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Viral Cultures for Coronavirus Disease 2019 Infectivity Assessment: A Systematic Review

Tom Jefferson,[1] Elisabeth A. Spencer,[2] Jon Brassey,[3] and Carl Heneghan[2]

[1]Department of Continuing Education, University of Oxford, Oxford, United Kingdom; [2]Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, United Kingdom; and [3]Trip Database Ltd, Bristol, United Kingdom

(See the Editorial Commentary by Doshi and Powers on pages e3900–1.)

Background. We aimed to review the evidence from studies relating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) culture with the results of reverse-transcription polymerase chain reaction (RT-PCR) and other variables that may influence the interpretation of the test, such as time from symptom onset.

Methods. We searched LitCovid, medRxiv, Google Scholar, and the World Health Organization coronavirus disease 2019 (COVID-19) database for COVID-19 up to 10 September 2020. We included studies attempting to culture or observe SARS-CoV-2 in specimens with RT-PCR positivity. Studies were dual-extracted and the data summarized narratively by specimen type. Where necessary, we contacted corresponding authors of included papers for additional information. We assessed quality using a modified Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS 2) risk-of-bias tool.

Results. We included 29 studies reporting attempts at culturing, or observing tissue infection by, SARS-CoV-2 in sputum, nasopharyngeal or oropharyngeal, urine, stool, blood, and environmental specimens. The quality of the studies was moderate with lack of standardized reporting. The data suggest a relationship between the time from onset of symptom to the timing of the specimen test, cycle threshold (Ct), and symptom severity. Twelve studies reported that Ct values were significantly lower and log copies higher in specimens producing live virus culture. Two studies reported that the odds of live virus culture were reduced by approximately 33% for every 1-unit increase in Ct. Six of 8 studies reported detectable RNA for >14 days, but infectious potential declined after day 8 even among cases with ongoing high viral loads. Four studies reported viral culture from stool specimens.

Conclusions. Complete live viruses are necessary for transmission, not the fragments identified by PCR. Prospective routine testing of reference and culture specimens and their relationship to symptoms, signs, and patient co-factors should be used to define the reliability of PCR for assessing infectious potential. Those with high Ct are unlikely to have infectious potential.

Keywords. COVID-19; mode of transmission, viral culture; symptom onset to test date; polymerase chain reaction; SARS-CoV-2.

Effective prevention and management of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections relies on our capacity to identify those who are infected or potentially infectious. In the absence of predictive clinical signs or symptoms, the major means of detection is testing using reverse-transcription quantitative polymerase chain reaction (RT-qPCR) [1–3].

The test amplifies genomic sequences identified in specimens and is highly sensitive, being capable of generating observable signals from specimens containing minute amounts of matching genomic sequence. Amplification of genomic sequence is measured in cycle threshold (Ct), each cycle being a cutoff for positive detection. There may be a correlation between Ct values from respiratory specimens, symptom onset to test (STT) date, and positive viral culture. Evidence suggests the lower the Ct value and the shorter the STT, the higher the infectious potential [4] If this is so, we should be able to identify those with the highest infectious potential.

Identification of a whole virion (as opposed to fragments) and proof that the isolate is capable of replicating its progeny in culture cells is the closest we are likely to get to a gold standard [5]. RT-qPCR cannot distinguish between the shedding of live virus or of viral fragments with no infectious potential, and it cannot measure the quantity of live virus present in a person's excreta. Although viral culture is difficult, time consuming, and requires specialized facilities, it potentially represents the best indicator of infection and infectious potential. We therefore set out to review those studies attempting viral culture, regardless of specimen type tested. We investigated the probability of successful culture with time from STT and Ct. We also examined the relationship between specimen Ct and infectious potential.

METHODS

We searched 4 databases: LitCovid, medRxiv, Google Scholar, and the World Health Organization COVID-19 database, using the terms "viral culture" or "viral replication" and associated

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Correspondence: T. Jefferson, Department of Continuing Education, University of Oxford, Oxford, United Kingdom (tom-jefferson@conted.ox.ac.uk).

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synonyms on 10 September 2020. For relevant articles, citation matching was undertaken and relevant results identified.

We included studies reporting attempts to culture SARS-CoV-2 and those which also estimated the potential infectivity of the isolates or observed tissue infection by SARS-CoV-2 and related them to other clinical variables such as STT date and patient characteristics. Isothermal methods of detection are not included in our review, as they do not provide a Ct value.

One reviewer extracted data for each study and a second reviewer checked the extraction. Heterogeneity and lack of detail of some of the reported data in the included studies prevented pooling. We tabulated data and summarized it descriptively by specimen: fecal, respiratory, environment, or mixed. Where possible, we also reported the duration of detectable RNA and the relationship of PCR Ct and $\log_{10}$ copies to positive viral culture.

Where necessary, we contacted corresponding authors of the cited papers for additional information. We assessed quality using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS 2) risk of bias tool, simplified because the included studies were not designed as primary diagnostic accuracy studies [6]. Our methods are more fully described in our protocol (published on 4 July 2020 and updated on 5 October 2020) [7].

RESULTS

We identified 145 possible articles for inclusion and after screening; 29 full texts were read and included (see the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [PRISMA] [8] flowchart in Figure 1). One unpublished study was not included as no permission was given by the authors. The included studies were published in 30 articles, 4 of which were on preprint servers (see the Supplementary Data for the list of Supplementary References). The characteristics of each study are shown in Table 1. All included studies were case series of moderate quality (Table 2). We could not identify a protocol for any of the studies. All had been made public in 2020. We received 5 author responses regarding clarifying information (see Acknowledgments).

Studies Using Fecal Specimens

Nine studies assessed viral viability from fecal specimens positive for SARS-CoV-2 based on RT-PCR result [Supplementary References 10, 11, 13, 17, 22, 23, 25–27]. One study reported infecting ferrets with stool supernatant [Supplementary Reference 10]; 2 reported visual growth in tissue [Supplementary References 19, 22] and 4 reported achieving viral replication [Supplementary References 13, 23, 24, 26]. In 1 additional study, the methods were unclear [Supplementary Reference 28].

Studies Using Respiratory Specimens

Seventeen studies reported attempting viral isolation and culture from respiratory specimens [Supplementary References 3, 4, 6–10, 13–16, 18, 21–23, 26, 27]. One study successfully cultured 26 of 90 nasopharyngeal specimens; positive cultures were observed only up to day 8 post–symptom onset [Supplementary Reference 7]. Another study obtained cultures from 31 of 46 nasopharyngeal and oropharyngeal specimens [Supplementary Reference 3]. The largest study came from the La Scola group publications [Supplementary Reference 15] with positive cultures from 1941 of 3790 specimens. Another study of United Kingdom healthcare workers during a period of low viral circulation isolated SARS-CoV-2 from 1 of 19 specimens [Supplementary Reference 5].

Two more studies reported a clear correlation between symptoms onset, date of sampling, Ct, and likelihood of viral culture [Supplementary References 18, 21].

One study [Supplementary Reference 14] of nasopharyngeal specimens from 638 patients aged <16 years reported achieving culture from 12 of the 23 (52%) who tested positive for SARS-CoV-2 with a Ct of around 28. Gniazdowski et al [Supplementary Reference 8] assessed RNA and infectious virus detection in 161 nasopharyngeal specimens from hospitalized COVID-19 patients. Positive culture was associated with Ct values of 18.8 ± 3.4 (median, 18.7); negative culture was associated with mean Ct values 27.1 ± 5.7 (median, 27.5). More than 90% of the virus isolates were obtained from specimens with a Ct value <23.

Basile et al [Supplementary Reference 4] reported 24% culture positivity, with specimens significantly more likely to be positive from the intensive care unit. A report by the Korean Centers for Disease Control failed to grow live viruses from 108 respiratory specimens from "re-positives"—that is, people who had tested positive after previously testing negative [Supplementary Reference 12].

