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Logistic advantage of two-step screening strategy for SARS-CoV-2 at airport quarantine

Isao Yokota a,*, Peter Y. Shane b,c, Takanori Teshima b,d,e,**

a Department of Biostatistics, Hokkaido University Faculty of Medicine, Sapporo, Japan
b International Medical Department, Hokkaido University Hospital, Sapporo, Japan
c Clinical Research and Medical Innovation Center, Hokkaido University Hospital, Sapporo, Japan
d Division of Laboratory and Transfusion Medicine, Hokkaido University Hospital, Sapporo, Japan
e Department of Hematology, Hokkaido University Faculty of Medicine, Sapporo, Japan

ABSTRACT

Background: Airport quarantine is required to reduce the risk of entry of travelers infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, it is challenging for both high accuracy and rapid turn-around time to coexist in testing; polymerase chain reaction (PCR) is time-consuming with high accuracy, while antigen testing is rapid with less accuracy. However, there are few data on the concordance between PCR and antigen testing.

Methods: Arrivals at three international airports in Japan between July 29 and September 30, 2020 were tested for SARS-CoV-2 using self-collected saliva by a screening strategy with initial chemiluminescent enzyme immunoassay (CLEIA) followed by confirmatory nucleic acid amplification tests (NAAT) only for intermediate antigen concentrations.

Results: Among the 95,457 persons entering Japan during the period, 88,924 (93.2%) were tested by CLEIA, and 0.29% (254/88,924) were found to be SARS-CoV-2 antigen positive (≥4.0 pg/mL). NAAT was required for confirmatory testing in 0.58% (513/88,924) with intermediate antigen concentrations (0.67–4.0 pg/mL) whereby the virus was detected in 6.6% (34/513). This two-step strategy reduced the utilization of NAAT to one out of every 173 test subjects. The estimated performance of this strategy did not show significant increase in false negatives as compared to performing NAAT in all subjects.

Conclusions: Point of care testing by quantitative CLEIA using self-collected saliva is less labor-intensive and yields results rapidly, thus suitable as an initial screening test. Reserving NAAT for CLEIA indeterminate cases may prevent compromising accuracy while significantly improving the logistics of administering mass-screening at large venues.

1. Introduction

The current pandemic has forced many countries to introduce border closures to prevent the entry of infected travelers from regions where coronavirus disease-19 (COVID-19) is rife [1]–[3]. However, this decision has brought on heavy social and economic repercussions. Recently, countries have been increasingly accepting international flights, albeit with various testing requirements that commonly include quantitative reverse transcriptase polymerase chain reaction (PCR) before departure, temperature and symptom checks at airports, and PCR upon arrival [4–7]. Consequently, the increasing volume of international travellers has presented new logistic challenges. Specifically, the “gold standard” of detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by PCR using nasopharyngeal swab (NPS) samples requires trained professionals in full protective gear to collect specimens one person at a time [8,9]. Furthermore, the time required for laboratory analysis presents another bottleneck whereby passengers may spend hours in transit among potential infectees.

Solutions to improve the efficiency of mass-screening for SARS-CoV-2 include the replacement of NPS samples with self-collected saliva thereby eliminating specialized medical personnel and allowing simultaneous parallel sample collection [10,11]. We and others have shown...
that the accuracy of paired samples of self-collected saliva and NPS are equivalent in large scale direct comparative studies, with true concordance probability of these tests estimated at 0.998 (90%CI:0.996–0.999) [12–14]. However, although PCR is highly accurate and reliable, results may take 24–48 h to return. Such delays may lead to further transmission of disease [15], especially in the confines of airport transit. Additional advantage may be conferred by using reverse transcriptase loop-mediated isothermal amplification (LAMP) at the point-of-care (POC) instead of PCR, reducing the laboratory turnaround time to 30 min [14,16]. The results of LAMP and PCR showed good concordance with Kendall’s coefficient of concordance \( W = 0.98 \) (\( n = 44 \)) and perfect concordance in a separate cohort of 1763 persons (4 positives and 1759 negatives) [14]. Significantly, we recently reported that a novel quantitative antigen test using chemiluminescent enzyme immunoassay (CLEIA) and PCR provided concordant results in 2020 (98.2%) out of 2056 persons [17]. Since CLEIA utilizes a fully automated system to detect SARS-CoV-2 nucleoproteins in 30 min, we proposed its use as the first-line testing modality. Accordingly, a novel two-step strategy has been implemented for mass-screening of SARS-CoV-2 at airport quarantines in Japan in which CLEIA was deployed as the initial test with confirmatory nucleic acid amplification test (NAAT) performed only for indeterminate results [17]. The aim of this study is to evaluate the utility of this two-step screening strategy in real-world implementation and to estimate its performance in several clinical scenarios.

