How to build a novel burst disease diagnostics for hunting potential infection source: the enlightenment from screening patients via combining viral and antibody testing at the COVID-19 pandemic early stage

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Abstract

Purpose: We aimed to summarize the diagnostic strategies used to identify potential patients with COVID-19 among inbound overseas travelers in Xiamen, China.

Methods: From October 2020 to December 2020, 180,000 inbound overseas travelers were subjected to government quarantine in Xiamen, China. We evaluated the screening efficiency of combining viral and antibody testing strategies to identify potential patients with COVID-19.

Results: The COVID-19 positive rate was 0.17% (306/180,000). There was no difference between the combined PCR and antibody and the PCR testing strategy for screening COVID-19(2.0 [IQR,2.0–3.0] vs. 2.0[IQR,2.0–2.0]; *P*= 0.175). The results for the combined screening strategy were available 4 days earlier than those for the PCR testing strategy (3.0 [IQR, 3.0–3.0] vs. 7.0[IQR, 7.0–14.0], *P*=0.000). Compared with the non-key screening population, the key screening population showed less PCR rounds (2.0 [IQR, 2.0–2.0] vs. 2.0[IQR, 2.0–3.0]; (*P*=0.008) and time (3.0[IQR, 3.0–3.0] vs. 7.0[IQR, 7.0–14.0]; *P*=0.000) for screening COVID-19.

Conclusion: Combining viral and antibody testing strategies is effective; it allows timely identification of the source of COVID-19 infection. Moreover, this strategy can be used for close contacts or sub-close contacts of patients with COVID-19 to reduce the risk of SARS-CoV-2 transmission.

Background

The pandemic of coronavirus disease 2019 (COVID-19) is an unprecedented global crisis with worldwide effects. Diagnostics have proven crucial to the response to this pandemic (1, 2). During the initial epidemic stages, molecular tests were based on the genome sequences of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). These tests are highly sensitive and specific; moreover, they were used as the reference standard for diagnosing active SARS-CoV-2 infection (1). Once the epidemic progressed to a pandemic, control programs began developing strategies for interrupting the transmission chains within communities by contact tracing, isolation and scaling up testing. Most countries found it challenging to scale up molecular testing due to high costs, shortages in trained staff, and global competition for reagents (3).

Many technologies have allowed the development of several novel diagnostic tests for COVID-19 screening. Viral and antibody testing are two diagnostic tests relevant to patient management and pandemic control for COVID-19. Viral testing, which can identify individuals with current SARS-CoV-2 infection, includes nucleic acid amplification tests (e.g., polymerase chain reaction [PCR]) that detect viral RNA as well as antigen tests that detect viral proteins. The PCR test for SARS-CoV-2 is the gold standard for confirming COVID-19 infection; however, false negatives may occur. The reliability of the PCR test for SARS-CoV-2 is subject to pre-analytical errors, including the quality of sample collection, the technology platform, and the designed primers; moreover, false negatives may result from clinical reasons such as pre-symptomatic or asymptomatic infections and variability in viral shedding(4).

Compared with molecular tests, antigen tests are more affordable and accessible; moreover, they can provide results within 15–20 min. However, antigen tests have a lower limit of detection (about 10^5–10^6 copies/mL) than molecular tests (about 10^2–10^3 copies/mL), which could result in missed diagnoses (5-8). Antibody testing can identify individuals exposed to the infection and is crucially involved in determining the true prevalence of COVID-19, especially for subclinical infections[9, 10]. However, antibody tests can be non-specific and yield false-positive results. Taken together, there remains no perfect test and identifying strategies for SARS-CoV-2 detection remains a critical element in the global strategy for controlling COVID-19.

After > 2 years into the COVID-19 pandemic, a testing strategy with clear goals can be adapted based on changes in the epidemiological situation, available tools and resources, and country- explicit context. During the initial pandemic stage, there was widespread confusion due to the poor understanding of the nature of the disease and the limited diagnostic capacity and capability (11). Gaining insight from applied COVID-19 testing strategies could inform approaches to future epidemics and pandemics. Accordingly, this study aimed to summarize the diagnostic strategies used to identify potential patients with COVID-19 among inbound overseas travelers in Xiamen, China. This could inform effective strategies for timely detection of the infection source, especially at the onset of a novel infectious disease.

