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Virological and Serological Assessment of US Army Trainees Isolated for Coronavirus Disease 2019

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Background. Laboratory screening for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a key mitigation measure to avoid the spread of infection among recruits starting basic combat training in a congregate setting. Because viral nucleic acid can be detected persistently after recovery, we evaluated other laboratory markers to distinguish recruits who could proceed with training from those who were infected.

Methods. Recruits isolated for coronavirus disease 2019 (COVID-19) were serially tested for SARS-CoV-2 subgenomic ribonucleic acid (sgRNA), and viral load (VL) by reverse-transcriptase polymerase chain reaction (RT-PCR), and for anti-SARS-CoV-2. Cluster and quadratic discriminant analyses of results were performed.

Results. Among 229 recruits isolated for COVID-19, those with a RT-PCR cycle threshold >30.49 (sensitivity 95%, specificity 96%) or having sgRNA $\log_{10}$ RNA copies/mL <3.09 (sensitivity and specificity 96%) at entry into isolation were likely SARS-CoV-2 uninfected. Viral load >4.58 $\log_{10}$ RNA copies/mL or anti-SARS-CoV-2 signal-to-cutoff ratio <1.38 (VL: sensitivity and specificity 93%; anti-SARS-CoV-2: sensitivity 83%, specificity 79%) had comparatively lower sensitivity and specificity when used alone for discrimination of infected from uninfected.

Conclusions. Orthogonal laboratory assays used in combination with RT-PCR may have utility in determining SARS-CoV-2 infection status for decisions regarding isolation.

Keywords. SARS-CoV-2; cycle threshold value; isolation; Army recruit; sgRNA

To avoid the spread of coronavirus disease 2019 (COVID-19), the disease caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2), in congregate settings that have a higher potential for transmission, multiple mitigation measures are needed [1,2]. A few nonmaterial interventions implemented by the US Army in the combat training environment included reductions in residential barrack size and training intake, restriction in movement after arrival, structuring small groups for all activities, screening for SARS-CoV-2 at entry and exit from 14-day quarantine at arrival, and isolation of recruits with suspected or confirmed COVID-19 based on medical evaluation and laboratory evidence of infection.

Laboratory evidence of SARS-CoV-2 infection supporting clinical diagnosis or screening in congregate settings can be achieved through molecular tests such as reverse-transcriptase polymerase chain reaction (RT-PCR) or antigen assays [3]. Automated RT-PCR assays are attractive for mass screening due to their accuracy and operational efficiency. However, the potential for persistence of test positivity long after active viral replication ceases has impeded its use for transmission-based precautions [4]. Prolonged RT-PCR test positivity has been reported to range from 2.4% to 69.2% among recovered COVID-19 patients [5]. However, viable virus could not be recovered in immunocompetent patients despite positive RT-PCR test results [6]. Furthermore, in contact tracing investigations, no infections were reported among close contacts

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exposed to index cases 5 days after symptom onset or among recovered index cases with prolonged PCR positivity [7, 8]. For this reason, the Centers for Disease Control and Prevention updated recommendations as of July 20, 2020 from a test-based strategy to end isolation to a time- or symptom-based strategy among immunocompetent individuals. Unlike RT-PCR-based tests, which require specialized equipment and personnel, antigen tests can be performed in point-of-care or home settings. However, test sensitivity can vary widely (by above 50%) depending on the test manufacturer, time course of infection, viral load, and whether people experienced symptoms or were asymptomatic [9]. Viral culture is a reliable way for determining infectivity to inform duration of isolation. However, time- and resource-intensive specialized laboratory requirements as well as limitations in culture sensitivity preclude its widespread use for screening, diagnosis, or infection control. Subgenomic ribonucleic acids (sgRNAs) are SARS-CoV-2 genomic RNA replication intermediates that are translated into structural spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins to form viral particles. Their presence and abundance are considered indicative of active replication [10]. Quantitative viral RNA and sgRNA have been reported to be good surrogate measures for infectivity among hospitalized patients [11–15]. Viral load (VL) ≥5 to 6 log copies/mL has been associated with culture-based isolation of SARS-CoV-2 from the respiratory tract; sgRNA demonstrated a 97% sensitivity, 94% positive predictive value, and greater odds (10.2; 95% confidence interval, 1.6–65.0) in detecting replication-competent virus compared with viral culture [13,16]. We followed recruits isolated for suspected or confirmed SARS-CoV-2 infection to evaluate whether discontinuation of isolation and entry into training was possible based on results from nonculture-based laboratory assays.

METHODS

Surveillance Design and Ethics

We surveyed recruits isolated for COVID-19 from October 14 to November 23, 2020 at a basic combat training (BCT) facility from entry (visit 1) until the end of isolation (visit 5). Recruits were isolated for COVID-19 for either testing positive by RT-PCR screening at entry or exit from initial arrival quarantine, or for having had exposure to COVID-19, or for clinical suspicion of COVID-19 upon medical evaluation. Screening assays in use by the facility during the surveillance period were GeneXpert (Cepheid, Sunnyvale, CA) and Panther Fusion SARS-CoV-2 (Hologic, Inc., San Diego, CA) assays. Upon arrival at the training facility, recruits underwent daily symptom and temperature checks, on the first day after arrival after which a quarantine or controlled-monitoring period of 14 days was enforced in groups (or "cocoons") of 30 to 60–65 recruits. Recruits identified or suspected of having COVID-19 were moved to separate housing for medical isolation. We collected nasopharyngeal swabs every 72 hours and blood specimens and self-administered questionnaires at entry and exit from isolation.