2. Methods

2.1. Design and population

The study cohort consisted of asymptomatic travelers arriving at three international airports between July 29 and September 30, 2020 who were able to provide self-collected saliva. Testing for SARS-CoV-2 using either self-collected saliva or NPS samples obtained by medical officers was mandatory for all international arrivals in Japan during the period. Due to logistic advantages, vast majority of tests were performed using self-collected saliva. Subjects who requested NPS sampling were excluded, and all test subjects were enrolled consecutively. Saliva samples were collected in a sterilized 15 mL polystyrene sputum collection tube (Toyo Kizai, Warabi, Japan) and all samples were analysed at the airport quarantine laboratories. This study was approved by the Institutional Ethics Board (Hokkaido University Hospital Division of Clinical Research Administration Number: 020–0116) and anonymously processed data were provided by the quarantine stations.

2.2. Interventions

The two-step screening strategy is the combination of an initial CLEIA test and the secondary NAAT test to confirm indeterminate CLEIA results [17]. Initially, all specimens were tested by CLEIA with positive and negative thresholds of 4.0 pg/mL and 0.67 pg/mL, respectively, as previously reported [17]. Concentrations in between the thresholds were considered indeterminate, and only these specimens underwent confirmatory testing by NAAT.

2.3. Definitions

Lumipulse SARS-CoV-2 Ag kit (Fujirebio, Tokyo, Japan), a sandwich CLEIA using monoclonal antibodies that recognize SARS-CoV-2 N–Ag on LUMIPULSE G1200 automated machine (Fujirebio), was used as previously described [17]. The detection range is between 0.01 pg/mL and 5000 pg/mL. Saliva was diluted 4-fold with phosphate buffered saline and centrifuged at 20,000 \( \times g \) for 5 min to remove cells and debris. RNA was extracted from 200 \( \mu \)L of the supernatant using QIAamp DSP Virus/Pathogen kit and QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Then, nucleic acids of SARS-CoV-2 were detected by either PCR or LAMP. PCR tests were performed as previously described [14]. The cycle threshold (Ct)-values were obtained using N2 primers (NIID_2019-nCOV_N_F2, NIID_2019-nCOV_N_R2) and a probe (NIID_2019-nCOV_N_P2). LAMP was carried out to detect SARS-CoV-2 RNA using Loopamp® 2019-SARS-CoV-2 Detection Reagent Kit (Eiken Chemical, Tokyo, Japan) and the Loopamp Real-time Turbidimeter (Eiken Chemical) as previously described [14].

