Health Information and Quality Authority

An tÚdarás Um Fhaisnéis agus Cáilíocht Sláinte

Evidence summary for the duration of infectiousness in those that test positive for SARS-CoV-2 RNA

15 September 2020

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Key points

- Public health interventions aim to minimise the burden of COVID-19 by reducing the spread of SARS-CoV-2. Important interventions that may be associated with specific durations of time include 'isolation' and 'restriction of movements'.
- Isolation (or self-isolation) is defined as separating those with symptoms of, or diagnosed with COVID-19, from people who are not infected.
- Isolation is distinct from restriction of movements (or quarantine), which is defined as separating and restricting the movements of people who were exposed or potentially exposed to COVID-19. This is performed as a precautionary measure to prevent transmission should exposed individuals later become diagnosed.
- Viral culture and contact tracing studies that report outcomes in relation to time since symptom onset or SARS-CoV-2 RNA detection can suggest the duration of potential infectiousness. Therefore, these can inform the duration of isolation required for patients who test positive for SARS-CoV-2 RNA.
- Viral culture studies measure the ability of SARS-CoV-2 to replicate in cultured cells. While a positive viral culture indicates potential infectiousness, risk of transmission (clinical infectivity) is also influenced by the viral titre, clinical and environmental factors, and behaviour of the infected individual.
- Contact tracing studies measure virus transmission between index cases (the first identified case in a cluster of persons infected) and close contacts.
- Fifteen studies were included in this evidence summary; 13 viral culture studies and two contact tracing studies.
- Across all 13 viral culture studies, there were at least 808 COVID-19 patients with culture attempted, at least 206 of which cultured SARS-CoV-2.

- For five of the 13 viral culture studies, each of which predominantly included patients with mild-to-moderate disease, the last day on which SARS-CoV-2 was cultured occurred within the first 10 days since onset of symptoms.
- For another five viral culture studies, each of which predominantly included mild-to-moderately ill patients, positive culture growth was identified beyond day 10 for approximately 3% of patients. However, this figure is based on several assumptions and the true estimate in the overall population is unknown.
- The remaining three viral culture studies presented results for patients with severe or critical disease at the time of sampling. SARS-CoV-2 growth was identified up to 32 days after symptom onset in one of these studies.
- Two of the 13 studies identified patients who were viral culture positive for a prolonged period (up to day 18 or day 20, respectively) and noted that these patients were immunosuppressed.
- There was insufficient evidence regarding the duration of infectiousness in children or asymptomatic individuals testing positive for SARS-CoV-2.
- One contact tracing study included 22 laboratory-confirmed COVID-19 secondary cases paired with laboratory-confirmed index cases. The other contact tracing study included 41 laboratory-confirmed or probable COVID-19 secondary cases paired with laboratory-confirmed index cases.
- Both contact tracing studies found no evidence of laboratory-confirmed onward transmission when close contacts were first exposed more than five days after symptom onset in the index case.
- The majority of evidence in this review comes from studies of low quality design, and there were important limitations associated with all studies.
- The evidence to date suggests that patients with mild-to-moderate COVID-19 symptoms are unlikely to be infectious beyond 10 days from onset of symptoms. However, evidence from a limited number of studies indicates that patients with severe-to-critical symptoms and or those who are immunocompromised, may be infectious for 20 days or more.

Evidence summary for the duration of infectiousness in those that test positive for SARS-CoV-2 RNA

The Health Information and Quality Authority (HIQA) has developed a series of 'Evidence Summaries' to assist the Expert Advisory Group (EAG) in supporting the National Public Health Emergency Team (NPHET) in their response to COVID-19. These summaries are based on specific research questions. This evidence summary was developed to address the following research question:

What is the duration of infectiousness in those that test positive for SARS-CoV-2 RNA?

The aim of this evidence summary is to inform the duration of isolation for those that test positive for SARS-CoV-2 RNA.

Background

SARS-CoV-2 is a highly infectious virus that is responsible for tens of millions of cases of COVID-19 worldwide.[1] Reasons for the high degree of infectiousness of SARS-CoV-2 include; its novel presence in the human population, the lack of effective treatment and vaccine options, the high SARS-CoV-2 viral loads detectable in respiratory samples, the predominantly mild-to-moderate symptoms and the occurrence of transmission by those asymptomatic or early in the disease course, often pre-symptomatically.[2-6] An evidence summary previously conducted by HIQA found that the viral load of SARS-CoV-2 from respiratory tract samples peaks around symptom onset, or shortly thereafter.[3, 7] These findings underline the critical importance of rapid diagnosis, tracing and isolation of suspected COVID-19 cases.

The SARS-CoV-2 infection is diagnosed by the presence of viral ribonucleic acid (RNA), as detected by molecular testing, usually reverse transcription polymerase chain reaction (RT-PCR). However, detection of viral RNA does not necessarily mean that a person is infectious and able to transmit the virus to another person. Factors that determine transmission risk include; whether a virus is still replication-competent (or live); the titre of the replicative virus, whether the patient has symptoms, such as a cough which can spread infectious droplets; and the behaviour and environmental factors associated with the infected individual.[8]

The ability of SARS-CoV-2 to replicate in cultured cells serves as a surrogate marker of viral infectivity, and is generally considered the gold standard. It is more informative than the detection of viral RNA for the purpose of estimating the infectiousness of a patient and the duration of isolation required to minimise the risk of onward transmission.[1, 8-10] However, a SARS-CoV-2 positive culture does not

necessarily prove that the patient is infectious, but rather that they are potentially infectious. Therefore, supplementary evidence from epidemiological investigations is important in understanding the transmission dynamics between index cases and close contacts, in relation to time since symptom onset of the index case, and can be used to better understand the duration of infectiousness.[10]

With regards to public health measures, isolation (or self-isolation) is defined as separating those with symptoms of or diagnosed with COVID-19, from people who are not infected.[11] It is distinct from restriction of movements (or quarantine), which is defined as separating and restricting the movements of people who were exposed or potentially exposed to COVID-19, as a precautionary measure to prevent transmission should they later become diagnosed.[11]

Recommendations regarding the duration of isolation for those who test positive for SARS-CoV-2 RNA have evolved since the emergence of the virus in China in December 2019, and vary across jurisdictions. The initial recommendation by the World Health Organization (WHO) published on 12 January 2020, stated that COVID-19 patients could only be released from isolation if they were clinically recovered and received two negative RT-PCR results on sequential samples taken at least 24 hours apart.[8] However, given the challenges due to limited laboratory resources required for the conduct of these tests, along with the findings of prolonged viral RNA detection in otherwise well patients,[12] and re-detection of virus in discharged patients,[13] the WHO updated its recommendations on 27 May 2020.[8] The WHO now uses a symptom-based approach, and recommends that a COVID-19 patient (regardless of symptom severity) can end isolation, without requiring re-testing, 10 days after symptom onset and at least three additional days without symptoms. For example, if a patient had symptoms for two days, then the patient could be released from isolation after 13 days (10 days + 3 days = 13 days). Asymptomatic patients can end their isolation period 10 days after they first test positive for SARS-CoV-2 RNA.[8]

The European Centre for Disease Prevention and Control (ECDC) issued guidance on 8 April 2020, in the context of widespread community transmission, with recommendations stratified based on whether the COVID-19 case required hospitalisation. Specific guidance is also given for critical infrastructure responders (e.g., healthcare workers (HCWs)) and household contacts and carers of COVID-19 patients. Patients in the community with mild or asymptomatic disease can end isolation eight days after the onset of symptoms as long as there is resolution of fever and clinical improvement of other symptoms for at least three days. It is noted that HCWs meeting these criteria can return to work immediately, but should wear a surgical mask during work until 14 days from onset of symptoms has elapsed to protect vulnerable patients in their care. The guidance specifies that, if testing

capacity allows, isolation should be ended for a clinically recovered patient with two negative RT-PCR tests from respiratory specimens at 24 hours interval, at least eight days after onset of symptoms. Critical infrastructure responders, especially HCWs, are noted as a priority group for testing during the pandemic. For patients that require hospitalisation for suspected or confirmed COVID-19, discharge and isolation criteria are informed by clinical criteria, testing and hospitalisation capacity and the location to which the patient is discharged. Patients must, at a minimum, isolate for eight days after onset of symptoms provided there is resolution of symptoms as per those not initially hospitalised. The minimum duration extends to 14 days from symptom onset in those who initially had severe disease and in those who are immunocompromised.[14]

The Centers for Disease Control and Prevention (CDC) in the United States (US) updated their guidance on 20 July 2020, recommending that isolation can generally be discontinued (in patients with mild-to-moderate illness) 10 days after symptom onset and resolution of fever for at least 24 hours (without the use of fever-reducing medications) and with improvement of other symptoms.[15] This may be contrasted with the CDC's previously recommended period of isolation of 14 days. However, the CDC also now recommends that isolation may be required for up to 20 days after symptom onset in a limited number of severe cases. The CDC further recommends that for patients who are severely immunocompromised, a test-based strategy (using RT-PCR) may be considered to discontinue isolation, in consultation with infectious diseases experts. For all other patients, the CDC no longer recommend a test-based strategy to discontinue isolation.[10]

In the United Kingdom (UK), recommendations regarding the duration of isolation were updated on 30 July 2020.[16] The current recommendation is that 10 days of isolation is required for mild cases, including asymptomatic cases, managed in the community, whereas for patients who require hospitalisation, isolation can be discontinued after 14 days, provided that the clinical improvement criteria have been met.[16] The UK government previously recommended seven days of isolation for all COVID-19 cases, except for patients requiring critical care or those who were severely immunocompromised, in which case 14 days was recommended.[17]

The current Irish guidance recommends that COVID-19 patients should self-isolate for a minimum of 10 days from the onset of symptoms, the last 5 days of which should be without fever.[18] This guidance, issued on 14 September 2020, replaces guidance issued in March 2020. The previous guidance recommended a period of isolation of 14 days after symptom onset, provided patients were fever-free for five days.

In light of the evolving evidence base and the changing recommendations observed in different jurisdictions, the aim of this evidence summary was to inform the duration of isolation for those that test positive for SARS-CoV-2 RNA.

Methods

The processes as outlined in HIQA's *'Protocol for evidence synthesis support - COVID-19'*,[19] available on www.hiqa.ie, were followed throughout the conduct of this review. Relevant databases of published literature and pre-print servers were searched. Data published by national agencies are not included. This evidence summary includes all relevant evidence until 26 August 2020.

Given the inconsistent presentation of data across the studies, and the heterogeneity of methods used to culture virus, a quantitative synthesis of results could not be reliably performed. However, a narrative synthesis including graphical representation of findings, is presented below.

Results

Search results

Of 4,226 studies screened after duplicate removal, fifteen were included.[9, 20-33] In addition, unpublished data linked to one of the included studies[27] were also included.[34] Thirteen of these studies conducted SARS-CoV-2 viral culture (Appendix 1),[9, 20-22, 24-32] and two studies conducted contact tracing of case-contact pairs (Appendix 2).[23, 33] All 15 studies reported results in relation to time since symptom onset. No study was found that reported duration of infectiousness in relation to truly asymptomatic patients (that is, patients that never develop symptoms).

Population characteristics of included studies

Two studies each were conducted in the US,[20, 27] Taiwan,[23, 29] the UK[30, 33] and Germany;[24, 32] one study each was conducted in Switzerland,[28] Australia,[21] Canada,[22] Spain,[25] South Korea,[26] Hong Kong,[9] and the Netherlands.[31]

Of the 13 SARS-CoV-2 viral culture studies, 11 were case series[9, 20-22, 25-28, 30-32] and two were case reports.[24, 29] The contact tracing studies were both described as case-ascertained studies.[23, 33]

Across all 13 viral culture studies, SARS-CoV-2 culture was attempted from samples collected from at least 808 COVID-19 patients, with SARS-CoV-2 growth observed in at least 206 of these. A total of at least 1,652 samples had SARS-CoV-2 culture attempted and at least 413 of these were positive. Five studies did not clearly provide information on the number of samples and or patients included.[21, 22, 25, 29, 32]

Details of numbers of samples and patient numbers for virus cultivation are provided in Table 1.

The number of patients in each study in whom SARS-CoV-2 viral culture was attempted, ranged from one[24, 29] to 253,[30] with a median (and interquartile range (IQR)) of 29 (9-111) patients per study. The number of positive SARS-CoV-2 cultures from respiratory tract samples, in each of these 13 studies, ranged from two[24] to 133,[30] with a median (IQR) of 21 (11.25-50.75) positive cultures per study.

