Towards sensitive and accurate interpretation of molecular testing for SARS-CoV-2: essential knowledge and clinical implications

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Summary

Sensitive molecular diagnostics and correct test interpretation are crucial for accurate COVID-19 diagnosis and thereby essential for good clinical practice. Furthermore, they are a key factor in outbreak phases where active case finding in combination with isolation and contact tracing are crucial for outbreak control. As more countries are on the brink to enter new phases in the outbreak response, we reviewed current published knowledge on kinetics of SARS-CoV-2 RNA molecular detection in a wide range of clinical samples of 10625 COVID-19 patients in different categories, to support clinical and public health decision making. We outline knowledge gaps and directions for future studies.

Introduction

In 2019 a novel human pathogenic coronavirus, SARS-CoV-2, emerged in Wuhan, China,[1] leading to a worldwide outbreak, declared a public health emergency of international concern on 30 January 2020[2] and a pandemic on 11 March 2020.[3]

SARS-CoV-2 is a positive-stranded RNA virus from the species Severe acute respiratory syndrome-related coronavirus, *Sarbecovirus* subgenus, genus *Betacoronavirus*, family *Coronaviridae*. The species contains a wide range of bat and human viruses including SARS-CoV that caused an outbreak in 2002/2003. The SARS-CoV-2 origins are still unknown, but zoonotic transmission, with bats (in particular *Rhinolophus* spp.) as probable primary reservoir and other animals as intermediate hosts, is considered the most likely route.[4,5]

In the context of the current pandemic, rapid and reliable laboratory diagnosis is essential for detection, confirmation, and ruling out of cases, clinical management and hospital infection prevention measures, source and contact tracing, and (lifting of) isolation measures. Laboratory testing plays a critical role in surveillance to guide public health response as a whole. Nucleic acid amplification tests became the first line of COVID-19 testing recommended by WHO.[6] Serological tests are also needed, and are yet in development.[7,8]

Here, we reviewed the current knowledge on the laboratory aspects of COVID-19 diagnostics with focus on SARS-CoV-2 molecular assays to support the laboratory response for clinical case management and to inform Public Health control.

Methods

Search strategy and selection criteria

We searched Pubmed, medRxiv and bioRxiv with keyword "coronavirus" limiting to results published December 2019 to April 2020, identified studies on SARS-CoV-2 (incl. name variations) in humans (all ages), written in English, Chinese or French, and excluded reviews, viewpoints or news. This yielded 450 publications for abstract and full text screening. Finally, we included 90 studies reporting on SARS-CoV-2 molecular detection and/or virus isolation in COVID-19 cases. We scanned literature cited in these articles for additional sources of information, and reviewed SARS-CoV-2 laboratory protocols listed on the WHO and national infectious diseases institutes websites.

We aimed to summarize the current information on SARS-CoV-2 kinetics in relation to clinical syndrome, in different bodily fluids, while also noting any specifics in vulnerable groups (pregnant women, children and immuno-compromised individuals). We compiled a dataset of 10625 COVID-19 cases (Supplementary Dataset) and additionally aggregated data from 200 adults with data points from 2506 samples with known collection day post illness onset (dpi) (Figure, Table 1 and Supplementary Table). Due to varying disease severity definitions, we opted for a simplified approach: patients with symptoms or clinical course described as mild, moderate, common, typical, are referred to as "mild"; while severe, critically ill and/or admitted to intensive-care units (ICU), as "severe". We summarised available SARS-Co-V-2 PCR protocols by country of first publication (Table 2).

SARS-CoV-2 kinetics and shedding in bodily fluids

Respiratory shedding

Key points

- SARS-CoV-2 replication in throat and diagnostic use of upper respiratory tract sampling in the early infection days (regardless of severity of symptoms)
- Viral RNA loads peak within the first infection days in the upper and later in the lower respiratory tract; need for standardisation of viral load testing and reporting
- SARS-CoV-2 RNA detectable from respiratory samples up to 6 weeks in mild and 8 weeks in severe cases, and beyond symptoms resolution
- Insufficient systematic comparisons between all respiratory samples types; higher viral loads in sputum than swabs, nasopharyngeal swabs more sensitive than oropharyngeal swabs
- CT findings could precede viral RNA detection in the upper respiratory tract

