



Research Article

S-IgG Assay is a Good Replacement of the High Risk, Time Costly Virus Neutralization Assay

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Abstract

Coronavirus disease (COVID-19) is caused by a newly discovered virus, SARS-COV-2. About 21 million people were infected and 766 thousand confirmed death. The only hope left is vaccine. Whether a vaccine could work or not is a problem waiting for answer. To find the best method that could predict the protective immunity after SARS-COV-2 virus infection, a comparative study of one virus neutralizing experiment and four ELISA kits was conducted. In one hospital from 164 Covid-19 patients that were discharged, 97.56% serum had neutralization activity and 93.29% had anti-S IgG. In another group of 184 persons that had closely contacted with covid-19 patients, 35.9% had neutralization activity and 34.7% had anti-S IgG. The S-IgG assay shows 93.5% agreement with the virus neutralization experiment, which indicates that more than 90% SARS-COV-2 virus neutralization activity is related to the S protein IgG. S-IgG assay could be used to replace the high risk, time costly virus neutralization assay.

Keywords: COVID-19; Immunity; Pandemic

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Citation: Liang L, Huang H, Zheng H, Zhang H, Zhou H, et al. (2020) S-IgG Assay is a Good Replacement of the High Risk, Time Costly Virus Neutralization Assay. J Clin Immunol Immunother 6: 042.

Received: September 16, 2020; **Accepted:** October 02, 2020; **Published:** October 09, 2020

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Introduction

While the worldwide spreading of SARS-COV-2 virus continues, the appearance of population immunity is expected after infection whether symptomatic or not. There are two options for preventing COVID-19 outbreaks. One is the social segregation model adopted by China. Everyone was required to stay at home for one to two months. Another one is the herd immunity model adopted by some European countries [1]. Not long ago, British Prime Minister Boris Johnson (healed after being infected with this virus) and his government scientific adviser publicly announced that herd immunity may be the ultimate methods to end this pandemic. But if the battle will be won by spreading immunity, which method could be used to detect the immunity after infection? Can we issue a safe passport while this immunity tests are positive? These are questions without an answer yet. The search for a rapidly available, cost-sensitive and accurate test remains active.

The sensitivity of virus nucleic acid detection is affected by sampling, operation, and the quality of detection reagents. Only about 40-70% of infected people test positive [2]. Especially those with mild and asymptomatic infections, which are difficult to be monitored [3]. Moreover, the detection of viral nucleic acid can only prove the presence of the virus, and does not necessarily reflect the patient's immune status. Immunity to viruses is divided into cellular immunity and humoral immunity. Cellular immunity is composed of memory T cells, NK cells and macrophages and has not been studied in this article. Our research focuses on serological antibody testing and virus neutralization.

The serum virus neutralization assay is a serological test to detect the presence and functionality of the antibodies. It only detects antibodies that can block virus replication. Although neutralization assay is a highly sensitive and specific test, it only could be carried in a bio safety level 3 facility because of the handling of an active virus and it requires 5 to 7 days for the cell culture and virus amplification. It is for all these that could not be used as screening test.

Currently, there are many antibody detection methods on the market, ELISA, colloidal gold, or chemiluminescence [4,5]. Detection antibodies include IgG, IgM, IgA and total antibodies [6-9]. The antigen used for detection is mainly N proteins and S proteins of the virus. For the S proteins could be divided into full-length and fragments of receptor binding domain [4]. Since there is no unified standard, it is difficult to analyze and compare the test results. It is unclear which method we should use to reflect the real immunity to the virus [7,8].

To answer these questions, this study compared four commercially available kits, the viral N protein IgG and IgM ELISA kit, the viral S protein IgG and IgM ELISA kit; and a virus neutralization experiments.

Materials and Methods

Recruitment of patients and specimen collection

Samples were collected from CDC. Totally there are 164 samples from positive Covid-19 patients confirmed by RT-PCR, male 94 (age range 1 to 69) and female 70 (age range 2 to 75). Covid-19 patient's closely contacted groups have 184 samples, male 102 (age range 1 to 85) and female 82 (age range 10 months to 80). Health control group have 300 samples, male 269 (age 11-59) and female 31 (age range 21 to 62). All samples were leftover serums of other experiments.

COVID-19 S-IgG and S-IgM ELISA ASSAY kits were provided from Leide Biosciences Co., Ltd., (#2022-96 and #2023-96)

S protein was expressed in 293T cells and purified by Ni-sepharose. The purified S protein-based ELISA kits were used for the detection of IgG and IgM antibody against SARS-CoV-2 S protein. 5µl serum samples were diluted to 500µl and 100µl were used for test. According to the instruction of the kits, the cutoff value of S-IgG and S-IgM both were 0.3 of OD 450-630.

COVID-19 N-IgG and N-IgM ELISA ASSAY kits were provided by Zhongshan Biological Engineering Co., Ltd (# Y20200304). The recombinant nucleocapsid (rN) protein based ELISA kits were used for the detection of IgG and IgM antibody against SARS-CoV-2 N protein. According to the instruction of the kits, the cutoff value of N-IgM and N-IgG were 0.15 and 0.3 respectively. When A450-630 was below the cutoff value, the test was considered negative, and when A450-630 was greater than or equal to the cutoff value, the test was considered positive.

Vero-E6 cell line purchased from ATCC (CRL-1586)

SAS-COV2 virus come from Guangdong CDC (Gisaid library code is EPI_ISL_403934. Named as 20SF014/vero-E6/3. Plate reader iMark is from Bio-Rad.