In the contact tracing study by Cheng et al., 100 confirmed COVID-19 cases and 22 paired secondary cases identified from 2,761 close contacts were included.[23] In the contact tracing study from Public Health England by Lopez Bernal et al., data were reported for 269 lab-confirmed COVID-19 primary cases (who lived with at least one other person) and their 472 household contacts, 161 of whom were found to be probable (N=96) or confirmed (N=65) secondary cases. Of the 161 probable or confirmed cases, 41 were identified as having a point source exposure (known contact with the index case of maximum one day) and for whom analysis of timing of exposure relative to the primary case symptom onset could be undertaken.[33]

Six studies included only hospitalised patients,[9, 24, 26, 29, 31, 32] four studies included both inpatients and outpatients,[21, 25, 27, 28] two studies included home quarantine patients who were close contacts of COVID-19 cases,[23, 33] one study included nursing home residents,[20] and one study included patients from a range of clinical scenarios (community and healthcare worker surveillance, symptomatic persons tested as part of early epidemic response, and samples acquired in outbreak investigations).[30] One study included samples from patients that were analysed at a provincial public health laboratory, but did not specify the exact setting for these patients.[22]

Seven studies included patients with diverse COVID-19 disease severity (ranging from mild to critical),[9, 20, 21, 23, 25, 26, 30] with one of these studies providing separate results for patients with mild and severe disease.[25] Five studies included only patients with mild disease,[24, 27-29, 32] and one study included only patients with severe or critical disease.[31] Two studies did not describe the severity of COVID-19 disease of included patients.[22, 33] One case report described SARS-CoV-2 viral culturing in a hospitalised, immunocompromised heart transplant patient with mild COVID-19 symptoms.[24] This particular patient was originally hospitalised for heart transplantation, and subsequently became infected with COVID-19 four months later while still hospitalised due to complications arising from the operation.[24] Another case report described SARS-CoV-2 viral culturing in a hospitalised patient (no co-morbidity details provided) also with mild COVID-19.[29]

Eleven of the 15 studies included only adults;[9, 20-22, 24-27, 29, 31, 32] however, for one of these studies it is unclear whether patients as young as eight years old were included due to inconsistencies in the study report.[21] Three studies included both adults and children[23, 30, 33] and one study included only children under the age of 16.[28]

Twelve studies only included COVID-19 patients after onset of symptoms.[9, 21, 22, 24-29, 31-33] Three studies additionally included patients before they become symptomatic (that is, pre-symptomatic).[20, 23, 30] The earliest SARS-CoV-2 viral culture attempt was 13 days before symptom onset,[30] whereas the latest SARS-CoV-2 culture attempt was 67 days after symptom onset.[9] The contact tracing study by Cheng et al. included contacts who had their first day of exposure to the index case between four days before and up to 26 days after symptom onset in the index case. Contacts were subsequently followed for 14 days after the last exposure to the index case.[23] The contact tracing study by Lopez Bernal et al. included household contacts who were exposed to the index case at any time between symptom onset and 14 days thereafter.[33] Follow-up of the contacts was limited to 14 days from onset of symptoms in the index case.[33] Although asymptomatic patients were included in three studies, no information was provided from these studies that could be used to inform the potential duration of infectiousness of asymptomatic patients. In other words, results from serial sampling and attempted culturing in asymptomatic patients were not reported,[20, 30] and the contact tracing study reported that no onward transmission occurred from the nine asymptomatic patients, despite 91 close contacts being identified.[23]

Table 1: Details of numbers of samples and patients for SARS-CoV-2 cultivation (positive cultures/attempted) and days on which culturing attempted and successful.

	Numbers of samples and patients (culture positive/attempted) and associated sample incubation duration			Days since symptom onset on which virus cultivation attempted		Days since symptom onset on which virus culture positive	
	N culture positive samples/ N samples where culture attempted	**N patients culture positive / N patients in whose samples culture was attempted**	**Duration (days) for which cultured cells incubated**	**First day attempted**	**Last day attempted (or further information)**	**Minimum**	**Maximum**
Studies including symptomatic period only							
Basile	56/234 (23.9%)	NR/195	5	0	29	0	18
Bullard	26/90 (28.9%)	NR	4	0	21	0	7
Decker	2/2 (100%)	1/1 (100.0%)	NR		*Only attempted on days 18 and 21.*	18	21
Folgueira	49/106 (46.2%) *Mild: 18/50* *Severe: 31/56*	NR/105 *Mild: NR/50* *Severe: NR/55*	5	1	*At least day 32*	1	 10 *(mild)* 32 *(severe)*
Jeong	3/9 (33.3%) *(respiratory samples)*	2/5 (40.0%)	4	8	30	11	15
Kujawski **CDC unpublished (Midgley et al., Kujawski group)**	13/17 (76.5%) *respiratory samples)* *Unclear from Figure*	9/9 (100.0%) *Unclear*/14†	NR NR	0 0	8 30	0 0	8 8
L'Huillier	12/23 (52.2%)	12/23 (52.5%)	6	0	5	1	5

Liu	NR/NR	1/1 (100%)	NR	NR	*>18 days (exact days not reported)*	NR	18
Perera	16/68 (23.5%)	16/35 (45.7%)	6 days (72 hours followed by an additional 72 hours)	0	67	0	7
Van Kampen	62/690 (9.0%)	23/129 (17.8%)	7 days	0	39	0	20
Wölfel	9/34 (26.5%) *respiratory (43 samples in total)**	NR/9	6 days	3	13	3	8
Studies including pre-symptomatic and symptomatic period							
Arons	32/55 (58%)	31/43 (72.1%)	NR	-7	13	-6	13 *(where all symptoms considered)* 9 *(fever, cough, shortness of breath only)*
Singanayagam	133/324 (41%)	111/253 (44%)	Up to 14 days	-13	60	-13	12

NR: Not reported
*Note that these figures are estimated from the number of markers displayed on Figure 1d in Wölfel et al.; it is possible that the numbers are underestimated due to potential overlay of markers.
†CDC unpublished data by Midgley et al. includes all patients included in Kujawski et al. plus 5 additional patients.

Molecular and viral culture methods used in included studies

All 15 studies used RT-PCR to diagnose COVID-19,[9, 20-33] however one study also used clinical symptoms to diagnose COVID-19 in the absence of RT-PCR testing.[33] For SARS-CoV-2 culturing, Vero E6 cells were only used in five studies,[9, 25, 28, 30, 32] Vero CCL 81 cells were only used in four studies,[20, 22, 26, 27] Vero E6 and LLC-MK2 cells were both used in one study,[29] Vero clone 118 cells only were used in one study,[31] and Vero C1008 cells were only used in one study.[21] One study did not provide information on the cell lines used for culturing.[24]

All 13 SARS-CoV-2 viral culturing studies used upper respiratory tract samples (such as nasopharyngeal and oropharyngeal) for culturing.[9, 20-22, 24-32] Additionally, eight of these studies also used lower respiratory tract samples (such as sputum and bronchial aspirates) for culturing.[9, 21, 22, 25, 27, 29, 31, 32]

The duration of incubation to observe for cytopathic effects (CPE) in cultured cells ranged from four[22, 26] to 14 days[30] with a median (IQR) of six (5-6) days (Table 1). Two studies described performing a second passage of the sample to observe a CPE.[9, 28]

SARS-CoV-2 growth was confirmed by means of just RT-PCR of supernatant fluid in seven studies.[9, 20, 21, 26-28, 32] SARS-CoV-2 growth was confirmed by just immunofluorescence* in two studies,[30, 31] and alongside RT-PCR of supernatant fluid in another study.[25] One study described confirming positive culture growth using microscopy, but provided no further information on this process.[22] Two studies did not provide any information regarding how culture growth was confirmed.[24, 29]

Serial sampling until SARS-CoV-2 replication in cell culture was no longer observed, was undertaken in only two studies.[21, 29] Serial sampling was attempted in five other studies.[9, 20, 24, 31, 32] However, for three of these studies reporting a maximum duration of SARS-CoV-2 culture positive, it is unclear whether viral culture of later samples from the same patient was attempted, (that is continued serial culture of SARS-CoV-2 culture positive patients until a negative culture obtained) or if the data were truncated.[9, 31, 32] Furthermore, the other two of these five studies only attempted viral culture at two time points, and hence infectious virus beyond these dates cannot be ruled out.[20, 24] Unpublished data linked to one of the included studies[27] appear to report serial sampling for viral culturing linked to 14 patients, where samples were collected every 2-3 days, up to a maximum of 30 days after symptom onset. However, as noted, these data are not yet formally published.[34] Four studies did not conduct serial sampling and instead attempted viral culturing

* Immunofluorescence is an approach using antibodies chemically labelled with fluorescent dyes to visualise antigens using fluorescence microscopy.

using sample(s) from patients at one time point only.[25-28] In two studies it is unclear whether serial sampling was conducted.[9, 30]

Viral culture study findings

Maximum day post-symptom onset during which positive virus cultures could be obtained

The first and last days, post-symptom onset, on which virus culture was attempted, are compared visually with the first and last days on which SARS-CoV-2 culture positives were observed, in Figure 1 for each individual study and are also listed in Table 1.

For five of the 13 studies, the last day on which a SARS-CoV-2 culture positive was observed occurred within the first 10 days since onset of symptoms.[9, 22, 27, 28, 32] One of these studies, the sole study including only children, did not appear to attempt viral culture beyond day five.[28] For the most part, the clinical severity of patients was mild-to-moderate in these five studies, though Perera et al. noted that three of the 35 patients in the study had been critically ill, while the remainder had mild disease or had been asymptomatic.[9]

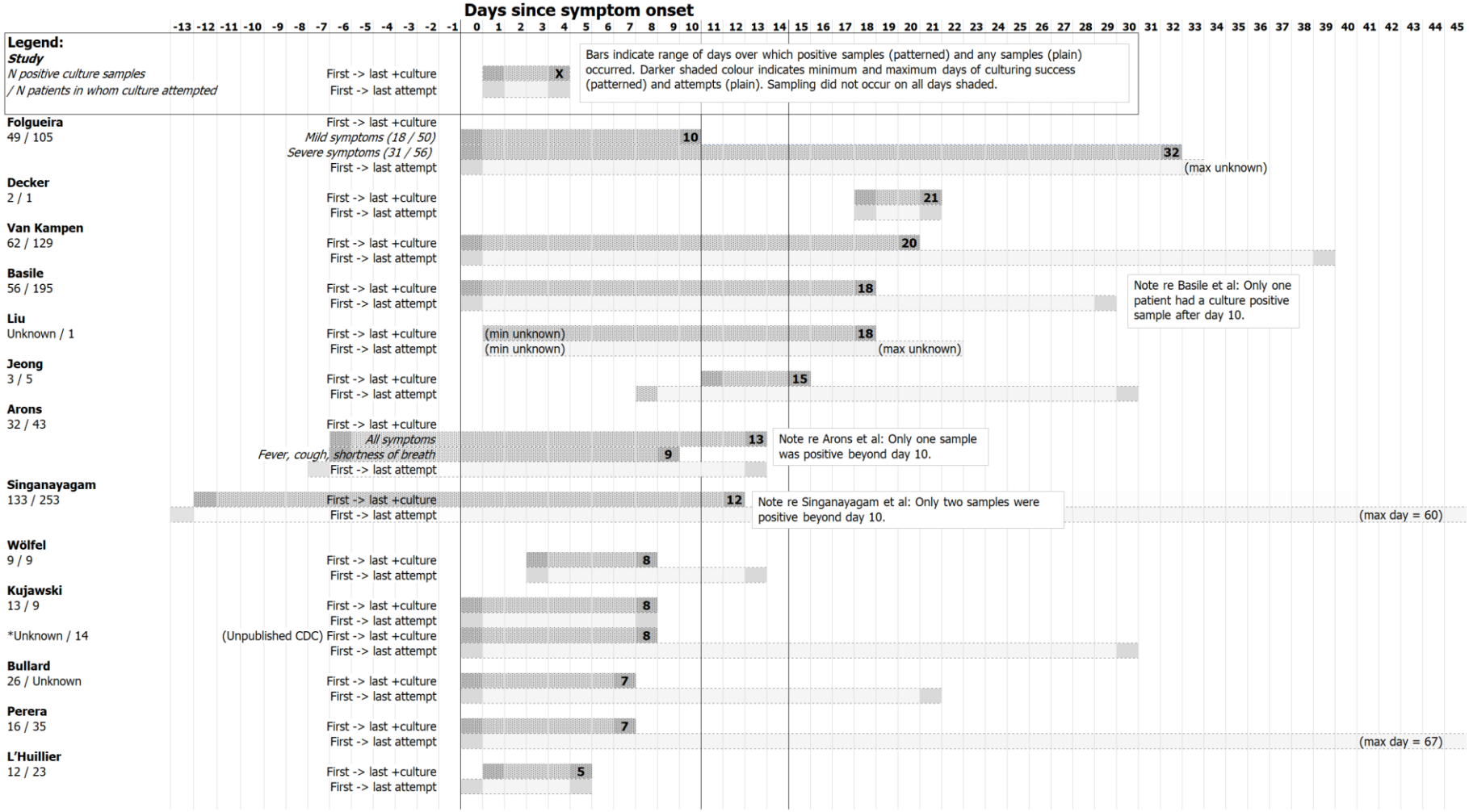
In two studies, the last day on which a SARS-CoV-2 culture positive was observed occurred between 10 and 14 days post onset of symptoms.[20, 30] Singanayagam (N=133 SARS-CoV-2 culture positive samples from 253 patients) obtained only two culture positive samples between days 10 and 14 (one each on day 11 and day 12). The authors also noted that more than half of the samples had been collected at least eight days from symptom onset, and that 21% of these samples were SARS-CoV-2 culture positive, the majority of which (25 of 27) were positive between eight and 10 days after symptom onset. None of these late culture-positive samples included patients who were immunosuppressed or severely ill.[30] In Arons et al., one of 53 samples (for which the date of symptom onset was known) produced a SARS-CoV-2 culture positive on day 13; SARS-CoV-2 was not observed in other samples collected after day 10, though no viral culture was attempted beyond day 13.[20] There was also some uncertainty with regards to symptom onset in this study, with the longest time between the onset of typical COVID-19 symptoms (that is, fever, cough or shortness of breath) and detection of SARS-CoV-2 culture positive recorded at nine days, but 13 days after onset of any symptom associated with COVID-19.

