Evaluation of the Clinical Performance and Clinical Validity of the Absoludy COVID-19 Ag Rapid and INIST COVID-19 Ag Rapid

Study No.: EONE-CTC-201106-01

As an Aid in the Diagnosis of COVID-19

PREFACE

The company, Absology Co., Ltd. (referred to Absology, hereinafter) was established in Korea in 2017 as a research-based company in biotechnology area. The company has developed innovative technologies on microfluidics and immunoassay applying properties like active flow microfluidic channel (on/off valve), dual & combination tests, vivid expressed GUI, and quantitative results allowing precise diagnosis. From these technologies, the company starts to establish its capability to develop next generation technologies through developing femtogram(sub-picogram) level detection for early disease diagnosis, high-sensitive ELISA by proprietary PIFA technology (Photooxidation induced fluorescence amplification), and flexible platform for any kinds of ELISA protocol and so on. Continuous invest will make development on new IVD reagents, and Absology will realize user satisfaction in IVD market.

Absology provides and ensures quality of product and process by compliance to international standards though the activities of design, development, manufacture and distribution service according to the processes of the Quality Management System as required by EN ISO13485:2016, cGMP, IVDD 98/79/EC and KGMP (Korea Good Manufacturing Practices) procedures that comply with. Absology has been audited by TÜV SÜD, and has received official certification according to EN ISO 13485:2016/AC:2019.

Sponsor/Manufacturer

Absology Co., Ltd.
#1303, Digital Empire "B"
Simin-daero 383, Dongan-Gu,
Anyang-Si, Gyeonggi-Do, 14057
REPUBLIC OF KOREA
Telephone: +82-31-348-8637
Facsimile: +82-31-348-8635
E-mail: sales@absology.co.kr

European Authorized Representative

MT Promedt Consulting GmbH
Altenhofstrasse 80
66386 St. Ingbert
GERMANY
Telephone: +49-6894-581020
Facsimile: +49-6894-581021
Email: info@mt-procons.com

Device Information

Proprietary Name: Absoludy COVID-19 Ag Rapid & INIST COVID-19 Ag Rapid

1. Purpose of the Study

Purpose: To evaluate the clinical performance and clinical validity of the Absoludy COVID-19 Ag Rapid and INIST COVID-19 Ag Rapid comparing the results with predicate device (Allplex ™ 2019-nCoV Assay.)

2. Investigational Site Information

Institute	Address	Telephone
EONE Laboratories	291, Harmony-ro, yeonsu-gu Incheon, Korea	1600-0021

3. Investigators Information

3.1 Principal Investigator

Name	Affiliation	Major	Title	Telephone
SON, JY	EONE Laboratories	Laboratory Medicine	Vice President	032-210-2153

3.2 Clinical Investigators

Name	Affiliation	Major	Title	Telephone
KIM, SI	EONE Laboratories	Clinical Physiology	Medical Laboratory Technologist	032-210-2317
KIM, SH	EONE Laboratories	Clinical Physiology	Medical Laboratory Technologist	032-210-2316

4. Investigational Device Manager, IDM, Information

Name	Affiliation	Title	Telephone
KIM, SH	EONE Laboratories	Medical Laboratory Technologist	032-210-2316
KIM, YS	EONE Laboratories	Medical Laboratory Technologist	032-210-2294

5. Sponsor Information

5.1 Sponsor

Affiliation	Name	Address	Telephone
Absology Co., Ltd.	JO, HS	#1303, Digital Empire “B” Simin-daero 383, Dongan-Gu, Anyang-Si, Gyeonggi-Do, 14057	031-348-8697

5.2 Clinical Research Associate, CRA

Affiliation	Name	Address	Telephone
Absology Co., Ltd.	Lee, JM	#1303, Digital Empire “B” Simin-daero 383, Dongan-Gu, Anyang-Si, Gyeonggi-Do, 14057	031-348-8697

6. Investigational Device

Name:
Absoludy COVID-19 Ag Rapid (ABCAR-020, ABCAR-005, ABCAR-001)
INIST COVID-19 Ag Rapid (MD4000201220, MD4000201205, MD4000201201)

Intended Use:
Absoludy COVID-19 Ag Rapid (ABCAR-020, ABCAR-005, ABCAR-001) and INIST COVID-19 Ag Rapid (MD4000201220, MD4000201205, MD4000201201) are intended for detection of specific antigens to SARS-CoV-2 virus (COVID-19) in human nasopharyngeal and oropharyngeal specimens following infection. This product is for professional use only.

