Clinical Performance Report

BIOCREDIT COVID-19 Ag

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Summary Clinical study Report

BIOCREDIT COVID-19 Ag is a rapid lateral flow immunochromatographic assay for the detection of SARS-CoV-2 antigen in human nasopharynx. Nasopharyngeal swab specimen is needed to perform the assay. Reading of the results is done visually i.e. subjectively read.

Performance evaluation (Total)

Total sensitivity and specificity are in the following.

BIOCREDIT COVID-19 Ag		**RT-PCR**		**Sensitivity (%)**	**Specificity (%)**
		Positive	**Negative**		
Ag	Positive	46	0	90.2% (46/51)	100% (136/136)
	Negative	5	136		
Total		51	136		

Sensitivity: 90.2% (46/51, 95% CI, 77.81% - 96.33%)

Specificity: 100% (136/136, 95% CI, 96.57% - 100%)

Positive predictive value (PPV): 100% (46/46, 95% CI, 90.40% - 100%)

Negative predictive value (NPV): 96.45% (136/141, 95% CI, 91.49% - 98.69%)

1) Clinical Evaluation 1

a. Test site: Seoul Asan Hospital(South Korea), Eunpyung St. Mary's Hospital(South Korea)

b. Test date: April 01, 2020

c. Specimen: 15 COVID-19 positive and 2 COVID-19 negative specimens

d. Test result: Summary of performance of BIOCREDIT COVID-19 Ag compare to PCR confirmed specimens

BIOCREDIT COVID-19 Ag		**RT-PCR**		**Sensitivity (%)**	**Specificity (%)**
		Positive	**Negative**		
Ag	Positive	12	0	80 (12/15)	100 (2/2)
	Negative	3	2		
Total		15	2		

Sensitivity: 80% (12/15, 95% CI, 51.37% - 94.69%)

Specificity: 100% (2/2, 95% CI, 19.79% - 100%)

2) Clinical Evaluation 2

a. Test site: Fundação Oswaldo Cruz, Brazil

b. Test date: June 18, 2020

c. Specimen: 11 COVID-19 positive and 109 COVID-19 negative specimens

d. Test result: Summary of performance of BIOCREDIT COVID-19 Ag compare to PCR confirmed specimens

BIOCREDIT COVID-19 Ag		RT-PCR		Sensitivity (%)	Specificity (%)
		Positive	Negative		
Ag	Positive	10	0	90.9% (10/11)	100% (109/109)
	Negative	1	109		
Total		11	109		

Sensitivity: 90.9% (10/11, 95% CI, 57.18% - 99.52%)

Specificity: 100% (109/109, 95% CI, 95.76% - 100%)

3) Clinical Evaluation 3

a. Test site: Infectious Clinical Hospital No. 2 of the Moscow City Health Department, Russia

(Web site: https://www.ikb2.ru/english)

b. Test date: June 15~18, 2020

c. Specimen: 25 COVID-19 positive and 25 COVID-19 negative specimens

d. Test result: Summary of performance of BIOCREDIT COVID-19 Ag compare to PCR confirmed specimens

BIOCREDIT COVID-19 Ag		RT-PCR		Sensitivity (%)	Specificity (%)
		Positive	Negative		
Ag	Positive	24	0	96 (24/25)	100 (25/25)
	Negative	1	25		
Total		25	25		

Sensitivity: 96% (24/25, 95% CI, 77.68% - 99.79%)

Specificity: 100% (25/25, 95% CI, 83.42% - 100%)

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Retrospective clinical trial to evaluate clinical performance of BIOCREDIT COVID-19 Ag using clinical specimens of nasopharyngeal swap obtained from symptomatic patients confirmed by RT-PCR

Catholic University, Eunpyung St. Mary's Hospital, South Korea

Seoul Asan Hospital, South Korea

Clinical Performance Report

BIOCREDIT COVID-19 Ag

List of Abbreviations

- COVID-19: Novel Coronavirus (SARS-CoV-2)
- WHO: World Health Organization
- SARS: Severe Acute Respiratory Syndrome
- MERS: Middle East Respiratory Syndrome
- RT-PCR: Real Time Polymerase Chain Reaction
- NP: Nasopharyngeal
- uL: microliter

1 Introduction

1.1 Background

The World Health Organization (WHO) has been declared pandemic, since the outbreak of the novel coronavirus (COVID-19) in Wuhan, China, the disease has spread rapidly to the world. While COVID-19 reports low-fatality rate compare to previous SARS or MERS, cases of significant respiratory failure requiring cases of significant respiratory failures requiring mechanical ventilation have been reported.

