Anti-SARS-CoV-2 IgA Identifies Asymptomatic Infection in First Responders

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Summary: In a serosurvey of high-risk first responders, detection of IgA in the absence of detectable IgG identified individuals with mild or asymptomatic infection which would have been missed based assessment of anti-spike or anti-nucleocapsid IgG.

Abstract

Background: IgA is an important component of the early immune response to SARS-CoV-2. Prior serosurveys in high-risk groups employing IgG testing alone have provided discordant estimates. The potential added benefit of IgA in serosurveys has not been established.

Methods: Longitudinal serosurvey of first responders (police, emergency medical service providers, fire fighters, and other staff) employing three serologic tests: anti-spike IgA, anti-spike IgG, and anti-nucleocapsid IgG correlated with surveys assessing occupational and non-occupational risk, exposure to COVID-19 and illnesses consistent with COVID-19.

Results: Twelve percent of first responders in Colorado at baseline and 22% at follow-up were assessed as having SARS-CoV-2 infection. Five percent at baseline and 6% at follow-up were seropositive only for IgA. Among those IgA positive only at baseline, the majority 69% had a positive antibody at follow-up. 45% of those infected at baseline and 33% at follow-up were asymptomatic. At all time points, the estimated cumulative incidence in our study was higher than that in the general population.

Conclusions: First responders are at high risk of infection with SARS-CoV-2. IgA testing identified a significant portion of cases missed by IgG testing and its use as part of serologic surveys may improve retrospective identification of asymptomatic infection.

Key Words: SARS-CoV-2, COVID-19, serosurvey, IgA, first responders, epidemiology, cumulative incidence

Background

Infection with SARS-CoV-2 is associated with a spectrum of illness from asymptomatic infection to severe pneumonia with acute respiratory distress syndrome (COVID-19). First responders are a recognized high-risk group for SARS-CoV-2 infection. Serologic surveys of first responders have shown variable results with some showing rates similar to the general population and others showing higher rates (see Table 1).[1-7] Some of this variability may relate to differences in access to personal protective equipment (PPE) and infection control policies.[8] Because high-risk groups such as older individuals and individuals with significant comorbidities may be underrepresented among first responders, the proportion of infections which are asymptomatic in this group may be higher.

Sensitive serologic assays have the potential to provide a measure of cumulative incidence of infection including both symptomatic and asymptomatic cases. A wide array of serologic tests have received Emergency Use Authorization (EUA) by the United States Food and Drug Administration (FDA).[9] These EUAs are based on validation studies conducted with confirmed positive COVID-19 cases.[10] In a multisite comparison of multiple serologic tests, the sensitivities and specificities of IgG tests ranged from 80% to 89% and 97% to 100%, respectively.[11] Given the potential for false positive tests when applied to low-risk populations, serosurvey strategies for these populations requiring concordant positivity of two tests with different antigenic targets have been recommended.[12, 13] In high-risk populations, however, the risk for false positive results will be lower and a single positive test may be sufficient to confirm infection.

In serosurveys, the observed prevalence of antibodies depends on the probability of a positive antibody following infection, the durability of the antibody response after infection, and the time elapsed between infection and the sample collection. Prior studies of the durability of antibody response have shown disparate results with some studies showing waning of response in as few as 2-3 months and others showing durable antibody responses up to 8 months after the onset of

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infection.[14-18] Persons with asymptomatic or mild infection in prior studies may have lower levels of IgG and may show a more rapid decay in antibody titers.[19-21]

Given that the upper respiratory tract is a primary point of infection for SARS-CoV-2, mucosal and systemic IgA plays an important role in host defense. Yu et al showed that seroconversion from IgM to IgA occurred 2 days after the onset of symptoms, and before the seroconversion from IgM and IgG.[22] IgA has also been shown to play an early role in virus neutralization.[23] In a longitudinal study, the median time to seroreversion for IgA was 70.5 days and 25% had seroreversion at 104 days or greater.[24] This finding aligns with other published longitudinal studies using assays for IgA targeting the S1 domain of Spike.[25, 26]

To characterize the risk of SARS-CoV-2 infection among first responders and the relative importance of IgA versus IgG testing in serologic monitoring, we conducted a longitudinal serosurvey among first responders in Colorado employing three serologic tests: anti-S1 domain of spike protein IgA, anti-S1 domain of spike protein IgG, and anti-nucleocapsid IgG and we correlated the serologic testing with participant surveys identifying times of exposure to persons with COVID-19 and illness potentially consistent with COVID-19.

