Clinical Performance Evaluation Report

Single-center, Randomized, Single-Blind comparison confirmatory clinical trial for evaluating clinical performance of the new Coronavirus (SARS-CoV 2) detection product, 'BioCore 2019-nCoV Real Time PCR Kit'

2020.03.31

	Name	Responsibility	Sign / Date
By	Chan-Ki Kim	Head of clinical performance test	2020.4.1

Seoul Clinical Laboratories

Contents

1. Title of clinical performance test

Single-center, Randomized, Single-Blind comparison confirmatory clinical trial for evaluating clinical performance of the new Coronavirus (SARS-CoV 2) detection product, 'BioCore 2019-nCoV Real Time PCR Kit'

2. The name and location of the clinical performance testing institution (hereinafter referred to as the testing institution)

Institution name	Location	Phone No,
Seoul Clinical Laboratories	24F, 13, Heungdeok 1-ro, Giheung-gu, Yongsin-si, Gyeonggi-do, 16954, South Korea	1800-0119

3. The name and position of the person in head of the clinical performance test, examiner, and the collaborators

3.1 Head of clinical performance test

Name	Department	Major	Position	Phone No.
Chang-Ki Kim	Seoul Clinical Laboratories	Laboratory Medicine	Ph.D. (M.D. of Laboratory medicine)	+82)-2-330-0562

3.1 Examiners of clinical performance test

Name	Department	Major	Position	Phone No.
Yeon-Su Seon	Seoul Clinical Laboratories	Clinical Pathology	Assistant manager	+82)-2-330-2106
Se-Won Kim	Seoul Clinical Laboratories	Biotechnology	Staff	+82)-2-330-2104

4. The name and position of the medical device manager for clinical performance testing

Name	Department	Major	Phone No.
Cho-Long Lee	Seoul Clinical Laboratories	The Science of Nursing	+82)-2-330-2105

5. The name and address of the person intending to conduct a clinical performance test

5.1 Referral Agency

Agency	CEO	Location	Phone No.
BioCore Co., Ltd. Biotechnology Division	Seung-Yong Hwang	#605, ACE Techno Tower 5th, 20, Digital-ro 31-gil, Guro-gu, Seoul, 08380, South Korea	+82)-2-2205-9942

5.2 Monitor personnel

Agency	Name	Location	Phone No.
BioCore Co., Ltd. Biotechnology Division	Ji-Yeon Park	#605, ACE Techno Tower 5th, 20, Digital-ro 31-gil, Guro-gu, Seoul, 08380, South Korea	+82)-2-2205-9942

6. Purpose and Introduction of clinical performance test

6.1 Purpose

This study was conducted to verify clinical effectiveness through clinical performance test. In order to verify the clinical effectiveness, we evaluated the clinical sensitivity and clinical specificity of BioCore 2019-nCoV Real Time PCR Kit developed by BioCore Biotechnology Division using sputum, oropharyngeal and nasopharyngeal swabs. In addition, we evaluated the conformity degree with the 'Emergency Use Authorization' product.

6.2 Introduction

Coronavirus Disease-19 (COVID-19) is a respiratory syndrome caused by SARS-COV-2 infection. Currently, it is classified as a new infectious disease syndrome in the first

class of infectious diseases in Korea. The propagation path is known as the spread of respiratory droplets due to coughing and sneezing, or through contact while touching contaminated objects and touching the eyes, nose, and mouth. The incubation period is known as 1 to 14 days (average 4 to 7 days). Symptoms include fever, boredom, cough, respiratory distress and pneumonia, and acute respiratory distress syndrome, ranging from mild to severe respiratory symptoms. Rarely, sputum, sore throat, headache, hemoptysis and nausea and diarrhea are known to appear. The fatality rate is known as 1-2%, but it is not clear. However, it can lead to severe or even death in the elderly, patients with reduced immune function, and patients with underlying diseases [1, 2]. Therefore, rapid diagnosis is necessary for proper treatment.