Methods

Study Population and Ethics Statement

We included 180,000 inbound overseas travelers in Xiamen, China, from March 2020 to December 2020. The participants' temperature and respiratory symptoms were recorded twice daily. SARS-CoV-2 RT-qPCR and/or tests for total antibodies against SARS-CoV-2 were performed using a defined process. Participants who tested positive for SARS-CoV-2 via RT-qPCR were diagnosed with COVID-19 based on epidemiologic and clinical evidence (12). Subsequently, they were admitted to the hospital for further observation and management. We recorded the participant's age, continent of origin, nationality, comorbidities, and SARS-CoV-2 RT-qPCR and total antibody results.

The Testing Strategy of Inbound Overseas Travelers

All inbound overseas travelers were subjected to a 14-day government quarantine, with the arrival date being considered as day 1. Two testing strategies were performed during different periods. From March 2020 to April 2020, a PCR testing strategy was applied. Briefly, each participant was placed in a separate room and tested for SARS-CoV-2 via RT-qPCR on days 1, 7, and 14. From May 2020 to December 2020, the strategy for combining PCR and antibody testing was applied. Briefly, each participant was placed in a separate room and tested for SARS-CoV-2 via RT-qPCR and total antibodies via chemiluminescence microparticle immunoassay on day 1 and day 7. Participants who tested positive for RT-qPCR (regardless of the antibody test results) were diagnosed with COVID-19. Among the remaining participants,

participants who tested negative in the antibody tests underwent PCR tests on days 7 and 14, while those who tested positive were assigned as a key screening population and underwent PCR tests at 2-day intervals. All participants with negative PCR test results were discharged on day 14 (Figure 1). The PCR test rounds were considered as the number of PCR tests performed for an individual during the 14-day quarantine period.

Nucleic Acid Amplification Tests

Nasopharyngeal and oropharyngeal swabs were obtained using the Tellgen platform (Tellegen, Shanghai, China) and tested via RT-PCR using the Daan 2019-nCoV RT-PCR Kit (Daan gene, Guangzhou, China) for the ORF1ab and N genes. The detection limit of the reagent was 500 copies/mL. The threshold cycle values for both the ORF1ab and N genes were ≤ 40 cycles. Samples positive for both genes were considered positive for SARS-CoV-2 RNA. Samples with either ORF1ab or N gene positivity were reexamined, with repeated positivity for the same gene indicating positivity for SARS-CoV-2 RNA.

Antibody Measurement

The total antibodies (Ab) against SARS-CoV-2 in plasma samples were tested using chemiluminescence microparticle immunoassay kits supplied by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, following the manufacturer's instructions. Briefly, the reagent was developed based on a double-antigen sandwich immunoassay, with the receptor-binding domain of the spike SARS-CoV-2 protein as the immobilized and horseradish peroxidase-conjugated antigen. The antibody titer was calculated based on the cutoff and was recorded as the cutoff index (COI). A COI < 1.00 and ≥ 1.00 was considered negative and positive, respectively.

Statistical Analysis

The Mann-Whitney U test was used for continuous variables with skewed distribution, while a $\chi 2$ test or Fisher's exact test was used for categorical variables. A two-sided *P*-value of < 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS statistics version 20 (SPSS Inc., Chicago, Illinois, USA).

Results

Characteristics of Participants with Covid-19

Among the 180,000 participants, 306 (0.17%) were diagnosed with COVID-19 (Figure 2), including 230 (75.2%) male and 76 (24.8%) female patients (ratio 3.0:1). Most (80.0%) participants diagnosed with COVID-19 were aged 18–50 years. Among them, 145 (47.4%) and 161 (52.6%) participants were further diagnosed with symptomatic and asymptomatic COVID-19, respectively. Additionally, 51.2%, 21.6%, and 20.3% of the patients came from Asia, Europe, and North America, respectively (Table 1).

Screening efficiency of dfferent the testing strategies

Among the 306 patients with COVID-19, excluding those screened on day 1 (n=121), there was no significant difference in the PCR rounds for the combined and PCR testing strategies for screening COVID-19 (P = 0.175). Further, the time until COVID-19 screening was 4 days earlier using the combined strategy (3.0 [IQR, 3.0–3.0]) than when using the PCR testing strategy (7.0 [IQR, 7.0–14.0], P = 0.000) (Table 2).

Screening efficiency for key screening population

The PCR rounds for screening COVID-19 were lower in the key than in the non-key screening population (P = 0.000). Further, the time until COVID-19 screening was 4 days earlier in the key population (3.0 [IQR, 3.0–3.0]) than in the non-key population (7.0 [IQR, 7.0–14.0], P = 0.000) (Table 3).

