Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #1 14 Oct 2020

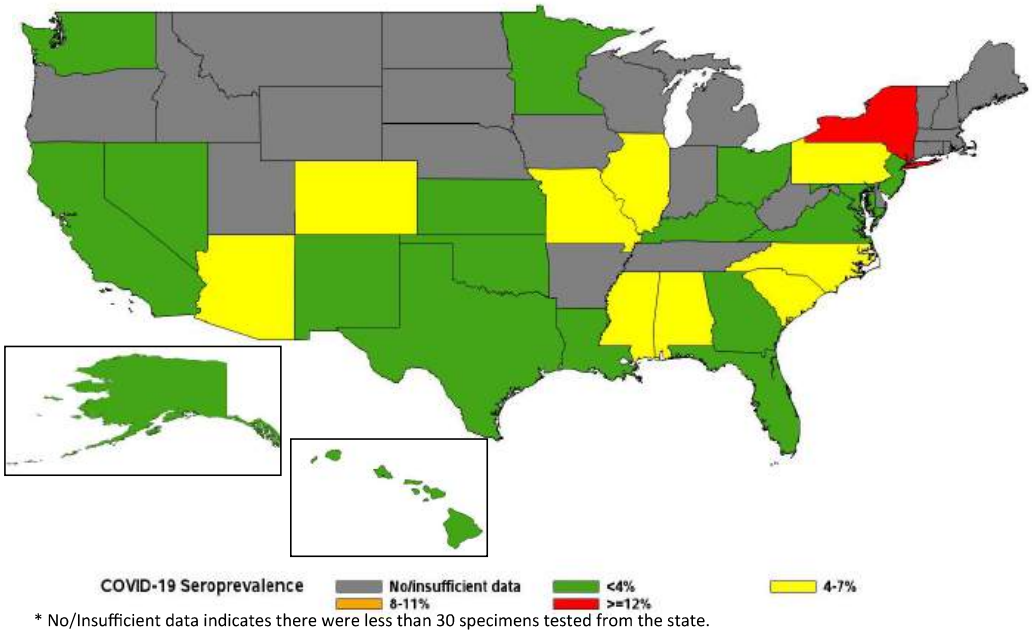
Executive Summary – Four thousand specimens were randomly selected from the 165,740 specimens submitted to the DoD Serum Repository (DoDSR) during the months of May and June in order to estimate the prevalence of SARS-CoV-2 antibodies among Service members. This prevalence gives a much more accurate estimation of total SARS-CoV -2 infections that have occurred among Service members (SM) than confirmed case counts, due to incomplete testing among asymptomatic and minimally symptomatic SARS-CoV-2 infections.

Specimens were tested on a multiplex assay for SARS-CoV-2 IgG antibodies. The overall seroprevalence rate among these samples was 2.88% (95% Confidence Interval (CI): 2.39, 3.44). The active component seroprevalence rate was 2.62% (95% CI: 2.079, 3.265), which was lower, but not statistically significantly different than the reserve component rate of 3.53% (95% CI: 2.53, 4.77). The confirmed number of COVID-19 cases increased from 2,831 (0.21%) on May 1, 2020 to 9,590 (0.72%) as of June 30, 2020 among the active component force with a rate at the midpoint of the sampling interval of 0.28% (3,787 cases) (N=1,333,977). Applying the active component seroprevalence rate to the total active component force results in an estimated 34,909 (95% CI: 27,730, 43,559) SARS-CoV-2 exposures among SM; a 9.2-fold higher case count compared to the reported number of confirmed cases. The presence of neutralizing antibodies, as reflected by microneutralization assay inhibition *in vitro* of SARS-CoV-2, was observed in 1.58% (95% CI: 1.21, 2.00) of the sample population (63 out of 4,000 specimens). Evidence of neutralization activity is more highly correlated with short-term immunity than other antibody tests.

The seroprevalence data from May and June shows a significant undercount of cases among the active component. The estimated total (true) number of infections is 7 to 11 times larger than confirmed case counts. The seroprevalence estimate for the July-August timeframe is underway. Multiple estimates of seroprevalence over time will provide greater confidence in the true case rates among active component Service members as well as the rate of change of infection among this component.

Additional details on rates by selected demographics and Geographic Combatant Command (GCC) and regions are shown in the tables and figure below.

Figure. Seroprevalence of SARS-CoV-2 Antibody among U.S. Service Members Sampled between May and June 2020 by State of Service Member's Unit



* No/Insufficient data indicates there were less than 30 specimens tested from the state.

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #1 14 Oct 2020

- SARS-CoV-2 antibody seroprevalence was similar between NORTHCOM (2.87%) and EUCOM (2.83%).
- INDOPACOM had a much lower seroprevalence (0.47%) than other geographic combatant commands with data, but this finding did not reach statistical significance.
- Seroprevalence estimates could not be calculated for CENTCOM, AFRICOM, and SOUTHCOM due to insufficient specimens collected in those areas of responsibility.

Table 1. Seroprevalence of SAR-CoV-2 Antibody by Geographic Combatant Command (GCC)

GCC	Seroprevalence Estimates (95% CI)
NORTHCOM	2.87 (2.35, 3.47)
EUCOM	2.83 (0.78, 7.70)
INDOPACOM	0.47 (0.02, 2.42)
CENTCOM	-
AFRICOM	-
SOUTHCOM	-

- Navy SMs had the highest seroprevalence (4.11%) compared to other services; which is primarily due to two large outbreaks between April and June. However, the Navy estimate was not statistically significantly higher than the other services.
- Reserve and Guard SMs had a higher seroprevalence than the active component; however these differences were not statistically significant.
- SAR-CoV-2 antibody seroprevalence was statistically significantly higher among SMs of Black and Hispanic race/ethnicity (5.05% and 4.22%, respectfully), compared to Whites (1.98%).

Table 2. Seroprevalence of SARS-CoV-2 Antibody by Service, Component and Race/ Ethnicity

Variables	Seroprevalence Estimates (95% CI)
Service	
Army	2.80 (2.15, 3.57)
Air Force	2.03 (1.16, 3.32)
Marine Corps	2.67 (1.34, 4.64)
Navy	4.11 (2.77, 5.82)
Component	
Active	2.62 (2.089, 3.27)
Guard	3.44 (2.20, 5.10)
Reserve	3.64 (2.25, 5.67)
Race/Ethnicity	
White	1.98 (1.46, 2.63)
Black	5.05 (3.53, 7.00)
Hispanic	4.22 (2.82, 6.03)
Other	2.28 (1.17, 4.13)

- SARS-CoV-2 antibody seroprevalence was similar between males and females.
- No trend of seroprevalence with age was observed. Male SMs who were 55 years of age or older had a high seroprevalence estimate (10.00%), but there were only 20 individuals in this group, which results in very wide 95% CIs.

Table 3. Seroprevalence of SARS-CoV-2 Antibody by Age and Sex

Variables	Seroprevalence Estimates (95% CI)	
Age Category	**Males**	**Females**
All	2.84 (2.30, 3.46)	3.02 (1.99, 4.48)
17-24	2.65 (1.91, 3.63)	3.53 (1.92, 5.88)
25-34	3.22 (2.29, 4.39)	3.31 (1.66, 6.11)
35-44	2.79 (1.59, 4.48)	1.11 (0.06, 5.71)
45-54	1.21 (0.21, 4.26)	<0.01 (0, 11.81)
55+	10.00 (1.81, 31.61)	<0.01 (0, 77.64)

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #1 14 Oct 2020

Study Methodology:

The Armed Forces Health Surveillance Division (AFHSD) utilized data from the Defense Medical Surveillance System (DMSS) and serum specimens from the DoDSR to conduct this study. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) conducted the SARS-CoV-2 serological testing of the DoDSR specimens.

Specimen Selection and Testing: DMSS data were used to identify specimens in the DoDSR with a collection date on or after May 01, 2020. For each 2-month selection window (May 01, 2020 – June 30, 2020 for the initial selection and then consecutive months following), 4,000 specimens (approximately 2,000 specimens per month) were randomly selected from specimens with a collection date during that window. After selection, specimens were aliquoted and delivered to USAMRIID for testing.

Samples were tested via a multiplexed, magnetic bead-based immunoassay optimized to identify SARS-CoV-2 IgG antibodies. The assay was developed at USAMRIID and run on the Luminex MAGPIX system. The Luminex assay utilizes 3 recombinant SARS-CoV-2 viral targets for detection including the full-length S-protein as well as the S1 and Receptor Binding Domain segments. Samples are considered positive when demonstrating sufficient reactivity to at least 2 of the 3 targets. A 95% MFI cutoff for all 3 targets was determined from assay validation using known positive and negative samples. For this study, positive samples underwent additional testing via a SARS-CoV-2 specific microneutralization assay to evaluate the sample for the presence of functional antibodies that block viral replication in cell culture, which may be indicative of a protective capacity of the antibody response.

Data Analysis: Results from the Luminex assay were merged with the demographic and location data from DMSS for the analysis. The overall seroprevalence of SARS-CoV-2 antibody (number of SAR-CoV-2 antibody positive specimens divided by the number of specimens tested multiplied by 100) and 95% confidence intervals (95% CI) were calculated for each sampling group. Stratified seroprevalence estimates for demographic and location specific variables were also calculated. To evaluate the overall estimated number of SAR-CoV-2 infections among the total active component force, the seroprevalence estimate among the active component sample was multiplied by the total active component force population number at the end of the surveillance window. This estimate was divided by the total number of reported COVID-19 cases to estimate the fold increase between reported and estimated infections based on seroprevalence.

Limitations: There are limitations to these data that should be considered when interpreting the results:

- The USAMRIID SARS-CoV-2 Luminex assay has 91% sensitivity and 98% specificity. Therefore, some serology results may be false positive results (the test result is positive, but the individual does not have SARS-CoV-2 antibodies) or false negative results (the test result is negative, but the individual does have SARS-CoV-2 antibodies).
- The sample size for this effort was calculated based on estimating the seroprevalence in the active force. Therefore, subgroup analyses of certain demographic categories or locations may be limited by insufficient numbers of individuals to calculate a precise estimate of the seroprevalence of SARS-CoV-2 antibodies in that group. The 95% CI will provide a measure of how precise the estimate is for that group. If the sample size for a specific group is too small, then seroprevalence data will not be provided since an accurate estimate cannot be determined.
- The fraction of infected individuals that develop detectable SARS-CoV-2 antibodies and the duration that those antibodies remain detectable has not been clearly defined to date. Therefore, the seroprevalence rate reported here may underestimate the total number infected. This effect is expected to become more pronounced over time, as the antibody response among those who were infected during the earlier months of the pandemic will fade over time.

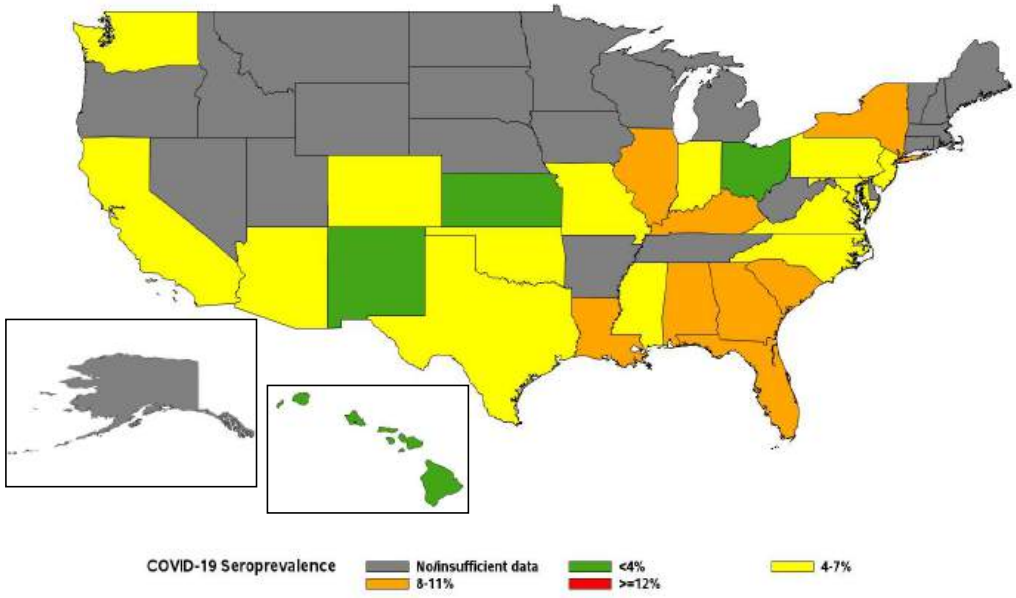
Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #2 20 Nov 2020

Executive Summary – Four thousand specimens were randomly selected from the 209,635 specimens submitted to the DoD Serum Repository (DoDSR) during the months of July and August in order to estimate the prevalence of SARS -CoV-2 antibodies among Service members (SM). This prevalence gives a much more accurate estimation of total SARS-CoV-2 infections that have occurred among Service members (SM) than confirmed case counts, due to incomplete testing among asymptomatic and minimally symptomatic SARS-CoV-2 infections.

Specimens were tested on a multiplex assay for SARS-CoV-2 IgG antibodies. The overall seroprevalence rate among these samples was 5.75% (95% Confidence Interval (CI): 5.05, 6.51); this is double the seroprevalence rate for the May/June specimens (2.88%). The active component seroprevalence rate was 5.49% (95% CI: 4.67, 6.41), which was lower, but not statistically significantly different than the reserve component rate of 6.30% (95% CI: 5.03, 7.78). The reported number of confirmed COVID-19 cases increased from 11,871 (0.88%) on July 1, 2020 to 31,106 (2.3%) as of August 31, 2020 among the active component force with a rate at the midpoint of the sampling interval of 1.45% (19,568 cases amongst 1,348,603 SM). Applying the active component seroprevalence rate to the total active component force results in an estimated 74,038 (95% CI: 62,990, 86,474) SARS-CoV-2 infections among SM; an estimated 3.8-fold higher "true" case count compared to the reported number of confirmed cases. Because the duration of antibody detection is unknown, this estimate of true to confirmed cases likely represents a lower limit of the actual ratio. The presence of neutralizing antibodies, as reflected by microneutralization assay inhibition *in vitro* of SARS-CoV-2, was observed in 3.83% (95% CI: 3.26, 4.46) of the sample population (based on 153 positives out of 4,000 specimens). Evidence of neutralization activity is more highly correlated with short-term immunity than other antibody tests.

The seroprevalence data from July and August continues to show a significant undercount of cases (73.6% less than the "true" count) among the active component. The estimated total ("true") number of infections is almost 4 times larger than the reported number of confirmed cases. The seroprevalence testing for the September to mid-October timeframe is underway. Multiple estimates of seroprevalence over time will provide greater confidence in the true case rates among active component SM as well as the rate of change of infection among this component.

Figure. Seroprevalence of SARS-CoV-2 Antibody among U.S. Service members Sampled between July and August 2020 by State of Service member's Unit



* No/Insufficient data indicates there were less than 30 specimens tested from the state.

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #2 20 Nov 2020

- SARS-CoV-2 antibody seroprevalence was highest in NORTHCOM (6.00%) compared to EUCOM (1.64%) and INDOPACOM (2.79%), but these differences did not reach statistical significance.
- Seroprevalence estimates could not be calculated for CENTCOM, AFRICOM, and SOUTHCOM due to insufficient specimens collected in those GCCs.

Table 1. Seroprevalence of SAR-CoV-2 Antibody by Geographic Combatant Command (GCC)

GCC	Seroprevalence Estimates (95% CI)
NORTHCOM	6.00 (5.26, 6.80)
EUCOM	1.64 (0.08, 8.43)
INDOPACOM	2.79 (1.11, 6.22)
CENTCOM	-
AFRICOM	-
SOUTHCOM	-

- Army SM had the highest seroprevalence (6.40%) compared to other services; however, there were no statistically significant differences between the Service estimates.
- Reserve SM had a higher seroprevalence than the active and guard component; however, these differences were not statistically significant.
- SARS-CoV-2 antibody seroprevalence was highest among Hispanic SM (9.08%), followed by Black SM (7.00%); however, these estimates were not statistically significantly higher than those for SM of white or other race/ethnicity (4.74% and 3.87%, respectively).

Table 2. Seroprevalence of SARS-CoV-2 Antibody by Service, Component and Race/Ethnicity

Variables	Seroprevalence Estimates (95% CI)
Service	
Army	6.40 (5.38, 7.52)
Air Force	4.79 (3.44, 6.48)
Marine Corps	5.80 (3.97, 8.22)
Navy	4.83 (3.33, 6.73)
Component	
Active	5.49 (4.67, 6.41)
Guard	5.11 (3.63, 6.98)
Reserve	7.79 (5.77, 10.29)
Race/Ethnicity	
White	4.75 (3.91, 5.70)
Black	7.00 (5.18, 9.24)
Hispanic	9.08 (7.04, 11.44)
Other	3.87 (2.33, 6.20)

- SARS-CoV-2 antibody seroprevalence was similar between males and females.
- No trend of seroprevalence with age was observed. Male SM who were 17-24 years of age had the highest seroprevalence estimate (6.92%); however, this was not statistically different than for other sex/age categories.