The remaining six studies, comprising two case reports and four case series, achieved SARS-CoV-2 culture positives between days 15 and 32 post-symptom onset.[21, 24-26, 29, 31] Three of the four case series included samples from over 100 patients.[21, 25, 31] Two of these studies included results for patients with severe

disease.[25, 31] Folgueira et al. (N=105 patients) presented the maximum day on which a SARS-CoV-2 culture positive was observed for each of patients with mild symptoms (max day: 10) and severe symptoms (max day: 32).[25] Van Kampen et al. (N= 129 patients) observed SARS-CoV-2 culture positives up until day 20. This study notably included only severely or critically ill patients, and included a large proportion of patients (23%) who were immunocompromised.[31] The third large case series, Basile et al. (N=195), reported that the maximum day on which a SARS-CoV-2 culture positive was obtained was day 10 for all but one patient (maximum day: 18).[21] The fourth case series, Jeong et al. (N=9) obtained positive cultures for two patients; the patient with a SARS-CoV-2 culture positive (saliva sample) on day 15 was noted to be critically ill at the time of sampling.[26] Both of the case reports which identified SARS-CoV-2 culture positives beyond day 14 were of hospitalised patients with mild disease,[24, 29] although one of the case reports described an immunocompromised patient who had recently had a heart transplantation.[24]

The viral titres of samples collected on the maximum day on which SARS-CoV-2 culture positives were observed for each study, are outlined in Appendix 1. Of note, lower cycle threshold (Ct) values infer higher viral titres, and Ct values greater than 40 (or sometimes greater than 35) are generally considered a negative RT-PCR test result. Of the six included studies that examined the relationship between higher viral titre and successful culture of SARS-CoV-2, all six found a positive correlation.[9, 21, 22, 30-32] Singanayagam et al. (N=324 samples) estimated that the probability of successfully culturing SARS-CoV-2 from samples with a Ct value greater than 35 was 8.3% (95% CI: 2.8%–18.4%).[30] Basile et al. (N=234 samples) concluded that any clinical sample with a Ct value of ≥37 was not indicative of viable (or transmissible) virus.[21] Bullard et al. (N=90 samples) estimated that for every one unit increase in Ct value, the odds of a positive SARS-CoV-2 culture decreased by 32%.[22] While Folgueira et al. reported a SARS-CoV-2 positive culture on day 32 post symptom onset in a patient with severe COVID-19, they observed a very low viral titre, with a Ct value of 39.1.[25] Hence it is unlikely that the patient was infectious at this particular time.

Figure 1: Days since symptom onset at which virus culture attempts (pale grey) and successful virus culturing (dark grey) took place in each study, displayed in descending order of last day of successful virus culturing



*Unpublished data from CDC website (Midgley et al.); Kujawski et al. comprises a subset of these data.

Summary estimates, including probability of virus culturing

Two studies reported a median value and interquartile range for the timing of SARS-CoV-2 culture positive results since symptom onset,[30, 31] the results for which differed significantly with their differing study populations. Singanayagam et al.,[30] which included data for pre-symptomatic patients and those with predominantly mild to moderate disease, presented a median of four days (IQR 1-8, range -13 to 12) while Van Kampen et al.,[31] which included only patients who were sampled in the context of severe or critical disease, reported a median of eight days (IQR 5-11, range 0 to 20) post-symptom onset.

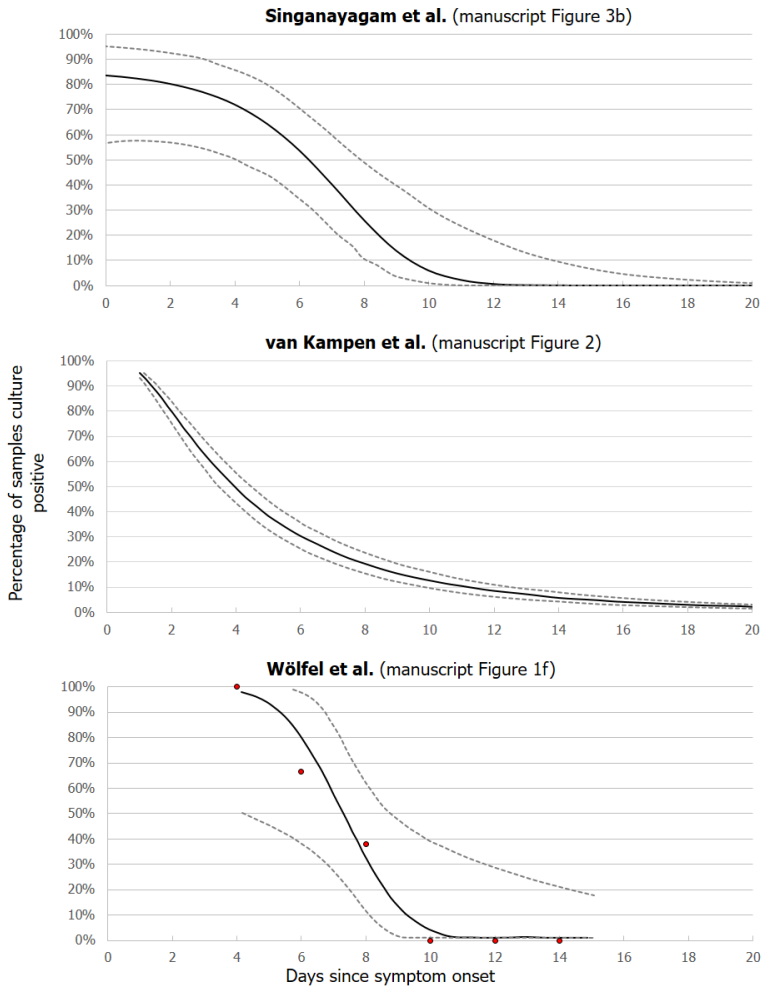
Three studies presented graphical estimates of the probability of achieving a SARS-CoV-2 culture positive versus the number of days since symptom onset.[30-32] These graphical estimates and information regarding the corresponding number of samples and patients are reproduced in Figure 2. Van Kampen et al.[31] and Wölfel et al.[32] both presented probit (dose-response)[†] distributions for this outcome and estimated the day post-symptom onset when the probability of a positive SARS-CoV-2 culture fell below 5%. These results were as follows: Van Kampen et al., 15.2 days (95% CI 13.4-17.2);[31] Wölfel et al., 9.8 days (95% CI 8.5-21.8).[32] Singanayagam et al.[30] presented the results of a mixed effects logistic regression analysis (to account for the clustering of samples within patients); estimates of the percentage of samples with potentially infectious SARS-CoV-2 were presented for each of days seven to 15 post-symptom onset, and are reproduced in Table 2. At 10 days post-symptom onset, the probability of a sample with potentially infectious SARS-CoV-2 was 6% (95% CI 0.9-31.2); however, these data carry a high degree of uncertainty, as shown by the wide confidence intervals.

[†] Probit distributions result from a type of statistical model that estimates the relationship between two factors, one of which is an 'all or nothing' response (e.g. positive viral culture either occurs or does not occur) and the other being a factor that stimulates that response, or lack thereof (e.g. time since symptom onset). In this case, the model calculates values for the number of days since symptom onset which correspond to the probability of positive viral culture.

Table 2: Estimated percentage of samples with infectious SARS-CoV-2 7–15 days after symptom onset (adapted from Singanayagam et al.)

Day post symptom onset	Estimated % culture positive (95% CI)		Observed number of samples culture positive	Observed number of samples tested
7	40.1	(22.8 - 60.4)	10	14
8	25.8	(11.0 - 49.4)	9	33
9	13.7	(3.7 - 39.6)	10	34
10	6.0	(0.9 - 31.2)	6	23
11	2.2	(0.2 - 23.9)	1	6
12	0.7	(0.0 - 17.9)	1	3
13	0.2	(0.0 - 13.1)	0	4
14	0.03	(0.0 - 9.4)	0	2
15	0.006	(0.0 - 6.7)	0	2
			Total: 37	Total: 121

Figure 2: Statistical curves presented in three studies which attempted to model the probability of successful virus culture versus duration of days of symptoms.[30-32] (Adapted from original curves in the manuscripts



Mixed effects logistic regression analysis.

In 246 samples where time to symptom onset was known:
- 103/246 samples were culture positive.
- 81/176 patients had positive samples.
- Cases predominantly had mild to moderate COVID-19.

At ten days post symptom onset the probability of isolation success declined to 6.0% (95% CI: 0.9–31.2%)

Probit distribution.

- 62/690 samples were culture positive.
- 23/129 patients had positive samples.
- All cases had severe or critical COVID-19.

A probability of isolation success of <5% was estimated to occur at 15.2 days post symptom onset (95% CI 13.4-17.2).

Probit distribution.

Circles are marker points (depicting individual samples).

- 9/34 samples were culture positive.
- Up to 9 patients had positive samples.
- All cases had mild COVID-19.

A probability of isolation success of <5% was estimated to occur at 9.8 days post symptom onset (95% CI 8.5-21.8).

While unpublished, Midgley et al. additionally presented a Kaplan-Meier analysis of time to inability to culture SARS-CoV-2 following illness onset.[34] This analysis represents an expanded analysis of the results reported by Kujawski et al.,[34] including a greater number of patients from the same setting (n=14 versus n=12 (9 of whom had samples attempted for culture)). Midgley et al. reported that the probability of successful SARS-CoV-2 culture fell to 50% at day four after illness onset, to 20% at day eight, and approached zero after day nine; the total number of samples tested is not known.

Analysis and interpretation of viral culture study findings

Data for maximum day of positive viral culture

Several organisations suggest a period of isolation for COVID-19 patients of a minimum of 10 days since the onset of symptoms (see Background).[8, 10, 16] As such, day 10 represents an important reference timepoint when considering the results of the viral culture studies included within this review.

While eight studies identified SARS-CoV-2 culture positives on days beyond day 10 post-symptom onset, these results require contextualisation. Important aspects to consider include the number of samples and patients in whom this outcome occurred with respect to the overall number of samples and patients tested, and the patient populations included.

In total, these eight studies included at least 337 SARS-CoV-2 culture positive samples obtained from 732 patients.[21, 24-26, 29-31] In four of the eight studies, only one patient was SARS-CoV-2 culture positive growth beyond day 10,[20, 21, 24, 29] and a further study identified only two samples (of 133 positive samples) resulting in SARS-CoV-2 culture growth beyond day 10.[30] As such, these five studies resulted in a maximum of six reported patients who were SARS-CoV-2 culture positive beyond day 10 (assuming conservatively that the two positive samples beyond day 10 in Singanayagam et al. arose from two unique patients).[30] These five studies in total included 223 SARS-CoV-2 culture positive samples from 493 patients in whom culture was attempted (Table 1).

While these five studies in total reported a maximum of six patients who had SARS-CoV-2 culture positive samples beyond day 10,[20, 21, 24, 29, 30] the total number of patients with positive samples is unknown (Table 1). However, among these five studies, the proportion of patients for whom SARS-CoV-2 culture positives were obtained ranged from 44%[30] to 100%[24, 29] (the latter representing case reports) (Table 1). Applying a worst case scenario by adopting the lower proportion, which is, assuming a positivity rate among patients of 44%, this results in an estimated

minimum of 217 patients across these five studies. As such, six patients among 217 patients (maximum proportion of approximately 3%) could be have replicating SARS-CoV-2 in samples collected beyond day 10 post-symptom onset.

Within the remaining three studies, wherein the maximum day of positive culturing exceeded day 10, it is less clear how many patients resulted in this outcome.[25, 26, 31] Folgueira et al.[25] and Van Kampen et al.[31] noted that patients with this outcome were severely or critically ill at the time of sampling. The remaining study, Jeong et al., included two patients with this outcome, one of whom was critically ill at the time of sampling (day 15), and the other was severely ill, but recovering at the time of sampling (day 11).[26] As such, patients with severe and critical illness may represent a relevant subgroup within the data.

Probability estimate data

Two of three published studies, both of which largely included patients with mild-to-moderate disease,[30, 32] and additional unpublished estimates associated with one of the included studies,[27, 34] estimated that the probability of culturing SARS-CoV-2 is low and falls below 6% at day ten. The remaining study which modelled the probability of virus culturing versus time, suggested that the probability of a SARS-CoV-2 positive culture would fall below 5% at day 15.[31] However, this latter study uniquely included only hospitalised patients with severe or critical illness.

Singanayagam et al., the viral culture study in our review with the greatest number of patients, and which predominantly included patients with mild-to-moderate disease, reported numerical estimates of the probability of a sample producing a SARS-CoV-2 positive culture at each day from day seven onwards.[30] These data carry a high degree of uncertainty, as shown by wide confidence intervals, but showed a steep decline in probability of culturing SARS-CoV-2 over time. For example, the estimated percentage of samples culture positive on day eight stood at 25.8% (95% CI 11.0-49.3) while the corresponding figure for day 10 was 6.0% (95% CI 0.9-31.2).

Contact tracing study findings

Cheng et al.[23] undertook a contact tracing study of 100 confirmed COVID-19 cases (ranging from asymptomatic to critical disease) in Taiwan. Among the 2,761 close contacts of the 100 COVID-19 cases, there were 22 paired index-secondary cases. This study found that the overall secondary clinical attack rate was 0.7% (22 cases from 2,761 contacts; 95% CI, 0.4%-1.0%). The attack rate was higher among the 1,818 close contacts whose exposure to index cases started within five days of index case symptom onset (22 cases from 1,818 contacts; 1.0%; 95% CI, 0.6%-1.6%) compared with those who were exposed later (0 cases from 852 contacts; 0%; 95%

CI, 0%-0.4%). The 299 close contacts with exclusively pre-symptomatic exposure (up to four days before symptom onset in the index case) were also found to be at risk of infection (2 cases from 299 contacts; 0.7%; 95% CI, 0.2%-2.4%). The authors of this study concluded that there is a relatively short period of infectiousness of SARS-CoV-2, with high transmissibility from four days before and up until five days after symptom onset. Based on the lack of onward transmission when close contacts were first exposed more than five days after symptom onset in the index case, the authors deduce that there is a lower transmission risk at the later stage of the disease.[23]

Lopez Bernal et al.[33] undertook a contact tracing study of 269 lab-confirmed COVID-19 cases in the UK, who lived with at least one other person. Local health protection teams contacted household contacts of these patients daily for 14 days after onset of symptoms in the index case. In total, 472 household contacts were identified. Of these 472 contacts, 65 (13.8%) had a laboratory-confirmed diagnosis of COVID-19 using RT-PCR testing, and another 96 (20.3%) had probable COVID-19 based on the onset of symptoms of fever, anosmia (that is, loss of smell) or respiratory symptoms. The remaining 311 contacts were classified as non-cases as they did not experience any symptoms suggestive of COVID-19 within 14 days of symptom onset in the index case. The household secondary attack rate was found to be 37% (95% CI, 31%-43%) including both laboratory-confirmed or probable secondary cases.