We reviewed 89 studies with respiratory sampling for 10393 COVID-19 cases, including data on nasopharyngeal/midturbinate/nasal (NP) swabs (≥5146 cases), oropharyngeal/throat (OP) swabs (≥2249), mixed NP and OP swabs (≥2), sputum (≥258), bronchoalveolar lavage fluid (≥11), and other respiratory specimens (endotracheal aspirate, bronchoalveolar swab, n=17) (Supplementary Dataset).[1,9-96]

Lower respiratory tract (LRT) bronchoalveolar lavage (BAL) sampling allowed the initial and subsequent virus culture of SARS-CoV-2.[1,44,95] Almost all BAL specimens described in peer-reviewed literature had detectable viral RNA regardless of timing of sampling, disease severity or co-morbidities and were useful for ultimate confirmation of difficult cases.[49,69,70,73,95] Virus isolation was successful from NP and OP swabs 4 dpi[33] in a mild case.[36]

In a German study of 9 mild COVID-19 cases, SARS-CoV-2 was isolated up to 8 dpi from both upper respiratory tract (URT) swabs (16%) and sputum samples (83%) with viral loads (VL) $>10^6$ copies/ml. Furthermore, the authors detected viral subgenomic messenger RNAs (sgRNA) which led them to conclude there was ongoing viral replication in the throat up to 5 dpi. Sequencing data also showed continuous presence of two genotypes of SARS-CoV-2 differing by a single mutation in the throat and lungs samples of a patient.[71]

Despite the growing amount of literature, only one study documented NP swabs, OP swabs, and sputum, collected sequentially in the same 16 patients,[39] while 2 studies described an upper respiratory specimen paired with sputum for a total of 11 cases.[41,71] Nevertheless, we aggregated the data from different observational studies with available infection timeline to provide a summary (Figure, Table 1 and Supplementary Table). SARS-CoV-2 RNA was detected in NP and OP swabs from symptoms onset up to 42 dpi in mild cases[59] and 50 dpi in severe ones.[39] Sputum yielded positive results up to 27 dpi in mild cases,[71] but 55 dpi in severely ill[39] (Figure). Several studies reported SARS-CoV-2 RNA detection from the URT for a median period of 10-20 days.[27,48,50,76,86,92,93] with a prolonged one seen in severe cases.[27,50,92] SARS-CoV-2 RNA was detectable in URT samples well beyond waning of respiratory symptoms.[17,18,43,71] SARS-CoV-2 remained detectable in OP swabs ≥2 weeks, (nine mild cases) and in sputum >3 weeks (six mild cases) despite symptoms resolution.[71] Chen and colleagues described recurring SARS-CoV-2 RNA positivity in OP swabs of a patient until 30 dpi (VL $4{\cdot}56 \times 10^2$ copies/mL), well after pneumonia resolution and hospital discharge.[18] They did not study if infectious virus could be detected. A Nanchang study (21 mild, ten severe cases) observed clearance in NP swabs within 10 dpi in 90% of the mild cases compared to continuous RNA detection >10 dpi in all severe cases.[50] Feng and colleagues detected SARS-CoV-2 RNA in NP swabs of 24 mild cases for 16±7 days

compared to 22±4 days in eight ICU patients.[27] A Wuhan study aggregating retrospective observations from 191 hospitalised adults reported a median duration of URT viral RNA detection of 20 (IQR 16-23) dpi with continuous detection until death in non-survivors and ranges 8-37 dpi in survivors.[93] Another study including 66 COVID-19 cases found a median of 9·5 (6-11) dpi until the first negative results in OP swabs.[48]

Duration of shedding may be related to patient's general health condition: in a Wuhan-based study 27 out 56 mild cases had prolonged SARS-CoV-2 RNA detection >24 days in NP/OP swabs, associated with old age and comorbidities. The proportion of positive respiratory samples decreased from 89% to 66%, 32%, 5%, and 0% in weeks 2-6 since symptoms onset.[76] Xu and colleagues summarised respiratory samples data from 113 patients and observed a median RNA detection for 17 (IQR 13-22) dpi. Prolonged detection ≥15 dpi was associated with males, old age, hypertension, severe illness upon admission, invasive mechanical ventilation, and corticosteroid treatment.[81]

