Serological and RT-PCR Surveillance for COVID-19 in an Asymptomatic US Army Trainee Population

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Background. Significant variability exists in the application of infection control policy throughout the US Army initial entry training environment. To generate actionable information for the prevention of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/coronavirus disease 2019 (COVID-19) transmission among new recruits, active enhanced surveillance was conducted for evidence of and exposure to SARS-CoV-2/COVID-19.

Methods. We serially tested recruits with a reverse transcriptase polymerase chain reaction (RT-PCR) COVID-19 and/or total antibody to SARS-CoV-2 tests at days 0, 14, and week 10 upon arrival for basic combat training at a location in the Southern United States.

Results. Among 1403 recruits who were enrolled over a 6-week period from August 25 through October 11, 2020, 84 recruits tested positive by RT-PCR, with more than half (55%, 46/84) testing positive at arrival and almost two-thirds (63%, 53/84) also testing seropositive at arrival. Similarly, among an overall 146 recruits who tested seropositive for SARS-CoV-2 during the period of observation, a majority (86%) tested seropositive at arrival; no hospitalizations were observed among seropositive recruits, and antibody response increased at week 10.

Conclusions. These findings that suggest serological testing may complement current test-based measures and provide another tool to incorporate in COVID-19 mitigation measures among trainees in the US Army.

Keywords. COVID-19; serology; asymptomatic; Army; recruit.
after the onset of the pandemic following a temporary halt in training in April 2020 for branches of service to develop COVID-19 prevention protocols [16]. Individuals who enlist in military service undergo intense collective initial entry training in close living quarters with other trainees for several weeks.

US Army training facilities have implemented routine viral screening of new recruits for rapid and early detection and isolation of SARS-CoV-2-infected cases [17]. Although the US Food and Drug Administration (FDA) recommends screening using Emergency Use Authorization (EUA)—approved diagnostic tests that have high sensitivity and rapid turnaround times, high-throughput requirements for screening at training facilities necessitate use of multiple molecular diagnostic assays with varying sensitivity and turnaround times. As of April 2, 2021, >150 commercial COVID-19 tests have received FDA EUA approval, with sensitivity ranging from 80% to 100% and specificity from 92% to 100% [18]. Due to the complexity of choosing the most appropriate COVID-19 tests, the Centers for Disease Control and Prevention (CDC) recommends selection of an assay based on 3 testing scenarios: diagnosis, screening, or public health surveillance [19]. To validate and identify potential gaps in testing and safety protocols, the Walter Reed Army Institute of Research (WRAIR), in collaboration with the Army Public Health Center (APHC) and with the endorsement of the US Army Center for Initial Military Training (CIMT), conducted a public health surveillance activity at a single recruit training facility among a cohort of recruits using standardized laboratory tools of measurement.

METHODS

Surveillance Site
A basic combat training (BCT) facility in the Southern United States was chosen for enhanced public health surveillance. Several control measures such as arrival quarantine, COVID-19 testing for early detection and isolation of cases, contact tracing, social distancing, and usage of face masks were in effect among new recruits during the period of surveillance. Upon arrival at the training facility, recruits were assigned into groups/cocoons during 14-day quarantine in which daily symptom and temperature checks were conducted. In addition, the training facility collected nasopharyngeal swabs upon arrival and at exit from quarantine to screen for COVID-19 using available molecular assays. Both nares were swabbed using the same swab the first morning after arrival. Recruits who tested positive at either quarantine screening test or who had an elevated body temperature or acknowledged having COVID-19 symptoms were removed from their cocoon and placed in isolation for 14 days.

Surveillance Design and Population
New recruits arriving to start BCT were surveyed for SARS-CoV-2 3 times from August 25 to December 11, 2020: time point 1—at arrival before entry into initial quarantine; time point 2—before exit from quarantine 10–14 days after arrival; and time point 3—at graduation from BCT ~10–12 weeks after arrival. Enrollment for the surveillance cohort took place 1–2 days every week from August 26 to October 11, 2020, from cocoons consisting of up to 60 recruits each to reach a target cohort size of 1500.