Methods

Study Design:

This study was a longitudinal serosurvey of first responders conducted in Colorado between May of 2020 and March of 2021 with serologic data correlated with longitudinal assessment of exposures, periods of illness and confirmed cases of COVID-19.

Recruitment and Consent:

Outreach occurred by email to agency staff as well as through direct communication via agency leadership. REDCap questionnaires were used for all data collection. [27] Interested persons were provided a link to an online screening questionnaire which included a link to review an electronic copy of the consent form. Subjects were consented, paper consents were signed, and phlebotomy was performed at scheduled events at each participating agency. Individuals who had not completed the online screening could complete the screening process on site.

Questionnaires:

All participants completed an intake questionnaire assessing their occupational and home risk for infection and any history of COVID-19. Individuals were categorized by their job role into the groups Police, Firefighters, EMS (emergency medical personnel), or Other, with those in the Other category being individuals who worked in ancillary job roles for participating agencies. Participants were surveyed regarding their prior exposures to persons with COVID-19, prior confirmed diagnosis of COVID-19, access to PPE, patterns of PPE utilization as part of their job role, and proportion of time spent in direct contact with the public. A follow-up survey was sent to assess periods of illness prior to baseline testing for which the participant took leave from work as well as periods of illness when the participant were either not tested or tested negative for SARS-CoV-2 infection. Individuals with reported illness or COVID-19 exposures after the baseline questionnaires were invited to complete supplemental questionnaires.

Serological Assays:

Blood samples were collected, serum was isolated on site and samples were frozen for serologic testing at baseline and at follow-up after 2-3 months. All samples were tested at ICON Laboratories using the Abbott Architect Anti-SARS-CoV2 chemiluminescent microparticle immunoassay (MIA) for IgG (anti-nucleocapsid protein, anti-N), Euroimmun Anti-SARS-CoV-2 ELISA for IgG (anti-S1 domain of

Spike, anti-S), and the Euroimmun Anti-SARS-CoV2 ELISA for IgA (anti-S1 domain of Spike). Results

for the anti-N IgG were categorized as positive based on an S/C index cut off of greater than or equal

to 1.4. Anti-S IgG and IgA were categorized as positive based on cutoffs of greater than or equal to

1.4 based on manufacturer specifications.[28]

Endpoint Definitions:

At baseline and follow-up, participants were categorized as having prior illness if they reported

an episode with symptoms potentially consistent with COVID-19 including fevers, typical respiratory

symptoms, or diarrhea with onset prior to the sample collection. Participants with a history of

confirmed COVID-19 were categorized as having prior COVID-19 for the time of a sample collection if

the onset of symptoms or test date was prior to the visit. Participants were categorized as having

prior exposure to COVID-19 if they reported an episode of quarantine from work starting prior to the

collection.

To assess the spectrum of illness associated with infection with SARS-CoV-2, we divided

participants into 6 categories based on serology, illness history and exposure: Definite COVID-19,

Probable COVID-19, Possible COVID-19, Definite Asymptomatic Infection, Possible Asymptomatic

Infection and No Evidence of COVID-19 or SARS-CoV-2 infection (see Table 2). The presence of

antibodies without report of prior COVID-19 diagnosis or periods of illness was defined as

asymptomatic infection. For those with no follow-up assessment, categorization was based on

baseline data. Given the potential for waning of antibody response, if an individual had a higher-

level categorization at baseline than follow-up, the baseline categorization was retained.

