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Research Article

Expanded Clinical Evaluation of the CLUNGENE® Rapid COVID-19 Antibody Test

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ABSTRACT

Background: COVID-19 antibody testing has been shown to be predictive of prior COVID-19 infection and an effective testing tool [1]. The CLUNGENE® SARS-COV-2 VIRUS (COVID-19) IgG/IgM Rapid Test Cassette was evaluated for its utility to aide healthcare professionals.

Method: Two studies were performed using the CLUNGENE® Rapid Test: 1. An expanded Point-of-Care (POC) study at two clinical sites to evaluate 99 clinical subjects: 62 positive subjects and 37 negative subjects were compared to RT-PCR, PPA, and NPA (95% CI). Sensitivity was calculated from blood collection time following symptom onset. 2. A cross-reactivity study was performed to determine the potential for false positive results from other common infections.

Results: Specificity of subjects with confirmed negative COVID-19 by RT-PCR was 100% (95% CI, 88.4%-100.0%). Sensitivity of subjects with confirmed positive COVID-19 by RT-PCR was 96.77% (95% CI, 88.98%-99.11%). In the cross-reactivity study, there were no false positive results due to past infections or vaccinations unrelated to the SARS-CoV-2 virus.

Conclusion: There is a need for a rapid, user-friendly, and inexpensive on-site monitoring system for diagnosis. The CLUNGENE® Rapid Test is a useful diagnostic test provides results within 15 minutes without high complexity laboratory instrumentation.

Keywords: COVID-19 Antibody testing; COVID-19 Immunity; COVID-19 serology; CLUNGENE®

INTRODUCTION

The COVID-19 pandemic SARS-CoV$_2$ COVID-19 has infected over 140 million people worldwide and caused approximately 3.89 million deaths as of June 23, 2021 [1,2], although some studies suggest that the actual number of global COVID-19 deaths may be about 6.9 million, more than double the recorded amount [3-5]. In response to the pandemic, the US Food and Drug Administration (FDA) authorized the use of COVID-19 serological tests through Emergency Use Authorizations (EUA) to make COVID-19 *in vitro* diagnostic tests widely available to help identify individuals with an adaptive immune response indicating recent or prior infection [6]. Serology tests or Immunoassays play a significant role in the fight against COVID-19 [7,8]. A prior history of SARS-CoV-2 infection is associated with a lower risk of infection, with an estimated protective effect of up to seven (7) months following primary infection; this supports the conclusion that convalescent plasma with specific antibodies to SARS-CoV-2 has powerful antiviral activity which can reduce the viral load and mortality in patients with active COVID-19 infection [9-11].

There is an urgent need for a rapid, user-friendly, and inexpensive on-site monitoring system for diagnosis [12]. The CLUNGENE® SARS-COV-2 VIRUS (COVID-19) IgG/IgM Rapid (15 minute) Test Cassette has been commercially available in the U.S. under an FDA approved Emergency Use Authorization (EUA201121) [13]

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and Europe (CE Mark reference 02PBJ267 dated March 9, 2020). The CLUNGENE® test has been previously studied, including the use of the test in the offices of general practitioners, evaluating the presence of antibodies in convalescent plasma donors, and how the test performs at a point of care facility [14,15]. The aim of this research is to better understand the sensitivity and specificity of the CLUNGENE® assay and the potential for false positive results related to infections or vaccinations not linked to the SARS-CoV-2 virus.