Discussion

The sudden COVID-19 onset required countries to rapidly develop strategies for viral containment. Among the critical elements of any response strategy is widespread testing. Testing is crucial in reducing the risk of COVID-19 transmission and managing the pandemic. Further, widespread testing allows prompt diagnosis, isolation, and treatment of individuals who have contracted COVID-19. Viral RNA detection through PCR assays has been the gold standard for early and precise detection. Despite the high analytical sensitivity, the real-world performance of PCR testing is unsatisfactory. Specifically, numerous suspected patients have to be tested for several days using multiple samples before confirming the diagnoses; additionally, during the waiting time, it might not be possible to assign priority to relevant treatments and quarantine management strategies(13). Comparisons among radiological, clinical, and PCR findings demonstrate these challenges. In one study, 35% of patients with positive computed tomography scan findings were negative on RNA PCR tests performed upon admission (14). Accordingly, timely COVID-9 diagnosis has become among the bottlenecks in adapting relevant actions for limiting the damage of the current outbreak. Our findings showed that combining PCR and antibody testing allowed 4-day earlier screening of patients with COVID-19 compared with PCR testing alone. Specifically, the combination strategy allowed timely isolation of patients with COVID-19 without increasing the PCR rounds.

The current COVID-19 pandemic has increased focus on routine asymptomatic testing strategies that could prevent sustained transmission in hospitals and other defined settings with at-risk individuals(15). Testing has been used at an unprecedented scale in settings outside health care for screening to protect clinically vulnerable individuals; to release people from quarantine at border crossings; and to facilitate safe environments for resuming cultural, social, and economic activities in communities (3). Accordingly, repeated PCR testing for population screening of asymptomatic individuals can be used to limit the spread of SARS-CoV-2 (16). However, PCR involves stringent requirements for its application; moreover, countries require sufficient capacity for conducting PCR tests to control the outbreak. In case of restricted capacity, inconsistent supplies, or delayed results, an alternative strategy should be adapted. Compared with PCR testing, antibody testing has the advantages of a faster turn-around time, high

throughput, and lower workload. A positive antibody test is not strictly indicative of a current COVID-19 infection; instead, serology tests may be used in population screening, modeling disease spread in the community, and staff surveillance. Additionally, the required performance criteria may vary across the different settings. Zhao et al. reported that combining molecular and antibody testing in the second week after symptom onset increased the COVID-19 detection rate by as much as 40% (17). Notably, in our study, although the PCR testing frequency was increased for the key screening population, this population showed a lower number of PCR rounds and detection time than the non-key screening population. This indicates that our strategy of combining PCR and antibody testing is effective by allowing timely detection of the source of infection.

Limitations

The study to describe an effective strategy involving the combination of viral and antibody testing to screen potential patients with COVID-19. However, this study has several limitations. First, we did not compare the public health measures and perform further effect analyses. Second, due to biosafety issues and equipment availability, we did not evaluate the virus activity, sequences, or SARS-CoV-2 transmission in positive patients.

Conclusions

In conclusion, combining viral and antibody testing is an effective strategy for timely detection of the infection source. Additionally, it can be used for close contacts or sub-close contacts of patients with COVID-19 to reduce the risk of SARS-CoV-2 transmission.

Declarations

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation..

Author Contributions

TCY, YYC, MLT and LLLin, designed the study and drafted the manuscript, TCY, and LLLin critical review and revision of the manuscript. YYC, MLT performed experiments. HLZ and LLLiu were responsible for statistical analysis. All authors agree to be accountable for the content of the work. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

Ethics approval

This study was approved by the Institutional Ethics Committee of Zhongshan Hospital, Xiamen University, which waived the requirement for written informed consent due to the emergent nature of COVID-19.

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Tables

Table 1. Participant demographics

Characteristics	Total	PCR testing strategy	Combining PCR and antibody strategy	Statistical analysis*
	COVID-19 n (%) n=306	COVID-19 n (%) n=20	COVID-19 n (%) n=286	
Sex				
Male	230(75.2%)	14(70.0%)	216 (75.5%)	χ2=0.306, *P*=0.595
Female	76(24.8%)	6(30.0%)	70 (24.5%)	
Age				
0~17	13(4.0%)	1(5.0%)	12(4.2%)	χ2=0.042, *P*=0.980
18~50	244(80.0%)	16(80.0%)	228(79.7%)	
≥ 50	49(16.0%)	3(15.0%)	46(16.1%)	
Types of infection				
Symptomatic	145(47.4%)	9(45.0%)	136(47.6%)	χ2=0.490, *P*=0.825
Asymptomatic	161(52.6%)	11(55.0%)	150(52.4%)	
Continent of departure				
Asia	157 (51.2%)	9(45.0%)	148(51.7%)	χ2=7.405, *P*=0.318
Europe	66(21.6%)	4(20.0%)	62(21.7%)	
North America	62(20.3%)	4(20.0%)	58(20.3%)	
South America	7(2.3%)	1(5.0%)	6(2.1%)	
Africa	10(3.3%)	1(5.0%)	9(3.1%)	
Oceania	4(1.3%)	1(5.0%)	3(1.1%)	

* Fisher's exact test was used to compare the portion of different characteristics between PCR testing strategy and combining PCR and antibody testing strategy group.