In the studies that provide quantitative results (n = 19), [16,18,34,36,37,39,41,47,50,55,56,59,60,71,85-87,91,96] the highest VL in URT specimens were reported in the early days of the disease,[34,36,39,41,50,71,86,96] also before development of LRT symptoms.[34,41] A study of nine mild cases reported SARS-CoV-2 RNA detection from all NP and OP swabs in the first 5 dpi with (average VL $6{\cdot}76x10^5$ copies/swab and maximum $7{\cdot}11x10^8$ copies/swab), whereas detection rate in subsequent swabs was only 40% reaching up to 28dpi (average VL $3{\cdot}44x10^5$ copies/swab).[71] He and colleagues reported high VL for 414 OP swabs from 94 patients in the early days of infection and gradual decrease until about 21 dpi with no difference when stratified by sex, age, or disease severity.[34] However, VL in sputum of 22 mild cases reached a peak in week 2 since symptoms onset and were significantly lower than those of 74 severe cases.[92]

Comparing respiratory specimens, higher VL were reported for sputum than respiratory swabs.[16,39,55,71,87] A study using RT-PCR and droplet digital PCR found both significantly higher positive rates and average VL in sputum (66% and 17429±6920 copies/test) compared to OP swabs (37% and 2552±1965 copies/test) and NP swabs (16% and 651±501 copies/test).[87] For 16 critically ill patients sputum and endotracheal aspirate samples all had detectable SARS-CoV-2 RNA at levels significantly higher than NP and OP swabs with positivity rates of 81% and 63% respectively.[39] In nine mild cases up to 5 dpi, the maximum SARS-CoV-2 VL in sputum ($2{\cdot}35x10^9$ copies/mL) was higher compared to respiratory swabs ($7{\cdot}11x10^8$ copies/swab). However, examining paired sputum and swab samples 2-4 dpi in seven patients showed higher virus concentration in swabs (two cases),sputum (two cases), and similar virus concentrations in both for the remaining five cases.[71]

Clearly, choosing the most appropriate respiratory specimen depends on timing in the infection course. However, well-documented studies comparing all respiratory and other sample types collected in a known timeline are limited. A preprint study in Guangdong on 866 respiratory samples from 213 symptomatic cases (37 severe), showed that apart from BAL, sputum is the most sensitive sample type for COVID-19 laboratory diagnosis, followed by NP swabs.[85] In the period 0-7 dpi, the highest positivity rate was observed in sputum (severe cases 89%, mild cases 82%), followed by NP swabs (73%, 72%) and OP swabs (60%, 61%). In the period 8-14 dpi the same order in positivity rates was observed: sputum (severe cases 83%, mild cases 74%), NP swabs (72%, 54%), and OP swabs (50%, 30%), as for $\geq$ 15 dpi: sputum (61%, 43%), NP swabs (50%, 55%), and OP swabs (37%, 11%). Sputum samples 0-7 dpi also yielded the lowest median Ct values (25 in severe cases, 28·5 in mild cases). BAL samples 8-14 dpi were positive for SARS-CoV-2 RNA in 12 severe cases and negative for three mild cases. Beyond 15 dpi the study showed 79% positivity in BAL in

severe cases.[85] Further peer-reviewed data was also in favour of sputum, or alternatively NP swabs.[49,69] A retrospective study of 4880 cases in Wuhan found 38% positive rate for 4818 NP and OP swabs compared to 49% for 57 sputum specimens and 80% for 5 BAL.[49] Another Chinese study including 205 COVID-19 cases yielded overall 1070 samples with the following positivity rates: 93% for 15 BAL samples, 72% for 104 sputum specimens, 63% for 8 NP swabs, 46% for 13 fibrobronchoscope brush biopsy samples, 32% for 398 OP swabs, 29% for 153 feces samples, 1% for 107 blood samples.[69] Only BAL specimen sequencing, and BAL and sputum samples PCR could confirm SARS-CoV-2 co-infection with influenza A in an ICU patient, whereas repeated NP swabs were negative.[73] Although sputum might seem like sample of choice, it was described that only a third of 1099 COVID-19 patients had productive cough,[28] suggesting that in practice NP swabs would be preferable in most cases. A higher sensitivity for NP swabs in comparison to OP swabs was observed as well in other studies and case reports.[46,96] A study with sequential sampling in 18 patients (72 NP and 72 OP swabs) showed higher VL in the nose than the throat.[96]