MATERIAL AND METHODS

Study-1

Design: In an initial study, a single Point-of-Care (POC) facility was used to estimate the sensitivity and specificity of the CLUNGENE® test [16]. The study was expanded to a second independent site. The two sites were Sharp Healthcare, a not-for-profit multi-center regional health care group located in San Diego, CA and Alivio Medical Center, an urgent care/primary care center in Indianapolis, IN. The study was approved by the Sharp HealthCare Institutional Review Board (study identifier: IgG/IgM COVID19 2005801). Samples used for RT-PCR were nares swabs. Positive subjects were symptomatic for SAR-CoV-2 Virus infected and confirmed with RT-PCR positive tested nares swabs. Negative subjects were asymptomatic, from high-risk areas, and confirmed with negative RT-PCR tested nares swabs. Finger prick whole blood samples were used for SARS-COV-2 Virus IgG/IgM detection. The comparator method for RT-PCR were either Cobas Roche SARS-COV_2 RT-PCR or Thermo Fisher TaqPath COVID-19 Combo Kit. Trained operators with no prior information about each subject drew samples. Subject inclusion criteria included individuals with a confirmed COVID-19 test result by SARS-CoV-2 RT-PCR. Subjects were excluded if they were unable to provide informed consent due to mental or cognitive disabilities.

Methods: The CLUNGENE® Point-of-Care test was run according to the manufacturer's instructions. See Figure 1. The test result was read after 15 minutes. Days from symptom onset were captured from an electronic medical record which documented self-reported data from patients reporting on the number of days they had been sick at time of study enrollment.

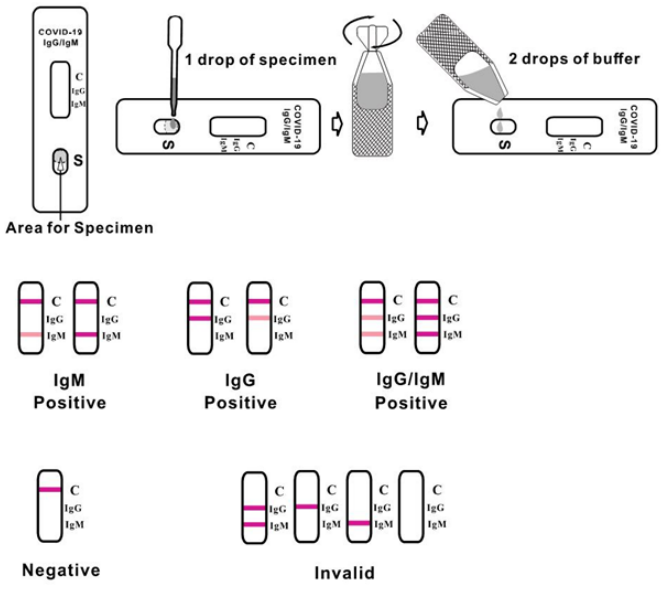

Figure 1: CLUNGENE® Point-of-Care test manufacturer's instructions.

Categorical variables were compared using the chi-squared or Fisher exact test, and continuous variables were compared using the Student t-test or Mann-Whitney U test, as appropriate. All tests were two tailed, and $p<0.05$ was considered statistically significant. SPSS Statistics, IBM SPSS software, version 27.0 (SPSS, Inc., Chicago, IL) was used for all calculations.

Results: An analysis was run on 99 patients who completed the study (Tables 1a-1d).

Thirty-seven (37) patients who had negative COVID-19 RT-PCR were tested and found antibody negative using the CLUNGENE® test (95% CI, 90.60%-100.00%). Three (3) out of four (4) or 75% of RT-PCR positive COVID-19 subjects tested prior to day 7 of symptom onset were antibody positive (95% CI, 30.06%-95.44%). Twenty-three (23) or 100% of RT-PCR positive COVID-19 subjects tested positive between day 8 and 14 from symptom onset (95% CI, 85.69%-100.00%). 33 out of 35 or 94.28% of RT-PCR positive COVID-19 subjects tested positive after day 14 from symptom onset were antibody positive (95% CI, 30.06%-95.44%). In all 62 patients with confirmed COVID-19 with RT-PCR, the combined sensitivity of IgM and IgG was 96.77% (95% CI, 88.98%-99.11%), meaning there as 96.77 positive agreement between a positive RT-PCR test and a positive antibody test. The specificity was 100% (95% CI, 88.4-100.0%), meaning there was 100% agreement between a negative RT-PCR test and 100% negative antibody result. These results are displayed in Tables 2a and 2b.

