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When to test for COVID-19 using real-time reverse transcriptase polymerase chain reaction: a systematic review

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ABSTRACT

Objectives: The aim of this study was to evaluate the time in days between symptom onset and first positive real-time reverse transcriptase polymerase chain reaction (RT-PCR) result for COVID-19.
Methods: This systematic review was conducted in the MEDLINE (PubMed), Embase, and Scopus databases using the following descriptors: "COVID-19", "SARS-CoV-2", "coronavirus", "RT-PCR", "real time PCR", and "diagnosis".
Results: The included studies were conducted in 31 different countries and reported on a total of 6831 patients. The median age of the participants was 49.95 years. The three most common symptoms were fever, cough, and dyspnea, which affected 4012 (58.68%), 3192 (46.69%), and 2009 patients (29.38%), respectively. Among the 90 included studies, 13 were prospective cohorts, 15 were retrospective cohorts, 36 were case reports, 20 were case series, and six were cross-sectional studies. The overall mean time between symptom onset and positive test result was 6.72 days. Fourteen articles were analyzed separately for the temporal profile of RT-PCR test results; the best performance was on days 22-24, when 98% of test results were positive.
Conclusion: These findings corroborate the RT-PCR COVID-19 testing practices of some health units. In addition, the most frequently described symptoms of these patients can be considered the initial symptoms of infection and used in decision-making about RT-PCR testing.

Introduction

By the end of 2019, a new enveloped RNA betacoronavirus was identified as responsible for episodes of a novel type of pneumonia in Wuhan, China. Called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it quickly spread to regions beyond China and later caused a global pandemic (Phan et al., 2020). In February 2020, the World Health Organization (WHO) named the disease caused by this pathogen COVID-19. COVID-19 has a varied clinical spectrum (World Health Organization, 2020b). Affected patients may be asymptomatic or develop various symptoms and complications, ranging from cough, myalgia, and headache to secondary infections, shock, and respiratory failure. More than 250 million cases had been reported by November 2021, with more than 5 million deaths (Huang et al., 2020; Li et al., 2021). In this scenario, it is clear that early diagnosis is important to focus conduct and achieve favorable prognosis.

According to the WHO, the gold standard for diagnosing COVID-19 is real-time reverse transcriptase polymerase chain reaction (RT-PCR) testing of nasopharyngeal and oropharyngeal mucus samples (Patel and Jernigan, 2020; CDC, 2021b). The alternative, bronchoalveolar lavage, is not recommended because of the aerosols it produces, which put patients and health professionals at risk (Pascarella et al., 2020). Antigen testing is a faster alternative, but it is less sensitive than RT-PCR; it can be used provided that the eval-

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uator is alert to false-positive results. Furthermore, negative antigen test results do not rule out SARS-CoV-2 infection unless there is low clinical suspicion (Dinnes et al., 2020). Finally, serological testing can detect antibodies generated by a previous or current infection that has lasted at least 15 days; it does not have great clinical importance in acute infections, and the antibodies do not guarantee immunity against future infections (Cheng et al., 2020).

SARS-CoV-2 consists of approximately 15 genes, some of which have been chosen and tested as targets for RT-PCR assays: E (envelope), N (nucleocapsid), RdRp (RNA-dependent RNA polymerase), nsp10 (nonstructural protein 10), and nsp14 (nonstructural protein 14) (Rai et al., 2021). Several protocols have been developed to optimize testing and decrease false- negative rates. The WHO and the United States (US) Centers for Disease Control and Prevention (CDC) have established two protocols as standards for molecular diagnosis; these have been adopted by regulatory agencies worldwide (World Health Organization, 2020a). The Charité protocol (WHO) uses the E, N, and RdRp genes as a detection model, while the CDC protocol targets the N1 and N2 genes (Fang et al., 2020).

Regarding the time between infection and positive test results, it is estimated that the mean incubation time of the virus is five days and that patients generally become positive 3 days after symptom onset and negative approximately 15 days after that (Quesada et al., 2021). However, several studies have shown that the viral load remains for a longer period and may be a contamination risk (Walsh et al., 2020). Therefore, with this review, we evaluated data on the number of days between COVID-19 symptom onset and the first positive RT-PCR results. Our review could help determine the best point at which to apply the RT-PCR test and encourage further dialog about this issue.

Methods

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Page et al., 2021) and was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42021293746).

Search strategy

A systematic search was conducted in the MEDLINE (PubMed), Embase, and Scopus databases using the following descriptors: "COVID-19", “SARS-CoV-2”, “coronavirus”, “RT-PCR”, “real time PCR”, and “diagnosis”. The search strategies were adapted by using comparable search elements for each of the databases and were selected according to the consistency and relevance of the titles of the first 50 articles in relation to the topic of this review. Searches were limited to publications from January 1, 2019 to July 27, 2021. No other filters were used.

Study selection

First, Mendeley software and manual evaluation were used to identify duplicate articles. Potentially eligible articles were assessed manually and independently by two authors (HCVSV and TRA). Disagreements were resolved by a third reviewer (JPG). During screening, articles were selected by reading the title and abstract. The inclusion criterion was the use of RT-PCR to diagnose COVID-19. The exclusion criteria were a) comparative studies of tests/techniques for detecting COVID-19; b) animal studies; and c) reviews or guidelines.

Studies not excluded during screening were read in their entirety by one of four authors (HCVSV, JPG, TRA, and VW.). When doubts arose regarding the inclusion or exclusion of an article, they were discussed jointly and assessed by two other researchers (DRM and GGZ). The criteria for full reading were a) use of RT-PCR to diagnose COVID-19; b) publication in English, Portuguese, or Spanish; c) classification as an observational study; d) reporting of the time between symptom onset and positive RT-PCR results; e) collection of test samples by professionals; and f) use of at least one of the following test sample types: nasopharyngeal, oropharyngeal, sputum, or bronchoalveolar lavage. The exclusion criteria for full reading were the same as those used in the initial screening.