Introduction and Principle:
Absoludy COVID-19 Ag Rapid is based on immunochromatography for the qualitative determination of SARS-CoV-2 virus specific antigens in human nasopharyngeal and oropharyngeal swab specimen.
The monoclonal antibodies against SARS-CoV-2 virus are immobilized onto a membrane as capture antibodies in the Test region (T). When the nasopharyngeal/ oropharyngeal swab specimens resuspended in extraction buffer solution are added to the sample well, the specimen starts to react with the gold-conjugate that SARS-CoV-2 virus specific antibodies were bound on colloidal gold. The mixture moves along the membrane by capillary action and reacts with fixed SARS-CoV-2 virus antibodies in the Test region (T). When SARS-CoV-2 virus is present in a sample, different intensity of red band appears on Test region of the membrane depending on the amount of SARS-CoV-2 virus present in the sample. The colorless of band in the Test region (T) indicates a negative result.

7. Predicate Device

Name: Allplex™ 2019-nCoV Assay

Intended Use:
The Allplex™ 2019-nCoV Assay is an *in vitro* diagnostic (IVD) real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the qualitative detection of SARS-CoV-2 viral nucleic acids in human nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, midturbinate and sputum specimens from individuals who are suspected of COVID-19 by their health care provider. Testing is limited to U.S. laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests. Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to

report all positive results to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

8. Sample Selection Criteria

8.1 Inclusion Criteria:

Remaining specimens for which the results of the novel coronavirus (SARS CoV-2) PCR test have been completed by the EONE Laboratories, and meets the requirements of Chapter 2, Article 7, Clause 3, 4,5 of the In Vitro Diagnostic Medical Devices Act

1) Positive sample: Human antigens residual sample collected from subjects confirmed as COVID-19 positive using AllplexTM 2019-nCoV (RT-qPCR) assay at the EONE Laboratories
2) Negative sample: Human antigens residual sample collected from subjects confirmed as COVID-19 negative using AllplexTM 2019-nCoV (RT-qPCR) assay at the EONE Laboratories
3) Residual samples kept frozen at -70℃ or lower for long-term storage after diagnosis.
4) Specimen with guaranteed anonymization

8.2 Exclusion Criteria:

1) Residual samples that do not fall under Inclusion Criteria.
2) Residual samples contaminated with microorganisms.
3) Residual samples that cannot guarantee stability due to poor storage conditions

9. Sample Size Determination

9.1 Sample Number

Total 142 samples previously tested with predicate device for confirmation of COVID-19 positive or negative. (Positive: 50, Negative: 92)

9.2 Rationale of the Sample Size

The main purpose of this study is to validate the clinical efficacy of the Absoludy COVID-19 Ag by evaluating the clinical sensitivity and clinical specificity. Therefore, the sample size is determined to prove the clinical sensitivity and clinical specificity.

Considering target clinical sensitivity as 85% with 70% confidence interval, level of significance for both sides as less than 5%, and the calculation resulted in 44.4 Considering drop-out rate over 10%, 50 of COVID-19 positive samples total is required for the target clinical sensitivity.

Considering target clinical specificity as 96% with 90% confidence interval, level of significance for both sides as less than 5%, and the calculation resulted in 83.6. Considering drop-out rate as 10%, 92 of COVID-19 positive samples total is required for the target clinical specificity.

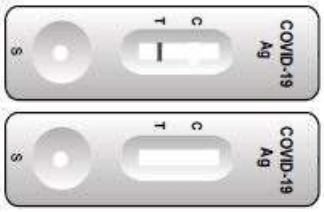
10. Investigational Device Test Procedure

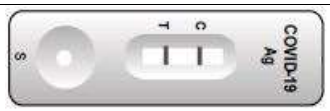
10.1 Test Procedure

1) This performance evaluation uses the remaining samples after COVID-19 test.
2) Freeze-stored residual samples are completely thawed at room temperature for about 15 to 30 minutes before starting the performance evaluation and shaked it gently before used for evaluation.
3) Take 100μL of the remaining samples and mix them with 100μL of the sample extraction solution.
5)Close the sample extraction solution tube containing mixed samples by covering the filter cap.
(4) Open the aluminum pouch and take out the inspection device and place it in a flat area.
8) Drop 4 drops (100 μL) of the samplet on the drop site (S) of the test device. At this time, hold the sample extract tube upright.
9) Visually read the results within 15 minutes after starting the inspection. The results after 15 minutes do not read.

10.2 Interpretation of Assay

1) Control (C) Line means that the test is working properly.
2) Test(T) Line indicates the test result.

No.	C Line	T Line	Test Result Interpretation	
1	not present	Any	Invalid Test. The specimen must be retested with another Absoludy COVID-19 Ag Rapid device.	
2	+	-	Valid Test. Negative for antigen for SARS-CoV-2.	

3	+	+	Valid Test. Positive for antigen for SARS-CoV-2.	

※ The directions may not have been followed correctly or the test device may have been deteriorated. It is recommended that the specimens be re-tested with the new device.