Patient Consent
This enhanced surveillance activity was determined by the Walter Reed Army Institute of Research’s human subject protection office (No. 2790) to be a public health activity not requiring informed consent.

Data and Specimen Collection
In addition to the sampling for quarantine screening performed by the training facility, the enhanced surveillance team collected paired nasopharyngeal and blood specimens at the first 2 time points and blood specimens at the third time point. Nasopharyngeal specimens were collected separately from quarantine sampling to avoid inefficient specimen yields for both testing efforts. Recruits were asked to complete self-administered hard copy questionnaires at the first and third time points. Questionnaires elicited information such as demographics, location of residence before arrival, and respiratory symptoms experienced before arrival for training or since arrival at the BCT and while in training. Existing electronic data for recruits such as laboratory and demographic records were obtained from a longitudinal medical surveillance system (Defense Medical Surveillance System, Armed Forces Health Surveillance Division).

Laboratory Methods
Nasopharyngeal swabs were tested for SARS-CoV-2 RNA using a real-time RT-PCR qualitative nucleic acid amplification test (NAAT) assay (Panther Fusion SARS-CoV-2, Hologic, San Diego, CA, USA). Upon collection, nasopharyngeal swabs were placed in Hologic specimen transport media (STM) and shipped overnight to the WRAIR HIV Reference and Diagnostics Laboratory (HDRL, Silver Spring, MD, USA) in UN3373 2°C–8°C temperature-controlled shippers (MAXQ MaxPlus specimenshipper, Stillwater, OK, USA).

Sera were tested for total antibody to spike (S) glycoprotein with a chemiluminescent immunoassay (CLIA, Ortho Clinical Diagnostics VITROS Anti-SARS-CoV-2 Total test, Creative Testing Solutions, Tampa, FL, USA) [20, 21]. Specimens with a signal-to-cutoff (s/co) ratio value of ≥1.00 were considered reactive [22]. Reactive specimens were confirmed using a SARS-CoV-2 Reporter Viral Particle Neutralization (RVPN) assay (Vitalant Research Institute, San Francisco, CA, USA) [20]. Due to possible cross-reactivity with other non-SARS-CoV-2 coronavirus on RVPN
testing, s/co values ≥12.0 provided a surrogate measure of neutralizing titers based on FDA-approved cutoff thresholds for qualifying convalescent plasma units for viral immuno-therapy [23].

Data Analysis

Positivity rates for laboratory test results at each time point were calculated by dividing the total number of positive results by the total number of tests performed and assessed for statistical significance (α = 0.05, WINPEPI) [24]. Both the training facility’s quarantine and enhanced surveillance test results were considered in determining RT-PCR positivity. Change from negative to positive RT-PCR results was considered an incident infection. Box plots were constructed to describe the distribution of results from RT-PCR, serology, and RVPN testing for all 3 time points. A change in results from nonreactive to reactive was considered seroconversion. Data collected from questionnaires were double-entered into Research Electronic Data Capture (REDCap), compared for discrepancies, resolved by review, summarized, and described. All data management and analysis were conducted using SAS, version 9.4 (Cary, NC, USA), unless indicated otherwise.

RESULTS

A total 2689 nasopharyngeal swabs and 3918 serologic specimens were collected from 1403 recruits enrolled over 7 consecutive weeks and followed before graduation from training in an interval that averaged 67.6 (range, 65.0–74.0) days. Among those enrolled, 99 (7%) recruits were lost to follow-up between the first 2 time points (mean [range], 13.0 [12–16] days), and a total of 190 (13%) from the first to last time points. Overall, 43 (3%) recruits were lost to follow-up due to isolation for COVID-19 test positivity. There were no statistical differences in demographics between those lost to follow-up and those who were surveyed (chi-square test, P > .05). Over half (55%) of recruits were between the ages of 17 and 20 years (mean [range], 21.8 [17.0–44.0] years) at arrival for training, 75% were male, almost half (47%) were White, 19% were of Hispanic ethnicity, and a majority (46%) were from the southern region of the United States (Table 1). Recruits reported sharing quarters with an average (range) of 34.6 (0–100) other recruits upon arrival. Only 1 recruit (out of 1364) reported having any respiratory symptoms in the 14 days preceding arrival. At the end of follow-up, a majority (90%, 1124/1244) reported having no respiratory symptoms since arrival for training.