Data extraction

Four authors were responsible for data extraction (HCVSV, JPG, TRA, and VW), and questions were discussed jointly with two other researchers (DM and GZ). Microsoft Excel was used for this step. The following data were extracted: study design; population characteristics (country, sample size, sex, age, symptoms, and comorbidities); RT-PCR characteristics (detection protocol used and type of test sample); time from symptom onset to diagnosis (1 to 7 days, 8 to 14 days, or 15 or more days); and temporal profile of the RT-PCR tests. When throat swabbing was reported, it was considered an oropharyngeal sample. Tables and figures were developed to summarize the data on demographics, time from symptom onset to diagnosis, and temporal profile of the RT-PCR tests.

Evidence level classification

The evidence level of each study was assessed using the Oxford Centre for Evidence-Based Medicine (Howick et al., 2011) and Grading of Recommendations Assessment, Development and Evaluation (GRADE) systems (Guyatt et al., 2011).

Results

Study Selection

A total of 6096 articles were found. After excluding duplicates, 2875 articles remained and 2155 of these were excluded. After screening, 720 articles were selected for full reading, and 625 of these were excluded. Thus, 90 articles were included in this systematic review. Five articles selected for full reading were not accessible. The PRISMA flow diagram (Figure 1) illustrates the study selection process.

Population characteristics

The included studies were conducted in 31 different countries, the most prevalent of which were China (21 studies), the US (seven studies), Japan (seven studies), Italy (six studies), Brazil (five studies), India (five studies) and France (four studies). The remaining countries are listed in Table 1 . The studies included a total of 6831 patients (3584 men and 3155 women); five articles (92 patients) did not report the sex of the participants (Table 1). The ages of the participants ranged from 5 months to 101 years, with a median age of 49.95 years. Two studies did not report the ages of the participants, and two others did not do so clearly (Table 1).

Patient symptoms were described in 78 articles (86.66%), encompassing 5743 participants (3102 men, 2581 women, and 60 unreported). The three most frequent symptoms were fever, cough, and dyspnea, which affected 4012 (58.68%), 3192 (46.69%), and 2009 patients (29.38%), respectively (Supplementary material).

RT-PCR characteristics

Only articles that reported COVID-19 diagnosis by RT-PCR were selected. Studies reporting other diagnostic test types were ex-

Table 1
Study characteristics.

Study	Population Characteristics			RT-PCR Characteristics		Study design / Evidence level		
	Country	N	Gender (M/F)	Protocol	Sample Type	Study design	GRADE	Oxford
Xu et al. (2020)	China	7	3 / 4	Chinese CDC	NPS; anal swabs	Prospective cohort	Low	2
De Clercq et al. (2022)	Belgium	7	3 / 4	E gene	BAL	Prospective cohort	Low	2
Biguenet et al. (2021)	France	453	146 / 307	Charité	NPS	Prospective cohort	Low	2
Chas et al. (2021)	France	247	76 / 171	-	NPS	Prospective cohort	Low	2
Stockdale et al. (2021)	United Kingdom	293	115 / 178	Chinese CDC	NOS	Prospective cohort	Low	2
Kim et al. (2020)	South Korea	172	66 / 106	Charité	NPS	Prospective cohort	Low	3
Xiao et al. (2020)	China	301	154 / 147	Chinese CDC	NOS	Retrospective cohort	Low	3
Lo et al. (2020)	China	9	-	Chinese CDC	NPS; fecal and sputum samples	Retrospective cohort	Low	3
Yu et al. (2020)	China	76	38 / 38	Chinese CDC	NOS; sputum samples	Prospective cohort	Low	3
Xie et al. (2020)	China	21	13 / 8	-	OPS; sputum samples	Retrospective cohort	Low	3
Tan-Loh and Cheong, (2021)	Malaysia	39	-	-	NPS	Retrospective cohort	Low	3
İşlek and Balcı, (2022)	Turkey	183	74 / 109	Chinese CDC	NOS	Cross-sectional	Low	3
Xia et al. (2020)	China	114	69 / 45	Chinese CDC	NOS	Retrospective cohort	Low	3
Cai et al. (2020)	China	13	9 / 4	Chinese CDC	NPS; anal swabs	Retrospective cohort	Low	3
Vlek et al. (2021)	Holland	25	-	E gene	NOS	Cross-sectional	Low	3
Williams et al. (2020)	Australia	54	33 / 21	Charité	NP, sputum, BAL and TA samples	Prospective cohort	Low	3
Tsukagoshi et al. (2021)	Japan	148	83 / 65	CDC	NPS	Retrospective cohort	Low	3
Gu et al. (2021)	China	155	87 / 68	-	OPS	Retrospective cohort	Low	3
Yamamoto et al. (2021)	Japan	5	4 / 1	-	NPS; sputum samples	Retrospective cohort	Low	3
Schmidt Fernandes et al. (2021)	Brazil	114	25 / 89	-	OPS	Cross-sectional	Low	3
Basille et al. (2021)	France	5	5 / 0	-	NPS	Retrospective cohort	Low	3
Chen et al. (2021)	China	1589	942 / 647	-	NPS	Retrospective cohort	Low	3
Trunfio et al. (2020)	Italy	93	63 / 30	-	NOS	Retrospective cohort	Low	3
Garibaldi et al. (2021)	Brazil	3	-	-	NOS	Cross-sectional	Low	3
de la Calle et al. (2021)	Spain	455	255 / 200	N gene	NPS	Retrospective cohort	Low	3
Bullard et al. (2021)	Canada	36	22 / 14	E, RNAseP	NPS	Prospective cohort	Low	3
Acharya et al. (2021)	USA	122	62 / 60	-	NPS	Prospective cohort	Low	3
Flores-Silva et al. (2021)	Mexico	1072	697 / 375	-	NPS	Cross-sectional	Low	3
Patel et al. (2021)	USA	146	81 / 65	CDC	NOS	Retrospective cohort	Low	3
Ji et al. (2021)	China	631	344 / 287	-	OPS; sputum samples	Retrospective cohort	Low	3
Sakanashi et al. (2021)	Japan	7	-	-	NPS	Prospective cohort	Low	3
Rodríguez-Grande et al. (2021)	Spain	10	4 / 6	Chinese CDC	NPS	Prospective cohort	Low	3
Buonafine et al. (2020)	Brazil	125	50 / 75	-	NPS	Cross-sectional	Low	3
Fox-Lewis et al. (2020)	New Zealand	9	-	E gene	NOS	Prospective cohort	Low	3
Hase et al. (2020)	Japan	1	0 / 1	-	Sputum samples	Case report	Very low	4
Tajima et al. (2020)	Japan	1	1 / 0	CDC	NOS; saliva samples	Case report	Very low	4
Chen et al. (2020a)	China	1	1 / 0	Chinese CDC	NPS	Case report	Very low	4
Yuan et al. (2020)	China	6	2 / 4	Chinese CDC	NPS; fecal samples	Case series	Very low	4
Tang et al. (2021)	China	2	2 / 0	-	NPS; BAL	Case series	Very low	4
Lv et al. (2020)	China	1	1 / 0	Chinese CDC	OPS	Case report	Very low	4
Shao et al. (2020)	China	1	1 / 0	Chinese CDC	NPS	Case report	Very low	4
Marando et al. (2020)	Switzerland	1	0 / 1	-	BAL	Case report	Very low	4
Xing et al. (2020)	China	2	1 / 1	Chinese CDC	OPS	Case series	Very low	4
dos Reis et al. (2020)	Brazil	3	0 / 3	-	NPS	Case series	Very low	4
Gualano et al. (2020)	Italy	1	0 / 1	-	BAL	Case report	Very low	4