Person-to-person spread is thought to occur mainly via respiratory droplets, and even asymptomatic carriers can spread the virus, posing challenges in containing widespread of the disease. Non-severe symptoms of COVID-19, which includes fever, cough, malaise, may overlap with symptoms of common cold and influenza infection.

Real Time Reverse-Transcription Polymerase Chain Reaction (qRT-PCR) is the current standard for COVID-19 diagnosis. Nasopharygeal swab (and/or from oropharyngeal/mid turbinate) samples are analyzed using qRT-PCR. These qRT-PCR tests, however, are expensive and requires dedicated laboratory and specimen handling capability, with turnaround time in hours if not days. This delay in diagnosis leads to increased resource utilization in some cases and cross-contamination in others. Furthermore, qRT-PCR analysis heavily relies on the presence of viral genome in sufficient quantities at the sample collection site that can be amplified.

There have been efforts to produce accurate yet faster results via less invasive sampling measures. Also, Immunochromatographic Assay, which detects presence of COVID-19 viral antigen, has been developed. These Assays are inexpensive, do not require laboratory or special equipment and have turnaround time of under 10 minutes. These kits have potential benefit of large-scale testing without the significant resource burden on healthcare. This is crucial because hospitals are overwhelmed with increased resource utilization, limited medical equipment including personal protection equipment (PPE), and rapid diagnosis providing appropriate resource triage.

Here we aim validate the sensitivity and specificity of these point of care immunochromatographic COVID-19 antigen test kits by comparing its result against standard RT-PCR.

1.1 Objectives

The goal of the study is to validate the sensitivity and specificity of BIOCREDIT COVID-19 Ag kits compared to standard of care (RT-PCR) in patients infected with COVID-19 virus. The results from this study will be used to verify that antigen test kits can accurately identify patients with COVID-19 infections.

2 Device Description

2.1 BIOCREDIT COVID-19 Ag Kit

BIOCREDIT COVID-19 Ag kit is a point-of-care (POC) in vitro diagnostic test based on immunochromatographic assay. It is designed for qualitative detection of serum Novel Coronavirus (COVID-19) antigens (nucleocapside proteins), which are markers of novel coronavirus infection. It is a small sized standalone single use kit.

Operation of the device is fully automated and requires minimal sample handling. After sample has been collected (either via nasopharyngeal swab or saliva specimen), samples are mixed with assay diluent and 3-4 drops (90- 150 uL) of this mixture is added to device sample well. Device will then produce result within 8 minutes. Photographic evidence of result can be obtained for study, and once the test is complete, the single use device can be discarded in a biosafety Sharps Container.

2.2 BIOCREDIT COVID-19 Ag Kit Mechanism

BIOCREDIT COVID-19 Ag kit mechanism is based on lateral flow immunochromatographic assay. The nitrocellulose membrane strip within the device contains test line pre-coated with anti-SARS-CoV-nucleocapside protein (NP) for capture of SARS-CoV-2. The captured SARS-CoV is also detected by mouse anti-SARS-CoV·NP-gold conjugate for visualization. When sample is added to device sample pad, it moves through the nitrocellulose membrane by capillary action and contact the immobilized antibody coated test region. Should the sample contain specific COVID-19 antigen, immunocomplex will form revealing visible colored conjugate. Control region contains colored conjugate regardless of test specimen composition.