Table 3. Seroprevalence of SARS-CoV-2 Antibody by Age and Sex

Variables	Seroprevalence Estimates (95% CI)	
Age Category	**Males**	**Females**
All	5.82 (5.05, 6.67)	5.45 (3.96, 7.31)
17-24	6.92 (5.70, 8.31)	6.08 (3.89, 9.01)
25-34	5.46 (4.22, 6.96)	5.93 (3.39, 9.54)
35-44	4.33 (2.77, 6.42)	3.26 (0.89, 8.87)
45-54	2.96 (1.17, 6.59)	<0.01 (0, 14.53)
55+	6.25 (1.12, 19.58)	<0.01 (0, 52.71)

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #2 20 Nov 2020

Study Methodology:

The Armed Forces Health Surveillance Division (AFHSD) utilized data from the Defense Medical Surveillance System (DMSS) and serum specimens from the DoDSR to conduct this study. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) conducted the SARS-CoV-2 serological testing of the DoDSR specimens.

Specimen Selection and Testing: DMSS data were used to identify specimens in the DoDSR with a collection date on or after May 01, 2020. For each 2-month selection window (May 01, 2020 – June 30, 2020 for the initial selection and then consecutive months following), 4,000 specimens (approximately 2,000 specimens per month) were randomly selected from specimens with a collection date during that window. After selection, specimens were aliquoted and delivered to USAMRIID for testing.

Samples were tested via a multiplexed, magnetic bead-based immunoassay optimized to identify SARS-CoV-2 IgG reactivity. The assay was developed at USAMRIID and run on the Luminex MAGPIX system. The Luminex assay utilizes 3 recombinant SARS-CoV-2 viral targets for detection including the full-length S-protein as well as the S1 and Receptor Binding Domain segments. Samples are considered positive when demonstrating sufficient reactivity to at least 2 of the 3 targets. A 95% MFI cutoff for all 3 targets was determined from assay validation using known positive and negative samples. For this study, positive samples underwent additional testing via a SARS-CoV-2 specific microneutralization assay to evaluate the sample for the presence of functional antibodies that block viral replication in cell culture, which may be indicative of a protective capacity of the antibody response.

Data Analysis: Results from the Luminex assay were merged with the demographic and location data from DMSS for the analysis. The overall seroprevalence of SARS-CoV-2 antibody (number of SAR-CoV-2 antibody positive specimens divided by the number of specimens tested multiplied by 100) and 95% confidence intervals (95% CI) were calculated for each sampling group. Stratified seroprevalence estimates for demographic and location specific variables were also calculated. To evaluate the overall estimated number of SAR-CoV-2 infections among the total active component force, the seroprevalence estimate among the active component sample was multiplied by the total active component force population number at the end of the surveillance window. This estimate was divided by the total number of reported COVID-19 cases to estimate the fold increase between reported and estimated infections based on seroprevalence.

Limitations: There are limitations to these data that should be considered when interpreting the results:

- The USAMRIID SARS-CoV-2 Luminex assay has 91% sensitivity and 98% specificity. Therefore, some serology results may be false positive results (the test result is positive, but the individual does not have SARS-CoV-2 antibodies) or false negative results (the test result is negative, but the individual does have SARS-CoV-2 antibodies).
- The sample size for this effort was calculated based on estimating the seroprevalence in the active force. Therefore, subgroup analyses of certain demographic categories or locations may be limited by insufficient numbers of individuals to calculate a precise estimate of the seroprevalence of SARS-CoV-2 antibodies in that group. The 95% CI will provide a measure of how precise the estimate is for that group. If the sample size for a specific group is too small, then seroprevalence data will not be provided since an accurate estimate cannot be determined.
- The fraction of infected individuals that develop detectable SARS-CoV-2 antibodies and the duration that those antibodies remain detectable has not been clearly defined to date. Antibody titers are known to decrease over time following infection, and because the duration of antibody detection is unknown, the above estimate of true to confirmed cases represents a lower limit of the actual ratio. This effect is expected to become more pronounced over time, as the antibody response among those who were infected during the earlier months of the pandemic will fade over time. Additional studies on antibody persistence will be necessary to further refine

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #3 27 Jan 2021

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Executive Summary

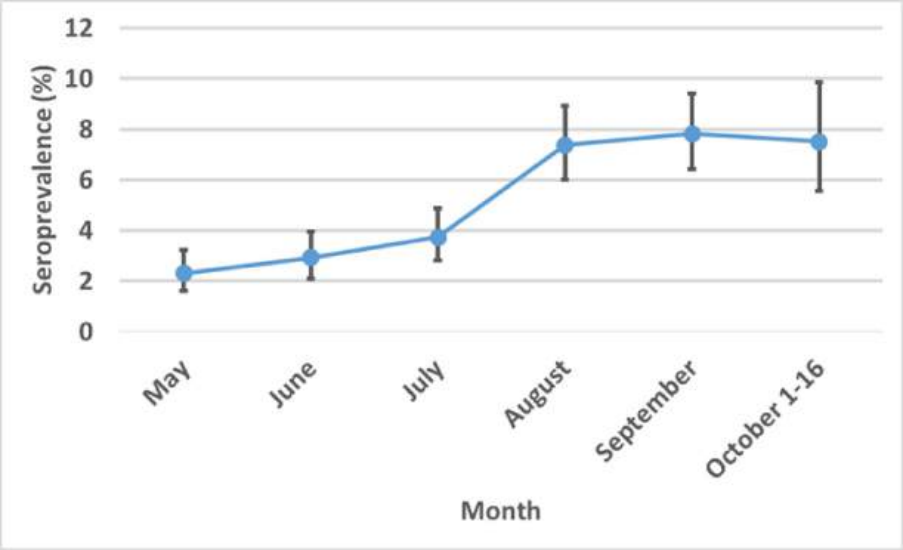
Three thousand specimens were randomly selected from the 172,629 specimens submitted to the DoD Serum Repository (DoDSR) with collection dates between 01 Sep 2020 and 16 Oct 2020 in order to estimate the prevalence of SARS-CoV-2 antibodies among Service members (SM). This prevalence gives a much more accurate estimation of total SARS-CoV-2 infections that have occurred among SM than confirmed case counts, due to incomplete testing among asymptomatic and minimally symptomatic SARS-CoV-2 infections.

Specimens were tested on a multiplex assay for SARS-CoV-2 IgG antibodies. **The overall seroprevalence rate among these samples was 7.73% (95% Confidence Interval (CI): 6.80, 8.74); this was a 34% higher seroprevalence rate than for the July/August specimens (5.75%).** The active component seroprevalence rate was 7.72% (95% CI: 6.60, 8.98), which was similar to the reserve/guard component rate of 7.75% (95% CI: 6.02, 9.54). The reported number of confirmed COVID-19 cases increased from 26,015 (2.00%) on September 1, 2020 to 34,719 (2.60%) as of October 16, 2020 among the active component force with a rate at the midpoint of the sampling interval of 2.24% (29,840 cases amongst 1,333,814 SM). **Applying the active component seroprevalence rate to the total active component force results in an estimated 102,970 (95% CI: 88,044, 119,820) SARS-CoV-2 infections among SM; an estimated 3.5-fold higher "true" case count compared to the reported number of confirmed cases.** Because the duration of antibody detection is unknown, this estimate of true to confirmed cases likely represents a lower limit of the actual ratio.[1] **The presence of neutralizing antibodies, as reflected by microneutralization assay inhibition *in vitro* of SARS-CoV-2, was observed in 6.17% (95% CI: 5.34, 7.09)** of the sample population (based on 185 positives out of 3,000 specimens). Evidence of neutralization activity is more highly correlated with short-term immunity than other antibody tests.

The SARS-CoV-2 seroprevalence data from May to mid-October among the active component force showed an increase from 2.3% to 7.5%. (Figure 1). Applying these seroprevalence rates to the monthly total active component force provided a similar trend in total estimated cases and showed a continued undercounting of confirmed cases (Figure 2). Undercounting of cases was most pronounced early in the pandemic with the estimated number of infections in May 2020 being 9.4 times larger than the reported number of confirmed cases, compared to 3.1 times larger in the first half of October 2020. The point estimate for seroprevalence decreased slightly between September and the first half of October. Future reports will include samples from later in October that will provide a more complete seroprevalence estimate for that month. Given the wide confidence interval for the current October point estimate, this cannot at this time be interpreted as a true downward trend in seroprevalence.

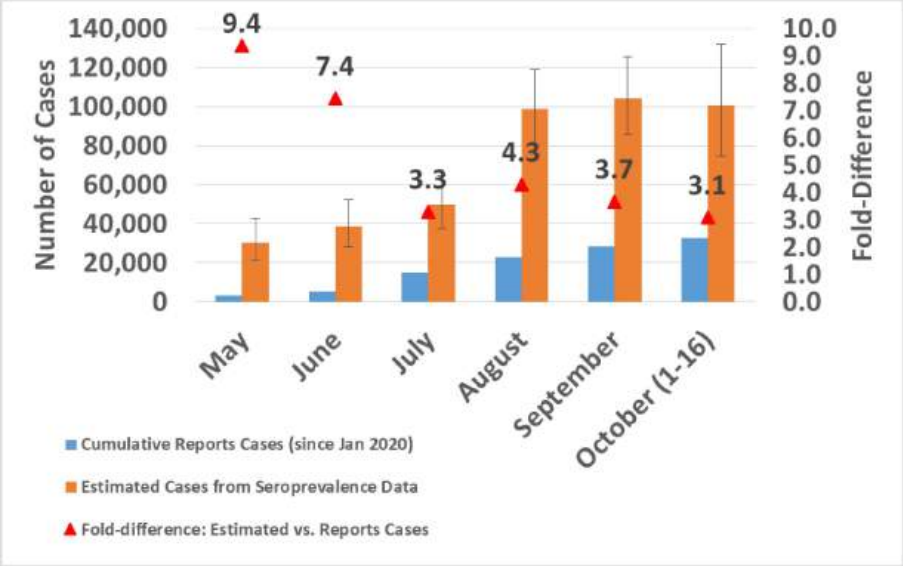
Additional details on rates by selected demographics and Geographic Combatant Command (GCC) and regions for September and mid-October are shown in additional tables and figures in this report.

Figure 1. Monthly Seroprevalence of SARS-CoV-2 Antibody among Active Component U.S. Service Members from 01 May 2020 to 16 October 2020



Bars represent 95% confidence intervals for the seroprevalence estimates.

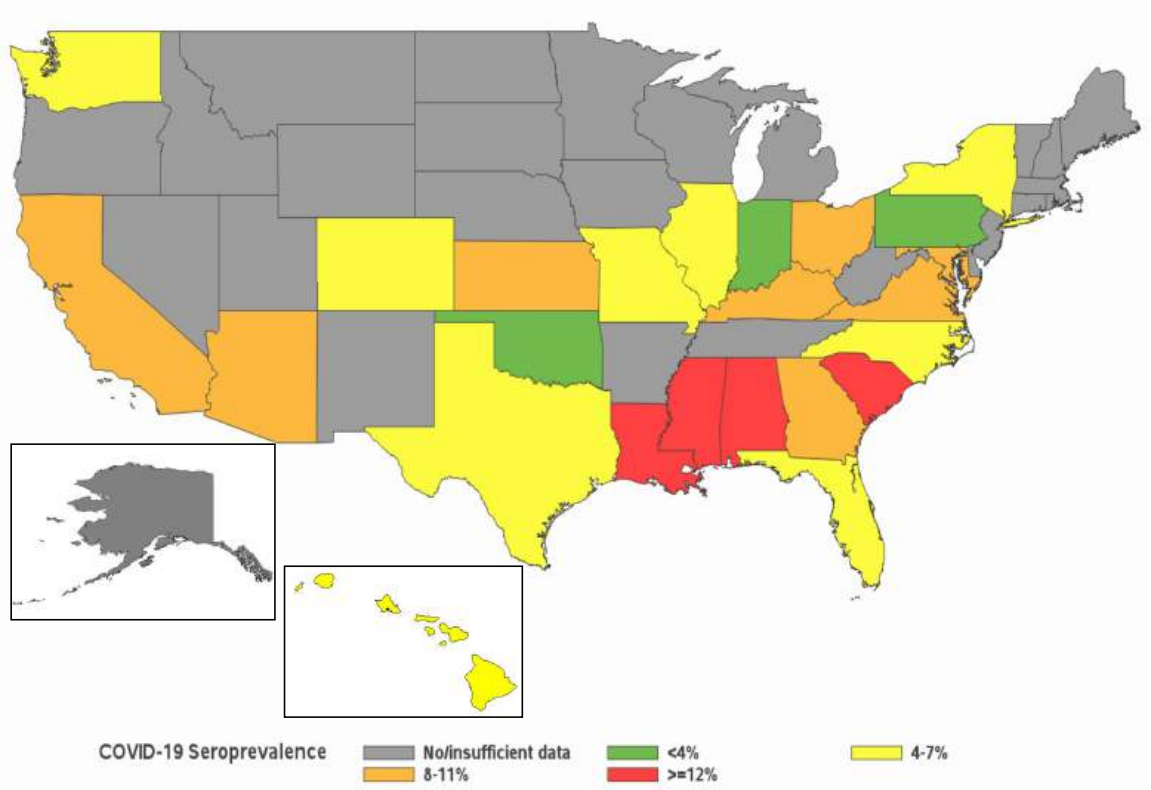
Figure 2. Seroprevalence Estimated Number of SARS-CoV-2 Infections Compared to the Cumulative Reported Number of Confirmed Cases by Month: Active Component Force, May 2020—October 16 2020



1. Vertical lines represent 95% confidence intervals for the estimated number of cases.
2. Reported cases are cumulative from January 2020 to the month of interest.
3. Estimated cases are based on the seroprevalence estimate for each month multiplied by the total active force during that same month.
4. Fold-difference is calculated by dividing the estimated case count by reported case count.

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #3 27 Jan 2021

Figure 3. Seroprevalence of SARS-CoV-2 Antibody among U.S. Service Members Sampled between 01 September and 16 October 2020 by State of Service Member's Unit



* No/Insufficient data indicates there were less than 30 specimens tested from the state.

For the September 1—October 16, 2020 sampling, among states with sufficient data:

- SARS-CoV-2 seroprevalence was highest (>12%) in the southern states Louisiana, Mississippi, Alabama, and South Carolina.
- SARS-CoV-2 seroprevalence was lowest (<4%) in Oklahoma, Indiana, and Pennsylvania.

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #3 27 Jan 2021

Results from the September 1—October 16, 2020 Sampling

- SARS-CoV-2 antibody seroprevalence was highest in NORTHCOM (8.00%) compared to EUCOM (3.70%) and INDOPACOM (3.42%), but these differences did not reach statistical significance.
- Seroprevalence estimates could not be calculated for CENTCOM, AFRICOM, and SOUTHCOM due to insufficient specimens collected in those GCCs.

Table 1. Seroprevalence of SARS-CoV-2 Antibody by Geographic Combatant Command (GCC): September 1-October 16, 2020

GCC	Seroprevalence Estimates (95% CI)
NORTHCOM	8.00 (7.02, 9.06)
EUCOM	3.70 (0.19, 17.6)
INDOPACOM	3.42 (1.36, 7.63)
CENTCOM	-
AFRICOM	-
SOUTHCOM	-

- Marine Corps SM had the highest seroprevalence (9.09%) compared to other services; however, there were no statistically significant differences between the Service estimates.
- Reserve SM had a higher seroprevalence than the active and guard component; however, these differences were not statistically significant.
- SARS-CoV-2 antibody seroprevalence was highest among Black SM (12.85%), which was statistically significantly higher than White SM (6.42%). Hispanic SM (7.46%) and SM of other race/ethnicities (6.94%) were also lower than Black SM; however these estimates did not reach statistically significant difference.

Table 2. Seroprevalence of SARS-CoV-2 Antibody by Service, Component and Race/Ethnicity: September 1-October 16, 2020

Variables	Seroprevalence Estimates (95% CI)
Service	
Army	8.26 (6.91, 9.76)
Air Force	6.01 (4.32, 8.14)
Marine Corps	9.09 (6.21, 12.77)
Navy	7.51 (5.61, 9.87)
Component	
Active	7.72 (6.60, 8.98)
Guard	6.99 (5.09, 9.38)
Reserve	8.67 (6.37, 11.55)
Race/Ethnicity	
White	6.42 (5.31, 7.68)
Black	12.85 (10.11, 16.05)
Hispanic	7.46 (5.36, 10.11)
Other	6.94 (4.44, 10.29)

- SARS-CoV-2 antibody seroprevalence was similar between males and females.
- SARS-CoV-2 antibody seroprevalence by age was highest among males and females aged 17-24 years (8.77% and 10.60%, respectively). No statistically significant differences with age and sex were seen.

