Clinical Performance Report

<Evaluation of Point-of-Care Tests for antigen of COVID-19>

IRB No. KNUH-2020-07-012

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1. Title

Evaluation of Point-of-Care Tests for antigen of COVID-19

2. IRB approval No. / Date

KNUH-2020-07-012 / 2020. 07. 24

3. Purpose of Clinical Research

1) 1st & 2nd evaluation: The purpose of this report is to evaluate clinical performance by comparing the performance of the reference test method approved by FDA under Emergency Use Authorization [CFX96 Realtime PCR system test method using high-risk infectious gene test reagent, Allplex 2019-nCoV Assay (manufacture: Seegen)] and test reagent [GENEDIA W COVID-19 Ag (manufacturer: GCMS)]. The specimens were used remaining samples to be discarded after the SARS-CoV-2 diagnosis.
2) 3rd evaluation: The purpose of this report is to evaluate clinical performance by comparing the performance of the reference test method approved by FDA under Emergency Use Authorization [CFX96 Realtime PCR system test method using high-risk infectious gene test reagent, STANDARD M nCoV Real-Time Detection kit (manufacture: SD BIOSENSOR)] and test reagent [GENEDIA W COVID-19 Ag (manufacturer: GCMS)]. The specimens were used remaining samples to be discarded after the SARS-CoV-2 diagnosis.

4. Test Period

1st evaluation: July 24, 2020 ~ July 27, 2020
2nd evaluation: August 22, 2020
3rd evaluation: September 22, 2020

5. Test Method

- The specimens with positive or negative COVID-19 confirmed were used visiting Kangwon University Hospital, remaining samples to be discarded after the examination. These blinded specimens could be used for 1 year when stored at the freezer (Below -20℃).
- Specimen type: lower respiratory tract(sputum specimens), upper respiratory tract (Nasopharyngeal swab)
- Blinded remaining samples were delivered to the investigator with randomly assigned.
- Clinical evaluation investigator conducted test according to each product

manufacturer's method, wrote CRF (Case Report Form) and delivered the CRFs to principle Investigator.

- Principle investigator compared test result.
  - Test Kit : GCMS, GENEDIA W COVID-19 Ag
  - Reference Test Kit : Seegen, Allplex™ 2019-nCoV Assay (1st & 2nd evaluation)
  - Reference Test Kit : SD BIOSENSOR, STANDARD M nCoV Real-Time Detection kit (3rd evaluation)

(1) Blinded Clinical Specimens

After the regular examination, the remaining samples were blinded (only written identification number) and aliquoted in 1.5 ml microtube by the principal investigator (Inbeom Seo). These specimens were delivered to the investigator (Hana Kim) and only identification number and test results were opened, patient information such as gender, name and age were not opened at all.

(2) Identification Code

Blinded remaining samples were conducted single-blind method by the principal investigator and investigator encoded identification code regardless of positive/negative information of specimens.

The method of assigning a unique clinical sample number is by using the formula for generating a random number table in the Excel program and assigns a new unique number for clinical trials randomly assigned from 1to 1650 by placing the samples in order and substituting the Excel formula. Blinded samples are delivered to the investigator.

Excel formula: INT((RAND()*(last value-start value+1))+start value

(3) Result Analysis

1) 1st & 2nd evaluation

Compared with the measured values of GENEDIA W COVID-19 Ag (the test reagent) and Allplex™ 2019-nCoV Assay (Seegene, Reference test kit). In this study, we compare the results of medical devices for clinical trials with the results performed for diagnostic purposes.

2) 3rd evaluation

Compared with the measured values of GENEDIA W COVID-19 Ag (the test reagent) and STANDARD M nCoV Real-Time Detection kit (SD BIOSENSOR, Reference test kit). In this study, we compare the results of medical devices for clinical trials with the results performed for diagnostic purposes.

