Tobias Bleicker[1], Sebastian Brünink[1], Julia Schneider[1], Marie Luisa Schmidt[1], Daphne GJC Mulders[3], Bart L Haagmans[3], Bas van der Veer[4], Sharon van den Brink[4], Lisa Wijsman[4], Gabriel Goderski[4], Jean-Louis Romette[6], Joanna Ellis[7], Maria Zambon[7], Malik Peiris[5], Herman Goossens[8], Chantal Reusken[4], Marion PG Koopmans[3], Christian Drosten[1]

1. Charité – Universitätsmedizin Berlin Institute of Virology, Berlin, Germany and German Centre for Infection Research (DZIF), Berlin, Germany



E: envelope protein gene; M: membrane protein gene; N: nucleocapsid protein gene; ORF: open reading frame; RdRp: RNA-dependent RNA polymerase gene; S: spike protein gene.

- Establishment and validation of a diagnostic workflow for 2019-nCoV screening and specific confirmation
- Recommendation: to use the E gene assay as the first-line screening tool, followed by confirmatory testing with the RdRp gene assay

Corman et al, Eurosurveillance, 2020

RT-PCR tests

- **Laboratory-Based Molecular Testing**
  - After specimen collection, samples undergo RNA extraction followed by qualitative RT-PCR for target detection (results within 3-4 hours)
  - 28 Commercial SARS–CoV-2 in vitro diagnostic assays given an EUA from the FDA as of 4 April 2020

- **Point-of-Care Molecular Diagnostics**
  - Low-complexity, rapid (results within 1 hour)

Comparison of 7 commercially available RT-PCR kits for the detection of SARS-CoV-2

Manufacturer	Country	Catalog number	Storage condition	Regulatory status	Target gene(s)
Altona Diagnostics	Germany	821003	−20 °C	RUO[b]	E[a], S
BGI	China	MFG030010	−20 °C	CE-IVD	ORF1ab
CerTest Biotec	Spain	VS-NCO213L	RT	CE-IVD	ORF1ab, N
KH Medical	Korea	RV008	−20 °C	CE-IVD	RdRp, S
PrimerDesign	England	Z-Path-COVID-19-CE	−20 °C[c]	CE-IVD	RdRp
R-Biopharm AG	Germany	PG6815RUO	−20 °C	RUO[d]	E
Seegene	Korea	RP10244Y	−20 °C	CE-IVD	RdRp, N, E[a]

All RT-PCR kits performed satisfactorily:

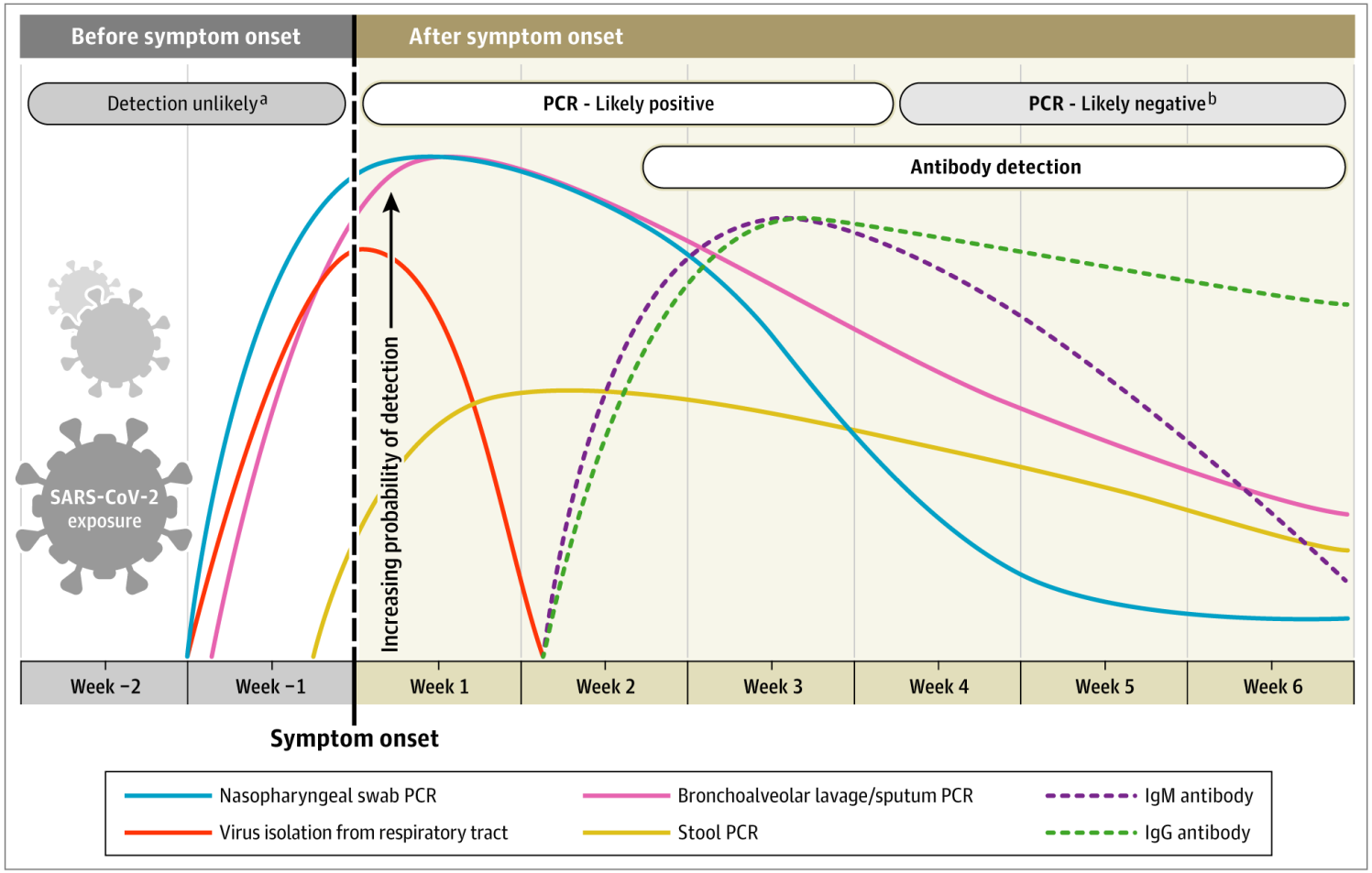
- PCR efficiency (≥96%)
- Estimated LOD95% varied within a 6-fold range between kits (3.8-23 copies/ml)

Puck B. van Kasteren, et al. J Clin Virol. 2020

The sensitivities of the RT-PCR tests are comparable according to comparison studies

- **Generally very sensitive and specific**
  - If an RT-PCR is positive, the result is most likely correct
    - the only case of false positive could be happening if a non-positive sample is contaminated by viral material, during test processing for instance
  - False negative results are also possible but are most frequently the result of a wrong patient sampling (up to 30%)
    - swabs not pushed far enough in the patients' nasopharynx for instance
    - inadequate time of sampling

