Viral dynamics in the Upper Respiratory Tract (URT) of SARS-CoV-2

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SUMMARY

To date, research on viral shedding (VS), live virus isolation and infection status remains ongoing as scientists and clinicians attempt to better understand the coronavirus disease of 2019 (COVID-19) pandemic. Viral RNA detection at different stages of the disease, quantitative changes and patterns of viral persistence and clearance all provide context for the pathogenesis and transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Given the highly infectious nature of SARS-CoV-2 and its impact on the global population and economy, clinicians continue to seek the best methods for controlling its spread, and data on public health preventative measures continue to emerge. In this paper we review the available evidence on the viral dynamics of SARS-CoV-2 in the URT to determine a timeline for infection based on molecular and viral culture findings and to assess the significance of persistently positive results.

Keywords: viral shedding, viral load, viral culture, SARS-CoV-2, upper respiratory tract.

INTRODUCTION

Since its origins in Wuhan, China in December 2019, the COVID-19 pandemic continues to claim a devastatingly high human toll with over 25 million cases and >800,000 deaths globally to date (September 2020) [1]. The fear of an anticipated second wave in the autumn-winter of 2020 is making the whole planet unsettled [2]. Known to primarily target the respiratory system, SARS CoV-2 is now widely acknowledged for its ability to affect multiple organ systems in adults, children and adolescents [3, 4]. The spectrum of disease severity varies from asymptomatic infection to mild upper respiratory tract illness, severe viral pneumonia with respiratory failure, Acute Respiratory Distress Syndrome (ARDS) and death [3]. In some extreme cases, lung transplantation has been pursued as a last resort [5]. Given SARS-CoV-2's rapid spread, a comprehensive examination of the available evidence on viral dynamics in the upper respiratory tract (URT) and duration of infectiousness that could effectively inform public health interventions of infection prevention, contact tracing, isolation and quarantine, is vital and timely.

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MATERIALS AND METHODS

We conducted a systematic search of PubMed, Science direct and Google scholar for articles reporting on viral shedding, viable virus isolation and culture of SARS-CoV-2 in the URT. We then extracted and synthesized data on duration of viral shedding in the URT. Specimens focused on included nasopharyngeal (NP), nasal, oropharyngeal (OP), throat and salivary samples.

Our search included the following terms within the title, abstract and author keywords: "viral dynamics" "SARS-CoV-2 in the upper respiratory tract", "viral load of SARS-CoV-2", "viral RNA shedding of SARS-CoV-2", "nasopharyngeal samples in COVID-19", "oropharyngeal samples in COVID-19"," throat swabs in COVID-19", "saliva samples in COVID-19", "virus culture and RT-PCR for SARS-CoV-2", "culture of live SARS-CoV-2".

We had no start date or country requirements and limited our search to papers published in English, with one paper translated from Chinese using Google translate. Our search included case reports, case series, original studies and letters of correspondence reporting small clinical studies (Table 1).

Viral dynamics in the Upper respiratory tract (URT) – Evidence so far

SARS-CoV-2, the causative agent of the COVID-19 pandemic, is a betacoronavirus, a positive single-stranded RNA virus which anchors to angiotensin-converting enzyme 2 (ACE2) receptor as its main portal of entry [6, 7]. ACE2 receptors are broadly expressed in respiratory epithelium, alveolar monocytes and macrophages [7]. The ACE2 receptor and the TMPRSS2 activating protease are prevalent in goblet and ciliated cells lining the nasal epithelium of the upper respiratory tract (URT). Hence, the URT serves as a portal of entry, colonization and active replication for SARS-CoV-2 [7]. Of all the cells in the upper airways, these notably possessed the highest levels of SARS-CoV-2 viral proteins in infected individuals [7, 8].

Studies of SARS-CoV-2 infection in the URT describe changes in viral behavior, using viral load (VL) as an indicator of infection status (Table 1). VL as quantified by cycle threshold (Ct) value, is the number of cycles required to reach a threshold of detection. Ct provides cut-off values for positive and negative samples and context for disease severity, with greater Ct values indicating weakly positive samples. Patterns of viral shedding (VS) can be followed to determine symptom onset, duration, transmission potential, viral clearance and recovery.

