Evaluation of the Clinical Performance and Clinical Validity of the Absoludy COVID-19 IgM/IgG Rapid (INIST COVID-19 IgM/IgG Rapid)

Study No.: MDCTC-20-030Version 1.0

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 IgM/IgG Rapid
INIST COVID-19 IgM/IgG Rapid

1. Purpose of the Study

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

2. Investigational Site Information

Institute	Address	Telephone
Seoul Clinical Laboratories	A-dong, Heungdeok IT valley, 13, Heungdeok 1-ro, Giheung-gu, Yongin-si, Gyeonggi-do, Korea, SCL	1800-0119

3. Investigators Information

3.1 Principal Investigator

Name	Affiliation	Major	Title	Telephone
KIM, CK	Seoul Clinical Laboratories	Laboratory Medicine	Professor	02-330-2207

3.2 Clinical Investigators

Name	Affiliation	Major	Title	Telephone
SEON, YS	Seoul Clinical Laboratories	Clinical Physiology	Investigator	02-330-2106
KIM, SW	Seoul Clinical Laboratories	Biotechnology	Investigator	02-330-2104

4. Investigational Device Manager, IDM, Information

Name	Affiliation	Major	Telephone
LEE, CR	Seoul Clinical Laboratories	Nursing	02-330-2105

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	031-348-8704

5.2 Clinical Research Associate, CRA

Affiliation	Name	Address	Telephone
Absology Co., Ltd.	SEO, JS	#1303, Digital Empire “B” Simin-daero 383, Dongan-Gu, Anyang-Si, Gyeonggi-Do, 14057	031-348-8704

6. Investigational Device

Name: Absoludy COVID-19 IgM/IgG Rapid (ABCVR-025)
INIST COVID-19 IgM/IgG Rapid (MD30002012)

Intended Use:
COVID-19, colloquially known as coronavirus, is the rapidly spreading respiratory viral
infectious disease. SARS-CoV-2 is the strain of coronavirus that causes coronavirus disease 2019 (COVID-19). SARS-CoV-2 is a positive-sense single-stranded RNA virus. It is contagious in humans, and the World Health Organization (WHO) has designated the ongoing pandemic of COVID-19 a Public Health Emergency of International Concern. The virus can be transmitted through airborne droplets to cause infection. Serological detection is a common method for monitoring to follow a typical seroconversion and immunoglobulin class switching time course, according to preliminary data. Generally, COVID-19 IgM starts to appear at 3 days after initial exposure and remain in the circulation for about 30-60 days. COVID-19 IgG raises at around 7 days, peaks at 2-3 weeks, and persists for life. The Absoludy COVID-19 IgM/IgG Rapid (ABCVR-025) and INIST COVID-19 IgM/IgG Rapid (MD30002012) is a chromatographic immunoassay kit for rapid qualitative determination for COVID-19 immune response.
Absoludy COVID-19 IgM/IgG Rapid (ABCVR-025) and INIST COVID-19 IgM/IgG Rapid (MD30002012) is based on the principle of an immunochromatography in vitro test for the qualitative determination of SARS-CoV-2 virus (COVID-19) specific IgM or IgG in whole blood, serum, or plasma (EDTA, Li-heparin, or Citrate.) When the specimens are added to the sample well and sample diluent is added to the sample pad, they move to the conjugate pad and re-suspend the recombinant SARS-CoV-2 virus antigen-gold conjugate. The mixtures move along the membrane by capillary action and react with the anti-human IgM or IgG antibodies immobilized in two lines on the test reaction zone. If antibodies against COVID-19 are present enough in the sample, a colored band of COVID-19 IgM or IgG in the test reaction zone will be appeared. If there are no antibodies against COVID-19 or not sufficient in the sample, the area will remain colorless. The sample continues to move to the control reaction zone and forms a red or purple color, indicating the test is working properly and the result is valid.

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 Information

The Absoludy COVID-19 IgM/IgG Rapid (INIST COVID-19 IgM/IgG Rapid) can be used for detecting SARS-CoV-2 antibodies in human serum, plasma or whole blood that has been freshly collected or previously refrigerated or frozen. Collection of specimen and the required storage conditions are described in the following sections.