Nalla AK , et al. *J Clin Microbiol*. 2020

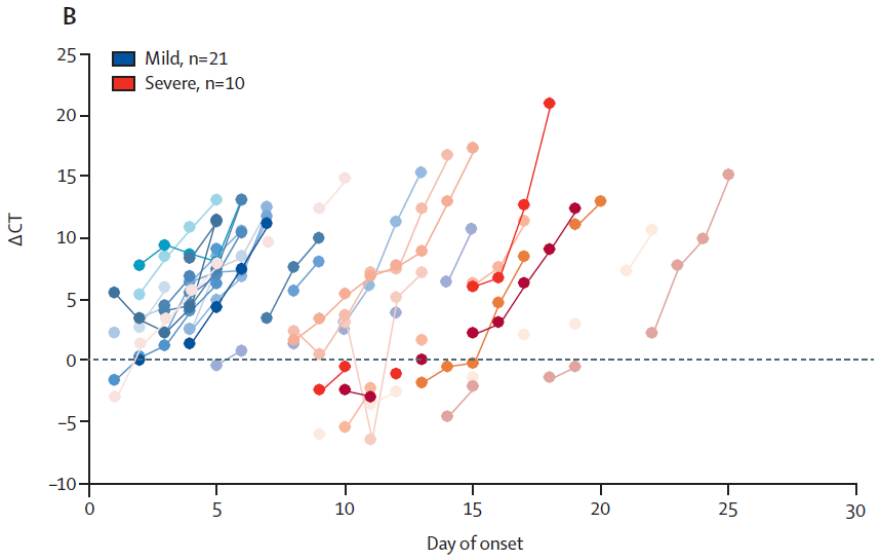


In most individuals with symptomatic COVID-19 infection, viral RNA in the nasopharyngeal swab as measured by the cycle threshold (Ct) becomes **detectable as early as day 1 of symptoms and peaks within the first week of symptom onset**.

This positivity starts to decline by week 3 and subsequently becomes undetectable.

Relationship between viral load and disease severity

- Prospective study, including 76 patients (Liu et al, The Lancet Inf. Dis., 19 March 2020)
  - Nasopharyngeal samples
  - 30 (39%) severe cases and 46 (61%) mild cases
  - The Ct is the number of replication cycles required to produce a fluorescent signal, with lower Ct values representing higher viral RNA loads.



- ΔCt values of severe cases < ΔCt values of mild cases at the time of admission
- **The mean viral load of severe cases was around 60 times higher than that of mild cases**

90% of mild cases were repeatedly testing negative on RT-PCR by day 10 post-onset,

By contrast, all severe cases still tested positive at or beyond day 10 post onset.

Patients with severe COVID-19 tend to have a high viral load and a long virus-shedding period

- The Ct values obtained in severely ill hospitalized patients are lower than the Ct values of mild cases, and PCR positivity may persist beyond 3 weeks after illness onset when most mild cases will yield a negative result.

- **However, a "positive" PCR result reflects only the detection of viral RNA and does not necessarily indicate presence of viable virus**

- In some cases, viral RNA has been detected by RT-PCR even beyond week 6 following the first positive test.
  - A few cases have also been reported positive after 2 consecutive negative PCR tests performed 24 hours apart. It is unclear if this is a testing error, reinfection, or reactivation.

The timeline of PCR positivity is different in specimens other than nasopharyngeal swab

- Prospective study including 67 CoVid+ (Tan et al, MedRXiv, March 2020)

Table 2. Characteristics and duration of SARS-CoV-2 RNA shedding in clinical specimens

Sample type	Total patients	Total samples	Positive (%)	Viral shedding model, no./total no. (%)				Duration time of virus from illness onset, days — median (range)*				Still positive after NS reached undetectable
				positive in continuous samples	fluctuated positive	single positive	negative the whole course	total	Severe	non-Severe	*p*-value	
Nasopharyngeal swab	67	377	63/67 (94.0)	31/67 (46.3)	27/67 (40.3)	5/67 (7.4)	4/67 (6.0)	12 (3-38)	14 (5-38)	11 (3-28)	0.054	na
Sputum	61	221	58/61 (95.1)	50/61 (82.0)	6/61 (9.8)	2/61 (3.3)	3/61 (4.9)	19 (5-37)	23 (6-37)	16 (5-33)	0.068	28/46 (60.9)
Stool	62	220	45/62 (72.6)	19/62 (30.6)	5/62 (8.1)	22/62 (35.5)	16/62 (25.8)	18 (7-26)	19.5 (14-26)	18(7-25)	0.492	14/46 (30.4)
Urine	64	231	12/64 (18.8)	1/64 (1.6)	0	11/64 (17.2)	52/64 (81.2)	na	na	na	na	na
Plasma	63	211	9/63 (14.3)	1/63 (1.6)	2/63 (3.2)	6/63 (9.5)	54/63 (85.7)	na	na	na	na	na
Any sample type	67	1260	67/67 (100.0)	na	na	na	na	22 (3-38)	23 (7-38)	20 (3-33)	0.023	na

* Duration time for nasopharyngeal swab, sputum, and stool were evaluated in patients with continuous positive samples; NS: nasopharyngeal swab; na: not applicable.

