HIGH THROUGHPUT SARS-COV-2 ANTIBODY TEST VALIDATION AT A CLIA CERTIFIED LABORATORY

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INTRODUCTION

SARS-CoV-2 coronavirus disease-2019 (COVID-19) was first identified in late December 2019 (Zhu, 2019). Chinese health authorities investigated a cluster of atypical pneumonia cases occurring primarily in individuals who had visited a seafood and wet market in Wuhan, Hubei Province, China. Patients reported fever and cough, and most developed chest discomfort and/or respiratory distress, with a diagnosis of pneumonia being made by chest radiographs and/or computed tomographic scan (Zhu, 2019). Pneumonia appears to be the most frequent serious manifestation of infection, characterized primarily by fever, cough, dyspnea, and bilateral infiltrates on chest imaging (Guan, 2020, Huang 2020, Chen 2020, Wang 2020). There are no specific clinical features that can reliably distinguish COVID-19 from other viral respiratory infections. Hence there is a need for scalable, effective, and accurate screening tests for COVID-19.

Current tests for SARS-CoV-2, the virus that causes COVID-19, assess the presence of the virus (viral nucleic acid or antigens) through specimens obtained from saliva or nasal or oral swabs, using Polymerase Chain Reaction (PCR) methodology. However, these tests cannot identify people who were infected, recovered, and have cleared the virus from their bodies. Serology testing, on the other hand, can identify previous infection by detecting antibodies to SARS-CoV-2 (Wang, 2020). Serology testing for antibodies, especially for neutralizing antibodies (NAbs), is important because it measures the active immune response of an individual against any virus (Coughlan, 2012). NAbs prevent reinfections by blocking entry of a virus to the cell or killing the virus by opsonization (Coughlan, 2012). In particular, serum IgG NAbs, play a major role in neutralization of COVID-19 (Deng, 2020). A combination of antigen, cytokine and antibody testing are important in diagnosis, treatment and management of patients and antibody testing is essential in confirming the diagnosis and determining the immune status of the patient (Zhang, 2020).

Studies of COVID-19 antibodies are sparse and vary somewhat in documenting antibody responses. In a study from Beijing, China (Guo, 2020), the median time to IgM and IgA antibody detection after onset of symptoms was 5 days, while IgG was detected at 14 days, with a positive rate of 85.4%, 92.7% and 77.9% respectively. In another study by Zheng et al, IgM antibodies start increasing at day 9 and peak at day 18. SARS-CoV-2 specific IgG begins increasing from day 9 to day 15 and persists at elevated level from day 15 to 39. The positive rate for IgG reached 100% around 20 days after onset of symptoms (Zheng, 2020). Long et al, reported that the median day of seroconversion for both IgG and IgM was 13 days after onset of symptoms. IgG was positive in 100% patients (19/19) and antibody levels plateaued within 6 days of seroconversion (Long, 2020).

Zhao et al, noted the presence of antibodies was <40% among patients in the first 7 days of illness, then rapidly increased to 100% 15 days after onset (Table 1). In contrast, the positive rate of viral RNA decreased from 66.7% (58/87) in samples collected before day 7 to 45.5% (25/55) from days 15 to 39. Combining RNA and antibody detections significantly improved the sensitivity of pathogenic diagnosis for COVID-19 patients even in early phase one week from onset. Moreover, a higher titer of Ab was independently associated with worse clinical classification. In addition to antibodies, the measurement of cytokines and other biomarkers of lung injury can be helpful in management of COVID-19 patients. An extreme response from a patient's immune system, termed "cytokine storm" is associated with disease severity and poor prognosis in COVID-19 patients. Continuous high levels of the cytokines are associated with disease deterioration and fatal outcome (Mehta, 2020).

These studies illustrate the value of antibody and cytokine detection in COVID-19 patients over the course of SARSCoV-2 infection. Antibodies offers additional information that is vital for contact tracing, management of infected populations and identification of individuals in whom the COVID-19 infection has been resolved and have some protection from the virus. A compilation of studies and internal data is presented graphically in Figure 1.