Ladhani et al [Supplementary Reference 16] reported a successful culture rate of 87 of 158 RT-PCR–positive nasopharyngeal specimens from 6 nursing homes in London.

Studies Using Environmental Specimens

Two possible (the text is unclear) positive cultures were obtained from 95 environmental specimens in 1 study that assessed aerosol and surface transmission potential of SARS-CoV-2 [Supplementary Reference 20]. No viruses could be grown from specimens from 7 areas of a large London hospital from specimens with a cutoff RT-PCR Ct >30 [Supplementary Reference 29].

Ahn and colleagues [Supplementary Reference 1] failed to grow live virus from an unspecified number of air specimens from isolation rooms of patients with severe COVID-19, but were able to grow virus from swabs of handrails and the external surfaces of intubation cannulae.

Mixed Sources

Some studies labeled as mixed-source specimens are also reported by individual specimen in this text.

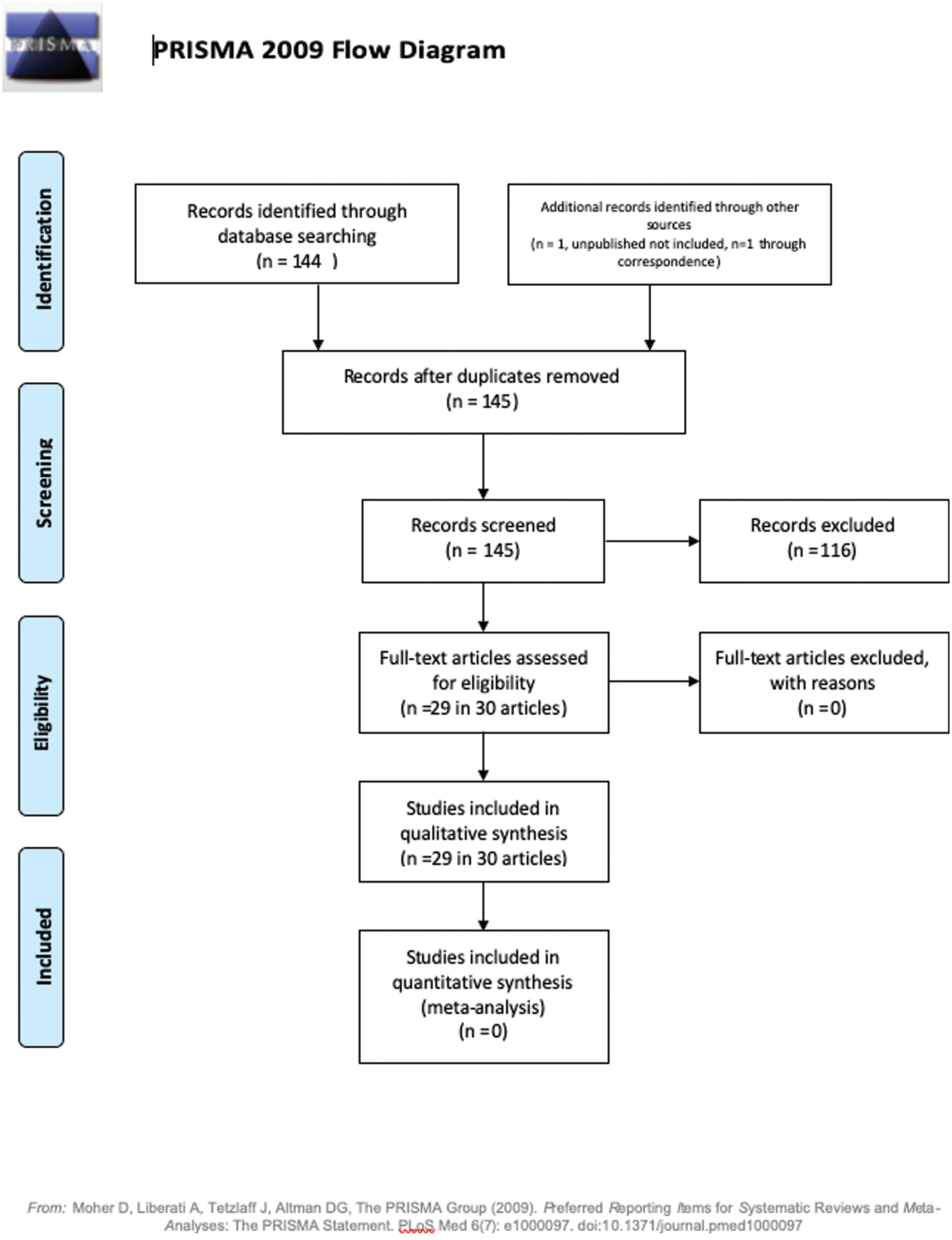

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart.

Eight studies reported viral culture from mixed sources: 12 oropharyngeal, 9 nasopharyngeal, and 2 sputum specimens [Supplementary Reference 9]; 1 stool specimen and an unreported number of other specimens [Supplementary Reference 10]; from saliva, nasal swabs, urine, blood, and stool collected from 9 COVID-19 patients and a possible specimen stool culture [Supplementary Reference 23]; 9 nasopharyngeal, oropharyngeal, stool, serum, and urine specimens [Supplementary Reference 13]; and 7 sputum specimens, 3 stool specimens, and 1 nasopharyngeal specimen of 11 patients [Supplementary Reference 26]. In the study by Yao [Supplementary Reference 26], all specimens had been taken within 5 days of symptom onset and there was a relationship between copy thresholds and cytopathic effect observed in infected culture cells.

Table 1. Characteristics of Included Studies

Study [Supplementary Reference]	Specimens (Source)	Specimens, No. [SST]	Culture Methods	Culture Positive	Additional Notes
Ahn 2020 [S1]	Air and surfaces of isolation room of 3 patients with severe COVID-19	48 [not reported]	Only positive samples (Ct ≤35 for the RdRp and E genes) were cultured in Vero E6 cells. Ten-fold dilutions of the SARS-CoV-2 supernatants from the environmental specimens were used. The inoculated cultures were grown in a humidified 37°C incubator with 5% CO_2. After 72 h, areas of cell clearance with crystal violet staining were used to demonstrate the CPE. In the presence of CPE, detection of nucleic acid of SARS-CoV-2 by real-time RT-PCR in the supernatant was performed to confirm a successful culture.	External surfaces of intubation cannulae and surfaces in the room of patient not intubated	No air specimens grew virus. Ct values of specimens that grew virus were uniformly low, <30, except in 1 case.
Andersson 2020 [S2]	20 RT-PCR–positive serum specimens, selected at random from a COVID-19 specimen bank, representing specimens from 12 individual patients (4 individuals were represented at 2 timepoints), collected at 3–20 d following onset of symptoms	20 serum specimens from 12 hospitalized COVID-19 patients	Specimens VC1–VC20 were provided blinded for viral culture experiments. 50-μL aliquots of specimens VC1–VC20 were separately added to 2.4×10^5 Vero E6 cells in 24-well plates. Cells were propagated in DMEM supplemented with 10% FBS. Viral growth assays were done in DMEM supplemented with 1% FBS, glutamine, and penicillin/streptomycin, according to published methods. In parallel, wells of the same number of cells were cultured in triplicate without virus challenge but with 50 μL control serum (VC21), or in duplicate with a stock of Victoria/01/2020 SARS-CoV-2 passage 4 (Oxford) at calculated 10-fold serial dilutions per well of 78, 7.8, 0.78, and 0.078 plaque-forming units in 50 μL of control serum (VC21). Wells were observed daily for CPE, and 50-L specimens were taken for viral RNA extraction on day 3 postchallenge. On day 4, 50-L aliquots of supernatants from cells challenged with VC1–20 were "blind passaged" to fresh cells, and the remaining supernatants were harvested and stored separately at –80°C for future analysis. After a further 3 d, CPE was recorded, if any, for second-passage cultures.	0 of 20 serum specimens produced positive viral culture	Serum specimens
Arons 2020 [S3]	NP and OP swabs	48 real-time RT-PCR–positive specimens [for asymptomatic, median 4 d, Ct 23.1]	All real-time RT-PCR–positive specimens shipped to US CDC for viral culture using Vero CCL-81 cells. Cells showing CPE were used for SARS-CoV-2 real-time RT-PCR to confirm isolation and viral growth in culture.	31 (no relation to symptoms presence. Culturable virus isolated from 6 d before to 9 d after symptom onset)	
Basile 2020 [S4]	234 specimens, 228 (97%) from the upper respiratory tract (sputum, NP swabs, bronchial lavage from 195 individuals with COVID-19)	Specimens from routine laboratory tests or from patients admitted to ICU or from a physician request [mean 4.5 days, 0–18, only 1 day to day 18]	Probe targets for PCR included E, RdRp, N, M, and ORF1ab for specimens from ICU patients and 1 to 4 E, RdRp, N, and Orf1ab for all other specimens. After stabilization at 4°C, specimens were inoculated into Vero E6 cells and incubated at 37°C in 5% CO_2 for 5 d (days 0–4). Cultures were observed daily for CPE. CPE, when it occurred, took place between days 2 and 4. Day 4 was chosen for terminal sampling.	Culture positivity rate was 56 (24%) and significantly more likely positive in ICU patients compared with other inpatients or outpatients and significantly more likely positive in specimens from inpatients.	The highest Ct value with a successful culture was 32 (N gene target). A Ct cutoff of ≥37 was not indicative of viable virus.

