Clinical Performance Study Report

A single-site, single-blind, randomized, retrospective clinical performance study of 'All*Check* COVID19 Ag(AccuFind COVID19 Ag)' – an investigational medical device designed to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in human upper respiratory specimens (nasopharyngeal and oropharyngeal swabs) for assessment of clinical sensitivity and clinical specificity in comparison with "Allplex™ 2019-nCoV Assay" – a reagent authorized for emergency use.

Study No.	**MDCTC-20-053**
Version No.	**1.0**
Version Date	**September 23 2020**
Sponsor	**<CALTH>**
Term	**September 18 – 22 2020**
Report Written by	**Se Won Kim, Sub-investigator /s/** 2020.10.7
Principal Investigator	**Chang Ki Kim, Principal Investigator /s/** 2020.10.7

SCL Quality, Service, Research (재)서울의과학연구소 Seoul Clinical Laboratories

원본대조필 날 짜 2020.10.7 담당자 김세원 서 명

Table of Contents

1. Title of Study

A single-site, single-blind, randomized, retrospective clinical performance study of 'All*Check* COVID19 Ag(AccuFind COVID19 Ag)' – an investigational medical device designed to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in human upper respiratory specimens (nasopharyngeal and oropharyngeal swabs) for assessment of clinical sensitivity and clinical specificity in comparison with "Allplex™ 2019-nCoV Assay" – a reagent authorized for emergency use.

2. Name and Address of Research Institute (hereinafter referred to as the "Institute")

Name	Address	Contact
Seoul Clinical Laboratories	24th Floor, Tower Building, Heungdeok IT Valley, 13, Heungdeok 1-ro, Giheung-gu, Yongin-si, Gyeonggi-do, Korea	1800-0119

3. Name and Duty of Principal Investigator, Sub-investigator and Person-in-charge

3.1 Principal Investigator

Name	Affiliation	Major	Duty	Contact
Chang Ki Kim	Seoul Clinical Laboratories	Laboratory Medicine	Specialist	02-330-2207

3.2 Sub-investigator

Name	Affiliation	Major	Task	Contact
Yeon Su Seon	Seoul Clinical Laboratories	Clinical Pathology	Implementation of Research	02-330-2106
Se Won Kim	Seoul Clinical Laboratories	Bioengineering	Implementation of Research	02-330-2104

3.3 Co-investigator

Name	Affiliation	Major	Task	Contact
Hannah Kim	Seoul Clinical Laboratories	Clinical Pathology	Sampling and anonymization	02-330-2105

4. Name and Address of Investigational Medical Device Administrator

Name	Affiliation	Major	Contact
Yeon Su Seon	Seoul Clinical Laboratories	Clinical Pathology	02-330-2106

5. Name and Address of Sponsor

5.1 Sponsor

Name	CEO	Address	Contact
<CALTH>	Dong Ho Lee	Suite 321, Corporate Growth Center, 54, Changeop-ro, Sujeong-gu, Seongnam-si, Gyeonggi-do, South Korea	031-754-0320

5.2 Monitoring Agent

Name	Name	Address	Contact
<CALTH>	Keum Hee Jung	Suite 321, Corporate Growth Center, 54, Changeop-ro, Sujeong-gu, Seongnam-si, Gyeonggi-do, South Korea	031-754-0320

6. Evaluation purpose and Background

6.1 Purpose

The investigational device (study reagent) - 'All*Check* COVID19 Ag(AccuFind COVID19 Ag)' - intended to be used for immunological assay of high-risk infectious agents deploys immunochromatographic assay (ICA) for qualitative assessment of SARS-CoV-2 in human upper respiratory specimens (nasopharyngeal and oropharyngeal swabs). The purpose of this study is to assess clinical effectiveness of the investigational medical device in comparison with the control device - 'Allplex™ 2019-nCoV Assay' - authorized for emergency use.