We developed "BioCore 2019-nCoV Real Time PCR Kit" that can diagnose SARS-COV-2 to help in the treatment of corona virus infection through accurate diagnosis of COVID-19. This clinical test is conducted to confirm whether the clinical performance of the 'BioCore 2019-nCoV Real Time PCR Kit' is equivalent to the 'Allplex ™ 2019-nCoV Assay' which is already approved with 'Emergency Use Authorization'.

6.3 Licensed Similar Products

Country	Manufacturer	Product Name	License
South Korea	Kogenebiotech	Powerchek™ 2019-nCoV Real-time PCR kit	Emergency Use Authorization Product (approval date: 2020.02.04)
South Korea	Seegene Inc.	Allplex™ 2019-nCoV Assay	Emergency Use Authorization Product (approval date: 2020.02.12)
South Korea	SD BIOSENSOR Inc.	STANDARD M n-CoV Real-Time Detection Kit	Emergency Use Authorization Product (approval date: 2020.02.27)
South Korea	SolGent Co., Ltd.	DiaPlexQ™ Novel Coronavirus(2019-nCoV) Detection Kit	Emergency Use Authorization Product (approval date: 2020.02.27)
South Korea	BioSewoom Inc.	Real-Q 2019-nCOV Detection Kit	Emergency Use Authorization Product (approval date: 2020.03.13)

7. Summary of in vitro diagnostic medical device for clinical performance test

7.1 Test Equipment

7.1.1 Item name (Item grade): High-risk infectious agent genetic test reagent (3)

7.1.2 Model name: BioCore 2019-nCoV Real Time PCR Kit

7.1.3 Manufacturer: BioCore Co., Ltd. Biotechnology Division

7.1.4 Purpose of use

This in vitro diagnostic medical device helps the diagnosis of new coronavirus infection (COVID-19) by qualitatively detecting the gene (N gene and RDRP gene) of new coronavirus (Corona 19, 2019-nCoV) using Reverse-transcription Real-time Polymerase Chain Reaction in samples of suspected patients with respiratory infections (sputum, oropharyngeal and nasopharyngeal swabs).

7.1.5 Target diseases and Indications

Target diseases: Coronavirus Disease-19 (COVID-19), the Respiratory syndrome

7.2 Control Equipment

7.2.1 Item name (Item grade): High-risk infectious agent genetic test reagent (3)

7.2.2 Model name: Allplex™ 2019-nCoV Assay

7.2.3 Manufacturer: Seegene Inc.

7.2.4 Purpose of use

In vitro diagnostic medical device that qualitatively detects the gene (E gene, RdRp gene, N gene) of corona19 virus (SARS-CoV-2) in samples (sputum, oropharyngeal and nasopharyngeal specimens) of patients with suspected respiratory infections

7.2.5 Target diseases and Indications

Target diseases: Coronavirus Disease-19 (COVID-19), the Respiratory syndrome

8. Subject of clinical performance test

8.1 Selection Criteria

8.1.1 Specimen selection criteria

1) An anonymized sample among the residual specimen of sputum, oropharyngeal and nasopharyngeal swabs used for the purpose of diagnosing SARS-CoV-2, the causative virus of coronavirus Disease-19, from the Seoul Clinical Laboratories. Also, these specimen meet the requirements of Article 24, Paragraph 1, 2 of the Enforcement Rule of the Medical Device Act

2) Sputum, oropharyngeal and nasopharyngeal throat swabs that were anonymized and stored frozen at - 70 ℃ after the IRB approval of the Seoul Clinical Laboratories

3) Residual specimens with a minimum volume of 1 ㎖ or more for nucleic acid extraction

8.1.2 Exclusion Criteria

1) Residual specimens that do not meet the subject's 'Selection Criteria'

2) Specimens that cannot be verified for stability due to be stored at refrigeration and room temperature for at least 1 day