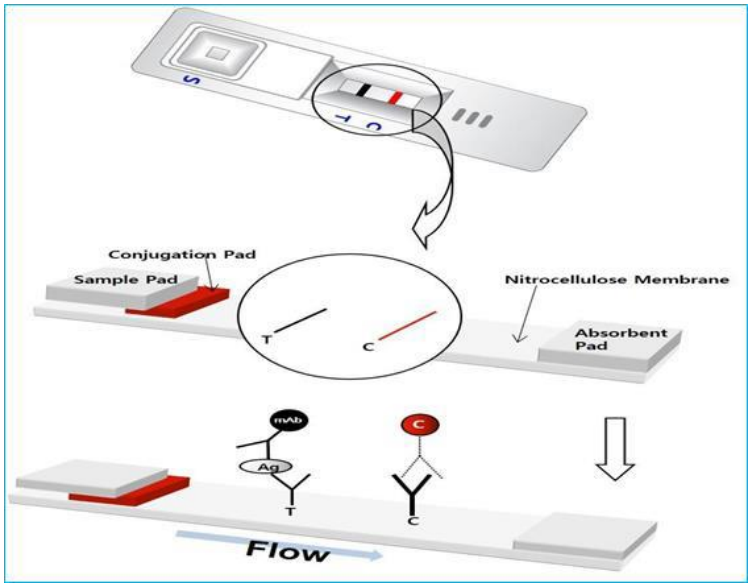

Figure 1. BIOCREDIT COVID-19 Ag kit mechanism

Interpretation of result is shown in Figure 2.

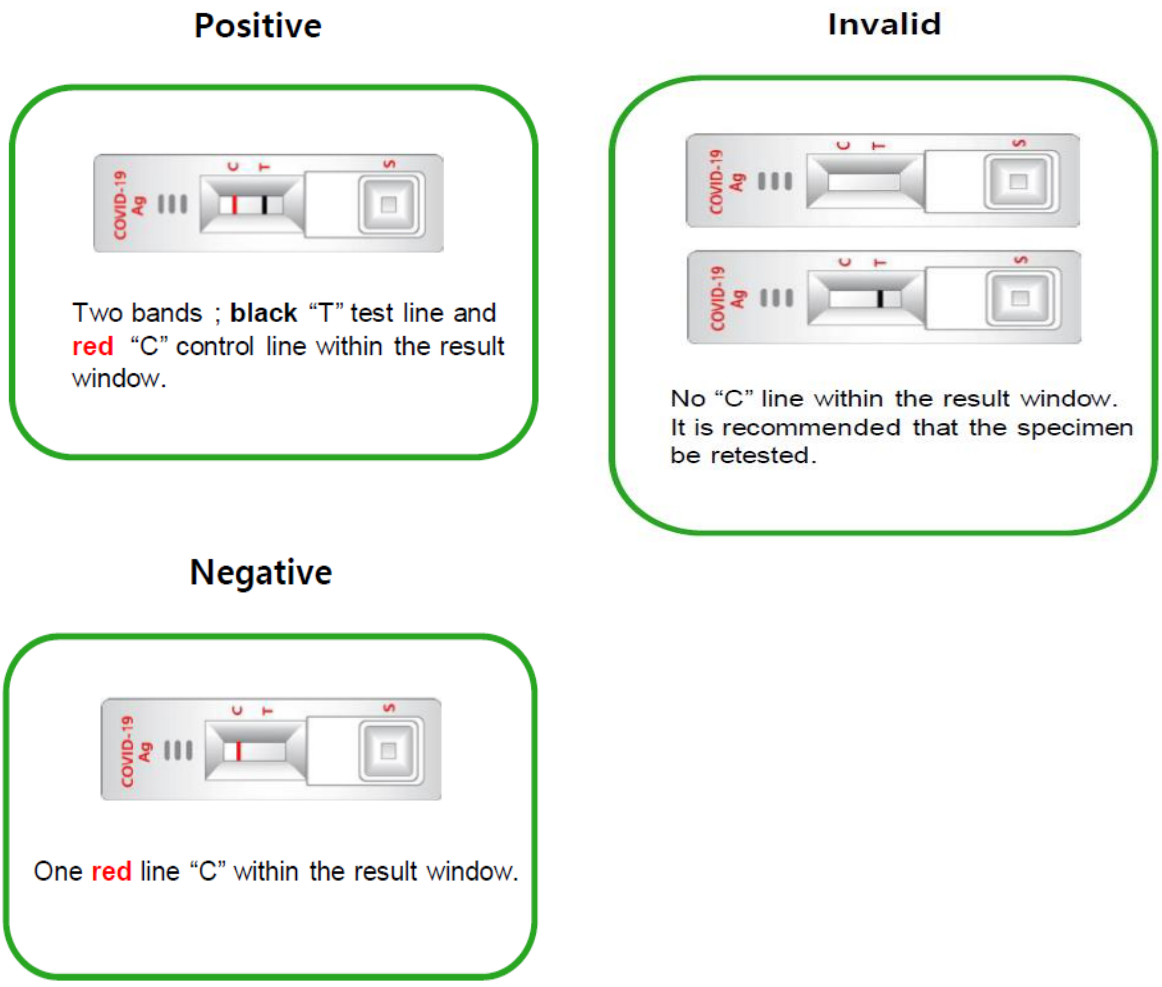

Figure 2. BIOCREDIT COVID-19 Ag kit's interpretation of results.