6.2 Background

A viral disease that comes from the specific variety of coronavirus, COVID-19 – also known as Wuhan pneumonia as named after where it occurred or Novel Coronavirus Infectious Disease - first occurred in December 2019 when this pandemic disease was known as contagious respiratory disease of unknown cause. Later known to be occurred by the specific variety of coronavirus like 2003's SARS-CoV and 2012's MERS-CoV on January 7 2020, COVID-19 went epidemic, became subject to public health emergencies of international concern (PHEIC) as declared by WHO on January 30 2020 and ended up became pandemic on March 11 2020 also as declared by WHO (www.who.int).

To prevent use of any name that may stigmatize certain communities or animal groups, WHO on February 11 2020 entitled this novel coronavirus disease as COVID-19 to stand for Corona Virus

Disease 2019, replacing all other varieties like Wuhan pneumonia. In Korea, Korea Centers for Disease Control and Prevention decided to call COVID-19 as Corona-19 locally.

COVID-19 has majorly been a contagious respiratory infection, known to be spread by droplet transmission (upon coughing or sneezing) or direct contact (of eyes, nose or mouth with contaminated hands). Symptoms of COVID-19 include, without limitation, fever, fatigue, malaise, cough, dyspnea, pneumonia and acute respiratory distress syndrome, varying severity, occasionally accompanying sputum, sore throat, headache, haemoptysis, nausea or diarrhea. The novel coronavirus of COVID-19 can penetrate the lung to occur hyperthermia, cough or dyspnea that resembles pneumonic symptoms, leading to alveolar damages and death by respiratory failure in the extreme case. The infection fatality rate of COVID-19 sits around 1 to 2%, not confirmative yet. Most death or severe cases are old patients, immunocompromised patients and patients with underlying diseases (Korea Centers for Disease Control and Prevention).

A specific type of ssRNA based virus falling under *Coronaviridae* family and *Coronavirinae* subfamily, SARS-CoV-2 being a Genus *Betacoronavirus* was found for the first time in February 2020 as a new NP (nucleocapsid protein) virus (Figure 1). It is to not that a coronavirus is made of different structural proteins; SP(spike protein), EP(envelope protein) and MP(membrane protein). A SARS-CoV-2 disease was entitled as COVID-19 as mentioned above. As of April 23, a total of 2,440,713 patients contracted COVID-19, 170,433 out of whom were dead. In Korea, a total of 10,702 patients contracted COVID-19, 240 out of whom were dead. (http://ncov.mohw.go.kr/)

While PCR testing is capable of confirming COVID-19 infection by taking advantage of its sensitivity that is highest among other test methods, complexity of testing, needs for highly skilled specialists and expensive test devices, and extensive period of testing that takes from 5 hours up to a week make the conventional PCR testing not an optimal test method for detection of COVID-19.

With the above being said, <CALTH>'s immunochromatographic assay-based rapid test kit for detection of COVID-19 antibodies takes advantage by shortening the testing where patients come up, receive testing and get the results back right away.

With clinical effectiveness of <CALTH>'s 'All*Check* COVID19 Ag(AccuFind COVID19 Ag)' researched in terms of the kit's ability to detect COVID-19-related markers , the clinical performance results are provided in this report.

6.3 Similar Medical Devices Authorized

Country	Manufacturer	Product	Authorization
Korea	Green Cross Medical Science Corp.	GENEDIA W COVID-19 Ag	*In Vitro* # Heo20-756
Korea	Sugentech, Inc.	SGTi-flex COVID-19 Ag	*In Vitro* # Heo20-675
Korea	GenBody, Inc.	Genbody COVID-19 Ag	*In Vitro* # Heo20-558
Korea	BIONOTE Co., LTD.	NowCheck COVID-19 Ag Test	*In Vitro* # Heo20-385
Korea	RapiGEN Inc.	BIOCREDIT COVID-19 Ag	*In Vitro* # Heo20-305
Korea	SD Biosensor, INC.	STANDARD™ Q COVID-19 Ag Test	*In Vitro* # Heo20-217

7. Investigational *In Vitro* Medical Device Outline

7.1 Investigational Medical Device (Study Reagent)

1) Name: All*Check* COVID19 Ag(AccuFind COVID19 Ag)

2) Class: 3

3) Model: CHR11

4) Manufacturer: <CALTH>

5) Dose form: Card type, in CALTH's package

6) Indication: Qualitative assessment of antibodies against COVID-19 in human upper respiratory specimens (nasopharyngeal and oropharyngeal swabs), by way of Immunochromatographic assay (ICA).