(*continued on next page*)

Table 1 (*continued*)

Study	Population Characteristics			RT-PCR Characteristics		Study design / Evidence level		
	Country	N	Gender (M/F)	Protocol	Sample Type	Study design	GRADE	Oxford
Elkhaled et al. (2020)	Qatar	1	1 / 0	Chinese CDC	NPS	Case report	Very low	4
Ata et al. (2020)	India	1	1 / 0	-	NPS	Case report	Very low	4
Soetisna et al. (2021)	Indonesian	1	1 / 0	-	NOS	Case report	Very low	4
Lamounier et al. (2020)	Brazil	1	0 / 1	-	NOS	Case report	Very low	4
Zhai and Zhang, (2020)	China	1	1 / 0	-	OPS	Case report	Very low	4
Islam et al. (2021)	Bangladesh	4	3 / 1	-	NPS	Case series	Very low	4
Waked et al. (2020)	Lebanon	1	1 / 0	-	BAL	Case report	Very low	4
Abid et al. (2021)	USA	3	3 / 0	-	BAL	Case series	Very low	4
Marza et al. (2021)	Romenia	1	0 / 1	-	NOS	Case report	Very low	4
Alsaud et al. (2021)	Qatar	5	5 / 0	-	NPS	Case series	Very low	4
Zheng et al. (2021)	China	2	0 / 2	-	OPS	Case series	Very low	4
Liang et al. (2020)	China	1	1 / 0	-	OPS	Case report	Very low	4
Ng et al. (2021)	Australia	1	1 / 0	-	BAL	Case report	Very low	4
Matsumura et al. (2020)	Japan	2	2 / 0	-	NPS	Case series	Very low	4
Condé et al. (2020)	Guinea	2	1 / 1	-	NPS	Case series	Very low	4
Gahide et al. (2020)	France	3	1 / 2	-	NPS	Case series	Very low	4
Khayat et al. (2021)	Saudi Arabia	1	0 / 1	-	NPS	Case report	Very low	4
Akca et al. (2020)	Turkey	1	1 / 0	-	BAL	Case series	Very low	4
Hegde et al. (2020)	USA	1	1 / 0	-	NPS	Case report	Very low	4
Akyala et al. (2020)	Nigeria	4	3 / 1	-	NPS	Case report	Very low	4
Malik et al. (2020)	USA	1	1 / 0	-	NPS	Case report	Very low	4
Páez-Velásquez et al. (2021)	Mexico	1	1 / 0	-	OPS	Case report	Very low	4
Kaya Tutar et al. (2020)	Turkey	3	2 / 1	-	NP swab	Case series	Very low	4
Luvira et al. (2020)	Thailand	3	3 / 0	Chinese CDC	NOS; sputum samples	Case series	Very low	4
Mahmoudi et al. (2020)	Iran	1	0 / 1	-	OPS	Case report	Very low	4
Bennasrallah et al. (2020)	Tunisia	1	0 / 1	-	OPS	Case series	Very low	4
Iancu et al. (2020)	Romania	1	0 / 1	-	NPS	Case report	Very low	4
Kariyappa et al. (2021)	India	1	0 / 1	-	NOS	Case report	Very low	4
Suryana K, (2021)	Indonesian	1	1 / 0	-	NPS	Case report	Very low	4
d'Orsi et al. (2021)	Italy	1	1 / 0	-	NPS	Case report	Very low	4
Kaushik et al. (2021)	India	1	1 / 0	-	NPS	Case report	Very low	4
Loconsole et al. (2021)	Italy	3	2 / 1	N, ORF1ab, S	NPS	Case series	Very low	4
Katti et al. (2021)	India	1	1 / 0	-	OPS	Case report	Very low	4
Birlutiu et al. (2021)	Romania	1	0 / 1	-	NPS	Case report	Very low	4
Novkovic and Cekerevac, (2021)	Serbia	1	0 / 1	-	NPS	Case report	Very low	4
Wan et al. (2021)	USA	1	1 / 0	Chinese CDC	NPS	Case report	Very low	4
Amaravathi et al. (2021)	India	1	1 / 0	-	NPS	Case report	Very low	4
Bourgonje et al. (2021)	Holland	3	2 / 1	-	NPS	Case series	Very low	4
Uda et al. (2021)	Japan	1	1 / 0	-	NPS; fecal samples	Case report	Very low	4
Baek et al. (2021)	South Korea	1	1 / 0	-	NPS; sputum and PF samples	Case report	Very low	4
Feng et al. (2020)	China	1	1 / 0	-	NOS	Case report	Very low	4
Chu et al. (2020)	USA	1	1 / 0	-	NOS	Case series	Very low	4
Paoli et al. (2020)	Italy	1	1 / 0	-	NOS	Case series	Very low	4
Persiano et al. (2020)	Italy	1	0 / 1	-	NPS	Case report	Very low	4
Zhang et al. (2020)	China	3	3 / 0	-	OPS	Case series	Very low	4
Total		**6831**	**3584 / 3155**					

BAL, bronchoalveolar lavage; CDC, US Centers for Disease Control and Prevention; Chinese CDC, Chinese Center for Disease Control and Prevention; F, female; M, male; NOS, nasopharyngeal/oropharyngeal swab; NP, nasopharyngeal; NPS, nasopharyngeal swab; OPS, oropharyngeal swab; PF, Pleural fluid; TA, tracheal aspirate.

