Comparative assessment of multiple SARS-CoV-2 antibody and neutralization assays from blood samples in COVID-19 infected patients

Robin Dewar[1]; Christina Trevino[2]; Perrine Lallemand[1]; Helene Highbarger[1], Tarek Elbeik[2]; Tauseef Rehman[1]; Michael Holbrook[3]; Connie Schmaljohn[3]; Cliff Lane[4]; Aarthi Chary[2]; Mark Holodniy[2,5]

[1]Virus Isolation and Serology Laboratory, Leidos Biomedical Research, Inc., Frederick National Laboratory, Frederick, MD; [2]Public Health Surveillance and Research, Department of Veterans Affairs, Palo Alto, CA
[3]NIAID Integrated Research Facility, Frederick, MD; [4]Division of Clinical Research, NIAID, NIH, Rockville, MD; [5]Stanford University, Palo Alto, CA

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BACKGROUND

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, COVID-19) has caused a world-wide pandemic. Diagnosis is usually made by an RT-PCR test from a respiratory sample. Many assays are available for antibody detection or assessment, including rapid, enzyme immunoassays (EIA) and neutralization. However, characterization of the antibody immune response is not well documented and the clinical significance of COVID antibodies remains largely unknown. In addition, comparison of results across different assay formats using identical samples has not been rigorously studied, making clinical interpretation of serologic tests difficult.

METHODS

Serum or plasma samples collected from 4/14-9/3/2020 from patients who were positive for SARS-CoV-2 by EUA authorized RT-PCR assays from nasopharyngeal specimens and control serum samples collected from patients between 2007-2018, where tested with the following COVID-19 antibody tests: LFA rapid tests (RightSign IgM/IgG, BTNX Rapid Response IgM/IgG), and EIA tests (BioRad Platelia SARS-CoV-2 Total antibody-IgG/IgM/IgA assay; EuroImmun SARS-CoV-2 IgG, IgA, and IgM assays; and InBios IgM and IgG assays). Results were recorded as positive, negative, or equivocal. Additionally, SARS-CoV-2 antibody neutralization was assessed on matched samples as adapted from previously published work (1-2). Neutralizing titer was defined as the reciprocal of the highest dilution of serum or antibody which neutralized 50% (NT_{50}) or 100% (NT_{100}) of virus infected cells.

RESULTS

326 samples (range, 1-56) from 40 SARS-CoV-2 positive patients and 77 single control samples were tested. Average number of days serum was collected after RT-PCR positivity was 13 days (range -7 to 129 d). Sensitivity and Specificity for each assay and overall is presented in Table 1. Temporal concordance among IgG and IgM assays are presented in Table 2. Five patients were negative in all assays in serial samples collected within one week of PCR positivity.

Contact: Mark Holodniy, MD, Department of Veterans Affairs, Mark.holodniy@va.gov

RESULTS Cont'd

Antibody results (IgA, IgG and/or IgM) from the EIA or Lateral Flow assay were compared to matched plasma/serum used for a SARS-CoV-2 Neutralization Assay (n=121) from 30 COVID-19 diagnosed patients. Of the 19 samples with no detectable antibody (IgA, IgG and/or IgM) in any test, 8 had an NT_{50} ranging from 1:40 to 1:320 plasma dilution factor and no NT100 was detected at any dilution tested. Of 102 samples with detectable antibody (IgA, IgG and/or IgM) in any assay, 10 samples showed no NT_{50} or NT_{100} response, and 92 samples had an NT_{50} ranging from 1:40 to 1:1280 plasma dilution factor. Of these 92 samples, only 17 had an NT_{100} ranging from 1:40 to 1:160 plasma dilution factor.