Of the 161 laboratory-confirmed or probable secondary cases, 41 had a point source exposure (that is, a maximum exposure window of one day) to an index case and data available to allow analysis of timing of exposure. Laboratory-confirmed and probable secondary cases considered together (N=41) were exposed a mean of 2.37 days (standard deviation (SD) 3.36) and a median of one day (interquartile range (IQR) 0-4) after symptom onset in the index case; exposure ranged from 0 days to the maximum follow up of 14 days. As identification of contacts was limited to 14 days from onset of symptoms in the index case, no information was provided regarding secondary cases in household contacts outside this exposure window. Similarly, of the contacts identified in the 14-day window, follow-up was truncated at 14 days from symptom onset in the index case, so no information was provided regarding secondary cases that may have arisen after this follow-up period.

Restricting to lab-confirmed secondary cases only (N=12), exposure occurred a mean of 1.33 days (SD 1.61) and a median of one day (IQR 0-1.25) after symptom onset in the index case, ranging from 0-5 days. In contrast, non-cases were exposed a mean of 2.71 days (SD 2.74) and a median of two days (IQR 0-5) after symptom

onset in the index case, ranging from 0-9 days. No analysis was undertaken in this study to determine whether these time differences were statistically significant.[33]

Methodological quality of included studies

There are some important methodological limitations associated with all of the included studies. All 13 SARS-CoV-2 culturing studies were retrospective in nature and were either case series[9, 20-22, 25-28, 30-32] or case reports,[24, 29] where patients do not appear to have been selected or sampled systematically. Hence, the majority of evidence in this review comes from studies of low quality design. Importantly, it would appear that most studies did not specifically set out to identify the temporal relationship between time post-symptom onset and presence of infectious virus, but rather reported culture positivity based on samples collected for other purposes.[22, 25-28, 30, 32] Thereby, some uncertainty remains regarding the true duration of infectiousness, as the upper limit of outliers may not have been fully clarified. Recall bias is another issue which may have important implications on outcomes across all studies, as patients may not have been able to accurately determine when symptoms began, particularly if they were quite mild and non-specific.[35] Reporting of outcomes was also notably ambiguous in one particular study, where there were conflicting findings reported between the main text and supplementary material.[20]

There are some concerns regarding the inconsistent approaches used for cell culturing among studies, and the interpretation of findings by the study authors. If, as noted in some studies,[9, 28] a second passage of the sample is required to observe a CPE, this suggests a very low replicative viral load in the original sample and may reflect that the individual from whom the sample was collected is unlikely to be infectious at the time of sampling.[36] Furthermore, given that longer periods for a CPE to develop are indicative of a lower viral replicative titre in the original sample,[37] some studies which incubate the cell lines for prolonged periods and only observed a CPE towards the end of this period, may also be overestimating the clinical significance of the findings.[30]

The size of included studies varied substantially from single case reports[24, 29] to large case series[30] or contact tracing studies.[23, 33] Particular caution is required when generalising findings from the case reports as these are likely reporting extreme cases.[24, 29] However, the larger studies, particularly those with a broad inclusion criteria,[23, 30] may have wider generalisability. Of note, the contact tracing study by Cheng et al. was generally well-conducted due to its prospective nature, the systematic matching of index cases and close contacts over time, and the follow up of contacts for 14 days after last exposure to the index case to assess for symptom onset.[23]

A de-novo tool to quality appraise case reports and case series was also used.[19] This tool identified other concerns regarding the limited reporting of demographic information,[21, 22, 29, 30, 32] the potential limited applicability to the Irish healthcare system, due to different treatment practices and patient populations,[9, 20, 23, 24, 26, 27, 29] the unclear criteria for selection of cases,[9, 22, 25, 26, 31, 32] the non-consecutive inclusion of cases,[9, 21, 22, 25, 26, 31, 32], uncertainty regarding the appropriateness of statistical analysis[9, 26, 30-32] and the inconsistent use of RT-PCR testing for diagnosis.[33] Four of the studies included in this review are published as pre-prints, so have not yet been formally peer-reviewed, raising additional concerns about overall quality and the potential for results to change prior to formal publication.[21, 25, 31, 33]

Discussion

Thirteen SARS-CoV-2 viral culture studies of various quality and size, and two large contact tracing studies were included in this evidence summary. The evidence to date from viral culture studies, would appear to suggest that patients with mild-to-moderate COVID-19 symptoms are unlikely to be infectious beyond 10 days from symptom onset. Evidence from large contact tracing studies[23, 33] appear to support this finding. However, evidence from a limited number of studies indicates that patients with severe-to-critical symptoms, and or those who are immunocompromised, may be infectious for a prolonged period, possibly for 20 days or more.[24, 25, 31] Viral culture studies suggested a steady decline in the probability of identification of infectious virus in the first 10 days following symptom onset; the largest included study estimated the probability as falling from 40% on day seven to 26% on day eight, 14% on day nine and 6% by day 10. However, these results were subject to considerable uncertainty, and the study may have overestimated the clinical significance of findings.[30] Notably, there are important methodological limitations associated with all included studies. The number of studies for consideration remains few in number and sample sizes are often small; hence it is essential that the findings from these studies are interpreted in light of these caveats. Viral culture studies measure the ability of SARS-CoV-2 to replicate in cultured cells. While a positive viral culture indicates potential infectiousness, risk of transmission (clinical infectivity) is also influenced by the viral titre, clinical and environmental factors, and behaviour of the infected individual.

One of the notable findings from two included studies was that patients who were found to be SARS-CoV-2 culture positive for a prolonged period were immunosuppressed.[24, 31] In particular, one of the case reports describes a mild clinical course of COVID-19 in a heart transplant recipient on immunosuppressant therapies (mycophenolate mofetil, ciclosporin and prednisone) who had SARS-CoV-2 culture growth 21 days after symptom onset.[24] Although the evidence pertaining to

prolonged infectiousness in immunosuppressed populations is very limited, these findings do raise important research and policy questions. Immunosuppressant therapies have been observed to delay the clearance of SARS-CoV-2 viral RNA in previous studies.[38, 39] However, some of these agents are also being investigated as treatments for severe and critical COVID-19 to modulate the hyperinflammatory response (for example, tocilizumab).[40, 41] Little is known regarding the presentation and course of disease in patients who are immunocompromised; it is unknown whether all patients who are in an immunocompromised state necessarily present with severe COVID-19 disease, or how important the degree of immunosuppression is for clearance of the virus.[41] More evidence is required to understand the relationship between immunosuppression and the duration of infectiousness of SARS-CoV-2.

Only one study provided information on SARS-CoV-2 culturing specifically in children.[28] However, as viral culturing was only attempted once per patient, up until a maximum of five days post-symptom onset, this study provides insufficient information to inform policy decisions surrounding isolation requirements specifically for children testing positive for SARS-CoV-2 RNA. Children have generally been under-represented in COVID-19 studies to-date, although this may be a function of testing practices which initially typically prioritised those with more severe symptoms, healthcare workers and those residing in long term care settings. Given reports of milder symptoms in children, during the earlier stages of the pandemic they were less likely to be tested and diagnosed.[42] As children return to school en masse, increased testing and cases may occur in these settings, thus more data may emerge regarding the potential duration of infectiousness in this population.

The findings from this evidence summary are largely in agreement with three previous reviews conducted in this area,[43-45] along with an evidence summary conducted by HIQA on the related topic of SARS-CoV-2 viral load.[3, 7] All of these reviews concluded that infectiousness generally declines 7-10 days after symptom onset, and point to uncommon outlier cases where this duration is exceeded. It is important to note that there is substantial overlap of studies included within these reviews, hence it is not surprising that the conclusions are similar.[3, 7, 43-45] Another common finding across several of these reviews was the prolonged duration of viral RNA detection, sometimes for 2-3 months after onset of symptoms, along with cases of positive re-detection after a patient has recovered.[3, 7, 43, 45] Hence, patients are likely not infectious for the entire duration of virus detection as the presence of viral RNA may not represent transmissible live or replication-competent virus.[3] These findings support the WHO policy to no longer require two consecutive negative RT-PCR tests, taken 24 hours apart, in order to end patient isolation.[8]

The findings from this evidence summary largely concur with the CDC recommendations regarding the duration of isolation for adults with COVID-19.[10] The CDC conclude that *"persons with mild to moderate COVID-19 remain infectious no longer than 10 days after symptom onset. Persons with more severe to critical illness or severe immunocompromise likely remain infectious no longer than 20 days after symptom onset."*[10] The CDC also point to the lack of equivalent data from children and infants and the need for more data regarding immunocompromised individuals. As new evidence emerges, recommendations regarding the duration of isolation may change.

The majority of evidence in this review comes from studies of low quality design, where determining the duration of infectiousness may not have been the primary objective and viral culturing methods were not consistent. Furthermore, the inconsistent reporting of data precluded any meaningful quantitative synthesis. Notably, viral culture studies are inherently fraught with challenges,[46] but the requirement for biosafety level three (BSL-3) facilities is a particular barrier to research. Further work is required to develop standardised methods to optimise culturing of SARS-CoV-2.

Future research should be prospective in design, following a large cohort of COVID-19 patients with a broad spectrum of disease and inclusive of sub-populations of interest (such as children, asymptomatic and immunosuppressed patients). Patients should be regularly and systematically sampled and SARS-CoV-2 virus culturing attempted using standardised methods, until virus can no longer be successfully isolated.[44] The time between sample inoculation and the detection of viral growth should be reported for all studies, as this is a reflection of the viral load of infectious virus in the original sample. Future research should also consider the viral titre of samples when interpreting SARS-CoV-2 culture. The findings of such viral culture studies should be supplemented with epidemiological evidence of onward transmission at different time points, as derived from well-conducted and large contact tracing studies. A core outcome set should be measured and reported, stratified by different sub-populations, thus enabling better synthesis by systematic reviewers to better facilitate interpretation and decision-making by policy-makers.[47]

Conclusion

The evidence to date suggests that patients with mild-to-moderate COVID-19 symptoms are unlikely to be infectious beyond 10 days from symptom onset. However, evidence from a very limited number of studies indicates that patients with severe-to-critical symptoms and or those who are immunocompromised, may be infectious for 20 days or more.

There are important methodological limitations associated with all included studies, these types of studies are still relatively few in number, and sample sizes are often small; hence it is essential that the findings from these studies are interpreted in light of these caveats. Viral culture studies measure the ability of SARS-CoV-2 to replicate in cultured cells. While a positive viral culture indicates potential infectiousness, risk of transmission (clinical infectivity) is also influenced by the viral titre, clinical and environmental factors, and behaviour of the infected individual. Large, prospective cohort studies with systematic and repeated sampling and viral culturing, supplemented with epidemiological evidence of onward transmission at different time points, are required to better ascertain the duration of infectiousness, particularly in children, asymptomatic and immunocompromised patients.

Appendix 1: Viral culture studies

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Arons **USA** **Case series** **https://www.nejm.org/doi/full/10.1056/NEJMoa2008457**	**Population setting:** 76 residents in a skilled nursing facility. 48 (63%) testing positive and 28 (37%) testing negative. Of these 48 positive residents, 27 (56%) were asymptomatic at the time of testing; 24 subsequently developed symptoms. Hence 24 were pre-symptomatic and 3 were asymptomatic. **Demographics:** *Sex:* Male, 28 (37%) Female, 48 (63%) *Age:* Mean (±SD), 76.8±10.5 years‡ **Co-morbidities of SARS-CoV-2 positive (n=48):** Any coexisting condition, 47 (98%) Chronic lung disease, 18 (38%) Diabetes, 18 (38%) Cardiovascular disease, 39 (81%) Cerebrovascular accident, 19 (40%)	**Molecular test methods**	**Viral culture methods**	**Viral culture outcomes**	**Other relevant findings**
		Molecular Test: rRT-PCR **Thresholds:** Ct values <40 = positive **Gene Targets:** N1 and N2 genes **Sample site(s):** NP and or OP	**Cell line:** Vero-CCL-81 cells **Medium and additives:** NR **Incubation process:** NR **Determination of positive culture growth:** Cells showing CPE were used for SARS-CoV-2 rRT-PCR to confirm isolation and viral growth in culture. **Sample site(s):** NP and or OP **Timing of samples:** Includes pre-	**Total number of samples where culture attempted (n=43 patients):** 55 **Positivity rate** *All samples: 32/*55 (58%)† ▪ 10 of 16 with typical ▪ symptoms ▪ 3 of 4 with atypical symptoms, ▪ 17 of 24 who were pre-symptomatic ▪ 1 of 3 who remained asymptomatic *Where date of typical symptom onset known (n=55 samples from 43 residents)* Day 5≠: None tested Day 6: 0/2 (0%) Day 7: 2/2 (100%) Day 8: 0/1 (0%) Day 9: 1/3 (33%) Day 10: None tested Day 11: 0/1 (0%) Day 12: None tested *Where date of any symptom onset known (n=53 samples from 42 residents)*	More than half of residents testing positive on initial testing were asymptomatic or pre-symptomatic. The lowest viral load (Ct value) for which there was positive culture growth was 34.3 (and this occurred in a pre-symptomatic resident).