Statistical Methods

The proportion of participants with antibody positivity was assessed at baseline and follow-up,

stratified by job role and history of exposure, illness, and COVID-19 diagnosis. Cumulative incidence

at each time point was assessed comparing multiple criteria including: any serologic positivity,

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positivity by each individual assay, any positive IgG, and positivity of all 3 tests for a given blood collection. For individuals who received the Pfizer or Moderna COVID-19 mRNA vaccines prior to follow-up, they were considered IgG positive only if positive for anti-N IgG given the potential for positive anti-S IgG elicited by the vaccine. For each job role, the total number of person-years at risk was calculated and the incidence per 100 person-years (PY) was calculated based on positivity by any of the 3 tests.

Given the potential for false positive IgA tests resulting from the lower specificity of the IgA assay, the positive predictive value (PPV) of the test was estimated based the reported sensitivity of 99% and specificity of 93.7% combined with the seroprevalence in the sample.[29] The true seroprevalence was assumed to be the combination of those positive by anti-S and/or anti-N IgG plus the proportion of true positives among those individuals positive only for IgA. The estimated PPV and number of participants with true positive IgA results was calculated for a range of potential seroprevalences from 8% to 25%. For baseline and follow-up, the estimated true seroprevalence was selected based on the level of seroprevalence for which the estimated seroprevalence equaled the observed seroprevalence when adjusting the observed number of participants positive by IgA only by the corresponding PPV of the test.

The assessment of cumulative incidence by each criteria was further stratified by history of prior exposure, illness, or confirmed diagnosis of COVID-19. The duration of time between illness or exposure and collection was calculated based on the last prior diagnosis or exposure date. The duration of time at risk was defined for the first blood collection as the number of days between 2/1/2020 and the date of the first visit. The duration of time at risk for the second collection was defined as the number of days between the first and second visits. For each participant, the time to an event consistent with COVID-19 was assessed based on the first occurrence of either confirmed COVID-19 infection or new seropositivity. Kaplan-Meier estimates of the proportion uninfected at each time point compared to estimated community prevalence of persons infected with

asymptomatic or symptomatic infection with SARS-CoV-2 based on modeling from the Colorado School of Public Health.[30]

Data management, statistical analyses, and figure generation were performed using SAS® Version 9.4 (SAS Institute Inc., Cary, NC, USA). Chi-square testing was used to assess differences in the proportion serologically positive across work setting and by exposure and illness categories. Assessment of statistically significant differences between incidence rates by job role was performed using Poisson regression (PROC Genmod). Kaplan Meier estimates were generated using PROC Lifetest.

IRB approval was obtained from the Western IRB, protocol # 20201662.

Results

Intake questionnaires were completed baseline samples were collected for 1007 first responders. This included 414 Firefighters, 241 EMS, 201 Police, and 151 Other. Of the initial sample, 2 subjects had missing results for anti-N IgG and 1 subject for anti-S IgG and anti-S IgA. Of the 1007 with baseline results, 783 (78%) completed follow-up testing including 341 Firefighters (82%), 165 EMS (68%), 149 Police (74%), and 128 Other (85%).

Across all roles, participants were predominantly male, Caucasian, non-Hispanic, with median age by occupational role between 34 (EMS) and 47 (Other) (see Table 3). Greater than 90% of Firefighters and EMS reported access to both N95 respirators and eye protection and over 80% reported access to surgical masks as well. Reported access was slightly lower among Police with 78% reporting access to N95 respirators, 80% to eye protection, and 82% to surgical masks. Those reporting Other roles did not report access to PPE. Firefighters reported being unable to socially distance at work 40% of the time compared to 50% for EMS and 38% for Police. Greater than 70% of Firefighters, EMS, and Police reported that they would inform a supervisor if they experienced a COVID-19 exposure.

Eighteen percent of participants reported illness either prior to the baseline visit or during the follow-up period. Confirmed COVID-19 was less common, occurring in 5% of participants overall. Eight percent of participants reported periods of quarantine due to exposure to persons with COVID-19. For all diagnostic criteria, the percent positivity at baseline was similar across job roles with no statistically significant differences between the groups. IgG positivity was identified in 5-7% of first responder roles at baseline and 9% of Other. Higher percentages of IgG positivity were seen at follow-up with 11% positive among Firefighters, 14% positive among EMS and 15% positive among Police and 16% positive among Other. The estimated cumulative incidence overall at baseline was 12% with a resulting PPV for a positive an IgA test at baseline of 68%. The estimated cumulative incidence of SARS-CoV-2 infection at follow-up was 22% with an associated PPV for an IgA test at follow-up of 82%. The incidence per 100 PY at baseline was 26 for Firefighters, 30 for EMS, 28 for Police and 30 for Other. The incidence at follow-up was higher with 45 cases per 100 PY among Firefighters, 43 among EMS, 33 among Police and 30 among Other.