(4) Specimen collection and storage method

1) Selection Criteria

- Among the samples confirmed positive and negative for Coronavirus Infectious Disease-19 after a regular test was requested at Kangwon National University Hospital, the remaining samples scheduled to be discarded after all requested tests are completed. Respiratory specimens (upper and lower respiratory tract)
- The remaining specimens include upper and lower respiratory tract specimens stored at the freezer under the -20 ℃ for re-examination. In this study, specimens were stored in a freezer at -80℃.
- Nasopharyngeal swab specimens were eluted in Viral Transport Media (VTM) (Noble Bioscience, Inc). Sputum specimens were not used VTM.

2) Exclusion Criteria

When the remaining specimen diagnosis (RT-PCR) information was uncertain.

When the remaining specimen quantity was under minimum (0.1 mL).

When the remaining specimen was not stored below -20℃.

(5) Test method of test reagent

1) After putting the sample into the sample extract so that it can be sufficiently extracted, turn the drip stopper and lock it.
2) By removing the aluminum pouch, take out the inspection device and place it on a flat area.
3) 3 drops are plated on the drop site.
4) The reading time is 10 minutes after dropping the specimen and if it can be determined as positive from 5 minutes.

(6) Number of Final specimens

1) 1st & 2nd evaluation: Specimens were confirmed by Allplex™ 2019-nCoV Assay (Seegen) if positive or negative.

2) 3rd evaluation: Specimens were confirmed by STANDARD M nCoV Real-Time Detection kit (SD BIOSENSOR) if positive or negative.

➔ 102 positive, 129 negative, and total of 231 specimens were used in this clinical evaluation.

Evaluation	Specimen Type	Positive	Negative
1st evaluation	Human respiratory specimen (Sputum specimens and Nasopharyngeal swab)	21	129
2nd evaluation	Human respiratory specimen (Nasopharyngeal swab)	3	0
3rd evaluation	Human respiratory specimen (Nasopharyngeal swab)	78	0
Total		102	129

(7) Confidential

All specimen lists and data analysis conducted using Microsoft office excel or word program and locked. Principle investigator and investigator only person who knows the password.

6. Evaluation Method

(1) Test interpretation

GENEDIA W COVID-19 Ag was used the principle of Immunochromatographic Assay (ICA) and the result was judged based on the measured value of GENEDIA W COVID-19 Ag.

Interpretation of result	Measured value (Device)
Positive	Color bands appear in both the test line (T) and the control line (C)
Negative	No color band on the test line (T) and only the control line (C) appears
Invalid	No color band on the control line (C)

(2) Observation test method

Reference test kits (Allplex™ 2019-nCoV Assay or STANDARD M nCoV Real-Time Detection kit) were real-time reverse transcription PCR method and GENEDIA W COVID-19 Ag, using Immunochromatographic Assay (ICA), and reference test kit test results were

compared.

(3) Statistical exclusion criteria

If the control line (C) does not appear as a result of the measured values of GENEDIA W COVID-19 Ag produced by using Immunochromatographic Assay (ICA), the specimen was exclusion.

7. Evaluation and Interpretation Method (According to the statistical method)

(1) Results by days since symptom onset

Days Since Symptom Onset	Confirmation Test Positive (+)	GENEDIA W COVID-19 Ag Positive (+)
-	A	B

- PPA (Positive predictive agreement) = B / A x 100

(2) Clinical sensitivity / Specificity

Clinical sensitivity and specificity for COVID-19 : Compare with the reference test kit(with confirmation test) with a 95% confidence interval.

		Confirmation Test Result	
		Positive	Negative
GENEDIA W COVID-19 Ag	Positive	C	D
	Negative	E	F

- Clinical Sensitivity: Positive confirmed by a confirmation test and test kit
  Sensitivity = C / (C+E) x 100
- Clinical Specificity: Negative confirmed by a confirmation test and test kit
  Specificity = F / (D+F) x 100

(3) Agreement ratio and Cohen's Kappa

- Total Agreement: (C+F) / (C+D+E+F) x 100
- Cohen's Kappa: Cohen's kappa (k) shall be used as a statistical way to measure agreement of comparative test results of medical devices for clinical study and control test, and it is evaluated to be 0 or 1. If k=0, it is to evaluate how they are completely different. If k=1, it is to evaluate how they are completely agreed. Cohen's kappa is known with the following criteria. It is required to see if 0.8 or above is derived by calculating Cohen's kappa (k). (Valid if k is greater than 0.8).