	Renal disease, 18 (38%) Received haemodialysis, 3 (6%) Cognitive impairment, 28 (58%) Obesity, 11 (23%) **Clinical characteristics SARS-CoV-2 positive (n=48):** *Presentation (1st test)* <u>Typical symptoms:</u> Fever, 5 (10%) Cough, 16 (33%) Shortness of breath, 4 (8%) <u>Atypical symptoms:</u> Sore throat, 3 (6%) Chills, 0 (0%) Confusion, 2 (4%) Rhinorrhoea or congestion, 1 (2%) Myalgia, 0 (0%) Dizziness, 2 (4%) Malaise, 6 (13%) Headache, 1 (2%) Nausea, 3 (6%) Diarrhoea, 2 (4%) No symptoms, 27 (56%) (3 of whom remained asymptomatic 7 days later) **Clinical severity**: Diverse severity. Of the 57 residents with SARS-CoV-2 infection, 11 had been hospitalized (3 in the ICU) and 15 had died (mortality, 26%). However 3 remained asymptomatic.		symptomatic and symptomatic periods. From 7 days before symptom onset to 13 days after symptom onset. **Serial sampling for culture attempts:** Yes, but only done twice for 12 residents (taken 7 days apart).	Day 5: None tested Day 6: 0/1 (0%) Day 7: 2/2 (100%) Day 8: None tested Day 9: 1/4 (25%) Day 10: None tested Day 11: 0/1 (0%) Day 12: None tested Day 13: 1/1 (100%) **Max time since symptom onset of positive culture (and Ct Value, if reported):** *Asymptomatic:* NR *Symptomatic (typical symptoms):* 9 days (Ct = 23.6)†, but not tested on later samples. *Symptomatic (typical or atypical symptoms):* 13 days (Ct = 15.2)†, but not tested on later samples. **Earliest positive culture:** 6 days before symptom onset (all symptoms).	

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Basile **Australia** **Case series** **https://www.medrxiv.org/content/10.1101/2020.07.14.20153981v1**	**Population setting:** 195 COVID-19 patients (including 178 outpatients, 5 ICU patients and 12 non-ICU inpatients) **Demographics:** *Age:* Median (range)◇, 40 (18-78) years *Sex:* Male, 147 (75%) Female, 48 (25%) **Co-morbidities:** NR **Clinical characteristics:** NR **Clinical severity:** Diverse severity.	**Molecular test methods** **Test:** RT-PCR **Thresholds:** NR **Gene Targets:** E, RdRp, N, M, and ORF1ab for samples from ICU patients; and between one and four of E, RdRp, N and Orf1ab for all other samples. **Sample site(s):** URT (NP and combined nose and throat swabs) and LRT (sputum, endotracheal aspirate, non-bronchoscopic bronchoalveolar lavage).	**Viral culture methods** **Cell line:** Vero C1008 cells (Vero 76, clone E6, Vero E6 [ECACC 85020206]) **Medium and additives:** Cells were seeded with DMEM supplemented with 9% FBS. The media was changed within 12 hours for inoculation media containing 1% FBS with the addition of penicillin, streptomycin and amphotericin B deoxycholate to prevent microbial overgrowth, and then inoculated with clinical sample. **Incubation process:** The inoculated cultures were incubated at 37°C in 5% CO_2 for 5 days (day 0 to day 4) **Determination of positive culture growth:** Cells were inspected daily for CPE. SARS-CoV-2-induced CPE was confirmed by PCR of	**Viral culture outcomes** **Total number of samples where culture attempted (n=195 patient):** 234 (11 from ICU patients, 42 from non-ICU hospitalised patients and 181 from outpatients) **Positivity rate** *All samples:* 56/234 (24%) ▪ 28/181 (15%) outpatients ▪ 19/42 (45%) non-ICU hospitalised ▪ 9/11 (82%) ICU *Positivity rate per day (excluding 56 samples where time of symptom onset unknown:* Day 5: 5/9 (56%) Day 6: 5/6 (83%)	**Other relevant findings** SARS-CoV-2 was only successfully isolated from samples with Ct_{sample} values <32. SARS-CoV-2 was significantly more likely to be isolated from samples collected from inpatients and ICU patients compared with outpatients, and in samples with lower Ct_{sample} values. The highest Ct value in a clinical sample that was successfully cultured was 32 with the N gene target. The authors conclude that any clinical sample with a Ct value of ≥37 was not indicative of viable virus. Whilst 1 patient continued to be culture positive to day 18 post-symptom onset, no others were positive beyond 10 days after

			culture supernatant. Viral culture supernatant was collected on days 1, 2, 3 and 4 for the ICU cohort and on day 4 for all other samples. Day four was chosen for terminal sampling as this was the optimal time for reading the endpoint as determined by the $TCID_{50}$ assay. Where no CPE was documented, PCR was performed on day 4 to confirm absence of virus replication. Ct values of the day 4 PCR ($Ct_{culture}$) and the PCR of the original clinical sample (Ct_{sample}) were compared, and positive cultures were defined as a Ct_{sample} - $Ct_{culture}$ value of ≥3. **Sample site(s):** Respiratory (97% URT) **Timing of samples:** Symptomatic only. Day 0-29 **Serial sampling for culture attempts:** Yes.	Day 7: 9/12 (75%) Day 8: 2/2 (50%) Day 9: 2/6 (33%) Day 10: 2/4 (50%) Day 11: 0/4 (0%) Day 12: 0/2 (0%) Day 13: 0/1 (0%) Day 14: 0/2 (0%) Day 15: 0/2 (0%) Day 16: 0/6 (0%) Day 17:3/4 (75%) Day 18: 1/12 (8%) Day 19-29: 0/77 (0%) **Max time since symptom onset of positive culture (and Ct Value, if reported):** 18 days for 1 patient only (sample site unknown; VL/Ct: NR; later samples tested). **Earliest positive culture growth:** 0 days **Median time since symptom onset of all positive cultures:** 3.5 days	symptom onset. Cultures were significantly more likely to be positive from samples collected within the first week after symptom onset when compared to the second week (80% vs 45%, p=0·002), and from samples collected in the second week compared to the third week (45% vs 4%, p<0·001).

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Bullard **Canada** **Case series** **https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa638/5842165**	**Population setting:** 90 SARS-CoV-2 samples from an unknown number of patients, analysed at a provincial public health laboratory. **Demographics:** *Age:* Median (IQR), 45 (30-59) years *Sex:* Male, 44 (49%) Female, 46 (51%) **Co-morbidities:** NR **Clinical characteristics:** NR **Clinical severity:** NR	**Molecular test methods** **Test:** RT-PCR **Thresholds:** NR **Gene Targets:** E gene. **Sample site(s):** NP or ETT	**Viral culture methods** **Cell line:** Vero CCL-81 cells **Medium and additives:** Vero cells maintained in MEM supplemented with 5% FBS, 1% penicillin/streptomycin, 0.5microgram/mL Amphotericin B and 1% L-glutamine, were seeded into 96 well plates at 70% confluency. Using dilution blocks, patient samples were serially diluted 10-fold from 10^{-1} to 10^{-8} in MEM supplemented with 2% FBS, 1% penicillin/streptomycin, 0.5microgram/mL Amphotericin B and 1% L-glutamine. **Incubation process:** Dilutions were placed onto the Vero cells in triplicate and incubated at 37°C with 5% CO_2 for 96 hours **Determination of positive culture growth:** Following incubation of 4 days,	**Viral culture outcomes** **Total number of samples where culture attempted (number of patients unknown):** 90 **Positivity rate** *All samples:* 26/90 (29%) *Positivity rate per day:* Day 5: 30% Day 6: None tested Day 7: 10% **Max time since symptom onset of positive culture (and Ct Value, if reported):** Day 7 Ct/VL = NR Later samples tested, but unclear if from the same patient. **Earliest positive culture growth:** Day 0	**Other relevant findings** There was no growth in samples with a Ct >24 or symptom to test time ≥8 days. The probability of obtaining a positive viral culture peaked on day 3 and decreased from that point Multivariate logistic regression using positive culture as a predictor variable (binary result) and STT, age and gender as independent variables showed Ct as being significant (OR 0.64 95% CI 0.49-0.84, p<0.001). This implies that for every one unit increase in Ct, the odds of a positive culture decreased by 32%. Increasing symptom to test time was also significantly associated with a negative culture (OR 0.63, 95% CI 0.42-0.94, p=0.025). For

			CPE was evaluated under a microscope and recorded. **Sample site(s):** NP or ETT **Timing of samples:** Symptomatic period only (day 0 to day 21). **Serial sampling for culture attempts:** Unclear.		every 1 day increase in STT, the odds ratio of being culture positive was decreased by 37%. Authors conclude that infectivity of patients with Ct >24 and duration of symptoms ≥8 days may be low.

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Decker **Germany** **Case report** **https://onlinelibrary.wiley.com/doi/epdf/10.1111/ajt.16133**	**Population setting:** 1 hospitalised patient who had underwent heart transplant surgery **Demographics:** 62-year-old male **Clinical characteristics:** *Presentation* Fever (39.9°C), tachycardia and sore throat. **Co-morbidities:** Arrhythmogenic cardiomyopathy of right ventricle, respiratory failure, renal failure. **Clinical Severity**: Mild COVID-19.	**Molecular test methods**	**Viral culture methods**	**Viral culture outcomes**	**Other relevant findings**
		Test: RT-PCR **Thresholds:** NR **Gene Targets:** NR **Sample site(s):** Throat	**Cell line:** NR **Medium and additives:** NR **Incubation process:** NR **Determination of positive culture growth:** NR **Sample site(s):** Throat swabs. **Timing of samples:** Includes symptomatic (and convalescent) period only. Day 18 -21) **Serial sampling for culture attempts:** Yes. But only done twice.	**Total number of samples where culture attempted (n=1 hospitalised patient):** 2 **Positivity rate** *All samples*: 2/2 (100%) ▪ Day 18: positive ▪ Day 21: positive **Max time since symptom onset of positive culture (and Ct Value, if reported):** Virus culture on days 18 and 21 confirmed active virus replication. Day 18 VL = 6.4log_{10} copies/Ml Day 20 VL = 6.8log_{10} copies/Ml **Earliest positive culture:** Day 18.	Despite moderate inflammatory response in the presence of immunosuppression, SARS-CoV-2 RNA concentration did not decline significantly in comparison to the onset of infection. Authors concluded that detectable viral RNA in the oropharyngeal swab in heart transplant patient with only mild symptoms at day 35 may hint at a possible delayed PCR conversion and possible prolonged infectivity of immunosuppressed patients, thus requiring specific isolation measures in this cohort. However, patients with low copy numbers in quantitative RT-PCR may not be as infectious.

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Folgueira **Spain** **Case series** **https://www.medrxiv.org/content/10.1101/2020.06.10.20127837v1**	**Population setting:** 105 COVID-19 patients (including 55 hospitalised patients) providing 106 samples. **Demographics:** *Age, Median (IQR):* ▪ Mild disease, 42 (32-50) years. ▪ Severe disease, 64 (53-78) years *Sex:* ▪ Mild disease Male, 11 (22%) Female, 39 (78%) ▪ Severe disease Male, 36 (65%) Female, 19 (35%) **Co-morbidities:** NR	**Molecular test methods** **Test:** rRT-PCR **Thresholds:** NR **Gene Targets:** E gene **Sample site(s):** NP and bronchial aspirates	**Viral culture methods** **Cell line:** Vero E6 cells **Medium and additives:** An aliquot (250µl) of the residual sample was decontaminated using gentamicin and amphotericin B, and inoculated in 24-well plates onto Vero E6 cells and cultured in Medium 199 supplemented with L-glutamine and 10% of foetal bovine serum. **Incubation process:** Plates were incubated in a CO_2 5% atmosphere for 5 days. **Determination of positive culture growth:** The development of CPE was examined daily. SARS-CoV-2 CPE specificity was confirmed by immunofluorescence (shell-vial technique) with a human serum with a high titter of anti-RBD IgG as primary antibody, and a FITC-labelled anti-human IgG as secondary antibody. Additionally, upon CPE observation, culture supernatants were collected	**Viral culture outcomes** **Total number of samples where culture attempted (n = 105 patients):** 106 **Positivity rate** *All samples:* 49/106 (46%) *Positive rate per week after symptom onset:* ▪ Mild disease Week 1: 17/24 (71%) Week 2: 1/26 (4%) ▪ Severe disease Week 1: 14/25 (56%) Week 2: 9/15 (60%) Week 3: 6/10 (60%) Week 4 and afterwards: 2/6 (33%) **Max time since symptom onset of positive culture (and Ct Value, if reported):** Mild disease – 10 days (Ct = 37.6)[†] Severe disease – 32 days (Ct = 39.1)[†]	**Other relevant findings** Persistent SARS-CoV-2 replication could be demonstrated in severe COVID-19 cases for periods up to 32 days after the onset of symptoms and at high Ct values. In outpatients, CPE was detected in 71% (17/24) of the samples obtained in the first week after the onset of symptoms. In this group of mild COVID-19 cases the maximal STT of a CPE positive sample during follow up was 10 days. In hospitalised patients with severe COVID-19, the virus was viable in a total of 55% (31/56) of samples: 56% (14/25) of those obtained in the first week, in 60% (9/15) in the second week, in 60% (6/10) in the third week and in 33% (2/6) of samples obtained beyond

	Clinical characteristics: NR **Clinical severity:** Mild (did not require hospitalisation), 50 (48%) Severe pneumonia (hospitalised), 55 (52%)		from each well, and rRT-PCR performed and confirmed to be positive at least 3 Ct lower than that of the original sample. **Sample site(s):** NP and bronchial aspirates **Timing of samples:** Symptomatic period (from day 1 to at least day 32 after symptom onset). Outpatients consulted for their symptoms at the beginning of the clinical symptoms (median: 3 days, IQR: 3-2), while severe cases attended the Emergency Department after a variable time from the onset of symptoms (median: 6 days, IQR: 10-4). **Serial sampling for culture attempts:** No.	**Earliest positive culture growth:** Day 1 after symptom onset. **Median time since symptom onset of all positive cultures:** NR	the third week STT. There were significant differences in positive culture growth between mild and severe patients at moderate or low viral loads (Ct > 26): 22/46 (48%) versus 7/38 (18%), ($p<0.01$), respectively. Hence in patients with severe forms of COVID-19, viral replication can be detected even with moderate or low viral load for a prolonged period of time. The authors conclude that severity of disease is more important than time since symptom onset or viral load in determining viral viability.

