Determining SARS-CoV-2 infection rates by nucleocapsid seropositivity in a vaccinated population with high seroprevalence

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Introduction

At the pandemic onset, we developed an ELISA assay to 3 SARS-CoV-2 antigens: spike (S), its receptor binding domain (RBD) and nucleocapsid (N)[1,2]. To avoid false positives for SARS-CoV-2 seropositivity, we required samples to be positive for at least two different antigens. Once vaccinations targeting spike were introduced, estimates of natural infection relied on N only.

Objective

To reassess the seropositivity threshold for an anti-N ELISA assay set early in the pandemic to improve the assay's predictive value for measuring SARS-CoV-2 infection in a vaccinated population with high seroprevalence.

Methods

Samples were analyzed in an automated chemiluminescent ELISA for total IgG antibodies to N, S or RBD. Raw values were normalized to a standard to create relative ratios (RRs) and converted to BAU/mL values. Receiver operating characteristics (ROC) curve analysis was performed. Results for N were stratified by vaccination status, time from infection and variant of concern.

Results

Figure 1. Density distribution of known negatives & positives.

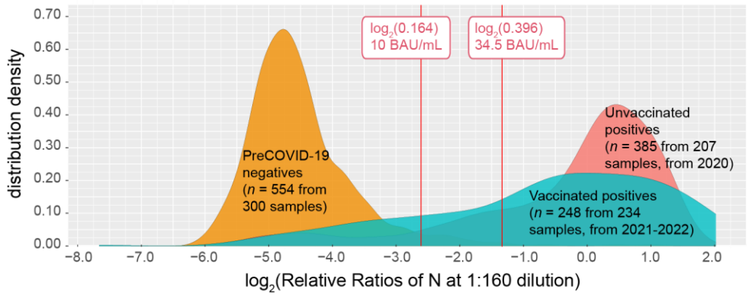


Table 1. Specificity & sensitivity at different N BAU/ml thresholds.

Plasma/Serum	Unvaccinated		Vaccinated	
	N (34.5)	N (10)	N (34.5)	N (10)
Specificity	99%	96%	99%	96%
Sensitivity	80%	91%	71%	84%
DBS[1]	Unvaccinated		Vaccinated	
	N (34.5)	N (11.4)	N (34.5)	N (11.4)
Specificity	99%	90%	99%	90%
Sensitivity	92%	97%	63%	88%

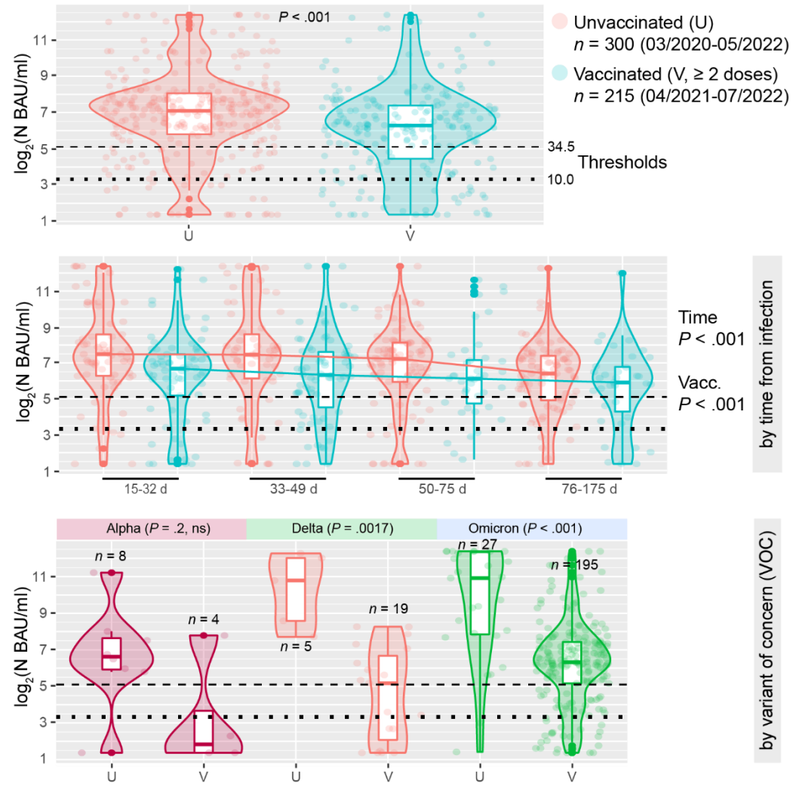
[1]DBS negatives (n = 187 from 89 samples), unvaccinated positives (n = 194 from 97 samples and vaccinated positives (n = 631 from 618 samples)

Table 2. Predictive Values by % Seroprevalence for Plasma/Serum.

% Seroprevalence	Predictive Value	Vaccinated	
		N (34.5)	N (10)
0.7%	Positive (PPV)	41.8%	14.1%
	Negative (NPV)	99.8%	99.9%
	PPV * NPV	0.42	0.14
35%	Positive (PPV)	98.2%	92.6%
	Negative (NPV)	86.6%	91.6%
	PPV * NPV	0.85	0.85
80%	Positive (PPV)	99.8%	98.9%
	Negative (NPV)	46.5%	59.4%
	PPV * NPV	0.46	0.59

At seroprevalence higher than 35%, the best predictive value for N for plasma/serum is at a lower threshold of 10 BAU/mL.

Figure 2. Convalescent N antibody levels by vaccination status.



Conclusions

- In vaccinated individuals, calls for SARS-CoV-2 infection are now reliant on N, but anti-N antibody levels and seroconversion rates decrease in vaccinated individuals.
- Alternative lower thresholds for both plasma/serum and DBS have been established to improve N's predictive value.

References

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2 Isho, B. et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Science immunology 5, doi:10.1126/sciimmunol.abe5511 (2020).

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