Below are instructions for collecting and storing samples. Standard blood collection techniques are as follows:

▪ Collect blood by venipuncture technique into a standard tube designed for the collection of whole blood which will then be allowed to clot to allow isolation of serum or into a tube with lithium heparin, citrate or K_2-EDTA as an anti-coagulant which will then be centrifuged to separate the plasma from the formed elements of the blood.
▪ Follow the manufacturer's instructions with regard to the handling of the tubes after the blood collection.
▪ After allowing the tube to sit undisturbed for sufficient time for the clot to form (serum samples) (refer to the manufacturer's instructions for the suggested time), or immediately in the case of plasma samples, centrifuge the tube for at least 10 minutes at 3000 rpm.
▪ Collect the clear layer of serum or plasma into a capped aliquot tube for immediate use, or store at 2-8˚C for 3 days, -20˚C for up to 20 days or place in long term storage at
-70˚C for a period of time commiserate with the stability of the SARS-CoV-2 antibodies.
Note: Serum or plasma specimen samples' stability has been shown for more than ten years when samples are tightly sealed and stored at a temperature colder than -70˚C. Frozen samples should be thawed at room temperature before use. Avoid multiple freeze-thaw cycles. Grossly turbid samples should not be used for testing purposes.

9 Control Mechanism

Internal Control:
Internal control is processed along with the specimen on the device. The internal control listed below must generate expected results in order for a test to be considered valid. The C lines should appear for every test and checks that flow of reagents is satisfactory. If the C line does not appear, the test is invalid.

External Control:
Good Laboratory Practices and laboratory regulations require the external quality control should be performed with the following situations:
▪ New lot of materials
▪ New calibration
▪ Maintenance procedure of main components
▪ Routine maintenance requirement set by the laboratory
Every laboratory should determine the frequency of external quality control and identify a control material appropriate for the Absoludy COVID-19 IgM/IgG Rapid (INIST COVID-19 IgM/IgG Rapid.) When identifying the external quality control material, concentration points of the SARS-CoV-2 antibodies from the lowest to the highest, as well as concentration points that are near the cut-off value of the Absoludy COVID-19 IgM/IgG Rapid (INIST COVID-19 IgM/IgG Rapid) should be considered.

10 Investigational Device Test Procedure

10.1 Test Procedure

※ Allow the specimens and unopened test devices to room temperature prior to use.
1. Remove the test device from the sealed pouch, and place it on a clean and flat surface.
2. Transfer 10μℓ of prepared specimen in the sample well (S) of the test device using specimen collection tube provided in the kit or precise micropipette not provided.
3. Add 3 drops (about 100μℓ) of sample diluent into the diluent well of the test device.
4. Read the test result at 10~15 minutes. Do not read the test result after 20 minutes.
※ Caution: Perform the test immediately after removing the test device from the foil pouch.

10.2 Interpretation of Assay

1. Control (C) band means that the test is working properly.
2. Test (’1’ and ‘2’) band indicates the test result.

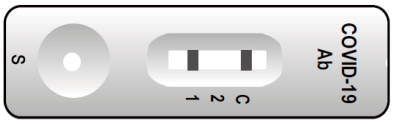
➢ Negative

The presence of only ‘C’ band indicates a negative result.

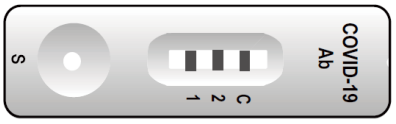
➢ Positive

1) IgM Positive: Control band and Test band 2(T2) appear

2) IgG Positive: Control band and Test band 1(T1) appear

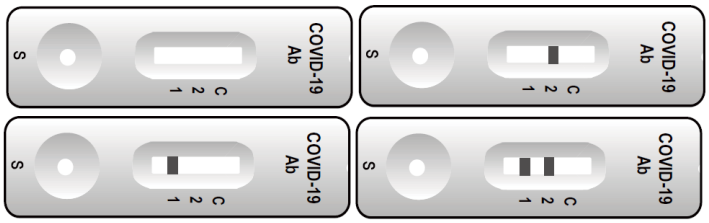


3) IgM and IgG Positive: Control, Test 1(T1), and Test 2(T2) bands appear



➢ Invalid

If control (C) band is not appeared in the result window after performing the test, the result is considered invalid.



※ 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.