Neutralization experiment

The SARS-COV-2 virus 20SF014/vero-E6/3 is isolated from a COVID-19 patient in Shenzhen in February. Stock virus was amplified by grown in Vero E6 cells. The virus stock was titrated by serial dilute by 10-fold. A confluent monolayer of Vero E6 cells was infected with 100 µl of each dilution in quadruplicate in 96-well plates. Cytopathic effect (CPE) was observed under microscope seven days after inoculation. The endpoint dilution leading to CPE in 50% of inoculated wells was designated as one 50% tissue culture infecting dose (TCID50).

Serial four-fold dilutions of heat-inactivated sera were made. The serum dilutions 240µl were mixed with equal volumes of 100 µl TCID 50 of SARS-CoV-2 as indicated. After 2 h of incubation at 37°C, 5% CO2 incubator, 100 µL of the virus-serum mixture was added in quadruplicate to Vero E6 cell mono layers in 96-well microtiter plates. Then, an additional 100 µL of culture medium IMM was added to each well and the plates incubated for 7 days at 37°C in 5% CO2 in a humidified incubator. A serial 10-fold dilution of the virus: 100µl TCID50/50 µL, 10 µl TCID50/50 µl, 1 µL TCID50/50 µL and 0.1 TCID50/50 µL was made as control and loading into 8 wells of 96-well microtiter plates with additional 150 µL of culture

medium MM. The CPE was read at 7 days post infection. The highest serum dilution that completely protected the cells from CPE in half of the wells was taken as the neutralizing antibody titer. More than 4 times dilution could protect the cells from virus infection was set as the cutoff of the positive. These procedures were carried in a biosafety level 3 facility.

ELISA binding assay

Each serum or plasma sample was tested at a dilution of 1:100 in sample dilution ELISA buffer (Supplied with each ELISA kits) and added to the ELISA wells of each plate for 1 h shaking with 250rpm at room temperature (or keep stand still at 37°C, follow the instruction of menu). After 5 times washing with washing buffer, horseradish peroxidase (HRP)-conjugated goat anti-human IgG or HRP-conjugated goat anti-human IgM were added for 30 minutes at shaking with 250rpm at room temperature (or keep stand still at 37°C, following the instructions). The ELISA plates were then washed five times with washing buffer. Subsequently, 100 µL of HRP substrate was added into each well. After 15 min incubation, the reaction was stopped by adding 50 µL of stop solution and read optical density (OD) within 15 minutes at 450nm and 630nm on iMark microplate reader (BioRad). Comparing the sample OD value with the Reference Control's OD value, it is positive when the S/CO is greater than 1.

Statistical Analysis

Correlations between serums ELISA OD, detection rates were assessed using Pearson's correlation coefficients.

Result

1. Performance comparison between four Covid-19 antibody assay kits and one neutralization assay. Four ELISA kits, Anti-N IgG, Anti-N-IgM, Anti-S-IgG, Anti-S-IgM were used for testing. The Positive group was formed by 164 hospital-released Covid-19 patients. Covid-19 patients closely contacted group had 184 cases, and the control group had 300 cases. They were tested by these five assays for sensitivity and specificity. In the positive group, the detection rate of Anti-N IgG and neutralization experiments were the highest, both reaching 97.6%. In the closely contacted group, the detection rate of Anti-N IgG was 46.3%, the detection rate of neutralization experiment was 35.9%, and the detection rate of Anti-S-IgG was 34.7%. In the control group, the detection rate of Anti-N-IgG was 2.3% and the detection rate of Anti-N-IgM was 1%, but the detection rates of S-IgG, S-IgM were zero (Table 1) (Supplementary Table 1).

Assays	Positive Group (n=164) Positive rate (%)	Closely contacted group (n=184) Positive rate (%)	Negative control group (n=300) Positive rate (%)
N-IgG (>0.5)	160/164(97.6%)	75/184(46.3%)	2.3%
N-IgM (>0.3)	102/164(62.2%)	47/184(25.5%)	1%
S-IgG (>0.3)	153/164(93.9%)	64/184(34.7%)	0
S-IgM (>0.3)	104/164(63.4%)	37/184(20.1%)	0
neutralization experiment	160/164(97.6%)	66/184(35.9%)	0

Table 1: Four Covid-19 ELISA kits: N-IgG, N-IgM, S-IgG, S-IgM and neutralization experiment were tested with 164 positive patients' group, 184 closely contacted group and 300 negative control group. Positive rates were compared as below.

2. In the 2X2 factorial design, the Anti-S-IgG ELISA and the neutralization assay were used to conduct a comparative study on 164 people that had closely contacted with covid-19 patients. Their positive agreement rate was 59/64 (92.2%), negative agreement rate was 113/120 (94.2 %). The total agreement rate was 172/184 (93.4%) (Table 2).

		Neutralization Assay		
		+	-	Total
S-IgG ELISA	+	59	5	64
	-	7	113	120
	Total	66	118	184

Table 2: 2X2 factorial design of neutralization assay and S-IgG assay in the Covid-19 closely contacted group.

Positive agreement rate: 59/64=92.2%; Negative agreement rate: 113/120=94.2%; Positive predictive value, PPV=59/64=92.2%; Negative predictive value, NPV=113/120=94.2%; Total agreement rate: 172/184=93.4%

3. In the virus-neutralization assay, 164 samples of patients confirmed with Covid-19 and 184 samples of closely contacted person were tested. It was found that 160/164 (97.6%) of the confirmed patients did have virus-neutralizing capacity when discharged from the hospital. In the close contact group, 66/186 (35.9%) samples had virus neutralization activity (Figure 1) (Supplementary Table 2).