Table 1. Continued

Study [Supplementary Reference]	Specimens (Source)	Specimens, No. [SST]	Culture Methods	Culture Positive	Additional Notes
Borczuk 2020 [S5]	Postmortem lung tissue from 68 elderly deaths (median age, 73)	Six	When a CPE was seen, the Vero cell culture supernatant was passed to a fresh Vero cell culture tube to ensure reproducibility. SARS-CoV-2 in the supernatant was further confirmed by RT-PCR.	6	No Ct reported. In 1 case, virus grew on day 26 from symptom start.
Brown 2020 [S6]	Combined viral throat and nose swab from each participant (n = 1152)	Healthcare workers in 6 UK hospitals	Specimens were sent on the same day for detection of SARS-CoV-2 RNA by RT-PCR to the PHE national reference laboratory (5 hospitals) or 1 hospital laboratory. The PHE laboratory used an Applied Biosystems 7500 FAST system targeting a conserved region of the SARS-CoV-2 ORF1ab gene. The hospital laboratory used a different CE-IVD kit, targeting 3 SARS-CoV-2 genes (RdRp, E, and N). Both PCRs had internal controls. Viral culture of PHE laboratory positives was attempted in Vero E6 cells with virus detection confirmed by CPE up to 14 d postinoculation.	SARS-CoV-2 was isolated from only 1 of 19 (5%) cultured specimens. Ct value of 26.2.	Symptoms in the past month were associated with 3-fold increased odds of testing positive (aOR, 3.46 [95% CI, 1.38–8.67]; $P = .008$). 23 of 1152 participants tested positive (2.0%) with a median Ct of 35.70 (IQR, 32.42–37.57).
Bullard 2020 [S7]	NP or ETT from COVID-19 patients (mean age 45 y)	90 [0–7 d]	NP swabs and ETT specimens in viral transport media were stored at 4°C for 24–72 h until they were tested for the presence of SARS-CoV-2 RNA using real-time RT-PCR targeting a 122-nt portion of the envelope gene (E gene). Dilutions were placed onto the Vero cells in triplicate and incubated at 37°C with 5% CO_2 for 96 h. Following incubation of 4 d, CPE was evaluated under a microscope and recorded.	26	The range of symptom onset to negative PCR was 21 d. Within this period, positive cultures were only observed up to day 8 post–symptom onset.
Gniazdowski 2020 [S8]	161 probably NP specimens	161 cases with positive PCR [not reported]	Ct values were calculated of only 1 gene target per assay: the Spike (S) gene for the RealStar SARS-CoV-2 and the nonstructural protein 1 (Nsp) 2 gene for the NeuMoDx SARS-CoV-2 assays. Genome sequencing was carried out. Incubation of the inoculum in Vero E6 cells cultured at 37°C was observed for 4 d for CPE and immunofluorescence used to identify viral presence.	Unclear; possibly 47 isolates	Positive culture was associated with Ct values of 18.8 ± 3.4. Infectious viral shedding occurred in specimens collected up to 20 d after the first positive result in symptomatic patients. Mean and 184 median Ct values associated with recoverable virus were 18.8 ± 3.4 and 18.17, respectively, which was significantly lower than the mean and median Ct values that did not correlate with infectious virus recovery: 27.1 ± 5.7 and 27.5, respectively. PCR results should be interpreted alongside symptoms.
Huang 2020 [S9]	OP or NP swabs, or sputum	60 specimens from 50 cases [3.4 days mean but see Table 1 for freeze-thaw cycle delays]	SARS-CoV-2 cDNA was prepared using RNA extracted from the specimens of the first patient with confirmed COVID-19. Reverse transcription was performed using the MMLV reverse-transcription kit. All procedures for viral culture were conducted in a biosafety level 3 facility. Vero-E6 and MK-2 (ATCC) cells were maintained in a virus culture medium and the cells were maintained in a 37°C incubator with daily observations of the CPE.	Obtained 23 isolates from different specimen types (12 from OP, 9 from NP, and 2 from sputum)	Specimens with high copy numbers of the viral genome, indicative of higher viral load, were more likely to be culturable.

Table 1. Continued

Study [Supplementary Reference]	Specimens (Source)	Specimens, No. [SST]	Culture Methods	Culture Positive	Additional Notes
Jeong 2020 [S10]	NP/OP swabs, saliva, urine, and stool	5 patients	Specimens positive by quantitative PCR were subjected to virus isolation in Vero cells. Urine and stool specimens were inoculated intranasally in ferrets and they evaluated the virus titers in nasal washes on 2, 4, 6, and 8 dpi. Immunofluorescence antibody assays were also done.	NP/OP saliva, urine, and stool Specimens were collected between days 8 and 30 of the clinical course. Viable SARS-CoV-2 was isolated from 1 NP/OP swab. Ferrets inoculated with patient urine or stool were infected. SARS-CoV-2 was isolated from the nasal washes of the 2 urine-treated ferrets and 1 stool-treated ferret.	Viral loads in urine, saliva, and stool specimens were almost equal to or higher than those in NP/OP swabs. After symptom resolution, patients shed viable virus in their saliva and urine up to day 15 of illness.
Kim 2020 [S11]	Unclear. Possibly 323 serum 247 urine and 129 stool specimens	74 COVID-19 hospital patients	RT-PCR was performed on the target genes were E and RdRp. Cell culture was performed in a level 3 facility by inoculum into CaCo-2 cell line after stabilization at 4°C and harvested after 5 d and the supernatant after centrifugation was reinoculated for another 5 d and assessed with RT-PCR.	No viral growth was detected in any specimen despite a positive RT-PCR very soon after admission	
Korean Center for Disease Control 2020 [S12]	Respiratory swab specimens for individuals testing positive after having previously tested positive, then negative	108 specimens	Methods not reported.	0/108 respiratory specimens	This report does not report the laboratory methods used.
Kujawski 2020 [S13]	NP, OP, stool, serum, and urine specimens	12 patients had initial respiratory specimens collected	SARS-CoV-2 real-time RT–PCR Ct values of virus isolated from the first tissue culture passage were 12.3 to 35.7 and for 1 patient, virus isolated from tissue culture passage 3 had a titer of 7.75×10^6 median tissue culture infectious dose/mL; these data were likely more reflective of growth in tissue culture than patient viral load.	Viral culture was attempted on initial respiratory specimens from 9 patients and was successful in all 9, including 2 patients who not hospitalized	Viable SARS-CoV-2 was cultured at day 9 of illness (patient 10), but was not attempted on later specimens. SARS-CoV-2 real-time RT–PCR Ct values of virus isolated from the first tissue culture passage were 12.3 to 35.7. Mean Ct values in positive specimens were 17.0 to 39.0 for NP, 22.3 to 39.7 for OP, and 24.1 to 39.4 for stool. All blood and urine isolates were negative. Ct values of upper respiratory tract specimens were lower in the first week of illness than the second in most patients.
L'Huillier 2020 [S14]	NP swabs in 638 patients aged <16 y in Geneva Hospital	23 (3.6%) tested positive for SARS-CoV-2; median age 12 y (range, 7 d to 14.9 y) [1–4]	Observation of CPE on days 2, 4, and 6 of inoculum in Vero cells in 2 passages.	12 (52% of PCR positive)	Ct was ~28 for the children whose specimens grew viable viruses.