Table 3. Seroprevalence of SARS-CoV-2 Antibody by Age and Sex: September 1-October 16, 2020

Variables	Seroprevalence Estimates (95% CI)	
Age Category	**Males**	**Females**
All	7.76 (6.75, 8.89)	7.59 (5.53, 10.06)
17-24	8.77 (7.16, 10.64)	10.60 (6.97, 15.3)
25-34	7.86 (6.14, 9.84)	7.98 (4.73, 12.29)
35-44	5.91 (3.83, 8.65)	1.98 (0.35, 6.59)
45-54	4.55 (2.00, 9.56)	0.00 (0.00, 20.12)
55+	5.56 (0.28, 26.6)	0.00 (0.00, 40.61)

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #3 27 Jan 2021

Study Methodology:

The Armed Forces Health Surveillance Division (AFHSD) utilized data from the Defense Medical Surveillance System (DMSS) and serum specimens from the DoDSR to conduct this study. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) conducted the SARS-CoV-2 serological testing of the DoDSR specimens.

Specimen Selection and Testing:

DMSS data were used to identify specimens in the DoDSR with a collection date on or after May 01, 2020 for each 6-8 week selection window. The previous reports have covered May 01, 2020 – August 31, 2020, while the current analysis includes samples from September 01—October 16 2020. Approximately 2,000 specimens per month were randomly selected from specimens with a collection date during each window. Due to limited availability of October samples during the current collection period, 1,000 samples from the first half of October are included in this analysis. After selection, specimens were aliquoted and delivered to USAMRIID for testing.

Samples were tested via a multiplexed, magnetic bead-based immunoassay optimized to identify SARS-CoV-2 IgG reactivity. The assay was developed at USAMRIID and run on the Luminex MAGPIX system. The Luminex assay utilizes 3 recombinant SARS-CoV-2 viral targets for detection including the full-length S-protein as well as the S1 and Receptor Binding Domain segments. Samples are considered positive when demonstrating sufficient reactivity to at least 2 of the 3 targets. A 95% MFI (mean fluorescence intensity) cutoff for all 3 targets was determined from assay validation using known positive and negative samples. For this study, positive samples underwent additional testing via a SARS-CoV-2 specific microneutralization assay to evaluate the sample for the presence of functional antibodies that block viral replication in cell culture, which may be indicative of a protective capacity of the antibody response.

Data Analysis:

Results from the Luminex assay were merged with the demographic and location data from DMSS for the analysis. The overall seroprevalence of SARS-CoV-2 antibody (number of SARS-CoV-2 antibody positive specimens divided by the number of specimens tested multiplied by 100) and 95% confidence intervals (95% CI) were calculated for each sampling group. Stratified seroprevalence estimates for demographic and location specific variables were also calculated. To evaluate the overall estimated number of SARS-CoV-2 infections among the total active component force, the seroprevalence estimate among the active component sample was multiplied by the total active component force population number at the middle point of the surveillance window. This estimate was divided by the total number of reported COVID-19 cases to estimate the fold increase between reported and estimated infections based on seroprevalence.

Limitations:

There are limitations to these data that should be considered when interpreting the results:

- The USAMRIID SARS-CoV-2 Luminex assay has 91% sensitivity and 98% specificity. Therefore, some serology results may be false positive results (the test result is positive, but the individual does not have SARS-CoV-2 antibodies) or false negative results (the test result is negative, but the individual does have SARS-CoV-2 antibodies).
- The sample size for this effort was calculated based on estimating the seroprevalence in the active force. Therefore, subgroup analyses of certain demographic categories or locations may be limited by insufficient numbers of individuals to calculate a precise estimate of the seroprevalence of SARS-CoV-2 antibodies in that group. The 95% CI will provide a measure of how precise the estimate is for that group. If the sample size for a specific group is too small, then seroprevalence data will not be provided since an accurate estimate cannot be determined.
- The fraction of infected individuals that develop detectable SARS-CoV-2 antibodies and the duration that those antibodies remain detectable has not been clearly defined to date. Antibody titers are known to decrease over time following infection, and because the duration of antibody detection is unknown, the above estimate of true to confirmed cases represents a lower limit of the actual ratio.[1] This effect is expected to become more pronounced over time, as the antibody response among those who were infected during the earlier months of the pandemic will fade over time. Additional studies on antibody persistence will be necessary to further refine these estimates.

References:

1. Mariën J, Ceulemans A, Michiels J, Heyndrickx L, Kerkhof K, Foque N, Widdowson M-Alain, Mortgat L, Duysburgh E, Desombere I, Jansens H, Van Esbroeck M, Ariën KK, Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay, Journal of Virological Methods (2020), doi: https://doi.org/10.1016/j.jviromet.2020.114025.

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #4 19 Mar 2021

Controlled by: Office of the Under Secretary of Defense for Personnel and Readiness
Controlled by: Office of the Assistant Secretary of Defense for Health Affairs
CUI Categories: Agricultural & Health Operations, Mission Assurance
Distribution/Dissemination Control: NONE
POC: LTC Kevin Taylor/kevin.m.taylor4.mil@mail.mil

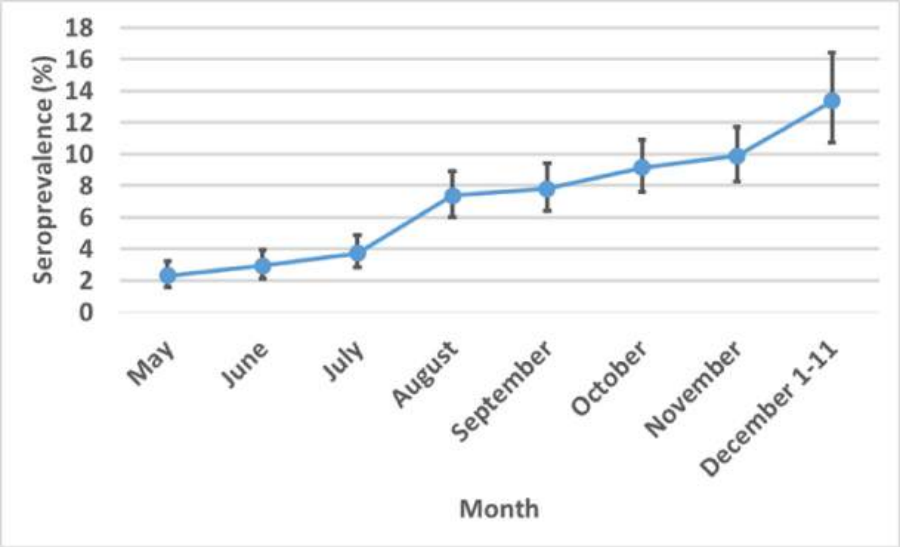
Executive Summary

Four thousand specimens were randomly selected from the 177,759 specimens submitted to the DoD Serum Repository (DoDSR) with collection dates between October 17, 2020 and December 11, 2020 in order to estimate the prevalence of SARS-CoV-2 antibodies among Service members (SM). This prevalence gives a much more accurate estimation of total SARS-CoV-2 infections that have occurred among SM than confirmed case counts, due to incomplete testing among asymptomatic and minimally symptomatic SARS-CoV-2 infections. While the information presented in this report illustrates rising seroprevalence among SMs over a 32 week period and more accurately demonstrates the impact of SARS-CoV-2 across the DoD, it should be noted that it only includes specimens obtained prior to mid-December and does not incorporate findings from much of the recent wave of cases experienced across the U.S.

Specimens were tested on a multiplex assay for SARS-CoV-2 IgG antibodies. **The overall seroprevalence rate among these samples was 11.70% (95% Confidence Interval (CI): 10.73, 12.73); this was a 51% higher seroprevalence rate than for the September/mid-October specimens (7.73%).** The active component seroprevalence rate was 10.96% (95% CI: 9.75, 12.26), which was lower, but not statistically significantly different than the reserve/guard component rate of 12.85% (95% CI: 11.23, 14.61). The reported number of confirmed COVID-19 cases increased from 34,699 (2.60%) on October 17, 2020 to 65,187 (4.88%) as of December 11, 2020 among the active component force with a rate at the midpoint of the sampling interval of 3.53% (47,150 cases amongst 1,334,775 SM). **Applying the active component seroprevalence rate to the total active component force results in an estimated 147,497 (95% CI: 131,149, 164,990) SARS-CoV-2 infections among active component SM; an estimated 3.1-fold higher "true" case count compared to the reported number of confirmed cases.** Because the duration of antibody detection is unknown, this estimate of true to confirmed cases likely represents a lower limit of the actual ratio.[1] **The presence of neutralizing antibodies, as reflected by microneutralization assay inhibition *in vitro* of SARS-CoV-2, was observed in 10.15% (95% CI: 9.24, 11.12) of the sample population (based on 406 positives out of 4,000 specimens).** Evidence of neutralization activity is more highly correlated with short-term immunity than other antibody tests.

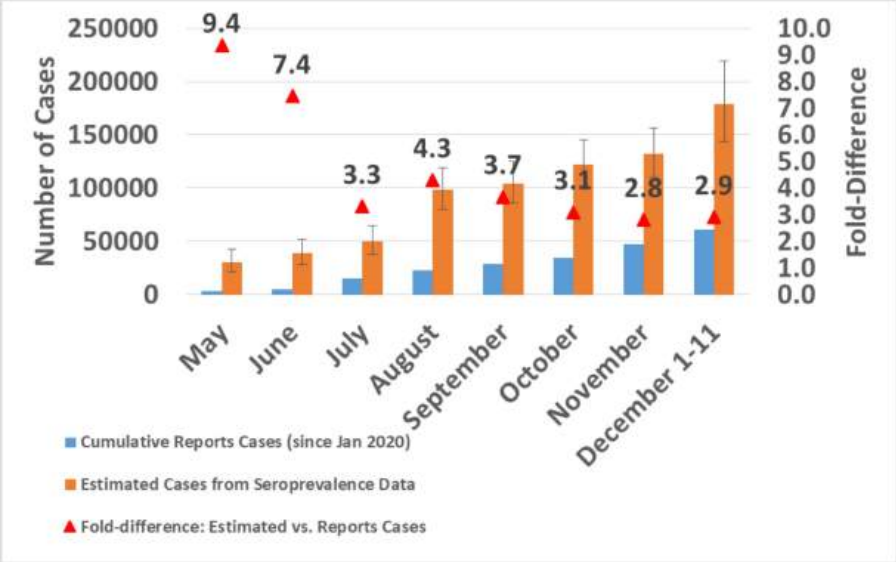
The SARS-CoV-2 seroprevalence data from May to December 11, 2020 among the active component SM showed an increase from 2.3% to 13.4%. (Figure 1). Applying these seroprevalence rates to the monthly total active component force provided a similar trend in total estimated cases and showed a continued undercounting of confirmed cases (Figure 2). Undercounting of cases was most pronounced early in the pandemic with the estimated number of infections in May 2020 being 9.4 times larger than the reported number of confirmed cases, compared to 2.9 times larger in the first half of December 2020. The higher undercounting of cases early in the pandemic may be due to a lack of and access to available testing and the absence of routine screening of DoD populations such as new accessions, which was implemented later in the pandemic. The continued discrepancy between reported case counts and seroprevalence estimates of case counts is most likely due to mild and asymptomatic cases.

Figure 1. Monthly Seroprevalence of SARS-CoV-2 Antibody among Active Component Service Members from May 01, 2020 to December 11, 2020



Bars represent 95% confidence intervals for the seroprevalence estimates.

Figure 2. Seroprevalence Estimated Number of SARS-CoV-2 Infections Compared to the Cumulative Reported Number of Confirmed Cases by Month: Active Component Force, May 01, 2020 to December 11, 2020



1. Vertical lines represent 95% confidence intervals for the estimated number of cases.
2. Reported cases are cumulative from January 2020 to the month of interest.
3. Estimated cases are based on the seroprevalence estimate for each month multiplied by the total active force during that same month.
4. Fold-difference is calculated by dividing the estimated case count by reported case count.

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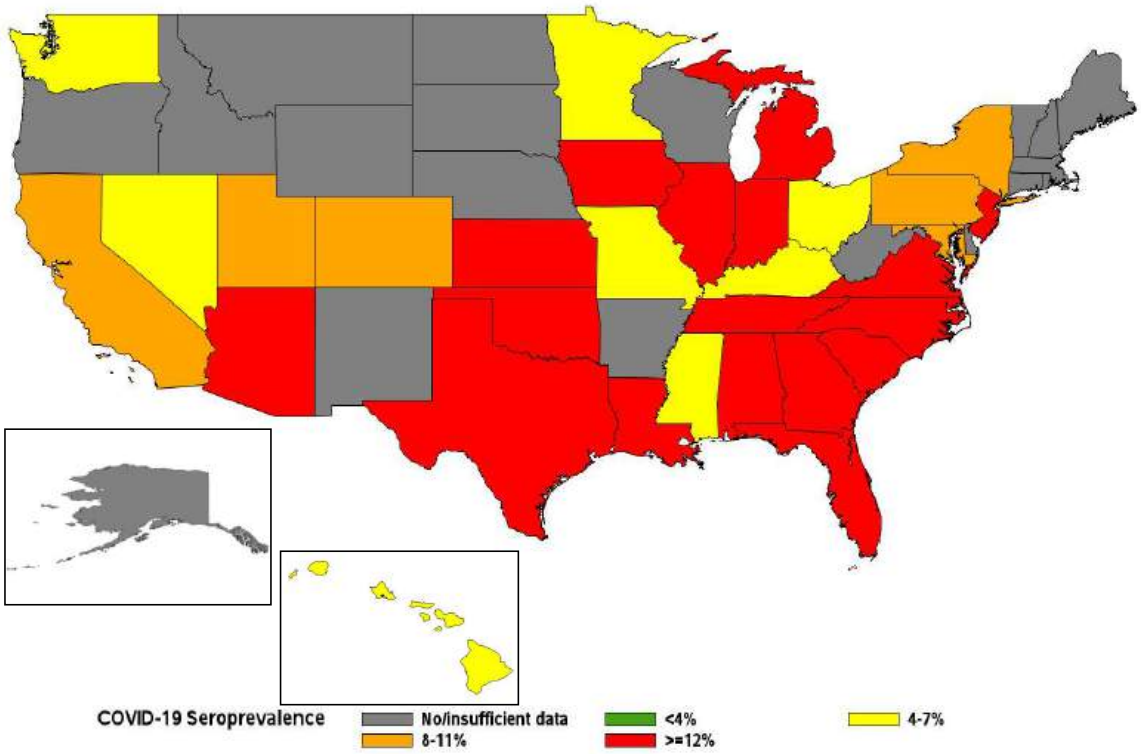
Figure 3. Seroprevalence of SARS-CoV-2 Antibody among U.S. Service Members Sampled between October 17, 2020 to December 11, 2020 by State of Service Member's Unit



* No/Insufficient data indicates there were less than 30 specimens tested from the state.

For the October 17—December 11, 2020 sampling:

- The number of states with SARS-CoV-2 seroprevalence greater than or equal to 12% increased from only 4 states (all in the Southeast) for the September/October sample to 17 states during the October/December sample.
- All states with sufficient data were at or above 4% SARS-CoV-2 seroprevalence.

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Results from the October 17-December 11, 2020 Sampling

- SARS-CoV-2 antibody seroprevalence continued to be highest in NORTHCOM (11.94%) compared to EUCOM (5.26%) and INDOPACOM (5.80%), but these differences did not reach statistical significance.
- Seroprevalence estimates could not be calculated for CENTCOM, AFRICOM, and SOUTHCOM due to insufficient specimens collected in those GCCs.

Table 1. Seroprevalence of SARS-CoV-2 Antibody by Geographic Combatant Command (GCC): October 17-December 11, 2020

GCC	Seroprevalence Estimates (95% CI)
NORTHCOM	11.94 (10.91, 13.05)
EUCOM	5.26 (1.45, 14.35)
INDOPACOM	5.80 (2.58, 10.98)
CENTCOM	-
AFRICOM	-
SOUTHCOM	-

- Marine Corps SM continued to have the highest seroprevalence (17.05%) compared to other services and nearly doubled from the last sample. The Marine Corps seroprevalence was statistically significantly higher than the Army and Air Force.
- Reserve SM continued to have a higher seroprevalence than the active and guard component; however, these differences were not statistically significant.
- SARS-CoV-2 antibody seroprevalence was highest among Black SM (15.71%), which was statistically significantly higher than White SM (10.31%). Hispanic SM (13.54%) and SM of other race/ethnicities (9.98%) were also lower than Black SM; however these estimates did not reach statistically significant difference.