SARS-CoV-2 shedding potential in asymptomatic and pre-symptomatic individuals needs to be elucidated.[34,66,97,98] Multiple studies worldwide reported SARS-CoV-2 RNA detection in respiratory samples from cases with epidemiological link and no[16,35,52,54,66,96] (6 adults, 3 children) or mild/non-specific symptoms[13,16,35,38,41,42,47,57,58,61,66,88] (16 adults). One described SARS-CoV-2 RNA detection in OP swabs for 17 days in an otherwise asymptomatic patient.[52] At this stage, it is unclear whether SARS-CoV-2 affects the upper or the lower respiratory tract first, or maintains independent replication in both sites. Thus, choosing between NP and OP swabs, or sputum as sampling strategy should be done with purpose (general population screening or confirmation of suspected cases) and potential infection timeline in mind. URT sampling would be the preferable in the early infection days,

especially in asymptomatic or mildly symptomatic suspected cases, whereas lower respiratory sampling provides more reliable confirmation in advanced COVID-19 with lungs involvement. Cases with epidemiologic link, radiologic findings, and initial negative result should be monitored further by PCR and evaluated in conjunction with their clinical presentation.[9,31,32,37,72,79,82] The discrepancy between URT and LRT test results has triggered discussion about lack of sensitivity of PCR testing. In a study among 1014 patients in Wuhan 59% (95%CI, 56%-62%) of OP swabs were positive whereas 88% (95%CI, 86%-90%) had chest CT findings within a median of one day, consistent with earlier resolution of viral replication in URT than in LRT samples. Furthermore, 308 patients (75%) had negative PCR results in conjunction with radiologic findings and 14 out 15 cases with CT findings tested positive on a follow-up PCR within a mean of 5 days, a finding that may be more difficult to explain.[9] Another study that aggregated data on PCR results and CT imaging showed all 167 patients had a positive OP swab by the end of their hospitalisation.[79] A multicentre study of 80 COVID-19 cases (no critically ill) reported the following positivity rates in repeated OP and/or NP swabs collection until confirmation: 51% upon first test, 38% on the second and 11% on the third PCR test.[72]

Gastro-intestinal shedding

Key points

- SARS-CoV-2 isolation from feces and RNA detection regardless of gastrointestinal symptoms
- Faecal sampling not recommended for diagnostic screening (unless laboratory diagnosis of suspected cases with negative respiratory tract results)

- Prolonged gastro-intestinal viral RNA detection up to three weeks, well after respiratory tract clearance and symptoms resolution in some patients

We reviewed 31 studies providing data on gastro-intestinal (GI) sampling in ≥527 COVID-19 patients, including stool specimens (≥401 cases), anal/rectal swabs (≥145), and others (endoscopic samples, n=14) (Supplementary Dataset).[15,16,23,25-28,36,39-41,45,48,53,55,56,59,64,68,69,71,77,78,80,84,86,89-92,99]

SARS-CoV-2 was isolated from stool sample 15 dpi from a COVID-19 patient with severe pneumonia[99] and from two patients without diarrhoea.[69] In a study involving nine mild cases, virus isolation was unsuccessful in stool samples 6-12 dpi from 4 patients, and no virus replication evidence was found through sgRNA assays despite detectable high VL.[71] SARS-CoV-2 nucleocapsid protein was detected in the cytoplasm of gastric, duodenal, and rectum glandular epithelial cells in one patient.[77] The gastric fluid samples of six out 13 critically ill patients were positive for SARS-CoV-2 RNA.[39]

GI disease has been described for some COVID-19 patients.[16,20,21,36,37,57,67] Though there was evidence of GI SARS-CoV-2 RNA detection, it was not necessarily in cases with GI symptoms.[78] Examining studies with available timeline of sampling (Figure, Table 1 and Supplementary Table) SARS-CoV-2 RNA detection was reported between 3 and 50 dpi in stool samples of 12 mild[36,71,86] and 11 severe cases,[39,77,86] regardless of presence of diarrhoea. Anal swabs had detectable SARS-CoV-2 RNA between 3 and 45 dpi in three mild[56,59] and seven severe cases.[23,39] No systematic comparison between viral detection in stool and anal swabs was available.