2.4. Statistical analysis

We compared the estimated effectiveness of three mass-screening strategies at border quarantine: the two-step strategy, NAAT only for all entrants (without CLEIA), and test-free entry, expressed as the rate of false negatives per 100,000 persons and the number of NAATs performed. The rate of false negatives by NAAT was estimated by \( p \times FN/Pos \) where \( p \) is the assumed proportion of the test population with positive NAAT, and \( FN/Pos \) defined as the ratio of false negatives to all positives (i.e. the ratio of undetected infectees to persons diagnosed as infected). Four scenarios with \( p \) values of 0.1%, 0.2%, 0.5%, and 1% were used for analyses, with a factor of 0.76 (probability of CLEIA positivity given NAAT positivity) applied to the \( p \) values for the two-step strategy, consistent with our previous report [17]. \( FN/Pos \) was set at 0.4 in accordance to a recent report showing 136 and 52 positive results at airport screening and during post-entry compulsory quarantine, respectively [18]. Since no other reliable reference for \( FN/Pos \) was available in the literature, additional analyses were performed for \( FN/Pos \) hypothetically set at 1.0 and 2.0. The impact of 14-day quarantine was calculated in all cases as a linear variable of adherence rates with all false negatives becoming apparent with adherence rate of 100%. By expressing \( a \) as the probability of CLEIA-positive given NAAT-positive, the rate of false negatives by CLEIA may be calculated as \( p \times FN/Pos \times (1 - a) \). All statistical analyses were conducted by R 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

88,924 persons screened by the two-step strategy using self-collected saliva accounted for 93.2% of all arrivals to the three international airports in Japan during the period. Initial CLEIA was found to be positive in 254 (0.29%) persons (Fig. 1). The 513 samples (0.58%) with antigen concentrations in the indeterminate range (between 0.67 pg/mL and 4.00 pg/mL) proceeded to NAAT with 34 (6.6%) positive results. 254 (99.2%) of the 288 positive results were diagnosed by the initial CLEIA, with only 34 (11.8%) diagnosed by NAAT. On the other hand, of the 88,636 persons who tested negative, NAAT was performed in only 479 (0.54%). The median [IQR] antigen concentrations were 9.70 [4.98–34.11] pg/mL and 0.10 [0.01–0.19] pg/mL in the NAAT-positive and -negative samples, respectively. In specimens negative by NAAT, the frequency of high antigen concentrations monotonically approached zero, while NAAT positives did not follow any trend (Fig. 2).

Comparing the effectiveness of the three strategies, the number of false negatives was greater in the two-step strategy compared to NAAT only in all scenarios of NAAT positivity, although both tests reduced false negative rates by more than half compared to test-free entry (Table 1). Conversely, the two-step strategy allowed the reduction of NAAT by approximately 95% compared to when NAAT was used in all persons. For example, in the scenario when \( p = 0.1\% \), the NAAT only strategy detected 40 false negatives by 100,000 NAATs, whereas the two-step strategy resulted in 64 false negatives but only required 549 NAATs to be performed. Furthermore, as the majority of CLEIA indeterminates were NAAT negative, the number of NAATs needed did not significantly increase with varying scenarios of \( p \).

When \( FN/Pos \) was set at 0.4, 0.1, and 2.0 in the scenario where \( p = 0.1\% \), the ratio of false negatives by NAAT only: two-step: free entry was 40 : 64 : 140 (i.e. 1 : 1.6 : 3.5), 100 : 124 : 200 (i.e. 1 : 1.2 : 2.0), and 200 : 224 : 300 (i.e. 1 : 1.1 : 1.5), respectively, showing increasingly diminished
relative difference in efficacy between the three screening strategies (Table 2). Regardless of FN/Pos, post-screening 14-day quarantine substantially reduced imported false negatives, although the efficacy of quarantine was highly dependent on the degree of adherence (Table 2).

4. Discussion

Although PCR is a standard of care for the detection of SARS-CoV-2, mandatory mass-screening should ideally avoid time-consuming and labor-intensive procedures. In this regard, quantitative antigen test by CLEIA is rapid albeit with slightly less accuracy than PCR [17]. Therefore, our two-step strategy combined the utility of initial CLEIA with the accuracy of NAAT only for diagnosis of indeterminate cases. This two-step strategy has been adopted in quarantine stations at the international airports in Japan, especially for the prevention of long waiting times spent in closed spaces in crowds and close-contact situations. Here, we showed the benefits of this strategy using 88,924 samples, providing numerical estimates of undetected infectees under various circumstances. The quantitative antigen testing allows for setting appropriate positive and negative thresholds to freely define the indeterminate range with a trade-off; a wider range improves test performance but at the expense of increasing the requirement of confirmatory NAAT. These thresholds may be altered to suit different clinical situations [19], most importantly the local prevalence of disease.