The positive predictive value can be calculated but the result is dependent on the prevalence of disease in the community. If a test for a disease has 96.77% sensitivity and 100% specificity, and the disease prevalence is 10%, the positive predictive value (PPV) is 100% and the negative predictive value (NPV) is 99.64%. At the time of this publication, the positivity rate for the nares SARS-CoV-2 RT-PCR tests was between 8.2% to 10% in San Diego, CA (San Diego County) and Indianapolis, IN (Marion County) [17]. Both PPV and NPV were calculated using a free statistical calculator from MedCalc [18].

Study-2

Design: An initial cross-reactivity study was performed in which various common infectious agents were tested for potential false positive results (Table 3). All tests were negative. In addition, as recommended by the US Food and Drug Administration [19], a follow up study tested the cross-reactivity of the CLUNGENE® device to antibodies to common coronaviruses that are not SARS-CoV-2: Anti-229E, anti-NL63, anti-OC43, anti-HKU1; and those for which there is a high rate of vaccinations and/or infection in the US: anti-Haemophilus influenzae IgG and IgM. The testing was performed at Medcomp Sciences, an independent clinical medical laboratory [20].

Methods: The CLUNGENE® Point-of-Care test was run according to same manufacturer's instructions as was performed with study #1. Samples (50) used for the cross-reactivity study were obtained from Trina Bioreactives, Ag [21]. The samples were collected under a protocol approved by the ethics committee of the National Medical Association, Baden-Wurttemburg, Germany (file #F-2012-027, "Plasma Samples for Studies").

Test results were read after 15 minutes. Five (5) serum samples positive for IgM and five for IgG were analyzed in three (3) separate

Table 1a: IgG positive percent agreement.

Days post symptom onset	#PCR total positive	Candidate Device Results		
		IgG positive results	IgG PPA	95% CI
0~7	4	2	50.00%	15.00%-85.00%
8~14	23	23	100.00%	85.69%-100.00%
≥ 15	35	33	94.28%	81.39%-98.42%
Total	62	58	93.55%	84.55%-97.46%

Table 1b: IgM positive percent agreement.

Days post symptom onset	#PCR total positive	Candidate Device Results		
		IgM positive results	IgM PPA	95% CI
0~7	4	3	75.00%	30.06%-95.44%
8~14	23	21	91.30%	73.21%-97.58%
≥ 15	35	27	77.14%	60.98%-87.93%
Total	62	51	82.26%	70.96%-89.79%

Table 1c: IgG/IgM combined antibody positive percent agreement.

Days post symptom onset	#PCR total positive	Candidate Device Results		
		IgG/IgM combined antibody positive results	IgG/IgM combined antibody PPA	95% CI
0~7	4	3	75.00%	30.06%-95.44%
8~14	23	23	100.00%	85.69%-100.00%
≥ 15	35	34	97.14%	85.47%-99.49%
Total	62	60	96.77%	88.98%-99.11%

Table 1d: Negative percent agreement.

#PCR total negative results	Candidate Device Results		
	IgG negative results	IgG NPA	95% CI
37	37	100.00%	90.60%-100.00%

Table 2a: Line table data site 1.