11 Sample Selection Criteria

11.1 Inclusion Criteria:

- Positive sample: Patients who have undergone a real-time Reverse Transcription Polymerase Chain Reaction (RT-qPCR) test for the purpose of diagnosis of COVID-19 at the Seoul Clinical Laboratories
- Negative sample: A normal individual who was requested for test for another purpose before the first confirmed case of COVID-19 in South Korea
- Leftover samples stored below -70C after diagnosis
- The anonymization of sample

11.2 Exclusion Criteria: Fulfilling any of the following items will be excluded from clinical trial sample.

- The residual sample with unclear clinical diagnosis of COVID-19 / The residual sample cannot be judged as positive or negative of COVID-19 infection
- The residual sample volume does not meet the minimum requirement for analysis
- Sample that contaminated from heat treatment, bacterial contamination, microbial contamination, foreign substances and all other reasons
- The sample that has undergone the freezing and thawing process more than three times

In addition to the above, the residual sample that are considered to be inadequate for this study by the medical director or the person in charge of the examination

12 Sample Size Determination

12.1 Sample Number

Total 133 samples previously tested with predicate device for confirmation of COVID-19 positive or negative. (Positive: 41, Negative: 92)

12.2 Rationale of the Sample Size

The main purpose of this study is to validate the clinical efficacy of the Absoludy COVID-19 IgM/IgG and INIST COVID-19 IgM/IgG 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 86% with 70% confidence interval, level of significance for both sides as less than 5%, and allowable error as 1.5%, the calculation resulted in 36.8. Considering drop-out rate as 10%, 41 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 allowable error as 1.5%, the calculation resulted in 36.8. Considering drop-out rate as 10%, 41 of COVID-19 positive samples total is required for the target clinical specificity.

13 Results of the Study

<Total Results of Clinical Study>

Method		Predicate Device		
		Positive	Negative	Total
Absoludy COVID-19 IgM/IgG Rapid	Positive	37	2	39
	Negative	4	90	94
Total		41	92	133

- Clinical Sensitivity: 90.24 % (37/41)
- Clinical Specificity: 97.82% (90/92)

<Test Results of Clinical Study: Collected Specimens after several days from PCR confirmation>

Days from PCR confirmation	Method		Predicate Device	
			Positive	Total
≤7	Absoludy COVID-19 IgM/IgG Rapid	Positive	21	22
		Negative	1	
8~14		Positive	11	13
		Negative	2	
≥15		Positive	5	6
		Negative	1	
Total			41	41

- Clinical Sensitivity of the days for ≤7: 95.45 % (21/22)
- Clinical Sensitivity of the days for 8~14: 84.61 % (11/13)
- Clinical Sensitivity of the days for ≥15: 83.33 % (5/6)

14 Conclusion

This clinical study was performed to evaluate the sensitivity and specificity of the trial medical device ‘Absoludy COVID-19 IgM / IgG Rapid,’ and ‘INIST COVID-19 IgM/IgG Rapid’ which detects IgM and IgG antibodies for novel coronavirus (Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) in human serum or plasma leftover samples. 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 antibody positive and negative specimens were used for the study, and the study was performed with single site, single blinded, and randomized method. 133 patient specimens, 41 SARS-CoV-2 antibodies positive and 92 SARS-CoV-2 antibody negative specimens were tested for the study. Clinical agreement data was analyzed giving 90.24% clinical sensitivity and 97.82% clinical specificity in detecting total IgM/IgG antibodies.

Appendix 1) This is the original cover page of the clinical study report written by the principal investigator at the Seoul Clinical Laboratory.

임상적 유효성평가 결과 보고서

사람의 혈청 또는 혈장 잔여검체를 대상으로 신종 코로나바이러스(Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2)에 대한 IgM과 IgG를 검출하는 시험용 의료기기 "Absoludy COVID-19 IgM/IgG Rapid"의 성능을 긴급사용승인시약 "Allplex™ 2019-nCoV Assay"과 비교하기 위한 단일기관, 단일눈가림, 무작위배정, 후향적으로 이루어지는 임상적 유효성 평가

2020. 05. 24. - 2020. 06. 01

	성명	담당 업무	서명 / 날짜
작성	김창기	시험책임자	2020.6.4

(재)서울의과학연구소