- Median duration of RNA SARS-CoV-2 shedding are in days 12 (3-38), 19 (5-37) and 18 (7-26) in nasopharyngeal swabs, sputum and stools, respectively.
- PCR positivity declines more slowly in sputum and may still be positive after nasopharyngeal swabs are negative

Virus isolation in culture was not successful beyond day 8 of illness onset

- Detailed virological analysis of 9 cases, providing proof of active virus replication in upper respiratory tract tissues
  - Pharyngeal virus shedding was very high during the first week of symptoms
  - Infectious virus isolated in 83% of samples during first 7 days from throat- and lung-derived samples and never after.
  - No infectious virus isolated from stool samples
- This correlates with the decline of infectivity beyond the first week

Wolfel et al, Nature, 2020

Bronchoalveolar lavage fluid specimens showed the highest positive rates of RT-PCR

- Retrospective study including 205 patients, 19% of patients had severe illness (1070 specimens) (Wang et al, JAMA, Mars 2020)

Table. Detection Results of Clinical Specimens by Real-Time Reverse Transcriptase–Polymerase Chain Reaction

Specimens and values	Bronchoalveolar lavage fluid (n = 15)	Fibrobronchoscope brush biopsy (n = 13)	Sputum (n = 104)	Nasal swabs (n = 8)	Pharyngeal swabs (n = 398)	Feces (n = 153)	Blood (n = 307)	Urine (n = 72)
Positive test result, No. (%)	14 (93)	6 (46)	75 (72)	5 (63)	126 (32)	44 (29)	3 (1)	0
Cycle threshold, mean (SD)	31.1 (3.0)	33.8 (3.9)	31.1 (5.2)	24.3 (8.6)	32.1 (4.2)	31.4 (5.1)	34.6 (0.7)	ND
Range	26.4-36.2	26.9-36.8	18.4-38.8	16.9-38.4	20.8-38.6	22.3-38.4	34.1-35.4	
95% CI	28.9-33.2	29.8-37.9	29.3-33.0	13.7-35.0	31.2-33.1	29.4-33.5	0.0-36.4	