3) Specimens with less than 1 ㎖ insufficient to diagnose

8.2 Number of Target Samples

1) Sputum

- Positive samples: 20, Diluted positive samples: 20, Negative samples: 40

cf) Diluted positive samples: To compare the sensitivity among products, we selected 5 positive samples and diluted them into two concentrations (① 1/2 dilution ② 1/10 dilution) to perform 2 repetition tests for each concentration. (5 samples x 2 dilution concentrations x 2 repeated tests)

2) Oropharyngeal and nasopharyngeal throat swabs

- Positive samples: 20, Diluted positive samples: 20, Negative samples: 40

cf) Diluted positive samples: To compare the sensitivity among products, we selected 5 positive samples and diluted them into two concentrations (① 1/2 dilution ② 1/10 dilution) to perform 2 repetition tests for each concentration. (5 samples x 2 dilution concentrations x 2 repeated tests)

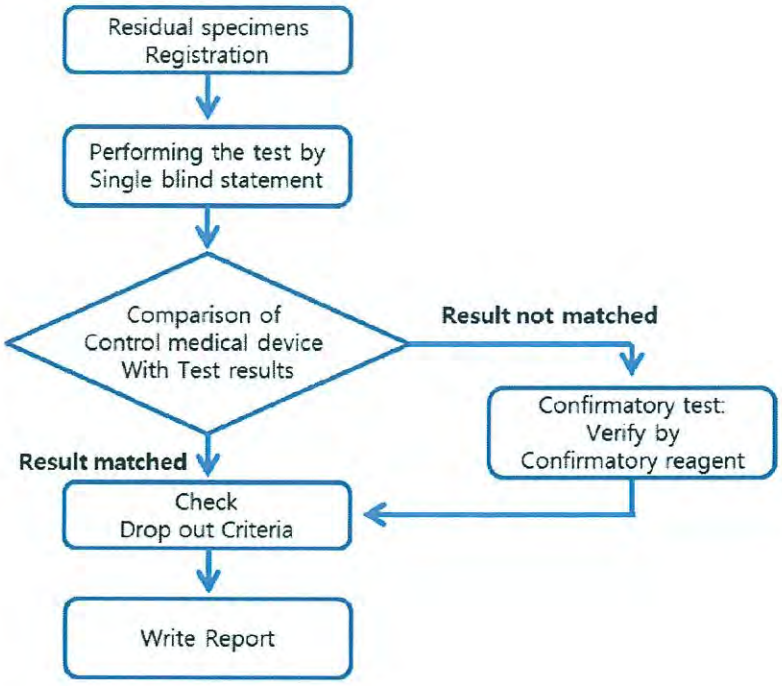
8.2.1 Basis for calculating the number of subjects

Refer to the sample applied for clinical performance by referring to the application data for the "Corona19 Diagnostic Medical Device for Export" by the Convergence Innovation Product Support Group of the Ministry of Food and Drug Safety.[4)]

However, there are no papers discussing the clinical performance of the corona-19 virus diagnostic kit within a few months after the first recognition and diagnosis of the corona-19 virus. Therefore, the number of subjects to be used in this clinical test was referred to the Q & A guideline of the COVID-19 (SARS-CoV-2 or 2019-CoV) test of the Korean Society for Diagnostic Medicine. They recommend that the each number of positive and negative samples should be parallel tested with at least 10. [5)]

9. Procedure of Clinical performance test

9.1 Summary



9.1.1 Comparison Test

1) Test reagent: BioCore 2019-nCoV Real Time PCR Kit

(BioCore Co., Ltd. Biotechnology Division)

Medical Device: CFX96™ System, Bio-rad

2) Control reagent: Allplex™ 2019-nCoV Assay (Seegene Inc.)