Among those with no reported periods of illness or exposure, antibody testing was completely negative in 90% of cases at baseline at 86% of cases at follow-up (see Table 4). Fifteen percent before adjustment for false positive IgA and 12% after adjustment were positive for at least one antibody in the absence of either reported illness or exposure. Thirteen percent were positive by IgA and 6% were positive for at least one IgG. Positivity of at least one antibody was identified at baseline in 43% (41% adjusted) of those with prior exposure and 41% (36% adjusted) of those with prior illness. IgA positivity at baseline was identified in 36% of those with prior exposure and 35% of those with prior illness. Among those with a history of COVID-19, 75% (73% adjusted) had at least one positive antibody at baseline with 43% positive by all three tests, 68% for at least one IgG, and 61% positive for IgA. At follow-up, among those with no history of illness or exposure, 14% (13% adjusted) had at least one positive antibody with 8% positive for at least one IgG and 13% positive for IgA. IgA positivity was found at follow-up for 42% of participants with prior exposure and 39% of those with prior illness. Among those with a history of confirmed COVID-19 at follow-up, 84% (82%

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adjusted) had at least one positive antibody with 76% positive for at least one IgG and 75% positive for IgA.

Figure 1 shows the proportion of participants with indication of infection by reported testing or positive serology in comparison to estimated proportion in the community who have recovered from infection. At each time point, the proportion of positivity in first responders with evidence of prior infection is higher than the modeling estimate for the state with a notable increase in differential observed starting in June to September based on the cases among first responders identified from baseline serologic testing.

For most with definite or probable COVID-19 at baseline the pattern of antibodies was consistent between baseline and follow-up. Among those negative for all antibodies at baseline, 95% remained negative at follow-up testing. Among the 56 with at least one positive IgG at baseline, 87% had at least one positive IgG at follow-up. Among those positive by all assays at baseline, 79% remained positive by all assays at follow-up.

Among all those with isolated IgA positivity at baseline, 69% had a positive antibody at follow-up and 56% remained positive by IgA alone. Thirteen percent had at least one positive IgG and 5% were positive on all three tests. Among those with documented prior illness, 22% were IgG positive as compared to 8% of those with no reported prior illness (p=0.07). Changes in IgA ratio between baseline and follow-up draws stratified by disease status are shown in Figure 2. Adjusting for false positive IgA tests, 45% of those classified as Definite, Probable, or Possible SARS-CoV-2 infection at baseline were asymptomatic, including 13% categorized as having Definite Asymptomatic Infection and 32% having Possible Asymptomatic infection based on isolated IgA positivity. At follow-up, 33% were asymptomatic with 5% categorized as having Definite Asymptomatic Infection and 28% Possible Asymptomatic Infection.

Discussion

This study identified a high cumulative incidence of SARS-CoV-2 infection among first responders in Colorado with rates as high 12% at baseline and 22% at follow-up as measured by seropositivity of one or more antibody tests (IgA, Anti-S IgG, Anti-N IgG). Of high risk first responders, 8% were seropositive for only anti-spike IgA as compared to 6% positive for anti-S or anti-N IgG. Among those with positive IgA only, the majority remained positive at follow-up. Individuals with positive IgA were more likely to report prior respiratory illness.

A multisite serosurvey from the period between March and May of 2020 reported population rates of only 6.9% in the most highly impacted areas such as New York.[31] The prior serosurvey of Colorado first responders required concordance of two positive IgG tests with an overall proportion positive of 4%. This proportion is similar to the proportion observed in our study positive by all 3 assays. It is also similar to the results of a life-insurance applicant survey from September of 2020 in which 4.1% of applicants from Colorado tested positive for anti-N IgG and the estimates of positivity based on modeling.[30, 32]. All the contemporaneous first responder surveys were based on testing exclusively for IgG, suggesting that these prior surveys may have significantly underestimated the rate of SARS-CoV-2 exposure and infection.