This activity was a subproject of an enhanced surveillance project determined by the Walter Reed Army Institute of Research's (WRAIR) human subject protection office (No. 2790) as a public health activity and does not require informed consent [17].

Laboratory Methods

Nasopharyngeal swabs were shipped overnight to the WRAIR (Diagnostics and Countermeasures Branch [DCB], Silver Spring, MD) for qualitative RT-PCR testing (Panther Fusion SARS-CoV-2 assay, ORF1ab gene targets). All residual specimens were tested for sgRNA and VL (laboratory-developed assay [WRAIR, DCB], E gene target) using methods reported previously [18]. In brief, RNA extracted from 200 µL transport medium using the EZ1 DSP Virus kit (QIAGEN, Valencia, CA) was used for reverse transcription, amplification, and quantification of sgRNA and RNA VL (TaqPath 1-Step RT-qPCR; Life Technologies, Thermo Fisher Scientific, Inc., Waltham, MA). Total viral genomic RNA (VL) and sgRNA values were extrapolated from an E gene calibration curve. Limit of detection (LOD) was 450 copies/mL for both VL and sgRNA assays. Blood specimens processed for serum were tested for total antibody (VITROS Anti-SARS-CoV-2 Total test, CLIA [Ortho Clinical Diagnostics, Rochester, NY], spike glycoprotein target) at a commercial laboratory (Creative Testing Solutions, Tampa, FL). Reactive specimens (signal-to-cutoff [S/CO] ratio ≥1.00) were reflexed to a pseudovirus-based neutralization assay (SARS-CoV-2 reporter viral particle neutralization; Vitalant Research Institute, San Francisco, CA) for confirmation.

Data Management and Analysis

Entry questionnaires elicited demographic, symptom, and exposure information before isolation, whereas exit questionnaires elicited symptom information during isolation. Supplemental demographic and laboratory electronic records were obtained from the Defense Medical Surveillance System (Armed Forces Health Surveillance Division, Silver Spring, MD).

Descriptive statistics were used to characterize demographic features of recruits at arrival for training and serial measurements of total VL, sgRNA, and total antibody levels during isolation. Differences in laboratory measurements by self-reported symptoms status were assessed using Kruskal-Wallis test for continuous variables. Exploratory cross-sectional analysis of laboratory results at visit 1 was performed by means of unsupervised hierarchical agglomerative cluster analysis using Ward's distance method. This analysis grouped recruits into clusters based on laboratory results at baseline and the longitudinal pattern of biomarkers. Spaghetti line plots and linear

mixed models (with adjustment for correlation from repeated measurements) were performed to assess for differences in trajectories across visits among the clusters identified at baseline. Baseline or cross-sectionally derived clusters were compared with longitudinally derived clusters with clusters identified at baseline considered as definitive; the comparison revealed a concurrence proportion of >90% existed in these cluster definitions. Quadratic discriminant analysis (QDA) identified optimal discriminatory thresholds to distinguish recruits who likely were SARS-CoV-2-infected from those who likely were uninfected. Before QDA, clusters having similar trajectories based on results from analysis of line plots and linear models were combined and classified as infected or uninfected. Results from QDA were used to calculate sensitivities and specificities for serial and parallel testing [19]. A 2-sided $P < .05$ was considered statistically significant.

Although the Panther-Fusion SARS-CoV-2 assay received emergency use authorization (EUA) as a qualitative assay by the US Food and Drug Administration (FDA), the instrument generates quantitative cycle threshold (Ct) values that can be used as inverse estimations of viral load. Negative results from the Panther Fusion assay, for which no Ct values were generated, were coded a numeric value of 42 to avoid having missing values in statistical analysis; the highest Ct value for positive Panther Fusion SARS-CoV-2 results in this analysis was 41.9. Viral load, sgRNA, and S/CO ratio results were log base 10 ($\log_{10}$) transformed for normalization in analysis. Viral load and sgRNA results of "Target not detected" were coded zero for descriptive statistics and 1 for $\log_{10}$ transformation. All data management and analysis were conducted using SAS 9.4 Software and R Studio 4.0.3.

