Summary of Allplex™ 2019-nCoV Assay performance data

A. Analytical Specificity

Cross-reactivity studies were performed to demonstrate that the test does not react with related pathogens (Table 1) that are reasonably likely to be encountered in the clinical specimen. In addition, the pathogens listed in Table 2, were also wet tested.

Table 1. List of Pathogens analyzed in silico

Other high priority pathogens from the same genetic family	**High priority pathogens likely in the circulating area**
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g. EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Streptococcus salivarius

Table 2 below lists 61 pathogens, and 1 pooled nasal swab wash evaluated by Wet Testing. The bacterial species were tested at ≥ 1 x 10^6 CFU/mL, and viral species at ≥ 1 x 10^5 PFU/mL or 1 x 10^6 genome copies/rxn

Table 2. List of Pathogens evaluated by Wet Testing

No.	**Usage**	**Pathogen**	**Source**	**Isolate No.**
1	*Exclusivity*	human coronavirus HKU1	*Korean isolate*	
2	*Exclusivity*	human coronavirus OC43	ATCC	VR-1558
3	*Exclusivity*	human coronavirus NL63	*Korean isolate*	
4	*Exclusivity*	human coronavirus 229E	ATCC	VR-740
5	*Exclusivity*	human Severe Acute Respiratory Syndrome, SARS	*Korean isolate*	
6	*Exclusivity*	human Middle East Respiratory Syndrome Coronavirus: MERS-CoV	*Korean isolate*	
7	*Exclusivity*	influenza A virus (H1N1)	ATCC	VR-95
8	*Exclusivity*	Influenza A virus (H3N2)	ATCC	VR-547

No.	Usage	Pathogen	Source	Isolate No.
9	*Exclusivity*	influenza B virus	ATCC	VR-523
10	*Exclusivity*	Human Rhinovirus 1	KBPV	VR-81
11	*Exclusivity*	Rhinovirus 21	KBPV	VR-40
12	*Exclusivity*	Human rhinovirus type 90	ATCC	VR-1291
13	*Exclusivity*	Human rhinovirus type 16	ATCC	VR-283
14	*Exclusivity*	Human rhinovirus type 42	ATCC	VR-338
15	*Exclusivity*	Human rhinovirus type 8	ATCC	VR-488
16	*Exclusivity*	Human rhinovirus type 14	ATCC	VR-284
17	*Exclusivity*	Human enterovirus type 68	ATCC	VR-1826
18	*Exclusivity*	Human enterovirus type 70	ATCC	VR-836
19	*Exclusivity*	Human enterovirus type 71	ATCC	VR-784
20	*Exclusivity*	human respiratory syncytial virus A	ATCC	VR-26
21	*Exclusivity*	human respiratory syncytial virus B	ATCC	VR-955
22	*Exclusivity*	Parainfluenza 1 virus	ATCC	VR-1380
23	*Exclusivity*	Human parainfluenza virus 2	ATCC	VR-92
24	*Exclusivity*	Human parainfluenza virus 3	ATCC	VR-93
25	*Exclusivity*	human parainfluenza 4 virus 4a	ATCC	VR-1378
26	*Exclusivity*	Human parainfluenza virus 4b	ATCC	VR-1377
27	*Exclusivity*	Human Metapneumovirus (MPV)	KBPV	VR-87
28	*Exclusivity*	Human adenovirus 1	ATCC	VR-1
29	*Exclusivity*	Human adenovirus 11	KBPV	VR-63
30	*Exclusivity*	Human adenovirus 18	ATCC	VR-1095
31	*Exclusivity*	Human adenovirus 23	ATCC	VR-1101
32	*Exclusivity*	Human adenovirus 3	ATCC	VR-3
33	*Exclusivity*	Human adenovirus 4	ATCC	VR-1572
34	*Exclusivity*	Human adenovirus 8	ATCC	VR-1368
35	*Exclusivity*	Human adenovirus type 31	ATCC	VR-1109
36	*Exclusivity*	Human adenovirus type 40	ATCC	VR-931
37	*Exclusivity*	Human adenovirus type 5	KBPV	VR-61
38	*Exclusivity*	Human adenovirus type 35	ATCC	VR-718
39	*Exclusivity*	*Legionella pneumophila Serotype 2*	ATCC	33154
40	*Exclusivity*	*Legionella pneumophila subsp. fraseri Serotype 4*	ATCC	33156
41	*Exclusivity*	*Legionella pneumophila Serotype 7*	ATCC	33823
42	*Exclusivity*	*Legionella pneumophila Serotype 10*	ATCC	43283
43	*Exclusivity*	*Legionella pneumophila Serotype 11*	ATCC	43130
44	*Exclusivity*	*Legionella pneumophila Serotype 12*	ATCC	43290
45	*Exclusivity*	*Legionella pneumophila Serotype 13*	ATCC	43736
46	*Exclusivity*	*Legionella pneumophila Serotype 14*	ATCC	43703
47	*Exclusivity*	*Legionella pneumophila subsp. fraseri Serotype 15*	ATCC	35251
48	*Exclusivity*	*Mycoplasma pneumoniae*	ATCC	15293
49	*Exclusivity*	*Mycoplasma pneumoniae M129-B7*	ATCC	29342
50	*Exclusivity*	*Chlamydophila pneumoniae*	ATCC	53592
51	*Exclusivity*	*Bordetella pertussis*	ATCC	BAA-589
52	*Exclusivity*	*Pseudomonas aeruginosa (Z139; VIM-1)*	Zeptometrix	801908
53	*Exclusivity*	*Mycobacterium tuberculosis*	ATCC	25177
54	*Exclusivity*	*Haemophilus influenzae*	ATCC	51907
55	*Exclusivity*	*Streptococcus pneumoniae*	KCCM	40410