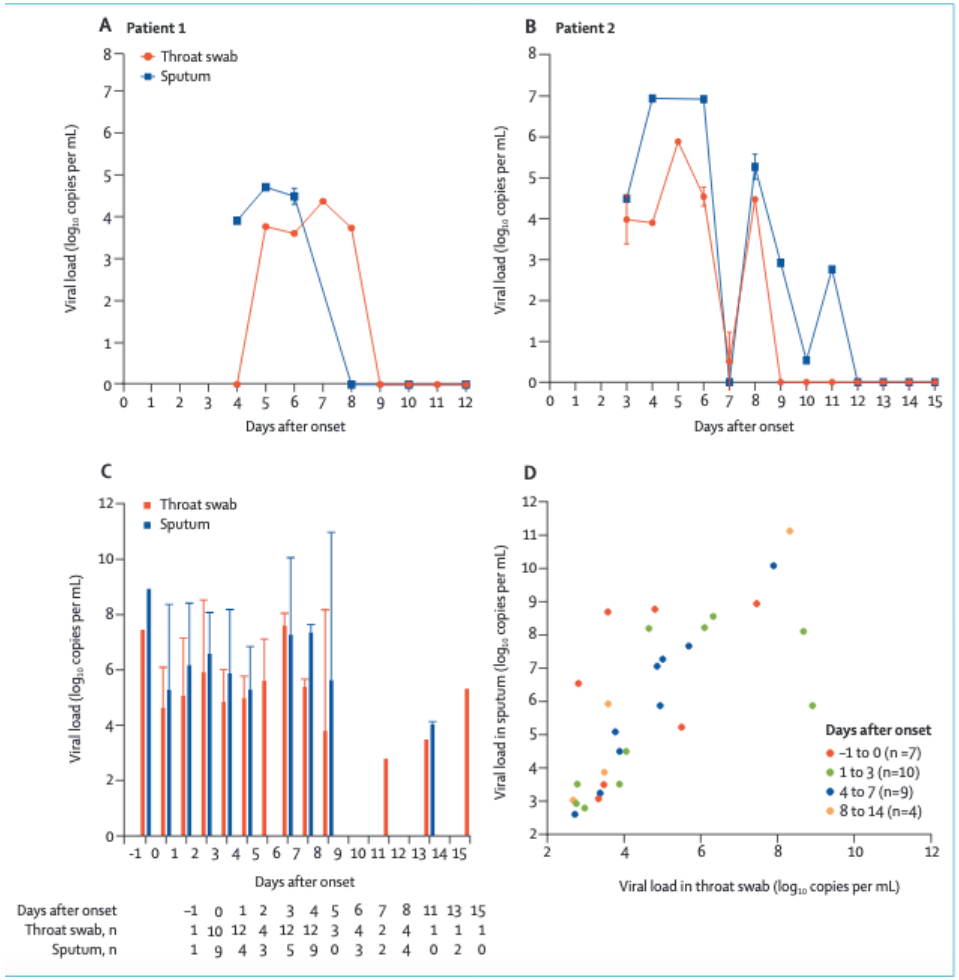
Abbreviation: ND, no data.

Between respiratory samples: **Bronchoalveolar lavage fluid specimens showed the highest positive rates (14 of 15; 93%)**, followed by sputum (72 of 104; 72%), nasal swabs (5 of 8; 63%), fibrobronchoscope brush biopsy (6 of 13; 46%), pharyngeal swabs (126 of 398; **32%**).

Higher viral load in sputum than in throat and nasal swabs

- Retrospective study including 82 patients (Pan et al, The Lancet, April 2020)
  - **Peak of viral load at 5-6 days after symptoms onset**
  - Sputum generally show higher viral load than throat sample
  - Median of 7.99 10^4 in throat
  - Median of 7.52 10^5 in sputum

- VL in sputum > VL in throat and nasal swabs (confirmed by Yu and al, Clin Inf. Dis. 28 March 2020)



Viral antigen detection

- Lateral flow immunoassay
- May be performed using swabs similar to those currently used in RT-PCR
- Would be quick to run (< 15 minutes) and could be used at the point-of-care (no need for a lab)
- Still require to have their performance assessed (as of 8 April 2020, 5 viral antigen tests received a CE IVD marking).
- Need high enough presence of the surface proteins to be detectable – means they have a **higher chance of false negatives than PCR tests**
- Would need to be implemented with clear guidance on correct interpretation

Conclusions

- RT-PCR is the recommended test for diagnosis of COVID-19
  - Symptomatic and close contacts
    - *Testing, tracking and tracing*
  - Nasopharyngeal swabs
  - Semi-automated, high throughput systems, POC (1h30 to 4h)
  - Generally very sensitive and specific
    - Sensitivity depends on quality, type of samples and disease stage

Conclusions

- RT-PCR
  - Detectable as early as day 1 of symptoms
  - Peaks within the first week of symptom onset
  - Longer shedding in sputum and stools, than in nasopharyngeal swabs
  - Patients with severe disease tend to have a high viral load and a long virus-shedding period

- Antigen
  - Needs further evaluation to be implemented with clear guidance on correct interpretation

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