In general, viral RNA is detectable 2-3 days prior to symptom onset, peaks with the start of symptoms, usually in the first week, and then trends down over the following days to weeks [8-12]. During periods of overt symptoms, an individual is known to be infectious. However, high viral titers in the absence of symptoms are now known to reflect active viral replication and these individuals can actively transmit the virus [13-17]. Pre-symptomatic transmission from the URT and infection following exposure to contacts with mild prodromal symptoms have been confirmed by findings of a nonsynonymous nucleotide polymorphism between infectees in at least one setting [13]. Alternative modes of asymptomatic transmission may also exist. An interesting case of an asymptomatic 8-year-old

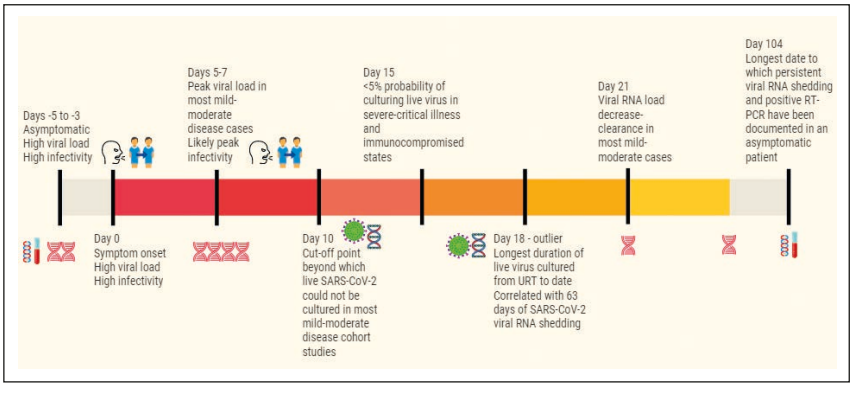

Figure 1 - Timeline of reported symptoms, viral RNA changes and live viral cultures [8-17, 23, 28, 30, 37, 38].

Table 1 - Summary of salient clinical details from studies on SARS-CoV-2 viral RNA and viral culture.

Citation	*Sample size (no.)*	*Gender distribution*	*Country*	*Population*	*Specimen type*	*Disease severity*	*Longest duration of VS via RT-PCR/days*	*Live virus cultures*
Wölfel R et al. [8]	9	N/S	Germany	Contacts of index case	Pharyngeal swabs	All mild-moderate	28	Virus cultured up to day 8
To KKW et al. [9]	23	Female - 10 Male - 13	Hong Kong	Hospitalized patients	Posterior OP saliva	Mild (13); Severe (10)	25	Attempted but results not reported
Zou L et al. [10]	18	Female - 9 Male - 9	China	Visitors to Wuhan, infectees within family clusters	Nasal and throat swabs	Asymptomatic (1); Mild to moderate (14); Severe (3)	21	Attempts not reported
Kujawski S et al. (COVID Investigating Team) [11]	12	Female - 4 Male - 8	USA	Visitors to Wuhan and their contacts	NP, OP swabs, Sputum	Mild (5); Moderate (7)	32 - NP; 36 - OP; 29 - sputum	Live virus cultured on days 1-5,8,9
He X et al. [12]	94	Female - 47 Male - 47	China	Hospitalized population	Throat swabs	Mild (27); Mild to severe (5); Moderate (47); Moderate to severe (14)	Approximately 21	Attempts not reported
Boehmer et al. [13]	16	Female - 4 Male - 12	Bavaria	Epidemiologically linked cluster	NP, OP swabs	Mostly mild; 2 developed signs of pneumonia	N/S	Attempts not reported
Arons et al. [15]	57	N/S	USA	Skilled nursing facility residents	NP, OP swabs	11 hospitalized in ICU; 15 died	N/S	Virus cultured 6 days before symptom onset to 9 days after
Rothe C et al. [16]	1	1 - male, others N/S	Germany	Exposure to index case	NP swabs, sputum	Mild	N/S	Attempts not reported
Jiang X et al. [18]	1	Female	China	Symptomatic admission	NP swabs, anal swabs	Asymptomatic	0 on NP (URT) swab, positive for 42 days on anal swab	Attempts not reported
Kim JY, Ko JH et al. [19]	2	Patient 1: Female Patient 2: Male	Korea	Patient 1: 35-year-old Chinese Patient 2: 55-year-old Korean	NP, OP samples, sputum	Patient 1: Oxygen therapy, ground glass opacities on CT Patient 2: - Mild symptoms	Patient 1: Day 14; Patient 2: Day 25	Attempted but unsuccessful
Kim ES et al. [20]	28	Female - 13 Male - 15	Korea	Hospitalized population	Respiratory samples - N/S; likely NP, OP or sputum	Mild/ moderate (22); Severe (6)	20; 18.5 days to off-isolation	Attempts not reported