RESULTS

We collected 1168 nasopharyngeal swabs from 235 recruits who were isolated for suspected or confirmed COVID-19 from October 14 to November 23, 2020. A total of 227 recruits screened RT-PCR positive at either entry or exit from initial arrival quarantine; 117 (52%) were screened using Panther Fusion assay and 110 (48%) by GeneXpert assay. Among 8 recruits who screened negative, 2 recruits had laboratory evidence of COVID-19 during follow-up; 1 had high SARS-CoV-2 antibody levels at visit 1 and another had quantifiable viral load. Therefore, the analysis reported here included 229 recruits. Seven recruits were lost to follow-up before completing their final visit. Recruits were followed an average 12.4 days (Table 1). A majority (204, 89%) of recruits were isolated within 1–4 days (mean 3.1) of screening positive (Table 1). Most recruits were male (79%), from southern US states (54%), and aged an average of 22.6 years (range, 18.0–39.0). Less than one third (31%) reported experiencing COVID-19-like symptoms either before or during isolation (Table 1). Most common symptoms reported were runny nose (12%), cough (12%), sore throat (11%), and loss of smell (11%).

Table 1. Characteristics of 229 Recruits Isolated for COVID-19, October 14–November 23, 2020

Characteristic	Overall ($n = 229$) n (%)	
Age, Years		
17–20	120	(52)
21–25	50	(22)
26+	59	(26)
Sex		
Female	49	(21)
Male	180	(79)
Race/Ethnicity		
White	91	(40)
Black	49	(21)
Hispanic	57	(25)
Other	13	(6)
Unknown	19	(8)
Region of Residence Before Arrival		
South	123	(54)
Midwest	45	(20)
West	32	(14)
Northeast	25	(11)
Territory	1	(0)
Unknown	3	(1)
Any Reported Symptoms Before or During Isolation		
Yes	72	(31)
No	157	(68)
Timing of Symptoms		
Both before and during isolation	24	(10)
Before isolation only	39	(17)
During isolation only	9	(4)
No symptoms reported	157	(68)
Type of Symptoms[a]		
Runny nose	28	(12)
Cough	27	(12)
Sore throat	26	(11)
Loss of smell	25	(11)
Headache	20	(9)
Shortness of breath	20	(9)
Fatigue	15	(7)
Loss of taste	13	(6)
Chills	12	(5)
Malaise	12	(5)
Muscle aches	11	(5)
Days to diagnosis at visit 1, median, mean, std (range)	3.0, 3.1, 1.7	(1.0–10.0)
1–4	204	(89)
5–7	18	(8)
8–10	7	(3)
Duration/days of follow-up, median, mean, std (range)	13.0, 12.4, 1.1	(4.0–14.0)
4–10	8	(3)
11–14	221	(97)

Abbreviations: COVID-19, coronavirus disease 2019; std, standard deviation.

[a]Other less frequently (<5%) reported symptoms included nausea 9 (4), joint ache 8 (3), abdominal pain 7 (3), loss of appetite 7 (3), diarrhea 6 (3), feverish 6 (3), fever 5 (2), and vomiting 2 (1).