Table 2. Seroprevalence of SARS-CoV-2 Antibody by Service, Component and Race/Ethnicity: October 17-December 11, 2020

Variables	Seroprevalence Estimates (95% CI)
Service	
Army	11.65 (10.37, 13.03)
Air Force	9.12 (7.07, 11.49)
Marine Corps	17.05 (13.48, 21.12)
Navy	11.39 (9.12, 14.00)
Component	
Active	10.96 (9.75, 12.26)
Guard	12.56 (10.48, 14.89)
Reserve	13.25 (10.79, 16.07)
Race/Ethnicity	
White	10.31 (9.11, 11.62)
Black	15.71 (13.01, 18.70)
Hispanic	13.54 (11.08, 16.26)
Other	9.98 (7.26, 13.26)

- SARS-CoV-2 antibody seroprevalence was similar between males and females.
- SARS-CoV-2 antibody seroprevalence by age continued to be highest among males and females aged 17-24 years (14.22% and 14.29%, respectively). No statistically significant differences with age and sex were seen.

Table 3. Seroprevalence of SARS-CoV-2 Antibody by Age and Sex: October 17-December 11, 2020

Variables	Seroprevalence Estimates (95% CI)	
Age Category	**Males**	**Females**
All	11.39 (10.32, 12.54)	12.92 (10.70, 15.43)
17-24	14.22 (12.32, 16.26)	14.29 (10.75, 18.37)
25-34	9.79 (8.14, 11.64)	13.90 (10.22, 18.37)
35-44	10.64 (8.37, 13.29)	7.04 (3.61, 12.44)
45-54	5.82 (3.00, 10.12)	13.33 (4.69, 29.46)
55+	5.00 (0.26, 23.88)	50.00 (2.53, 97.47)

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #4 19 Mar 2021

Study Methodology:

The Armed Forces Health Surveillance Division (AFHSD) utilized data from the Defense Medical Surveillance System (DMSS) and serum specimens from the DoDSR to conduct this study. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) conducted the SARS-CoV-2 serological testing of the DoDSR specimens.

Specimen Selection and Testing:

DMSS data were used to identify specimens in the DoDSR with a collection date on or after May 01, 2020 for each 6-8 week selection window. The previous reports have covered May 01, 2020 – October 16, 2020, while the current analysis includes samples from October 17—December 11, 2020. Approximately 2,000 specimens per month were randomly selected from specimens with a collection date during each window. The current selection period sampled 1,000 specimens for the last half of October, 2,000 specimens from November, and 1,000 specimens from the first part of December, as specimens were only available in the DoDSR through December 11, 2020. After selection, specimens were aliquoted and delivered to USAMRIID for testing.

Samples were tested via a multiplexed, magnetic bead-based immunoassay optimized to identify SARS-CoV-2 IgG reactivity. The assay was developed at USAMRIID and run on the Luminex MAGPIX system. The Luminex assay utilizes 3 recombinant SARS-CoV-2 viral targets for detection including the full-length S-protein as well as the S1 and Receptor Binding Domain segments. Samples are considered positive when demonstrating sufficient reactivity to at least 2 of the 3 targets. A 95% MFI (mean fluorescence intensity) cutoff for all 3 targets was determined from assay validation using known positive and negative samples. For this study, positive samples underwent additional testing via a SARS-CoV-2 specific microneutralization assay to evaluate the sample for the presence of functional antibodies that block viral replication in cell culture, which may be indicative of a protective capacity of the antibody response.

Data Analysis:

Results from the Luminex assay were merged with the demographic and location data from DMSS for the analysis. The overall seroprevalence of SARS-CoV-2 antibody (number of SARS-CoV-2 antibody positive specimens divided by the number of specimens tested multiplied by 100) and 95% confidence intervals (95% CI) were calculated for each sampling group. Stratified seroprevalence estimates for demographic and location specific variables were also calculated. To evaluate the overall estimated number of SARS-CoV-2 infections among the total active component force, the seroprevalence estimate among the active component sample was multiplied by the total active component force population number at the middle point of the surveillance window. This estimate was divided by the total number of reported COVID-19 cases to estimate the fold increase between reported and estimated infections based on seroprevalence.

Limitations:

There are limitations to these data that should be considered when interpreting the results:

- The USAMRIID SARS-CoV-2 Luminex assay has 91% sensitivity and 98% specificity. Therefore, some serology results may be false positive results (the test result is positive, but the individual does not have SARS-CoV-2 antibodies) or false negative results (the test result is negative, but the individual does have SARS-CoV-2 antibodies).
- The sample size for this effort was calculated based on estimating the seroprevalence in the active force. Therefore, subgroup analyses of certain demographic categories or locations may be limited by insufficient numbers of individuals to calculate a precise estimate of the seroprevalence of SARS-CoV-2 antibodies in that group. The 95% CI will provide a measure of how precise the estimate is for that group. If the sample size for a specific group is too small, then seroprevalence data will not be provided since an accurate estimate cannot be determined.
- The fraction of infected individuals that develop detectable SARS-CoV-2 antibodies and the duration that those antibodies remain detectable has not been clearly defined to date. Antibody titers are known to decrease over time following infection, and because the duration of antibody detection is unknown, the above estimate of true to confirmed cases represents a lower limit of the actual ratio.[1] This effect is expected to become more pronounced over time, as the antibody response among those who were infected during the earlier months of the pandemic will fade over time. Additional studies on antibody persistence will be necessary to further refine these estimates.

References:

1. Mariën J, Ceulemans A, Michiels J, Heyndrickx L, Kerkhof K, Foque N, Widdowson M-Alain, Mortgat L, Duysburgh E, Desombere I, Jansens H, Van Esbroeck M, Ariën KK, Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay, Journal of Virological Methods (2020), doi: https://doi.org/10.1016/j.jviromet.2020.114025.

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Controlled by: Office of the Under Secretary of Defense for Personnel and Readiness
Controlled by: Office of the Assistant Secretary of Defense for Health Affairs
CUI Categories: Agricultural & Health Operations, Mission Assurance
Distribution/Dissemination Control: NONE
POC: LTC Kevin Taylor/kevin.m.taylor4.mil@mail.mil

Executive Summary

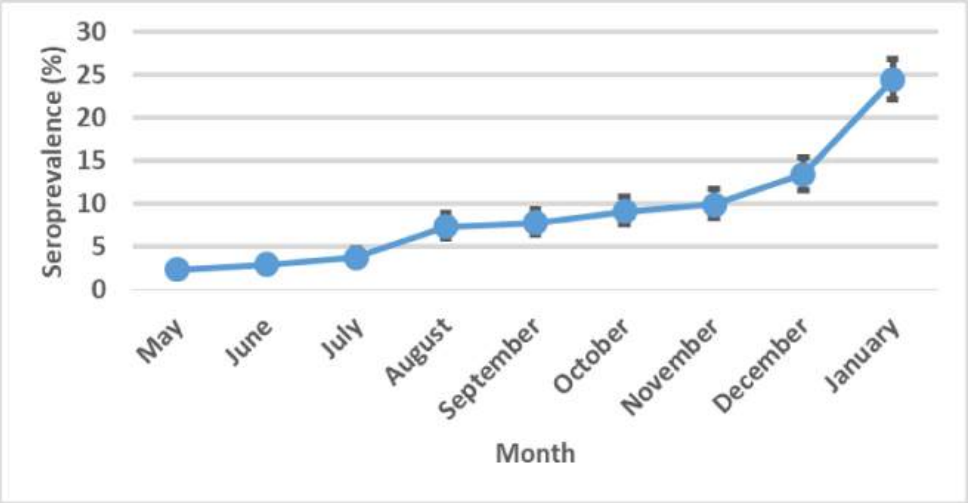
Three thousand specimens were randomly selected from the 116,825 specimens submitted to the DoD Serum Repository (DoDSR) with collection dates between December 12, 2020 and January 31, 2021 in order to estimate the prevalence of SARS-CoV-2 antibodies among Service members (SM). This prevalence gives a much more accurate estimation of total SARS-CoV-2 infections that have occurred among SM than confirmed case counts, due to incomplete testing among asymptomatic and minimally symptomatic SARS-CoV-2 infections. While the information presented in this report illustrates rising seroprevalence among SM over a 39-week period and more accurately demonstrates the impact of SARS-CoV-2 across the DoD, it should be noted that it only includes specimens obtained prior to February 2021 and does not incorporate findings from much of the recent wave of cases experienced across the U.S.

Specimens were tested on a multiplex assay for SARS-CoV-2 IgG antibodies. **The overall seroprevalence rate among these samples was 21.97% (95% Confidence Interval (CI): 20.51, 23.49); this was a 86% higher seroprevalence rate than for the mid-October to mid-December specimens (11.79%).** The active component seroprevalence rate was 20.61% (95% CI: 18.83, 22.48), which continued to be lower, but not statistically significantly different than the reserve/guard component rate of 24.37% (95% CI: 21.84, 27.05). The reported number of confirmed COVID-19 cases increased from 64,788 (4.81%) on December 12, 2020 to 100,347 (7.45%) as of January 31, 2021 among the active component force with a rate at the midpoint of the sampling interval of 6.11% (82,267 cases amongst 1,347,117 SM). **Applying the active component seroprevalence rate to the total active component force results in an estimated 277,764 (95% CI: 253,637, 302,772) SARS-CoV-2 infections among active component SM (20.62% of the active component force); an estimated 3.4-fold higher "true" case count compared to the reported number of confirmed cases.** Because the duration of antibody detection is unknown, this estimate of true to confirmed cases likely represents a lower limit of the actual ratio.[1] **The presence of neutralizing antibodies was observed in 20.93% (95% CI: 19.49, 22.43) of the sample population.** This was based on a 10% sample of the multiplex positive specimens which underwent additional testing by microneutralization assay inhibition in vitro. Of the 64 multiplex positive specimens tested, 61 were positive on the microneutralization assay, which was extrapolated to the full sample size to give an estimated 628 positive for neutralizing antibodies out of 3,000 specimens. Evidence of neutralization activity is more highly correlated with short-term immunity than other antibody tests.

The SARS-CoV-2 seroprevalence data from May 01, 2020 to January 31, 2021 among the active component SM showed an increase from 2.3% to 24.5%. (Figure 1). Applying these seroprevalence rates to the monthly total active component force provided a similar trend in total estimated cases and showed a continued undercounting of confirmed cases (Figure 2). Undercounting of cases was most pronounced early in the pandemic with the estimated number of infections in May 2020 being 9.4 times larger than the reported number of confirmed cases, compared to 3.6 times larger in January 2021. The higher undercounting of cases early in the pandemic may be due to a lack of and access to available testing and the absence of routine screening of DoD populations such as new accessions, which was implemented later in the pandemic. The continued discrepancy between reported case counts and seroprevalence estimates of case counts is most likely due to mild and asymptomatic cases.

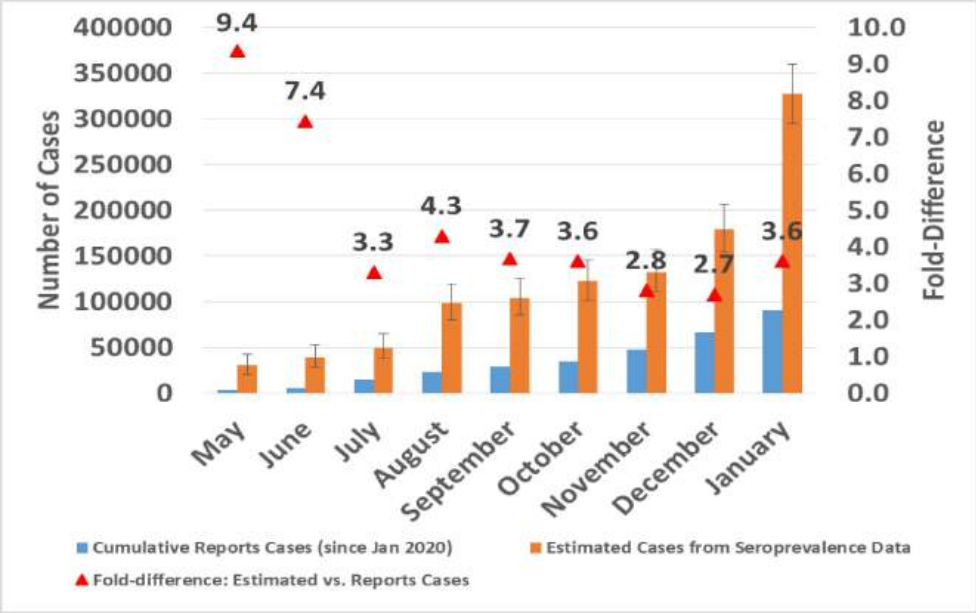
Additional details on rates by selected demographics and Geographic Combatant Command (GCC) and regions for the December 12, 2020—January 31, 2021 sample are shown in additional tables and figures in this report.

Figure 1. Monthly Seroprevalence of SARS-CoV-2 Antibody among Active Component Service Members from May 01, 2020 to January 31, 2021



Bars represent 95% confidence intervals for the seroprevalence estimates.

Figure 2. Seroprevalence Estimated Number of SARS-CoV-2 Infections Compared to the Cumulative Reported Number of Confirmed Cases by Month: Active Component Force, May 01, 2020 to January 31, 2021



1. Vertical lines represent 95% confidence intervals for the estimated number of cases.
2. Reported cases are cumulative from January 2020 to the month of interest.
3. Estimated cases are based on the seroprevalence estimate for each month multiplied by the total active force during that same month.
4. Fold-difference is calculated by dividing the estimated case count by reported case count.

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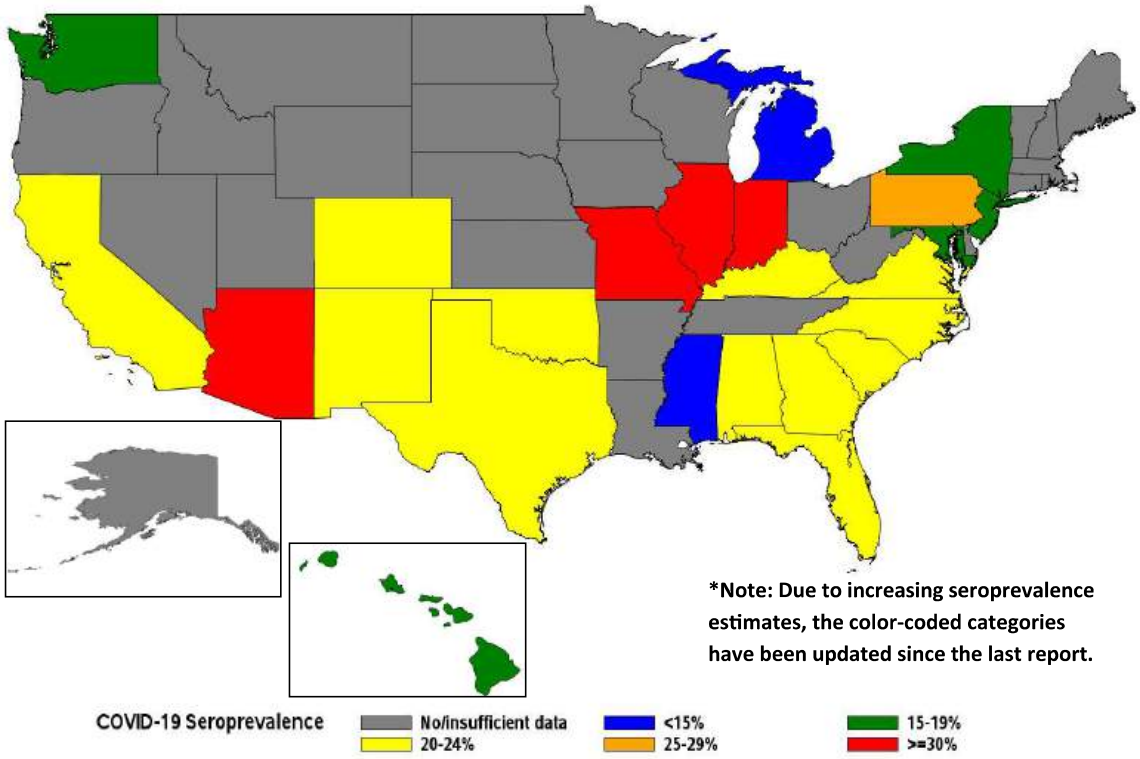
Figure 3. Seroprevalence of SARS-CoV-2 Antibody among U.S. Service Members Sampled between December 12, 2020—January 31, 2021 by State of Service Member's Unit



* No/Insufficient data indicates there were less than 30 specimens tested from the state.