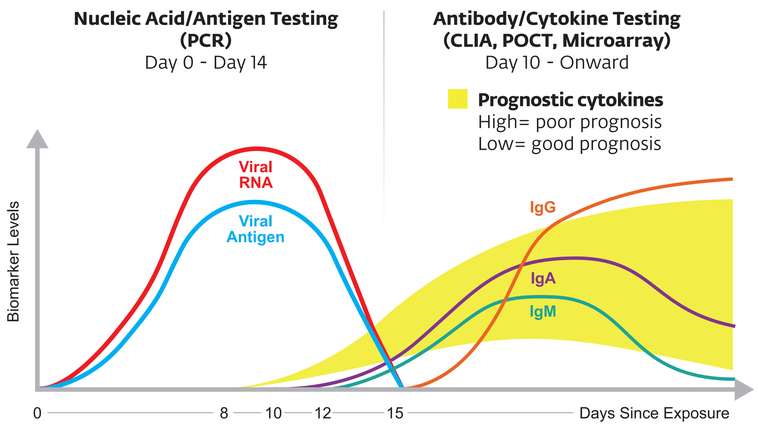

Figure 1. KSL Biomarker Profile: virus, antibodies and cytokines.

METHODS

Clinical Study: Sensitivity of the KSL Coronavirus Disease (SARS-CoV-2/COVID-19) Antibody Chemiluminescence Immunoassay was determined by testing 59 PCR confirmed SARS-CoV-2 patients for IgA, IgG and IgM antibody reactivity (separate results for each antibody). Specificity was determined by testing 865 negative samples along with 252 SARS-CoV-2 PCR positive patient samples. Longitudinal Study: Five of the PCR positive patients from the sensitivity study were repeatedly tested over multiple days to determine the average number of days when antibodies appeared after the beginning of clinical symptoms. In another study, the kits were evaluated based upon comparison with at least 34 known antibody positive samples from competitor kits and 48 antibody negative samples (collected prior to November 2019). All assays were run on the Kaeser 1000 Chemiluminescence automatic analyzer.

RESULTS

Sensitivity

59 PCR confirmed patient sera samples were tested on the KSL SARS-CoV-2 Antibody Tests. A description of the 59 PCR positive patients along with the percent positivity for different sample draw dates is shown below.

			IgA		IgG		IgM	
Stage of Disease	Days Since 1st Symptoms	# Patients	# Pos	% Pos	# Pos	% Pos	# Pos	% Pos
Acute phase (early)	≤ 7 days	10	7	70.0%	7	70.0%	6	60.0%
Recovery period (medium)	8-14 days	19	17	89.5%	18	94.7%	16	84.2%
Cure discharge period (late)	≥ 15 days	30	30	100.0%	30	100.0%	29	96.7%

Specificity

Specificity was determined by studying 865 SARS-CoV-2 PCR negative patient samples tested along with 252 SARS-CoV-2 PCR positive patient samples for a total of 1117 patient samples tested.

	IgA Antibody		IgG Antibody		IgM Antibody	
Sensitivity	**91.5%**	95% CI 80.6%-96.8%	**93.2%**	95% CI 82.7% - 97.8%	**86.4%**	95% CI 74.5% - 93.6%
Specificity	**98.8%**	95% CI 99.1%-100.0%	**99.2%**	95% CI 98.31% - 99.7%	**99.4%**	95% CI 98.5% - 99.8%

Longitudinal Study

Five of the PCR positive patients from the sensitivity study were repeatedly tested over multiple days to determine the average number of days when antibodies appeared after the beginning of clinical symptoms. The median number of days for antibody positivity following the appearance of symptoms was 6 days for IgA and 8 days for IgG and IgM.

# of Days Until Antibody Positivity Following the Appearance of Symptoms

Patient	IgA	IgG	IgM
No. 1	8	8	8
No. 2	4	8	4
No. 3	6	6	6
No. 4	11	11	15
No. 5	5	12	12
Average	7	9	9
Median	6	8	8
Range	4 - 11	6 - 12	6 - 15
Still Positive	> 50 days	> 50 days	> 50 days

Another Study

In another study, the kits were evaluated based upon comparison with known antibody positive samples and antibody negative samples (collected prior to November 2019) based upon competitor kits. The positive and negative percent agreement values are shown below.