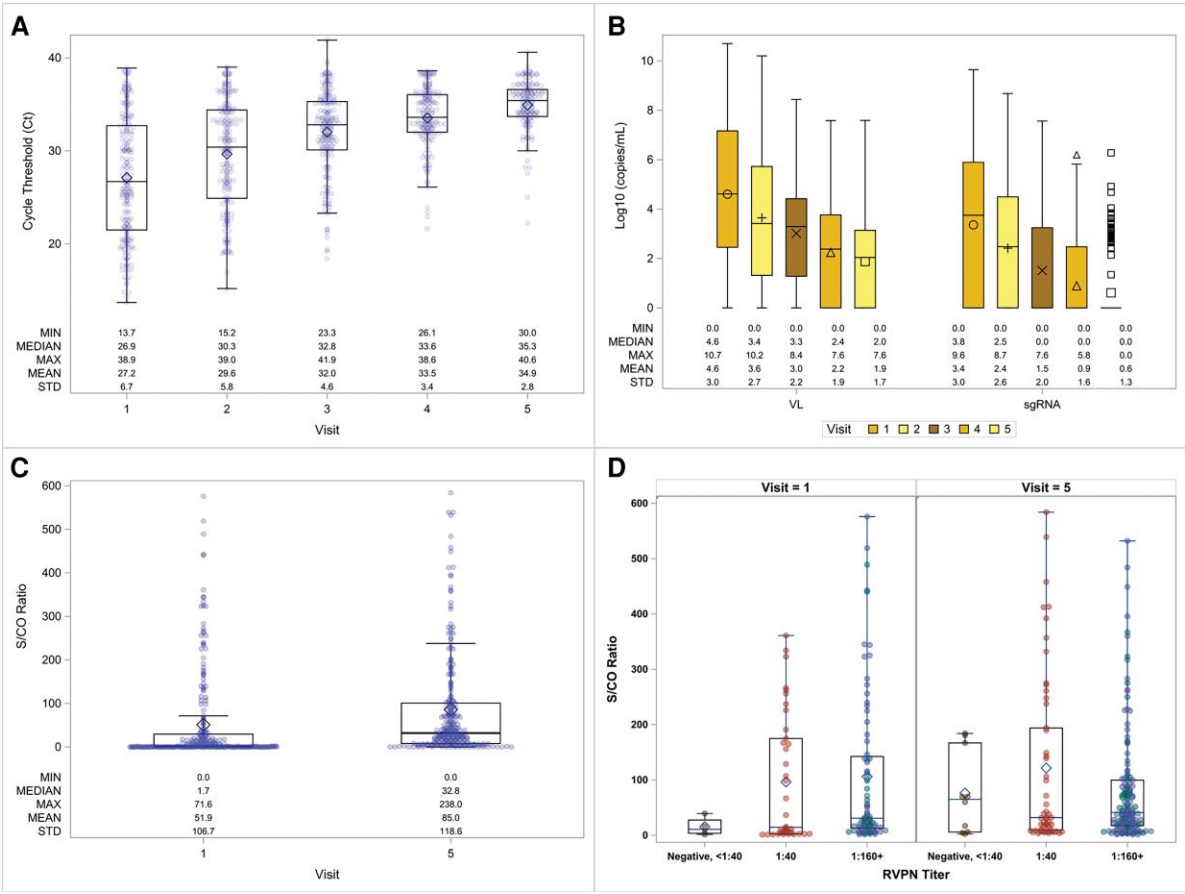

Figure 1. Box plots and summary statistic tables of the distributions of laboratory markers by visit among recruits during coronavirus disease 2019 isolation, October 14–November 23, 2020: (*A*) cycle threshold (Ct) value, (*B*) viral load (VL) and subgenomic ribonucleic acid (sgRNA) values (log_{10} copies/mL), (*C*) signal-to-cutoff (S/CO) ratio values, (*D*) S/CO ratio values by reporter pseudovirus plaque neutralization (RVPN) titer. MAX, maximum; MIN, minimum; STD, standard deviation.

At visit 1, almost one fifth (48, 21%) of recruits tested negative on Panther Fusion, 87 (38%) and 38 (17%) had undetectable sgRNA and viral load results, respectively, and 119 (52%) were seropositive. Overall, Panther Fusion RT-PCR positivity decreased during follow-up by 20% from visit 1 (181, 79%) to visit 5 (138, 59%) with an average 9.3-fold increase in Ct values (Figure 1A, Supplementary Figure 1). Correspondingly, sgRNA and viral load levels during follow-up decreased an average 2.9-fold and 2.8-fold, respectively (Figure 1B, Supplementary Figure 1). The proportion of recruits who seroconverted increased 39% during follow-up, from 52% ($n = 119$ of 228) at visit 1 to 91% (198 of 216) at visit 5 with an average 36.4-fold increase in S/CO ratio (Figure 1C, Supplementary Figure 1). Among recruits who were seropositive, the proportion of recruits with neutralizing titer results ≥1:160 rose minimally from 64% (76 of 118) at visit 1 to 68% (135 of 197) at visit 5.

All laboratory markers differed significantly by self-reported symptoms (Kruskal-Wallis test, $P < .05$) (Figure 2, Supplementary Table 1). Overall, Ct and S/CO ratio values across visits were lower for recruits who reported any symptoms compared to those who did not (mean: Ct = 30.1 vs 31.8, S/CO = 45.7 vs 78.6, respectively). In addition, overall sgRNA and viral load levels across visits were higher for recruits with symptoms compared to those who were asymptomatic (mean log_{10} copies/mL: sgRNA = 2.4 vs 1.5, VL = 3.9 vs 2.8, respectively).

In cluster analysis of laboratory results at visit 1, recruits were grouped into 4 clusters: A, B, C, and D (Figure 3). Panther Fusion RT-PCR Ct values correlated strongly with sgRNA and viral load levels (r = −.92 and r = −.96, respectively) (Supplementary Figure 2). Recruits in clusters B and D generally had similar profiles across visits with undetected (zero) or low sgRNA (mean, .1–1.1, log_{10} copies/mL) and viral load levels (mean, .5–3.2 log_{10} copies/mL) and high Panther-Fusion Ct values (mean, 34.5–42.0) (Figure 4, Supplementary Table 2). Results from fitting linear mixed-effect models indicated the slopes of Ct, sgRNA, and viral load differed among clusters across visits ($P < .05$) with clusters B and D having similar predicted slopes for sgRNA levels (Supplementary Figure 3). As a