Table 2 Screening efficiency of different the testing strategies

* Mann-Whitney U test was used to compare the different groups.

Table 3 Screening efficiency for key screening population

	Combining PCR and antibody strategy （n=179）	PCR testing strategy （n=6）	Statistical analysis*
PCR round for screening COVID-19 Round, median (IQR)	2.0 (2.0-2.0)	2.0 (2.0-3.0)	*P*=0.175
Time for screening COVID-19 Day, median (IQR)	3.0 (3.0-3.0)	7.0(7.0-14.0)	*P*=0.000

	Key screening population (n=140)	Non-key screening population (n=39)	Statistical analysis*
PCR round for screening COVID-19 Round, median (IQR)	2.0(2.0-2.0)	2.0(2.0-3.0)	*P*=0.000
Time for screening COVID-19 Day, median (IQR)	3.0(3.0-3.0)	7.0(7.0-14.0)	*P*=0.000

* Mann-Whitney U test was used to compare the different groups.

Figures

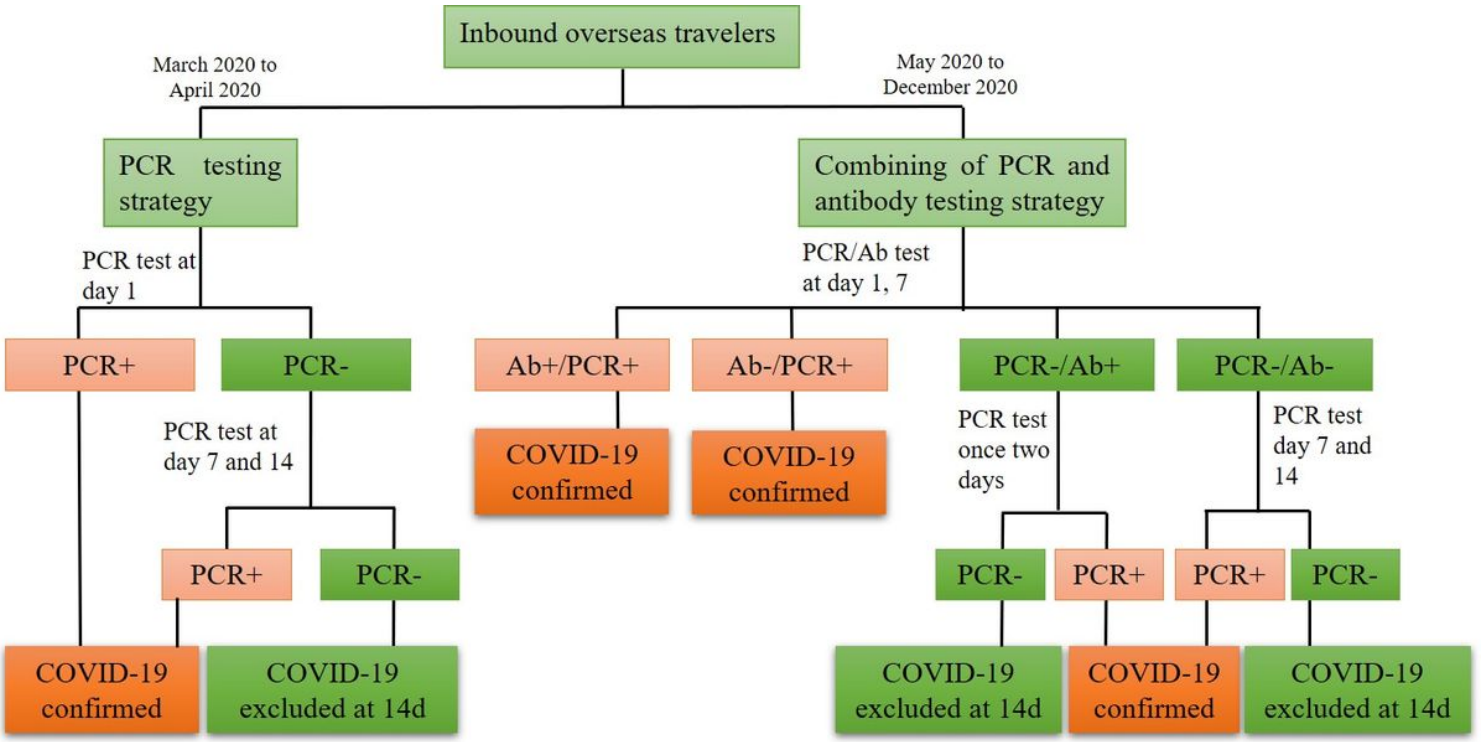

Figure 1

The testing strategy of inbound overseas travelers during different period

PCR, polymerase chain reaction; Ab, antibody; +, positive for the test; -, negative for the test.