3 Clinical Performance Design

This is multiple site, single-blind, and retrospective study to validate the sensitivity and specificity of the BIOCREDIT COVID-19 Ag kits versus the RT-PCR in patients with COVID -19 infection. The hospitals were selected because it is currently admitting and actively testing for patients with COVID-19 infection or those who are suspected to have COVID-19 infection.

Patients who present to hospitals, signs and symptoms that are concerning for COVID-19 infection undergo nasopharyngeal swab for PCR test. Using BIOCREDIT COVID-19 Ag kit, we will compare the device results with diagnosis of COVID-19 using standard method (RT-PCR) from these patients.

3.1 Sample types

Patients with suspicion of COVID-19 infection undergo RT-PCR as a standard of care. Patients who are PCR positive case participants.

Collect nasopharyngeal swab for use on BIOCREDIT COVID-19 Ag.

Clinical Centers	Type of Specimen	Number of specimens	Comments
Catholic University of Korea, Eunpyung St. Mary's Hospital	UTM (nasopharyngeal collection)	Positive: 3 Negative: 2	RT-PCR confirmed for positive specimens
Seoul Asan Hospital	UTM (nasopharyngeal collection)	Positive: 12 Negative: 0	

3.2 Inclusion Criteria

The inclusion criteria for the test are:

- Subject has undergone RT-PCR test for COVID-19 diagnosis.

3.3 Exclusion Criteria

The exclusion criteria for the test are:

- Subject is unable to provide informed consent prior to performing any test related procedures.
- Subject is affected by conditions that, in the opinion of the clinical team, may pose additional risks. These include patients who are known to be coagulopathic or have history of significant nosebleed that would pose increased risk for nasopharyngeal swab.

3.4 Sample Exclusion Criteria

Samples will be excluded from the analysis data set if any of the test requirements or manufactural guidelines were not fulfilled. Manufacturer guideline states that collected specimen should be in room temperature.

4 Procedure

4.1 BIOCREDIT COVID-19 Ag Kit Training

Prior to subject enrollment, training will be provided to ensure that the designed instrument operators are proficient with the use of the BIOCREDIT COVID-19 Ag kit. Proficiency will be demonstrated by reproducing test kits without invalid results (control line is visible).

4.2 Sample Source and handling

Per discussion with manufacturer, source of samples for this study will be from nasopharyngeal swab.

Sample Source	Collection Method	Handling
Nasopharynx	Gently insert a nasopharyngeal swab into the nasal cavity until the resistance is met at the level of turbinate. Rotate softly and withdraw the swab. Make sure the tip of the swab is wet. Insert the specimen swab into manufacturer provided assay diluent tube.	Perform test immediately after collection or can be stored at 2~8℃ up to 12 hours or at -20℃ or below up to 24 hours.

4.3 Sample Analysis

Each sample from above sources will be analyzed on the BIOCREDIT COVID-19 Ag kit as follows:

- Tests should be performed immediately after collection. However, if the collected specimens were stored in refrigerated condition, leave the samples in room temperature for 15 to 30 minutes prior to use.
- Open the sealed pouch and place the device on a clean, dry, and level surface.
- Invert the assay diluent tube and gently squeeze it to draw 3~4 drops (90~150$\mu\ell$) into a sample well.
- Read the result at 8 minutes. Do not read results after 8 minutes.
- Properly discard device and diluent tube in a biohazard container.

5 Materials

The following materials are required for the performance of the study described in this document. The study sites will be responsible for ensuring the materials are handled per the manufacturer's recommended instructions for use.

