Test Report

Title:	**Analytical Performance Study**
Product:	SGTi-flex COVID-19 IgM/IgG
Date:	Jun. 12, 2020

Protocol No. P-LA-771-01

Revision History		
Rev.0	Apr. 07, 2020	First study after design
Rev.1	Jun. 12, 2020	Addition of sensitivity, potential cross-reactive and interfering substances

Prepared by/ date	Reviewed by/ date	Approved by/ date
Kiyoung Park		Eunkyung Kim
Jun. 12, 2020		Jun. 12, 2020

Table of Content

1. Purpose

The analytical performance studies were carried out for SGTi-flex COVID-19 IgM/IgG Test as design verification according to the Analytical Performance Study Protocol, P-LA-771-01 / Rev.01 / Jun. 01, 2020

2. Test location and duration

2.1 Test location: Laboratory room #216, Sugentech, Inc. Migun Techno World 2-cha
2.2 Test duration: Mar.30 – Jun. 10, 2020

3. Responsibilities

3.1 Study Coordinator: Kiyoung Park / Preparation of test protocol & test report
3.2 Overall Supervisor: Eunkyung Kim / Final review and approval
3.3 Researcher: Kiyoung Park, Paul Kim / Performance of testing

4. Test Results – Sensitivity study

4.1 Test device

Table 1. Test device

Product Name	SGTi-flex COVID-19 IgM/IgG
Lot No.	COVT20101
Manufacturing date	Mar. 25, 2020

4.2 Test Materials

(1) Commercial SARS-CoV-2 IgM/IgG positive sample confirmed by real time RT-PCR was used.
(2) One batch is used for the testing and each testing is to be performed 20 times repeatedly.

4.3 Test date : Jun 09, 2020

4.4 Tested by : Kiyoung Park / Dept. of R&D
Confirmed by : Eunkyung Kim / Dept. of R&D

4.5 Results

Table 2. Test result of sensitivity SGTi-flex COVID-19 IgM/IgG

Dilution Factor	Result_IgM		Result_IgG	
	Number of Positive/all	% Positive rate	Number of Positive/all	% Positive rate
1X	20/20	100	20/20	100
1/2X	20/20	100	20/20	100
1/4X	20/20	100	20/20	100
1/8X	20/20	100	20/20	100
1/16X	20/20	100	20/20	100
1/32X	20/20	100	20/20	100
1/64X	2/20	10	20/20	100
1/128X	0/20	0	2/20	10
COVN-01	0/20	0	0/20	0

4.6 Conclusion

It may vary depending on the sample being tested, but the sensitivity of SGTi-flex COVID-19 IgM/IgG was 1/32X for IgM and 1/64X for IgG based on the IgM/IgG positive sample of patient confirmed by real time RT-PCR used in this test.

5. Test Results - Specificity study

5.1 Test device

Table 3. Test device

Product Name	SGTi-flex COVID-19 IgM/IgG
Lot No.	COVT20101
Manufacturing date	Mar. 25, 2020

5.2 Test Materials

(1) Various commercial plasma were prepared at the original concentration. The potential cross-reactive substances are as follows:

Table 4. Commercial serum and plasma

No.	Supplier	Analytical reactive substance	Number of Samples
1	Trina	Adenovirus IgM	6
2	Trina	Adenovirus IgG	6
3	Trina	Enterovirus	1

4	Trina	Measles IgM	6
5	Trina	Measles IgG	6
6	Trina	Mumps IgM	5
7	Trina	Mumps IgG	6
8	Trina	Parainfluenza	1
9	Seracare	Epstein-Barr Virus (EBV) VCA IgM	1
10	Seracare	Epstein-Barr Virus (EBV) VCA IgG	1
11	Seracare	Cytomegalovirus IgM	1
12	Seracare	Cytomegalovirus IgG	1
13	Seracare	Varicella Zoster Virus (VZV) IgM	1
14	Seracare	Varicella Zoster Virus (VZV) IgG	1
15	Seracare	Mycoplasma IgM	1
16	Seracare	Mycoplasma IgG	1
17	Seracare	Chlamydia IgM	1
18	Seracare	Chlamydia IgG	1
19	LEE biosolution	Rheumatoid Arthritis	2
20	Trina	Autoimmune Control	3
21	Trina	anti-HCV	3
22	Trina	VZV IgM/IgG Positive	3
23	Trina	anti-HBs positive (total)	5
24	Trina	anti-HIV-I Virus Type 1	5
25	Trina	Influenza A virus (H1N1+H3N2) IgM	5
26	Trina	Influenza A virus (H1N1+H3N2) IgG	5
27	Trina	Influenza B virus (Yamagata+Victoria) IgM	5
28	Trina	Influenza B virus (Yamagata+Victoria) IgG	5
29	Trina	Enterovirus group A IgM	5
30	Trina	Enterovirus group A IgG	5
31	Trina	ds-DNA	5
32	Trina	Parainfluenza virus IgM	5
33	Trina	Parainfluenza virus IgG	5
34	Trina	Respiratory syncytial virus IgM	5
35	Trina	Respiratory syncytial virus IgG	5