For the December 12, 2020—January 31, 2021 sampling:

- Please note the categories for the color coding have been updated since the last report.
- The majority of states with available data had a SARS-CoV-2 seroprevalence of 20% or greater, with 4 of those states having a seroprevalence equal to or greater than 30%.
- All states with sufficient data were at or above 10% SARS-CoV-2 seroprevalence.

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Results from the December 12, 2020—January 31, 2021 Sampling

- SARS-CoV-2 antibody seroprevalence continued to be highest in NORTHCOM (23.09%) compared to INDOPACOM (13.61%).
- Seroprevalence estimates could not be calculated for EUCOM, CENTCOM, AFRICOM, and SOUTHCOM due to insufficient specimens collected in those GCCs.

Table 1. Seroprevalence of SARS-CoV-2 Antibody by Geographic Combatant Command (GCC): December 12, 2020—January 31, 2021

GCC	Seroprevalence Estimates (95% CI)
NORTHCOM	23.09 (21.52, 24.71)
EUCOM	-
INDOPACOM	13.61 (8.59, 20.22)
CENTCOM	-
AFRICOM	-
SOUTHCOM	-

- The Army had the highest seroprevalence (25.17%) compared to other services, however it was only statistically significantly higher than the Air Force.
- Reserve SM continued to have a higher seroprevalence than the active and guard component; however, these differences were not statistically significant.
- SARS-CoV-2 antibody seroprevalence was highest among Hispanic SM (27.46%) compared to the other race/ethnicity categories and was statistically significantly higher than White SM (20.80%) and other race/ethnicity SM (18.38%).

Table 2. Seroprevalence of SARS-CoV-2 Antibody by Service, Component and Race/Ethnicity: December 12, 2020—January 31, 2021

Variables	Seroprevalence Estimates (95% CI)
Service	
Army	25.17 (22.74, 27.74)
Air Force	17.45 (14.94, 20.20)
Marine Corps	23.51 (19.48, 27.92)
Navy	20.86 (17.79, 24.24)
Component	
Active	20.61 (18.83, 22.48)
Guard	24.92 (21.50, 28.59)
Reserve	23.71 (20.05, 27.69)
Race/Ethnicity	
White	20.80 (18.91, 22.80)
Black	23.19 (19.52, 27.19)
Hispanic	27.46 (23.62, 31.62)
Other	18.38 (14.53, 22.77)

- SARS-CoV-2 antibody seroprevalence was similar between males and females.
- No statistically significant differences in SARS-CoV-2 antibody seroprevalence were seen between age/sex categories.

Table 3. Seroprevalence of SARS-CoV-2 Antibody by Age and Sex: December 12, 2020—January 31, 2021

Variables	Seroprevalence Estimates (95% CI)	
Age Category	**Males**	**Females**
All	22.06 (20.43, 23.77)	21.58 (18.36, 25.13)
17-24	22.82 (20.21, 25.65)	24.89 (19.72, 30.73)
25-34	22.5 (19.86, 25.30)	19.63 (14.57, 25.44)
35-44	19.76 (16.07, 23.92)	17.65 (10.93, 26.26)
45-54	22.39 (15.82, 30.10)	25.00 (11.38, 44.46)
55+	11.11 (2.01, 32.61)	0.00 (0.00, 63.16)

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #5 28 Apr 2021

Study Methodology:

The Armed Forces Health Surveillance Division (AFHSD) utilized data from the Defense Medical Surveillance System (DMSS) and serum specimens from the DoDSR to conduct this study. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) conducted the SARS-CoV-2 serological testing of the DoDSR specimens.

Specimen Selection and Testing:

DMSS data were used to identify specimens in the DoDSR with a collection date on or after May 01, 2020 for each 6-8 week selection window. The previous reports have covered May 01, 2020 – December 11, 2020, while the current analysis includes samples from December 12, 2020—January 31, 2021. Approximately 2,000 specimens per month were randomly selected from specimens with a collection date during each window. The current selection period sampled 1,000 specimens for the last half of December and 2,000 specimens from January. After selection, specimens were aliquoted and delivered to USAMRIID for testing.

Samples were tested via a multiplexed, magnetic bead-based immunoassay optimized to identify SARS-CoV-2 IgG reactivity. The assay was developed at USAMRIID and run on the Luminex MAGPIX system. The Luminex assay utilizes 3 recombinant SARS-CoV-2 viral targets for detection including the full-length S-protein as well as the S1 and Receptor Binding Domain segments. Samples are considered positive when demonstrating sufficient reactivity to at least 2 of the 3 targets. A 95% MFI (mean fluorescence intensity) cutoff for all 3 targets was determined from assay validation using known positive and negative samples. For this study, positive samples underwent additional testing via a SARS-CoV-2 specific microneutralization assay to evaluate the sample for the presence of functional antibodies that block viral replication in cell culture, which may be indicative of a protective capacity of the antibody response. Due to the increasing number of positive specimens by the multiplex assay, only a 10% sample of the positives were tested by microneutralization assay for the December 12, 2020—January 31, 2021 sample.

Data Analysis:

Results from the Luminex assay were merged with the demographic and location data from DMSS for the analysis. The overall seroprevalence of SARS-CoV-2 antibody (number of SARS-CoV-2 antibody positive specimens divided by the number of specimens tested multiplied by 100) and 95% confidence intervals (95% CI) were calculated for each sampling group. Stratified seroprevalence estimates for demographic and location specific variables were also calculated. To evaluate the overall estimated number of SARS-CoV-2 infections among the total active component force, the seroprevalence estimate among the active component sample was multiplied by the total active component force population number at the middle point of the surveillance window. This estimate was divided by the total number of reported COVID-19 cases to estimate the fold increase between reported and estimated infections based on seroprevalence.

Limitations:

There are limitations to these data that should be considered when interpreting the results:

- The USAMRIID SARS-CoV-2 Luminex assay has 91% sensitivity and 98% specificity. Therefore, some serology results may be false positive results (the test result is positive, but the individual does not have SARS-CoV-2 antibodies) or false negative results (the test result is negative, but the individual does have SARS-CoV-2 antibodies).
- The sample size for this effort was calculated based on estimating the seroprevalence in the active force. Therefore, subgroup analyses of certain demographic categories or locations may be limited by insufficient numbers of individuals to calculate a precise estimate of the seroprevalence of SARS-CoV-2 antibodies in that group. The 95% CI will provide a measure of how precise the estimate is for that group. If the sample size for a specific group is too small, then seroprevalence data will not be provided since an accurate estimate cannot be determined.
- The fraction of infected individuals that develop detectable SARS-CoV-2 antibodies and the duration that those antibodies remain detectable has not been clearly defined to date. Antibody titers are known to decrease over time following infection, and because the duration of antibody detection is unknown, the above estimate of true to confirmed cases represents a lower limit of the actual ratio.[1] This effect is expected to become more pronounced over time, as the antibody response among those who were infected during the earlier months of the pandemic will fade over time. Additional studies on antibody persistence will be necessary to further refine these estimates.

References:

1. Mariën J, Ceulemans A, Michiels J, Heyndrickx L, Kerkhof K, Foque N, Widdowson M-Alain, Mortgat L, Duysburgh E, Desombere I, Jansens H, Van Esbroeck M, Ariën KK, Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay, Journal of Virological Methods (2020), doi: https://doi.org/10.1016/j.jviromet.2020.114025.

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #6 10 June 2021

Controlled by: Office of the Under Secretary of Defense for Personnel and Readiness
Controlled by: Office of the Assistant Secretary of Defense for Health Affairs
CUI Categories: Agricultural & Health Operations, Mission Assurance
Distribution/Dissemination Control: NONE
POC: LTC Kevin Taylor/kevin.m.taylor4.mil@mail.mil

Executive Summary

Four thousand specimens were randomly selected from the 202,788 specimens submitted to the DoD Serum Repository (DoDSR) with collection dates between February 1, 2021 and March 31, 2021 in order to estimate the prevalence of SARS-CoV-2 antibodies among Service members (SM). This prevalence gives a much more accurate estimation of total SARS-CoV-2 infections that have occurred among SM than confirmed case counts, due to incomplete testing among asymptomatic and minimally symptomatic SARS-CoV-2 infections.

Specimens were tested on a multiplex assay for SARS-CoV-2 IgG antibodies. **The overall seroprevalence rate among these samples was 40.01% (95% Confidence Interval (CI): 38.53, 41.88); this was a 82% higher seroprevalence rate than for the mid-December 2020 through January 2021 specimens (21.97%).** The active component seroprevalence rate was 38.68% (95% CI: 36.83, 40.56), which continued to be lower, but not statistically significantly different than the reserve/guard component rate of 42.74% (95% CI: 40.08, 45.43). **The notable rise in seroprevalence in February through March was driven largely by vaccinations rather than new SARS-CoV-2 infections.**

The reported number of confirmed COVID-19 cases increased from 99,903 (7.46%) on February 01, 2021 to 114,947 (8.60%) as of March 31, 2021 among the active component force with a rate at the midpoint of the sampling interval of 8.16% (109,134 cases amongst 1,337,895 SM). In order to compare reported numbers to SARS-CoV-2 infection driven seroprevalence, individuals who had received at least one dose of a COVID-19 vaccine prior to the serum collection date were excluded from the comparison analysis. **Applying the active component seroprevalence rate of non-vaccinated individuals (24.20%) to the total active component force resulted in an estimated 323,771 (95% CI: 299,391, 349,024) SARS-CoV-2 infections among active component SM; an estimated 3.0-fold higher "true" case count compared to the reported number of confirmed cases.** Because the duration of antibody detection is unknown, this estimate of true to confirmed cases likely represents a lower limit of the actual ratio.[1] **An estimated 38.80% (95% CI: 37.29, 40.33) of the sample population also demonstrated neutralizing antibody reactivity.** This was based on a 10% sample of the multiplex positive specimens which underwent additional testing by microneutralization assay inhibition in vitro. Of the 161 multiplex positive specimens tested, 156 (96.9%) were positive on the microneutralization assay, which was extrapolated to the full sample size to give an estimated 1552 positive for neutralizing antibodies out of 4,000 specimens. Evidence of neutralization activity is more highly correlated with short-term immunity than other antibody tests.

The SARS-CoV-2 seroprevalence data from May 2020 to March 2021 among the active component SM showed an increase from 2.3% to 24.2% for non-vaccinated service members. Seroprevalence among all active component SM (vaccinated and unvaccinated) was 43.4% in March 2021 (Figure 1). Applying these seroprevalence rates to the monthly total active component force provided a similar trend in total estimated cases and showed a continued undercounting of confirmed cases (Figure 2). Undercounting of cases was most pronounced early in the pandemic with the estimated number of infections in May 2020 being 9.4 times larger than the reported number of confirmed cases, compared to 3.0 times larger in March 2021. The higher undercounting of cases early in the pandemic may be due to a lack of and access to available testing and the absence of routine screening of DoD populations such as new accessions, which was implemented later in the pandemic. The continued discrepancy between reported case counts and seroprevalence estimates of case counts is most likely due to mild and asymptomatic cases.

Additional details on rates by selected demographics and Geographic Combatant Command (GCC) and regions for the February 1, 2021—March 31, 2021 sample are shown in additional tables and figures in this report.

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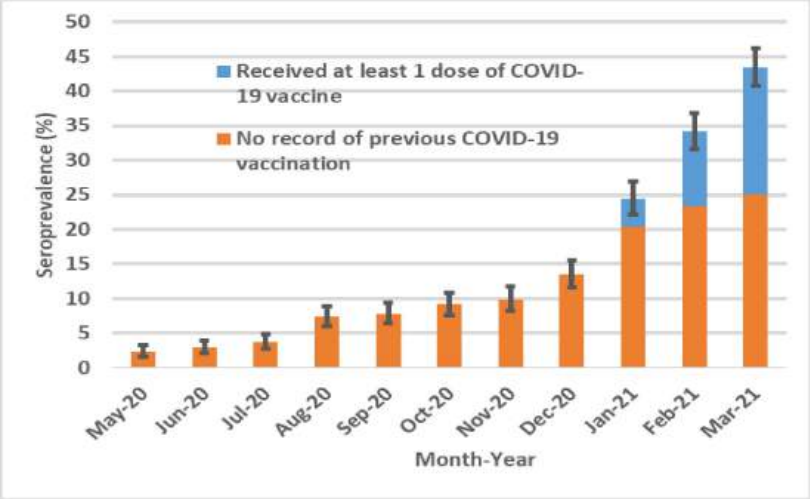
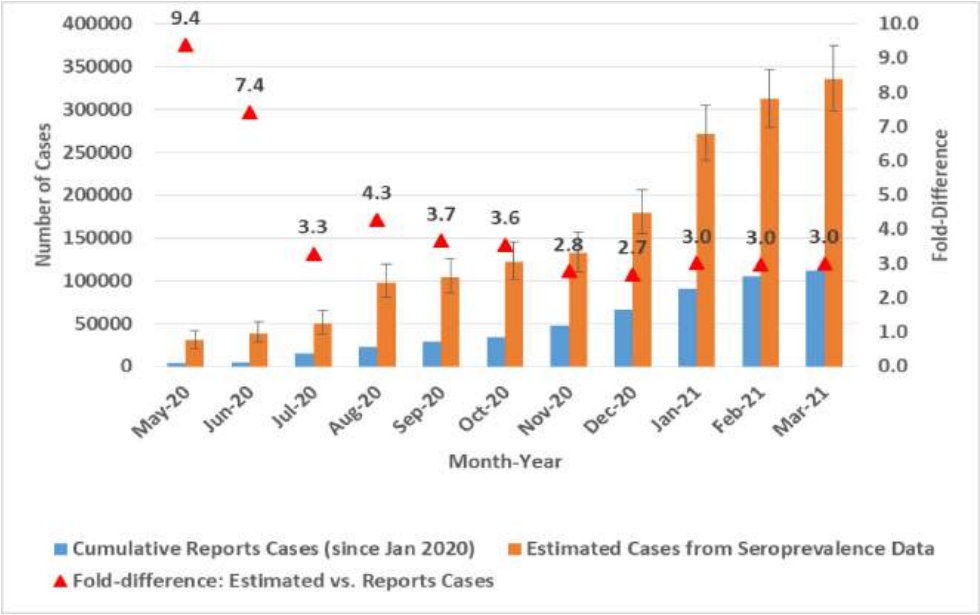
Figure 1. Monthly Seroprevalence of SARS-CoV-2 Antibody among Active Component Service Members from May 01, 2020 to March 31, 2021



Bars represent 95% confidence intervals for the overall seroprevalence estimate.

Figure 2. Seroprevalence Estimated Number of SARS-CoV-2 Infections Compared to the Cumulative Reported Number of Confirmed Cases by Month: Active Component Force, May 01, 2020 to March 31, 2021



1. Vertical lines represent 95% confidence intervals for the estimated number of cases.
2. Reported cases are cumulative from January 2020 to the month of interest.
3. Estimated cases are based on the seroprevalence estimate for each month (Feb /Mar restricted to non-vaccinated individuals) multiplied by the total active force during that same month.
4. Fold-difference is calculated by dividing the estimated case count by reported case count.

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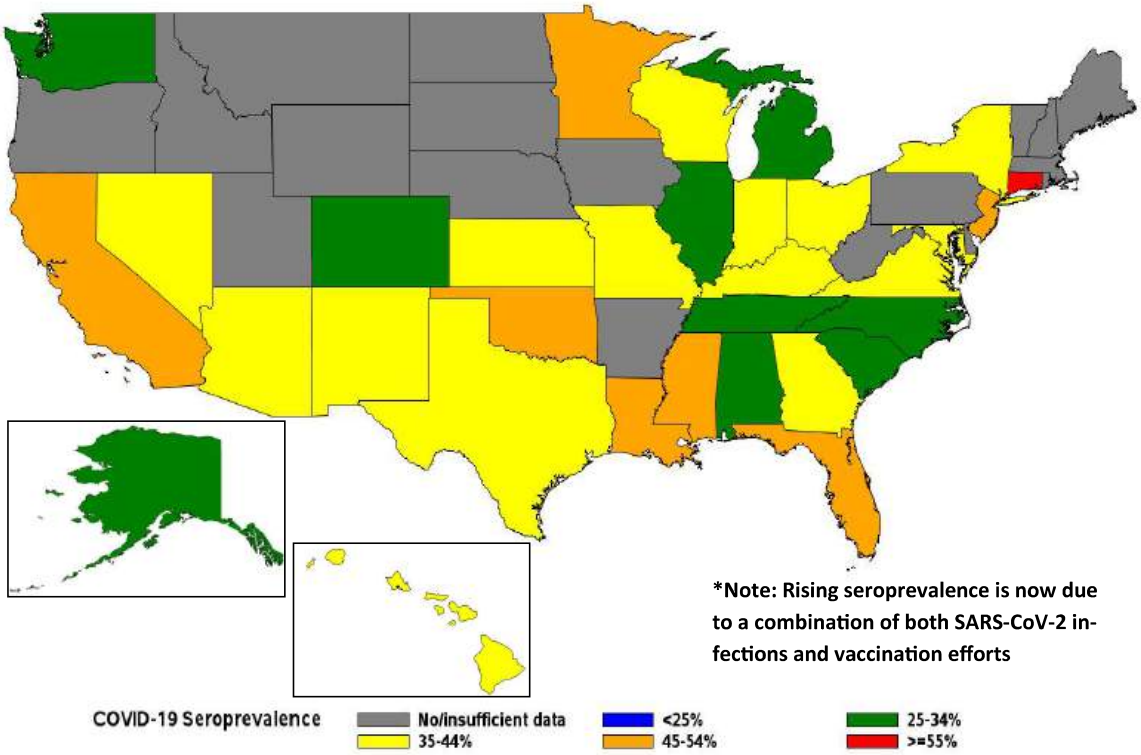
Figure 3. Overall Seroprevalence of SARS-CoV-2 Antibody among U.S. Service Members Sampled between February 1, 2021—March 31, 2021 by State of Service Member's Unit



* No/Insufficient data indicates there were less than 30 specimens tested from the state.