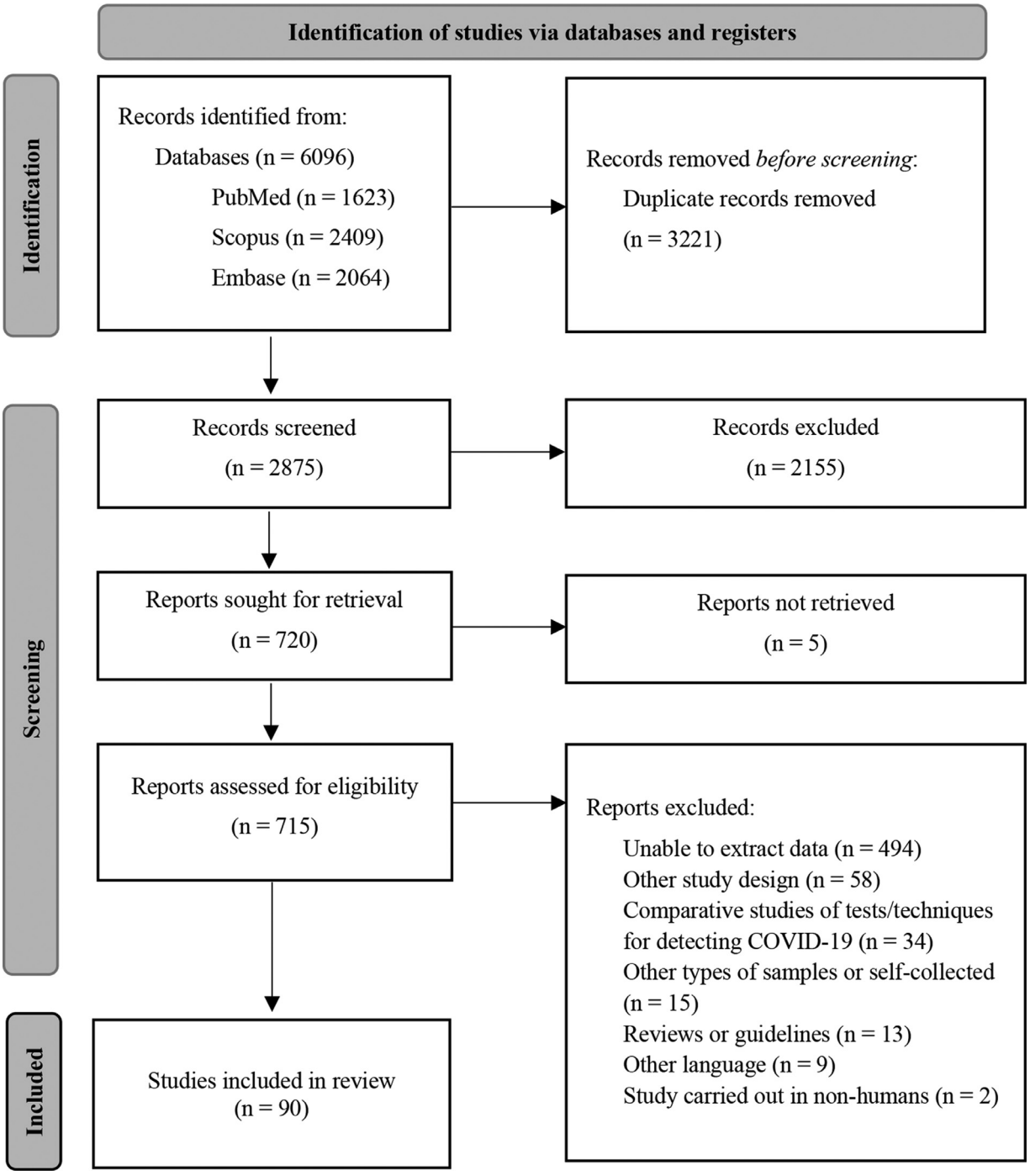

Figure 1. PRISMA flow diagram summarizing evidence search and study selection.

cluded. Among the 90 included articles, 29 reported the specific detection protocol used in RT-PCR testing. A total of 17 studies used the Chinese Center for Disease Control and Prevention protocol, which targets the ORF1ab and N genes or the ORF1ab and E genes. Three studies used the Charité protocol, which targets the E, N, and RdRp genes. Three studies used the US CDC protocol, which targets the N1 and N2 genes. Three studies described targeting only the E gene, one targeted the E and RNAseP genes, one targeted only the N gene, and another targeted the N, ORF1ab, and S genes as alternative protocols.

RT-PCR testing was performed using the following sample types in the 90 included studies: nasopharyngeal swabs (40 studies, 44.44%); oropharyngeal swabs (12 studies, 13.33%); bronchoalveolar lavage (seven studies, 7.77%); and nasopharyngeal/oropharyngeal swabs (16 studies, 17.77%). In addition, two studies used oropharyngeal and sputum samples, two studies used nasopharyngeal and anal swabs, two studies used nasopharyngeal and fecal samples, and two studies used nasopharyngeal and oropharyngeal swabs and sputum. One study only used sputum samples; one used nasopharyngeal swabs and sputum; one used nasopharyngeal and bronchoalveolar lavage; one used nasopharyngeal/oropharyngeal swabs and saliva; one used nasopharyngeal, fecal and sputum samples; and one used samples from the nasopharynx, sputum, bronchoalveolar lavage, and tracheal aspirate.

