Comparison of SARS-CoV-2 Live Virus Neutralization and Serologic Immunoassays

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BACKGROUND

Live virus micro-neutralization (MN) is the gold standard for quantifying the neutralizing titer (NT) of antibodies to SARS-CoV-2. However, performing MN is labor intensive and requires a biosafety level 3 laboratory, highly trained technicians, and expensive resources. We assessed the performance of 8 immunoassays which measure SARS-CoV-2 NT and compared them to gold standard MN results.

METHODS

Study Population:

- 269 individuals known to previously be SARS-CoV-2 PCR+ (i.e., convalescent individuals, <10% hospitalized)
- 200 pre-pandemic individuals

Microneutralization Assay:

MN was performed on all samples from convalescent individuals; results were classified as undetectable vs any detection of MN NT (NT<20 vs. NT>20), as well as high and low MN NT (NT>80 vs. NT<80) (Table 1).

Immunoassays:

All samples were completed on 3 Lateral Flow Immunoassays (FLAs; Wondfo Colloidal Gold, Wondfo Colored Microsphere, Wondfo Finecare) and 5 enzyme-linked immunoassays (ELISAs; ImmunoRank, GenScript, CUSABIO, Euroimmun NeutraLISA, Euroimmun QuantiVac) that reported to test for neutralizing antibodies to SARS-CoV-2.

Statistical Analysis:

Receiver operating curve analysis was used to assess accuracy for detecting levels of NT. The area under the curve (AUC) was calculated for the manufacturer's cut off and empirically to identify the best discriminatory cut off value. Cohen's kappa statistics were calculated to assess categorical agreement and Spearman's rank statistics were calculated to assess correlations. Sensitivity and Specificity for assays was calculated.

Table 1

nAb Categories	N	Percent
AUC <20	89	33.09
AUC >=20&<80	63	23.42
AUC >=80	117	43.49
Total	**269**	**100**