		IgA Antibody		IgG Antibody		IgM Antibody	
		Comparator		Comparator		Comparator	
		Pos	Neg	Pos	Neg	Pos	Neg
KSL	Pos	46	0	50	0	34	0
	Neg	5	48	2	48	1	48
	Total	51	48	52	48	35	48
		Positive % Agreement	90.2%	Positive % Agreement	96.2%	Positive % Agreement	97.1%
		Negative % Agreement	100.0%	Negative % Agreement	100.0%	Negative % Agreement	100.0%

		IgA Antibody		IgG Antibody		IgM Antibody	
		Comparator		Comparator		Comparator	
		Pos	Neg	Pos	Neg	Pos	Neg
KSL	Pos	46	0	50	0	34	0
	Neg	5	48	2	48	1	48
	Total	51	48	52	48	35	48
		Positive % Agreement	90.2%	Positive % Agreement	96.2%	Positive % Agreement	97.1%
		Negative % Agreement	100.0%	Negative % Agreement	100.0%	Negative % Agreement	100.0%

Cross reactivity

The assay was tested and found to be negative with sera containing antibodies to the following pathogens: Epstein-Barr virus (EBV), varicella-zoster virus (VZV), human cytomegalovirus (CMV), Mycoplasma pneumoniae, Toxoplasma Gondii, respiratory syncytial virus (RSV), herpes simplex virus Type 1 and Type 2. In addition to pathogen interference testing, antibody specificity was confirmed by testing strong positive SARS-CoV-2 sera from a different antibody. IgA SARS-CoV-2 antibodies were not detected in strong mono-positive IgG or strong mono-positive IgM samples. The assays also produced correspondingly negative results with strong positive sera of the other antibody isotypes.

Endogenous interference

Five samples were tested (2 Neg, 1 Weak Pos and 2 Moderated Pos) in duplicate on three kit lots. Samples with tested with spiked interferents and no interferents. The following concentrations of 20mg/dL bilirubin, 500mg/dL hemoglobin, 3000 mg/dL triglycerides, 12g/dL total protein, 200IU/mL Rheumatoid factor, 285 ng/mL human anti-mouse antibody, and 380 IU/mL anti-nuclear antibody did not interfere with assay results. There was 100% qualitative agreement among all samples.

Precision

Two moderate positive samples were tested in duplicate over 20 days with 2 runs per day using three kit lots at one testing site. All %CV were less than 10%.

		IgA Total Imprecision			IgG Total Imprecision			IgM Total Imprecision		
Kit Lot	Sample	Mean COI	Stnd Dev	CV	Mean COI	Stnd Dev	CV	Mean COI	Stnd Dev	CV
Lot 1	S1	1.81	0.15	8.1%	1.79	0.12	6.5%	1.79	0.14	7.6%
	S2	2.37	0.21	8.8%	2.35	0.21	8.8%	2.33	0.20	8.7%
Lot 2	S1	1.78	0.14	7.8%	1.79	0.14	7.6%	1.80	0.14	7.7%
	S2	2.38	0.20	8.3%	2.32	0.21	9.0%	2.36	0.20	8.4%
Lot 3	S1	1.78	0.13	7.5%	1.79	0.14	8.0%	1.80	0.14	7.8%
	S2	2.40	0.20	8.4%	2.34	0.20	8.5%	2.36	0.18	7.7%

Lot to Lot Reproducibility

Five samples (two negatives, 1 near the cutoff and 2 positives) were tested at one site on three kit lots over 20 days. There was 100% qualitative agreement among all samples for all three antibodies.

Matrix effect

Matched blood samples were collected with heparinized plasma, citrate plasma, sera (no additive) and serum separator tubes. 40 samples were tested in duplicate ranging from negative, low, medium and high positive. There was 100% qualitative agreement among all samples for all three antibodies.

Cutoff Determination

118 apparently normal individuals tested negative on a SARS-CoV-2 Receptor Binding Assay were tested on the KSL SARS-CoV-2 Antibody Tests to determine the Relative Light Units (RLU) where 95% of the patient samples would report as negative on the KSL SARS-CoV-2 Antibody Tests. The cutoffs were set at 40,000 RLU for IgA and IgM and 10,000 for IgG.

CONCLUSIONS

Clinical specificity greater than 98% with a large sample set (n=865)	99.4% IgM, 98.8% IgA, and 99.2% IgG	The KSL SARS-CoV-2 Antibody (IgM, IgA, IgG) Chemiluminescence Assay is a validated high throughput test in a CLIA certified high complexity laboratory cleared to offer patient testing as per Section IV.D of the FDA's Policy for Coronavirus Disease-2019 Tests
Clinical sensitivity greater than 90% with 59 PCR confirmed cases	97% IgM, 90%IgA and 96% IgG	
Precision and reproducibility less than 10%CV	8.7% IgM, 8.8% IgA, 9.0% IgG	

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