Table 1. Continued

Study [Supplementary Reference]	Specimens (Source)	Specimens, No. [SST]	Culture Methods	Culture Positive	Additional Notes
La Scola 2020 (Jaafar 2020) [S15]	NP swabs or sputum specimens Only NP specimens from the subsequent Jaafar et al letter	183 (4384 specimens from 3466 patients) [not reported]	From 1049 specimens, 611 SARS-CoV-2 isolates were cultured. 183 specimens testing positive by RT-PCR (9 sputum specimens and 174 NP swabs) from 155 patients were inoculated in cell cultures. SARS-CoV-2. RNA real-time PCR targeted the E gene. NP swab fluid or sputum specimen was filtered and then inoculated in Vero E6 cells. All specimens were inoculated between 4 and 10 h after sampling and kept at 4°C before processing. After centrifugation they were incubated at 37°C. They were observed daily for evidence of CPE. Two subcultures were performed weekly and scanned by electron microscopy and then confirmed by specific RT-PCR targeting E gene.	Of the 183 specimens inoculated in the studied period of time, 129 led to virus isolation. Of these, 124 specimens had detectable CPE between 24 and 96 h. The letter by Jaafar et al adds that 1941 SARS-CoV-2 isolate cultures were positive out of 3790 inoculated specimens. These could be seen after the first inoculation or up to 2 blind subcultures. At a Ct of >34, 2.6% of specimens yielded a positive culture.	There was a significant relationship between Ct value and culture positivity rate: Specimens with Ct values of 13–17 all had positive culture. Culture positivity rate decreased progressively according to Ct values to 12% at 33 Ct. No culture was obtained from specimens with Ct >34. The 5 additional isolates obtained after blind subcultures had Ct between 27 and 34, thus consistent with low viable virus load.
Ladhani 2020 [S16]	NP swabs	87 [residents post, pre, and symptomatic: 5 (6 to 3), 4 (2 to 11), 7 (10 to 4). Staff post, pre, and symptomatic: 7 (9 to 4), 3 (2 to 5), 5 (9 to 3)]	All SARS-CoV-2–positive specimens with a Ct value of <35 were incubated on Vero E6 mammalian cells and virus detection was confirmed by CPE up to 14 d postinoculation. Whole-genome sequencing was carried out on all RT-PCR–positive specimens.	87	Ct values <35 Higher Ct values (lower virus load) specimens were associated with decreasing ability to recover infectious virus from 100% (2/2) with Ct <20.00 to 17.0% (9/53) with Ct 30.00–34.99 ($\chi 2$ for trend, $P < .001$).
Lu 2020 [S17]	87 cases testing "re-positive" at RT-PCR	619 hospital discharges of which 89 re-tested positive after discharge	137 swabs and 59 serum specimens from 70 "re-positive" cases to assess the immunological and virologic characteristics of the SARS-CoV-2 "re-positive" cases. From 23 January, hospital dischargees followed a strict isolation protocol living, eg, in single dedicated hotel rooms, and went home only when nucleic acid tests were negative on both respiratory tract and digestive tract specimens. Specimens (NP, throat, and anal swabs), were collected for RT-PCR diagnosis at 7 and 14 d after discharge. Culture was performed by inoculating Vero E6 cells with patient specimen. CPE was observed daily at 7 d with a second round of passage.	No cultures were positive	"Re-positive" cases are unlikely to be infectious as no intact RNA single helix was detected or viral isolated grew.
	137 swabs (51 NP, 18 throat, and 68 anal)		RT-PCR diagnosis was performed out on RNA using 3 RT-PCR kits to conduct nucleic acid testing, in an attempt to avoid false negatives. Ct varied from 29 to 39 depending on gene and kit.		Prolonged detection of viral RNA is a challenge for public health interventions targeted at isolating infectious cases. "Re-positive" discharged cases are caused by intermittent shedding of cells containing remnant RNA.

Table 1. Continued

Study [Supplementary Reference]	Specimens (Source)	Specimens, No. [SST]	Culture Methods	Culture Positive	Additional Notes
Perera 2020 [W18]	68 specimens: NP aspirates combined with throat swab (n = 49), NP aspirate (n = 2), NP swab combined with throat swab (n = 3), NP swab (n = 2), sputum (n = 11), and saliva (n = 1)	35 patients, 32 with mild disease [1–67 d]	Specimens were tested for sub-genomic RNA with ≥5 $\log_{10}$ N gene copies/mL. The cDNA obtained was subjected to PCR (40 cycles). Vero E6 cells were seeded and incubated for 24 h in a CO_2 incubator. The culture medium was removed and 125 μL of the clinical specimen in virus transport medium was diluted and inoculated into 2 wells. After 2 h incubation in a CO_2 incubator at 37°C, the plates were incubated at 37°C in a CO_2 incubator. A specimen (100 μL) of supernatant was tested for a quantitative real-time RT-PCR at 0 and 72 h postinoculation. At 72 h, cells were scraped into the supernatant and transferred onto fresh cells in 24-well plates and monitored for an additional 72 h. A final quota of cells was collected for quantitative real-time RT-PCR. Cells were observed for CPE daily and harvested for testing if 25%–50% of cells showed a CPE.	Virus was isolated from 16 specimens for 16 patients out of a total of 35 specimens	Culturable SARS-CoV-2 and subgenomic RNA (good indicator of replication) was rarely detectable beyond 8 d after onset of illness, although virus RNA by RT-PCR remained for up to 70 d.
Qian 2020 [S19]	Rectal tissue obtained from a surgical procedure was available	1 [1 to 3 d postoperation]	Ultrathin sections of tissue fixed in epoxy resin on formvar-coated copper grids were observed under electron microscope under 200 kV. Immunohistochemical staining was used to establish expression and distribution of SARS-CoV-2 antigen.	1	No culture performed. Visualization of virions in rectal tissue and detection of SARS-CoV-2 antigen in the rectal tissue.
Santarpia 2020 [S20]	Windowsill and air, mean 7.3 specimens per room. The percentage of PCR-positive specimens from each room was 40%–100%	13 patients [days 5 to 9 and day 18 of isolation in a quarantine unit]	Vero E6 cells were used to culture virus from environmental specimens. The cells were cultured in DMEM supplemented with heat-inactivated FBS (10%), penicillin/streptomycin (10000 IU/mL and 10000 μg/mL), and amphotericin B (25 μg/mL).	Possibly 2 with weak CPE	Isolates were from days 5 and 8 of occupancy of hospital/isolation rooms.
Singanayagam 2020 [S21]	324 specimens: nose, throat, combined nose/throat, and NP swabs and aspirates	253 positive cases [–10 to 60 d]	Vero E6 cells were inoculated with clinical specimens and incubated at 37°C, 5% CO_2. Cells were inspected for CPE daily up to 14 d. Presence of SARS-CoV-2 was confirmed by SARS-CoV-2 nucleoprotein staining by enzyme immunoassay on infected cells.	133 (41%) specimens (from 111 cases)	RT-PCR Ct values correlate strongly with cultivable virus, ie, likelihood of infectiousness. Median Ct of all 324 specimens was 31.15. Probability of culturing virus declines to 8% in specimens with Ct >35 and to 6% 10 d after onset and was similar in asymptomatic and symptomatic persons. Asymptomatic persons represent a source of transmissible virus, but there is no difference in Ct values and culturability by age group.
Wang 2020 [S22]	Bronchoalveolar fluid, sputum, feces, blood, and urine specimens from hospital inpatients with COVID-19	1070 specimens collected from 205 patients with COVID-19	Real-time RT-PCR targeting the ORF1ab gene of SARS-CoV-2; Ct values of real-time RT-PCR were used as indicators of the copy number of SARS-CoV-2 RNA in specimens with lower Ct values corresponding to higher viral copy numbers. A Ct value <40 was interpreted as positive for SARS-CoV-2 RNA.	Live SARS-CoV-2 was observed in the stool specimen from 2 patients who did not have diarrhea	The details of how the specimens were cultured were not reported.