Initial CLEIA has excellent specificity with the upper cutoff value at 4.00 pg/mL, as increasing antigen concentrations of NAAT negative samples consistently approach zero (Fig. 2b). Assuming that the frequency continues to decrease by one for every 0.5 pg/mL, 36 NAAT-negative samples would be included between 4 pg/mL and 8 pg/mL, giving specificity as high as 99.96% (=88,636/(88,636 + 36)). To further reduce the false positive rates, the upper cutoff may be set at higher values, but at the expense of increased requirement for confirmatory NAAT. For example, raising the upper cut-off from 4 pg/mL to 8 pg/mL would increase the number of indeterminate results and hence the number of NAAT from 513 to 603 (Fig. 2a). The main objective of screening for SARS-CoV-2 is to detect all transmissible persons, and this has been a great challenge with presymptomatic false negative PCR at 67% one day prior to and 38% on the day of symptom onset [20]. Therefore, in order to accurately identify presymptomatic infectees, pre-departure testing should be conducted several days before departure. Assuming that all passengers were asymptomatic with negative results before departure, infectees will most likely be in the latent phase at the time of screening. Given the median incubation period of 5 days [21–23], testing five days prior to departure should reveal half the infections (i.e. FN/Pos = 1) as false positives are very rare. Shorter intervals between pre-departure testing and inbound screening would yield more presymptomatic false negatives, and reduce operational test sensitivity [3, 20]. Illustrating this point, our results showed the ratio of false negatives comparing NAAT only to the two step strategy was 40 : 64 (i.e. 1 : 1.6) and 200 : 224 (i.e. 1 : 1.1) setting FN/Pos at 0.4 and 2.0, respectively. This trend was consistently seen in all scenarios of p and between any two strategies, indicating the vulnerability of depending on any test at a single time-point.

Regardless of testing strategy, post-screening quarantine performed very well at limiting import cases of false negatives in any scenario. However, perfect adherence to two weeks of compulsory isolation by all travelers is unrealistic, with detrimental psychological, social, and economic impact for those who do comply [24] [26]. Recently, Wells et al. reported that testing on day 6 may reduce the duration of a 14-day quarantine by 50% while effectively preventing expected transmission events [27]. As with pre-departure, the timing of testing is essential as infected individuals very early in the incubation period may not be detected due to low viral loads. Nevertheless, mass screening at airport has benefits in reducing false negatives, especially in combination with well-timed pre-departure testing (i.e. when FN/Pos is small). Furthermore, screening is useful in monitoring the dynamics of test positivity,
which may influence various immigration and health policies as well as suggest the possibility of viral mutations.

The main limitation of our study was the lack of clinical information after screening to assess the rates of observed false positivity. Post-screening longitudinal investigation after real-world mass-screening was simply out of the scope of this study. An additional limitation was that the probability of CLEIA-positive given NAAT-positive could not be validated, as NAAT was not performed for CLEIA-negative samples (antigen levels below 0.67 pg/mL). Finally, although not truly a limitation of our study, we alluded to NAAT positivity as infectiousness, whereas this may not be true in cases of high Ct values [28,29].

In summary, we examined the data from mass screening of 88,924 persons at airport quarantines and showed the effectiveness of the two-step strategy. We believe the logistic advantage of reducing the burden of NAAT to one in 173 subjects far outweigh the cost of slightly higher imported cases of false negatives. Two-step testing by CLEIA followed by NAAT is effective in real-world situations, especially when combined with appropriately timed pre-departure testing and/or with quarantine optimized with repeat testing.