7.1.1 Method of Use

1) Preparing samples

Put a cotton swab into an oral / nasal cavity, roll gently for about 4 to 5 times to get oropharyngeal / nasopharyngeal swabs and remove.

2) Transport and storage of specimens

① If possible, it is advised that testing immediately follows sampling.

② If testing is not performed immediately, swabs must be stored in properly capped Viral Transport Media (VTM).

③ If stored in VTM, it is advised that swabs are stored under extremely low temperature (-70℃) (or in frozen carbon dioxide or liquid nitrogen. Make sure swabs shall not be stored for in excess of 7 days in refrigerated settings. (Storage in general-purpose freezer at around -20℃ is not advised.)

④ Avoid re-freezing any thawed component to preserve sensitivity. If swabs are need frozen, dispense swabs by proper amount before brought frozen.

3) Test method

① Ensure all components of the Kit thawed under room temperature at least 15 to 30 minutes prior to testing.

② Put a cotton swab into an oral / nasal cavity, roll gently for about 4 to 5 times to get oropharyngeal / nasopharyngeal swabs and remove. Take 60㎕ swab in VTM back and mix with the 60㎕ swab just collected.

③ Unseal moistureproof (aluminum) pouch and put the device on an even surface.

▶ Device sensitivity may be affected when stored in moist condition. Use the device immediately when unsealed.

④ Drip 100㎕ prepared swab on the designated spot (S) on the device.

⑤ Read in 10 to 15 minutes.

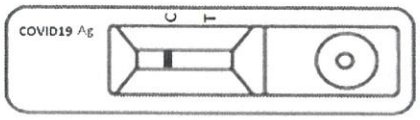
※ For accuracy's sake, a result read after 15 minutes does not count.

4) Quality control

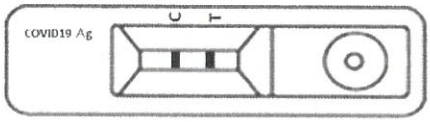
For a result to be counted valid, Control Line (C) color must be either violet or purple.

5) Judgment

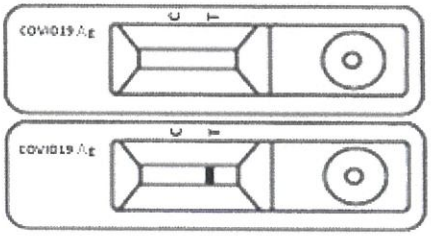
① NEG: Color line only on Control Line (C)



② POS: Color line on both Control Line (C) and Test Line (T)



③ Invalid: No color line on both Control Line (C) and Test Line (T), or no color line on Control Line (C)



7.2 Reagent in Control Medical Device for *In Vitro* Diagnosis (Control Reagent)

1) Name: Allplex™ 2019-nCoV Assay

2) Classification (Class): Reagent for Genetic Testing of High-risk Infectants (3)

3) Medical Device for *In Vitro* Diagnosis: CFX96™ Dx System (#Su In 10-205, Gene Amplifier, Bio-rad)

7.2.1 Purpose of Use

The control medical device is a medical device for diagnosis of COVID-19 in *in vitro* settings by qualitatively detecting 2019-nCoV genes (E genes, N genes, RdRp genes) using real-time reverse-transcription PCR in samples (sputum, oropharyngeal and nasopharyngeal swabs, nasopharyngeal aspirate, bronchoalveolar lavage) of suspected patients of respiratory infection diseases.

7.2.2 Method of Use

1) Real-time PCR

① Set reagents ready on ice. Mix all of the following reagents in a 1.5ml tube:

<One-step RT-PCR Mastermix by Reaction Count (in μℓ)>

No. of Reactions	1	2	3	4	5
2019-nCoV MOM	5	10	15	20	25
RNase-free Water	5	10	15	20	25
5X Real-time One-step Buffer	5	10	15	20	25
Real-time One-step Enzyme	2	4	6	8	10

② Gently shake for about 5 times or vortex and bring to gentle centrifugation.