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Jeong **South Korea** **Case series** **https://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(20)30427-4/fulltext**	**Population setting:** Five hospitalised patients **Demographics:** *Age:* Median (range), 63 (51-63) range *Sex:* Male, 3 (60%) Female, 2 (40%) **Clinical characteristics:** Pneumonia (mild, severe or critical). **Co-morbidities:** Ischaemic heart disease, 1 (20%) Schizophrenia, 2 (40%) **Clinical Severity**: Three severe, One mild, One critical	**Molecular test methods** **Test:** RT-PCR **Thresholds:** Limit of viral RNA detection qRT-PCR 0.3 log_{10} copies/mL per reaction. **Gene Targets:** S gene **Sample site(s):** NP/OP swabs, saliva, urine and faecal samples	**Viral culture methods** **Cell line:** Vero cell (ATCC, CCL-81) **Medium and additives:** MEM (Lonza) with 8% heat-inactivated FBS (Gibco) and antibiotics. The infection of Vero cells with each sample was carried out in phosphate-buffered saline containing 50 μg/mL DEAE dextran and 2% FBS. **Incubation process:** Cells were monitored daily for 4 days to examine the CPE. Thermal cycling was performed using the following conditions: initial denaturation at 95°C for 3 minutes and then 35 cycles of 95°C for 30 seconds, 56°C for 30 seconds and elongation at 72°C for 40 seconds, and final elongation step at 72°C for 5 minutes. **Determination of positive culture growth:** To confirm virus isolation, RT-PCR was performed on supernatants from infected cell cultures using S gene-specific primer sets.	**Viral culture outcomes** **Total number of samples where culture attempted (n=5 hospitalised patients):** 17 (9 respiratory samples) **Positivity rate** *All samples:* 3/17 (18%) (1 NP/OP swab and 2 saliva). *Only respiratory samples: 3/9 (33%)* **Max time since symptom onset of positive culture (and Ct Value, if reported):** Day 15 from symptom onset (VL, 1.44 ± 0.40 log_{10} copies/Ml) Not tested on later samples Patient in critical condition with respiratory failure and septic shock. **Earliest positive culture:** Day 11.	**Other relevant findings** Authors found that patients after symptom resolution shed the viable virus in their saliva and urine up to day 15 of illness. Although they failed to directly demonstrate the presence of the viable virus in urine and faecal samples using cell culture isolation, when they used a stool sample from a COVID-19 patient to inoculate naive ferrets they could isolate SARS-CoV-2 from two out of two ferrets. Authors concluded that symptom-recovered COVID-19 patients shed infectious SARS-CoV-2 in various clinical samples, including urine and stool, as well as in respiratory secretions.

			Sample site(s): NP/OP, urine, saliva, stool **Timing of samples:** Includes symptomatic period only (Days 8-30 of illness). **Serial sampling for culture attempts:** No.		

Author Country Study design Study URL	Population setting	Test Methods		Outcomes	
Kujawski (and linked unpublished data by Midgley) **USA** **Case series** **https://www.nature.com/articles/s41591-020-0877-5**	**Population setting:** 12 patients with COVID-19. **Demographics:** *Age:* Median (range), 53 (21-68) years *Sex:* Male, 8 (67%) Female, 4 (33%) **Clinical characteristics:** *Initial presentation*: Cough, 8 (67%) Fever, 7 (58%) Fatigue, 5 (42%) Shortness of breath, 1 (8%) Sore throat, 1 (8%) Headache, 3 (25%) Runny nose, 1 (8%) Chills, 1 (8%) Diarrhoea, 1(8%) Nausea, 1 (8%) **Co-morbidities:** Any underlying condition, 5 (42%)	**Molecular test methods**	**Viral culture methods**	**Viral culture outcomes**	**Other relevant findings**
		Test: rRT-PCR **Thresholds:** NR **Gene Targets:** NR **Sample site(s):** NP, OP, sputum, serum, urine, stool.	**Cell line:** Vero CCL-81 cells **Medium and additives:** Clinical samples were diluted twofold across a 96-well plate in serum-free DMEM supplemented with 2× penicillin–streptomycin and 2× amphotericin B. Vero CCL-81 cells were trypsinized and re-suspended in DMEM + 10% FBS + 2× penicillin–streptomycin + 2× amphotericin B at 2.5×10^5 cells per ml. **Incubation process:** Humidified 37°C incubator with 5% CO_2 (unclear	**Total number of samples where culture attempted (n=9 patients, including 7 hospitalised patients):** 17 **Positivity rate** *All samples:* 13/17 (76%) All 9 patients had at least 1 positive sample. *Positivity rate per day since symptom onset:* Day 5: None tested Day 6: None tested Day 7: 1/3 (33%) Day 8: 2/2 (100%) **Max time since symptom onset of positive culture (and Ct Value, if reported):** Day 8 from NP sample (Ct = 14.6) (but not tested on later samples). **Earliest positive culture:** Day 1 **Linked unpublished data by Midgley et al.** Correspondence with author (Dr Claire Midgley on 25 August 2020) provided supplementary viral culture data based on the 12 included patients plus an additional 2 patients. Notably, the researchers were able to isolate live virus in the	SARS-CoV-2 rRT–PCR Ct values of virus isolated from the first tissue culture passage ranged from 12.3–35.7. For one patient, virus isolated from tissue culture passage 3 had a titre of 7.75×10^6 median tissue culture infectious dose per ml; these data were likely more reflective of growth in tissue culture than patient viral load.

	Cardiac disease, 2 (17%) Hypertension, 2 (17%) Diabetes Mellitus, 1 (8%) Chronic lung disease, 1 (8) High cholesterol, 2 (17%) Fatty liver disease, 1 (8%) Hepatitis B, 1 (8%) **Clinical severity:** Mild to moderate, 12 (100%) (not defined) Hospitalised, 7 (58%) Requiring mechanical ventilation, 0 (0%)		duration). **Determination of positive culture growth:** When CPE was observed, presence of SARS-CoV-2 was confirmed by rRT–PCR. **Sample site(s):** NP, OP and sputum **Timing of samples:** Includes symptomatic period only. Between days 0-8 after symptom onset **Serial sampling for culture attempts:** No. First sample only.	respiratory tract up to and including 8 days post-onset, but not afterwards. The patients were all mild to moderately ill, and typically had samples collected every 2-3 days. The authors concluded that the last probability of successful isolation falls to 50% at day 4 after illness onset and to 80% at day 8. After day 9, probability approaches zero.	

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
L'Huillier **Switzerland** **Case series** **https://wwwnc.cdc.gov/eid/article/26/10/20-2403_article**	**Population setting:** 23 hospitalised symptomatic neonates, children and teenagers with lab-confirmed COVID-19 (aged <16 years) **Demographics:** *Age:* Median (IQR), 12 (3.8-14.5) years *Sex:* Female, 12 (52%) Male, 11 (48%) **Co-morbidities:** NR **Clinical characteristics:** *Presentation* Cough, 16 (70%) Nasal discharge, 16 (70%) Shortness of breath, 7 (30%) Fever, 15 (65%) Arthralgia, 5 (22%) Myalgia, 7 (30%) Nausea, 3 (13%) Vomiting, 2 (9%) Diarrhoea, 5 (22%)	**Molecular test methods**	**Viral culture methods**	**Viral culture outcomes**	**Other relevant findings**
		Test: RT-PCR **Thresholds:** NR **Gene Targets:** E gene **Sample site(s):** NP	**Cell line:** Vero E6 cells **Medium and additives:** Vero E6 cells were seeded at 8 × 10^4 cells/well in a 24-well plate and inoculated with 200µL of viral transport medium the following day. **Incubation process:** Cells were inoculated for 1 hour at 37°C; inoculum was removed; cells were washed once with phosphate buffered saline; and regular cell growth medium containing 10% foetal calf serum was added (max duration of 6 days). **Determination of positive culture growth:** Cells were observed on days 2, 4, and 6 for CPE	**Total number of samples where culture attempted (n=23 patients, including 7 hospitalised patients):** 23 **Positivity rate** *All samples:* 12/23 (52%) Day 5: 1/1 (100%) **Max time since symptom onset of positive culture (and Ct Value, if reported):** Day 5 from NP sample, VL = 1.4 × 10^5 viral RNA copies/mL (but not tested on later samples). **Earliest positive culture:** Day 1	Virus isolation was successful in 12/23 (52%) of the children. The youngest patient that SARS-CoV-2 was isolated from was a 7-day old neonate. No correlation between disease presentation and success of virus isolation was observed. Virus isolation was successful from NP samples from all age groups, with a median initial VL of 1.7x10^8 copies/ml (mean 7.9x10^8, IQR 4.7x10^6-1.0x10^9). Viral loads were higher in NP samples that were culture positive compared with those that were culture negative. Sex, age, duration of symptoms, clinical diagnosis, symptoms, and likelihood of admission did not differ between patients with and without isolation. The authors concluded that initial viral loads at diagnosis in

	Abdominal pain, 6 (26%) Anosmia, 4 (17.4%) Headache, 10 (44%) Fatigue, 12 (52%) Rash, 4 (17%) **Clinical Severity**: Most patients were managed as outpatients and self-isolated at home, 7 were hospitalised.		by light microscopy. The supernatant was harvested at first observation of CPE or, if no CPE occurred, on day 6. SARS-CoV-2 replication in all positive isolates was confirmed by a second passage. For a second passage, 20µL supernatant of CPE-positive samples was transferred onto new Vero E6 cells. Supernatant was collected after inoculation and on observation of CPE and confirmed isolation of replication competent SARS-CoV-2 by an increase in viral RNA. **Sample site(s):** NP **Timing of samples:** Included symptomatic period only. Between days 0-5 of symptom onset (median of 2 days after symptom onset). **Serial sampling for culture attempts:** No.		symptomatic children is comparable to those seen in adults. Infectious virus isolation success was largely comparable to that of adults, although two samples yielded an isolate at a lower viral load ($1.2x10^4$ and $1.4x10^5$ copies/ml) than what was observed in adults.

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Liu **Taiwan** **Case report** **https://www.ncbi.nlm.nih.gov/pubmed/32283147**	**Population setting:** 1 hospitalised patient **Demographics:** 50-year-old female **Clinical characteristics:** *Presentation* Acute onset fever **Co-morbidities:** NR **Clinical Severity**: Mild	**Molecular test methods** **Test:** RT-PCR **Thresholds:** NR **Gene Targets:** RdRp, E and N genes **Sample site(s):** Sputum (or gargle when not available), throat, stool and plasma.	**Viral culture methods** **Cell line:** Vero E6 and LLC-MK2 cells **Medium and additives:** NR **Incubation process:** NR **Determination of positive culture growth:** NR **Sample site(s):** Sputum (or gargle when not available) and throat. **Timing of samples:** Includes symptomatic (and convalescent) periods. Range of sampling period not reported. **Serial sampling for culture attempts:** Yes. Every second day.	**Viral culture outcomes** **Total number of samples where culture attempted (n=1 hospitalised patient):** NR **Positivity rate** *All samples:* NR *Throat sample:* All negative beyond time of admission. *Sputum (or gargle):* Positive detection up to day 18 since symptom onset (exact timing of samples NR). **Max time since symptom onset of positive culture (and Ct Value, if reported):** Day 18 from sputum sample (VL = $10^{5.3}$ viral RNA copies/Ml (E gene)[†]. Later samples tested, but unclear how many. **Earliest positive culture:** NR	**Other relevant findings** SARS-CoV-2 persisted to be detectable on 63rd day of symptoms, despite sero-conversion (antibody unspecified) on day 10 of symptom onset. Authors concluded that the contagious period of COVID-19 might last more than one week after "clinical recovery".