Table 1. Description of Demographic Characteristics, SARS-CoV-2 RT-PCR, and Serology Test Positivity (With 95% CIs) by Time Point of Follow-up, 1403 Recruits, August 25–December 11, 2020

| Description | Time Pointa |
|-------------|-------------|
|              | Arrival (n = 1403) | Exit Quarantine (n = 1304) | Graduation (n = 1213) |
| Age, years  |              |              |                        |
| 17–20       | 775 (55)     | 719 (55)     | 661 (54)               |
| 21–25       | 371 (26)     | 345 (26)     | 322 (26)               |
| 26+         | 251 (18)     | 238 (18)     | 229 (19)               |
| Male sex    | 1058 (75)    | 985 (75)     | 933 (77)               |
| Race/ethnicity |              |              |                        |
| White       | 666 (47)     | 610 (47)     | 561 (46)               |
| Black       | 311 (22)     | 298 (23)     | 275 (23)               |
| Hispanic    | 265 (19)     | 251 (19)     | 238 (20)               |
| Other       | 104 (7)      | 92 (7)       | 87 (7)                 |
| Region of residence |              |              |                        |
| South       | 653 (46)     | 615 (47)     | 562 (46)               |
| Midwest     | 275 (20)     | 251 (19)     | 238 (20)               |
| West        | 257 (18)     | 238 (18)     | 226 (19)               |
| Northeast   | 189 (13)     | 177 (14)     | 167 (14)               |
| Territory   | 10 (1)       | 9 (1)        | 9 (1)                  |
| SARS-CoV-2 RT-PCR-positive, surveillance testb | 41/1386 (3.0, 2.2–4.0) | 42/1303 (3.2, 2.4–4.3) | -                  |
| SARS-CoV-2 RT-PCR-positive, quarantine screening testb | 16/1371 (1.2, 0.7–1.8) | 27/1270 (2.1, 1.4–3.0) | -                  |
| SARS-CoV-2 seropositivityb | 126/1403 (9.0, 7.6–10.6) | 113/1304 (8.7, 7.2–10.3) | 115/1211 (9.5, 7.9–11.2) |
| SARS-CoV-2 seroincidenceb | - | 2/1193 (0.2, 0.0–0.6) | 18/1114 (1.6, 1.0–2.5) |
| RVPN        |              |              |                        |
| Positive, 1:40 to 1:2560 | 103/124 (83) | 78/98 (80) | 49/58 (84) |
| Negative, <1:40 | 21/124 (17) | 20/98 (20) | 9/58 (15) |

Abbreviations: RT-PCR, reverse transcription polymerase chain reaction; RVPN, Reporter Viral Particle Neutralization assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

*No. (%) unless otherwise noted.

*No. (%; 95% CI).
**Laboratory Results**

Overall, 6% (84/1399; 95% CI, 4.8–7.3) of recruits tested positive by RT-PCR assay, with half (55%, 46/84) testing positive at arrival (Table 1). Existing quarantine screening at arrival/exit from quarantine identified 51% (43/84) of infections, whereas enhanced surveillance testing identified an additional 65% (55/84) of infections (Table 2; Supplementary Table 1). However, an analysis of cycle threshold (Ct) values of infections missed by quarantine screening indicated that all (except for 1 with a Ct value of 25.2) had Ct values >33 and almost three-fourths (71%, 39/55) were seropositive (Table 2). Quarantine screening identified 18% (15/84) of infections not detected by enhanced surveillance testing. Data were not available to assess Ct values of infections missed by enhanced surveillance testing, but serology testing indicated that 33% (5/15) were seropositive (Table 2).