36	Trina	Rotavirus IgM	5
37	Trina	Rotavirus IgG	5
38	Trina	Rhinovirus group A IgM	5
39	Trina	Rhinovirus group A IgG	5

(2) The tests were performed according to the instructions for use.
(3) Each testing was performed 3 times repeatedly.

5.3 Test date : Mar. 31, 2020, Jun. 10, 2020

5.4 Tested by : Kiyoung Park / Dept. of R&D
Confirmed by : Eunkyung Kim / Dept. of R&D

5.5 Results
The result of reactivity data is summarized at Table 5.

Table 5. Test result of reactivity

No.	Analytical reactive substance	Number of Samples	Number Reactive	Number Non-reactive
1	Adenovirus IgM	6	0	6
2	Adenovirus IgG	6	0	6
3	Enterovirus	1	0	1
4	Measles IgM	6	0	6
5	Measles IgG	6	0	6
6	Mumps IgM	5	0	5
7	Mumps IgG	6	0	6
8	Parainfluenza	1	0	1
9	Epstein-Barr Virus (EBV) VCA IgM	1	0	1
10	Epstein-Barr Virus (EBV) VCA IgG	1	0	1
11	Cytomegalovirus IgM	1	0	1
12	Cytomegalovirus IgG	1	0	1
13	Varicella Zoster Virus (VZV) IgM	1	0	1
14	Varicella Zoster Virus (VZV) IgG	1	0	1
15	Mycoplasma IgM	1	0	1
16	Mycoplasma IgG	1	0	1
17	Chlamydia IgM	1	0	1
18	Chlamydia IgG	1	0	1

19	Rheumatoid Arthritis	2	0	2
20	Autoimmune Control	3	0	3
21	anti-HCV	3	0	3
22	VZV IgM/IgG	3	0	3
23	anti-HBs positive (total)	5	0	5
24	anti-HIV-I Virus Type 1	5	0	5
25	Influenza A virus (H1N1+H3N2) IgM	5	0	5
26	Influenza A virus (H1N1+H3N2) IgG	5	0	5
27	Influenza B virus (Yamagata+Victoria) IgM	5	0	5
28	Influenza B virus (Yamagata+Victoria) IgG	5	0	5
29	Enterovirus group A IgM	5	0	5
30	Enterovirus group A IgG	5	0	5
31	ds-DNA	5	0	5
32	Parainfluenza virus IgM	5	0	5
33	Parainfluenza virus IgG	5	0	5
34	Respiratory syncytial virus IgM	5	0	5
35	Respiratory syncytial virus IgG	5	0	5
36	Rotavirus IgM	5	0	5
37	Rotavirus IgG	5	0	5
38	Rhinovirus group A IgM	5	0	5
39	Rhinovirus group A IgG	5	0	5

5.6 Conclusion

The results showed that the SGTi-flex COVID-19 IgM/IgG had no cross-reactivity with samples containing IgM or IgG antibodies to other viruses, bacteria as well as autoantibodies.

6. Test Results – Interference (1)

6.1 Test device

Table 6. Test device

Product Name	SGTi-flex COVID-19 IgM/IgG
Lot No.	COVT20101
Manufacturing date	Mar. 25, 2020

6.2 Test Materials

(1) The negative reference material (COVN-01) and a positive reference materials (IgM : COVM-01, COVM-03, IgG : COVG-01, COVG-03) manufactured according to SGTi-flex COVID-19 IgM/IgG Standard Material Manufacture and Maintenance (SOP-QC-219) were used.
(2) Various concentrations of potential interfering substances were prepared in negative and positive reference material. All those concentrations of the interfering substances are assured as higher than the recommended levels by the CLSI guideline, EP07-A2.