No.	Subject ID	Age	Gender (F/M)	Whole blood specimen collection date	Days after symptom onset	CLUNGENE® rapid test result (Pos/Neg)		PCR test date	PCR confirmation result
						IgM	IgG		
1	007sgh	66	F	2020.06.01	/	Neg	Neg	2020.05.30	Neg
2	008sgh	65	F	2020.06.01	/	Neg	Neg	2020.05.29	Neg
3	009sgh	72	M	2020.06.01	/	Neg	Neg	2020.05.31	Neg
4	010sgh	67	F	2020.06.05	/	Neg	Neg	2020.05.27	Neg
5	011sgh	44	M	2020.06.05	/	Neg	Neg	2020.06.01	Neg
6	012sgh	31	F	2020.06.05	/	Neg	Neg	2020.06.01	Neg
7	013sgh	77	F	2020.06.05	/	Neg	Neg	2020.06.01	Neg
8	024smh	37	M	2020.06.05	11	Neg	Pos	2020.06.02	Pos
9	025sgh	89	M	2020.06.05	/	Neg	Neg	2020.06.11	Neg
10	023sgh	91	F	2020.06.06	/	Neg	Neg	2020.06.09	Neg
11	024sgh	81	M	2020.06.08	/	Neg	Neg	2020.06.09	Neg
12	026sgh	59	M	2020.06.08	/	Neg	Neg	2020.06.11	Neg
13	019sgh	69	F	2020.06.09	20	Neg	Pos	2020.05.23	Pos
14	028sgh	58	M	2020.06.10	/	Neg	Neg	2020.06.11	Neg
15	021sgh	63	F	2020.06.11	13	Neg	Pos	2020.06.06	Pos
16	027sgh	77	F	2020.06.11	/	Neg	Neg	2020.06.01	Neg
17	029sgh	29	M	2020.06.12	8	Pos	Pos	2020.06.08	Pos
18	033sgh	69	M	2020.06.12	/	Neg	Neg	2020.06.05	Neg
19	034sgh	53	M	2020.06.12	/	Neg	Neg	2020.06.11	Neg

20	035sgh	77	M	2020.06.12	/	Neg	Neg	2020.06.11	Neg
21	036sgh	74	M	2020.06.12	/	Neg	Neg	2020.06.10	Neg
22	037sgh	30	F	2020.06.12	/	Neg	Neg	2020.06.09	Neg
23	038sgh	22	M	2020.06.12	/	Neg	Neg	2020.06.06	Neg
24	001sgh	38	M	2020.06.15	26	Pos	Pos	2020.05.23	Pos
25	006sgh	53	M	2020.06.18	23	Neg	Pos	2020.05.29	Pos
26	027smh	47	M	2020.06.18	14	Pos	Pos	2020.06.14	Pos
27	039sgh	60	M	2020.06.18	13	Pos	Pos	2020.06.14	Pos
28	043sgh	22	F	2020.06.18	18	Neg	Pos	2020.06.14	Pos
29	040sgh	70	M	2020.06.19	17	Pos	Pos	2020.06.12	Pos
30	044sgh	32	F	2020.06.19	/	Neg	Neg	2020.06.17	Neg
31	045sgh	66	M	2020.06.19	/	Neg	Neg	2020.06.10	Neg
32	046sgh	65	M	2020.06.19	/	Neg	Neg	2020.06.09	Neg
33	050sgh	31	M	2020.06.26	8	Pos	Pos	2020.06.18	Pos
34	051sgh	44	M	2020.06.26	10	Pos	Pos	2020.06.21	Pos
35	052sgh	35	M	2020.06.26	7	Pos	Pos	2020.06.19	Pos
36	055sgh	57	M	2020.06.26	/	Neg	Neg	2020.06.21	Neg
37	056sgh	69	F	2020.06.26	/	Neg	Neg	2020.06.22	Neg
38	057sgh	67	M	2020.06.26	/	Neg	Neg	2020.06.21	Neg
39	058sgh	59	F	2020.06.26	/	Neg	Neg	2020.06.17	Neg
40	059sgh	39	M	2020.06.26	6	Neg	Neg	2020.06.20	Pos
41	060sgh	28	F	2020.06.26	/	Neg	Neg	2020.06.24	Neg
42	061sgh	51	F	2020.06.26	/	Neg	Neg	2020.06.23	Neg
43	062sgh	71	F	2020.06.26	11	Pos	Pos	2020.06.23	Pos
44	072sgh	62	M	2020.07.02	17	Pos	Pos	2020.06.15	Pos
45	031smh	31	F	2020.07.02	22	Pos	Pos	2020.06.11	Pos
46	066sgh	43	F	2020.07.02	15	Pos	Neg	2020.06.24	Pos
47	068sgh	60	M	2020.07.02	12	Pos	Pos	2020.06.25	Pos
48	075sgh	62	F	2020.07.02	7	Pos	Pos	2020.06.25	Pos
49	081sgh	73	M	2020.07.09	9	Pos	Pos	2020.07.04	Pos
50	082sgh	63	M	2020.07.09	11	Pos	Pos	2020.07.03	Pos
51	084sgh	68	M	2020.07.09	15	Pos	Pos	2020.07.02	Pos
52	088sgh	56	F	2020.07.09	10	Pos	Pos	2020.07.07	Pos
53	090sgh	88	F	2020.07.09	20	Neg	Pos	2020.07.07	Pos
54	089sgh	56	F	2020.07.17	16	Pos	Pos	2020.07.04	Pos
55	094sgh	19	F	2020.07.17	15	Pos	Pos	2020.07.11	Pos
56	096sgh	36	M	2020.07.17	27	Pos	Pos	2020.07.14	Pos
57	097sgh	unknown	F	2020.07.17	/	Neg	Neg	2020.07.14	Neg
58	098sgh	unknown	M	2020.07.17	/	Neg	Neg	2020.07.08	Neg
59	099sgh	48	F	2020.07.21	9	Pos	Pos	2020.07.18	Pos
60	100sgh	48	F	2020.07.21	14	Pos	Pos	2020.07.17	Pos
61	101sgh	72	M	2020.07.21	16	Neg	Neg	2020.07.25	Pos
62	053sgh	50	F	2020.07.23	27	Neg	Pos	2020.06.26	Pos
63	073sgh	38	M	2020.07.23	34	Pos	Pos	2020.06.30	Pos