For the February 1, 2021—March 31, 2021 sampling:

- Please note the categories for the color coding have been updated again since the last report.
- The majority of states with available data had a SARS-CoV-2 seroprevalence of 35-44%. One state, Connecticut, had a seroprevalence of ≥55%.
- All states with sufficient data were at or above 25% SARS-CoV-2 seroprevalence.

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Results from the February 1, 2021—March 31, 2021 Sampling

- SARS-CoV-2 antibody seroprevalence was highest in INDOPACOM (44%), but was not statistically significantly higher than NORTHCOM (40%) or EUCOM (34%).
- Seroprevalence estimates could not be calculated for CENTCOM, AFRICOM, and SOUTHCOM due to insufficient specimens collected in those GCCs.
- Among non-COVID-19-vaccinated individuals, the NORTHCOM SARS-CoV-2 antibody seroprevalence was 30% (29, 32). The other GCCs had insufficient specimens for a non-vaccinated seroprevalence estimate.

Table 1. Overall Seroprevalence of SARS-CoV-2 Antibody by Geographic Combatant Command (GCC): February 1, 2021—March 31, 2021

GCC	Overall Seroprevalence Estimates (95% CI)
NORTHCOM	39.87 (38.29, 41.47)
EUCOM	33.96 (22.23, 48.08)
INDOPACOM	43.96 (36.75, 51.38)
CENTCOM	-
AFRICOM	-
SOUTHCOM	-

- The Navy had the highest overall seroprevalence (44%), however it was only statistically significantly higher than the Air Force. When restricted to non-COVID-19-vaccinated subjects, the Army had the highest seroprevalence (34%), possibly indicating a higher rate of infection among Army compared to other services.
- Guard SM had a statistically significant higher overall seroprevalence (47%) than both the active (39%) and reserve (38%) component. This was true as well when restricting to non-COVID-19-vaccinated subjects.
- Overall SARS-CoV-2 antibody seroprevalence continued to be highest among Hispanic SM (44%) compared to the other race/ethnicity categories and was statistically significantly higher than Black SM (35%).

Table 2. Seroprevalence of SARS-CoV-2 Antibody by Service, Component and Race/Ethnicity: February 1, 2021—March 31, 2021

Variables	Overall Seroprevalence Estimates (95% CI)	Seroprevalence Estimates among Non-COVID-19 Vaccinated Subjects (95% CI)
Service		
Army	40.26 (38.04, 42.52)	33.89 (31.60, 36.22)
Air Force	34.85 (31.70, 38.07)	22.72 (19.71, 26.02)
Marine Corps	41.88 (37.55, 46.29)	27.46 (23.12, 32.08)
Navy	44.33 (40.80, 47.91)	26.09 (22.51, 29.95)
Component		
Active	38.68 (36.83, 40.56)	24.20 (22.38, 26.09)
Guard	46.67 (43.06, 50.27)	43.39 (39.66, 47.17)
Reserve	37.83 (33.97, 41.82)	31.95 (28.06, 36.07)
Race/Ethnicity		
White	40.09 (38.05, 42.18)	28.51 (26.42, 30.69)
Black	35.08 (31.25, 39.02)	28.19 (24.37, 32.21)
Hispanic	43.57 (39.87, 47.34)	33.57 (29.68, 37.59)
Other	40.91 (36.80, 45.08)	29.28 (25.18, 33.75)

- Overall SARS-CoV-2 antibody seroprevalence was similar between males and females.
- No statistically significant differences in overall SARS-CoV-2 antibody seroprevalence were seen between age/sex categories.
- Similar patterns were also seen when restricting to the non-COVID-19-vaccinated subjects.

Table 3. Seroprevalence of SARS-CoV-2 Antibody by Age and Sex: February 1, 2021—March 31, 2021

Variables	Overall Seroprevalence Estimates (95% CI)	
Age Category	**Males**	**Females**
All	40.17 (38.47, 41.88)	39.59 (36.14, 43.07)
17-24	37.00 (34.32, 39.74)	38.25 (32.65, 44.02)
25-34	40.86 (38.08, 43.70)	35.24 (30.10, 40.77)
35-44	41.48 (37.60, 45.49)	47.83 (39.42, 56.20)
45-54	51.63 (43.49, 59.53)	57.58 (40.12, 73.25)
55+	54.05 (37.55, 69.36)	50.00 (2.53, 97.47)

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Study Methodology:

The Armed Forces Health Surveillance Division (AFHSD) utilized data from the Defense Medical Surveillance System (DMSS) and serum specimens from the DoDSR to conduct this study. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) conducted the SARS-CoV-2 serological testing of the DoDSR specimens.

Specimen Selection and Testing:

DMSS data were used to identify specimens in the DoDSR with a collection date on or after May 01, 2020 for each 6-8 week selection window. The previous reports have covered May 01, 2020 – January 31, 2021, while the current analysis includes samples from February 1, 2021—March 31, 2021. Approximately 2,000 specimens per month were randomly selected from specimens with a collection date during each window. The current selection period sampled 2,000 specimens from February and 2,000 specimens from March. After selection, specimens were aliquoted and delivered to USAMRIID for testing.

Samples were tested via a multiplexed, magnetic bead-based immunoassay optimized to identify SARS-CoV-2 IgG reactivity. The assay was developed at USAMRIID and run on the Luminex MAGPIX system. The Luminex assay utilizes 3 recombinant SARS-CoV-2 viral targets for detection including the full-length S-protein as well as the S1 and Receptor Binding Domain segments. Samples are considered positive when demonstrating sufficient reactivity to at least 2 of the 3 targets. A 95% MFI (mean fluorescence intensity) cutoff for all 3 targets was determined from assay validation using known positive and negative samples. For this study, positive samples underwent additional testing via a SARS-CoV-2 specific microneutralization assay to evaluate the sample for the presence of functional antibodies that block viral replication in cell culture, which may be indicative of a protective capacity of the antibody response. Due to the increasing number of positive specimens by the multiplex assay, only a 10% sample of the positives were tested by microneutralization assay for the February 1, 2021—March 31, 2021 sample.

Data Analysis:

Results from the Luminex assay were merged with the demographic and location data from DMSS for the analysis. The overall seroprevalence of SARS-CoV-2 antibody (number of SARS-CoV-2 antibody positive specimens divided by the number of specimens tested multiplied by 100) and 95% confidence intervals (95% CI) were calculated for each sampling group. Stratified seroprevalence estimates for demographic and location specific variables were also calculated. To evaluate the overall estimated number of SARS-CoV-2 infections among the total active component force, the seroprevalence estimate among the active component sample was multiplied by the total active component force population number at the middle point of the surveillance window. This estimate was divided by the total number of reported COVID-19 cases to estimate the fold increase between reported and estimated infections based on seroprevalence.

Limitations:

There are limitations to these data that should be considered when interpreting the results:

- The USAMRIID SARS-CoV-2 Luminex assay has 91% sensitivity and 98% specificity. Therefore, some serology results may be false positive results (the test result is positive, but the individual does not have SARS-CoV-2 antibodies) or false negative results (the test result is negative, but the individual does have SARS-CoV-2 antibodies).
- The sample size for this effort was calculated based on estimating the seroprevalence in the active force. Therefore, subgroup analyses of certain demographic categories or locations may be limited by insufficient numbers of individuals to calculate a precise estimate of the seroprevalence of SARS-CoV-2 antibodies in that group. The 95% CI will provide a measure of how precise the estimate is for that group. If the sample size for a specific group is too small, then seroprevalence data will not be provided since an accurate estimate cannot be determined.
- The fraction of infected individuals that develop detectable SARS-CoV-2 antibodies and the duration that those antibodies remain detectable has not been clearly defined to date. Antibody titers are known to decrease over time following infection, and because the duration of antibody detection is unknown, the above estimate of true to confirmed cases represents a lower limit of the actual ratio.[1] This effect is expected to become more pronounced over time, as the antibody response among those who were infected during the earlier months of the pandemic will fade over time. Additional studies on antibody persistence will be necessary to further refine these estimates.
- While the Luminex assay is able to detect antibodies specific to SARS-CoV-2 infection that would not be present due to vaccination alone, the analysis of seroprevalence in vaccinated vs. unvaccinated individuals was based on vaccination data acquired from the electronic health record of study subjects.

References:

1. Mariën J, Ceulemans A, Michiels J, Heyndrickx L, Kerkhof K, Foque N, Widdowson M-Alain, Mortgat L, Duysburgh E, Desombere I, Jansens H, Van Esbroeck M, Ariën KK, Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay, Journal of Virological Methods (2020), doi: https://doi.org/10.1016/j.jviromet.2020.114025.

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #7 21 July 2021

Controlled by: Office of the Under Secretary of Defense for Personnel and Readiness
Controlled by: Office of the Assistant Secretary of Defense for Health Affairs
CUI Categories: Agricultural & Health Operations, Mission Assurance
Distribution/Dissemination Control: NONE
POC: LTC Kevin Taylor/kevin.m.taylor4.mil@mail.mil

Executive Summary

Two thousand specimens were randomly selected from the 113,301 specimens submitted to the DoD Serum Repository (DoDSR) with collection dates between April 1, 2021 and April 30, 2021 in order to estimate the prevalence of SARS-CoV-2 antibodies among Service members (SM). This prevalence gives a much more accurate estimation of total SARS-CoV-2 infections that have occurred among SM than confirmed case counts, due to incomplete testing among asymptomatic and minimally symptomatic SARS-CoV-2 infections.

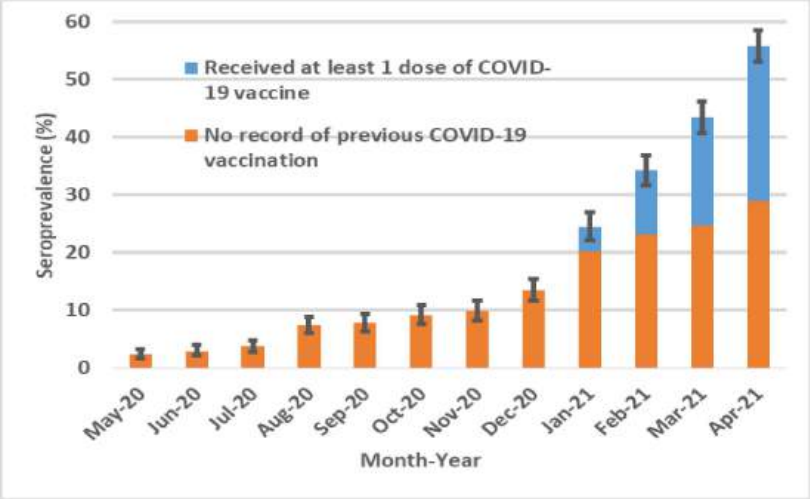
Specimens were tested on a multiplex assay for SARS-CoV-2 IgG antibodies. **The overall seroprevalence rate among these samples was 55.95% (95% Confidence Interval (CI): 53.75, 58.13); this was a 40% higher seroprevalence rate than for the February-March 2021 specimens (40.01%).** The active component seroprevalence rate was 55.92% (95% CI: 53.17, 58.63), which was not statistically significantly different than the reserve/guard component rate of 56.01% (95% CI: 52.26, 59.71). **The continued rise in seroprevalence in April was driven largely by vaccinations rather than new SARS-CoV-2 infections.**

The reported number of confirmed COVID-19 cases increased from 115,136 (8.61%) on April 01, 2021 to 121,515 (9.08%) as of April 30, 2021 among the active component force with a rate at the midpoint of the sampling interval of 8.85% (118,431 cases amongst 1,337,895 SM). In order to compare reported numbers to SARS-CoV-2 infection driven seroprevalence, individuals who had received at least one dose of a COVID-19 vaccine prior to the serum collection date were excluded from the comparison analysis. **Applying the active component seroprevalence rate of unvaccinated individuals (28.99%) to the total active component force resulted in an estimated 387,856 (95% CI: 345,082, 432,466) infections among active component SM; an estimated 3.3-fold higher "true" case count compared to the reported number of confirmed cases.** Because the duration of antibody detection is unknown, this estimate of true to confirmed cases likely represents a lower limit of the actual ratio.[1] **An estimated 52.95% (95% CI: 50.73, 55.16) of the sample population also demonstrated neutralizing antibody reactivity.** This was based on a 10% sample of the multiplex positive specimens which underwent additional testing by microneutralization assay. Of the 112 multiplex positive specimens tested, 106 (94.64%) were positive on the microneutralization assay, which was extrapolated to the full sample size to give an estimated 1,059 positive for neutralizing antibodies out of 2,000 specimens. Evidence of neutralization activity is more highly correlated with short-term immunity than other antibody tests.

The SARS-CoV-2 seroprevalence data from May 2020 to April 2021 among the active component SM showed an increase from 2.3% to 29.0% for unvaccinated SM. Seroprevalence among all active component SM (vaccinated and unvaccinated) was 55.92% in April 2021 (Figure 1). Applying these seroprevalence rates to the monthly total active component force provided a similar trend in total estimated cases and showed a continued undercounting of confirmed cases (Figure 2). Undercounting of cases was most pronounced early in the pandemic with the estimated number of infections in May 2020 being 9.4 times larger than the reported number of confirmed cases, compared to 3.3 times larger in April 2021. The higher undercounting of cases early in the pandemic may be due to a lack of and access to available testing and the absence of routine screening of DoD populations such as new accessions, which was implemented later in the pandemic. The continued discrepancy between reported case counts and seroprevalence estimates of case counts is most likely due to mild and asymptomatic infections in individuals that did not report for medical care and/or testing.

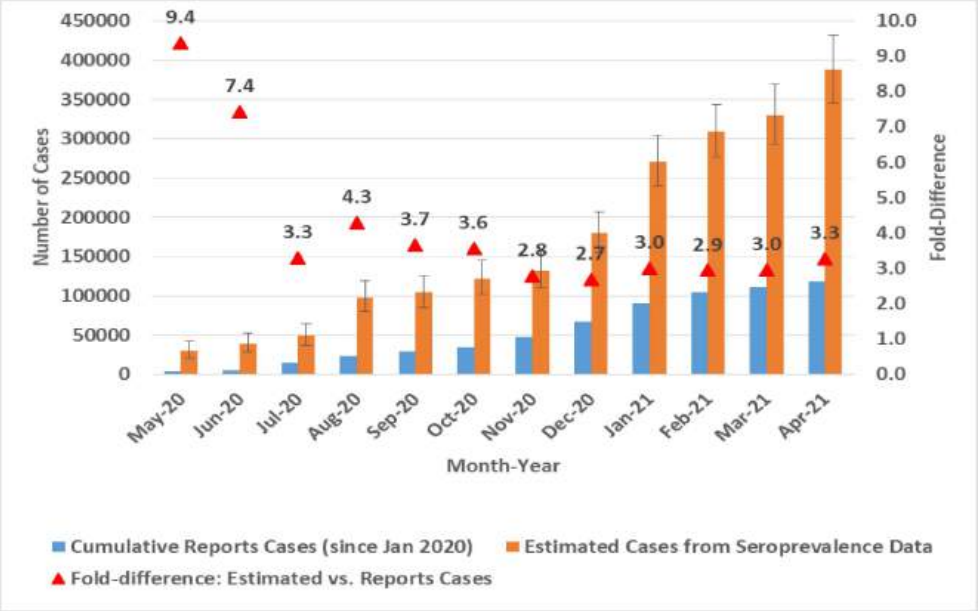
Additional details on rates by selected demographics and Geographic Combatant Command (GCC) and regions for the April 1, 2021—April 30, 2021 sample are shown in additional tables and figures in this report.