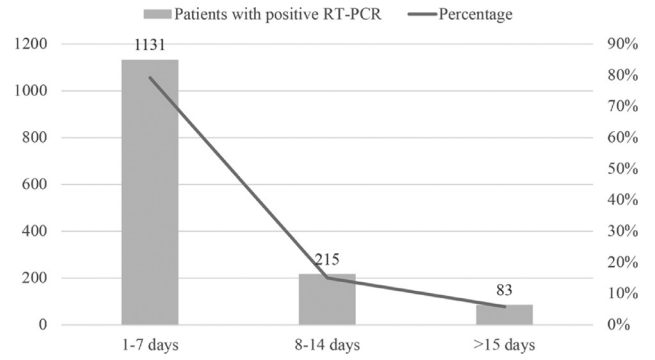

Figure 2. Time from symptom onset to diagnosis. Shown are the numbers of patients with positive RT-PCR test results at different times after onset of symptoms. RT-PCR, real-time reverse transcriptase polymerase chain reaction.

Finally, one study detected SARS-CoV-2 in pleural fluid, apart from nasopharyngeal and sputum samples.

Time from symptom onset to diagnosis

Only studies reporting the time between COVID-19 symptom onset and the first positive RT-PCR results were included. An overall average was calculated, grouping the results into the following periods: 1 to 7 days, 8 to 14 days, and 15 days or more. In the 90 included studies (6831 patients), the overall average was 6.72 days, with time gap described for 1429 patients; in 1131 patients (79.15%) it was 1 to 7 days, in 215 patients (15.05%) it was from 8 to 14 days, and in 83 patients (5.8%) it was 15 or more days (Table 2 and Figure 2). Altogether, in 65 studies the average time was 1 to 7 days (72.22%), in 17 studies it was 8 to 14 days (18.89%), and in eight studies it was 15 days or more (8.89%) (Table 2 and Figure 2).

Temporal profile of RT-PCR tests

We analyzed 14 articles separately (Bennasrallah et al., 2020; Chen et al., 2020a; Hase et al., 2020; Luvira et al., 2020; Lv et al., 2020; Shao et al., 2020; Xia et al., 2020; Xing et al., 2020; Xu et al., 2020; Tajima et al., 2020; Sakanashi et al., 2021; Uda et al., 2021; Wan et al., 2021; Zheng et al., 2021) that reported two or more RT-PCR tests on upper or lower respiratory tract specimens after symptom onset. Figure 3 shows positivity over time, with data regarding how many tests were performed and how many of these were positive in each period. The best performance occurred on days 22-24 after symptom onset, when 98% of the test results were positive. One study (Xiao et al., 2020) reported the results 7 days apart, so they were analyzed separately. This study used oropharyngeal swabs, resulting in 137 of 140 tests (97.9%) positive in the first 7 days, 152 of 221 tests (68.8%) positive between days 7 and 14, 127 of 350 tests (36.3%) positive between days 14 and 21, and 97 of 307 tests (30%) positive between days 21 and 28 (Figure 3).

Study designs and evidence level

Of the 90 included studies, 13 were prospective cohort studies (14.44%), 15 were retrospective cohort studies (16.66%), 36 were case reports (40%), 20 were case series (22.22%), and six were cross-sectional studies (6.66%). According to the GRADE system, 56 articles had a very low evidence level and 34 had a low evidence level. None of the included studies had a moderate or high evidence level. According to the Oxford system, 56 articles were level 4, 29 were level 3, and five were level 2. None of the included studies was level 1 (Table 1).

Studies in the low evidence category (level 2)

In total, five studies, all prospective cohorts, were defined as evidence level 2 (low). These five studies included a total of 1007 participants. Only one study described longitudinal testing follow-up. The gene detection protocol was reported in four of these studies; two used the Chinese Center for Disease Control and Prevention protocol (5, 76), one used the Charité protocol, and one used a protocol based on E gene detection. Regarding sample type, three studies (5, 49, 57) used only nasopharyngeal samples, one (40) used only bronchoalveolar samples, and one (76) used nasal and oropharyngeal samples. The median time from symptom onset to diagnosis was 5.287 days.

Studies in the low evidence category (level 3)

A total of 29 articles that fulfilled the inclusion criteria were classified in the evidence level 3 (low). They included 13 retrospective cohorts, eight cross-sectional studies, and eight prospective cohorts.

Thus, not all articles provided standardized data; therefore, we compiled the available data. Most of the studies used the Charité protocol, which targets genes E, N and RdRp; the US CDC protocol, which targets genes N1 and N2; or the Chinese Center for Disease Control and Prevention protocol, which targets genes ORF1ab and N to diagnose COVID-19. Unfortunately, because many articles did not report which protocol was used, we cannot accurately describe which genes were targeted.

Although some studies also used urine, blood, stool, sperm, or saliva as test samples, we included only data generated using approved methods for RT-PCR testing for COVID-19: nasopharyngeal swabs, oropharyngeal swabs, sputum, and bronchoalveolar lavage. Most studies with a low evidence level (level 3) used nasopharyngeal and oropharyngeal swabs, although some used sputum.

In 26 studies that reported a total of 5496 positive COVID-19 cases, the median time from symptom onset to diagnosis was 6.68 days. It is worth mentioning that three articles (267 patients) also provided longitudinal data about days since symptom onset: 241 patients had a positive result within seven days, 24 patients had a positive result between days eight and 14, and two patients did not have a positive result until day 15.

Studies in the very low evidence category (level 4)

A total of 56 articles that fulfilled the inclusion criteria were classified in the very low evidence category (level 4). These were mostly case reports (36 articles) and case series (20 articles). Although the majority did not report which RT-PCR protocol was used, two studies, nine studies, and one study reported using the primer sets recommended by the US CDC, the Chinese Center for Disease Control and Prevention, and an internal laboratory protocol, respectively. Regarding the sample type, 46 studies used nasopharyngeal swabs; 17 used oropharyngeal swabs; eight used bronchoalveolar lavage; five used sputum; and one used an anal swab, pleural fluid, pharyngeal swab, semen, and urine. In these studies, the time between symptom onset and diagnosis was reported for 87 patients, with a median of 5.93 days, ranging from zero (same day as onset) to 28 days.