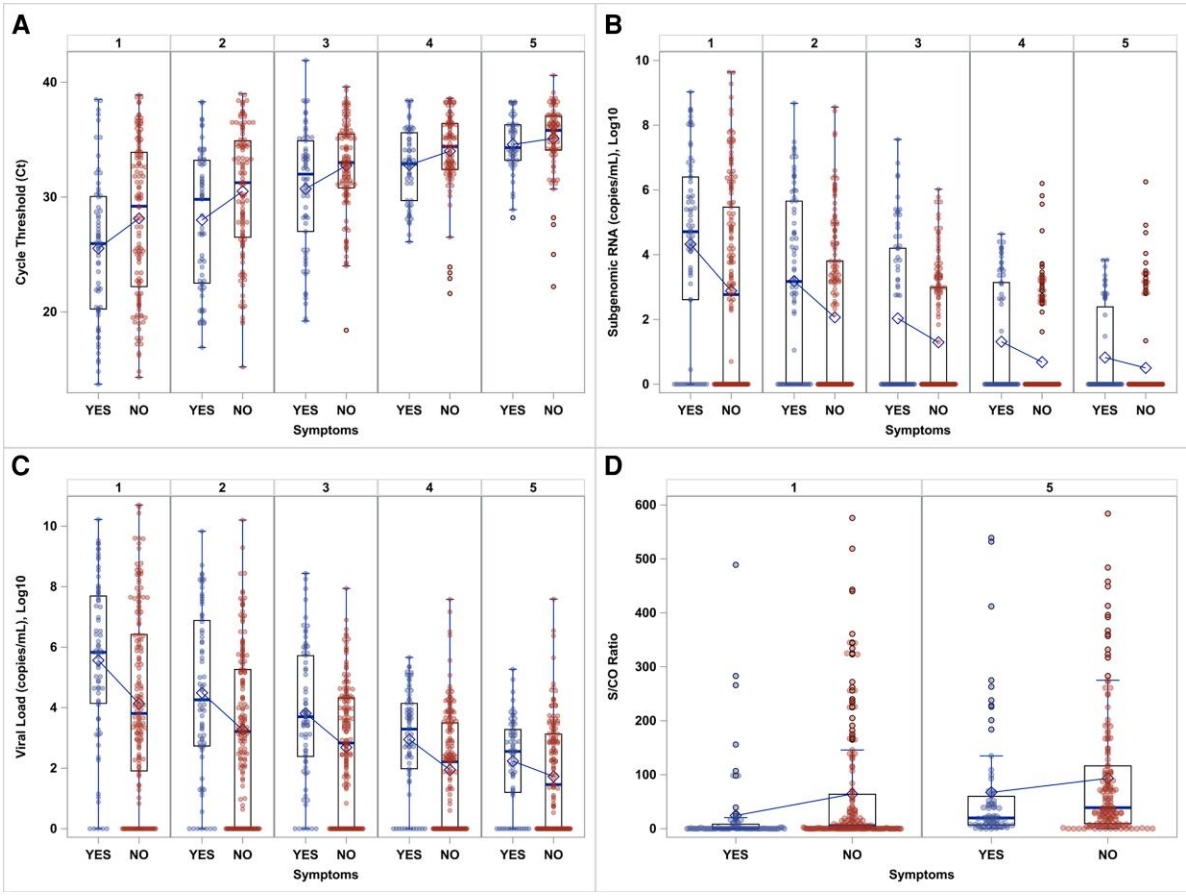

Figure 2. Summary statistics of laboratory markers by symptoms and by visit among recruits during coronavirus disease 2019 isolation, October 14–November 23, 2020: (*A*) cycle threshold value, (*B*) subgenomic ribonucleic acid values (log_{10} copies/mL), (*C*) viral load levels (log_{10} copies/mL), (*D*) signal-to-cutoff ratio values.

result, recruits in clusters B and D were grouped as one and considered likely SARS-CoV-2 uninfected and likely to have controlled and cleared the virus, whereas recruits in clusters A and C were considered infected and grouped as one.

Quadratic discriminant analysis identified 2 of 4 markers as having the highest sensitivity and specificity for distinguishing infected from uninfected groups (Table 2). A Panther Fusion Ct value below 30.49 or sgRNA above 3.09 log10 copies/mL (1230.27 copies/mL) were suggestive of SARS-CoV-2 infection at similar and relatively high sensitivity (95%–96%) and specificity (96%) (Table 2). Comparatively, antibody tests had the lowest sensitivity (83%–93%) and specificity (79%–89%) for potentially discriminating SARS-CoV-2 infected from uninfected at an optimal threshold of less than S/CO ratio 1.38 (log_{10} 0.14). In applying sensitivity and specificity results from QDA, parallel testing using RT-PCR and another assay improved sensitivity (99.2%–99.8%) compared to using RT-PCR alone (95%) (Table 3), and serial testing increased the specificity of RT-PCR (99.2%–99.8%) when combined with another assay versus using RT-PCR testing alone (96%) (Table 3).

DISCUSSION

Recruits isolated for COVID-19 who were predominantly asymptomatic were evaluated for viral genomic and sgRNA levels every 3 days for 2 weeks, alongside total SARS-CoV-2 antibody response at entry and exit from isolation. Our analysis suggests that Ct values from qualitative RT-PCR testing may have utility in guiding infection control measures within a congregate setting such as basic combat training. Specifically, testing SARS-CoV-2 screen-positive specimens with an additional orthogonal assay may increase capacity to identify recruits who could proceed to training from those who should be isolated.