**Author contributions**

IY, PYS and TT provided statistical analysis and drafted the manuscript and all authors reviewed critically and approved the final manuscript.

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**Declaration of interests**

IY reports a policy research grant from the Ministry of Health, Labour and Welfare, Japan, during the conduct of the study; and personal fees from Chugai Pharmaceutical, AstraZeneca, Japan Tobacco Pharmaceutical Division, and Nippon Shinyaku, outside the submitted work. PYS reports personal fees from AYUMI Pharmaceutical, Japan Pharmaceutical Manufacturers Association, Alexion Pharmaceuticals, and Kyowa Kirin, outside the submitted work. TT reports policy research grant from the Ministry of Health, Labour and Welfare, Japan, during the conduct of the study; personal fees from Merck Sharp & Dohme, Takeda Pharmaceutical, Pfizer Japan, and Bristol Myers Squibb, grants and personal fees from Kyowa Hakko Kirin, grants, personal fees, and non-financial support from Novartis Pharma, grants from Chugai Pharmaceutical, Sanofi, Astellas Pharma, Teijin Pharma, Fuji Pharma, Nippon Shinyaku, the Japan Society for the Promotion of Science (Grants-in-Aid for Scientific Research), and the Center of Innovation Program of the Japan

**Table 1**

The effectiveness of three mass-screening strategies in a test population of 100,000 persons (when FN/Pos\(^a\) = 0.4). The two-step strategy reduced the number of NAATs performed by approximately 95% compared to NAAT only, with an increase in false negatives by only 24 per 100,000 persons. Both NAAT only and two-step performed significantly better than free entry at limiting the number of false negatives.

| p       | NAAT only |  Two-step\(^b\) | Free entry |
|---------|-----------|----------------|------------|
|         | NAAT | Pos | FN | NAAT | Pos | FN | NAAT | Pos | FN | NAAT | Pos | FN |
| 0.1%    | 100,000 | 100 | 40 | 549 [548–550] | 76 [68–83] | 64 [57–72] | 0 | 0 | 140 |
| 0.2%    | 100,000 | 200 | 80 | 558 [556–559] | 152 [136–166] | 128 [114–144] | 0 | 0 | 280 |
| 0.5%    | 100,000 | 500 | 200 | 583 [579–587] | 380 [340–415] | 320 [285–360] | 0 | 0 | 700 |
| 1.0%    | 100,000 | 1000 | 400 | 626 [617–634] | 750 [680–820] | 640 [579–720] | 0 | 0 | 1400 |

FN/Pos: ratio of false negatives to positives; p: proportion of NAAT positivity; NAAT: number of nucleic acid amplification test performed; Pos: number of positive results; FN: number of false positives.

\(^a\) FN/Pos is the ratio of infected persons who test negative to all test positives.

\(^b\) Estimated when the probability of CLEIA-positivity given NAAT-positivity is 76% in point estimates (90% credible interval between 68% and 83%).

Fig. 2. Barplots of viral antigen concentrations
(a) The frequency of viral antigen concentrations of the entire test population sorted by final diagnosis by the two-step strategy (288 positives and 88,636 negatives). (b) The frequency of antigen concentration in 513 persons judged to be indeterminate by initial CLEIA. NAAT was only performed for CLEIA results with antigen concentrations between 0.67 and 4.0 pg/mL. The frequency of NAAT negative samples consistently approach zero with increasing antigen concentrations, while NAAT positives did not show any trend.
Table 2
Imported false negative (IFN) cases adjusted by 14-day quarantine in various settings of FN/Pos. Combining post-screening quarantine further decreased the number of imported cases false negatives depending on the adherence rate. The operational sensitivity of any strategy diminished with greater values of FN/Pos, with converging relative differences between the three strategies.