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Citation	*Sample size (no.)*	*Gender distribution*	*Country*	*Population*	*Specimen type*	*Disease severity*	*Longest duration of VS via RT-PCR/days*	*Live virus cultures*
Lui Y et al. [21]	76	Female - 28 Male - 48	China	Hospitalized population	NP swabs	Mild (46); Severe (30); ICU treatment in severe group - 23/30	>10 in severe cases	Attempts not reported
Lui G et al. [22]	11	Female - 4 Male - 7	Hong Kong	Hospitalized population	NP and throat swabs, sputum and tracheal aspirate	Mild/ moderate (6); Severe/critical (5)	>14; up to day 29 in a critical and moderate patient	Attempts not reported
Xiao AT et al. [23]	56	Female - 22 Male - 34	China	Hospitalized population	NP swabs	Mild - moderate	All patients tested negative by week 6, about 42 days	Attempts not reported
Xu T et al. [24]	51	Female - not listed Male - 10 (imported), 7 (secondary), 8 (tertiary)	China	Laboratory confirmed patients	Throat swabs	Mild - moderate	Imported - >14; Secondary - >14; Tertiary - 14	Attempts not reported
Zhou F et al. [25]	191	Female - 72 Male - 119	China	Hospitalized population	Throat swabs	General (72); Severe (66); Critical (53)	37 - severe disease	Attempts not reported
Fang Z et al. [26]	32	Female - 16 Male - 16	China	Hospitalized population	Nasal swabs, saliva	ICU (8); Non-ICU (24)	Nasal swabs: ICU - 22.25 +/- 3.62; Non-ICU - 15.67 +/- 6.68	Attempts not reported
Miyamae et al. [27]	23	Female - 13 Male - 10	Japan	Cruise ship travellers	OP and nasal swabs	Asymptomatic and mild disease	37 - mild disease	Attempts not reported
Lui WD et al. [28]	1	Female	China	Symptomatic patient admission	Throat gargles	Mild	63 - mild disease	Cells cultured up to day 18
Molina et al. [30]	1	Female	USA	Asymptomatic pregnant female	NP swab	Asymptomatic for duration of pregnancy and testing	34 days postpartum; 104 days from initial positive test	Attempts not reported
Yu F et al. [31]	76	Female - 50%; Male - 50%	China	Laboratory confirmed patients	Throat swabs, nasal swabs, sputum	Mild - 77.6% ; Severe - 22.4%	N/S	Attempts not reported
Zheng S et al. [32]	96	Female - 38 Male - 58	China	Hospital clinical database	Saliva	Mild (22); Severe (74)	Mild - 21; Severe - 30	Attempts not reported
Bunyavanich et al. [33]	305	Female - 49.2%; Male - 48.9%	USA	Asthma study cohort	Nasal epithelium via cytology brushing	N/S	N/S	Attempts not reported

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Citation	Sample size (no.)	Gender distribution	Country	Population	Specimen type	Disease severity	Longest duration of VS via RT-PCR/days	Live virus cultures
Wang W et al. [36]	205	Female - 32%; Male - 68%	China	Hospital clinical database	Pharyngeal and nasal swabs, BAL and fibrobroncho-scopic biopsies	19% - severe illness	N/S	Live virus detected in respiratory tract samples (7), feces (5) and blood (2)
Bullard et al. [37]	90	Female - 51%; Male - 49%	Canada	Public health database	NP, ETT, OP samples	Not detailed through case data	N/S	Live virus detected up to day 8
van Kampen JJA et al. [38]	129	Female - 43 Male - 86	The Netherlands	Hospital clinical database	URT swabs - N/S, likely NP and OP	Medium care - Intensive care (89)	20	Virus cultured up to 17.2 days post-symptom onset
Harcourt J et al. [39]	1	N/S	USA	Traveller from Wuhan	NP, OP samples	N/S	N/S	Yes; CPE 2 days post-inoculation
Matsuyama S et al. [40]	7	N/S	Japan	Laboratory confirmed cases	Throat swabs and sputum	N/S	N/S	CPE at 2-3 days post inoculation in Vero E6/ TMPRSS2 cells from 5/7 specimens
Park WB et al. [42]	1	Female	Korea	Symptomatic visitor from Wuhan	OP swab	N/S; possibly moderate-severe	N/S	CPE seen 3 days after culturing blind OP culture supernatant
La Scola B et al. [43]	155	N/S	France	Hospital clinical database	NP swabs	N/S	20	Virus cultured until day 8; CPE seen from 24-96 hours post-inoculation
To KKW et al. [44]	12	Female - 5 Male - 7	Hong Kong	Hospitalized population	Serial saliva samples	N/S	11	Viral cultures were positive for 3 patients and negative for 2 at the time of writing
Calderaro et al. [47]	1	Male	Italy	Symptomatic baby, 7 months old	Nasal and pharyngeal swabs; NP aspirate	Mild	17	Grown on day 10-post-symptom onset
Perera APM et al. [48]	35	N/S	Hong Kong	Laboratory confirmed cases	Throat, NP, saliva, sputum	Asymptomatic (3); Mild (29); Critical (2); Died (1)	>50	Detected up to day 8