Table 1. Sensitivity and Specificity Across Assays

BioRad		EuroImmun		InBios		BTNX		RightSign	
POSITIVES									
Total Ab (+):	268	IgG (+):	239	IgG (+):	266	IgG (+):	268	IgG (+):	244
Total Ab (-):	55	IgG (-):	84	IgG (-):	57	IgG (-):	58	IgG (-):	82
Sensitivity:	83%	Sensitivity:	74%	Sensitivity:	82%	Sensitivity:	82%	Sensitivity:	75%
TOTAL:	323	TOTAL:	323	TOTAL:	323	TOTAL:	326	TOTAL:	326
		IgM (+):	119	IgM (+):	241	IgM (+):	240	IgM (+):	199
		IgM (-):	204	IgM (-):	82	IgM (-):	86	IgM (-):	127
		Sensitivity:	37%	Sensitivity:	75%	Sensitivity:	74%	Sensitivity:	61%
		TOTAL:	323	TOTAL:	323	TOTAL:	326	TOTAL:	326
		IgA (+):	214						
		IgA (-):	108						
		Sensitivity:	67%						
		TOTAL:	320						
NEGATIVE CONTROLS									
Total Ab (+):	0	IgG (+):	2	IgG (+):	0	IgG (+):	0	IgG (+):	0
Total Ab (-):	64	IgG (-):	62	IgG (-):	64	IgG (-):	77	IgG (-):	77
Specificity:	100%	Specificity:	97%	Specificity:	100%	Specificity:	100%	Specificity:	100%
Total:	64	Total:	64	Total:	64	Total:	77	Total:	77
		IgM (+):	0	IgM (+):	0	IgM (+):	0	IgM (+):	0
		IgM (-):	64	IgM (-):	64	IgM (-):	77	IgM (-):	77
		Specificity:	100%	Specificity:	100%	Specificity:	100%	Specificity:	100%
		Total:	64	Total:	64	Total:	77	Total:	77
		IgA (+):	0						
		IgA (-):	64						
		Specificity:	100%						
		Total:	64						

Overall Sensitivities:

BioRad	83%
EuroImmun IgG	74%
EuroImmun IgM	37%
EuroImmun IgA	68%
InBios IgG	82%
InBios IgM	75%
BTNX IgG	82%
BTNX IgM	74%
RighSign IgG	75%
RightSign IgM	67%
Avg Sensitivity:	**71%**

Overall Specificities:

BioRad	100%
EuroImmun IgG	97%
EuroImmun IgM	100%
EuroImmun IgA	100%
InBios IgG	100%
InBios IgM	100%
BTNX IgG	100%
BTNX IgM	100%
RightSign IgG	100%
RightSign IgM	100%
Avg Specificity:	**99.7%**

Table 2. Temporal Concordance among IgM and IgG assays

		Number of IgM Assays Concordant (N=4)			
Days from First PCR Positive	N	4	3	2	1
(-7) - 2	32	81% (26)	16% (5)	0% (0)	3% (1)
3 - 5	28	54% (15)	18% (5)	14% (4)	14% (4)
6 - 10	24	46% (11)	33% (8)	17% (4)	4% (1)
11 - 15	42	48% (20)	40% (17)	5% (2)	7% (3)
16 - 20	33	30% (10)	52% (17)	18% (6)	0% (0)
21 - 25	29	28% (8)	52% (15)	21% (6)	0% (0)
26 - 30	30	40% (12)	53% (16)	3% (1)	3% (1)
31 - 35	20	25% (5)	60% (12)	10% (2)	5% (1)
36 - 40	17	24% (4)	71% (12)	6% (1)	0% (0)
41 - 45	9	0% (0)	56% (5)	44% (4)	0% (0)
46 - 50	14	21% (3)	21% (3)	57% (8)	0% (0)
51 - 55	10	0% (0)	20% (2)	70% (7)	10% (1)
56 - 129	12	17% (2)	8% (1)	25% (3)	50% (6)

		Number of IgG Assays Concordant (N=4)			
Days from First PCR Positive	N	4	3	2	1
(-7) - 2	32	91% (29)	3% (1)	0% (0)	6% (2)
3 - 5	28	75% (21)	7% (2)	4% (1)	14% (4)
6 - 10	24	58% (14)	4% (1)	21% (5)	17% (4)
11 - 15	42	79% (33)	17% (7)	0% (0)	5% (2)
16 - 20	33	67% (22)	21% (7)	12% (4)	0% (0)
21 - 25	29	66% (19)	28% (8)	7% (2)	0% (0)
26 - 30	30	87% (26)	13% (4)	0% (0)	0% (0)
31 - 35	20	95% (19)	5% (1)	0% (0)	0% (0)
36 - 40	17	100% (17)	0% (0)	0% (0)	0% (0)
41 - 45	9	100% (9)	0% (0)	0% (0)	0% (0)
46 - 50	14	93% (13)	7% (1)	0% (0)	0% (0)
51 - 55	10	100% (10)	0% (0)	0% (0)	0% (0)
56 - 129	12	100% (12)	0% (0)	0% (0)	0% (0)

CONCLUSIONS

- Overall specificity across assays was 99%
- Overall sensitivity across assays was 71%; and ranged from 37%-83%; reflective of sample timing, with IgG testing and certain platforms performing better than others
- IgG results were more concordant across assays and across time than IgM assays
- Among tested samples, neutralization titer was low and may reflect disease outcome

LIMITATIONS

- Limited number of inpatient-only COVID-19 infected patients; some had only one sample tested
- Heterogeneous timepoint intervals sampled across patients
- Reduced sample volume limited testing some samples with all assays
- Not all samples were tested for NT

REFERENCES

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