③ Dispense 17μℓ One-step RT-PCR Mastermix to each PCR tube.

④ Add 8μℓ nucleic acids, 8μℓ 2019-nCoV PC and 8μℓ 2019-nCoV NC to each tube where One-step RT-PCR Mastermix is dispensed.

⑤ Cap the PCR tube and gently centrifuge.

⑥ If the precipitation is not observed, perform centrifugation over at higher rpm and for extensive time.

⑦ Perform PCR immediately upon completion of centrifugation.

2) Getting Real-time PCR Equipment Ready

① Protocol Setup

A. In the main menu, go to File → New → Protocol to access Protocol Editor.

B. In Protocol Editor, Set the reaction protocol conditions as follows:

C. Click Sample Volume and enter 25μℓ.

D. Click OK to access Experiment Setup.

② Plate Setup

A. In Experiment Setup, go to Plate tab and click Create New to access Plate Editor.

B. Click on Select Fluorophores and choose the desired fluorescent materials to be used (FAM, HEX, Cal Red610, Quasar670).

C. Select Wells, and choose Sample Type among other dropdown menus.

- Unknown: For study group.

- Negative Control: For negative control group.

- Positive Control: For positive control group.

D. In the selected Wells, check the fluorescent materials to be used (FAM, HEX, Cal Red610, Quasar670).

E. In each selected Well, enter Sample Name and press enter to confirm.

F. In the main menu for Plate Editor, Go to Settings and select appropriate Plate Size (96well) and Plate Type (BR White).

G. Confirm by clicking on OK and save as a new plate file.

H. Experiment Setup is accessed just like that.

I. Click on Next to Start Run.

③ Click on Start Run.

A. On Start Run tab in Experiment Setup, click on Close Lid to close the lid shut.

B. Click on Start Run.

C. To save a run file on Document folder or any other folder in the desired directory, define the file name and click on SAVE. Device starts running as set.

3) Database Interpretation

[INTERPRETATION]

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
IC (HEX)	+/-	+/-	+/-	+/-	+/-	+	-
E gene (FAM)	+	+/-	-	+/-	+	-	-
RDRP gene (Cal Red 610)	+	-	+	+	-	-	-
N gene (Quasar 670)	+	+	+	-	-	-	-
Result Interpretation	2019-nCoV Detected	Inconclusive Result*			2019-nCoV Not Detected, Sarbecovirus Detected.	Negative	Invalid

* 1) Testing needs performed over at higher nucleic acid concentration.

2) Result of testing needs interpreted by way of sequencing.

[Cut-off]

Ct value	Result

≤ 40	Valid
> 40 or N/A	Invalid

Testing needs performed over if IC Ct > 40.

8. Term of Clinical Performance Study

8.1 Inclusion Criteria

8.1.1 Donor

1) Positive samples: Samples of patients tested (RT-qPCR) COVID-19 positive in Seoul Clinical Laboratories
2) Negative samples: Samples of healthy subjects tested (RT-qPCR) COVID-19 negative in Seoul Clinical Laboratories

8.1.2 Sample

1) Positive samples: Upper respiratory (nasopharyngeal and oropharyngeal swabs) of donors satisfying all criteria under 8.1.1 1)
2) Negative samples: Upper respiratory (nasopharyngeal and oropharyngeal swabs) of donors satisfying all criteria under 8.1.1 2)
3) Samples stored in VTM
4) Residual samples stored at the temperature of or below -70℃ after the point of diagnosis made
5) Anonymous samples
6) Samples sufficient for analysis in terms of volume

8.2 Exclusion Criteria

8.2.1 Donor

Donors not having been tested for COVID-19 infection yet.