The specificity of the Euroimmun IgA ELISA among 30 samples from laboratory confirmed SARS-CoV-2 infected persons and 80 pre-pandemic samples submitted to the US FDA for Emergency Use Authorization was 91.2%.[33] In a subsequent validation study based on 100 samples from laboratory confirmed SARS-CoV-2 infected persons and 300 pre-pandemic samples, the specificity of the Euroimmun IgA ELISA was found to be higher at 93.7% for IgA compared to 91.7% found for IgG (anti-S).[29] These validation studies are conducted with known positive cases and collected at a fixed interval after onset of symptoms typically within 2-4 weeks.

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The performance of these tests when applied in individuals who are well as part of serosurveys has not been well described. In a longitudinal study of 1013 German healthcare workers, IgA positivity was identified in 6.8% of workers as compared IgG positivity which was found in only 2.1%.[34] In this study, IgA remained more common at 10-week follow-up seen in 10.4% or participants as compared to IgG in 1.6% with 6% of those IgA positive at baseline developing IgG positivity by the time of follow-up.   This study reported a lower specificity of IgA testing at 73% but treated all positives as true positives. In our analysis using the more specific manufacturer recommended cutoffs for positive for the IgA assay, we equally found significant numbers of individuals positive for IgA in the absence of IgG positivity with a slightly higher percentage (12%) developing IgG positivity at follow-up. It is notable on our sample that the estimated 35% false positive rate for the IgA test is similar to the proportion of individuals (31%) who were positive by IgA alone at baseline and negative on all tests at follow-up suggesting that this may be a potential indicator of false positive tests.

The significant portion of cases identified by isolated IgA positivity in this cohort is notable and highlights the potential importance of IgA in longitudinal serosurveys.   In prior surveys, as much as 45% of individuals infected with SARS-CoV-2 have been asymptomatic.[35] Much of this data derives from investigation of confined cohorts, however in a large serosurvey from Iceland 43% of those positive by IgG had had no symptoms of illness.[36] In our sample of high-risk first responders, 45% of those infected at baseline and 33% at follow-up were asymptomatic with 13% of those infected at baseline and 5% at follow-up identified by IgA positivity alone.

Limitations

Follow-up was only available for 783 participants, limiting the sample for evaluation of serostatus over time. Despite this, the associations between antibody response and exposure and illness were consistent between baseline and follow-up. Early in the pandemic access to SARS-CoV-2 testing was limited. It is possible that some of those with reported illness may have had infections

with other circulating respiratory viruses. There was less circulation of these viruses between the period of the 1st and 2nd sample and the consistency of the identified associations between baseline and follow-up suggests that confounding by other viral infections in these analyses is limited.

Conclusions

Based on serologic testing, first responders are at higher risk of infection with SARS-CoV-2 relative to the general population. In this serosurvey, anti-spike IgA identified a significant number of cases missed by anti-S and anti-N IgG testing. Testing for IgA as part of serologic surveys is therefore important and may improve retrospective identification of asymptomatic or mildly symptomatic cases. Additional information is needed to clarify the level of protection conferred to those with isolated IgA positivity following infection with SARS-CoV-2.

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Table 1. Published serologic surveys for anti-SARS-CoV2 antibodies among first responders