The concentrations of the interfering substances are as follows:

Table 7. Concentrations of the interfering substances

No	Interfering substance	Supplier	Concentration	Comment
1	Albumin	Sigma, A9647	150 mg/ml	Intrinsic interfering substance, CLSI EP7-A02
2	Glucose	Sigma, G8270	1.2 mg/ml	Intrinsic interfering substance, CLSI EP7-A02
3	Hemoglobin	Sigma, H7379	20 mg/ml	Intrinsic interfering substance, CLSI EP7-A02
4	Bilirubin	Alfa Aesar, A17522	0.02 mg/ml	Intrinsic interfering substance, CLSI EP7-A02
5	HAMA	LEE biosolution, 991-24-S-HAMA	46 ng/mL	-
6	Triglyceride	Supelco, 17810	10 mg/mL	Intrinsic interfering substance, CLSI EP7-A02
7	Acetaminophen	Sigma, A7085	0.2 mg/mL	Exogenous interfering substance, CLSI EP7-A02
8	Acetylsalicylic acid	Sigma, A5376	0.7 mg/mL	Exogenous interfering substance, CLSI EP7-A02
9	Caffeine	Sigma, C1778	0.1 mg/mL	Exogenous interfering substance, CLSI EP7-A02
10	Ascorbic acid	Samchun, A1182	0.2 mg/mL	Exogenous interfering substance, CLSI EP7-A02

6.3 Test date : Apr. 01, 2020, Jun. 08, 2020

6.4 Tested by : Kiyoung Park / Dept. of R&D

Confirmed by : Eunkyung Kim / Dept. of R&D

6.5 Results

Test result of interfering substances data is summarized at Table 8.

Table 8. The result of interference test

No	Interfering Substance	specimen	Result		
			Visual reading		
			1st	2nd	3rd
1	Albumin	COVM-01	M+	M+	M+
		COVM-03	M+	M+	M+
		COVG-01	G+	G+	G+
		COVG-03	G+	G+	G+
		COVN-01	-	-	-
2	Glucose	COVM-01	M+	M+	M+
		COVM-03	M+	M+	M+
		COVG-01	G+	G+	G+
		COVG-03	G+	G+	G+
		COVN-01	-	-	-
3	Hemoglobin	COVM-01	M+	M+	M+
		COVM-03	M+	M+	M+
		COVG-01	G+	G+	G+
		COVG-03	G+	G+	G+
		COVN-01	-	-	-
4	Bilirubin	COVM-01	M+	M+	M+
		COVM-03	M+	M+	M+
		COVG-01	G+	G+	G+
		COVG-03	G+	G+	G+
		COVN-01	-	-	-
5	HAMA	COVM-01	M+	M+	M+
		COVM-03	M+	M+	M+
		COVG-01	G+	G+	G+
		COVG-03	G+	G+	G+
		COVN-01	-	-	-
6	Triglyceride	COVM-01	M+	M+	M+
		COVM-03	M+	M+	M+
		COVG-01	G+	G+	G+
		COVG-03	G+	G+	G+
		COVN-01	-	-	-

7	Acetaminophen	COVM-01	M+	M+	M+
		COVM-03	M+	M+	M+
		COVG-01	G+	G+	G+
		COVG-03	G+	G+	G+
		COVN-01	-	-	-
8	Acetylsalicylic acid	COVM-01	M+	M+	M+
		COVM-03	M+	M+	M+
		COVG-01	G+	G+	G+
		COVG-03	G+	G+	G+
		COVN-01	-	-	-
9	Caffeine	COVM-01	M+	M+	M+
		COVM-03	M+	M+	M+
		COVG-01	G+	G+	G+
		COVG-03	G+	G+	G+
		COVN-01	-	-	-
10	Ascorbic acid	COVM-01	M+	M+	M+
		COVM-03	M+	M+	M+
		COVG-01	G+	G+	G+
		COVG-03	G+	G+	G+
		COVN-01	-	-	-

6.6 Conclusion

The results showed that the SGTi-flex COVID-19 IgM/IgG had no interference by the potential interfering substances above which may exist in specimen, such as prescription/OTC drugs, and elevated levels of chemical and biological analytes.

7. Test Results – Interference (2)

7.1 Test device

Table 9. Test device

Product Name	SGTi-flex COVID-19 IgM/IgG
Lot No.	COVT20101
Manufacturing date	Mar. 25, 2020

7.2 Test Materials

(1) Ig free serum was used as a basic matrix.

(2) The concentration of Cross-reactive susbstances are as follows :

Table 10. Cross-reactive substances

No.	Potential cross-reactive substance	Test concentration	Supplier	Cat. No.
1	human IgG	5.5 mg/mL	Sigma	I2511
2	human IgM	1.2 mg/mL	Sigma	I8260
3	human IgA	1.1 mg/mL	Sigma	I4036
4	Immunoglobulin E (IgE)	13.3 IU/mL	NIBSC	11/234

7.3 Test date : Apr. 01, 2020

7.4 Tested by : Kiyoung Park / Dept. of R&D
Confirmed by : Eunkyung Kim / Dept. of R&D

7.5 Results

The result of interfering substances data is summarized at Table 11.