64	103sgh	29	M	2020.07.24	12	Pos	Pos	2020.07.15	Pos
65	104sgh	40	M	2020.07.24	21	Pos	Pos	2020.07.11	Pos
66	106sgh	45	M	2020.07.28	15	Neg	Pos	2020.07.22	Pos
67	113sgh	46	F	2020.07.31	7	Pos	Neg	2020.07.25	Pos
68	114sgh	79	F	2020.08.04	12	Pos	Pos	2020.07.30	Pos
69	110sgh	51	F	2020.08.05	15	Pos	Pos	2020.07.29	Pos
70	109sgh	63	M	2020.08.07	19	Pos	Pos	2020.07.25	Pos
71	119sgh	60	F	2020.08.08	10	Pos	Pos	2020.08.07	Pos
72	105sgh	38	M	2020.08.12	31	Pos	Pos	2020.07.20	Pos
73	120sgh	73	M	2020.08.13	22	Pos	Pos	2020.07.26	Pos
74	123sgh	47	M	2020.08.13	12	Pos	Pos	2020.08.10	Pos
75	124sgh	80	M	2020.08.13	30	Pos	Pos	2020.07.20	Pos
76	118sgh	94	F	2020.08.14	15	Neg	Pos	2020.07.31	Pos
77	117sgh	28	F	2020.08.14	14	Pos	Pos	2020.08.05	Pos
78	121sgh	62	F	2020.08.17	14	Pos	Pos	2020.08.12	Pos
30.06%-	30.06%-	30.06%-	30.06%-	30.06%-	30.06%-	30.06%-	30.06%-	30.06%-	30.06%-

Table 2b: Line table data site 2.