	Location	Dates	Assay	Anti S	Anti N	Police n (% positive)	Fire n (% positive)	EMS n (% positive)	Total
Sabourin et al.	Colorado	7/1/20-8/31/20	Local	Y	Y	125 (4%)	42 (2%)	-	264 (4%)
Sami et al.	New York City	5/18/20 - 7/2/2020	Ortho VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG Test	Y		9969 (19%)	4310 (21.2%)	1449 (38.3%)	22,647 (22.5%)
Akinbami et al.	Michigan	5/18/20 - 6/30/20	VITROS IgG	Y		785 (4.8%)	330 (2.0%)	1158 (7.1%)	16,397 (6.9%)
McGuire et al.	Minnesota	5/16/20-5/17/20	Vitros IgG	Y		163 (1.2%)	92 (1.1%)		255
Iwuji et	Texas	5/12/20	Abbott Architect		Y	2 (-%)	2 (-%)	1 (-%)	683

al.		- 5/13/20	IgG						(0.73 %)
Shukla et al.	Arizona	4/24/20 - 5/21/20	Ray Biotech Rapid IgG/IgM		Y	1,643 (1.52%)	1,713 (1.46%)	-	3,326 (1.5%
Tarabic hi et al.	Ohio	4/20/20 -6/2/20	Epitope IgG/IgM		Y	-	175 (13.3%)	105 (5.7%)	296 (5.4%)

Table 2. Categorization of SARS-CoV-2 infection and disease

		Baseline	Follow-Up
Definite COVID-19	1) Positive COVID-19 PCR or antigen test OR 2) Positive IgG antibody combined with history of exposure to a person with COVID-19 and a history of symptoms compatible with COVID-19	45 (4%)	55 (7%)
Probable COVID-19	1) Positive IgA or IgG antibody combined with history of symptoms compatible with COVID-19 OR 2) History of exposure to a person with COVID-19 AND history of symptoms compatible with COVID-19	9 (1%)	31 (4%)
Possible COVID-19	History of exposure to a person with COVID-19 and a history of symptoms compatible with COVID-19	14 (1%)	29 (4%)
Definite Asymptomatic SARS-CoV-2 Infection	Positive IgA or IgG antibody with history of exposure to a person with COVID-19 with no history of symptoms compatible with COVID-19 OR Positive IgG antibody without history of exposure to a person with COVID-19 and without a history of symptoms compatible with COVID-19	23 (2%)	10 (1%)
Possible	Positive IgA antibody alone without history of exposure to	82 (8%)	65 (8%)

Asymptomatic SARS-CoV-2 Infection	a person with COVID-19 and without a history of symptoms compatible with COVID-19		
No evidence of COVID-19 or SARS-CoV-2 Infection	All antibodies negative and no history of exposure to a person with COVID-19 and without a history of symptoms compatible with COVID-19	834 (83%)	593 (76%)

Table 3. Occupational and demographic factors by occupational role[*]

	Fire (n=414)	EMS (n=241)	Police (n=201)	Other (n=151)	Overall
Age median (IQR)	43 (13)	34 (18)	42 (15)	47 (18)	42(17)
Sex					
Male	377 (92%)	162 (70%)	143 (72%)	70 (47%)	752 (76%)
Female	34 (8%)	66 (29%)	55 (28%)	78 (53%)	233 (24%)
Transgender	1 (<1%)	2 (1%)	1 (<1%)	-	4 (<1%)
Race/Ethnicity					
Caucasian	380 (92%)	210 (87%)	183 (92%)	133 (90%)	906 (92%)
Black	6 (1%)	2 (1%)	4 (2%)	5 (3%)	17 (2%)
Asian	4 (1%)	1 (<1%)	3 (2%)	1 (1%)	9 (1%)
Other	17 (4%)	13 (7%)	5 (3%)	8 (5%)	53 (5%)
Hispanic	21 (5%)	18 (8%)	19 (10%)	16 (11%)	74 (8%)
PPE Available					
N95	394 (95%)	227 (94%)	157 (78%)	1 (<1%)	778 (77%)
Surgical mask	358 (86%)	214 (89%)	162 (81%)	1 (<1%)	735 (73%)
Eye protection	394 (95%)	227 (94%)	165 (82%)	1 (<1%)	787 (78%)
Unable to Distance at Work (% of time)	31% (40)	43% (50)	35% (38)	-	35% (38)
Episode of Quarantine	38 (9%)	14 (6%)	14 (7%)	10 (7%)	76 (8%)
Episode of Symptomatic	74 (18%)	36 (15%)	53 (26%)	25 (17%)	188 (19%)