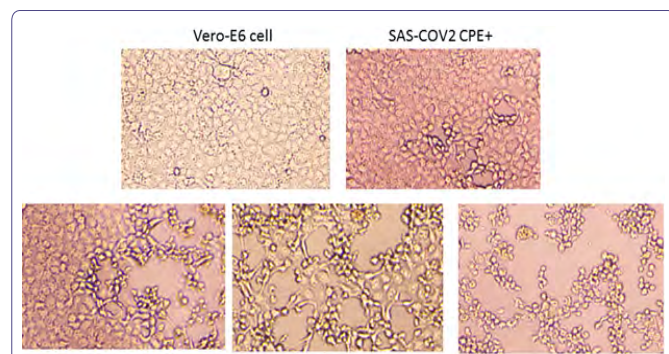


Figure 1: Neutralization experiment, SAS-COV2 virus infects Vero-E6 cells. There are four levels of Cytopathic effect (CPE). A confluent monolayer of Vero E6 cells was prepared in 96 well plate. Serial four-fold dilutions of heat-inactivated sera were made. The serum dilutions 240ul were mixed with equal volumes of 100 TCID50 of SARS-CoV-2 virus. After 2 h of incubation at 37°C, 5%CO₂ incubator, 100μL of the virus-serum mixture were added in quadruplicate to Vero E6 cell monolayers in 96-well microtiter plates. Then the plates were incubated 7 days at 37°C in 5% CO₂ in a humidified incubator. The CPE was read at 7 days post infection. The highest serum dilution that completely protected the cells from CPE in half of the wells was taken as the neutralizing antibody titre. More than 4 times dilution could protect the cells from virus infection was set as the cutoff of the positive. Below are 4 different levels of the virus infection.

+: 25% cells was infected; ++: 50% cells was infected; +++: 75% cells was infected; ++++:100% cells were infected.

4. Covid-19 patients closely contacted group was separately into virus neutralization activity positive group (66 people) and negative group (118 people). The positive coincidence rate of N-IgG and S-IgG was identical, 59/66 (89.4%). But in the negative group, the detection rate of anti-N-IgG was 16/118 (13.6%) where as anti-S-IgG was only 5/118 (4.2%). About 14/118(11.8%) people only produced antibodies against viral N protein (Figure 2).

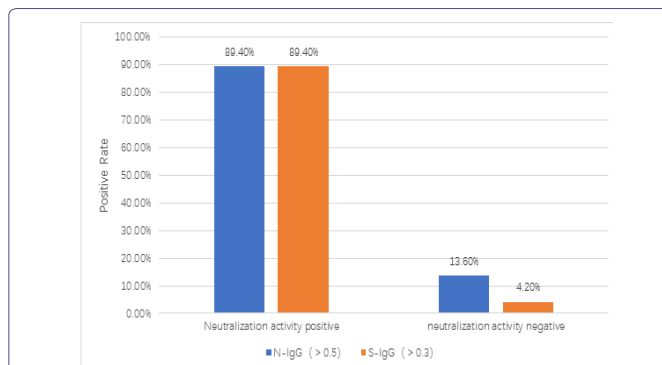


Figure 2: Comparing of N-IgG and S-IgG ELISA in the Covid-19 closely contacted group. Both assay had 59/66(89.4%) positive in the 66 neutralization positive samples. In the 118 persons which neutralization test negative group, N-IgG had 16 positive (about 13.6%) and S-IgG had 5 positive (about 4.2%). About 14 persons (11.8%) only produced antibody to the N protein of the SAS-COV2 virus.

Discussion

The global epidemic of SARS-COV-2 caused the world's largest public health crisis with over 21 million infections and 766 thousand deaths till now. To answer the question which method could be used to detect the immunity after infection, 164 covid-19 patients, 184 high-risk individuals and 300 healthcare personnel were tested. The S- IgG assay showed 93.5% agreement with the virus neutralization experiment. It could be used to replace the high-risk, time-costly virus neutralization experiment. Normally, measuring neutralization antibodies requires the use of live virus, cells, highly skilled operators, and complex laboratory procedures that are generally less sensitive and require several days to obtain results. Conventional virus neutralization tests require live coronaviruses and a bio safety level 3 laboratory for processing. This report provided a simple method to test.

Results also showed that viral N-protein induced the highest antibody response. In the Covid-19 confirmed patient group, the closely contacted group and the health control group, the positive rate of N-IgG was the highest, 97.6%, 46.3%, and 2.3%, respectively; and the detection rate of S-IgG was slightly lower, 93.9%, 34.7% and 0 respectively. The positive rates of neutralization experiment were 97.6%, 35.9% and 0, respectively. Although Anti-N-IgG had high detection rate, the Anti-N-IgG and Anti-N-IgM in the control group's detecting may be cross reactions owing to sequence the homology of the viral nucleoprotein, which need further confirm [10-16].

To answer the question whether each infection could produce protection immunity, a group of 184 people that had closely contacted with covid-19 patients were tested. Only 66 serum samples have neutralization activity. In those 118 persons that do not have neutralization activity, anti-N-IgG ELISA positive rate was 14/118 (11.8%), which suggests that some infection did not produce protection antibodies. Some of the covid-19 patients only produced anti-N protein antibodies and no anti-S antibodies 5/164 (3.05%). Some of them neither had antibodies against S protein nor antibodies against N protein 5/164 (3.05%). How did these patients recover from the virus attack is still unknown? Whether they are vulnerable to virus re-infection is also unknown.

The United States plans to conduct antibody testing for nearly 10 million people [15]. If the N protein antigen is used, their test result will not reflect whether the patients have protective antibodies or not. If only the S protein antigen is used, some infected patients will be reported as false negative. A better way is to analyze both: IgG against S and IgG against N proteins. Thus, the highest infection rate and the effective immunity rate could be obtained simultaneously.

Molecular assays have been the gold standard to directly detect for the presence of viral genetic material in infected individuals. However, insufficient viral RNA at the point of detection may lead to false negative result.