No.	Subject ID number	Age	Gender (F/M)	Whole blood specimen collection date	Days after symptom onset	CLUNGENE® rapid test result (Pos/Neg)		PCR test date	PCR confirmation result
						IgM	IgG		
1	C011	29	F	2020.12.12	14	Pos	Pos	2020.11.30	Pos
2	A005	44	F	2020.12.12	/	Neg	Neg	2020.12.06	Neg
3	C020	30	F	2020.12.12	14	Pos	Pos	2020.11.28	Pos
4	C012	52	M	2020.12.14	14	Pos	Pos	2020.12.05	Pos
5	C032	46	M	2020.12.14	/	Neg	Neg	2020.12.10	Neg
6	C033	18	M	2020.12.14	/	Neg	Neg	2020.12.10	Neg
7	C004	74	M	2020.12.19	26	Pos	Pos	2020.12.03	Pos
8	C030	28	M	2020.12.19	/	Neg	Neg	2020.12.16	Neg
9	C016	39	F	2020.12.21	19	Pos	Pos	2020.12.10	Pos
10	C031	18	F	2020.12.21	/	Neg	Neg	2020.12.16	Neg
11	C009	53	F	2020.12.29	18	Pos	Pos	2020.12.19	Pos
12	C010	55	M	2020.12.29	19	Pos	Pos	2020.12.19	Pos
13	C028	46	M	2020.12.29	24	Pos	Pos	2020.12.18	Pos
14	C005	19	F	2020.12.31	21	Pos	Pos	2020.12.18	Pos
15	C006	19	F	2020.12.31	21	Pos	Pos	2020.12.18	Pos
16	C007	42	F	2020.12.31	21	Pos	Pos	2020.12.18	Pos
17	C019	42	F	2020.12.31	15	Pos	Pos	2020.12.18	Pos
18	C026	51	F	2020.12.31	/	Neg	Neg	2020.12.31	Neg
19	C027	43	F	2020.12.31	/	Neg	Neg	2020.12.31	Neg
20	C029	54	M	2020.12.31	15	Pos	Pos	2020.12.26	Pos
21	C013	40	M	2021.01.04	28	Pos	Pos	2020.12.15	Pos

Table 3: Cross-reactivity study using the below list of common infectious agents.

Analog
Anti-HBe, anti-HBc and HBsAg
Anti- Hepatitis C Virus (HCV)
Anti-Human Immunodeficiency Virus (HIV)-1
Anti-HIV-2
Anti-influenza A IgG
Anti-influenza B IgG
Anti-influenza A IgM
Anti-influenza B IgM
Anti- Respiratory syncytial virus (RSV) IgG
Anti- Respiratory syncytial virus (RSV) IgM
Anti- Mycoplasma pneumoniae (MP) IgM
Anti- Chlamydia pneumoniae (CP) IgM
Human parainfluenza virus PCR positive (Paired Convalescent)
Anti- Treponema pallidum (TP)
Rheumatoid factor 122.00 IU/mL Rheumatoid factor 125.00 IU/mL Rheumatoid factor 144.00 IU/mL Rheumatoid factor 146.00 IU/mL Rheumatoid factor 158.00 IU/mL Rheumatoid factor 197.00 IU/mL Rheumatoid factor 310.00 IU/mL Rheumatoid factor 342.50 IU/mL Rheumatoid factor 347.00 IU/mL Rheumatoid factor 500.00 IU/mL Rheumatoid factor 521.00 IU/mL Rheumatoid factor 565.00 IU/mL Rheumatoid factor 796.00 IU/mL Rheumatoid factor 825.00 IU/mL
Antinuclear antibodies (ANA) 1:240
Common human pathogenic coronaviruses: HCoV -HKU1 HCoV -NL63 HCoV -OC43 HCoV -229E

CLUNGENE® Point-of-Care test lots with each of the below antibodies:

- anti-Haemophilus influenzae IgM
- anti-Haemophilus influenzae IgG
- IgG, anti-coronavirus 229E IgG
- anti-coronavirus NL63 IgG
- anti-coronavirus OC43 IgG
- anti-coronavirus HKU1 IgG
- anti-coronavirus 229E IgM
- anti-coronavirus NL63 IgM
- anti-coronavirus OC43 IgM
- anti-coronavirus HKU1 IgM

Cross reactivity was determined using Beckman-Coulter UniCel DxI Access Immunoassay System is an *in vitro* diagnostic device used for the quantitative, semi-quantitative, or qualitative determination of various analyte concentrations found in human body fluids (Table 4).