Table 1. Continued

Study [Supplementary Reference]	Specimens (Source)	Specimens, No. [SST]	Culture Methods	Culture Positive	Additional Notes
Wölfel 2020 [S23]	Saliva, nasal swabs, urine, blood, and stool	9 patients [2–4 d]	The average virus RNA load was 6.76×10^5 copies per the whole swab until day 5, and the maximum load was 7.11×10^8 copies per swab. The last swab specimen that tested positive was taken on day 28 after the onset of symptoms.	Yes in respiratory specimens, and indicative in stool	
Xiao 2020 [S24]	Esophageal, gastric, duodenal, and rectal tissues were obtained from 1 COVID-19 patient by endoscopy	1 plus an unknown additional number of fecal specimens from RNA-positive patients	Histological staining (H&E) as well as viral receptor ACE2 and viral nucleocapsid staining was performed.	1/1 RNA-positive patient. Positive staining of viral nucleocapsid protein was visualized in the cytoplasm of gastric, duodenal, and rectum glandular epithelial cells, but not in esophageal epithelium of the 1 patient providing these tissues. Additionally, positive staining of ACE2 and SARS-CoV-2 was also observed in gastrointestinal epithelium from other patients who tested positive for SARS-CoV-2 RNA in feces (results not shown).	Total specimen numbers are not reported.
Xiao 2020 [S25]	Serial feces specimens collected from 28 hospitalized COVID-19 patients; 3 specimens from 3 RNA-positive patients were tested for possible viral culture	3, one patient admitted day 7 postonset	Inoculation of Vero 6 cells. Ct values for the fecal specimen were 23.34 for the ORFlab gene and 20.82 for the nucleoprotein gene. A CPE was visible in Vero E cells 2 d after a second-round passage. The researchers negatively stained culture supernatant and visualized by transmission electron microscopy. Viral particles that were visible were spherical and had distinct surface spike protein projections, consistent with a previously published SARS-CoV-2 image.	Infectious virus was present in feces from 2 cases)	Selection of specimens is not entirely clear.
Yao 2020 [S26]	Sputum (n = 7), stool (n = 3), and NP (n = 1) specimens	11 patients admitted to hospital: 9 classified as serious or critical, 1 moderate, 1 mild symptoms [0–16 d]	The specimens of the 11 patients involved in this study were collected during the early phase of the COVID-19 outbreak in China, dates ranging from 2 January to 2 April 2020. All except 1 of the patients had moderate or worse symptoms. Three patients had comorbidities and 1 patient needed ICU treatment. Seven patients had sputum specimens, 1 NP, and 3 had stool specimens. The specimens were pre-processed by mixing with appropriate volume of minimum essential medium with 2% FBS, amphotericin B, penicillin G, streptomycin, and TPCK-trypsin. The supernatant was collected after centrifugation at 3000 rpm at room temperature. Before infecting Vero E6 cells, all collected supernatant was filtered using a 435 0.45-μm filter to remove cell debris etc. Vero E6 cells were infected with 11 viral isolates and quantitatively assessed their viral load at 1, 2, 4, 8, 24, and 48 h postinfection and their viral CPE at 48 and 72 h postinfection, and examined whether the viral isolates could successfully bind to Vero E6 243 cells as expected. Super-deep sequencing of the 11 viral isolates on the Novaseq 6000 platform was performed.	11 specimens taken up to 16 d from admission to hospital	Cultured viruses were inoculated in Vero cells. At 8 h postinfection, there was a significant decrease in Ct value (increases in viral load) for 5 isolates. At 24 h, significant decreases in the Ct values for all of the viral isolates were observed. Mutations of the viruses are also reported.

Table 1. Continued

Study [Supplementary Reference]	Specimens (Source)	Specimens, No. [SST]	Culture Methods	Culture Positive	Additional Notes
Young 2020 [S27]	NP swabs, stool, fresh urine	152 of 74 patients	Material from NP swabs was inoculated in Vero E6 cells in a Level 3 laboratory. Urine and stool specimens were collected and transported fresh for virus culture but stools were filtered before inoculation. Cells were cultured at 37°C for ≤7 d if CPE was observed by day 4 and confirmed by PCR.	21 NP specimens from 19 (14%) patients	No virus was isolated when the PCR Ct value was >30 or >14 d from symptom onset. Urine and stool specimens at admission did not grow virus.
Zhang 2020 [S28]	Stool	Unknown [not reported]	Vero cells were used for viral isolation from stool specimens of COVID-19 patients. A SARS-CoV-2 strain was isolated from a stool specimen of a laboratory-confirmed COVID-19 severe pneumonia case, who experienced onset on 16 January 2020 and from whom specimens were obtained on 1 February 2020. The interval between sampling and onset was 15 d. The full-length genome sequence indicated that the virus had high-nucleotide similarity (99.98%) to that of the first isolated novel coronavirus isolated from Wuhan, China. In the Vero cells, viral particles with typical morphology of a coronavirus could be observed under the electron microscope.	1	We do not know what influenced successful virus culture. eg, methods optimal, or concentration of virus optimal. More information needed.
Zhou 2020 [S29]	218 surface specimens; 31 air specimens	7 areas of large London hospital	RT-PCR with primers and probes for the envelope gene. Duplicate PCR was carried out and specimens were considered positive if both duplicates had Ct <40.4, or suspected if 1 of the 2 had Ct <40.4 (equivalent to 1 genome copy). For culture, Vero E6 and CaCo2 cells were used from air and environmental specimens using a method adapted from one previously used to culture influenza virus. On day 0 and after 5–7 d, cell supernatants were collected, and RT-qPCR to detect SARS-CoV-2 was performed as described above. Specimens with at least 1 log increase in copy numbers for the envelope gene (reduced Ct values relative to the original specimens) after 5–7 d propagation in cells compared with the starting value were considered positive by viral culture.	No cultures were positive	The predefined Ct cutoff was too high.