Discussion

For efficient containment of the COVID-19 pandemic, patients who are infected must be promptly identified and isolated. To more effectively identify patients who are infected, the time of COVID-19 testing can be optimized, resulting in fewer false negatives and false positives. An important, recognized way of do-

Table 2
Time from symptom onset to diagnosis in each included study.

Study	1-7 days	8-14 days	> 15 days	N	Mean
Xu et al. (2020)	7			7	1,3 days
De Clercq et al. (2022)				7	12 days (9-15)
Biguenet et al. (2021)				453	4 days (1-10)
Chas et al. (2021)				247	3,3 days (1-16)
Stockdale et al. (2021)	120	108	65	293	7 days
Kim et al. (2020)				172	14 days (8-17)
Xiao et al. (2020)				301	16 days (10-23)
Lo et al. (2020)	6	3		9	5,2 days
Yu et al. (2020)	76			76	4 days (2-6)
Xie et al. (2020)				21	145.64 hours (21-441)
Tan-Loh and Cheong, (2021)				39	3,4 days
İşlek and Balcı, (2022)	123			183	7,9 days
Xia et al. (2020)	89	23	2	114	6,7 days
Cai et al. (2020)	13			13	2 days (1.1-4)
Vlek et al. (2021)	25			25	1-2 days
Williams et al. (2020)	54			54	3 days (1-5)
Tsukagoshi et al. (2021)	148			148	<5 days
Gu et al. (2021)	101	53	1	155	6,8 days
Yamamoto et al. (2021)	1	3	1	5	10,6 days
Schmidt Fernandes et al. (2021)	114			114	2 days (1-4)
Basille et al. (2021)	2	2	1	5	10 days
Chen et al. (2021)				1589	6 days
Trunfio et al. (2020)				93	4,95 days (0-11)
Garibaldi et al. (2021)	3			3	1,33 days
de la Calle et al. (2021)				455	5 days
Bullard et al. (2021)	36			36	1 dia
Acharya et al. (2021)				122	5,3 days
Flores-Silva et al. (2021)				1072	7 days (6-10)
Patel et al. (2021)	146			146	2 days (1-4)
Ji et al. (2021)				631	7 days (4-10)
Sakanashi et al. (2021)				7	1,57 days
Rodríguez-Grande et al. (2021)	6		4	10	9,9 days
Buonafine et al. (2020)				125	6 days
Fox-Lewis et al. (2020)	5	3	1	9	8,4 days
Hase et al. (2020)	1			1	6 days
Tajima et al. (2020)	1			1	4 days
Chen et al. (2020b)		1		1	11 days
Yuan et al. (2020)	1			6	7 days
Tang et al. (2021)	1	1		2	7,5 days
Lv et al. (2020)			1	1	23 days
Shao et al. (2020)	1			1	4 days
Marando et al. (2020)		1		1	9 days
Xing et al. (2020)	1	1		2	7,5 days
dos Reis et al. (2020)	2	1		3	6,6 days
Gualano et al. (2020)			1	1	19 days
Elkhaled et al. (2020)	1			1	3 days
Ata et al. (2020)	1			1	1 day
Soetisna et al. (2021)			1	1	15 days
Lamounier et al. (2020)	1			1	4 days
Zhai and Zhang, (2020)	1			1	6 days
Islam et al. (2021)	4			4	4 days
Waked et al. (2020)		1		1	10 days
Abid et al. (2021)		3		3	8,6 days
Marza et al. (2021)	1			1	3 days
Alsaud et al. (2021)	4	1		5	7,5 days
Zheng et al. (2021)	1		1	2	17,5 days
Liang et al. (2020)	1			1	1 day
Ng et al. (2021)	1			1	1 day
Matsumura et al. (2020)	1	1		2	10 days
Condé et al. (2020)	2			2	1 day
Gahide et al. (2020)	3			3	1,3 day
Khayat et al. (2021)	1			1	1 day
Akca et al. (2020)		1		1	12 days
Hegde et al. (2020)		1		1	9 days
Akyala et al. (2020)	4			4	3-7 days
Malik et al. (2020)			1	1	21 days
Páez-Velásquez et al. (2021)	1			1	2 days
Kaya Tutar et al. (2020)	2	1		3	5,5 days
Luvira et al. (2020)	2	1		3	4,6 days
Mahmoudi et al. (2020)	1			1	5 days

(*continued on next page*)

Table 2 (*continued*)

Study	1-7 days	8-14 days	> 15 days	N	Mean
Bennasrallah et al. (2020)	1			1	3 days
Iancu et al. (2020)	1			1	2 days
Kariyappa et al. (2021)		1		1	10 days
Suryana K, (2021)	1			1	3 days
d'Orsi et al. (2021)			1	1	20 days
Kaushik et al. (2021)	1			1	2 days
Loconsole et al. (2021)	3			3	2,66 days
Katti et al. (2021)		1		1	10 days
Birlutiu et al. (2021)			1	1	28 days
Novkovic and Cekerevac, (2021)	1			1	7 days
Wan et al. (2021)	1			1	3 days
Amaravathi et al. (2021)	1			1	5 days
Bourgonje et al. (2021)	1	1	1	3	10,66 days
Uda et al. (2021)	1			1	2 days
Baek et al. (2021)	1			1	1 day
Feng et al. (2020)	1			1	6 days
Chu et al. (2020)	1			1	4 days
Paoli et al. (2020)	1			1	7 days
Persiano et al. (2020)		1		1	11 days
Zhang et al. (2020)	2	1		3	7,33 days
Total	1134	215	83	6831	604,96

Light gray squares represent contribution of study data for a given time range.
Dark gray squares represent study data that could not be grouped into any of time ranges.