All Quality Control (QC) materials are supplied and used as indicated by the manufacturer. A minimum of 2 levels of QC are processed per run in accordance with Westgard rules for 6-sigma quality requirements. QC results must fall within 3 standard deviations of historical data as recorded in Levy-Jennings plots by the system. All QC results are documented and verified by Clinical Laboratory Scientist before processing samples (Table 5).

Qualitative results are reported as Positive/Reactive or Negative/Non-Reactive, depending on whether the analyte in question is above or below the established signal cut-off value (S/CO). Each analyte tested may have a different cut-off value, but each cut-off value is determined during calibration of the instrument, using the value of the calibrator.

Results: The test results of negative quality control samples were all negative, and the test results of the positive quality control samples were all positive. The consistency rate of cross reactivity of negative samples was 100%.

RESULTS AND DISCUSSION

In the first study, the performance characteristics of CLUNGENE® were evaluated and showed a specificity of 100% and a sensitivity of 96.77%. In the second study, there were no false positive results

Table 4: Reagents: Ref# C58961 and C58957 Beckman Coulter immunoassay system.

Reagent	Reference number	Material type
Access SARS-CoV-2 IgG calibrator	Ref. No. C58963	
Access SARS-CoV-2 IgG calibrator	Ref. No. C58958	
Access SARS-CoV-2 IgG QC	Ref. No. C58964	Quality Control (QC)
Access SARS-CoV-2 IgM QC	Ref. No. C58959	Quality Control (QC)
Access substrate	Ref. No. 81906	Quality Control (QC)
Access wash buffer II	Ref. No. A16792	Quality Control (QC)
UniCel DxI wash buffer II	Ref. No. A16793	Quality Control (QC)

Table 5: Quality control for DXI600 analysis of cross reactivity.

Rack/Pos.	Sample ID patient/Lot ID	Type dilution	Test	Calibrator results[1]	RLUs[2]	Completion	Reagent
207/1	COV-2IgG QC1 922605	Serum	COV2G	Non-Reactive	0.02 S/CO	7143	04/27/2021 10:20 AM
207/2	COV-2IgG QC2 922605B	Serum	COV2G	Reactive	2.49 S/CO	995255	04/27/2021 10:20 AM
207/3	COV-2IgM QC1 922821	Serum	COV2M	Non-Reactive	0.16 S/CO	11220	04/27/2021 10:27 AM
			COV2M(2)	Non-Reactive	0.16 S/CO	11053	04/27/2021 10:27 AM
207/4	COV-2IgM QC2 922821	Serum	COV2M	Reactive	4.23 S/CO	301471	04/27/2021 10:28 AM
			COV2M(2)	Reactive	2.62 S/CO	186854	04/27/2021 10:27 AM

Abbreviations: [1]S/CO: Signal/Cutoff; [2]RLUs: Relative Light Units

due to past infections or vaccinations unrelated to the SARS-CoV-2 virus. These results are in line with the new European Commission's Medical Device Coordination Group requirements for rapid COVID-19 antibody tests and consistent with previously published results [22-26].

Antibody testing is a useful aid to confirm past infection [27]. Recent findings confirm that antibody testing is predictive of prior COVID-19 infection, and rapid screening methods--even from finger pricks--are effective testing tools [1]. However, we see the potential for a much broader use and recommend a combined approach that uses both RT-PCR and serological testing. The advantage of the CLUNGENE® antibody test is its simplicity since there is no need for specialized laboratory personnel to perform and interpret results. The low rate of false positivity makes this test ideal to rule in disease and eliminate the need for further RT-PCR testing if seroconversion occurs since the CLUNGENE® antibody test can diagnose most infected COVID-19 patients. If the test is negative a recommendation should be made to have a follow up RT-PCR test.