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Citation	*Sample size (no.)*	*Gender distribution*	*Country*	*Population*	*Specimen type*	*Disease severity*	*Longest duration of VS via RT-PCR/days*	*Live virus cultures*
Young BE et al. [50]	18	Female - 9 Male - 9	Singapore	Hospitalized patients and travelers to Wuhan	NP; ETT for 2 patients admitted to ICU	Mild-moderate; supplemental Oxygen (6), ICU (2), mechanical ventilation (1)	24 - Median duration of VS from 1st to last positive NP swab was 12 days (range, 1-24)	Viral culture results not available at the time of writing
Yang JR et al. [67]	1	Male	China	Symptomatic patient	Throat swab, saliva	N/S; possibly moderate-severe	>40 days; prolonged positivity after testing negative	Attempts not reported

Abbreviations: NP – Nasopharyngeal samples, OP – Oropharyngeal, ETT - Endotracheal tube aspirate, BAL - bronchoalveolar lavage, ICU – Intensive care unit, VS – viral shedding, CPE – Cytopathic effects, I/C – immunocompromised, I/S – immunosuppressed, N/S – Not specified

girl, with no URT symptoms who repeatedly tested negative by NP swab, but persistently positive by anal swabs for 42 days raises questions about transmission and detection which remain to be answered [18].

Within the URT, RT-PCR results vary with disease severity and progression. In mild disease with predominantly URT symptoms, the VL has been found to be both higher and lower than samples from the lower respiratory (LRT) [8, 10, 19]. Mild to moderate cases have demonstrated peak VL in the 1st week of illness, with prodromal symptoms associated with high VS in the URT until day 5. In contrast, VL has been found to be 60 times higher in severe disease, suggesting an association between higher VL, greater, prolonged infectivity and poorer clinical outcomes [20, 21]. A delayed VS peak in the 2nd week in severe disease compared to mild disease which has VL peaks in the 1st week of illness has been noted [21, 22].

Though VL decreases by the 3rd week post-symptom onset, prolonged VS can occur in all disease severities [23]. High VS and short incubation period were consistent with early transmission, secondary generation transmission and attack rate within family clusters and in imported cases [12, 24]. Third generation infectees showed a longer incubation period with an undetectable VL up to day 7 compared to imported and second-generation groups, suggesting a possible lower potency infection in those with a more protracted infection exposure history [24].

As mentioned, prolonged VS in the URT has been demonstrated in both mild and severe cases [25-29]. Notably, durations post-symptom onset of 25, 37, 63, 83 and 104 days have been reported, with 104 days as the longest duration thus far in a survivor [9, 27-30]. In severe disease, Zheng et al. found that VL peaked in the third week, versus the first week in mild disease. Older and male patients showed longer VS duration [31]. VS in nasal swabs was significantly longer than in blood or saliva, and higher VL have been found in the progressive versus recovery stage of disease [26, 32]. Here, it has been detected in higher concentrations in sputum than nasal samples, though unmatched samples and the lack of sensitivity of the Ct value to reflect low viral loads (Ct values between 34 and 38) has limited this claim [32]. With changes in disease severity from mild to severe, VL in the URT can undergo changes. A decrease in the URT and tandem increase in the LRT reflects both anatomical transition and a change in disease severity [8]. However, where VL decreases in both the URT and LRT, this can be confusing to clinicians, as patients may appear to show improvements in URT symptoms before more severe LRT ones develop [19]. Liu et al. noted that URT samples were more sensitive for virus detection early in the disease course when a patient was febrile compared to later stages when a patient is afebrile. In early disease however, LRT samples were barely available and throat wash gargles could be used as an alternative for diagnosis [28].

Patients with moderate disease have shown a similar initial pattern of viremia to those with

mild symptoms. In this subset, higher VL were found within the LRT versus the URT once the disease had progressed to the LRT. This change appeared to be driven by both VS and independent viral replication. Wölfel et al. demonstrated this through isolation of 2 distinct genomes from the throat and lung in an infected patient, indicating independent replication between the two sites [8].