Medical Device: CFX96™ System, Bio-rad

9.1.2 Confirmatory Test

1) Confirmatory reagent: Powerchek™ 2019-nCoV Real-time PCR kit

(Kogenebiotech)

Medical Device; ABI Prism 3130/3130XL Genetic Analyzer

9.2 Sample Screening

Residual specimens were collected from the lower respiratory tract (sputum) or upper respiratory tract (oropharyngeal swabs, nasopharyngeal swabs) and stored in COVID-19 at the Seoul Clinical Laboratories.

9.2.1 Sample Anonymization and Randomization

1) Anonymization method

The composition of the person in charge for this clinical performance test consisted of the head tester, examiner 1, examiner 2, the sample managing /screening manager and the medical device controller, and the statistician. The sample managing/screening personnel anonymized the subject information by assigning screening numbers (ex, OPT-001, OPT-002,...) to selected samples according to selection and exclusion criteria.

2) Blind Test method

Information about samples was conducted in blind state except the sample managing/screening manager and medical device controller. And in principle, they do not communicate with each other about the results.

In addition, the tester of the test reagent and the control reagent were placed separately so that the test results were not known to each other. The quality level of blindness was maintained by having independent statistician.

3) Termination of Blind Test

After the clinical performance test, the blind state was released.

4) Cancellation of Blind Test

Since it is a test using the residual sample, it was determined that the effect on the test subject is extremely weak and so is not applied.

5) Randomization method

Block Randomization program: Screening numbers were randomized using 'RESEARCH RANDOMIZER' (https://www.randomizer.org), and random numbers were assigned in a randomized order. The tester performed the test by providing only random number information.

9.2.2 Stop-Drop out Criteria

1) Stop Criteria

Because of the use of residual samples, there are no separate stop criteria and stop treatment.

2) Drop out Criteria

(1) When the results of the medical device used in test and control test are invalid,

Proceed the 'reanalysis', then if the control group and the experimental group fail to derive the analysis results, exclude the relevant experimental results and samples.

(2) If the direction or preparation method of the control test medical device and clinical performance test medical device is not followed, exclude the results and samples of the test.

(3) In case of the clinical evaluation is difficult to proceed due to contamination of the sample.

3) Drop out Treatment

(1) In case of abnormal reagent, request the company and exchange it for the same lot product.

(2) In case of a malfunction of a medical device, the medical device manufacturer must repair the device and then clinical evaluation should be performed.

(3) In the case of stop or drop out due to the subject's request or contamination of the sample, another subject sample should be collected according to the clinical performance test protocol and conduct the clinical performance test.

10. Result

10.1 Clinical performance test period

2020. 03. 30 – 2020. 03. 31

10.2 Target Samples

40 positive and 40 negative samples, the same quantity as the protocol, were used in this clinical performance test.

10.2.1 Practically Used Samples

1) A total of 160 samples were used, 80 upper respiratory tract samples and 80 lower respiratory tract samples were used for clinical performance tests.

2) In the case of the 40 positive samples, 20 samples (10 upper respiratory tract samples, 10 lower respiratory tract samples) were diluted 1/2 and 1/10 for further experiments.

Table. Types of samples used in this clinical performance test

Result	Dilution Fact	Pharyngeal swab	Sputum	Total
Negative	Undiluted	40	40	80
Positive	Undiluted	20	20	80
	Diluted (1/2)	10	10	
	Diluted (1/10)	10	10	
Total		80	80	**160**

(Unit : case)

10.3 Primary Validation

Comparison test		Confirmatory test	Result
Test reagent	**Control reagent**	**Confirmatory reagent**	
Positive: 80	Positive: 80	-	True Positive: 80
	Negative: 0	Positive: -	True Positive:
		Negative: -	False Positive
Negative: 80	Positive: 0	Positive: -	False Negative
		Negative: -	True Negative
	Negative: 80	-	True Negative: 80

(Unit : case)

10.3.1 Evaluation of Clinical Sensitivity

Clinical Sensitivity = True Positive/(True Positive + False Negative) X 100(%)

= 80/(80+0) X 100(%) = 100%

(95% Confidence interval: 95.42%-100.00% / Significance level = 0.05)