Serology testing also has the potential to monitor presence of antibodies. Studies confirm that a prior history of SARS-CoV-2 infection is associated with lower risk of infection with an estimated seven (7) month protective effect [9,10]. The association of SARS-CoV-2 Seropositive Antibody Test with Risk of Future Infection has now been established [28]. It is clear that titers of IgM and IgG antibodies against the receptor-binding domain (RBD) of the spike protein of SARS-CoV-2 decrease significantly between one (1) and seven (7) months and concurrently, neutralizing activity decreases [29]. Monitoring the presence of antibodies from past infection could assist health care professionals to assess likely presence of neutralizing activity and immunity when managing patient care.

Given widespread availability, COVID-19 serology testing, similar to other infectious diseases, can now become routine [30]. Consideration should be given to adding COVID-19 antibody testing to the WHO List of Essential *In vitro* Diagnostics (EDL) which now consists of 122 test categories, including most serious infectious diseases [31]. A rapid 15-minute COVID-19 assay offers the ability to do this testing quickly and efficiently—tests are now available for less than $5 and routinely reimbursed in the U.S. by public and private insurers [6].

In addition, the ability of the CLUNGENE® antibody test to detect antibodies to the coronavirus's spike protein's receptor binding domain means it has the potential to assess the efficacy of most vaccines as well as convalescent plasma therapy [32]. Countries in Europe are now using antibody testing to determine of a second COVID-19 vaccine dose is required if a patient has a prior infection based on a positive antibody test [33]. Recently airports and Blood Banks have been providing COVID-19 antibody testing services to determine whether a person has developed immunity to COVID-19 through vaccination or through contracting the virus previously [34,35]. Some countries, including China, require an antibody test. Limited evaluation of the CLUNGENE® antibody test has confirmed positive antibody test results following patients who have been vaccinated [36]. Pfizer's recent data suggests that its vaccine is efficacious for only 6 months and that a third shot within 12 months is likely needed [37]. Furthermore, there is a potential issue regarding vaccine efficacy for recipients who do not received a full dose; the US CDC estimates that 11% of vaccine who received a first dose did not receive a recommended second dose as of June 16, 2021 [38,39]. COVID-19 vaccination also fails to stimulate an immune response in many blood cancer patients or those otherwise immunocompromised [40,41]. Additional studies are needed to confirm the efficacy of serology testing to monitor vaccine effectiveness.

LIMITATIONS

Limitations of the study include a small sample size from two geographic areas. The study also did not include special groups such as pregnant women or children. The subjectivity of symptom reporting by patients can be a confounding factor in determining the duration of illness. Some patients may have been symptomatic for a different time period than they recalled.

CONCLUSION

In a pandemic crisis with significant economic and health implications, this study confirms the utility of serological testing for COVID-19 disease diagnosis providing rapid test results with a relatively high degree of sensitivity and specificity. Furthermore, given recent data regarding the relationship between positive serology and immunity, routine testing can be a useful tool to monitor antibody status for optimal patient care. Tests such as the CLUNGENE® SARS-COV-2 VIRUS (COVID-19) IgG/IgM Rapid Test Cassette can assist health care professionals to help identify individuals with an adaptive immune response indicating recent or prior infection as intended by the US FDA under an EUA.

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INSTITUTIONAL REVIEW BOARD STATEMENT

This was a formal IRB approved clinical study conducted within Sharp Healthcare, a not-for-profit multicenter regional health care group located in San Diego, California. Subjects were included if hospitalized or recently discharged following a SARS-CoV-2 RT-PCR nares test. A study protocol and an informed consent were initiated and approved by the Sharp Institutional Review Board. Subjects were included if >18 years of age and understood the study and its requirements. Patients who had impairment of cognition or decision-making capacity were excluded. Subjects were screened by research coordinators to determine if they had a nares SARS-CoV-2 RT-PCR test result and then consent was requested to enroll in the study.

INFORMED CONSENT STATEMENT

"Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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CONFLICTS OF INTEREST

Christopher C. Lamb, PhD, has worked with the manufacturers of SARS-CoV-2 tests for Emergency Use Authorization submissions to the US FDA. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing

of the manuscript, or in the decision to publish the results.

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