Prolonged GI SARS-CoV-2 RNA detection after resolution of respiratory symptoms and/or convalescence was observed in several studies,[25,48,71,77,89,92] though infectious virus shedding is still an outstanding question. In a German study stool samples remained RNA positive ≥21

dpi for 6 mild cases, including a patient with potential independent intestinal tract replication, suggested by the authors by comparison with the SARS-CoV-2 URT kinetics.[71] Zheng and colleagues reported a 59% positivity rate in 842 stool samples from 96 patients and a median viral RNA detection duration of 22 (IQR 17-31) dpi that was significantly longer than in sputum/saliva and serum samples.[92] In a cohort of 42 patients (11 severe cases) with GI symptoms, 28 cases (nine severe) had a median RNA detection period of 11 (IQR 7-13) dpi until first positive stool sample compared to 6·5 (IQR 3-7·25) dpi for OP swabs. More than half (n=18, 5 severe) remained stool positive for median 7 (IQR 6-10) days after negative OP swabs.[25] A total of 39 (53%) of 73 hospitalized patients had detectable SARS-CoV-2 RNA in stool and in 17 patients (20%) it remained positive after respiratory samples turned negative.[77] Another Chinese study on recovering patients (n=55) found a median of 11 (9-16) dpi until the first negative results in stool: 43 patients had a 2 (1-4) days median delay in clearance in feces compared to OP swabs, while in 12 patients both turned negative at the same time.[48]

Similar to observations for stool specimens, prolonged SARS-CoV-2 RNA detection was reported for anal swabs. Presence of SARS-CoV-2 RNA in anal swabs seemed linked to disease severity in a Guangzhou cohort (2 severe and 16 mild cases).[16] Zhang and colleagues found positive anal swabs in 4 out 16 patients upon hospitalization and 6 out 16 cases at day 5.[91] A Chinese study with discharge criteria of two consecutive negative OP swab and a negative anal swab, reported a median RNA detection duration of 12 (IQR 9-14, range 4-34) days for 24 patients.[59] SARS-CoV-2 RNA detection in anal swabs ≥17 days was observed in an asymptomatic patient.[52] Finally, SARS-CoV-2 RNA was detected in toilet hospital room sampling for one of three COVID-19 patients, who notably had no diarrhoea.[100]

Large well-documented cohort studies are needed to estimate the proportion of COVID-19 cases with continuous GI shedding and the SARS-CoV-2 VL levels over time. A 29% SARS-CoV-2 RNA positivity rate in stool samples was observed in a study aggregating data on 205 patients (unclear how many provided the analysed 153 stool samples).[69] Few studies (n=8) provided quantitative data on SARS-CoV-2 RNA detection in stool and anal swabs for 31 COVID-19 cases.[36,39,56,59,71,86,91] Furthermore, there was no significant difference between the VL in stool between 22 mild and 71 severe cases.[92] SARS-CoV-2 GI VL in adults seemed to be subjectively lower (higher Ct values) than in the respiratory tract in 22 cases, [36,39,56,71,86] and higher in two mild cases, [71] though meaningful conclusions cannot be drawn from such small sample sizes and non-systematic observations.

Viremia

Key points

- Blood sampling not recommended for initial diagnostics
- No evidence of SARS-CoV-2 isolation from blood nor blood-borne transmission
- SARS-CoV-2 RNA detection as sign of severe disease up to 4 weeks post symptoms onset and use as clinical monitoring tool

We reviewed 22 studies providing blood samples (whole blood, plasma or serum) data of ≥363 COVID-19 patients (Supplementary Dataset).[15,16,23,24,27,36,37,39-41,45,48,56,64,69,71,77,78,86,87,91,92] Systematic comparison of SARS-CoV-2 RNA detection in different types of blood samples was lacking. No virus isolation from blood samples was reported.