Factors with immunomodulating impact can affect VL and VS patterns. Older age has been correlated with higher VL, while immunosenescence and immunocompromise have been implicated as obstacles to mounting a full immune response, leading to a longer asymptomatic incubation period in older patients [9]. Co-morbidities have also been linked to poorer outcomes. In patients with mild illness with prolonged VS, older age, diabetes mellitus and hypertension were more prevalent [9, 15, 24-26]. In severe and critical disease, VL remained consistently high over the disease course [21, 26]. In patients who died, VL remained high into death [25]. Poor prognosis for in-hospital patients has been associated with older age, high SOFA score and d-dimer >1 kg/ml, while in the absence of immunomodulating factors, VL shows a pattern of increase and positive RT-PCR results up to the 3rd week of infection, with decreasing VL and negative results in the 3rd to 6th week [25, 13, 23].

Overall, the number of cases has been largely skewed towards adults, and the higher concentration of ACE-2 receptors in adult airways has been implicated [33,34]. However, Heald-Sargeant et al. have demonstrated higher VL in the nasopharynx of children 5 and under, suggesting that young children may be drivers of infection. The authors did not correlate high VL with live viral cultures at the time of writing this paper and did not confirm that high VL was linked to greater infectiousness. [35]

Though we have chosen to focus on URT findings, studies included here have also concurrently sampled the lower respiratory tract (LRT) (sputum), gastrointestinal (GI) tract (urine, stool, anal/rectal swabs) and blood [36]. We have also not specifically looked at how the VL is impacted by treatment, as this area deserves a more thorough review than we chose to focus on in this paper, neither did we take an in-depth review of the antibody response for the same reason.

Culturing SARS-CoV-2

Though viral RNA is used as a marker of viral replication, viral cultures are considered more specific and reliable indicators of live virus, with the ability to cause infection [36-38]. To this end, the ability to culture live SARS-CoV-2 is vital to understanding its transmission and infection patterns. A multitude of cell lines have been used to culture SARS-CoV-2, with VeroE6/TMPRSS2 cells showing the most replication and syncytium formation *in vivo* and *in vitro* [39, 40].

Like VL, variations in viral culture patterns have been found. For instance, the China Novel Coronavirus Investigating and Research Team found that SARS-CoV-2 was visible and demonstrated cytopathic effects (CPEs) 96 hours after inoculation on surface layers of human airway epithelial cells, while Harcourt et al. noted CPEs at 60 hours post-inoculation with a peak at 72 hours [41, 39]. Researchers from the first recorded patient case in Korea cultured SARS-CoV-2 on day 3 of the patient's illness from a blind oropharyngeal (OP) sample. Genome sequencing found it to be comparable to the original Wuhan SARS-CoV-2 genome [42].

Wölfel et al, La Scola et al. and the COVID-19 Investigation Team which reported on the first 12 American patients infected with SARS-CoV-2, also reported culturing live virus in under 10 days from NP or OP samples [8, 11, 43]. Bullard et al. correlated positive cultures with Ct values to day 8 post-symptom onset, with the probability of a positive culture peaking on day 3 and declining thereafter, while van Kampen et al. demonstrated that after day 15, the probability of culturing live virus in severe-critical and immunocompromised patients was <5% (Figure 1; Table 1) [37, 38]. One exception to these findings was a single case report by Liu et al. which reported live virus cultures up to 18 days post-symptom onset (Figure 1) [28].

Live virus has also been cultured from saliva samples. To et al. found that in 3 patients, live virus was cultured 2 days after hospitalization, indicating that saliva may act as a viral conduit from infector to infectee [44]. Serial saliva VL monitoring showed a declining trend with 11 days as the longest duration for positive RNA detection [44]. ACE2 receptor expression in the oral mucosa, particularly epithelial cells of the tongue, oral and gingival mucosa further supported salivary transmission, and studies utilizing salivary sam-

ples have successfully quantified viral RNA with RT-PCR testing [28, 44-46].

Relationship between viable virus and PCR measurements over the timeline from onset of symptoms

It is now known that prolonged VS does not always indicate live virus and therefore, infectiousness. Hence, to better understand the infectivity pattern of SARS-CoV-2, studies have assessed viable virus culture together with RT-PCR measurements (Table 1). Calderaro et al., who demonstrated viral cultures on day 10 post-symptom onset from a 7-week-old baby, highlighted the importance of viral culture in conjunction with RT-PCR to identify SARS-CoV-2. They note that in mild cases, it is the "only reference laboratory method able to reveal the presence of cytopathogenic viral agents and demonstrate their infectivity in cases of emerging viruses" [47].