5.1 materials included in purchases from RapiGEN

- the BIOCREDIT COVID-19 Ag Test Device
- Assay Diluent kits
- Nasopharyngeal swabs
- Instruction for Use

5.2 Materials to be Provided by Sites

- Biohazard container for disposing device/diluent tubes after running the test

6 Results

1) Clinical Evaluation 1

a. Test site: Eunpyeong St. Mary's Hospital, Seoul, Korea

b. Test date: Apr 1, 2020

c. Specimen: 3 COVID-19 positive and 2 COVID-19 negative specimens

d. Conditions of RT-PCR; PowerCheckTM 2019-nCoV Real-time PCR Kit (Kogenebiotech Inc., Korea); Cut-off value was determined as the detection of both target RNAs 35.0 Cycles of Threshold (CT) (CT > 35: Negative)

e. Test result: Summary of performance of BIOCREDIT COVID-19 Ag compare to PCR confirmed specimens

Catholic University of Korea, Eunpyung St. Mary's Hospital					**PCR data**		**BIOCREDIT COVID-19 Ag**	
Patient No.	Date of Symptom onset	Date of specimen collection	Specimen type	Day after onset	CT value	Results	Ag	Results
1	No data	No data	UTM	No data	E:16.76, R:17.20	Pos.	±	Pos.
2	No data	No data	UTM	No data	E:15.36, R:15.58	Pos.	+	Pos.
3	No data	No data	UTM	No data	E:18.18, R:18.55	Pos.	+	Pos.
4	No data	No data	UTM	No data	>40	Neg.	-	Neg.
5	No data	No data	UTM	No data	>40	Neg.	-	Neg.

2) Clinical Evaluation 2

a. Test site: Asan Medical Center, Seoul, Korea

b. Test date: Apr 9, 2020

c. Specimen: 12 COVID-19 positive

d. Conditions of RT-PCR; Allplex 2019-nCoV Kit (Seegene Inc., Korea); Cut-off value was determined as the detection of both target RNAs under 40.0 Cycles of Threshold (CT) (CT > 40: Negative)

e. Test result: Summary of performance of BIOCREDIT COVID-19 Ag compare to PCR confirmed specimens

Seoul Asan Hospital					**PCR data**		**BIOCREDIT COVID-19 Ag**	
Patient No.	Date of symptom onset	Date of Specimen collection	Specimen type	Day after onset	CT value	Results	Ag	Results
A001	No data	27 Feb. 2020	UTM	1	E:22.61, R:24.88 N:25.18	Pos.	w+	Pos.
	No data	29 Feb. 2020	UTM	3	E:32.5, R:33.09 N:35.24	Pos.	w+	Pos.
	No data	02 Mar. 2020	UTM	5	E:31.95, R:34.49 N:35.95	Pos.	-	Neg.
A002	No data	08 Mar. 2020	UTM	1	E:19.94, R:21.93 N:35.95	Pos.	w+	Pos.
	No data	10 Mar. 2020	UTM	3	E:22.57, R:24.53 N:26.79	Pos.	w+	Pos.
	No data	14 Mar. 2020	UTM	7	E:32.6, R:34.83 N:36.59	Pos.	-	Neg.
A003	No data	13 Mar. 2020	UTM	1	E:21.07, R:22.96 N:24.29	Pos.	-	Neg.
	No data	16 Mar. 2020	UTM	4	E:9.63, R:12.81 N:13.51	Pos.	+	Pos.

	No data	17 Mar. 2020	UTM	5	E:18.46, R:19.95 N:23.36	Pos.	w+	Pos.
A004	No data	28 Mar. 2020	UTM	1	E:20.35, R:21.93 N:23.99	Pos.	w+	Pos.
	No data	30 Mar. 2020	UTM	3	E:27.95, R:29.81 N:30.99	Pos.	w+	Pos.
	No data	03 Apr. 2020	UTM	7	E:29.99, R:31.69 N:32.26	Pos	w+	Pos.

7. Conclusion

1) 15 positive specimens which were confirmed by RT-PCR are tested with BIOCREDIT COVID-19 Ag kit, and all the specimens are identified as positive as well.
Relative Sensitivity (%) = 100 x (No. of specimens with positive results / No. of positive
specimen tested by RT-PCR)

2) 2 negative specimens collected by hospital are tested with BIOCREDIT COVID-19 Ag kit, and all the specimens are identified as negative as well.
Relative Specificity (%) = 100 x (No. of specimens with negative results/ No. of negative specimens)

Total sensitivity and specificity are in the following.

BIOCREDIT COVID-19 Ag		**RT-PCR**		**Sensitivity (%)**	**Specificity (%)**
		Positive	**Negative**		
Ag	Positive	12	0	80 (12/15)	100 (2/2)
	Negative	3	2		
Total		15	2		

8. Reference

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