Table 11. Test result of cross-reactivity

No.	Analytical reactive substances	Result					
		Visual reading					
		1st		2nd		3rd	
		M	G	M	G	M	G
1	human IgG	-	-	-	-	-	-
2	human IgM	-	-	-	-	-	-
3	human IgA	-	-	-	-	-	-
4	Immunoglobulin E (IgE)	-	-	-	-	-	-

6.6 Conclusion

SGTi-flex COVID19 IgM/IgG showed no cross-reactivity with human IgG, IgM, IgA and IgE.

8. Test Results – Class Specificity

8.1 Test device

Table 12 Test device

Product Name	SGTi-flex COVID-19 IgM/IgG
Lot No.	COVT20101
Manufacturing date	Mar. 25, 2020

8.2 Test Materials

(1) 5 COVID-19 IgM positive sera (M01 to M05) and 5 COVID-19 IgG positive sera (G01 to G05) were prepared. The positive sera were residual paired serum specimens from PCR-positive confirmed patients.
(2) The concentration of class specific susbstances to compete were as follows :

Table 13. Class specific substances

No.	Class specific substance	Test concentration	Supplier	Cat. No.
1	Anti-human IgM	1 mg/mL	Fapon	BRCIGMC105
2	Anti-human IgG	10 mg/mL	Fapon	BRCIGGS101

(3) As shown in the table below, M01 to M05 were spiked with anti-human IgM and G01 to G05 were spiked with anti-human IgG.

Table 14. Preparation of IgM sample (M01 to M05)

No.	Test (Anti-human IgM treatment)	Control (No Anti-human IgM treatment)
IgM Positive serum	10 µL	10 µL
Anti-human IgM	3 µL	0 µL
1X PBS	0 µL	3 µL

Table 15. Preparation of IgG sample (G01 to G05)

No.	Test (Anti-human IgG treatment)	Control (No Anti-human IgG treatment)
IgG Positive serum	10 µL	10 µL
Anti-human IgG	20 µL	0 µL
1X PBS	0 µL	20 µL

8.3 Test date : Apr. 20, 2020

8.4 Tested by : Kiyoung Park / Dept. of R&D
Confirmed by : Eunkyung Kim / Dept. of R&D

8.5 Results
The result of class specificity is summarized at Table 16and 17

Table 16. Test result of IgM class specificity

Sample	Replicates	Test Result	Control Result	IgM signal

ID		Anti-human IgM treatment (IgM/IgG)	No anti-human IgM treatment (IgM/IgG)	decrease
M01	1	-/-	+/-	yes
	2	-/-	+/-	yes
M02	1	+/++++	++/++++	yes
	2	+/++++	++/++++	yes
M03	1	-/++	+/++	yes
	2	-/++	+/++	yes
M04	1	weak pos/+++	+/+++	yes
	2	weak pos/+++	+/+++	yes
M05	1	weak pos/+++	+/+++	yes
	2	weak pos/+++	+/+++	yes

Table 17. Test result of IgG class specificity

Sample ID	Replicates	Test Result Anti-human IgG treatment (IgM/IgG)	Control Result No anti-human IgG treatment (IgM/IgG)	IgG signal decrease
G01	1	weak pos/weak pos	weak pos/++	yes
	2	weak pos/weak pos	weak pos/++	yes
G02	1	+/weak pos	+/+	yes
	2	+/weak pos	+/+	yes
G03	1	-/-	-/+	yes
	2	-/-	-/+	yes
G04	1	-/-	-/+	yes
	2	-/-	-/+	yes
G05	1	-/+	-/++	yes
	2	-/+	-/++	yes

8.6 Conclusion

To demonstrate that the assay accurately detects each antibody class (IgM and IgG), SGTi-flex COVID19 IgM/IgG was tested with specimens treated with the same antibody as the immobilized antibody.

Because the same anti-human IgM immobilized on the membrane was used for spiking, we predicted that the spiked anti-human IgM would bind COVID-19 IgM of sample and compete with the anti-human IgM immobilized on the membrane, resulting in a decrease in IgM signal. The same was predicted for IgG.

SGTi-flex COVID19 IgM/IgG showed 100% agreement with expected result to establish antibody class specificity. When reacting with anti-human IgM, the results

showed that the final IgG result was unaffected and the final IgM signal decreased. On the other hand, the results of the reaction with anti-human IgG showed that the final IgM results were not affected and the final IgG signal decreased.