| Prob. of NAAT positivity | 14-days Quarantine Adherence | IFNs/100,000 persons (when FN/Pos = 0.4) | IFNs/100,000 persons (when FN/Pos = 1.0) | IFNs/100,000 persons (when FN/Pos = 2.0) |
|--------------------------|-------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
|                          | NAAT only                     | Two-step                                 | Free entry                              | NAAT only                               | Two-step                                 | Free entry                              | NAAT only                               | Two-step                                 | Free entry                              |
| 0.1%                     | 0%                            | 40 [55–74]                              | 140                                     | 100                                     | 124 [117–132]                           | 200                                     | 200                                     | 224 [217–232]                           | 300                                     |
|                          | 25%                           | 30 [41–56]                              | 105                                     | 75                                      | 93 [88–99]                              | 150                                     | 150                                     | 168 [163–174]                           | 225                                     |
|                          | 50%                           | 20 [28–37]                              | 70                                      | 50                                      | 62 [59–66]                              | 100                                     | 100                                     | 112 [109–116]                           | 150                                     |
|                          | 75%                           | 10 [16–14]                              | 35                                      | 25                                      | 31 [29–33]                              | 50                                      | 50                                      | 56 [54–58]                              | 75                                      |
| 0.2%                     | 0%                            | 80 [110–148]                            | 280                                     | 200                                     | 248 [234–264]                           | 400                                     | 400                                     | 448 [434–464]                           | 600                                     |
|                          | 25%                           | 60 [93–111]                             | 310                                     | 150                                     | 186 [176–198]                           | 300                                     | 300                                     | 336 [326–348]                           | 450                                     |
|                          | 50%                           | 40 [75–74]                              | 140                                     | 100                                     | 124 [117–132]                           | 200                                     | 200                                     | 224 [217–232]                           | 300                                     |
|                          | 75%                           | 20 [28–37]                              | 70                                      | 50                                      | 62 [59–66]                              | 100                                     | 100                                     | 112 [109–116]                           | 150                                     |
| 0.5%                     | 0%                            | 200 [275–370]                           | 700                                     | 500                                     | 620 [585–660]                           | 1000                                   | 1000                                   | 1120 [109–116]                          | 150                                     |
|                          | 25%                           | 150 [206–278]                           | 525                                     | 375                                     | 465 [439–495]                           | 750                                     | 750                                     | 840 [814–870]                           | 1125                                    |
|                          | 50%                           | 100 [138–185]                           | 350                                     | 250                                     | 310 [293–330]                           | 500                                     | 500                                     | 560 [543–580]                           | 750                                     |
| 1.0%                     | 0%                            | 50 [60–69]                              | 175                                     | 125                                     | 155 [146–165]                           | 250                                     | 250                                     | 280 [271–290]                           | 375                                     |
|                          | 25%                           | 40 [70–74]                              | 140                                     | 100                                     | 124 [117–132]                           | 200                                     | 200                                     | 224 [217–232]                           | 300                                     |
|                          | 50%                           | 30 [75–74]                              | 105                                     | 75                                      | 93 [87–99]                              | 150                                     | 150                                     | 168 [163–174]                           | 225                                     |
|                          | 75%                           | 20 [75–74]                              | 70                                      | 50                                      | 62 [59–66]                              | 100                                     | 100                                     | 112 [109–116]                           | 150                                     |
| 0.75%                    | 0%                            | 100 [138–185]                           | 350                                     | 250                                     | 310 [293–330]                           | 500                                     | 500                                     | 560 [543–580]                           | 750                                     |

FN/Pos: ratio of false negatives to positives; NAAT: nucleic acid amplification test.

a FN/Pos is the ratio of infected persons who test negative to all test positives.

b Estimated when the probability of CLEIA-positivity given NAAT-positivity is 76% in point estimates (90% credible interval between 68% and 83%).

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CRediT authorship contribution statement

Isao Yokota: Conceptualization, Methodology, Software, R4.0.2, Data curation, Writing – original draft. Peter Y. Shane: Conceptualization, Writing – original draft. Takanoi Teshima: Conceptualization, Writing – original draft, Supervision.

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