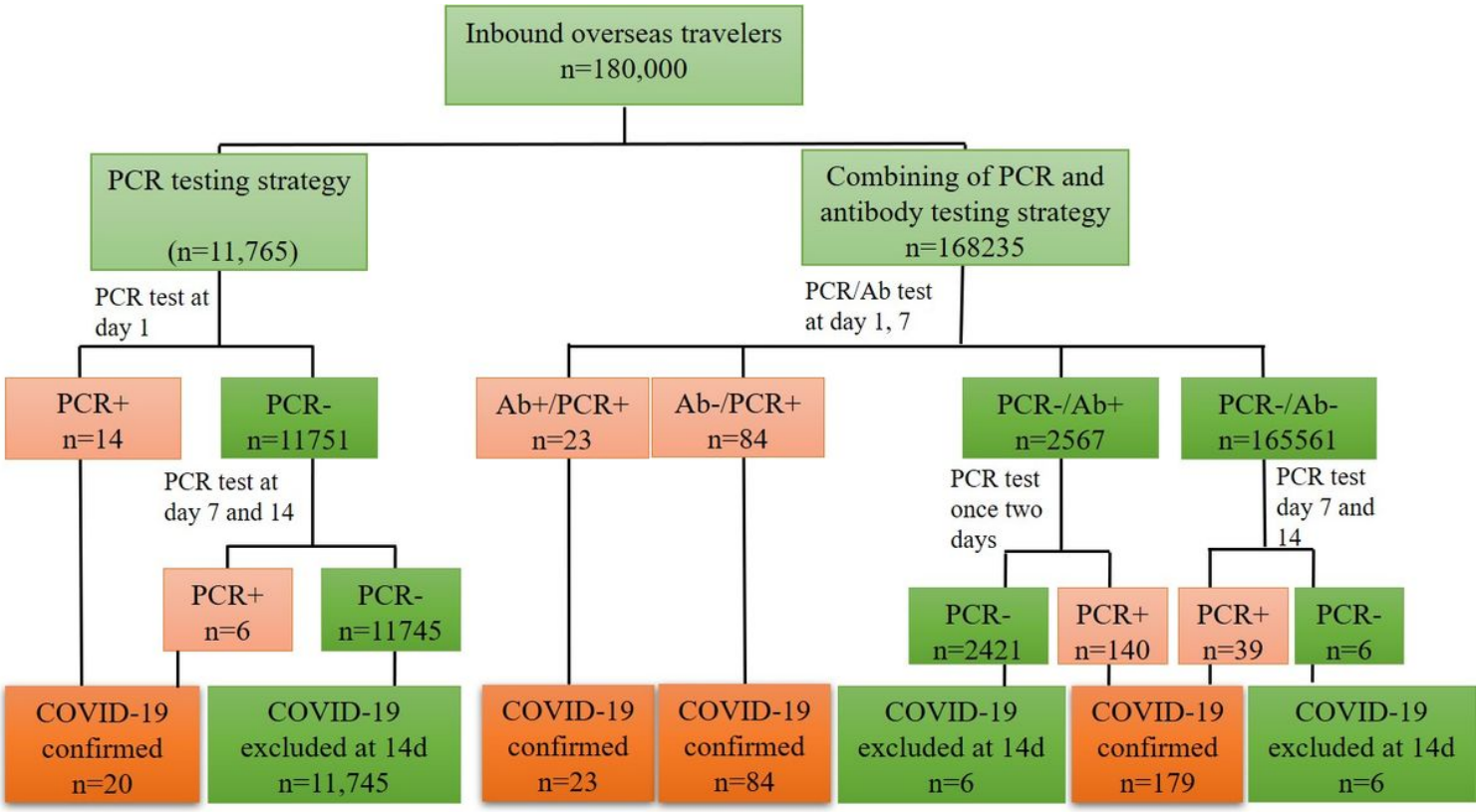

Figure 2

patients with COVID-19 were screening by different testing strategy

PCR, polymerase chain reaction; Ab, antibody; +, positive for the test; -, negative for the test.