Funding

This study was supported by grants from: The Key Research and Development Program of Guangdong Province (2019B111103001); Natural Science Foundation of Guangdong Province (2019A1515012121); The leading talents of Guangdong Province program (No.00201512); and the National Science and Technology Major Project (2018zx10732401). NIAID center of excellence for influenza research and surveillance (HHSN272201400006C).

Availability of Data and Materials

The datasets used and/or analyzed in this study are available from the corresponding author upon reasonable request.

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Supplementary Tables

编号	Sex	Age	S-IgG	S-IgM	N-IgG	N-IgM	Neutralition results
20SF1678	M	56	2.584	0.707	2.003	1.091	128
20SF1684	M	69	2.352	0.517	1.179	0.235	256
20SF1899	M	34	1.714	1.06	1.253	2.556	128
20SF2727S	F	29	0.334	0.581	0.737	1.102	64
20SF3034	M	57	2.298	0.792	0.961	0.601	128
20SF3847	M	29	0.377	0.727	2.090	0.607	64
20SF3850	M	69	2.748	0.338	3.811	0.515	64
20SF3851	F	27	1.33	0.382	0.041	0.021	32
20SF3894	M	57	2.202	0.494	2.558	0.294	32
20SF3899	M	29	0.182	0.767	1.866	0.391	8
20SF3902	M	69	2.722	0.46	2.553	0.348	32
20SF4001	M	59	3.221	1.064	4.067	1.590	1200
20SF4002	M	59	3.205	1.389	4.004	1.054	512
20SF4009	M	28	3.17	2.919	3.951	0.399	1024
20SF4211	M	37	3.154	0.479	0.800	0.156	64
20SF4212	M	26	2.642	0.456	2.454	0.382	32
20SF4216	M	3	2.888	0.133	1.555	0.220	32
20SF4217	M	10	1.676	0.339	0.429	0.174	32
nCoV-1005S	F	25	3.103	0.404	1.461	0.177	64
nCoV-1016S	M	35Y	0.976	0.818	0.477	0.321	16
nCoV-1023S	F	23Y	2.211	0.141	1.394	0.222	8
nCoV-1024S	M	12Y	2.312	0.151	0.608	0.084	16
nCoV-1038S	M	2Y	2.659	0.183	1.072	0.21	16
nCoV-1787S	M	9	3.187	0.266	1.113	0.018	32
nCoV-1969S	M	28	2.955	1.293	3.752	0.296	256
nCoV-2016S	M	41	3.2	2.148	3.158	0.694	512
nCoV-2019S	M	1	2.901	0.968	0.278	0.082	512
nCoV-2023S	M	6	2.74	0.105	1.709	0.046	32
nCoV-2027S	M	8	2.386	0.402	0.673	0.017	64
nCoV-2028S	M	37	3.094	0.827	1.883	0.302	256
nCoV-2053S	M	61	2.939	0.527	1.931	0.098	256
nCoV-2076S	F	37	1.865	0.678	0.352	-0.002	64
nCoV-2111S	M	28	2.773	1.452	1.569	0.032	128
nCoV-2112S	M	60	3.15	0.302	3.392	0.047	128
nCoV-2114S	M	46	3.149	0.331	1.010	0.034	64
nCoV-2116S	M	37	2.905	1.491	1.368	0.108	32
nCoV-2121S	F	29	3.174	0.455	2.714	0.248	128
nCoV-2127S	F	27	0.503	0.085	0.359	0.023	4
nCoV-2128S	M	60	2.718	0.399	1.717	0.107	16
nCoV-2129S	M	60	2.818	0.486	1.228	0.091	32
20SF015	F	68	2.999	1.377	3.114	0.477	1024
20SF020	F	68	3.146	0.762	2.591	0.33	256
20SF035	F	68	3.183	1.53	3.050	0.546	512
20SF087	F	68	3.192	1.862	2.549	0.269	512
20SF178	M	36	0.172	0.575			8
20SF365	M	37	0.267	0.182	0.040	0.03	4
20SF1466-3	F	25	2.645	1.032	1.706	0.39	512
20SF1677	F	56	3.275	1.001	3.410	0.902	128
20SF1679	M	61	0.192	0.224	2.994	0.604	16
20SF1680	M	24	2.313	0.727	1.906	0.959	16