Summarizing data on COVID-19 patients with known infection timeline (Figure, Table 1 and Supplementary Table) SARS-CoV-2 RNA was detected 3-18 dpi in 14 patients: 18 blood

samples of ten severe cases[16,23,39,77,86] and 4 samples of four mild cases respectively.[16,41,56] SARS-CoV-2 RNA presence in blood was linked with disease severity[23] and reported in further 52 severe cases.[24,27,37,64,91,92] Additionally, viral RNA was detected in blood samples from 32 mild cases[27,37,64,91,92] and 3 samples from unspecified cases.[69] SARS-CoV-2 RNA detection in blood might be useful as laboratory sign of deterioration in severe cases. Fang and colleagues detected SARS-CoV-2 RNA in blood samples from 16 mild cases for 10±6 days and seven ICU patients for 15±6 days.[27] A Chinese study reported a median viral RNA detection duration in serum of 16 (IQR 11-21) dpi, and 27% serum positivity rates in 22 mild cases compared to 45% in 74 severe cases. Detection rates peaked in weeks 2-3 since symptoms onset in all patients with detectable SARS-CoV-2 RNA (17 severe and 3 mild cases) and dropped to 11% (n=5) for severe cases and 0 for mild cases in week 4. However, VL had no significant difference between severe and mild cases.[92]

None of nine adults diagnosed with COVID-19 using OP swabs had detectable viral RNA in blood when tested with three different kits.[78] Although SARS-CoV-2 was detected and successfully isolated from respiratory samples, all 31 serum samples from nine mild cases tested negative.[71] Finally, none of the serum samples from 14 convalescent patients (no respiratory symptoms and two consecutive negative OP swabs) were positive for SARS-CoV-2 RNA despite simultaneous detection in OP swabs and stool.[48]

Other specimens: oral fluid, tears, urine, semen

Key points

- Oral fluid as self-collectable alternative to respiratory sampling
- SARS-CoV-2 RNA detection in oral fluid up to 4 weeks
- Rare RNA detection and no SARS-CoV-2 isolation in conjunctival secretions

- Limited SARS-CoV-2 RNA detection in urine and no virus isolation
- SARS-CoV-2 RNA detection in semen

Seven studies reported on oral fluid sampling (with varying collection methods) in 234 COVID-19 cases.[11,12,27,64,65,74,92] Self-collected deep throat (posterior oropharyngeal) saliva was suggested as alternative to sputum and yielded positive PCR results in 11 out 12 hospitalized COVID-19 patients in Hong Kong, as well as three positive and two negative virus cultures.[65] Further cohort of 23 patients with 173 saliva and endotracheal aspirate samples, studied by the same group, had median VL 5·2 log10 copies/mL (IQR 4·1–7·0) at presentation. The highest saliva VL were reported in week 1 since symptoms onset (20 patients), followed by gradual decline, and prolonged RNA detection ≥20 days (seven patients).[64] A pre-print US study including 44 cases reported comparable/superior sensitivity of saliva to NP swabs and higher SARS-CoV-2 saliva VL for 38 matched samples.[74] A study in Zhejiang confirmed COVID-19 in 96 patients by testing 668 sputum and 1178 saliva samples but did not specify samples types positivity rates separately. Taken together the latter declined from 95 to 54% in weeks 1-4 since symptoms onset with a median RNA detection duration of 18 (IQR 13-29) days.[92] In 25 cases SARS-CoV-2 RNA detection in the saliva was reported for 13±5 days in mild cases and 16·5±6 days in ICU patients.[27] SARS-CoV-2 was detected in all saliva samples collected by drooling technique from 25 severe cases,[11] including two patients with same-day negative respiratory sampling in NP and bronchoalveolar swabs.[11,12]

We reviewed four studies reporting conjunctival swab sampling in 94 COVID-19 cases.[27,39,60,75] SARS-CoV-2 RNA was detected, but virus not isolated, in the tears and conjunctival secretions of one mildly symptomatic patient with conjunctivitis,[75] while samples and cultures from 46 patients without ocular symptoms were negative.[60,75] SARS-

CoV-2 RNA detection in tears was also reported for one critically ill patient[39] and in 5 cases with unspecified disease severity.[27]

We reviewed 21 studies featuring urine samples from ≥291 patients.[15,16,25-27,36,39-41,45,48,55,56,64,69,71,77,78,86,87,92] None reported SARS-CoV-2 isolation from urine. SARS-CoV-2 RNA was only detected in the urine of four patients (three patients with positive sample upon OP swab turning negative),[48] at 7 dpi in one woman with positive OP swab,[56] and in one critically ill patient with suspected systemic COVID-19 infection.[39]