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Perera **Hong Kong** **Case series** **https://wwwnc.cdc.gov/eid/article/26/11/20-3219_article**	**Population setting:** 35 hospitalised patients (patients with prolonged virus shedding and patients readmitted because RT-PCR positivity was detected after discharge were oversampled). **Demographics:** *Age:* Median (range), 38 (17-75) years *Sex:* Male, 23 (66%) Female, 12 (34%) **Clinical characteristics:** NR **Co-morbidities:** Diabetes mellitus, hypertension, ischemic heart disease, atrial fibrillation, chronic obstructive airways	**Molecular test methods** **Test:** RT-PCR **Thresholds:** 1 log_{10} copies/mL **Gene Targets:** N **Sample site(s):** NP, throat, sputum, saliva.	**Viral culture methods** **Cell line:** Vero E6 cells (ATCC-ORL-1586) **Medium and additives:** Vero E6 cells were seeded at a cell count of 150,000 cells/well into 24-well tissue culture plates sufficient to give a subconfluent cell monolayer after incubation for 24 hours in a CO_2 incubator. The culture medium was removed and 125µL of the clinical sample in virus transport medium diluted 1:1 in DMEM containing 2% FBS (GIBCO) was inoculated into 2 wells. **Incubation process:** CO_2 incubator at 37°C. A sample (100µL) of supernatant was sampled for a qRT-PCR at 0 and 72 hours post-inoculation. At 72 hours, cells were scraped into the supernatant and	**Viral culture outcomes** **Total number of samples where culture attempted (n=35 hospitalised patient):** 68 **Positivity rate** *All samples:* 16/68 (24%) **Max time since symptom onset of positive culture (and Ct Value, if reported):** 7 days VL 7 log_{10} copies/Ml unclear if later samples tested† **Earliest positive growth:** 0 Days after symptom onset†	**Other relevant findings** A subset analysis was conducted for 42 samples collected from patients who did not receive antiviral drugs or samples that were collected before antiviral therapy. This sample included all 16 samples that were culture positive and 18 of 19 samples that were sgRNA positive. The median VL in culture-positive samples was 7.5 log_{10} copies/mL. Median viral RNA load (measuring sgRNA) in culture-positive samples was 7.54 log_{10} genome copies/mL and in culture-negative samples was 4.0 log_{10} genome copies/mL. Of the 16 culture positive samples, 15 (94%) had viral RNA load >6 log_{10} copies/mL. All were collected within the first 8 days of illness. However,

	disease, carcinoma of the lung, mild renal dysfunction, and chronic hepatitis B. **Clinical Severity**: Mild disease, 29 (83%) Asymptomatic, 3 (9%) Critically ill/died, 3 (9%)		transferred onto fresh cells in 24-well plates, followed by refeeding the cells with fresh culture medium and monitoring for an additional 72 hours. **Determination of positive culture growth:** A final aliquot was collected for qRT-PCR. Cells were observed for CPE daily and harvested for passage if 25%–50% of cells showed a CPE. **Sample site(s):** NP aspirates and throat swab samples (n = 46), NP aspirates (n = 2), NP swab samples and throat swab samples (n = 4), NP swab samples (n = 3), sputum (n = 11), and saliva (n = 2). **Timing of samples:** Includes symptomatic period only. The duration after onset of illness to sample collection ranged from 0 to 67 days after symptom onset.[†] 22% of samples (n=36) were collected 1-2 days after symptom onset. 40% (n=17) were collected 3-8 days after symptom onset and 24% (n=10) were collected >9		duration of illness in this subset was limited to 31 days. Five samples with viral load >6 log_{10} virus N gene copies/mL collected >50 days after onset of illness were negative by virus culture and virus sgRNA (all of these patients had received antiviral therapy). Detection of virus sgRNA was attempted for 33 of the 35 the clinical samples that had viral loads >5.0 log_{10} virus genome copies/mL. Virus sgRNA was detectable in 18 (82%) of 22 samples collected <8 days after symptom onset and in 1 (9%) of 11 samples collected >9 days after onset of disease. Virus was isolated from 12 of 17 samples with viral loads ≥7.0 log_{10} copies/mL, 3 of 11 samples with viral loads 6.0–6.99 log_{10} copies/mL, 1 of 7 samples with viral loads 5.0–5.99 log_{10} copies/mL, and 0 of 33 samples viral loads <5 log_{10} copies/mL. The authors concluded that patients with mild or

			days after symptom onset. **Serial sampling for culture attempts:** Yes. A total of 14 patients were sampled sequentially: 2–6 samples were collected from each patient at 3–4 day intervals. However unclear which patients had serial culturing undertaken.		moderate illness might be less contagious 8 days after symptom onset. Mildly ill patients who have clinically recovered and are not immunocompromised might be discharged from containment >9 days after symptom onset, as long as they are not being discharged into settings that contain other highly vulnerable persons (e.g. old age care homes).

<table>
<tr><th>Author
Country
Study design
Study URL</th><th>Population setting</th><th colspan="2">Test methods</th><th colspan="2">Outcomes</th></tr>
<tr><td rowspan="2">Singanayagam
UK
Case series
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.32.2001483</td><td rowspan="2">Population setting:
253 COVID-19 cases providing 324 samples. Range of clinical scenarios, including community and healthcare worker surveillance, symptomatic persons tested as part of early epidemic response, and samples acquired in outbreak investigations.
Demographics:
Sex: NR
Age: (N cases)
0-20 years: 14
21-40 years: 81
41-60 years: 140
61-80 years: 40
81-100 years: 49
Co-morbidities:
NR</td><td>Molecular test methods</td><td>Viral culture methods</td><td>Viral culture outcomes</td><td>Other relevant findings</td></tr>
<tr><td>Molecular Test:
RT-PCR
Thresholds:
NR
Gene Targets:
RdRp
Sample site(s):
Nose, throat, combined nose-and-throat and NP swabs, or NP aspirates.</td><td>Cell line:
Vero E6
Medium and additives:
NR
Incubation process:
Incubated at 37°C, 5% CO_2 for up to 14 days.
Determination of positive culture growth:
Inspected for CPE daily up to 14 days. Presence of SARS-CoV-2 confirmed by nucleoprotein staining by enzyme immunoassay on infected</td><td>Total number of samples where culture attempted:
324 samples from 253 cases.
Positivity rate
All samples: 133/324 (41%)
• Where cases asymptomatic: 21/62 (34%)
• Where cases symptomatic: 112/262 (43%)
• Where symptom to test interval known: 103/246 (42%).
Percentage of patients for whom virus could be isolated and percentage of patients asymptomatic, by age-group.
<table>
<tr><th>Age-group</th><th>N samples</th><th>Estimated[Δ] % culture-positive (95% CI)</th><th>Estimated[□]% asymptomatic (95% CI)</th></tr>
<tr><td>0-20</td><td>14</td><td>57.8% (26.7- 83.8)</td><td>14.3% (3.0-47.3)</td></tr>
<tr><td>21-40</td><td>81</td><td>43.2% (30.7-56.5)</td><td>17.5% (10.0-28.9)</td></tr>
<tr><td>41-60</td><td>140</td><td>37.7% (27.8-48.7)</td><td>13.6% (8.6-20.8)</td></tr>
<tr><td>61-80</td><td>40</td><td>41.3% (24.4-60.5)</td><td>17.5% (7.8-34.6)</td></tr>
<tr><td>81-100</td><td>49</td><td>32.1% (18.8-49.2)</td><td>40.8% (27.4-55.7)</td></tr>
</table>
[Δ]Random intercept logistic regression
[□]Cluster adjusted logistic regression
Observed and estimated probability of positive viral culture at each of days 7-15 (n=121 samples)</td><td>The culture positivity rate was significantly higher in week 1 than week 2 (74% versus 20%, p=0.002).

13 individuals who were asymptomatic at the time of sampling developed symptoms within 14 days of sampling and were classified as presymptomatic; 7 among these were culture-positive.</td></tr>
</table>

Clinical characteristics: Only severity reported.

Clinical severity: 233 cases (92%) were classified as non-severe (asymptomatic or mild-moderate) and 20 (8%) had severe illness (ICU admission and or fatal).

cells.

Sample site(s): URT

Timing of samples: Includes pre-symptomatic and symptomatic periods (ranging from 13 days before symptom onset to 60 days after symptom onset)[†]

Serial sampling for culture attempts: NR

Day post symptom onset	Estimated[¥] % positive (95% CI)	Observed samples culture positive/samples tested
7	40.1 (22.8-60.4)	10/14
8	25.8 (11.0-49.4)	9/33
9	13.7 (3.7-39.6)	10/34
10	6.0 (0.9-31.2)	6/23
11	2.2 (0.2-23.9)	1/6
12	0.7 (0.0-17.9)	1/3
13	0.2 (0.0-13.1)	0/4
14	0.03 (0.0-9.4)	0/2
15	0.006 (0.0-6.7)	0/2
		Total: 37/121

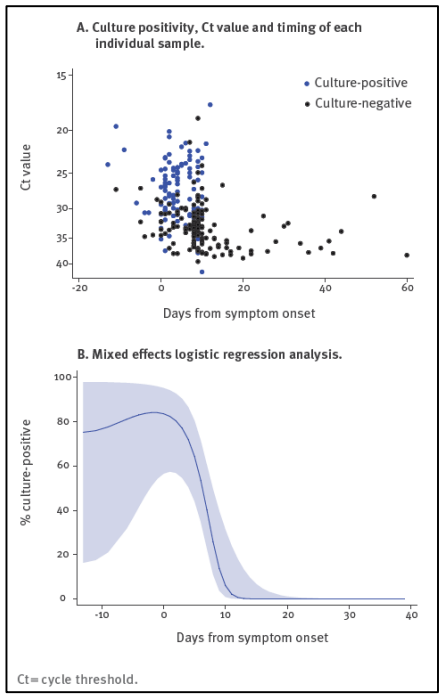
[¥]Mixed effects logistic regression. Estimated percentage of samples culture positive

Max time since symptom onset of positive culture (and Ct Value, if reported):
Max day of culture positivity: Day 12 (Ct =18.7)[†]
See scatterplot below displaying Ct value versus days on which positive culture achieved. In the first week of symptom onset (days -2 to 7), the geometric mean Ct was 28.8 (95% CI 27.76-28.61) while in the second week (days 8 to 14) the geometric mean Ct was 30.65 (95% CI 29.82-31.52). These values were significantly different (p<0.001).

Late culture positive samples:
More than half of the samples (n=130, 53% samples, derived from 91 cases) were received more than 7 days after symptom onset; 21% of these were culture positive and 25 out of these 27

No difference in culture positivity between asymptomatic and symptomatic cases (OR=0.66, 95% CI 0.34-1.31, mixed effects logistic regression model p=0.23).

A strong relationship between Ct value and ability to recover infectious virus was observed. The estimated OR of recovering infectious virus decreased by 0.67 for each unit increase in Ct value (95% CI:

				culture positive samples were taken between 8 and 10 days after symptom onset. None of these were in immunosuppressed or severely ill patients. **Median (IQR) time since symptom onset of all positive cultures:** 4 days (IQR: 1 – 8; range: -13 to 12). **Earliest positive culture:** 13 days before symptom onset. **Culture positivity and time between symptom onset and sample collection, for samples where the date of symptom onset was known:** (N= 246 samples from 176 symptomatic cases.)  	0.58–0.77) Virus propagation was successful from five of 60 samples with Ct > 35; all five were from symptomatic cases and none had severe illness. The estimated probability of recovery of virus from samples with Ct > 35 was 8.3% (95% CI: 2.8%–18.4%).

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Van Kampen **The Netherlands** **Case series** **https://www.medrxiv.org/content/10.1101/2020.06.08.20125310v1**	**Population setting:** 129 hospitalised patients with severe or critical COVD-19 providing 690 samples **Demographics:** *Sex:* Male, 86 (67%) Female, 43 (33%) *Age:* All: Median 65 (IQR 57-72) years Intensive care: 66 (57-72) Medium care: 63 (57-74) p-value ICU versus ward, p=0.90 **Co-morbidities:** 30 patients were immunosuppressed (23%) of whom 19 (15%) were non-severely immunocompromised and 11 (8.5%) were severely immunocompromised. **Clinical characteristics:** Median duration of illness (IQR): Overall: 18 days (13-21) Intensive care: 18 (13-22)	**Molecular test methods**	**Viral culture methods**	**Viral culture outcomes**	**Other relevant findings**
		Molecular Test: RT-PCR **Thresholds:** NR **Gene Targets:** NR **Sample site(s):** Swabs from URT and sputum from lower respiratory tract	**Cell line:** Vero, clone 118 **Medium and additives:** IMDM, L-glutamine, pencillin, streptomycin, amphotericin B, FBS. **Incubation process:** Samples cultured at 37°C and 5% CO_2 for 7 days. Each sample cultured in triplicate; 2 samples were fixed with ice-cold acetone after 24 hours and 48 hours, respectively, if CPE was visible. The remaining sample was scored for CPE on a daily basis for 7 days. **Determination of positive culture growth:** Cultures were regarded as negative if no CPE was visible during 7 days. Once CPE was visible, the presence of SARS-CoV-2 was confirmed with	**Total number of samples where culture attempted (n=129 patients):** 690 samples 127/690 (18%) were from immunocompromised patients. **Positivity rate** 62/690 samples (9%), 23/129 patients (18%). **Max time since symptom onset of positive culture (and Ct Value, if reported):** Max day: 20 (VL = 7.4 Log_{10} RNA copies/Ml; unclear if later sample tested).[†] Probit analysis showed a probability of ≤5% for isolating infectious SARS-CoV-2 when the duration of symptoms was 15.2 days (95% CI 13.4 – 17.2). **Median (IQR) time since symptom onset of all positive cultures:** 8 days (IRQ 5-11, range 0-20) **Earliest positive culture**	The median viral load was significantly higher in culture positive samples than in culture negative samples (8.14 versus 5.88 Log_{10} RNA copies/mL, p<0.0001) and the probability of isolating infectious SARS-CoV-2 was less than 5% when the viral load was below 6.63 Log_{10} RNA copies/mL (95% CI 6.24 – 6.91). A viral load exceeding 7 Log_{10} RNA copies/mL, less than 7 days of symptoms, absence of serum neutralizing antibodies and being immunocompromised were all associated with a positive virus culture in univariate analysis. After submitting all these

	Medium care: 15 (12-18) p-value ICU versus ward, p=0.009 **Clinical severity**: Intensive care: N=89 (69.0%) Medium care: N=40 (31.0%) 81 of the 89 patients in intensive care received mechanical ventilation (91% ICU patients). 8 of the intensive care patients (9%) and 35 of the medium care patients (88%) received supplemental oxygen. 14 patients died (11%), 11 in intensive care (12%) and 3 in medium care (8%)		immunofluorescent detection of nucleocapsid proteins. **Sample site(s):** Obtained from the lower respiratory tract (sputum) on the ICU (538/690 samples, 78%) and from the upper respiratory tract (swabs) on the ICU as well as on the medium care unit (152/690 samples, 22%). **Timing of samples:** Includes symptomatic period only (Ranging from 0-39 days after symptom onset)† **Serial sampling for culture attempts:** Yes. Total (mean) culture tests per person: All: 690 (5.2) Intensive care: 601 (6.8) Medium care: 89 (2.2)	**growth:** Day 0 since symptom onset†	variables into a multivariate analysis, only a viral load above 7 Log_{10} RNA copies/mL and absence of serum neutralizing antibodies were independently associated with isolation of infectious SARS-CoV-2 from the respiratory tract. Infectious virus could not be isolated from respiratory tract samples once patients had a serum neutralizing antibody titre of at least 1:80.