20SF1681	M	35	3.012	0.285	0.650	0.154	128
20SF1682	M	48	0.17	0.169	2.132	0.438	128
20SF2115	F	54	0.38	0.217	1.224	0.694	128
20SF2123	M	45	0.862	0.37	3.268	2.737	256
20SF2384	M	56	3.06	0.708	2.833	0.31	128
20SF2480	F	50	0.494	0.35	1.157	0.935	32
20SF2485	M	29	2.927	1.681	2.252	1.604	512
20SF2518	F	54	0.324	0.136	2.743	2.020	256
20SF2519	M	50	1.109	0.365	1.134	1.032	512
20SF2520	M	45	0.747	0.31	3.429	2.821	512
20SF2550			0.578	0.182	0.105	0.040	32
20SF2728S	F	55	0.272	0.259	2.481	0.546	64
20SF2729S	F	36	1.427	0.338	1.393	1.051	32
20SF2973S	M	48	2.871	2.02	1.179	1.798	256
20SF2974S	F	45	0.074	0.116	0.098	0.161	1
20SF3033	F	57	3.261	1.515	3.327	0.627	1024
20SF3035	M	61	3.226	2.344	3.637	2.004	512
20SF3036	M	24	3.098	1.42	1.960	0.468	128
20SF3046	M	48	3.168	0.729	2.618	0.456	512
20SF3780	F	24	0.579	0.602	2.272	0.693	128
20SF3784	F	24	1.284	0.819	1.700	0.427	128
20SF3791	F	25	0.167	0.237	0.026	0.02	1
20SF3815	M	52	2.972	0.492	3.748	2.383	512
20SF3833	M	50	0.406	0.2	0.746	0.356	8
20SF3835	M	50	3.194	0.865	3.969	1.693	64
20SF3848	F	60	3.186	0.375	3.832	1.367	1024
20SF3849	M	77	0.592	0.761	3.248	0.192	128
20SF3893	F	57	3.18	1.141	4.046	1.123	256
20SF3895	M	61	3.156	1.951	4.063	2.694	128
20SF3896	M	24	2.951	1.061	3.543	1.314	256
2135	M	57	1.423	0.309	0.733	0.024	16
2148	M	33	2.905	0.299	1.154	0.51	32
2206	M	25	3.097	0.153	2.777	0.095	64
2208	M	53	2.985	0.547	2.006	0.182	16
2209	M	6	3.09	0.214	1.844	0.006	32
2210	M	53	2.982	1.468	1.858	0.151	32
2211	M	28	3.149	0.165	2.784	0.097	16
2213	F	38	1.282	0.184	0.779	0.013	8
2214	F	35	2.88	0.275	2.320	0.014	32
2215	M	62	3.217	0.821	2.899	2.71	512
2219	M	38	3.211	0.746	1.965	0.424	128
2220	M	54	2.217	0.278	0.761	0.023	16
2221	M	38	1.547	0.124	0.431	0.024	8
2224	F	38	1.408	0.121	0.588	-0.002	8
2225	F	35	2.522	0.512	2.442	0.036	128
2226	M	62	(+)	0.797	2.647	2.447	512
2229	M	54	2.021	0.189	0.712	0.03	32
2230	F	50	3.134	0.521	2.049	2.849	64
2231	F	34	1.589	0.091	0.402	0.005	8
2232	M	25	3.163	0.195	2.469	-0.002	128
2233	F	25	3.068	0.222	1.689	0.019	32
2238	M	45	1.167	2.932	1.283	0.141	16

2239	F	6	1.684	0.166	1.064	0.004	32
2241	F	50	2.595	0.871	1.928	2.949	128
2242	F	34	1.45	0.072	0.429	0.004	8
2243	M	25	3.007	0.292	2.404	0.004	128
2245	M	53	3.037	0.694	1.506	0.118	32
2246	F	57	3.169	0.796	1.395	0.491	64
2248	F	50	3.154	0.144	1.344	0.246	32
2251	M	20	1.217	0.217	0.563	0.005	16
2252	M	45	1.494	2.127	1.329	0.151	128
2253	F	6	1.626	0.189	1.146	0.024	32
2256	M	36	2.851	0.177	1.507	0.185	16
2257	F	57	3.1	1.374	1.267	0.532	32
2258	F	35	3	0.244	2.432	0.017	128
2259	M	62	3.161	1.042	2.487	2.785	512
2262	M	54	1.022	0.51	0.657	0.001	32
2263	F	72	1.362	0.243	0.934	0.004	<4
2264	M	54	1.48	0.741	2.401	0.07	128
2521	M	36	2.828	0.324	0.844	0.055	32
2522	M	25	2.71	0.283	0.587	0.032	32
2524	M	62	3.184	2.784	0.380	0.018	128
2529	F	28	2.912	0.223	1.040	0.012	32
2532	F	50	2.439	0.834	0.298	0.789	32
2533	M	25	3.083	0.266	0.348	0.002	32
2536	M	54	1.9	0.968	1.052	0.01	32
2544	F	6	1.703	1.244	0.380	0.006	32
2546	M	62	3.464	2.644	0.736	0.047	256
2551	F	58	2.84	0.219	2.047	0.033	128
2552	F	75	3.079	0.267	1.331	0.12	128
2553	F	59	3.181	0.421	1.374	0.05	32
2554	F	57	3.132	0.342	0.717	0.004	256
2555	M	46	1.897	0.47	0.433	0.005	32

2559	F	57	3.177	1.367	0.342	0.184	64
2560	F	75	3.138	0.228	1.401	0.153	512
2561	F	59	3.21	0.544	1.378	0.031	128
2562	F	57	3.029	0.179	1.105	0.002	256
2563	M	58	1.193	0.449	1.266	0.002	64
2564	M	46	2.366	0.215	0.625	-0.009	8
2566	M	53	2.136	1.02	0.388	0.006	32
2568	M	36	2.511	0.267	0.728	0.034	32
2569	M	54	2.337	0.537	2.127	0.033	32
2570	M	38	1.319	0.108	0.409	0.04	8
2573	F	12	2.46	0.268	0.489	0.056	16
3897	M	35	3.011	0.269	2.974	0.267	512
3901	F	23	0.51	0.17	0.040	0.03	8
3903	F	60	3.294	0.202	3.398	0.630	512
3928	F	45	1.838	0.735	0.040	0.03	128
4005	F	22	2.22	0.58	3.881	0.888	128
4006	F	22	2.967	0.356	3.545	0.710	32
4083	F	62	2.686	0.453	4.176	0.549	128
4089	F	54	2.336	0.36	3.658	2.880	128
4109	M	61	3.12	1.388	4.008	2.312	1024
4111	F	60	3.255	0.424	3.851	1.740	1024
4210	F	25	3.159	0.834	3.313	1.543	1024
4213	F	27	0.539	0.156	1.848	1.185	8
4214	F	43	1.919	0.415	2.620	0.827	32
4241	F	24	0.083	0.09	0.353	0.194	1
4279	F	32	0.505	0.303	0.401	0.478	16
4280	M	37	0.451	0.293	2.175	0.574	32
4281	F	55	0.178	0.183	2.297	0.528	4
4353	F	54	2.394	0.199	3.948	2.999	128
4642			3.18	0.637	3.566	3.120	128
4647			0.065	0.096	0.040	0.03	4