10.3 Evaluation criteria, evaluation method and interpretation method of effectiveness (statistical analysis method)

1) Primary efficacy evaluation

(1) Evaluation Criteria

: Evaluation of clinical sensitivity and specificity

- Clinical sensitivity(Positive percent agreement):
  Rate of positive test results among people with specific diseases
- Clinical specificity(Negative percent agreement):
  Ratio of negative test results among people who do not have a specific disease
- Target value:

  Clinical sensitivity(Positive percent agreement): ≥85%(95% confidence interval)

  Clinical sensitivity(Negative percent agreement): ≥96%(95% confidence interval)

Clinical effectiveness is evaluated by confirming whether the lower limit of the 95% confidence interval of the clinical sensitivity and specificity identified as a result of the clinical performance test is higher than the lower limit of the confidence interval of the clinical target sensitivity and specificity.

(2) Assessment Methods

The test reagent and the control reagent positive or negative are confirmed to be matched.

Type	Predicate Device	Candidate Device		판정
		Control	Test	
Sample case 1	+	+	+	positive
Sample case 2	+	+	-	negative
Sample case 3	+	+	intermediate	false negative
Sample case 4	-	+	intermediate	false positive

Results		Predicate Device		
		Positive	Negative	Total
Candidate Device	Positive	A	B	A+B

	Negative	C	D	C+D
Total		A+C	B+D	A+B+C+D

-Clinical sensitivity = A / (A+C) x 100(%)
-Clinical specificity = D / (B+D) x 100(%)
Confirm that the clinical sensitivity and specificity of the Candidate device is higher than the target clinical performance.
The final result is presented by expressing the 95% confidence interval (Clopper-pearson exact method) as a percentage along with clinical sensitivity and specificity.

2) Secondary efficacy evaluation
(1) Evaluation Criteria: Cohen's kappa

Cohen's kappa(κ) is used as a statistical method to measure the degree of agreement. The evaluation of Cohen's kappa value is generally known as below table. Therefore, Cohen's kappa(κ) of 0.8 or more is set as the criterion for evaluating the correlation.

Kappa	Comments
Kappa ≤ 0	Agreement equivalent to chance
0.0 < Kappa ≤ 0.20	Slight agreement
0.2 < Kappa ≤ 0.40	Fair agreement
0.4 < Kappa ≤ 0.60	Moderate agreement
0.6 < Kappa ≤ 0.80	Substantial agreement
0.8 < Kappa ≤ 0.99	Near perfect agreement
1	Perfect agreement

(2) 14.2.1. Assessment Methods

Kappa = 2(AD-BC)/{(A+B)(B+D)+(A+C)(C+D)}
Mark the 95% confidence interval (Clopper-pearson exact method).

11. Results of the Study

Results		Predicate Device (Allplex™ 2019-nCoV Assay)		
		Positive	Negative	Total
Candidate Device (Absoludy COVID-19 Ag Rapid, INIST COVID-19 Ag Rapid)	Positive	50	0	50
	Negative	0	92	92
Total		50	92	142

- Positive Agreement: $a/(a+c) \times 100(\%)$
$= 50/(50+0) \times 100(\%)$
$= 100\%\ with\ 95\%\ confidence\ interval\ 0.93 - 1.00$

- Negative Agreement: $d/(b+d) \times 100(\%)$
$= 92/(0+92) \times 100(\%)$
$= 100\%\ with\ 95\%\ confidence\ interval\ 0.96 - 1.00$

- Total Agreement: $(a+d)/(a+b+c+d) \times 100(\%)$
$= (50+92)/(50+0+0+92) \times 100(\%)$
$= 100\%$

- Kappa: $2(ad-bc)/\{(a+b)(b+d)+(a+c)(c+d)\}$
$= 2(50 \cdot 92 - 0 \cdot 0)/\{(50+0)(0+92)+(50+0)(0+92)\}$
$= 9200/9200$
$= 1$

12. Conclusion

This clinical study was performed to evaluate the sensitivity and specificity of the trial medical device 'Absoludy COVID-19 Ag Rapid, INIST COVID-19 Ag Rapid' which detects antigen for novel coronavirus (Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) in human antigen residual samples in UTM. The comparator method used to establish clinical confirmation for the patients is "Allplex ™ 2019-nCoV Assay" manufactured by Seegene Inc., which is an EUA- authorized by US FDA. The retrospectively collected SARS-CoV-2 antigen positive and negative specimens were used for the study, and the study was performed with single site, single blinded, and randomized method. 142 patient specimens, 50 SARS-CoV-2 antigen positive and 92 SARS-CoV-2 antigen negative specimens were tested for the study.

Total agreement results were analyzed as below.
The total positive agreement showed 100% (with 95% confidence interval 0.93-1.00) total negative agreement showed 100% (with 95% confidence interval 0.96-1.00) and total agreement showed 100%. Agreement results of kappa method showed 1 which indicates "Perfect " agreement.