8.2.2 Sample

No sample falling under any of the below exclusion criteria shall be included in the study:

1) Samples not stored at the temperature of or below -70℃
2) Samples with unclear positivity/negativity to COVID-19
3) Insufficient samples for analysis in terms of volume
4) Any other samples with any clinically significant finding deemed, by either principal investigator or any sub-investigator, inappropriate for the study

8.3 Recruitment Target

A total of 100 samples (with 40 samples positive and 60 negative)

8.3.1 Ground of Recruitment Target

Database for Application for Authorization of "Exported Medical Device for Diagnosis of COVID-19," Innovative Combination Products Support Team, MFDS

9. Method of Clinical Performance Study

9.1 Design of Clinical Performance Study

Rate of agreement was assessed by comparing study reagent result and control reagent result of detecting SARS-CoV-2 infection. Samples were collected based on the inclusion criteria in Article 8.1., followed by randomization, blinding, randomization and detection of SARS-CoV-2 Ag in residual samples. Infection of SARS-CoV-2 infection was determined by comparing the result of above with control reagent result.

9.2 Sample Supply and Management Criteria

9.2.1 Method of Sample Supply

The residual samples of patients tested either COVID-19 positive or COVID-19 negative as a result of RT-qPCR using control reagent (Allplex™ 2019-nCoV Assay) in detection of SARS-CoV-2 - a causative virus.

9.2.2 Volume : 100㎕

9.2.3 Methods of Storage and Discarding

1) Samples collected based on the inclusion criteria in Article 8.1 were stored at temperature of -70℃ or lower, followed by thawing at 4℃ immediately prior to testing and, if left, brought to storage at temperature of -70℃ or lower.

2) Upon completion of all tests, samples must be discarded within a week under the principal investigator's supervision.

3) Governing law(s) and regulation(s) as well as guideline(s) shall apply to discarding of samples collected.

9.3 Methods of Anonymization, Blinding and Unblinding of Sample

9.3.1 Anonymization

Principal Investigator, Sub-investigator 1, Sub-investigator 2, Co-investigator and Medical Device Administrator comprise a team of this research. Principal Investigator, Sub-investigator 1, Sub-investigator 2, Co-investigator and Medical Device Administrator comprise a team of this research (e.g. KE-001, KE-002, ...) to each sample satisfying inclusion and exclusion criteria.

9.3.2 Blinding

As regards samples, all the above team members with the exception of Co-investigator and Medical Device Administrator must stay blind by not sharing any test result. Investigational reagent and control reagent must be tested by separate Sub-investigators to ensure they are oblivious of the result of the reagent not tested by themselves. Unblinding should take place once all clinical performance studies are complete. Unblinding should take place once all clinical effectiveness researches are complete.

9.4 Randomization Method

Randomization should take place as follows:

1) Block Randomization program: 'RESEARCH RANDOMIZER' (https://www.randomizer.org).

2) Random number table needs printed out upon completion of two rounds of randomization with the labeled screening number veiled.

Once screening numbers (e.g. KE-COV-001) are assigned to all samples, the Principal Investigator should break down these samples using the above-mentioned random number table and hand the breakdown to the sub-investigators to perform tests.

10. Discontinuation and Drop-off Criteria and Handling of Discontinued and Dropped-off Cases

10.1 Discontinuation Criteria

Tests use reagents and samples collected and stored, all in *in vitro* settings; no test is expected to be subject to discontinuation in the middle due to adverse reaction or side effect for safety of subjects. However, the Principal Investigator may suspend any test, in whole or in part, if deemed necessary in light of any result of clinical performance study by consulting the CALTH. The CALTH may suspend any test, in whole or in part, due to any safety or administrative reason.

10.2 Drop-off Criteria

If a sample is registered to the test by satisfying all criteria but failed to complete the test regardless of use of the investigational medical device, that sample is 'dropped off.' When falling under any of the following drop-off criteria, a sample must be dropped off of the test, in which case the concerned Sub-investigator must clearly set forth the reason for the drop-off in the case report form:

1) Error occurs in the results of any two tests performed;

2) Volume of any sample is insufficient to perform any test;

3) Sample was exposed to room temperature for in excess of 2 hours or to refrigerated (2~8℃) condition for in excess of 24 hours and thereby becomes inappropriate to be used to perform any test;

4) Sample failed to satisfy any inclusion / exclusion criterion is used;

5) Error is confirmed in screening number assigned to any sample;

6) Any result regarding confirmation of infectious disease is misstated under any standard test method; or

7) Sample is otherwise deemed inappropriate to be used or hindering performance of any clinical research.