8. Evaluation Result

(1) The number of specimen evaluation: Clinical sensitivity and specificity

Specimen	Positive	Negative
Human respiratory specimen (Sputum specimens)	18	0
Human respiratory specimen (Nasopharyngeal swab)	84	129

(2) Patient Demographics: The positive results broken down by age of the patients.

Age	GENEDIA W COVID-19 Ag		
	Total #	Positive	Prevalence
≤ 5 years	7	2	28.6%
6 to 21 years	26	1	3.8%
22 to 59 years	77	28	36.4%
≥ 60 years	121	58	47.9%

(3) Positive results broken down by days since symptom onset

Days Since Symptom Onset	RT-PCR Positive (+)	GENEDIA W COVID-19 Ag Positive (+)	PPA	95% Confidence Interval	
≤7	49	48	98%	89.1%	100.0%
8 to 14	22	17	77%	54.6%	92.2%
≥15	7	6	86%	42.1%	99.6%
Asymtomatic	24	18	75%	53.3%	90.2%

(4) Clinical sensitivity and specificity

1) In Sputum specimens

		Comparator (RT-PCR)		Total
		Positive	Negative	
GENEDIA W COVID-19 Ag	Positive	16	0	16
	Negative	2	0	2
Total		18	0	18

- Clinical sensitivity : 88.89% (95% CI : 65.3% - 98.6%)
- Total Agreement Ratio: 88.89%

2) In Nasopharyngeal swab

		Comparator (RT-PCR)		Total
		Positive	Negative	
GENEDIA W COVID-19 Ag	Positive	73	0	73
	Negative	11	129	140
Total		84	129	213

- Clinical sensitivity : 86.90% (95% CI : 77.8% - 93.3%)
- Clinical specificity : 100% (95% CI : 97.2% - 100%)
- Total Agreement Ratio: 94.8%

3) Clinical sensitivity and specificity in all specimen

		Comparator (RT-PCR)		Total
		Positive	Negative	
GENEDIA W COVID-19 Ag	Positive	89	0	89
	Negative	13	129	142
Total		102	129	231

- Clinical sensitivity : 87.25% (95% CI : 79.2% - 93.0%)
- Clinical specificity : 100% (95% CI : 97.2% - 100%)

(5) Agreement ration and Cohen's kappa value

1) Total Agreement Ratio : 94.4%

2) Cohen's Kappa : K=0.884 (High performance)

9. Conclusion

To confirm the clinical performance, GENEDIA W COVID-19 Ag was evaluated in Kangwon National University.

Total 231 specimens were used : 18 positive sputum specimens / 84 positive and 129 negative nasopharyngeal swabs. The specimens were confirmed RT-PCR assay.

The table below shows the positive results broken down by age of the specimens,

Age	GENEDIA W COVID-19 Ag (n= 231)		
	Total #	Positive	Prevalence
≤ 5 years	7	2	28.6%
6 to 21 years	26	1	3.8%
22 to 59 years	77	28	36.4%
≥ 60 years	121	58	47.9%

Positive results broken down by days since symptom onset :

Days Since Symptom Onset	RT-PCR Positive (+)	GENEDIA W COVID-19 Ag Positive (+)	PPA	95% Confidence Interval	
≤7	49	48	98%	89.1%	100.0%
8 to 14	22	17	77%	54.6%	92.2%
≥15	7	6	86%	42.1%	99.6%
Asymtomatic	24	18	75%	53.3%	90.2%

Overall data analysis is presented below

		Comparator (RT-PCR)		Total
		Positive	Negative	
GENEDIA W COVID-19 Ag	Positive	89	0	89
	Negative	13	129	142
Total		102	129	231

- Clinical sensitivity : 87.25% (95% CI : 79.2% - 93.0%)
- Clinical specificity : 100% (95% CI : 97.2% - 100%)
- Total Agreement Ratio : 94.4%
- Cohen’s Kappa : K=0.884 (High performance)