Figure 1. Monthly Seroprevalence of SARS-CoV-2 Antibody among Active Component Service Members from May 01, 2020 to April 30, 2021



Bars represent 95% confidence intervals for the overall seroprevalence estimate.

Figure 2. Seroprevalence Estimated Number of SARS-CoV-2 Infections Compared to the Cumulative Reported Number of Confirmed Cases by Month: Active Component Force, May 01, 2020 to April 30, 2021



1. Vertical lines represent 95% confidence intervals for the estimated number of cases.
2. Reported cases are cumulative from January 2020 to the month of interest.
3. Estimated cases are based on the seroprevalence estimate among unvaccinated individuals for each month multiplied by the total active force during that same month.
4. Fold-difference is calculated by dividing the estimated case count by reported case count.

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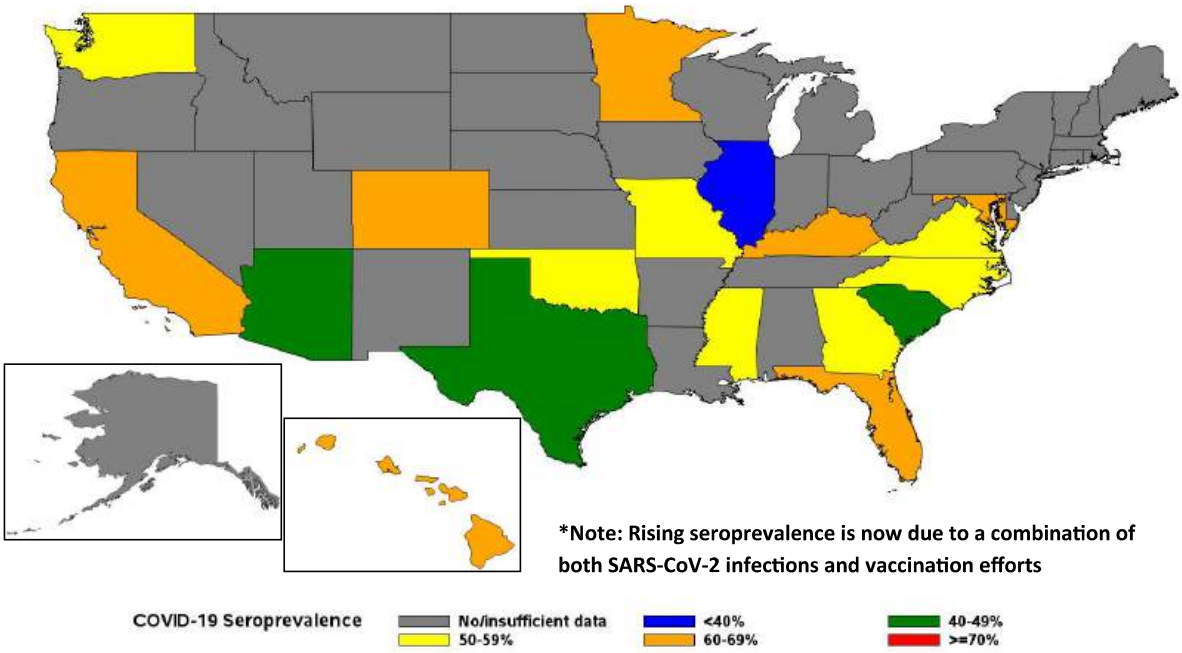
Figure 3. Overall Seroprevalence of SARS-CoV-2 Antibody among U.S. Service Members Sampled between April 1, 2021—April 30, 2021 by State of Service Member's Unit



* No/Insufficient data indicates there were less than 25 specimens tested from the state.

For the April 1, 2021—April 31, 2021 sampling:

- Please note the categories for the color coding have been updated again since the last report.
- The majority of states with available data had a SARS-CoV-2 seroprevalence of 50-69%.
- All states with sufficient data were at or above 35% SARS-CoV-2 seroprevalence.

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Results from the April 1, 2021—April 30, 2021 Sampling

- SARS-CoV-2 antibody seroprevalence was highest in EUCOM (57%), but was not statistically significantly higher than NORTHCOM (55%) or INDOPACOM (56%).
- Seroprevalence estimates could not be calculated for CENTCOM, AFRICOM, and SOUTHCOM due to insufficient specimens collected in those GCCs.
- Among unvaccinated individuals, the NORTHCOM SARS-CoV-2 antibody seroprevalence was 36% (33, 39). The other GCCs had insufficient specimens for an unvaccinated seroprevalence estimate.

Table 1. Overall Seroprevalence of SARS-CoV-2 Antibody by Geographic Combatant Command (GCC): April 1, 2021—April 30, 2021

GCC	Overall Seroprevalence Estimates (95% CI)
NORTHCOM	55.17 (52.82, 57.49)
EUCOM	57.14 (39.74, 73.48)
INDOPACOM	55.73 (46.92, 64.22)
CENTCOM	-
AFRICOM	-
SOUTHCOM	-

- The Navy continued to have the highest overall seroprevalence (64%), however it was only statistically significantly higher than the Army. When restricted to unvaccinated subjects, the Army had the highest seroprevalence (38%), but it was not statistically significantly different than the other services.
- Guard SM continued to have the highest seroprevalence overall (60%) and among unvaccinated subjects (47%), however it was only statistically significantly higher than the other components for the unvaccinated subject analysis.
- Overall SARS-CoV-2 antibody seroprevalence continued to be highest among Hispanic SM (61%), however this difference was not statistically significant in comparison to White and Black SM and could not be attributed to higher vaccination rates in Hispanics.

Table 2. Seroprevalence of SARS-CoV-2 Antibody by Service, Component and Race/Ethnicity: April 1, 2021—April 30, 2021

Variables	Overall Seroprevalence Estimates (95% CI)	Seroprevalence Estimates among Unvaccinated Subjects (95% CI)
Service		
Army	53.06 (49.78, 56.28)	38.06 (34.38, 41.85)
Air Force	54.73 (50.11, 59.36)	30.04 (24.83, 35.85)
Marine Corps	55.34 (48.29, 62.19)	28.13 (20.89, 36.62)
Navy	63.83 (59.12, 68.36)	32.34 (26.01, 39.25)
Component		
Active	55.92 (53.17, 58.63)	28.99 (25.79, 32.32)
Guard	60.43 (55.3, 65.35)	46.56 (40.42, 52.68)
Reserve	51.18 (45.85, 56.53)	38.25 (32.28, 44.4)
Race/Ethnicity		
White	57.87 (54.79, 60.91)	34.51 (30.79, 38.35)
Black	51.53 (45.75, 57.31)	34.45 (28.13, 41.11)
Hispanic	61.49 (55.84, 66.88)	38.33 (31.29, 45.81)
Other	49.6 (44.49, 54.7)	31.54 (26.07, 37.46)

- Overall SARS-CoV-2 antibody seroprevalence was similar between males and females.
- Overall seroprevalence among males aged 35-44 and 45-54 were statistically significantly higher than males aged 17-24 and 25-34. However, this was not found in the unvaccinated subject analysis (data not shown), indicating vaccination is driving this difference as opposed to infections.

Table 3. Seroprevalence of SARS-CoV-2 Antibody by Age and Sex: April 1, 2021—April 30, 2021

Variables	Overall Seroprevalence Estimates (95% CI)	
Age Category	**Males**	**Females**
All	55.38 (52.91, 57.83)	58.69 (53.79, 63.51)
17-24	47.64 (43.47, 51.88)	47.77 (39.78, 55.77)
25-34	54.33 (50.39, 58.2)	63.98 (56.25, 71.24)
35-44	63.61 (58.06, 68.91)	68.25 (55.63, 78.96)
45-54	80.21 (71.03, 87.3)	69.23 (41.34, 88.73)
55+	60 (28.29, 85)	100 (36.84, 100)

Department of Defense SARS-CoV-2 Seroprevalence Study – Interim Report #7 21 July 2021

Study Methodology:

The Armed Forces Health Surveillance Division (AFHSD) utilized data from the Defense Medical Surveillance System (DMSS) and serum specimens from the DoDSR to conduct this study. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) conducted the SARS-CoV-2 serological testing of the DoDSR specimens.

Specimen Selection and Testing:

DMSS data were used to identify specimens in the DoDSR with a collection date on or after May 01, 2020 for each 6-8 week selection window. The previous reports have covered May 01, 2020 – March 31, 2021, while the current analysis includes a total of 2,000 samples from April 1, 2021—April 30, 2021. After selection, specimens were aliquoted and delivered to USAMRIID for testing.

Samples were tested via a multiplexed, magnetic bead-based immunoassay optimized to identify SARS-CoV-2 IgG reactivity. The assay was developed at USAMRIID and run on the Luminex MAGPIX system. The Luminex assay utilizes 3 recombinant SARS-CoV-2 viral targets for detection including the full-length S-protein as well as the S1 and Receptor Binding Domain segments. Samples are considered positive when demonstrating sufficient reactivity to at least 2 of the 3 targets. A 95% MFI (mean fluorescence intensity) cutoff for all 3 targets was determined from assay validation using known positive and negative samples. For this study, positive samples underwent additional testing via a SARS-CoV-2 specific microneutralization assay to evaluate the sample for the presence of functional antibodies that block viral replication in cell culture, which may be indicative of a protective capacity of the antibody response. Due to the increasing number of positive specimens by the multiplex assay, only a 10% sample of the positives were tested by microneutralization assay for the April 1, 2021—April 30, 2021 sample.

Data Analysis:

Results from the Luminex assay were merged with the demographic and location data from DMSS for the analysis. The overall seroprevalence of SARS-CoV-2 antibody (number of SARS-CoV-2 antibody positive specimens divided by the number of specimens tested multiplied by 100) and 95% confidence intervals (95% CI) were calculated for each sampling group. Stratified seroprevalence estimates for demographic and location specific variables were also calculated. To evaluate the overall estimated number of SARS-CoV-2 infections among the total active component force, the seroprevalence estimate among the active component sample was multiplied by the total active component force population number at the middle point of the surveillance window. This estimate was divided by the total number of reported COVID-19 cases to estimate the fold increase between reported and estimated infections based on seroprevalence.

Limitations:

There are limitations to these data that should be considered when interpreting the results:

- The USAMRIID SARS-CoV-2 Luminex assay has 91% sensitivity and 98% specificity. Therefore, some serology results may be false positive results (the test result is positive, but the individual does not have SARS-CoV-2 antibodies) or false negative results (the test result is negative, but the individual does have SARS-CoV-2 antibodies).
- The sample size for this effort was calculated based on estimating the seroprevalence in the active force. Therefore, subgroup analyses of certain demographic categories or locations may be limited by insufficient numbers of individuals to calculate a precise estimate of the seroprevalence of SARS-CoV-2 antibodies in that group. The 95% CI will provide a measure of how precise the estimate is for that group. If the sample size for a specific group is too small, then seroprevalence data will not be provided since an accurate estimate cannot be determined.
- The fraction of infected individuals that develop detectable SARS-CoV-2 antibodies and the duration that those antibodies remain detectable has not been clearly defined to date. Antibody titers are known to decrease over time following infection, and because the duration of antibody detection is unknown, the above estimate of true to confirmed cases represents a lower limit of the actual ratio.[1] This effect is expected to become more pronounced over time, as the antibody response among those who were infected during the earlier months of the pandemic will fade over time. Additional studies on antibody persistence will be necessary to further refine these estimates.
- While the Luminex assay is able to detect antibodies specific to SARS-CoV-2 infection that would not be present due to vaccination alone, the analysis of seroprevalence in vaccinated vs. unvaccinated individuals was based on vaccination data acquired from the electronic health record of study subjects.

References:

1. Mariën J, Ceulemans A, Michiels J, Heyndrickx L, Kerkhof K, Foque N, Widdowson M-Alain, Mortgat L, Duysburgh E, Desombere I, Jansens H, Van Esbroeck M, Ariën KK, Evaluating SARS-CoV-2 spike and nucleocapsid proteins as targets for antibody detection in severe and mild COVID-19 cases using a Luminex bead-based assay, Journal of Virological Methods (2020), doi: https://doi.org/10.1016/j.jviromet.2020.114025.

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Executive Summary

Five thousand specimens were randomly selected from the 214,239 specimens submitted to the DoD Serum Repository (DoDSR) with collection dates between May 1, 2021 and July 15, 2021 in order to estimate the prevalence of SARS-CoV-2 antibodies among Service members (SM). This prevalence gives a much more accurate estimation of total SARS-CoV-2 infections that have occurred among SM than confirmed case counts, due to incomplete testing among asymptomatic and minimally symptomatic SARS-CoV-2 infections.

Specimens were tested on a multiplex assay for SARS-CoV-2 IgG antibodies. **The overall average seroprevalence rate among these samples (from May 1-July 15, 2021) was 71.38% (95% Confidence Interval (CI): 70.11, 72.63); this was a 28% relative increase in the seroprevalence rate since April 2021 (55.90%).** The active component seroprevalence rate was 72.65% (95% CI: 71.12, 74.12), which was statistically significantly higher than the reserve/guard component rate of 68.62% (95% CI: 66.62, 70.91). **Analysis of the most recent specimens obtained in this study from active component SM between July 1 and July 15, 2021 demonstrated a seroprevalence of 77.00% (95% CI: 74.26, 79.58). The continued rise in seroprevalence during the May 1 – July 15, 2021 timeframe was largely due to vaccinations, as this timeframes saw the lowest rates of new SARS-CoV-2 infections within DoD since the onset of the pandemic (Figure 1).**

An estimated 70.98% (95% CI: 69.70, 72.24) of the sample population also demonstrated neutralizing antibody reactivity. This was based on a 4% sample of the multiplex positive specimens which underwent additional testing by microneutralization assay. Of the 177 multiplex positive specimens tested, 176 (99.43%) were positive on the microneutralization assay, which was extrapolated to the full sample size to give an estimated 3,549 positive for neutralizing antibodies out of 5,000 specimens. Evidence of neutralization activity is more highly correlated with short-term immunity than other antibody tests.

The reported number of confirmed COVID-19 cases increased from 119,460 (8.85%) on May 01, 2021 to 128,919 (9.52%) as of July 15, 2021 among the active component force with a rate at the midpoint of the sampling interval of 9.13% (123,572 cases amongst 1,353,644 SM). **Applying the active component seroprevalence rate due to infection alone (16.33%) to the total active component force resulted in an estimated 221,050 (95% CI: 204,543, 238,319) infections among active component SM; an estimated 1.8-fold higher "true" case count compared to the reported number of confirmed cases.** Because the duration of antibody detection is unknown, this estimate of true to confirmed cases likely represents a lower limit of the actual ratio.[1] Applying these seroprevalence rates to the monthly total active component force provided a similar trend in total estimated cases and showed a continued undercounting of confirmed cases (Figure 2). Undercounting of cases was most pronounced early in the pandemic with the estimated number of infections in May 2020 being 9.4 times larger than the reported number of confirmed cases, compared to 1.7 times larger in July 2021. The higher undercounting of cases early in the pandemic may be due to a lack of and access to available testing and the absence of routine screening of DoD populations such as new accessions, which was implemented later in the pandemic.

Additional details on rates by selected demographics and Geographic Combatant Command (GCC) and regions for the May 1, 2021—July 15, 2021 sample are shown in additional tables and figures in this report.