Abbreviations: aOR, adjusted odds ratio; CDC, Centers for Disease Control and Prevention; CE-IVD, a different kit with European Certification of Conformity for In vitro Diagnostics; CI, confidence interval; CO_2, carbon dioxide; COVID-19, coronavirus disease 2019; CPE, cytopathic effect; Ct, cycle threshold; DMEM, Dulbecco's minimal essential medium; ETT, endotracheal; FBS, fetal bovine serum; H&E, hematoxylin and eosin; ICU, intensive care unit; IQR, interquartile range; NP, nasopharyngeal; OP, oropharyngeal; PHE, Public Health England; RT-PCR, reverse-transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; STT, symptom onset to test date; UK, United Kingdom; US, United States.

Table 2. Quality of Included Studies

Study [Supplementary Reference]	Description of Methods and Sufficient Detail to Replicate	Sample Sources Clear	Analysis and Reporting Appropriate	Is Bias Dealt With	Applicability
Ahn 2020 [S1]	Yes	Yes	Yes	Partly	Unclear
Andersson 2020 [S2]	Yes	Yes	Yes	Partly	Yes
Arons 2020 [S3]	Yes	Yes	yes	Yes	Unclear
Basile 2020 [S4]	Yes	Yes	Yes	Unclear	Unclear
Borczuk 2020 [S5]	Yes	Yes	Yes	Yes	Unclear
Brown 2020 [S6]	Yes	Yes	Yes	Unclear	Unclear
Bullard 2020 [S7]	Yes	Yes	Yes	Unclear	Unclear
Gniazdowski [S8]	Yes	Yes	Yes	Unclear	Unclear
Huang 2020 [S9]	Yes	Yes	Yes	Unclear	Unclear
Jeong 2020 [S10]	Yes	Yes	Yes	No	Unclear
Kim 2020 [S11]	No	No	No	Unclear	Unclear
Korean Centers for Disease Control [S12]	No	Partly	Partly	No	Unclear
Kujawski 2020 [S13]	Yes	Yes	Yes	Unclear	Unclear
L'Huillier 2020 [S14]	Yes	Yes	Yes	Unclear	Unclear
La Scola 2020 [S15]	Yes	Yes	Yes	Unclear	Unclear
Ladhani 2020 [S16]	Yes	Yes	Yes	Yes	Likely
Lu 2020 [S17]	Yes	Yes	Yes	Partly	Yes
Perera 2020 [S18]	Yes	Yes	Yes	Unclear	Unclear
Qian 2020 [S19]	Yes	Yes	Yes	Unclear	Unclear
Santarpia 2020 [S20]	Yes	Yes	Yes	Unclear	Unclear
Singanayagam [S21]	Yes	No	Yes	Unclear	Unclear
Wang 2020 [S22]	No	Yes	Yes	No	Unclear
Wölfel 2020 [S23]	Yes	Yes	Yes	Unclear	Unclear
Xiao 2020 [S24]	No	Yes	Yes	No	Unclear
Xiao 2020 [S25]	Yes	Yes	Yes	No	Unclear
Yao 2020 [S26]	Yes	Yes	Yes	Unclear	Unclear
Young 2020 [S27]	Yes	Yes	Yes	Yes	Yes
Zhang 2020 [S28]	Partly	Yes	Yes	No	Unclear
Zhou 2020 [S29]	Yes	Yes	Yes	Unclear	Unclear

Kim and colleagues reported no viral growth from an unclear number of serum, urine, and stool specimens, despite these specimens being collected soon after admission [Supplementary Reference 11]. Lu and colleagues also reported no viral growth; however, their specimens were from 87 cases that tested "re-positive" [Supplementary Reference 17].

One study [Supplementary Reference 27] reported 21 positive cultures from nasopharyngeal specimens of 19 hospitalized patients in Singapore, but no growth from specimens with a Ct value >30 or collected >14 days after symptoms onset. No culture was achieved from the urine or stool specimens.

Blood Cultures

In 1 study by Andersson et al [Supplementary Reference 2], 20 RT-PCR–positive serum specimens from 12 individual patients were selected at random from a COVID-19 specimen bank at 3–20 days following onset of symptoms. None of the 20 serum specimens produced a viral culture.

Postmortem Study

One study on alveolar specimens from 68 elderly deceased patients reported that postmortem studies on lung tissues from 6 cases were available for viral isolation. The evaluation showed viable SARS-CoV-2 in all 6 cases—in 1 case on day 26 from symptom onset [Supplementary Reference 6].

Duration of RNA Viral Detection

Table 3 shows that 9 studies reported on the duration of viral RNA detection as assessed by PCR for SARS-CoV-2 RNA [Supplementary References 7, 8, 10, 12, 13, 21, 24, 25, 27]. All 9 studies reported RNA detection for >7 days. Young et al [Supplementary Reference 27] reported that SARS-CoV-2 was detectable from nasopharyngeal swabs by PCR up to 48 days after symptom onset.

Live Viral Culture Window

The live viral culture time window was much shorter than for viral RNA identification, including reports of <8 days STT [Supplementary Reference 23] and Ct <24 [Supplementary Reference 7]. Median duration of viral RNA identification in

Table 3. Duration of Detectable Severe Acute Respiratory Syndrome Coronavirus 2 RNA in the Included Studies

Study [Supplementary Reference]	Duration of Detectable SARS-CoV-2 RNA as Assessed by PCR	Comments on the Clinical Course
Bullard [S7]	Specimens included in this study included those positive for SARS-CoV-2 by RT-PCR from day of symptom onset (day 0) up to 21 d post–symptom onset.	SARS-CoV-2 Vero cell infectivity of respiratory specimens from SARS-CoV-2–positive individuals was only observed for RT-PCR Ct <24 and symptom onset to test of <8 d.
Gniazdowski [S8]	Patients who received repeated testing with longitudinal positive results were tested within a time frame that ranged from <1 d to >45 d.	Four patients had infectious virus recovered from specimens collected up to 22 d after the first positive result. Many patients who tested negative for SARS-CoV-2 showed a subsequent positive result.
Jeong [S10]	Five PCR-positive patients, day 8 to day 30 after symptom onset.	Viable SARS-CoV-2 was demonstrated in saliva, urine, and stool specimens from COVID-19 patients up to days 11–15 of the clinical course.
Korean CDC [S12]	On average, it took 45 d (range, 8–82 d) from the initial symptom onset date to testing re-positive after discharge (based on 226 cases symptomatic at the time of initial confirmation).	This may indicate duration of viral RNA detection over a long period of time and inconsistently. These data may not be comparable with information from studies specifically observing the duration of viral RNA detection as an outcome. Time to retesting positive via PCR is reported among this specific group of individuals who retested positive by PCR.
Kujawski [S13]	Duration of SARS-CoV-2 detection by RT-PCR was 7–22 d.	First 12 identified patients in the United States. Respiratory specimens were collected between illness days 1 to 9 (median, day 4). All patients had SARS-CoV-2 RNA detected in respiratory specimens, typically for 2 to 3 wk after illness onset. Mean duration of fever was 9 d. Two patients received a short course of corticosteroids.
Singanayagam [S21]	SARS-CoV-2 viral load identified that the level of SARS-CoV-2 RNA in the upper respiratory tract was greatest around symptom onset, steadily decreased during the first 10 d after illness onset, and then plateaued up to day 21.	Probability of culturing virus declined to 8% in specimens with Ct >35 and to 6% 10 d after onset.
Xiao [S24]	The viral load was higher in feces than in respiratory specimens collected at multiple time points (17–28 d after symptom onset).	Isolation of virus from fecal specimens collected at later time points was not successful, although results for virus RNA remained positive, indicating only RNA fragments, not infectious virus, in feces of this patient collected at later time points of disease onset.
Xiao [S25]	The duration time of positive stool results ranged from 1 to 12 d.	17 (23%) patients continued to have positive results in stool after showing negative results in respiratory specimens.
Young [S27]	SARS-CoV-2 RNA was detectable from nasopharyngeal swabs by PCR up to 48 d after symptom onset.	Mean duration of viral RNA detection by PCR was 16.7 d (95% CI, 15.2–18.3). Cessation of viral RNA detection by PCR occurred in 4% by day 7, 30% by day 14, 78% by day 21, and 91% by day 28. There were no differences by disease severity. No virus was isolated when the PCR cycle threshold value was >30 or >14 d after symptom onset.