SARS-CoV-2 was not detected in semen collected during the recovery of 12 cases (11 mild, 1 asymptomatic), nor in the testis samples from a deceased severe case.[62] A recent study published after our review period (not included in Supplementary Dataset or elsewhere in the review) reported SARS-CoV-2 RNA detection in semen from six (four with acute COVID-19 and two recovering) out of 38 patients.[101]

Pregnancy and infancy, childhood

We reviewed 6 studies including 28 pregnant women and their infants.[19,22,45,51,63,68] No confirmed mother-to-child transmission was reported for these babies delivered mainly via Caesarean section. A single infant, delivered via section, isolated, and formula-fed, had a positive pharyngeal swab 36 hours after delivery. Cord blood and placenta tested negative, but it was uncertain whether it was a vertical transmission or nosocomial infection.[68] Altogether SARS-CoV-2 RNA was not detected in the amniotic fluid and umbilical cord blood in seven cases,[19,45] nor in four placental tissue samples.[22,45] Sampling of vaginal mucosa has not yet been reported and it is unknown whether shedding occurs in the birth canal during vaginal delivery. It is unclear whether SARS-CoV-2 could be transmitted through breastmilk either, so far it was not detected in samples from nine mothers.[19,40,45,68]

We reviewed 15 studies describing COVID-19 in 58 children.[15,16,21,30,40,53,54,66,68,72,75,80,83,84,90] Some studies included cases <18 years old grouped with adults and/or provided incomplete stratification by age. An asymptomatic 6-month infant maintained detectable SARS-CoV-2 RNA in NP swabs until 17 days of hospitalization and had a positive stool sample on day 9.[40] A case-report documented an episode of detectable SARS-CoV-2 RNA in blood and transient fever in an otherwise asymptomatic 6-month infant.[40] Prolonged GI SARS-CoV-2 RNA detection was observed in stool samples up to 35 dpi for 17 children aged 0·25-10 years[15,53,80,90] and in rectal swabs ≥3 weeks in ten children aged 0·17-15 years.[84] Higher SARS-CoV-2 VL in stool than OP swabs were reported for >20 days in an infant with mild COVID-19.[80] Eight children had higher average VL in anal swabs compared to NP swabs.[84]

Immuno-compromised

Data on SARS-CoV-2 shedding patterns in immuno-compromised individuals is lacking. A single case of co-infection in a person living with HIV was reported.[94] Evidence of endothelial cell infection and endothelitis was observed in 3 severe COVID-19 cases, incl. a renal transplant recipient.[102]

Discussion and knowledge gaps

Sensitive molecular diagnostics through PCR and correct test interpretation are crucial for accurate COVID-19 diagnosis and thereby essential for good clinical practice. Furthermore, they are a key factor in outbreak phases where active case finding in combination with isolation and contact tracing are crucial for outbreak control. We reviewed current knowledge on kinetics of SARS-CoV-2 RNA shedding in different clinical samples of 10625 COVID-19 patients to inform clinical and public health decision making.

Respiratory tract specimens are the samples of choice for wide-spread screening and clinical course monitoring purposes, as well as for discharge and de-isolation.[103,104] Further reports with well-documented sampling timepoints, comparing the different types of respiratory samples and their diagnostic window of use, are needed. Viral RNA concentrations in the URT peak in the early infection days, including in asymptomatic and mildly symptomatic cases. SARS-CoV-2 was successfully isolated from respiratory samples with data suggesting independent replication potential in both the upper and lower respiratory tract. As for convalescence, SARS-CoV-2 RNA detection in the respiratory tract can be prolonged. However, detection of viral genomes does not directly imply presence of infectious virus and thereby infectivity. More information on the presence of viable SARS-CoV-2 infectivity is urgently needed, especially with de-isolation strategies in mind.

Although faecal-oral transmission has not been confirmed, it cannot be firmly excluded due to evidence of SARS-CoV-2 isolation from the GI tract.[77,99] The detection of viable SARS-CoV-2 in faecal samples also has implications for the diagnostic possibilities of faeces tests. Laboratory diagnostic and safety protocols might need adjustments, for example by implementing inactivation steps and flow cabinet work. Stool and anal swabs are not samples of choice for screening purposes, though they might play a role in clinical monitoring. Some data suggested a shift from respiratory to gastrointestinal shedding in the course of COVID-19 infection or independent replication in the GI tract. More information is needed on the significance of SARS-CoV-2 detection in faecal samples and how it relates with the timing of convalescence, as well as to the viral shedding specifics in children.