numbers	Sex	Age	types	Neutralization results	S-IgG	S-IgM	N-IgG	N-IgM
nCoV-1042S			Close Contact	256	0.066	0.091	0.002	0
nCoV-1066S			Close Contact	16	0.06	0.072	0.004	0.004
nCoV-1071S			Close Contact	4	0.051	0.072	0.006	-0.002
nCoV-1085S	M	8	Close Contact	64	0.077	0.083	0.011	0.011
nCoV-1097S	F	40	Close Contact	1	0.044	0.048	0.009	0.007
nCoV-1100S	F	20	Close Contact	1	0.051	0.051	0.008	0.003
nCoV-1101S	M	27	Close Contact	1	0.146	0.052	0.374	0.325
nCoV-1102S	F	33	Close Contact	1	0.056	0.053	0.003	0
nCoV-1103S	M	24	Close Contact	1	0.109	0.055	0.006	0
nCoV-1115S	M	32	Close Contact	1	0.113	0.055	0.007	0.002
nCoV-1123S	F	24	Close Contact	1	0.111	0.055	0.016	-0.006
nCoV-1127S	F	39	Close Contact	1	0.155	0.056	0.006	0.006
nCoV-1131S	M	47	Close Contact	1	0.121	0.056	0.017	0.003
nCoV-1139S	M	51	Close Contact	1	0.06	0.056	0.289	0.268
nCoV-1170S	M	63	Close Contact	32	0.069	0.06	0.024	0.024
nCoV-1191S	M	29	Close Contact	1	0.08	0.057	0.053	0.027
nCoV-1219S	M	33	Close Contact	1	0.05	0.058	0.005	0.004
nCoV-1220S	F	9	Close Contact	1	0.055	0.058	0.008	0.003
nCoV-1221S	M	58	Close Contact	1	0.133	0.058	0.026	0.023
nCoV-1222S	F	58	Close Contact	1	0.073	0.058	0.297	0.007

nCoV-1223S	F	34	Close Contact	1	0.066	0.059	0.038	0.029
nCoV-1224S	F	12	Close Contact	1	0.156	0.059	0.047	0.002
nCoV-1225S	M	30	Close Contact	1	0.052	0.059	0.09	-0.004
nCoV-1226S	M	28	Close Contact	1	0.138	0.059	0.14	-0.001
nCoV-1227S	M	44	Close Contact	1	0.094	0.061	0.009	0.005
nCoV-1228S	M	22	Close Contact	1	0.18	0.061	0.095	0.008
nCoV-1229S	F	42	Close Contact	1	0.077	0.062	0.016	0.015
nCoV-1230S	M	34	Close Contact	1	0.117	0.062	0.133	0.005
nCoV-1231S	F	58	Close Contact	1	0.067	0.063	0.005	0.001
nCoV-1232S	M	59	Close Contact	1	0.061	0.063	0.41	0.006
nCoV-1233S	M	38	Close Contact	1	0.052	0.064	0.005	0.005
nCoV-1234S	M	38	Close Contact	1	0.095	0.065	0.123	0.449
nCoV-1235S	F	30	Close Contact	1	0.054	0.066	0.05	0.271
nCoV-1236S	F	39	Close Contact	1	0.087	0.067	0.014	-0.002
nCoV-1237S	M	71	Close Contact	1	0.069	0.068	0.009	0.005
nCoV-1238S	M	44	Close Contact	1	0.132	0.068	0.014	0.013
nCoV-1239S	M	85	Close Contact	1	0.134	0.069	0.006	0.003
nCoV-1240S	F	80	Close Contact	1	0.09	0.069	0.007	0.003
nCoV-1241S	M	32	Close Contact	1	0.098	0.07	0.004	-0.002
nCoV-1242S	M	7	Close Contact	1	0.053	0.07	0.045	0.026
nCoV-1243S	M	5	Close Contact	1	0.083	0.071	0.012	0.005
nCoV-1244S	M	55	Close Contact	1	0.149	0.071	0.023	0.011
nCoV-1245S	M	38	Close Contact	1	0.199	0.071	0.045	0.031
nCoV-1246S	M	26	Close Contact	1	0.057	0.071	0.159	0.006
nCoV-1247S	M	56	Close Contact	1	0.13	0.072	0.01	0.015
nCoV-1248S	M	25	Close Contact	1	0.13	0.072	0.026	0.005
nCoV-1249S	M	29	Close Contact	1	0.06	0.072	0.046	0.024
nCoV-1250S	M	40	Close Contact	1	0.063	0.073	0.024	0.006
nCoV-1251S	F	33	Close Contact	1	0.121	0.075	0.016	0.008
nCoV-1252S	M	53	Close Contact	1	0.078	0.075	0.26	0.152
nCoV-1255S	M	64	Close Contact	64	0.869	0.329	0.173	0.005
nCoV-1257S	F	27	Close Contact	1	0.071	0.076	0.003	0.013
nCoV-1258S	F	23	Close Contact	1	0.057	0.077	0.336	0.27
nCoV-1259S	F	23	Close Contact	1	0.073	0.078	0.014	0.026
nCoV-1260S	M	62	Close Contact	1	0.12	0.078	0.407	0.102
nCoV-1261S	M	68	Close Contact	1	0.136	0.079	0.68	0.153
nCoV-1262S	M	56	Close Contact	64	0.076	0.063	0.174	0.543
nCoV-1263S	M	68	Close Contact	1	0.064	0.08	0.051	-0.003
nCoV-1264S	F	62	Close Contact	16	2.852	0.32	0.267	0.003
nCoV-1265S	M	65	Close Contact	1	0.096	0.08	0.357	0.047
nCoV-1266S	M	67	Close Contact	1	0.103	0.081	0.005	0.002
nCoV-1267S	F	14	Close Contact	1	0.09	0.081	0.012	0.012
nCoV-1269S	F	54	Close Contact	1	0.07	0.081	0.019	-0.001
nCoV-1270S	M	72	Close Contact	1	0.139	0.081	0.042	0.003
nCoV-1271S	M	71	Close Contact	1	0.088	0.086	0.007	0.012
nCoV-1272S	M	53	Close Contact	32	1.484	0.731	0.318	0.064
nCoV-1273S	F	56	Close Contact	1	0.306	0.086	0.092	0.014
nCoV-1274S	F	57	Close Contact	1	0.086	0.086	0.303	0.251
nCoV-1276S	F	66	Close Contact	1	0.104	0.087	0.006	0
nCoV-1277S	F	66	Close Contact	1	0.075	0.087	0.114	0.222
nCoV-1278S	F	63	Close Contact	1	0.061	0.088	0.24	0.133
nCoV-1279S	M	65	Close Contact	1	0.082	0.088	0.56	0.006
nCoV-1280S	F	57	Close Contact	1	0.097	0.089	0	0.003
nCoV-1281S	M	73	Close Contact	1	0.098	0.089	0.187	1.046