Abbreviations: CDC, Centers for Disease Control; CI, confidence interval; PCR, polymerase chain reaction; RT-PCR, reverse-transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

culture was 4 days (interquartile range, 1–8 days) [Supplementary Reference 21].

Relationship Between RT-PCR Results and Viral Culture of SARS-CoV-2

Ten studies analyzed the relationship between Ct values and the possibility of culturing live virus [Supplementary References 4, 5, 7–9, 15, 16, 21, 23, 27] and 3 quantified the mean log copies of detected virus and live culture [Supplementary References 9, 14, 18] (Table 4). All reported that Ct values were significantly lower and log copies were significantly higher in those with live virus culture. Five studies reported no growth in specimens based on a Ct cutoff value [Supplementary References 5, 7, 9, 16, 27], ranging from Ct >24 [Supplementary Reference 7] to 35 [Supplementary Reference 15].

The estimated probability of recovery of virus from specimens with Ct=35 was 8.3% (95% confidence interval [CI], 2.8%–18.4%) [Supplementary Reference 21]. All donors above the Ct threshold of 35 (n = 5) producing live culture were symptomatic.

In 6 London nursing homes, there was no correlation between Ct values and symptoms in either residents or staff [Supplementary Reference 16], although nearly 50% of both categories were asymptomatic.

One study [Supplementary Reference 9] reported different cutoff thresholds depending on the gene fragment analyzed. No growth was found for the Nsp 12 fragment at Ct >31.5, whereas the value was higher for the N gene fragment (>35.2).

The odds for culturing live virus decreased by 0.64 for every 1-unit increase in Ct (95% CI, .49–.84; $P < .001$) [Supplementary Reference 7]; another study [Supplementary Reference 21] reported similar results in line with empirical evidence of an increased Ct of 0.58 per day since symptoms started [9].

Table 4. Relationship of Polymerase Chain Reaction Cycle Threshold and Log[10] Copies to Positive Viral Culture

	Specimen				Cycle Threshold			Log_{10} Copies			
Study [Supplementary Reference]	RT-PCR SARS-CoV-2–Positive Specimens, No.	Viral Culture Growth, No.	No Growth, No.	Gene Fragment Sampled on PCR Test	Positive Culture Ct Value	Negative Culture Ct Value	No Growth in Specimens Based on Ct	Log_{10} Copies Positive Culture (Unless Otherwise Stated)	Log_{10} Copies Negative Culture	No Growth Based on Log Copies	ORs for Viral Culture
Basile 2020 [S4]	234	56	178	E, RdRp, N, M, and ORF1ab for ICU patients	25.01	27.75	Ct >32 with the N gene target[a]	…	…	…	…
Brown 2020 [S5]	23	1	22	RdRp, E, and N	26.16	35.16 ± SEM 0.63	Ct >26.2	…	…	…	…
Bullard 2020 [S7]	90	26	64	E gene	17 (16–18; lower and upper limits)	27 (22–33)	Ct >24	…	…	…	OR, 0.64 (95% CI, .49–.84); $P < .001$) for every 1-unit increase in Ct
Gniazdowski 2020 [S8]	132	47	85	S, Nsp 2	Mean 12.8 ± 3.4; median 18.17	Mean 27.1 ± 5.7; median 27.5	Ct ≥23 yielded 8.5% of virus isolates	…	…	…	…
Huang 2020 [S9]	60	23	34	Nsp 12	Mean 23.9 ± SEM 0.78	Mean 29.26 ± SEM 0.78	Ct >31.47	Mean 7.37 ± SEM 0.20	Mean 5.98 ± SEM 0.18	…	…
		23	37	E	Mean 22.39 ± SEM 0.75	Mean 28.92 ± SEM 0.65	Ct >31.46	Mean 8.21 ± SEM 0.18	Mean 6.62 ± SEM 0.16	…	…
		21	31	N	Mean 27.29 ± SEM 0.77	Mean 31.49 ± SEM 0.59	Ct >35.2	Mean 7.87 ± SEM 0.21	Mean 6.70 ± SEM 0.17	…	…
L'Huillier 2020 [S14]	23[b]	12	11	…	…	…	…	Mean 7.9×10^8 (IQR, 4.7×10^6 to 1.0×10^9)	Mean 5.4×10^7 (IQR, 4.2×10^3 to 1.8×10^6)	…	…
La Scola 2020 (Jaafar 2020) [S15]	611 (3790)	129 (1941)	482 (1849)	E	…	…	Ct ≥34 (2.6% positives)	…	…	…	…
Ladhani 2020 [S16]	87	31	56	ORF1ab	100% cultures (2/2) with Ct <20.00 to 17.0% (9/53) with Ct 30.00–34.99	Cutoff >35	…	…	…	…	…
Perera 2020 [S18]	68	16	52	N	…	…	…	7.5[c]	3.8	<5.0	…
Singanayagam 2020 [S21]	324	133	191	Unclear	…	…	Ct > 35 probability of no growth was 8.3% (95% CI, 2.8%–18.4%)[d]	…	…	…	OR 0.67 for each unit increase in Ct value (95% CI, .58–.77)
Wölfel 2020 [S23]	45	9	36	E, subgenomic messenger RNA	…	…	…	…	…	…	…
Young 2020 [S27]	100	21	79	N, S, and ORF1ab	28.2 (24.3–33.3; lower and upper limits)	>30	…	…	…	…	…

Abbreviations: CI, confidence interval; Ct, cycle threshold; ICU, intensive care unit; IQR, interquartile range; OR, odds ratio; PCR, polymerase chain reaction; RT-PCR, reverse-transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SEM, standard error of the mean.

[a]No cytopathic effects visualized but a decrease in Ct values between the Ct of the original clinical specimen PCR (Ct specimen) and the terminal culture (day 4) supernatant PCR (Ct culture) of ≥3 (equivalent to a 1-log increase in virus quantity), ie, Ct specimen – Ct culture ≥3 = culture positive. The authors hypothesized that a Ct specimen minus Ct culture <3 was due to residual inoculated clinical specimen and not replicating virus.

[b]Twenty-three SARS-CoV-2–infected children.

[c]Of the 16 culture-positive specimens, 15 (94%) had viral RNA load >6 log_{10} copies/mL ($P < .01$). All of them were collected within the first 8 days of illness.

[d]All with Ct >35 (n = 5) were symptomatic.

DISCUSSION

The studies in this review attempted, and some successfully achieved, culture of SARS-CoV-2 in the laboratory, using a range of different specimens. There is evidence of a positive relationship between lower cycle count threshold, likelihood of positive viral culture, and date of symptom onset [10]. This is seen clearly in the 2 studies assessing the infectious potential of "re-positives"—that is, COVID-19 patients who had been discharged from hospital after testing negative repeatedly and who then tested positive after discharge [Supplementary References 12, 17].

Lu and colleagues considered 4 hypotheses for the origin of "re-positives" [Supplementary Reference 17]. On the basis of their evidence, they discarded reinfection and latency as explanations and concluded that the most plausible explanations were either contamination of the specimen by extraneous material or identification in the specimen of minute and irrelevant particles of dead SARS-CoV-2 representing virus long neutralized by the immune system.