SARS-CoV-2 RNAemia has been detected mainly in severe cases and blood sampling could be useful in monitoring hospitalized patients. At this stage there is no evidence to suggest SARS-CoV-2 could be transmitted through blood. More systematic data is needed to guide

blood and organ transplantation safety protocols.[105] Data on SARS-CoV-2 kinetics in vulnerable groups like immunocompromised patients is insufficient.

In conclusion, using SARS-CoV-2 molecular testing by amplification techniques to the maximum of its potential is a combination of choosing the most appropriate sample type, collected with adequate sampling technique, and with the infection timeline in mind. Further well-documented systematic studies are needed to characterise fully the COVID-19 clinical course and guide public health decisions concerning optimal testing strategies.

Contributors

KRS searched, compiled and organised the information, and wrote the manuscript drafts. AAE, LMK, AM, MPGK and CBEMR reviewed the evidence and agreed on the conclusions. KRS and CBEMR finalised the manuscript text. All authors reviewed and contributed to the final manuscript.

Declaration of interests

We declare no competing interests.

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Figure captions

Figure. Aggregated RT-PCR results in different sample types (n=2506) by days post illness onset in patients (n=200) with mild or severe COVID-19 symptoms.

Number of samples with and without detectable SARS-CoV-2 RNA are aggregated by sample type, collection day post illness onset and disease severity. Sampling for patients with symptoms or clinical course described in the original publications as mild, moderate, common or typical are shown in circles: total of 1412 samples from 159 adults. Sampling for severe or critically ill and/or admitted to the ICU are shown in squares: total of 1094 samples from 41 adults. Asymptomatic patients were excluded. Sample types are as follows: NP swab (nasopharyngeal/midturbinate/nasal swab), OP swab (oropharyngeal/throat swab), NP+OP swab (both swabs collected in one tube or results published aggregated), sputum (induced/spontaneous sputum), stool, anal swab (anal/rectal swab), blood (serum, plasma, whole blood or not specified), urine.

Tables

Table 1. Aggregated RT-PCR results in different sample types (n=2506) in patients (n=200) with mild or severe COVID-19 symptoms

	Positive results			Negative results		
Sample type	**Samples, n**	**Mild cases, n**	**Severe cases, n**	**Samples, n**	**Mild cases, n**	**Severe cases, n**
NP swab	383	52	22	193	36	23
OP swab	129	26	17	139	21	22
NP+OP swab*	197	67	1	296	67	0
All URT swabs	**709**	**132**	**27**	**628**	**114**	**28**
Sputum	374	35	24	131	12	17
Stool	163	12	11	100	16	17
Anal swab	19	3	7	65	8	17
All GIT samples	**182**	**15**	**14**	**165**	**24**	**21**
Blood	22	4	10	200	21	22
Urine	3	1	2	92	23	19

NP= nasopharyngeal/midturbinate/nasal. GIT=gastro-intestinal tract. OP= oropharyngeal/throat. URT=upper respiratory tract.
*Both swabs collected in one tube or results published aggregated

Table 2. SARS-CoV-2 RNA detection by PCR (adapted from the WHO COVID-19 Laboratory guidance [6])

Country	Institute	Date published or updated	Gene targets	Reference
China*	China CDC	24 Jan 2020 20 Feb 2020 28 Feb 2020	ORF1ab, N	28,95,106
China	CAMS & PUMC	24 Jan 2020	E	37
China	HKU-Shenzhen Hospital	24 Jan 2020	RdRP, S	16
China	WIV, CAS	3 Feb 2020	S	107
China	Wuhan University	12 Feb 2020	S	108
France*	Institut Pasteur	2 Mar 2020	RdRP, E	106
Germany*	Charité	17 Jan 2020 23 Jan 2020	E, RdRP	106,109
Hong Kong*	HKU	23 Jan 2020 31 Jan 2020	ORF1b, N	106,110
Japan*	NIID	24 Jan 2020	ORF1a, S, N	106
Singapore	NCID	28 Feb 2020 3 Mar 2020	N, ORF1ab, S	40,86
Thailand*	MOPH	23 Jan 2020	N	106
USA*	US CDC	25 Jan 2020 15 Mar 2020	N	106,111

* Shared via the WHO website

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