10.3 Handling Dropped-off Cases

Once falling under any drop-off criterion, the sample must be dropped off and the result must not be included in any statistical analysis, while the data related to the duly conducted clinical research and reason for drop-off are kept in writing and preserved and the test must be performed over by using a new sample.

11. Results of Research

11.1 Assessment Criteria

Agreement (in terms of agreement and rate of agreement) between study reagent and control reagent was assessed in this study.

11.2 Assessment and Interpretation

11.2.1 Assessment

Result		Control		
		Positive	Negative	Sum
Study	Positive	a	b	a+b
	Negative	c	d	c+d
	Sum	a+c	b+d	a+b+c+d

1) Agreement

Agreement is assessed in terms of Cohen's kappa; Kappa statistic must be at or in excess of 80%.

From statistical perspective, agreement is assessed in terms of Cohen's kappa(κ) – results generally interpreted as per the following criteria. To ensure good agreement, Kappa statistic - with 95% confidence interval defined along - must be at or in excess of 80%.

$$Kappa = 2(AD-BC)/\{(A+B)(B+D)+(A+C)(C+D)\}$$

Kappa	Interpretation
Kappa ≤ 0	Coincidence or less than likely
0.0 < Kappa ≤ 0.2	Poor agreement
0.2 < Kappa ≤ 0.4	Fair agreement

0.4 < Kappa ≤ 0.6	Moderate agreement
0.6 < Kappa ≤ 0.8	Substantial agreement
0.8 < Kappa ≤ 1.0	Good agreement

2) Rates of agreement in positive samples, negative samples and all samples are represented in terms of percentage, with 95% confidence interval presented along:

- PPA(%) = 100 x a / (a+c)
- NPA(%) = 100 x d / (b+d)
- Overall Rates of Agreement(%) = 100 x (a+d) / (a+b+c+d)

11.3 Term of Research

One month, starting the date of IRB approval

Term: September 18 – 22 2020

11.4 Samples Used

40 Positive samples and 60 negative samples – satisfying all the inclusion and exclusion criteria – were used for this research.

11.5 Results of Research

The study reagent [All*Check* COVID19 Ag(AccuFind COVID19 Ag)] results derived out of samples collected were compared with the control reagent (Allplex™ 2019-nCoV Assay) results derived out of the same samples. The results have it that only one positive sample (tested negative) out of the total of 100 samples discord with the corresponding investigational reagent samples, with the remaining 99 samples in agreement.

(In number of cases)

Effectiveness Results		Control (Allplex™ 2019-nCoV Assay)		Sum
		Positive	Negative	
Study Reagent (All*Check* COVID19 Ag)	**Positive**	39	0	39
	Negative	1	60	61
Sum		40	60	100

- PPA = a / (a+c) x 100(%)

= 39 / (39+1) x 100(%) = 97.50(%)

(95% CI 0.8712 – 0.9956)

- NPA = d / (b+d) x 100(%)

= 60 / (0+60) x 100(%) = 100.0(%)

(95% CI 0.9398 – 1.0000)

- Overall Rates of Agreement = (a+d) / (a+b+c+d) x 100(%)

  = (39+60) / (39+0+1+60) x 100(%) = 99.0(%)

  (95% CI 0.9455 – 0.9982)

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

  = 2(39*60-0*1)/{(39+0)(0+60)+(39+1)(1+60)} = 4680 / 4780 = 0.9791

  (95% CI 0.9746 – 0.9828)

12. Conclusion of Clinical Performance Study

The clinical performance of All*Check* COVID19 Ag(AccuFind COVID19 Ag) detecting SARS-CoV-2 in samples collected in upper respiratory tracts of the patients that were tested either positive or negative when using Allplex™ 2019-nCoV Assay (# Heo 20-119) is analyzed as follows:

In terms of rate of agreement, 97.50% (95% CI 0.8712– 0.9956) of positive samples, 100.0% (95% CI 0.9398 – 1.0000) of negative samples and 99.0% (95% CI 0.9455 – 0.9982) of all samples were in agreement with the corresponding investigational reagent results, representing 'good agreement' in terms of kappa statistic (kappa = 0.9791 (95% CI 0.9746 - 0.9828)).