Serology test results differentiated new from existing infections for recruits positive by quarantine screening and enhanced surveillance RT-PCR. Two-thirds (65%, 55/84) of recruits with infections identified by RT-PCR also tested seropositive, with almost all (96%, 53/55) testing seropositive at arrival (Figure 1). Among recruits (43/84) who tested RT-PCR-positive by existing quarantine screening and who tested seropositive at arrival (18/43), 14 (77%) had antibody levels indicative of neutralizing activity. Furthermore, among 37 recruits with apparent incident infection during the quarantine period by RT-PCR testing for enhanced surveillance or quarantine screening, 15 (41%) tested seropositive at arrival, whereas 22 (59%) tested seronegative both at arrival and before exit from quarantine. Therefore, only 22 were incident infections likely acquired during quarantine. This finding was supported by comparing Ct values for 27 of 37 recruits for whom Ct values were available by surveillance testing; a lower Ct value (median [IQR], 22.7 [21.6–34.3]) was observed for 12 seronegative recruits compared with a higher Ct value (median Ct [IQR], 37.1 [35.8–38.2]) for 15 seropositive recruits. Furthermore, new infections appeared to predominate in 1 cocoon. Fifteen of 22 infected recruits were from the same cocoon, 2 were identified from a separate second cocoon, and 1 was identified from a third; cocoon information was unavailable for 4 recruits.

Like RT-PCR results, seropositivity was highest (9%; 95% CI, 7.6%–10.6%) at arrival; an additional 2 (0.2%) recruits tested seropositive before exit from quarantine, and another additional 18 (1.6%) recruits had seroconverted in the interval from arrival to follow-up at graduation (Table 1). Among recruits who tested seropositive at either arrival or exit from quarantine, 39% (54/128) also tested positive by RT-PCR assays.

Serology testing indicated that SARS-CoV-2-seropositive recruits maintained a robust antibody response during training. Among recruits who tested seropositive at arrival, most (90%, 114/126) had antibody levels ≥12.0 (median [IQR], 136 [113–398]) (Figure 2A) and most (82%, 103/126) had neutralizing titers ranging from 1:40 to 1:2560 (Figure 2B). Moreover, in comparing serological test results at arrival/exit quarantine with follow-up testing at graduation, an almost 2-fold (median [IQR], 1.79 [1.3–2.8]) increase in antibody response was observed. Although a slight decline in mean values was observed for RVNP titer results from arrival to follow-up at graduation (mean, 142.0 vs 82.4, respectively; P = .16, Wilcoxon signed rank test), most recruits

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**Table 2.** Description of SARS-CoV-2 RT-PCR-Positive Results by Serology Results and Time Point of Follow-up, 84 Recruits, August 25–December 11, 2020

| Test Description | Time Point |
|------------------|------------|
|                  | Arrival (n = 46) | Exit Quarantine (n = 52) |
| RT-PCR+ only     | 30 (65)       | 25 (48)               |
| Median Ct (range)| 37.8 (25.2–41.4) | 37.0 (33.4–40.8)      |
| Quarantine screening only | 5 (11)       | 10 (19)               |
| Surveillance and quarantine screening | 11 (24)   | 17 (33)              |
| Median Ct (range) | 33.9 (21.9–38.6) | 23.5 (20.1–38.2)     |
| RT-PCR+ with serology+ | 25 (54)       | 22 (42)                |
| Median s/co (range) | 80.1 (6.8–456.0) | 110.5 (19.9–573.0)      |
| Quarantine screening only | 4 (9)        | 1 (2)                |
| Median s/co (range) | 59.5 (5.5–113.0) | 3870 (-)              |
| Surveillance and quarantine screening | 8 (17) | 7 (13)               |
| Median s/co (range) | 375 (4.8–240.0) | 203.0 (2.5–418.0)       |
| Surveillance, all | 33 (72)       | 30 (58)               |
| Median s/co (range) | 70.4 (4.8–456.0) | 1170 (2.5–573.0)       |
| Quarantine screening, all | 12 (26) | 8 (15)               |
| Median s/co (range) | 39.8 (4.8–240.0) | 223.5 (2.5–418.0)      |

Abbreviations: Ct, cycle threshold; RT-PCR, reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; s/co, signal-to-cutoff ratio.