No.	Usage	Pathogen	Source	Isolate No.
56	*Exclusivity*	*Streptococcus pyogenes*	ATCC	19615
57	*Exclusivity*	*Staphylococcus epidermidis*	KCCM	40416
58	*Exclusivity*	*Candida albicans*	KCCM	11282
59	*Exclusivity*	*Pneumocystis pneumonia jirovecii (PJP)*	*Korean isolate*	
60	*Exclusivity*	*Staphylococcus salivarius*	*Korean isolate*	
61	*Exclusivity*	Pooled human nasal wash	*Clinical sample*	

Allplex™ 2019-nCoV Assay was tested for cross-reactivity to 61 different pathogens. It was performed three times under the same conditions. As a result, E gene was detected with 'human Severe Acute Respiratory Syndrome, SARS', and none of the 60 pathogens generated detectable signals.

B. Sensitivity

Analytical Sensitivity - LoD(Limited of Detection) using CFX96™, CFX96 Touch™

Detection pathogen		Limit of Detection		Unit
		CFX96™	CFX96 Touch ™	
2019-nCoV	E gene	100	100	RNA Copies/rxn
	RdRP gene	100	100	RNA Copies/rxn
	N gene	100	100	RNA Copies/rxn

C. Clinical Evaluation

In this study, 300 left-over samples from Persons Under Investigation (PUI) testing were chosen based on the specimen selection criteria. In order to evaluate the clinical performance of Allplex™ 2019-nCoV Assay (Seegene), the generated real-time PCR results were compared to US FDA EUA cleared 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Panel (CDC).

The results from testing Upper Respiratory specimens including Nasopharyngeal + Oropharyngeal swabs shown in Table 3A generated a Positive Percent Agreement (PPA): 100.00% (49/49) [95% CI: 92.75% ~ 100.00%], and a Negative Percent Agreement (NPA): 94.00% (94/100) [95% CI: 87.40% ~ 97.77%].

Table 3 A: Upper Respiratory samples (Nasopharyngeal + Oropharyngeal swab) n=150

Upper Respiratory result		2019-Novel Coronavirus(2019-nCoV) Real-time RT-PCR Panel			
		2019-nCoV Detected	**Inconclusive**	**2019-nCoV Not Detected**	**Total**
Allplex™ 2019-nCoV Assay	**2019-nCoV Detected**	49	1	2	52
	2019-nCoV presumptive positive	0	0	4	4
	Negative	0	0	94	94
	Total	49	1	100	150

The results from testing Lower Respiratory specimens (Sputum) shown in Table 3 B, generated Positive Percent Agreement (PPA): 100.00% (49/49) [95% CI: 92.75% ~ 100.00%], and a Negative Percent Agreement (NPA): 97.87% 97.87% (92/94) [95% CI: 92.52% ~ 99.74%]

Table 3 B: Lower Respiratory samples (Sputum) n=150

Lower Respiratory result		**2019-Novel Coronavirus(2019-nCoV) Real-time RT-PCR Panel**			
		2019-nCoV Detected	**Inconclusive**	**2019-nCoV Not Detected**	**Total**
Allplex™ 2019-nCoV Assay	**2019-nCoV Detected**	49	1	1	51
	2019-nCoV presumptive positive	0	0	1	1
	Negative	0	0	92	92
	Total	49	1	94	144

By comparing the clinical efficacy with CDC's predicate, 100.00% (49/49) ([95% CI: 92.75% ~ 100.00%] Positive Percent Agreement (PPA) and 94.00% (94/100) [95% CI: 87.40% ~ 97.77%] Negative Percent Agreement (NPA) was obtained for upper respiratory samples. In case of lower respiratory samples, 100.00% (49/49) ([95% CI: 92.75% ~ 100.00%] Positive Percent Agreement (PPA) and 97.87% (92/94) [95% CI: 92.52% ~ 99.74%] Negative Percent Agreement (NPA) was obtained when compared with the CDC's predicate.

In conclusion, we confirm a high clinical concordance between Seegene's Allplex™ 2019-nCoV assay and CDC's FDA EUA cleared 2019-Novel Coronavirus(2019-nCoV) Real-time RT-PCR Panel.