Across studies, however, diverse relationships between viral culture and RT-PCR results have been obtained. For instance, some studies report the ability to culture live virus while others have not, despite ongoing detection of SARS-CoV-2 viral RNA [19]. Disease symptoms have also shown heterogenous alignment with both culture and RT-PCR results. For example, Kim et al. report ongoing RT-PCR positivity in patients with decreasing VL despite disease progression from URT to LRT symptoms, yet were unable to culture live virus on multiple attempts [19].

However, the absence of live virus in the presence of persistent viral RNA cannot definitively rule out infectivity. In light of this, the detection of subgenomic RNA (sgRNA) has been considered. sgRNA is a replicative viral intermediate that suggests that an active process is occurring where live virus has been cultured. Varied results linking the two have been obtained [8, 38, 48, 49]. Perera et al. found that both viral culture and sgRNA were rarely detectable beyond 8 days post-symptom onset but viral RNA was detected by RT-PCR for weeks post-illness. This correlated with viral RNA >6 $\log_{10}$ virus N gene copies/mL of clinical specimen [48]. It was found in 18 out of 22 specimens collected 8 days or fewer post-symptom onset while only in 1 sample 9 days post-disease onset, suggesting that infectiousness decreased over time as reflected by a decreased ability to culture virus [48]. Hamster studies demonstrated positive viral cultures on day 1 of inoculation, suggesting efficient transmission, but tested negatively on day 6 despite high correlating RT-PCR VL values [48]. Wölfel et al. detected sgRNA up to day 5 from throat samples demonstrating active viral tropism, but live virus was only cultured to day 8 despite high ongoing VL [8]. van Kampen et al. also found that sgRNA and Ct values correlated well, but noted that sgRNA did not improve the ability of the Ct value to predict culture positivity. sgRNA correlated poorly with live virus isolates and was detected even when cultures turned negative, suggesting that active viral replication could occur even when VS had stopped [38].

In the absence of live virus, viral transmission cannot be ruled out as the presence of live virus from URT samples has not yet been linked to infection transmission. Due to cost and limitations with laboratory equipment, viral cultures are not attempted by all studies. However, in correlating Ct values and live viral cultures, Bullard et al. detected no growth in samples with a Ct >24 or symptom onset to test (STT) >8 days [37]. By and large, high copy numbers and low Ct values are indicative of high VL and correlated with positive viral cultures. [21, 36, 37, 43, 50] La Scola et al. similarly demonstrated the ability to culture virus at Ct values 13-17 with a decrease in culture positivity with increasing Ct values, and no virus cultured at Ct values ≥34. Though PCR values continued to demonstrate viral RNA to day 20, live virus could not be cultured beyond day 8 [43].

Real time RT-PCR (rRT-PCR) has been attempted to hone sensitivity in virus detection. Zhu et al. demonstrated positive results on rRT-PCR assay together with virus isolation using human airway epithelial cells, Vero E6 and Huh-7 cell lines and Kujawski et al. successfully cultured SARS-CoV-2, and correlated virus isolates with VL via rRT-PCR [41, 11].

While most studies have demonstrated virus culture isolation under 10 days, Liu et al. found prolonged VS up to 63 days, and cultured SARS-CoV-2 up to day 18 in a patient with mild disease (Figure 1), suggesting contagiousness to at least 1-week post- "clinical recovery". [28] Arons et al. demonstrated viable virus 6 days pre-symptom onset to 9 days post-symptom onset in the presence of high VL in asymptomatic patients, with almost half of the patients testing positive being asymptomatic for COVID-19 [15].

Conundrum of persistently positive testing

It is not definitively known whether people who have clinically recovered from COVID-19 but have persistent VS are infectious. However, as of August 16th, the CDC's latest update states that a person who has recovered from COVID-19 may have low levels of virus in the body for up to 3 months after diagnosis [51].

In an immunocompetent host, after viral infection, antibodies formed against the virus become detectable and increase over time. These antibodies then prevent viral infection in *in vitro* studies. However, there is no conclusive evidence that antibodies developed after SARS-CoV-2 infection are protective. If such antibodies are protective, the antibody titers associated with protection from reinfection are not known [28, 52-55]. Recent studies have also shown that the antibody response can decrease 1-3 months after acute infection [56,57]. Additionally, the magnitude, persistence and durability of the immune response may vary from one person to another, with various factors (*e.g.*, age) influencing protection [9, 55, 58, 59]. Furthermore, true neutralizing antibodies to SARS-CoV-2 are generated

Table 2 - Clinical details of cases with confirmed re-test positives by RT-PCR.