Illness					
Confirmed COVID-19	20 (5%)	13 (5%)	12 (6%)	10 (7%)	55 (5%)
COVID-19 Vaccine Prior to Follow-up	9 (2%)	12 (5%)	6 (3%)	9 (6%)	36 (4%)

[*]No statistically significant difference identified between job roles

Table 4. Reported exposure and illness by antibody response[*]

	Baseline (n=1007)					Follow-Up (n=783)				
	No illness or Exposure (n=839)	Prior Exposure (n=44)	Prior Illness (n=155)	Prior COVID-19 (n=44)	Overall	No Illness or Exposure (n=592)	Prior Exposure (n=66)	Prior Illness (n=170)	Prior COVID-19 (n=51)	Overall
Any Positive	**82 (10%)**	**19 (43%)**	**64 (41%)**	**33 (75%)**	148 (15%)	**85 (14%)**	**31 (47%)**	**77 (45%)**	**43 (84%)**	165 (21%)
Adjusted Any Positive[**]	63 (7%)	18 (41%)	56 (36%)	32 (73%)	118 (12%)	78 (13%)	30 (45%)	74 (43%)	42 (82%)	154 (20%)
Euroimmun IgA (anti-S) Positive	**74 (9%)**	**16 (36%)**	**54 (35%)**	**27 (61%)**	129 (13%)	**79 (13%)**	**28 (42%)**	**66 (39%)**	**38 (75%)**	147 (19%)
IgA Only	**60 (7%)**	2 (5%)	**24 (15%)**	3 (7%)	85 (8%)	39 (7%)	4 (6%)	**19 (11%)**	4 (8%)	59 (7%)

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Adjusted IgA Only[**]	41 (5%)	1 (2%)	16 (10%)	2 (4%)	55 (5%)	32 (4%)	3 (5%)	16 (9%)	3 (6%)	48 (6%)
Any IgG	**22 (3%)**	**17 (39%)**	**40 (26%)**	**30 (68%)**	63 (6%)	**46 (8%)**	**27 (41%)**	**58 (34%)**	**39 (76%)**	106 (13%)
Euroimmun IgG (Anti-S) Positive	**17 (2%)**	**16 (36%)**	**38 (25%)**	**28 (64%)**	56 (6%)	**43 (7%)**	**26 (39%)**	**56 (33%)**	**38 (75%)**	100 (13%)
Abbot IgG (Anti-N) Positive	**12 (1%)**	**13 (30%)**	**33 (21%)**	**26 (60%)**	45 (4%)	**12 (2%)**	**19 (30%)**	**40 (24%)**	**30 (59%)**	53 (7%)
All Positive	**6 (<1%)**	**11 (25%)**	**24 (15%)**	**19 (43%)**	30 (3%)	8 (1%)	**17 (26%)**	**34 (20%)**	**27 (53%)**	42 (5%)

[*]Bolded values were statistically significant in bivariate comparisons with p<0.05. Positive results for Euroimmun IgA and IgG include those categorized as borderline or positive per manufacturer specifications.

[**]Estimates based on a positive predictive value of a positive IgA at baseline of 0.65 and at follow-up of 0.80. Tests of statistical association were not performed on the adjusted estimates.

Figure 1. Cumulative incidence of COVID-19 or new seropositivity among first responders compared to estimated proportion of individuals in the community who have recovered from symptomatic or asymptomatic infection. Community estimates are derived from modeling from the Colorado School of Public Health.