In our analysis, a cycle threshold below 30.49 on the Panther Fusion assay or sgRNA above 3.09 log_{10} copies/mL demonstrated the highest sensitivity and specificity for identification of active infection. This is consistent with reports of culture-based isolation of SARS-CoV-2 from respiratory tract specimens collected for surveillance/clinical care with corresponding RT-PCR cycle threshold values ranging from 24 to 32 [20–22]. Single timepoint testing has the limitation of discrimination of individuals in early phase of infection versus those recovering from infection. This limitation was addressed in

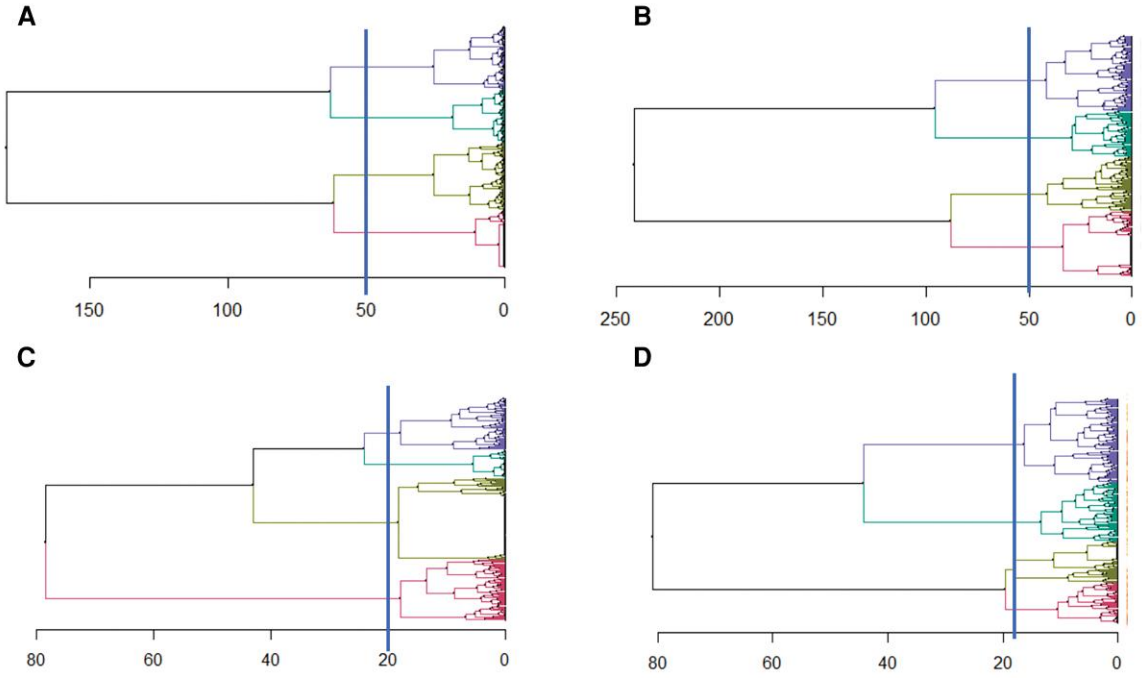

Figure 3. Dendrograms of hierarchical cluster analysis of laboratory results among 229 recruits at visit 1 and during follow-up. The vertical lines indicate the cut distance used to define clusters. Clusters A–D are color-coded in purple, aqua, gold, and red, respectively. (*A*) Cycle threshold values, visit 1, (*B*) cycle threshold values across visits, (*C*) subgenomic ribonucleic acid levels (copies/mL, $\log_{10}$) across visits, and (*D*) viral load levels (copies/mL, $\log_{10}$) across visits.

one longitudinal study among National Basketball Association personnel by identifying a patient's stage of infection from Ct values from a second RT-PCR test performed 2 days after an initial diagnostic RT-PCR [23]. Our analysis suggests that using a supplemental sgRNA or viral load test on the same screening sample or a conducting a serological test on a blood sample collected at the same test event may assist in discriminating infected from uninfected recruits. Using this method, up to 1 in 2 (43%–49%) recruits may have been able to avoid isolation and proceed to training. Although limitations to leveraging Ct values include a lack of direct comparability among test platforms, variability in preanalytical conditions, and lack of quantitative RT-PCR or sgRNA FDA EUA assays, using a discriminatory cutoff from RT-PCR in a setting where testing is standardized in conjunction with a supplementary test would mitigate unnecessary use of isolation resources and increase routing of personnel through basic combat training. In the absence of a Ct value, addition of a second orthogonal test such as sgRNA, viral load, or serology among recruits who screen positive may have value for medical isolation decision making.