nCoV-1282S	F	54	Close Contact	1	0.073	0.091	0.009	0.001
nCoV-1283S	M	27	Close Contact	1	0.108	0.092	0.009	0.006
nCoV-1284S	F	52	Close Contact	1	0.217	0.092	0.022	0.007
nCoV-1285S	F	48	Close Contact	1	0.137	0.092	0.026	0.036
nCoV-1286S	M	54	Close Contact	1	0.074	0.092	0.662	0.021
nCoV-1287S	F	28	Close Contact	1	0.091	0.095	0.01	0.005
nCoV-1288S	F	58	Close Contact	1	0.06	0.095	0.031	0.004
nCoV-1289S	F	44	Close Contact	1	0.159	0.096	0.002	0.002
nCoV-1290S	F	49	Close Contact	1	0.087	0.096	0.003	0.002
nCoV-1291S	M	43	Close Contact	1	0.054	0.098	0.002	0.003
nCoV-1292S	F	37	Close Contact	1	0.073	0.099	0.003	0.004
nCoV-1293S	M	53	Close Contact	1	0.224	0.1	0.126	0.165
nCoV-1294S	M	48	Close Contact	1	0.141	0.101	0.013	0.004
nCoV-1304S	M	1	Close Contact	32	0.507	0.47	0.357	0.056
nCoV-1306S	F	50	Close Contact	1	0.195	0.101	0.098	0.218
nCoV-1350S	M	36	Close Contact	32	1.188	0.088	0.374	0.023
nCoV-1437S	F	43	Close Contact	16	0.31	0.483	0.393	0.007
nCoV-1443S	M	31	Close Contact	1	0.183	0.102	0.003	-0.006
nCoV-1451S	M	12	Close Contact	64	1.168	0.227	0.42	0.015
nCoV-1464S	M	69	Close Contact	128	2.828	0.803	0.459	0.088
nCoV-1465S	F	47	Close Contact	16	1.988	0.167	0.488	0.145
nCoV-1466S	M	46	Close Contact	1	0.114	0.102	0.007	0.005
nCoV-1484S	F	36	Close Contact	16	1.58	0.236	0.522	0.203
nCoV-1485S	F	32	Close Contact	1200	3.146	0.407	0.531	0.084
nCoV-1486S	M	32	Close Contact	256	2.574	0.677	0.539	0.087
nCoV-1487S	F	33	Close Contact	16	1.02	0.137	0.632	0.034
nCoV-1507S	F	10 month	Close Contact	256	3.113	0.274	0.696	0.032
nCoV-1518S	F		Close Contact	32	0.482	1.013	0.735	0.044
nCoV-1520S	F	41	Close Contact	16	2.694	0.39	0.805	0.002
nCoV-1524S	F	71	Close Contact	8	2.211	0.108	0.828	0.012
nCoV-1526S	F	74	Close Contact	8	1.275	0.726	0.836	0.027
nCoV-1528S	M	12	Close Contact	32	1.503	0.278	0.842	0.156
nCoV-1530S	F	30	Close Contact	32	1.992	0.458	0.931	0.014
nCoV-1531S	F	36	Close Contact	32	2.69	0.231	0.982	0.316
nCoV-1532S	F	57	Close Contact	1200	1.198	0.154	1.103	0.17
nCoV-1536S	M	8	Close Contact	4	1.317	0.136	1.127	0.232
nCoV-1551S	F	19	Close Contact	256	2.827	0.25	1.134	0.038
nCoV-1554S	M	51	Close Contact	32	3.111	0.25	1.141	0.005
nCoV-1579S	M	16	Close Contact	8	2.132	0.164	1.155	0.087
nCoV-1581S	F	44	Close Contact	1024	2.319	0.323	1.172	0.131
nCoV-1584S	F	53	Close Contact	1	0.077	0.103	0.097	0.431
nCoV-1585S	F	53	Close Contact	64	2.955	0.173	1.403	0.019
nCoV-1594S	M	37	Close Contact	256	3.121	0.862	1.446	0.308
nCoV-1595S	M	8	Close Contact	256	(+)	(+)	1.475	0.025
nCoV-1596S	M	32	Close Contact	32	2.388	0.094	1.532	0.026
nCoV-1597S	M	58	Close Contact	128	3.154	1.687	1.588	0.003
nCoV-1598S	F	53	Close Contact	128	1.743	0.166	1.661	0.025
nCoV-1607S	M	38	Close Contact	1	0.371	0.107	0.003	0.002
nCoV-1608S	F	37	Close Contact	128	3.212	0.448	1.779	0.047
nCoV-1610S	M	5	Close Contact	4	3.039	3.014	1.91	0.88
nCoV-1611S	M	15	Close Contact	512	3.204	2.512	1.922	0.369
nCoV-1615S	M	28	Close Contact	128	3.254	1.959	1.964	3.058
nCoV-1617S	F	49	Close Contact	64	3.104	0.166	2.055	0.146
nCoV-1635S	F	17	Close Contact	128	2.134	0.503	2.126	0.645
nCoV-1636S	F	39	Close Contact	512	3.232	1.316	2.152	0.457