10.3.2 Evaluation of Clinical Specificity

Clinical Specificity = True Negative /(True Negative + False Positive) X 100(%)

= 80/(80+0) X 100(%) = 100%

(95% Confidence interval: 95.42%-100.00% / Significance level = 0.05)

10.4 Secondary Validation

10.4.1 Evaluation of Conformity degree

		Control reagent		Total
		Positive	Negative	
Test reagent	Positive	80	0	80
	Negative	0	80	80
Total		80	80	160

(Unit : case)

- **Total Conformity degree**

= (a+d)/(a+b+c+d) X 100(%) = (80+80)/(80+0+0+80) X 100(%) =100%

(95% Confidence interval, 97.72 ~ 100.0% / Significance level: 0.05)

- **Conformity degree of Positive = a/(a+c) X 100(%) = 80/(80+0) X 100(%) =100%**

(95% Confidence interval, 95.42 ~ 100.0% / Significance level: 0.05)

- **Conformity degree of Negative = d/(b+d) X 100(%) = 80/(0+80) X 100(%)=100%**

  (95% Confidence interval, 95.42 ~ 100.0% / Significance level: 0.05)

- **Kappa : 2(ad-bc)/{(a+b)(b+d)+(a+c)(c+d)}**

  = 2(80*80-0*0)/{(80+0)(0+80)+(80+0)(0+80)} = 12800 / 12800 = 1

  (95% Confidence interval, 1 ~ 1)

<Guideline for analyzing concordance rate by K value (Cohen, 1960)>

Kappa	Interpretation
Kappa ≤ 0	Less than chance agreement
0.0 < Kappa ≤ 0.2	Poor
0.2 < Kappa ≤ 0.4	Fair
0.4 < Kappa ≤ 0.6	Moderate
0.6 < Kappa ≤ 0.8	Substantial
0.8 < Kappa ≤ 1.0	Good

11. Conclusion

We evaluated the clinical sensitivity and specificity of the BioCore 2019-nCoV Real Time PCR Kit for the new coronavirus (SARS-CoV 2) by comparison with the licensed product, Allplex™ 2019-nCoV Assay (Seegene, No. 20-119). These kits were tested by clinical samples (nucleic acid) of the same new Coronavirus Disease (COVID-19).

As a result of testing 80 positive samples and 80 negative samples, the conformity degree was 100.0%, clinical sensitivity was 100.0% (95% confidence interval, 95.42 ~ 100.0%), and clinical specificity was 100.0% (95% confidence interval, 95.42 ~ 100.0%).

In addition, the result of BioCore 2019-nCoV Real Time PCR Kit did not derived the false positive and false negative of new Coronavirus Disease (COVID-19). And, the concordance rate with the licensed product Allplex™ 2019-nCoV Assay (Kappa = 1) was excellent. Therefore, BioCore 2019-nCoV Real Time PCR Kit is useful for detecting new coronavirus Disease (COVID-19).

12. References

1) Corona19. Korea Centers for Disease Control and Prevention http://ncov.mohw.go.kr/baroView.do?brdId=4&brdGubun=&dataGubun=&ncvContSeq=&contSeq=&board_id=
2) Severe acute respiratory syndrome-related coronavirus: The species and its viruses – a statement of the Coronavirus Study Group. Alexander E. Gorbalenya. 2020.02.07
3) Corona 19 gene detection test approved for emergency use Product information. KCDC
4) "Corona19 Diagnostic Medical Device for Export" Application for permission, 2020.02.19(Wed) the Convergence Innovation Product Support Group of the Ministry of Food and Drug Safety
5) COVID-19 (SARS-CoV-2 or 2019-nCoV) test Q&A, COVID-19 Diagnostic Test Management Committee Q&A version3 (2020.03.23)
6) Measurement of Inter-Rater Reliability in Systematic Review. Hanyang Med Rev, 2015, 35: 44-49

13. Appendix

- Case Report Form (Attachment)