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Wölfel **Germany** **Case series** **https://pubmed.ncbi.nlm.nih.gov/32235945/**	**Population setting:** 9 cases (samples taken from inpatients). **Demographics:** Described as young-to-middle-aged professionals. **Co-morbidities:** Hypothyroidism, 1 (11%) COPD, 1 (11%) Hypercholesterolemia, 1 (11%) None, 6 (67%) **Clinical characteristics:** *Initial presentation*: Cough, 6 (67%) Fever, 2 (22%) Diarrhoea, 2 (22%) Sinusitis, 4 (44%) Cephalgia, 1 %11%) Arthralgia, 1 (11%) Otitis, 1 (11%) Rhinitis, 1 (1%) No symptoms, 1 (11%). **Clinical severity:**	**Molecular test methods**	**Viral culture methods**	**Viral culture outcomes**	**Other relevant findings**
		Test: qRT-PCR **Thresholds:** 10^2 copies/ml **Gene Targets:** E- and RdRp **Sample site(s):** OP, NP, sputum urine, serum, stool.	**Cell line:** Vero E6 **Medium and additives:** Vero E6 cells were seeded on a 24-well plate at 3.5×10^5 cells/mL in DMEM containing 1% sodium pyruvate, 1% nonessential amino acids, 1% l-glutamine, and 10% FCS 1 day prior to inoculation. Sputum and swabs from patients were diluted in 2ml OptiPro serum-free medium. Vero E6 cells were inoculated with 100 µl processed patient sample at 37°C for 1 hour. Then, cells were washed once with phosphate-buffered	**Total number of samples where culture attempted (n=9 hospitalised patients):** 43 (34 respiratory samples) **Positivity rate** ▪ *All samples:* 9/43 (21%) ▪ *Respiratory samples:* 9/34 (26%) ▪ *Stool samples:* All negative ▪ *NP/OP swabs:* All negative ≥Day 5 ▪ *Sputum samples:* 5/14 (35.7%) positive ≥Day 5; *Positivity rate per day (respiratory samples only)* Day 5: 1/6 (17%) Day 6: 1/4 (25%) Day 7: 1/3 (33%) Day 8: 2/6 (33%) Day 9: 0/2 (0%) Day 10: 0/2 (0%) Day 11: 0/3 (0%) Day 12: 0/1 (0%) Day 13: 0/1 (0%) **Max time since symptom onset of positive culture**	Infectious virus was readily isolated from throat- and lung-derived samples, but not from stool samples in spite of high and prolonged viral RNA concentration. No infectious isolates were obtained from any sample taken after day 8 in spite of ongoing high viral loads. The success of virus isolation also depended on viral load: samples that contained $<10^6$ copies per ml (or copies per sample) never yielded an isolate. Levels of viral subgenomic mRNA were compared against viral genomic RNA in the same sample. In sputum samples taken on day 4 to day 9, during which time active replication in sputum was obvious in all patients as per longitudinal viral load courses, the ratios of mean normalized subgenomic mRNA per

	Mild (not defined).		saline and supplied with 500µl DMEM composite as described above, except for a reduced FCS content of 2% and 1% amphotericin B. **Incubation process:** Cells were controlled daily for CPE for 6 days. **Determination of positive culture growth:** Every 2 days or upon observation of cytopathogenic effects, 50µl of cell culture supernatant was subjected to viral RNA extraction and SARS-CoV-2 specific real-time RT-PCR using the SARS-2-CoV E assay. **Sample site(s):** OP, NP, sputum, stool. **Timing of samples:** Symptomatic only. From day 3 to day 13 after symptom onset. **Serial sampling for culture attempts:** Yes.	**(and Ct Value, if reported):** Day 8 from sputum sample (VL or Ct: NR). Later samples tested, but unclear if later samples from this same patient were tested. **Earliest positive culture:** Day 3 **Fig c Projected virus isolation success based on probit distributions. The inner lines are probit curves (dose–response rule). The outer dotted lines are 95% confidence interval.** Probit analysis showed a probability of viable culturing of ≤5% at 9.78 days post symptom onset (95% CI 8.45-21.78).	genome were about 0.4%. A decline occurred from day 10 to day 11. In throat swabs, all samples taken up to day 5 were in the same range, whereas no subgenomic mRNA was detectable in swabs thereafter. Together, these data indicate the active replication of SARS-CoV-2 in the throat during the first five days after the onset of symptoms. The authors suggest that early discharge with ensuing home isolation could be chosen for patients who are beyond day 10 of symptoms with less than 100,000 viral RNA copies per ml of sputum.

Key: ARDS, Acute respiratory distress syndrome; CI, Confidence intervals; CPE, Cytopathic effect; COVID-19, Coronavirus disease 2019; Ct, Cycle threshold; DEAE, Diethylaminoethyl; DMEM, Dulbecco's modified eagle medium; ETT, Endotracheal tube aspirate; FBS, Fetal bovine serum; FITC, Fluorescein isothiocyanate; ICU, Intensive care unit; IFA, Immunofluorescence antibody; IgG, Immunoglobulin G; IMDM, Iscove's modified Dulbecco's media; IQR, Interquartile range; LRT, Lower respiratory tract; MEM, Modified eagles medium; NP, Nasopharyngeal; NR, Not reported; OP, Oropharyngeal; OR, Odds ratio; PPE, Personal protective equipment; (q)(r)RT-PCR – (quantitative)(real-time) reverse transcriptase polymerase chain reaction; RdRp, Ribonucleic acid-dependent ribonucleic acid polymerase; RNA, Ribonucleic acid; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SD, Standard deviation; STT, Symptom onset to test; TCID50, Tissue Culture Infectious Dose 50 assay; URT, Upper respiratory tract; VL, Viral load; WHO, World Health Organization.

[†]Data extracted from graphs using webplot digitiser https://automeris.io/WebPlotDigitizer/
[≠]Positivity rate per day is only provided from 5 day after symptom onset onwards, in order to focus on the upper limit of positive culture growth
[◊]Inconsistency noted in this paper: lower range reported as 8 and 18 in different places, unclear which is correct
[‡]Data analysed using online data calculator https://www.statstodo.com/CombineMeansSDs_Pgm.php

Appendix 2: Contact tracing studies

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Cheng **Taiwan** **Prospective case-ascertained study** **https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/2765641**	**Population setting:** 100 confirmed COVID-19 cases and 22 paired cases (index-secondary cases) identified from 2,761 close contacts. **Demographics (n=100 confirmed cases):** *Age:* Median (range), 44 (11-88) years *Sex:* Male, 56 (56%) Female, 44 (44%) **Demographics (n=22 secondary cases):** NR **Co-morbidities** NR **Clinical characteristics:** NR **Clinical severity (WHO definition):** *Index cases (n=22)* Asymptomatic, 0 (0%) Mild, 4 (18%) Mild pneumonia, 5 (23%)	**Molecular test methods** **Test:** RT-PCR **Thresholds:** NR **Gene Targets:** NR **Sample site(s):** NR	**Epidemiological investigation methods** **Patient recruitment:** All contacts of the initial 100 confirmed cases in Taiwan were followed up until 14 days after their last exposure to the index case. **Definition of close contact:** Did not wear appropriate PPE while having face-to-face contact with a confirmed case for >15 minutes during the investigation period. **Investigation period:** For symptomatic patients this period ranged from time of symptom onset, or up	**Epidemiological investigation outcomes** **Secondary clinical attack rate (95% CI):** ▪ Exposure to index cases started within 5 days of symptom onset: 22 from 1818 close contacts, 1% (0.6%-1.6%) ▪ Exposure to index case started after 5 days of symptom onset: (0 cases from 852 close contacts, 0% (0%-0.4%) ▪ Time from onset of symptoms in index case to first exposure in close contact: ○ <0 days: 10 cases from 735 close contacts, 1% (0.5%-2.0%) ○ 0-3 days: 9 cases from 867 close contacts, 1% (0.5%-1.8%) ○ 4-5 days: 3 cases from 216 close contacts, 1%	**Other relevant findings** The overall secondary clinical attack rate was 22 of 2,761, or 0.7% (95% CI, 0.4%-1.0%). All of the 22 secondary cases had their first exposure before the sixth day of the index case's symptom onset. Hence, suggesting high transmissibility near, or even before symptom onset. The 735 contacts whose initial exposure occurred before symptom onset of the index case were also at risk, with a secondary clinical attack rate of 1% (95% CI, 0.5%-2.0%). The 299 contacts with exclusive pre-symptomatic exposures were also at risk (attack

	Severe pneumonia, 7 (32%) ARDS, Sepsis, 6 (27%) *Secondary cases (n=22):* NR		to 4 days before when epidemiologically indicated and finished at time of COVID-19 confirmation. For asymptomatic patients this period was based on date of confirmation and determined according to the epidemiological investigation.	(0.5%-4.0%) ○ 6-7 days: 0 cases from 119 close contacts, 0% (0%-3.1%) ○ 8-9 days: 0 cases from 449 close contacts, 0% (0%-0.9%) ○ >9 days: 0 cases from 284 close contacts, 0% (0%-1.3%)	rate, 0.7% [95% CI, 0.2%-2.4%]). None of the 9 asymptomatic case patients transmitted a secondary case. The authors suggest that the rapid reduction of transmissibility over time implies that prolonged hospitalisation of mild cases might not be necessary in large epidemics.

Author Country Study design Study URL	Population setting	Test methods		Outcomes	
Lopez Bernal **UK** **Prospective case-ascertained study** **https://www.medrxiv.org/content/10.1101/2020.08.19.20177188v1**	**Population setting:** Contact tracing study of 269 lab-confirmed COVID-19 primary cases, who lived with at least 1 other person, and their 472 household contacts, in whom 161 were found to be probable (n=96) or confirmed (n=65) secondary cases. Of the 161 probable or confirmed cases, a total of 45 were identified as having a point source exposure, that is, an exposure window of maximum 1 day, and were identified for analysis of timing of exposure relative to primary case symptom onset. **Demographics (n=472 household contacts):** *Age:* <19 years, 175 (37.1%) 19-64 years, 279 (59.1%) ≥ 65 years, 18 (3.8%) *Sex:* Male, 231 (48.9%) Female, 241 (51.1%) **Demographics (n=65 lab-confirmed secondary cases):** *Age:* <19 years, 9 (13.8%) 19-64 years, 50 (76.9%) ≥ 65 years, 6 (9.2%) *Sex:* Male, 31 (47.7%) Female, 34 (52.3%)	**Molecular test methods**	**Epidemiological investigation methods**	**Epidemiological investigation outcomes**	**Other relevant findings**
		Test: RT-PCR **Thresholds:** NR **Gene Targets:** NR **Sample site(s):** NR	**Patient recruitment:** All household contacts (in houses with at least two household members) of 269 confirmed cases co-habiting with others. **Definition of close contact:** Household contacts were defined as those living or spending significant time in the same household. **Investigation period:** 14 days after the onset of symptoms in the index case.	Among contacts with a point source exposure, 45 probable or lab-confirmed cases were identified. 41 had data available to allow analysis of timing of exposure. ▪ Probable and lab-confirmed secondary cases (N=41) were exposed a mean of 2.37 days (standard deviation (SD) 3.36) and a median of 1 day (interquartile range (IQR) 0-4) after symptom onset in the index case, ranging from 0-14 days. ▪ Restricting to lab-confirmed secondary cases only (N=12), exposure occurred a mean of 1.33 days (SD 1.61) and a median of 1 day (IQR 0-1.25) after symptom onset in the	Secondary attack rates (SARs) were lowest in contacts aged under 18 years or aged 65 years and over; however, these differences were not statistically significant.

	Demographics (n=96 probable secondary cases): *Age:* <19 years, 33 (34.4%) 19-64 years, 63 (65.6%) ≥ 65 years, 0 (0%) *Sex:* Male, 50 (52.1%) Female, 46 (47.9%) **Co-morbidities (n=472 household contacts):** Any comorbidity, 60 (12.7%) Asthma requiring medication, 25 (5.3%) Respiratory disease excluding asthma, 10 (2.1%) Diabetes, 7 (1.5%) Heart disease, 7 (1.5%) Immunodeficiency, 6 (1.3%) Malignancy, 5 (1.1%) Neurological disease, 5 (1.1%) Kidney disease, 4 (0.8%) Unknown, 35 (7.4%) **Clinical characteristics (n=472 household contacts):** *Presentation:* Cough, 26.4% Fatigue, 21.1% Headache, 19.3% Runny nose, 15.0% Sore throat, 14.5% Muscle ache, 14.5% Fever, 13.9% Sneezing, 13.6% Loss of appetite, 13.0% Anosmia, 11.2% Shortness of breath, 10.7% Joint ache, 9.3%			index case, ranging from 0-5 days. ▪ Non-cases were exposed a mean of 2.71 days (SD 2.74) and a median of two days (IQR 0-5) after symptom onset in the index case, ranging from 0-9 days. **Secondary clinical attack rate (95% CI):** ▪ The household SAR was 37% (95% CI, 31%-43%) including both confirmed or probable secondary cases. ▪ If restricted to confirmed secondary cases only, the SAR was 16% (95% CI, 11%-20%).	

	Nausea, 6.1% Diarrhoea, 5.7% Nose bleed, 2.0% Vomiting, 1.6% Rash, 0.9% Altered consciousness, 0.7% **Clinical severity:** NR				

Key: ARDS, Acute respiratory distress syndrome; CI, Confidence intervals; COVID-19, Coronavirus disease 2019; ICU, Intensive care unit; IQR, Interquartile range; NR, Not reported; OR, Odds ratio; PPE, Personal protective equipment; (q)(r)RT-PCR – (quantitative)(real-time) reverse transcriptase polymerase chain reaction; SAR, secondary attack rate; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SD, Standard deviation; UK, United Kingdom; WHO, World Health Organization.

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