Citation	*Country*	*Population*	*Clinical details*
Gousseff et al. [55]	France	COCLICO (COllaborative CLInician COVID-19) French study group meeting database	- 11 patients with re-test positive results - Median duration of symptoms was 18 [13-41] days for the first episode and 10 [7-29] days for the second for the 7 patients who eventually recovered - 2/11 patients died of ARDS recurrence and 1/11 of exacerbation of chronic right heart failure
Ye et al. [64]	China	Laboratory-confirmed COVID-19 patients	- 55 patients; 5/55 showed reactivation 4-17 days after initially testing negative for SARS-CoV-2 - No patients developed severe disease or died - 1/5 reactivated patients had a history of tuberculosis (2009)
Ravioli et al. [65]	Switzerland	2 cases: - 81-year-old female transfer from a Psychiatric institution - 77-year old hospital transfer	- Patient retested positive following negative test and symptom resolution - Approximately 5 weeks transpired between 1st and 2nd positive new test - The patient eventually died - Positive retest following serial negative testing and discharge from hospital - The authors believe that these cases were due to SARS-CoV-2 reactivation since cases in their region were low - Approximately 4 weeks transpired between 1st and 2nd new positive test
Loconsole et al. [66]	Italy	48-year-old male, symptomatic admission	- Patient re-presented with new onset symptoms following symptom resolution and negative test - Approximately 6 weeks transpired between 1st and 2nd new positive test - The patient was treated and recovered; his case was deemed a "re-activation of COVID-19 in an apparently cured patient in Italy"; episodes appear to have occurred 15 days apart
To KKW et al. [68]	China	33-year-old male symptomatic admission	- 142 days transpired between 1st and 2nd positive tests - Genome sequence comparisons between patient's initial and second infections showed them to be due to 2 different strains
Lu et al. [70]	China	Previously hospitalized and recovered cohort who tested re-positive in social isolation	- 87/169 patients who re-tested positive following discharge - Re-test positives appear to have occurred over a 1-month period following discharge - 59/87 showed neutralizing antibodies. No live virus was detected - 77/87 were asymptomatic on discharge and had previously tested negatively, while 10/77 had a nocturnal dry cough

against the spike protein. However, a lack of replication fidelity can lead to mutations in the spike protein and the production of antibody-resistant SARS-CoV-2 variants. Hence, neutralizing antibodies to one spike protein are unlikely to convey protection against others [60, 61].

In both hospitalized and non-hospitalized patients who have clinically recovered and tested negative, "rebound" positivity and "re-positive" test results are both confusing and concerning to clinicians in the absence of infection exposure (Table 2) [9, 22, 55, 58]. Experience with other viruses suggests it is unlikely that such individuals are infectious [62,63]. Usually the amount of detectable virus decreases over time. This decrease is associated with a decreased ability to recover replication competent virus after symptom onset [8, 10, 37, 43, 39, 51].

For patients with mild to moderate COVID-19, most studies report replication-competent virus detection up to 10 days after symptom onset. [8, 15, 37, 43]. In severe COVID-19 and immunocompromised patients, recovery of replication-competent virus between 10 and 20 days after symptom onset has been documented [38]. However, exceptions exist. One case report from Taiwan cites VS up to 63 days post-symptom onset and viral culture up to day 18 in a patient with a mild febrile illness. [28] Though Li et al. do not specify if this is found in the URT, they document VS lasting 83 days post-symptom onset [29]. Finally, the longest VS period we found was 104 days in a post-partum patient who acquired the disease during her pregnancy, and continued to shed virus post-partum. She remained asymptomatic for the entirety of her pregnancy post-detection [30].

"Rebound" positivity or obtaining positive results after testing negative is a growing phenomenon [9, 22]. Originally attributed to the intermittent VS of non-viable virus and viral debris, this rebound phenomenon also suggests that low levels of virus, below the RT-PCR detection threshold may continue to be secreted despite symptom resolution [13]. Delayed VS as seen in SARS, where low virus concentration in the URT led to initial false negative testing despite symptoms and a short incubation period which hindered detection, were thought to contribute [13].

Testing and processing factors can influence results and create false positives. For instance, Ct values are not a measure of viral burden, are not standardized by PCR platform, and have not been approved by the U.S. Food and Drug Administration for use in clinical management [49]. Therefore, although attempts to culture virus from URT specimens have been somewhat successful when Ct values are in high but detectable ranges, Ct values are not recommended by the Center for Diseases Prevention and Control (CDC) as a way to assess when a person is no longer infectious [49, 51]. Hence, false negatives in the face of low-grade persistent VS can go undetected. During specimen collection, the freezing and thawing of samples has also been found to alter results [45]. Yu et al. have appeared to mitigate against this with the use of digital drop PCR (ddPCR) which was found to be better at detecting low viral loads compared to conventional RT-PCR [31].