Rapid expansion in testing capability requires training protocols and precautions to avoid poor laboratory practice, which may not be possible in the time pressure of a pandemic. The evidence in this review shows that those with high Ct values are unlikely to have infectious potential.

Interpreting the results of RT-PCR requires consideration of patient characteristics such as symptoms and their severity, contact history, presence of preexisting morbidities and drug history, the Ct value, the number of days of STT, and the specimen donor's age [11, 12].

Several of our included studies assessed the relationship of these variables; there appears to be a time window during which RNA detection is at its highest with low Ct and higher possibility of culturing a live virus, with viral load and probability of growing live virus of SARS-CoV-2 peaking much sooner than that of severe acute respiratory syndrome (SARS) or Middle East respiratory syndrome (MERS) coronaviruses [11]. We propose that further work should be done on this with the aim of constructing an algorithm for integrating the results of PCR with other variables, to increase the effectiveness of detecting infectious patients.

PCR should be continuously calibrated against a reference culture in Vero E6 cells in which cytopathic effect has been observed [Supplementary Reference 6]. Confirmation of visual identification using methods such as an immunofluorescence assay may also be needed to aid diagnosis [5]. Henderson and colleagues have called for a multicenter study of all currently manufactured SARS-CoV-2 nucleic acid amplification tests to correlate the Ct values on each platform for patients who have positive and negative viral cultures. Calibration of assays could then be done to estimate virus viability from the Ct with some certainty [13].

Ascertainment of infectious potential is all the more important as there is good evidence of viral RNA persistence across a whole range of different viral diseases with little or no infectious potential in the postinfectious phase of MERS [14], measles [15], other Coronaviridae, hepatitis C virus, and a variety of animal RNA viruses [16].

In 1 COVID-19 (former) case, viral RNA was detectable until day 78 from symptom onset with a very high Ct [9] but no culture growth, implying a lack of infectious potential.

SARS-CoV-2 methods of cell culture vary and to our knowledge have not been standardized. Methods vary depending upon the selection of the cell lines and the collection, transport, and handling and maintenance of viable and healthy inoculated cells [17]. We therefore urgently recommend the development of standard culture methods and external quality assessment schemes for laboratories offering testing for SARS-CoV-2 [18, 19]. If identification of viral infectious potential relies on visual inspection of cytopathic effect, then a reference culture of cells must also be developed to test recognition against infected cells. Viral culture may not be appropriate for routine daily results, but specialized laboratories should use viruses as controls, perform complete investigations when needed, and store representative clinical strains whenever possible [17]. Current evidence is too limited to establish the feasibility of generating a universal Ct value, as this may change with circumstances (eg, hospital, community, cluster, and symptom level) and laboratory methods, so more information is urgently needed [20].

We suggest the WHO produce a protocol to standardize the use and interpretation of PCR and routine use of culture or animal models to continuously calibrate PCR testing, coordinated by designated Biosafety Level 3 laboratory facilities with inward directional airflow [21]. Further studies with standardized methods [20] and reporting are needed to establish the magnitude and reliability of this association.

The results of our review agree with the scoping review by Byrne and colleagues on infectious potential periods [22] and those of the living review by Cevik and colleagues [11]. The authors reviewed 79 studies on the dynamics, load, and RNA detection for SARS, MERS, and SARS-CoV-2 from symptom onset. They concluded that although SARS-CoV-2 RNA identification in respiratory (up to 83 days) and stool (35 days) can be prolonged, duration of viable virus is relatively short-lived (up to a maximum of 8 days from symptom onset). Those results are consistent with Bullard et al, who found no growth in specimens with a Ct >24 [Supplementary Reference 7] or when symptom onset was >8 days, and Wölfel et al [Supplementary Reference 23] who reported that virus could not be isolated from specimens taken after day 8 even among cases with ongoing high viral loads. The review by Rhee and colleagues reaches conclusion similar to ours [10].

The importance of symptom onset and reported PCR threshold is shown in a study that collected test data during a prospective household transmission study. The authors found that Ct values were lowest soon after symptom onset and correlated with time elapsed since symptom onset (within 7 days after symptom onset, the median Ct value was 26.5 compared with a median of 35.0 at 21 days after onset). Ct values were significantly higher among those participants reporting no symptoms, and lower in those reporting upper respiratory symptoms at the time of specimen collection [23].

The evidence is increasingly pointing to the probability of culturing live virus being related to the amount of viral RNA in the specimen and, therefore, inversely related to the Ct. Thus, detection of viral RNA per se cannot be used to infer infectiousness. Duration of excretion may also be linked to age, male sex, and possibly use of steroids and severity of illness.

Our review is limited by the lack of standardized reporting and lack of standard testing methods among the included studies [18]. Ct threshold reporting was inconsistent, preventing pooling or further in-depth analysis of the data, and insufficient clinical details were reported to define the possible role of asymptomatic or presymptomatic persons in transmission. The included studies were case reports or case series with a mixture of laboratory and clinical data, and variable in reporting the relation between donor characteristics and PCR results.

We may have missed some studies or new studies as they are published, and we aim to update this review with emerging evidence.

CONCLUSIONS

The evidence gathered in this review points to a relationship between the time from collection of a specimen to test, Ct, and symptom severity. We recommend that a uniform international standard for reporting of comparative SARS-CoV-2 culture with index test studies be produced. Particular attention should be paid to the relationship between the results of testing, clinical conditions and the characteristics of the source patients, description of flow of specimens, and testing methods. Defining cutoff levels predictive of infectious potential [24] should be feasible and is necessary for diagnosing viral respiratory infections using molecular tests.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. T. J. was in receipt of a Cochrane Methods Innovations Fund grant to develop guidance on the use of regulatory data in Cochrane reviews (2015–2018); was a member of 3 advisory boards for Boehringer Ingelheim (in 2014–2016); was a member of an independent data monitoring committee for a Sanofi Pasteur clinical trial on an influenza vaccine (2015–2017); and is occasionally interviewed by market research companies about phase 1 or 2 pharmaceutical products for which he receives fees (current). T. J. is also a relator in a False Claims Act lawsuit on behalf of the United States that involves sales of Tamiflu for pandemic stockpiling; if resolved in the United States' favor, he would be entitled to a percentage of the recovery. T. J. is also co-holder of a Laura and John Arnold Foundation grant for development of a Restoring Invisible and Abandoned Trials support center (2017–2020) and Jean Monnet Network Grant (2017–2020), for the Jean Monnet Health Law and Policy Network; is an unpaid collaborator to the project Beyond Transparency in Pharmaceutical Research and Regulation led by Dalhousie University and funded by the Canadian Institutes of Health Research (2018–2022); has consulted for Illumina LLC on next-generation gene sequencing (2019–2020); was the consulting scientific coordinator for the Health Technology Assessment Medical Technology program of the Agenzia per i Serivizi Sanitari Nazionali of the Italian Ministry of Health (2007–2019); is Director Medical Affairs for BC Solutions, a market access company for medical devices in Europe; is funded by the NIHR and the World Health Organization to update Cochrane review A122, "Physical interventions to interrupt the spread of respiratory viruses"; is funded by Oxford University to carry out a living review on the transmission epidemiology of COVID-19; and receives fees for articles published by *The Spectator* and other media outlets (since 2020). E. A. S. is Epidemiology and Evidence Synthesis Researcher at the Centre for Evidence-Based Medicine. J. B. is the Director of Trip Database Ltd, Lead for Knowledge Mobilisation at Public Health Wales (NHS), and an Associate Editor at BMJ Evidence-Based Medicine. J. B. is a shareholder in Trip Database Ltd. C. H. is Professor of Evidence-Based Medicine, Director of the Centre for Evidence-Based Medicine, and Director of Studies for the Evidence-Based Health Care Programme.

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