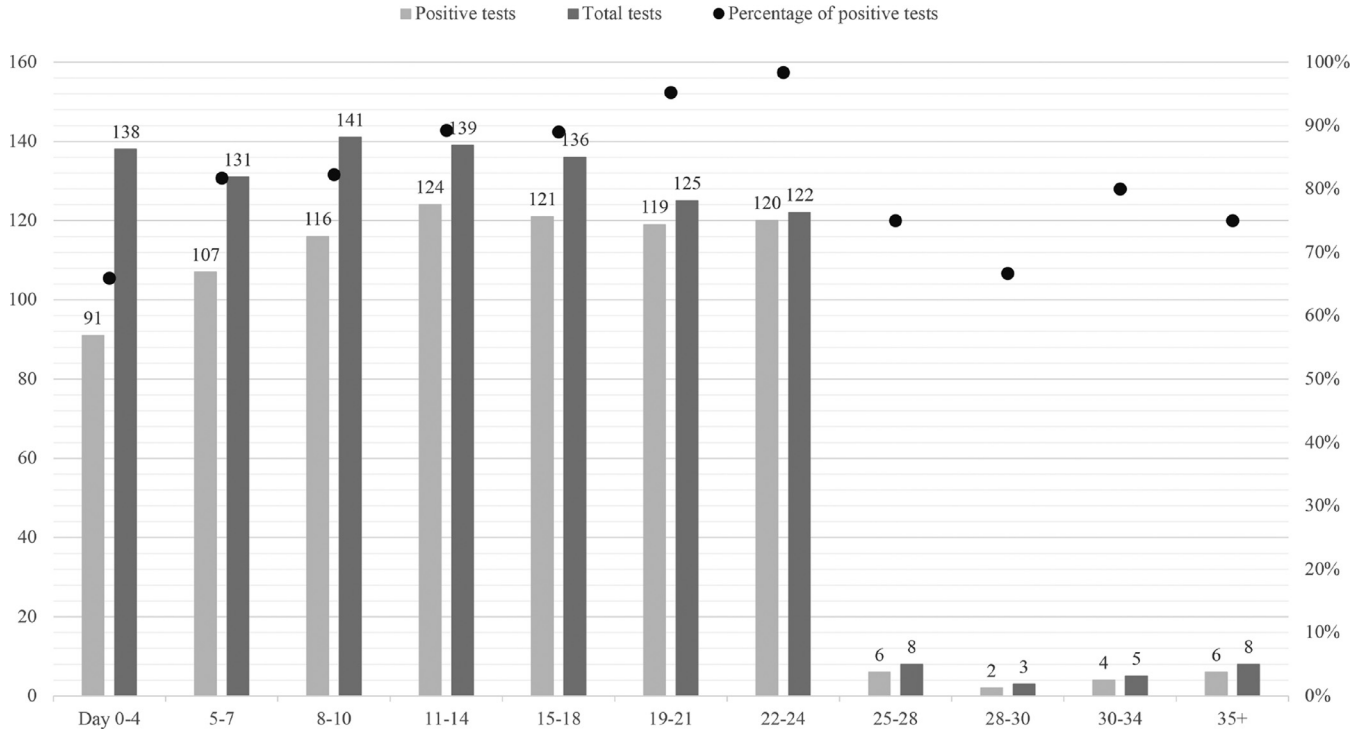

Figure 3. Temporal profile of real-time reverse transcriptase polymerase chain reaction (RT-PCR) tests. Shown are the numbers of patients with positive RT-PCR test results and total tests performed at different times after onset of symptoms.

ing this is to associate symptomatology with the optimal testing time in potential patients. Furthermore, chronic illnesses, such as systemic arterial hypertension, obesity, diabetes mellitus, and cardiovascular diseases, may also be associated with infection and morbidity (Ejaz et al., 2020; Sanyaolu et al., 2020). The preferred sample types for RT-PCR, identified by the WHO as the gold standards (World Health Organization, 2020a), are nasopharyngeal and oropharyngeal swabbing or lower respiratory tract samples, such as sputum and bronchoalveolar lavage, which are known to be even more sensitive than upper respiratory tract samples (Bwire et al., 2021).

At the beginning of the COVID-19 pandemic, the typical presentation of SARS-CoV-2 infection was determined to include mainly fever, dry cough, and dyspnea (Hui et al., 2020). Thus, in efforts to define which patients should be tested, these symptoms, especially in combination with anosmia and/or ageusia, were used to identify suspected COVID-19 infection (Guan et al., 2020; Vaira et al., 2020). However, some patients were asymptomatic, the clinical presentation of others was non-severe, and some patients developed severe symptoms or died (Chams et al., 2020; Chen et al., 2020b; Heymann and Shindo, 2020; Majumder and Minko, 2021). Therefore, as more studies became available, it became clear that

SARS-CoV-2 infection could cause a great variety of clinical symptoms and sequelae that are not fully comprehended to this day (Gavriatopoulou et al., 2020; Xiong et al., 2021). This situation poses a great challenge for diagnosing COVID-19 clinically.

In this systematic review, we found that the most common symptom type was systemic (fever), followed by pulmonary (cough and dyspnea). This clinical presentation agrees with the literature (Chen et al., 2020b; Jiang et al., 2020; Yang et al., 2020). In early 2020, Huang et al. reported on a cohort of 41 patients with COVID-19, finding that the most prevalent symptoms were fever, cough, and fatigue, with more than half of the patients developing dyspnea. Although anosmia and/or ageusia have been associated with COVID-19 (Lechien et al., 2020), our results indicate that, while important, they are not among the main symptoms reported here. Lee et al. (2020) reported that only 15.3% of 3191 patients diagnosed with COVID-19 presented with anosmia or ageusia. These symptoms have been associated with a variety of viral infections, and although they are neither highly prevalent nor pathognomonic, they are hypothesized to be more frequently observed with SARS-CoV-2 infection (Lee et al., 2020).

The difficulty in establishing an accurate and reliable clinical presentation, and consequent difficulty in determining which symptoms are caused by COVID-19 infection and which are due to other comorbidities and conditions, presents a confounding factor (Ejaz et al., 2020; Guan et al., 2020; Sanyaolu et al., 2020; Vaira et al., 2020). This leads to a certain overlap between symptoms that are and are not caused by COVID-19. In the present review, this confounding factor should be considered, especially for symptoms reported in fewer studies and by fewer participants.