nCoV-1643S	F	30	Close Contact	32	3.246	0.13	2.164	0.003
nCoV-1658S	M	57	Close Contact	1	0.155	0.108	0.019	0.006
nCoV-1674S	F	75	Close Contact	1	0.172	0.108	0.143	0.255
nCoV-1675S	F	48	Close Contact	1	0.089	0.111	0.297	0.256
nCoV-1676S	M	55	Close Contact	1	0.085	0.113	0.003	0.002
nCoV-1677S	F	67	Close Contact	256	3.237	1.122	2.175	0.234
nCoV-1678S	M	62	Close Contact	16	2.824	0.351	2.253	0.013
nCoV-1679S	F	62	Close Contact	128	3.177	0.564	2.263	0.034
nCoV-1680S	M	65	Close Contact	128	3.244	0.302	2.302	0.051
nCoV-1692S	F	34	Close Contact	1	0.138	0.114	0.011	0.016
nCoV-1693S	F	9	Close Contact	1	0.288	0.114	0.042	0.007
nCoV-1697S	F	38	Close Contact	128	3.213	1.652	2.385	0.322
nCoV-1699S	F	33	Close Contact	32	3.114	0.094	2.462	0.067
nCoV-1700S	F	4	Close Contact	16	3.333	0.518	2.474	0.21
nCoV-1702S	M	40	Close Contact	64	2.012	0.152	2.543	0.029
nCoV-1745S	M	31	Close Contact	1	0.119	0.115	0.138	0.275
nCoV-1785S	F	38	Close Contact	1	0.126	0.115	0.23	0.263
nCoV-1791S	F	60	Close Contact	64	2.817	0.247	2.719	1.13
nCoV-1808S	M	48	Close Contact	1024	0.126	0.112	2.737	0.786
nCoV-1823S	F	/	Close Contact	1	0.119	0.118	0.014	0.003
nCoV-1824S	F	/	Close Contact	1	0.217	0.119	0.018	0.009
nCoV-1825S	M	/	Close Contact	1	0.063	0.121	0.017	0.011
nCoV-1826S	M	/	Close Contact	1	0.199	0.124	0.023	0.01
nCoV-1827S	F	/	Close Contact	1	0.36	0.125	0.011	0.006
nCoV-1828S	F	/	Close Contact	1	0.076	0.126	0.01	0.003
nCoV-1829S	M	/	Close Contact	1	0.165	0.127	0.033	0.016
nCoV-1831S	F	47	Close Contact	1	0.14	0.127	0.275	0.078
nCoV-1832S	F	46	Close Contact	1	0.228	0.131	0.052	0.037
nCoV-1835S	F	38	Close Contact	1	0.104	0.134	0.243	0.278
nCoV-1880S	M	40	Close Contact	256	2.975	0.955	2.754	1.411
nCoV-1882S	M	65	Close Contact	256	3.204	0.788	2.756	1.151
nCoV-1883S	F	49	Close Contact	64	2.189	0.204	2.886	0.061
nCoV-1884S	F	36	Close Contact	128	3.236	0.452	2.919	2.513
nCoV-1887S	M	33	Close Contact	1	0.145	0.136	0.025	0.024
nCoV-1908S	M	65	Close Contact	1	0.296	0.138	0.002	0.001
nCoV-1913S	M	29	Close Contact	1200	3.253	3.178	2.943	3.061
nCoV-1915S	F	34	Close Contact	16	0.501	0.131	3.043	0.322
nCoV-1919S	M	4	Close Contact	128	1.834	0.972	3.045	0.193
nCoV-1981S	M	28	Close Contact	1	0.19	0.14	0.045	0.018
nCoV-1998S	M	5	Close Contact	64	1.864	2.303	3.057	0.973
nCoV-2017S	F	28	Close Contact	1	0.062	0.144	0.004	0.005
nCoV-2050S	F	24	Close Contact	512	3.127	0.753	(+)	0.93
nCoV-2182S	M	48	Close Contact	1	0.159	0.144	0.034	0.028
nCoV-2183S	M	32	Close Contact	1	0.236	0.148	0.009	0.006
nCoV-2184S	M	38	Close Contact	1	0.177	0.163	0.176	0.345
nCoV-2185S	M	25	Close Contact	1	0.162	0.168	0.098	0.003
nCoV-2186S	M	39	Close Contact	1	0.16	0.176	0.003	0.011
nCoV-2187S	M	36	Close Contact	1	0.209	0.181	0.085	0.235
nCoV-2188S	M	27	Close Contact	1	0.232	0.188	0.007	0.009
nCoV-2189S	M	57	Close Contact	1	0.528	0.212	0.257	0.011
nCoV-2190S	M	26	Close Contact	1	0.219	0.221	0.045	0.027
nCoV-2191S	M	56	Close Contact	1	0.219	0.235	0.137	0.005
nCoV-2192S	M	48	Close Contact	1	0.181	0.282	0.007	0.009
nCoV-2193S	M	44	Close Contact	1	0.792	0.336	0.498	0.534
nCoV-2194S	M	44	Close Contact	1	0.163	0.352	0.135	1.233



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