Figure 2: Euroimmun anti-S (IgA) serology evaluation at baseline and follow up. The semiquantitative ratio from the Euroimmun IgA serology test was plotted at baseline and at follow up. Subjects are grouped by the results of their COVID-19 assessment at the baseline collection: Definite COVID-19 (n=37), Probable COVID-19 (n=9), Possible COVID-19 (n=11), Definite Asymptomatic Infection (n=15), Possible Asymptomatic Infection (n=72), No Evidence of SARS-CoV-2 Infection (n=603). Lines connect paired results from a single subject. The color of the dot represents the qualitative test result. Participants who received SARS-CoV-2 vaccination prior to the follow-up collection were excluded.
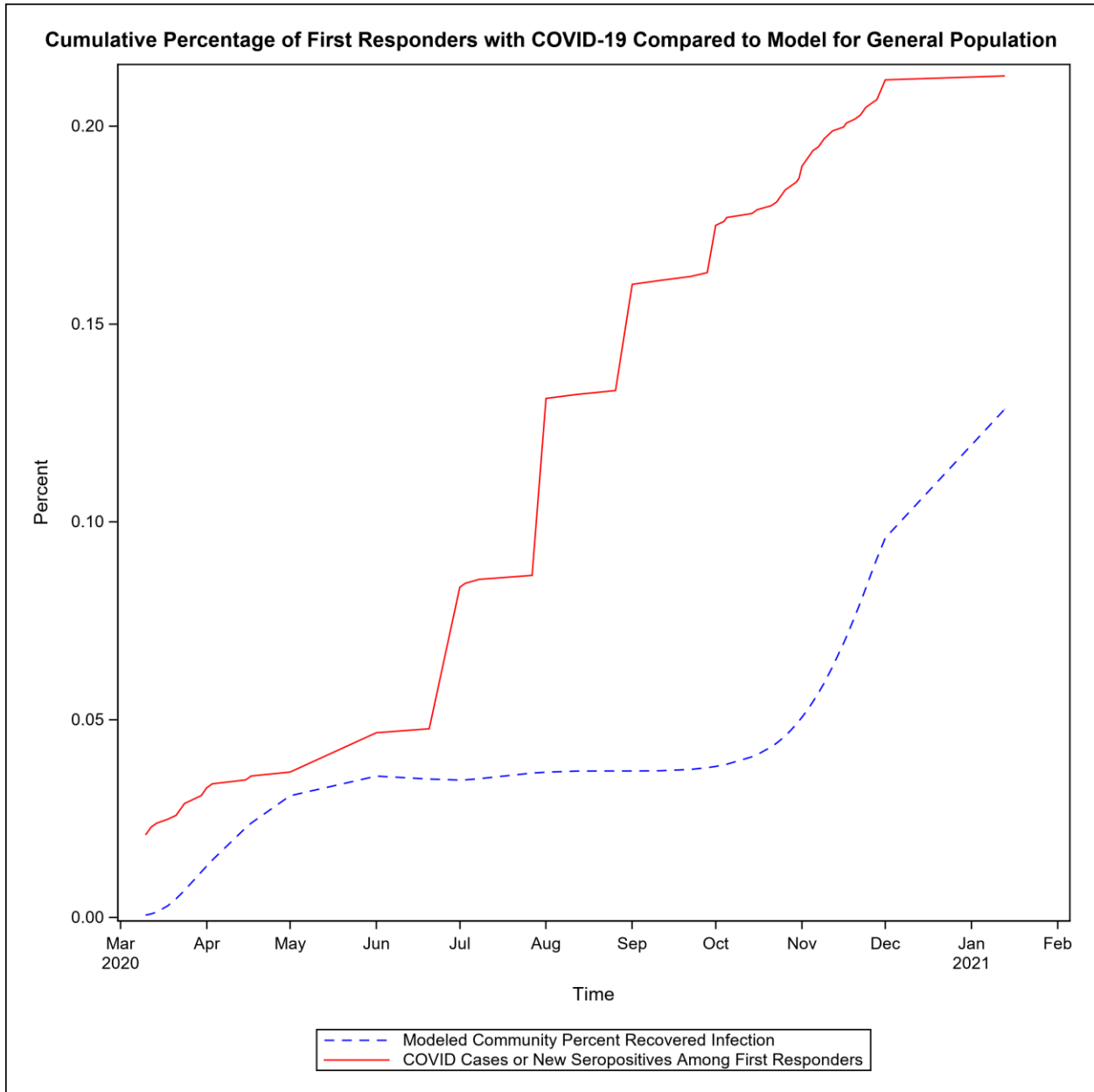
Figure 1

Cumulative Percentage of First Responders with COVID-19 Compared to Model for General Population

Legend:
- - - Modeled Community Percent Recovered Infection
—— COVID Cases or New Seropositives Among First Responders

Y-axis: Percent
X-axis: Time

Figure 2