Although our data imply that RT-PCR testing should be performed 6.72 days after the onset of symptoms, the results of studies included in this review are heterogeneous. This heterogeneity could be linked to a variety of causes, including differences in symptoms and particularly in the interpretation of the moment symptoms begin. Investigating healthcare professionals, Chas et al. (2021) and Schmidt Fernandes et al. (2021) discovered an average of 3.3 days (range 1-16 days) and 2 days (range 1-4 days), respectively between symptom onset and diagnosis by RT-PCR positive test result. This difference in relation to our findings may be explained by the ability of professionals, compared with the rest of the population, to identify symptoms earlier. Furthermore, there was considerable variation in the mean within the studies themselves.

On the other hand, the studies representing larger populations demonstrated means similar those identified in our review (Buonafine et al., 2020; Xia et al., 2020; Chen et al., 2021; Flores-Silva et al., 2021; Gu et al., 2021; Ji et al., 2021; Stockdale et al., 2021; İşlek and Balcı, 2022). A cross-sectional study of 1072 patients (Flores-Silva et al., 2021) found that the median time from symptom onset to diagnosis was 7 days, but the time varied from 6 to 10 days. In this study the clinical manifestations were mostly dyspnea, fever, and cough. A retrospective cohort study of 1589 patients (Chen et al., 2021) found that the median time from symptom onset to diagnosis was 6 days, and the clinical manifestations were similar to those in the aforementioned cross-sectional study (fever and cough). Ji et al. (2021) found a mean time of 7 days (range 6-10 days) in 631 patients; the most frequent symptom was fever, which was associated with disease severity. Therefore, we suggest testing during the period between 1 and 7 days after onset of symptoms.

Of note, certain studies report the average time between COVID-19 symptom onset and the first RT-PCR postive test result is more than 14 days (Kim et al., 2020; Xiao et al., 2020). In 301 patients hospitalized with COVID-19, the mean time between symptom onset and positive RT-PCR test result was 16 days (range 10-23 days). The median age of this population was 58 years, and the median period of SARS-CoV-2 RT-PCR positive test results was significantly longer in older (≥65 years) patients (Xiao et al., 2020). Kim et.al. (2020) reported a median of 14 days (range 8-17 days) between symptom onset and COVID-19 diagnosis. In this case, it is interesting to note that the population was limited to community facilities intended for the isolation of patients with mild symptoms of COVID-19. These differences in findings can be explained by sample variability. With advances in vaccination, it would be easier, although expensive, to follow patients with suspected COVID-19 symptoms and to test for COVID-19 over approximately 15 days after the first symptom to confirm or rule out the diagnosis.

SARS-CoV-2 causes systemic infection, affecting a wide variety of organs. For this reason, several sample types have been tested as targets for RT-PCR, including fecal samples, anal swabs, saliva, oropharyngeal and nasopharyngeal swabs, sputum samples and bronchoalveolar lavage (Bwire et al., 2021). These sample types were compared to find the most sensitive and effective; oropharyngeal and/or nasopharyngeal swabs, bronchoalveolar lavage, and sputum demonstrated the best results, and now are considered the gold standard by the WHO (World Health Organization, 2020a). In this review, we included data only from tests that used at least one of these gold-standard sample types, increasing the reliability of our findings.

During the COVID-19 pandemic, RT-PCR molecular diagnostic techniques focused on categorizing samples as having “detectable” or “undetectable” levels of distinct SARS-CoV-2 target genes, rather than on viral load quantification. Only 20 of the 90 papers in this systematic review provided cycle threshold (Ct) values or cutoffs for determining whether the virus was considered “detected”. In some cases, different detection protocols were used for individual sample Ct values and for the information about maximum Ct for a sample to be considered “detectable”. As a result, this data could not be pooled for review. According to the protocol established by the CDC (CDC, 2021a) and revised according to the protocol CDC-006-00019, Revision: 06 CDC/DDID/NCIRD/ Division of Viral Diseases Effective: 01/12/2020, when all controls exhibit the expected performance, a sample is considered negative if all amplification curves with the specific probes used present Ct values >40 and the reaction control curve (RNAseP) presents a Ct value <40. This analysis method was widely adopted for molecular diagnostics for SARS-CoV-2.

The COVID-19 pandemic has had a major impact on public health. The urgent need for empirical resources to support decision making led to certain limitations in this review, the foremost of which is that the evidence levels of the included studies were low or very low. We could find no studies with high or moderate levels of evidence that fulfilled the inclusion criteria. Most related studies in the literature lack the data necessary for a more critical analysis. In this systematic review, we did not consider the SARS-CoV-2 variants as variables in the analysis. As new variants of SARS-CoV-2 are being identified, it is important that reported symptoms and incubation times are considered and are used in decision making for RT-PCR testing.

Conclusion

Our systematic review assessed an important global issue – the time from COVID-19 symptom onset to the time when RT-PCR testing (the gold standard for SARS-CoV-2 diagnosis) has the highest probability of providing correct diagnosis. The included studies used different detection targets, each of which had been validated by a regulatory agency. Corroborating the practice of some health units, this review confirmed that the optimal time to perform RT-PCR testing is between the first and seventh days after symptom onset, with the highest positive result rate seen at a mean of 6.72 days. Among early symptoms of potential SARS-CoV-2 infection,

fever should be given highest consideration, followed by coughing and dyspnea. Onset of these symptoms can be used to count the number of days in which a patient has been symptomatic. Our results, which have identified the most relevant symptoms of infection and the time after symptom onset at which gold-standard testing should be performed, can shape medical practice, especially in countries with limited resources for RT-PCR testing. The results may also clarify parameters for patient monitoring and isolation, especially in the first seven days after infection is suspected.

Declarations of competing interest

The authors have no competing interests to declare.

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Data availability

The data are available from the corresponding author upon reasonable request.

Author contributions

PGS, DRM, GGZ, and JCC elaborated the idea and the design of the study. VW, JPG, TRA, and HCVSV selected studies and extracted data. PGS, DRM, and GGZ reviewed these procedures. PGS, VW, JPG, TRA, HCVSV, DRM, and GGZ performed analysis and interpretation of data and drafted the article. JCC revised it critically for important intellectual content. All authors approved the final version of the manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.06.037.

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