9. Test Results – Precision/Reproducibility

9.1 Test device

Table 18. Test device

Product Name	SGTi-flex COVID-19 IgM/IgG		
Lot No.	COVT20101	COVT20102	COVT20103
Manufacturing date	Mar. 25, 2020	Mar. 26, 2020	Mar. 27, 2020

9.2 Test Materials

The negative reference material (COVN-01) and a positive reference materials (COVM-01,02,03, COVG-01,02,03) manufactured according to SGTi-flex COVID-19 IgM/IgG Standard Material Manufacture and Maintenance (SOP-QC-219) were used.

9.3 Test date : Mar. 30, 2020

9.4 Tested by : Kiyoung Park / Dept. of R&D
Confirmed by : Eunkyung Kim / Dept. of R&D

9.5 Results

The Within-run performance data is summarized at Table 19
The Between-run performance data is summarized at Table 20
The Batch-to-batch performance data is summarized at Table 21

Table 19. Test result of within-run performance

Test	Reference material						
	COVM-01	COVM-02	COVM-03	COVG-01	COVG-02	COVG-03	COVN-01
1	M+	M+	M+	G+	G+	G+	-
2	M+	M+	M+	G+	G+	G+	-
3	M+	M+	M+	G+	G+	G+	-
4	M+	M+	M+	G+	G+	G+	-
5	M+	M+	M+	G+	G+	G+	-

6	M+	M+	M+	G+	G+	G+	-
7	M+	M+	M+	G+	G+	G+	-
8	M+	M+	M+	G+	G+	G+	-
9	M+	M+	M+	G+	G+	G+	-
10	M+	M+	M+	G+	G+	G+	-
11	M+	M+	M+	G+	G+	G+	-
12	M+	M+	M+	G+	G+	G+	-
13	M+	M+	M+	G+	G+	G+	-
14	M+	M+	M+	G+	G+	G+	-
15	M+	M+	M+	G+	G+	G+	-
16	M+	M+	M+	G+	G+	G+	-
17	M+	M+	M+	G+	G+	G+	-
18	M+	M+	M+	G+	G+	G+	-
19	M+	M+	M+	G+	G+	G+	-
20	M+	M+	M+	G+	G+	G+	-
21	M+	M+	M+	G+	G+	G+	-
22	M+	M+	M+	G+	G+	G+	-
23	M+	M+	M+	G+	G+	G+	-
24	M+	M+	M+	G+	G+	G+	-
25	M+	M+	M+	G+	G+	G+	-
26	M+	M+	M+	G+	G+	G+	-
27	M+	M+	M+	G+	G+	G+	-
28	M+	M+	M+	G+	G+	G+	-
29	M+	M+	M+	G+	G+	G+	-
30	M+	M+	M+	G+	G+	G+	-

Table 20.Test results of Between-run performance

Tested by	Repeat testing	Reference Material						
		COVM-01	COVM-02	COVM-03	COVG-01	COVG-02	COVG-03	COVN-01

Person 1	1st	M+	M+	M+	G+	G+	G+	-
	2nd	M+	M+	M+	G+	G+	G+	-
	3rd	M+	M+	M+	G+	G+	G+	-
	4th	M+	M+	M+	G+	G+	G+	-
	5th	M+	M+	M+	G+	G+	G+	-
Person 2	1st	M+	M+	M+	G+	G+	G+	-
	2nd	M+	M+	M+	G+	G+	G+	-
	3rd	M+	M+	M+	G+	G+	G+	-
	4th	M+	M+	M+	G+	G+	G+	-
	5th	M+	M+	M+	G+	G+	G+	-

Table 21.Test results of Batch-to-batch performance

Lot No.	Repeat testing	Reference Material						
		COVM-01	COVM-02	COVM-03	COVG-01	COVG-02	COVG-03	COVN-01
COVT 20901	1st	M+	M+	M+	G+	G+	G+	-
	2nd	M+	M+	M+	G+	G+	G+	-
	3rd	M+	M+	M+	G+	G+	G+	-
COVT 20902	1st	M+	M+	M+	G+	G+	G+	-
	2nd	M+	M+	M+	G+	G+	G+	-
	3rd	M+	M+	M+	G+	G+	G+	-
COVT 20903	1st	M+	M+	M+	G+	G+	G+	-
	2nd	M+	M+	M+	G+	G+	G+	-
	3rd	M+	M+	M+	G+	G+	G+	-

9.6 Conclusion

The results showed that the reproducibility of the SGTi-flex COVID-19 IgM/IgG was acceptable. Within-run, Between-run, Batch-to-batch performance results meet 100% of the acceptance criteria.