Figure 1

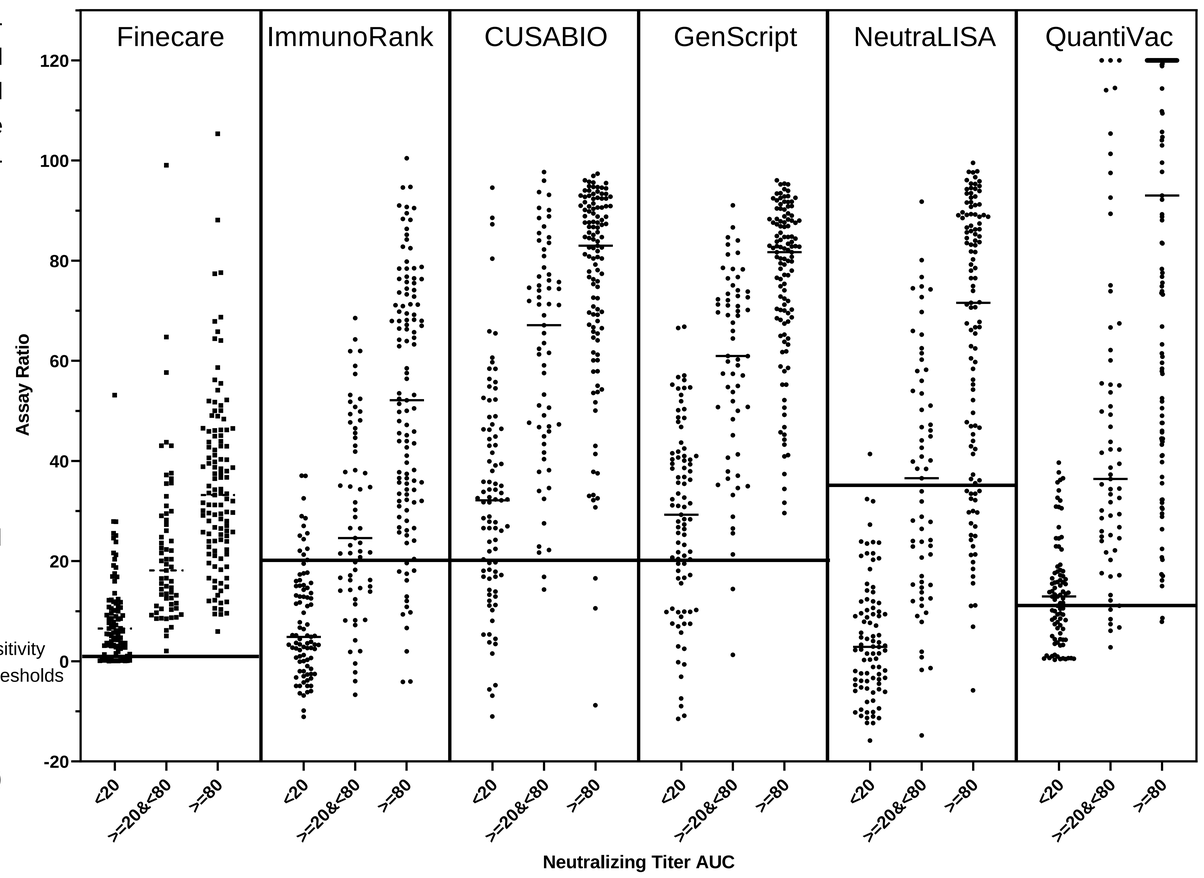


Table 2

	Manufacturer's Cutoff	Empirical Analysis			
	Any NT	Any NT	High NT	Agreement Analysis	
Serologic Assay	Cut off; AUC (95% CI)	Cut off; AUC (95% CI)	Cut off; AUC (95% CI)	Spearman's Rank	Cohen's Kappa
Wondfo Colloidal Gold	visual; 0.88 (0.85, 0.90)	-	-	-	0.33
Wondfo Colored Microsphere	visual; 0.87 (0.85, 0.90)	-	-	-	0.24
Wondfo Finecare	1.0; 0.87 (0.84, 0.89)	8.6; 0.97 (0.96, 0.98)	29.9; 0.94 (0.92, 0.96)	0.74	0.20
ImmunoRank	20.0; 0.87 (0.84, 0.90)	16.2; 0.97 (0.95, 0.98)	25.1; 0.94 (0.92, 0.96)	0.77	0.60
CUSABIO	20.0; 0.83 (0.80, 0.86)	46.7; 0.96 (0.94, 0.97)	64.1; 0.92 (0.89, 0.94)	0.73	0.34
GenScript	20.0; 0.89 (0.86, 0.91)	43.3; 0.98 (0.97, 0.99)	61.7; 0.95 (0.93, 0.97)	0.82	0.36
NeutraLISA	35.0; 0.90 (0.87, 0.93)	11.3; 0.97 (0.96, 0.99)	24.3; 0.94 (0.92, 0.97)	0.82	0.68
QuantiVac	35.2; 0.88 (0.86, 0.91)	64.7; 0.97(0.96, 0.99)	127; 0.95 (0.93, 0.97)	0.81	0.45

RESULTS

- Of the 269 convalescent plasma samples, 89 (33%) had MN NT values <20 (undetectable) and 117 (43%) >80 (high NT) (Table 1)
- Using the manufacturer's cutoffs, sensitivity for detection of samples with any NT ranged from 79% to 100%, and the false-positive rate (ie, classifying samples with undetectable NT as positive)
  - 72% to 84% for LFAs
  - 14% to 69% for the ELISAs
- For all assays except the ImmunoRank and NeutraLISA ELISAs, discrimination to identify samples with any NT was improved by raising the cut off values (Table 2)
- AUCs of ~0.94 to discriminate high NT samples could be achieved for all quantifiable assays using an adjusted cut off value
- Cohen's kappa statistic ranged from 0.20 to 0.69.
- Spearman's rank correlation between each assay and NT value ranged from 0.73 to 0.86
- Using the manufacturer's cutoffs, specificity on pre-pandemic samples was ≥98% for all assays except for CUSABIO which was 86%

CONCLUSIONS

The performance of immunoassays using manufacturer's cutoff to discriminate samples with any NT was accurate (AUC>0.83 for all assays), but could be improved by changing the cutoff. Identifying samples with high NT could be achieved using an alternative cutoff. Assays such as CUSABIO underperformed and resulted in increased false positive results within the pre-pandemic cohort. Others performed well at correctly classifying high NT samples.

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