*No. (%) unless otherwise indicated.

†Ct values were unavailable for RT-PCR test results.
(83%, 49/58) had neutralizing titers ranging from 1:40 to 1:640 at follow-up, and titers either remained the same or increased in more than half (54%, 26/48) of recruits (Figure 2B).

**DISCUSSION**

We conducted enhanced surveillance of a cohort of recruits arriving for training during August 26 to October 11, 2020. Despite a rapid rate of increase in COVID-19 in the United States during the summer of 2020, the prevalence of exposure to SARS-CoV-2 was not high among recruits. Our findings confirm that RT-PCR-based viral RNA testing is an effective screening measure to mitigate SARS-CoV-2 transmission during training. Moreover, our data suggest that antibody detection of COVID-19 may serve to complement viral RNA testing and could play a discriminatory role in test-based screening and isolation.

Universal arrival and quarantine exit screening in conjunction with other comprehensive COVID-19 mitigation measures, such as social distancing, mandatory face mask use, cocooning, and rapid isolation of cases in designated buildings, seemed to be effective in controlling SARS-CoV-2 transmission in the cohort under surveillance. While surveillance RT-PCR testing identified 65% more recruits with SARS-CoV-2 than quarantine screening assays, high Ct values for most suggest that these recruits were most likely in the recovery phase of their infection and unlikely to transmit SARS-CoV-2. Although the CDC does not recommend using Ct values generated from qualitative RT-PCR testing to quantify SARS-CoV-2 RNA levels in an individual as a measure of infectiousness [25], studies have reported on the usefulness of Ct values to assess infectious periods in the clinical course of infection [26, 27]. In a large surveillance study of both symptomatic and asymptomatic cases, Ct values strongly correlated with culturable virus, and the rate of culturable virus was similar among asymptomatic and symptomatic individuals [26]. Although there are several limitations to using Ct values such as lack of direct comparability between qualitative RT-PCR assays, using binary positive/negative results from such testing in conjunction with total antibody/pan Ig testing results warrants further investigation as a screening tool among recruits to reduce the threat of COVID-19 transmission.

Serological testing at the end of training suggests that screening strategies currently in use are effective at control. Molecular screening assays detected the bulk of infections (total 43) within the first 2 weeks of arrival compared with infections detected during training based on serology results (total 18). In a modeling study that evaluated mitigation strategies, the strategy of strict social distancing and mask-wearing alone was shown to prevent 87% of infections in congregate settings similar to basic training facilities, namely college campuses. The addition of laboratory screening every 3 days with a high-specificity (100%) laboratory test prevented 96% of total infections [28]. In another study that modeled 3 epidemic scenarios with varying test frequencies, sensitivity, and cost, test frequency, rapidity of resulting, and high test specificity, along with strict adherence
to other mitigation measures, were identified as more important than test sensitivity (assessed at >70%) in population-wide surveillance to achieve effective control of COVID-19 and avoid outbreaks [29].