To date, there is no definitive evidence that people who have clinically recovered from COVID-19 but have persistent VS have transmitted infection to others. Prolonged VS has been associated with independent risk factors including male sex, delayed admission to hospital after illness onset, invasive mechanical ventilation, corticosteroid treatment and old age [59, 67]. However, a recent surge of case reports detailing both symptomatic and asymptomatic repeat positive tests obtained days to months after negative results, have raised worrying questions [55, 64-66, 68, 70]. Though a confirmed pattern of SARS-CoV-2 reinfection has not yet been determined, whether re-positivity is due to persistent VS or true re-infection, has been queried with mixed findings [59, 64-67, 70]. To et al. report the first case of true reinfection, describing a patient who was tested positive on 2 distinct instances, 142 days apart and was found to be infected with 2 distinct SARS-CoV-2 strains [68]. Similar findings, though without genomic sequencing have been echoed by Gousseff et al., who report confirmed repeat positivity following initial disease resolution in their patient cohort and suggest that re-infection could have occurred. However, they hypothesize that viral reactivation, rebound phenomenon due to incomplete initial resolution and a second episode of viral replication or interference of the immune response by drugs, such as corticosteroids or underlying conditions needed to be first ruled out [55, 67]. Lu et al. report similar accounts of re-positivity in the face of neutralizing antibodies, but were unable to demonstrate live virus or full

genome sequencing, suggesting that viral debris may have caused their results [67, 69, 70]. Ye et al. reportedly demonstrated SARS-CoV-2 "reactivation" and cited host factors (sex, older age, comorbidity requiring immunosuppression), virologic factors (high baseline SARS-CoV-2 load and variable genotype) and degree and type of immunosuppression as factors that could influence this phenomenon [64, 66]. In their own analysis, Zhou et al. endorsed these factors, adding that testing and processing factors can also affect results [71]. Therefore, if a person who has recovered from COVID-19 has new symptoms of COVID-19, evaluation for reinfection of the individual must be done, especially if exposure to an infected contact has occurred (Table 2) [8, 15, 37, 51, 55, 64-66, 68]. A large contact tracing study demonstrated that high-risk household and hospital contacts did not develop infection if their exposure to a case patient started 6 days or more after the case patient's illness onset, though this remains to be replicated extensively [72]. Research continues to refine exposure-infection timelines, and mask wearing, social distancing, quarantining/self-isolation and diligent hand hygiene continue to be endorsed as protective measures in containing and reducing the spread of SARS-CoV-2 [73].

CONCLUSION

The compendium of data available on SARS-CoV-2 and COVID-19 continues to evolve, and with it, patterns of viral dynamics within the URT. Based on what is known, there are clear benefits to URT sampling including diagnostic accuracy, test cost-effectiveness and convenience. However, the exact periods of greatest infectivity remain unknown and due to the emerging data on disease patterns, the URT may be limited in certain instances. It is now known that VS does not equate to infectiousness. However, in cases where Ct values are low or equivocal, viral cultures have successfully confirmed infection, and as data on SARS-CoV-2 increases, the capacity for making projections on disease trajectory is becoming stronger, and public health measures continue to advance accordingly. Research studies will benefit from greater sample sizes and more reliable laboratory data, and while some researchers have sought to address this issue with multicentre sampling and proxy measures, striking a balance between sample size and producing timely data may remain a challenge until a more definitive reduction in cases is seen globally. Still, despite the need for more research to refine the current process, management strategies are already benefitting from the work that has been done, and we remain optimistic about future developments.

In conclusion:

1. High initial viral titers occur in the early stages of infection, even in asymptomatic/pre-symptomatic individuals, and can lead to transmission from the URT
2. Declining URT viral titers must be considered with caution. This may indicate the resolution of symptoms or herald progression to lower respiratory tract disease.
3. Viral shedding in the recovery period does not confirm or exclude infectiousness; live viral cultures should be sought for clarity. Imperfect but enlightening proxies of Ct value and sgRNA levels have also been used.
4. Factors associated with prolonged viral shedding include: older age, medical comorbidities, particularly diabetes and hypertension corticosteroid treatment male sex, delayed admission to hospital after onset of illness, invasive mechanical ventilation, severe SARS-CoV-2.
5. Repeat positive results include "rebound" positives, "reactivation" positives and the possibility of true re-infection. These can occur in the presence of neutralizing antibodies, raising questions about the value and duration of immunity from SARS-CoV-2.
6. Re-exposure post-recovery definitively requires re-testing.

Conflicts of interest
None

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