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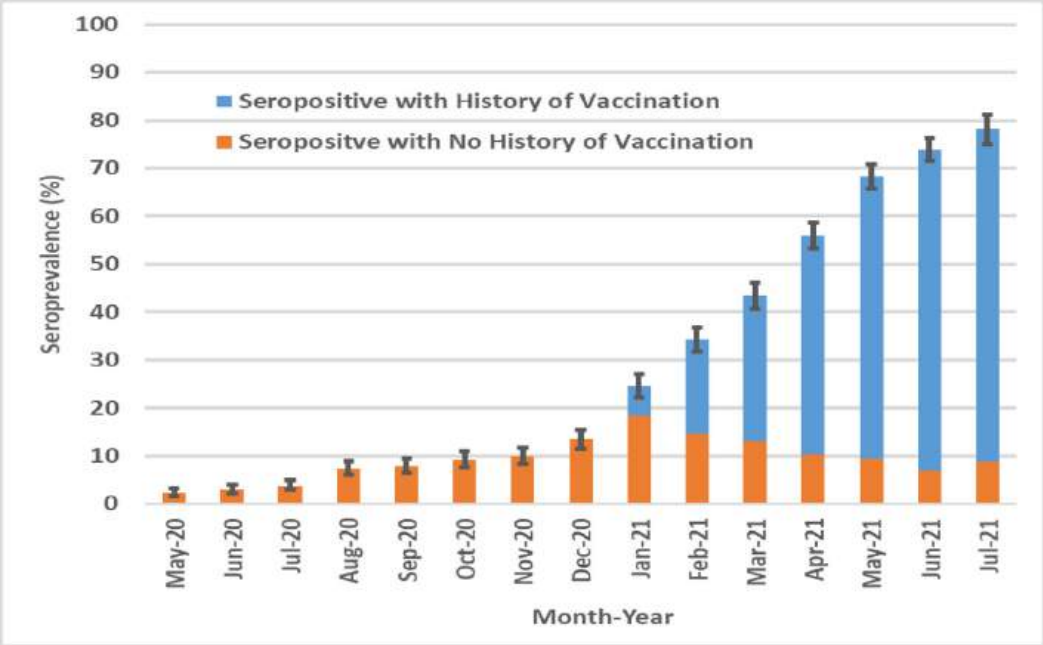
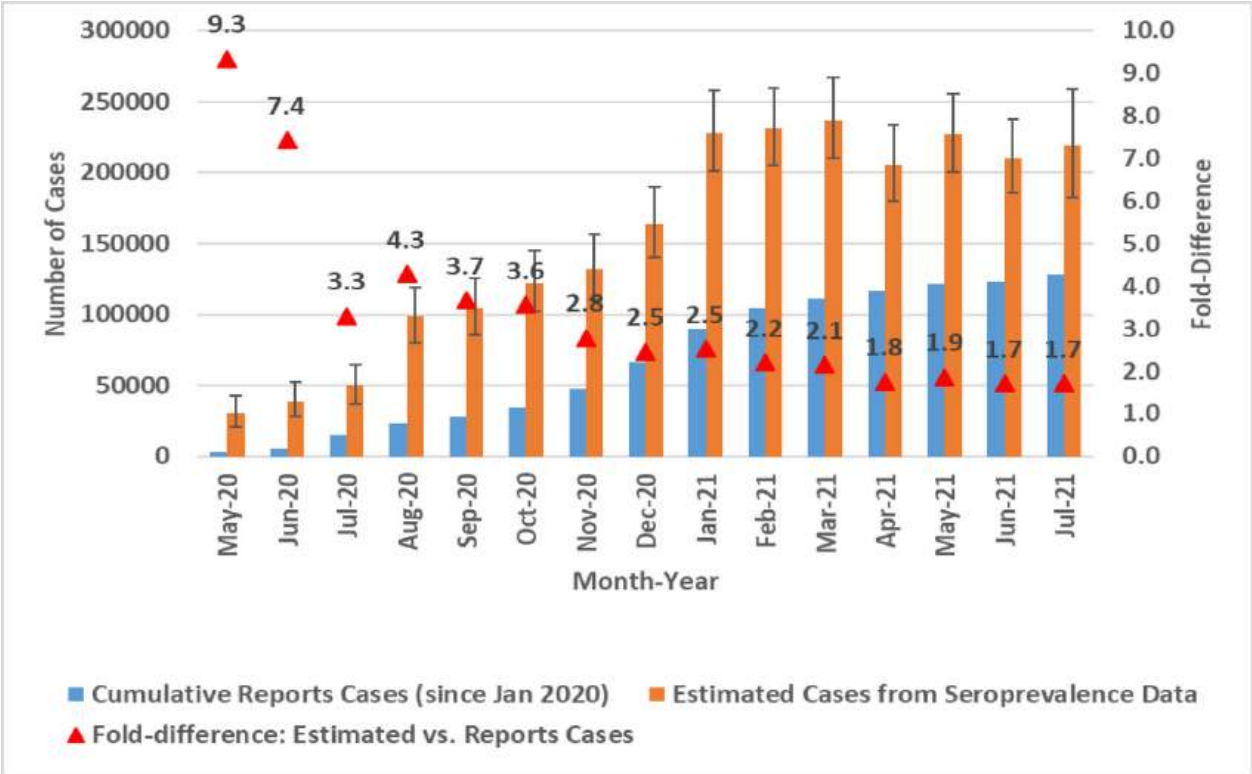
Figure 1. Monthly Seroprevalence of SARS-CoV-2 Antibody among Active Component Service Members from May 01, 2020 to July 15, 2021



Bars represent 95% confidence intervals for the overall seroprevalence estimate.

Figure 2. Seroprevalence Estimated Number of SARS-CoV-2 Infections Compared to the Cumulative Reported Number of Confirmed Cases by Month: Active Component Service Members, May 01, 2020 to July 15, 2021



1. Vertical lines represent 95% confidence intervals for the estimated number of cases.
2. Reported cases are cumulative from January 2020 to the month of interest.
3. Estimated cases are based on the seroprevalence estimate due solely to infection for each month multiplied by the total active force during that same month.
4. Fold-difference is calculated by dividing the estimated case count by reported case count.

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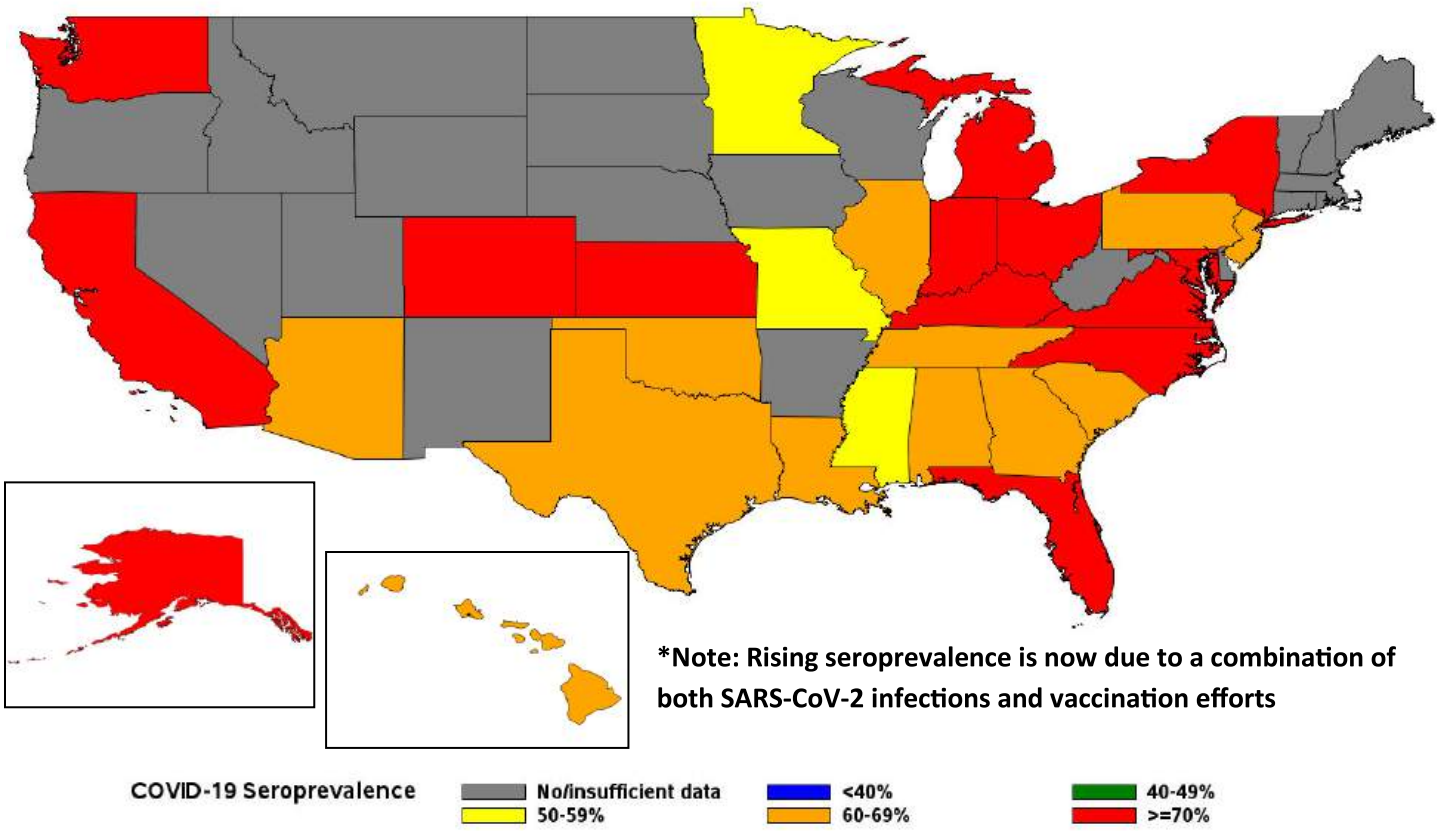
Figure 3. Overall Seroprevalence of SARS-CoV-2 Antibody among U.S. Service Members Sampled between May 1, 2021—July 15, 2021 by State of Service Member's Unit



* No/Insufficient data indicates there were less than 25 specimens tested from the state.

For the May 1, 2021—July 15, 2021 sampling:

- The majority of states with available data had a SARS-CoV-2 seroprevalence of 60%-69% (12 states) and ≥70% (14 states).
- All states with sufficient data were at or above 50% SARS-CoV-2 seroprevalence.

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Results from the May 1, 2021—July 15, 2021 Sampling

- SARS-CoV-2 antibody seroprevalence was highest in EUCOM (83%), but was not statistically significantly higher than NORTHCOM (71%) and INDOPACOM (76%).
- Seroprevalence estimates could not be calculated for CENTCOM, AFRICOM, and SOUTHCOM due to insufficient specimens collected in those GCCs.
- Among unvaccinated individuals, the NORTHCOM SARS-CoV-2 antibody seroprevalence estimate was 47% (45, 49). The other GCCs had insufficient specimens for an unvaccinated seroprevalence estimate.

Table 1. Overall Seroprevalence of SARS-CoV-2 Antibody by Geographic Combatant Command (GCC): May 1, 2021—July 15, 2021

GCC	Overall Seroprevalence Estimates (95% CI)
NORTHCOM	70.69 (69.35, 72)
EUCOM	82.76 (64.27, 92.95)
INDOPACOM	76.02 (69.99, 81.35)
CENTCOM	-
AFRICOM	-
SOUTHCOM	-

- The Navy continued to have a statistically significantly higher overall seroprevalence (77%) compared to the other services. However, among the unvaccinated, the Marine Corps had the highest seroprevalence (30%), which was statistically significantly higher than the Navy.
- Overall, seroprevalence was similar between the components. However, among the unvaccinated, the Guard had the highest seroprevalence (32%), which was statistically significantly higher than the active component, indicating a higher number of infections among the Guard components.
- Overall, individuals of white and black race had a statistically significant lower seroprevalence then Hispanics and Other race/ethnicities. However, among the unvaccinated, only individuals of white race had a significantly lower seroprevalence estimate.

Table 2. Seroprevalence of SARS-CoV-2 Antibody by Service, Component and Race/Ethnicity: May 1, 2021—July15, 2021

Variables	Overall Seroprevalence Estimates (95% CI)	Seroprevalence Estimates among Unvaccinated Subjects (95% CI)
Service		
Army	69.76 (68.07, 71.4)	27.96 (25.44, 30.58)
Air Force	68.12 (61.84, 73.96)	20.69 (12.82, 30.27)
Marine Corps	69.75 (66.09, 73.23)	30.08 (24.73, 35.85)
Navy	76.98 (74.49, 79.34)	19.35 (15.22, 24.12)
Component		
Active	72.65 (71.12, 74.12)	24.08 (21.66, 26.63)
Guard	68.92 (65.62, 72.08)	31.86 (27.09, 36.81)
Reserve	68.31 (64.9, 71.57)	29.13 (24.41, 34.19)
Race/Ethnicity		
White	69.92 (68.15, 71.65)	22.85 (20.33, 25.58)
Black	68.7 (65.49, 71.82)	31.49 (26.8, 36.43)
Hispanic	75.06 (72.04, 77.9)	30.82 (25.61, 36.43)
Other	76.1 (72.6, 79.33)	29.27 (23.3, 35.79)

- Overall SARS-CoV-2 antibody seroprevalence was similar between males and females.
- Overall seroprevalence among males aged 17-24 was statistically significantly lower than males in the other age groups, except for 55+. However, this was not found in the unvaccinated subject analysis (data not shown), indicating vaccination is driving this difference as opposed to infections.

Table 3. Seroprevalence of SARS-CoV-2 Antibody by Age and Sex: May 1, 2021—July 15, 2021

Variables	Overall Seroprevalence Estimates (95% CI)	
Age Category	**Males**	**Females**
All	71.83 (70.42, 73.22)	69.38 (66.41, 72.24)
17-24	66.89 (64.66, 69.06)	64.92 (60.37, 69.21)
25-34	73.46 (71.04, 75.75)	72.04 (66.92, 76.74)
35-44	78.25 (74.91, 81.34)	75.18 (67.48, 81.75)
45-54	81.99 (76.18, 86.89)	76.47 (59.07, 88.65)
55+	83.87 (66.62, 93.42)	85.71 (44.58, 99.27)

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Study Methodology:

The Armed Forces Health Surveillance Division (AFHSD) utilized data from the Defense Medical Surveillance System (DMSS) and serum specimens from the DoDSR to conduct this study. The U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) conducted the SARS-CoV-2 serological testing of the DoDSR specimens.

Specimen Selection and Testing:

DMSS data were used to identify specimens in the DoDSR with a collection date on or after May 01, 2020 for each 6-8 week selection window. The previous reports have covered May 01, 2020 – April 30, 2021, while the current analysis includes a total of 5,000 samples from May 1, 2021—July 15, 2021. After selection, specimens were aliquoted and delivered to USAMRIID for testing.

Samples were tested via a multiplexed, magnetic bead-based immunoassay optimized to identify SARS-CoV-2 IgG reactivity. The assay was developed at USAMRIID and run on the Luminex MAGPIX system. The Luminex assay utilizes 3 recombinant SARS-CoV-2 viral targets for detection including the full-length S-protein as well as the S1 and Receptor Binding Domain segments. Samples are considered positive when demonstrating sufficient reactivity to at least 2 of the 3 targets. A 95% MFI (mean fluorescence intensity) cutoff for all 3 targets was determined from assay validation using known positive and negative samples. After vaccinations became available to SM, the Luminex assay was used to also assess SARS-CoV-2 nucleoprotein IgG reactivity in addition to S-protein reactivity in order to distinguish seroprevalence from vaccination versus natural infection. Positive samples underwent additional testing via a SARS-CoV-2 specific microneutralization assay to evaluate the sample for the presence of functional antibodies that block viral replication in cell culture, which may be indicative of a protective capacity of the antibody response. Due to the increasing number of positive specimens by the multiplex assay, only a 4% sample of the positives were tested by microneutralization assay for the May 1, 2021—July 15, 2021 sample.

Data Analysis:

Results from the Luminex assay were merged with the demographic, location, and vaccination data from DMSS for the analysis. The overall seroprevalence of SARS-CoV-2 antibody (number of SARS-CoV-2 antibody positive specimens divided by the number of specimens tested multiplied by 100) and 95% confidence intervals (95% CI) were calculated for each sampling group. Stratified seroprevalence estimates for demographic and location specific variables were also calculated. To evaluate the overall estimated number of SARS-CoV-2 infections among active component SM, the seroprevalence rate due to infection among the active component sample was multiplied by the total active component force population number at the middle point of the surveillance window. This estimate was divided by the total number of reported COVID-19 cases to estimate the fold increase between reported and estimated infections based on seroprevalence. The seroprevalence rate due to infection was calculated by including individuals that were both S-protein and nucleoprotein positive on the Luminex assay. Since vaccination only results in S-protein reactivity, the presence of nucleoprotein reactivity in individuals that were seropositive was regarded as evidence of prior infection.

Limitations:

There are limitations to these data that should be considered when interpreting the results:

- The USAMRIID SARS-CoV-2 Luminex assay has 91% sensitivity and 98% specificity. Therefore, some serology results may be false positive results (the test result is positive, but the individual does not have SARS-CoV-2 antibodies) or false negative results (the test result is negative, but the individual does have SARS-CoV-2 antibodies).
- The sample size for this effort was calculated based on estimating the seroprevalence in the active force. Therefore, subgroup analyses of certain demographic categories or locations may be limited by insufficient numbers of individuals to calculate a precise estimate of the seroprevalence of SARS-CoV-2 antibodies in that group. The 95% CI will provide a measure of how precise the estimate is for that group. If the sample size for a specific group is too small, then seroprevalence data will not be provided since an accurate estimate cannot be determined.
- The fraction of infected individuals that develop detectable SARS-CoV-2 antibodies and the duration that those antibodies remain detectable has not been clearly defined to date. Antibody titers are known to decrease over time following infection, and because the duration of antibody detection is unknown, the above estimate of true to confirmed cases represents a lower limit of the actual ratio.[1] This effect is expected to become more pronounced over time, as the antibody response among those who were infected during the earlier months of the pandemic will fade over time. Additional studies on antibody persistence will be necessary to further refine these estimates.

References:

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