Other studies have reported mixed results as to the utility of sgRNA testing as a tool for guiding infection control decision making for isolation [24–26]. In a study Santos Bravo et al [24] conducted from February 25 to May 25, 2020 among 84 hospital healthcare workers (HCWs) assessing 2 positive swab specimens collected consecutively 7 days apart, sgRNA (E gene target) negativity correlated with RT-PCR Ct values $>$27.85 (positivity with Ct $<$24.38) and normalized viral load $\leq$1 $\log_{10}$ RNA copies/mL (positivity with $\geq$4 $\log_{10}$). In this study, 91.7% of HCWs were symptomatic. Santos Bravo et al [24] recognized the predictive value of Ct for an RT-PCR test standardized to gene targets and laboratory procedures used for sgRNA and normalized viral load tests. They concluded that sgRNA and normalized viral load supplemental tests may provide useful surrogates of infectivity for clinical decision making. However, in a study among 185 SARS-CoV-2-infected patients hospitalized from March 13 to June 10, 2020, Dimcheff et al [25] concluded that sgRNA correlated to total viral RNA and provided no additional utility in assessing infectivity than Ct values from RT-PCR for total RNA. They found that sgRNA was undetectable at Ct values of 32 and 35 for E and N genes, respectively, and total N/E gene $>$6.5 $\log_{10}$ copies/mL. Likewise, Verma et al [26] concluded that sgRNA had questionable utility to guide isolation; quantification of sgRNA (E and N genes) had no advantage over genomic RNA (N gene) because the rate of sgRNA decline was comparable to decline of genomic RNA. Their study was conducted among 205 COVID-19 patients enrolled from August 2020 to January 2021 during drug treatment trials, 96.1% of whom reported at least 1 COVID-19 symptoms. These findings may correspond to

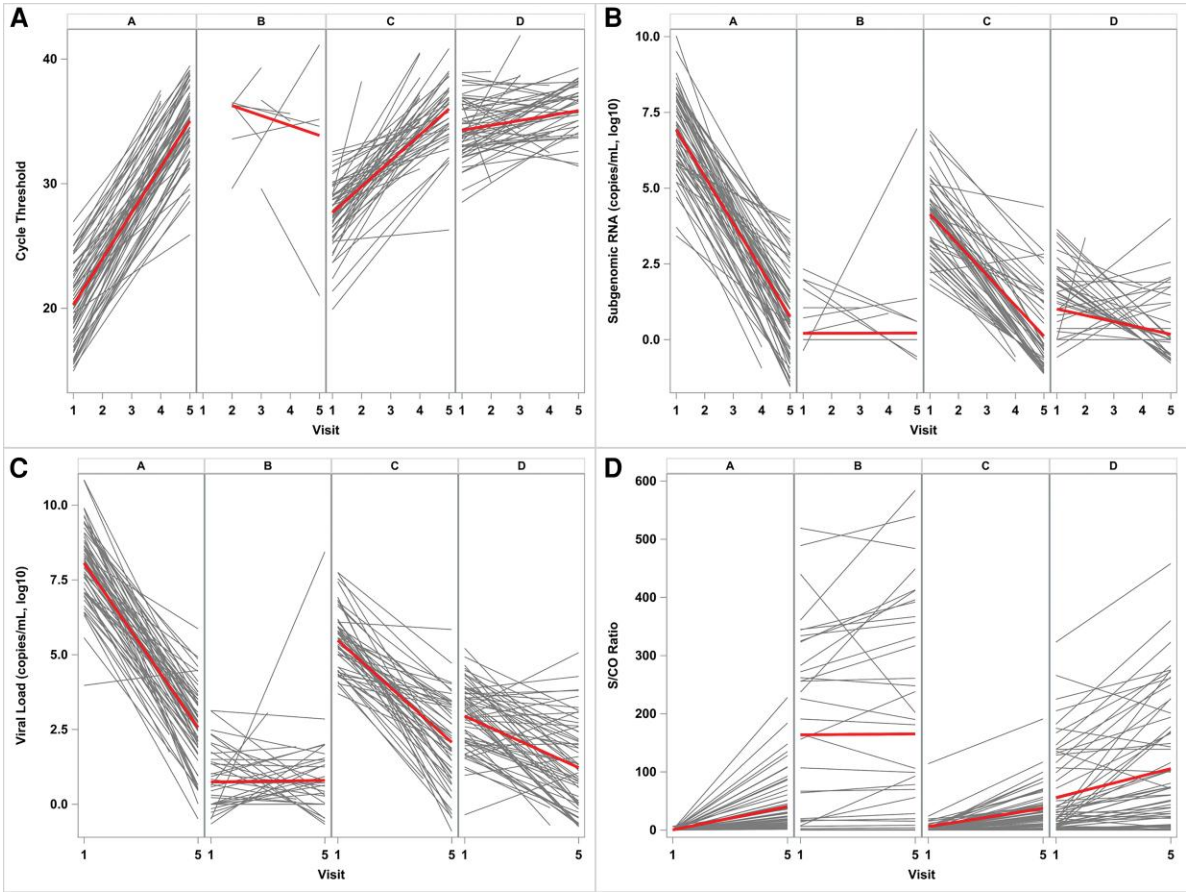

Figure 4. Laboratory results for each recruit plotted across visits with a red line displaying the average trend for each of 4 clusters (*A*–*D*). RNA, ribonucleic acid; S/CO, signal-to-cutoff.