From our analysis, serological results paired with status quo quarantine RT-PCR screening results indicate that 1 in 3 recruits who screened COVID-19-positive may have been able to proceed to training 2–4 weeks earlier. Furthermore, most recruits who tested seropositive at arrival had antibody levels suggestive of neutralizing activity throughout their training, which may be supportive of including serological testing as a decision-making tool to further sort recruits into test-based cohorts for training. Similar antibody levels have been used to qualify blood donation for clinical management of COVID-19 patients. In its EUA approval of emergency use of high-titer COVID-19 convalescent plasma (CCP) for treatment of hospitalized COVID-19 patients, the US FDA provided cutoff values for commercial serological assays approved for labeling of CCP; the revised EUA approval (dated March 9, 2021) listed an s/co ratio value of ≥9.5 on the Ortho VITROS Anti-SARS-CoV-2 immunoglobulin G (IgG) assay for qualifying CCP units; previous EUA approval (dated August 23, 2020) was for a cutoff of 12.0 [30]. Although the Ortho total Ig test used in this analysis is not FDA EUA–approved for qualifying CCP units, it was found to be equivalent to the FDA EUA–approved Ortho total IgG assay [31]. Moreover, initial reports suggest that seropositivity is associated with protection from reinfection [32–34]. In a longitudinal cohort study conducted by Lumley et al. among 12,541 health care workers in the United Kingdom who were followed for up to 31 weeks, only 2 reinfections were reported among 1265 antispike IgG–seropositive individuals who had an 8-fold lower PCR positivity during follow-up compared with antispike IgG–seronegative individuals [34]. Similarly, in the United States, Harvey et al. reported a 10-fold lower NAAT positivity during follow-up among seropositive individuals compared with seronegative individuals in an analysis of

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**Figure 2.** A, Frequency distribution of s/co ratio values for recruits who tested seropositive during follow-up. Mean (diamond marker), median (line), interquartile range (bottom and top of the box), range (whiskers), and reference s/co ratio value of 12.0 (red line) are shown. B, Frequency distribution of Reporter Viral Particle Neutralization (RVPN) titers for recruits who tested seropositive by time point of follow-up. C, Frequency distribution of s/co ratio values for recruits who tested seropositive by RVPN titers and by time point of follow-up. Abbreviations: RVPN, Reporter Viral Particle Neutralization assay; s/co, signal-to-cutoff ratio.
SARS-CoV-2 antibody and nucleic acid amplification testing (NAAT) performed by commercial laboratories [32].

While qualitative viral RNA testing is useful for early identification of cases, it has been shown to have limited use in ascertaining infection status among individuals who have recovered or have cleared the virus [25]. Although the immune response to SARS-CoV-2 can take 1–2 weeks [35, 36] and serological tests may not be useful for early identification of cases as IgG and immunoglobulin M (IgM) titers have been reported to peak 6 days after seroconversion [37], immunological tests for total IgG and IgM antibodies to nucleocapsid and spike structural SARS-CoV-2 proteins have shown a high level of accuracy for the diagnosis of COVID-19 [38]. Studies that have used CLIA tests to detect antibodies to spike protein have reported that IgA antibody levels peak before IgM and IgG antibodies, although reportedly such responses have been correlated with severity of disease [39]. Complementing viral RNA detection with antibody testing has been shown to increase diagnostic accuracy [40].

This analysis has limitations. As nasopharyngeal swabs were not collected at the same time or in the same media as the training facility, differences in quarantine screening vs enhanced surveillance RT-PCR results may be attributable to pre-analytical variations. As nasopharyngeal swabs were not collected after exit from quarantine or at the last follow-up, asymptomatic infections in this interval may have been underestimated using serological test results alone. Although the overall percentage of loss to follow-up was not significant demographically and attrition due to COVID-19 isolation was identified, other reasons for dropout were not captured due to logistical challenges.

In summary, preliminary results from surveillance of a cohort of recruits from their time of arrival to graduation from training indicate that serological testing paired with RT-PCR screening may have utility in reducing the number of recruits who are isolated and therefore have an impact on training throughput. Although it is unclear whether vaccine-induced immune responses will offer long-lasting protection from infection, results from this report suggest that if vaccine-induced antibody response is equivalent to antibody response from asymptomatic natural infection, then response will last through the duration of BCT. However, a combination of mitigation strategies may be needed to sustain control of the virus.

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

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