Table 2. Optimal Thresholds With Corresponding Sensitivity and Specificity Computed From Quadratic Discriminant Analysis for 4 Laboratory Measurements for Identifying SARS-CoV-2 Infection Among Recruits Isolated for COVID-19

Laboratory Marker (assay used)	Threshold (log base 10)	Decision Rule	Sensitivity	Specificity
Cycle threshold value (Hologic Panther Fusion assay)	30.49 (—)	Less	.95	.96
Subgenomic messenger RNA (laboratory developed assay)	1230.27 (3.09)	Greater	.96	.96
Viral load (laboratory-developed assay)	38 018.94 (4.58)	Greater	.93	.93
SARS-CoV-2 total antibody levels, signal-to-cutoff ratio (Vitros Anti-SARS-CoV-2 Total test)	1.38 (.14)	Less	.83	.79

Abbreviations: COVID-19, coronavirus disease 2019; RNA, ribonucleic acid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

severity of illness in the populations studied. To our knowledge, no previous studies have studied sgRNA levels to inform medical isolation in a young predominantly asymptomatic physically fit population.

Table 3. Sensitivity and Specificity of 2 Laboratory Tests Used in Parallel or in Series Using Sensitivity and Specificity Results From Quadratic Discriminant Analysis

Laboratory Assay	Sensitivity	Specificity
RT-PCR With Subgenomic RNA		
Series	.912	.998
Parallel	.998	.922
RT-PCR With Viral Load		
Series	.884	.997
Parallel	.997	.893
RT-PCR With Serology		
Series	.789	.992
Parallel	.992	.758

Abbreviations: RNA, ribonucleic acid; RT-PCR, reverse-transcription polymerase chain reaction.

Results from modeling studies indicate factors such as testing frequency, rapid result turnaround time, high test specificity, along with strict adherence to other mitigation measures are more important for effective population screening than test sensitivity alone [27, 28]. Serial antigen testing in congregate settings has been suggested for those individuals who test negative initially (avoiding the need for confirmatory nucleic acid testing) as well as to identify infected individuals rapidly and prevent transmission [29]. Compared with RT-PCR, rapid antigen tests have been reported to have a sensitivity ranging from 24.7% to 40.0% among asymptomatic SARS-CoV-2-infected adults [30–32]. Test sensitivity increased as viral load increased, with a reported 90.9% sensitivity for specimens with SARS-CoV-2 RNA viral load above 10^6 copies/mL, 100% sensitivity for VL of 10^8 copies/mL or above, and below 10% for low viral load specimens below 10^4 copies/mL [30, 32]. The limitation of diagnosis during the early phase of an infection from a single timepoint RT-PCT test apply to rapid antigen tests as well unless serial testing is conducted. However, increasing screen testing frequency among recruits entering BCT from biweekly to either weekly, every 3 days, or daily using antigen or RT-PCR assays may be logistically and financially prohibitive. Additional testing of a positive screening specimen may be a viable cost-effective alternative. Large-scale roll out of sgRNA and/or viral load assays is possible via an automated platform. The manual sgNRA and viral load assays used in this project have been validated on the Hologic Panther Fusion open access system using analyte-specific reagents for automated high-throughput leveraging armored RNA controls.

Our study has a few limitations. First, we surveyed recruits at entry into isolation and not at time of diagnosis. As a result, discriminatory thresholds identified in our analysis were not representative of infection status at diagnosis. However, standardized use of assays ensured comparability of results during follow-up. Second, screening assays in use had an estimated 9-fold difference in detection sensitivity with Cepheid GeneExpert having a lower limit of detection of 5400 nucleic acid detectable units (NDU)/mL compared with 600 NDU/mL for Panther Fusion [33]. It is possible that differential assay sensitivity may have led to misclassification of infection status. However, use of multiple assays may reduce misclassification and identification of a discriminatory threshold for infection status. Third, without normalization, the utility of a Ct value has afore-mentioned limitations of comparability across test platforms and assays. Nonetheless, the Ct value identified in this analysis may be a useful indicator for the Panther Fusion platform. Finally, because our survey was conducted in a time that predated the emergence of SARS-CoV-2 variants of concern (VOCs) and the roll out of vaccine campaigns, the application of our findings to later phases of the pandemic may need to be verified with further study in this population. The prominent viral mutations among VOCs have been identified in the spike gene region and have impacted assays that have targeted this region, whereas the sgRNA and viral load assays we used targeted the envelope gene [34, 35]. Test performance of the supplemental molecular assays was not compromised by circulating VOCs; in silico analysis of primers and probes used in sgRNA and VL assays targeted to the envelope gene region indicate no impact of VOCs. Seropositivity ascertained by the assay we used determined total antibody to the spike gene region and does not distinguish between antibody levels in response to vaccination versus infection, unlike serological assays that target the nucleocapsid (N) gene; the spike region is the antigenic target of many FDA-issued EUA vaccines. Nevertheless, a supplemental test that measures total anti-S antibody levels would be a useful marker for either vaccination or natural infection and assist in distinguishing individuals in early phase of an infection from those recovering from an infection.

CONCLUSIONS

In conclusion, even with the roll out of vaccination and booster campaigns, mitigation measures such as masking, quarantining, isolation of infected individuals, and laboratory screening are a mainstay for preventing the spread of SARS-CoV-2. Improving screening strategies to include identification of a standardized cycle threshold cutoff value for universally identifying SARS-CoV-2 infection status or addition of a supplemental test to laboratory screening algorithms may inform quarantine and isolation decisions.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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