RapidFor™ SARS-CoV-2 & Flu A/B Antigen Combo Test

Clinical Evaluation Report

Product Name: RapidFor™ SARS-CoV-2 & Flu A/B Antigen Combo Test (VSCD10)

Packing specification: 1 Test/Kit or 25 Tests/Kit

Clinical evaluation category: Comparison with clinical PCR results

Clinical evaluation place: Private Gelisim Laboratory

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1.OVERVIEW

1.1 Abstract

Objective:

The aim of the test is to see whether Vitrosens Biyoteknoloji's RapidFor™ SARS-CoV-2 & Flu A/B Antigen Combo Test's detection capability is comparable to clinical diagnostic standards when used in vitro for qualitative detection of SARS-CoV-2 and Flu A/B antigen in human nasopharyngeal (NP) and nasal (NS) swab samples. The results compared by real time PCR test by using nasopharyngeal swab samples.

Methods:

Methodological comparison arrangement and synchronized blind test.

1.2 Abbreviation

Severe Acute Respiratory Syndrome Coronavirus 2：SARS-CoV-2

2.MAIN CONTENT

2.1 Basic Content

2.1.1 Introduction

The novel coronaviruses belong to the β-genus, a positive strand RNA virus. SARS-COV-2 is an acute respiratory infectious disease which people are susceptible to infection. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be spread the virus. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue, loss of smell and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

Influenza is a highly contagious acute viral infection of the respiratory tract. It is a communicable disease easily transmitted from person to person through aerosol droplets excreted when sneezing and coughing. Common symptoms include high fever, chills, headache, cough, sore throat and malaise. The type A influenza virus is more prevalent and is the primary pathogen associated with serious epidemics. The type B virus causes a disease that is generally not as severe as that caused by the type A virus.

An accurate diagnosis of SARS-CoV-2 and influenza based on clinical symptoms is difficult because the initial symptoms of influenza are like those of numerous other illnesses. Therefore, it can be confirmed only by laboratory diagnostic testing. Early differential diagnosis of SARS-CoV-2 and influenza type A or type B can allow for proper treatment with appropriate antiviral therapy. Early diagnosis and treatment are of value in a clinical setting where accurate diagnosis can assist the healthcare professional with management of SARS-CoV-2 and influenza patients who are at risk for complications. The Combo Test is a rapid immunoassay to be used as an aid for the differential diagnosis of SARS-CoV-2 and influenza type A and type B.

RapidFor™ SARS-CoV-2 & Flu A/B Antigen Combo Test developed by Vitrosens Biyoteknoloji LTD. ŞTİ. is used to detection of the SARS-CoV-2 and Flu A/B antigen qualitatively in human nasopharyngeal and nasal swab samples.

2.1.2 Research purpose

To prove the capacity of detection of the RapidFor™ SARS-CoV-2 & Flu A/B Antigen Combo Test produced by Vitrosens Biyoteknoloji LTD. ŞTİ. is equal when used for qualitative detection of antigen in nasopharyngeal and nasal swab samples in vitro to similar products on the market.

2.1.3 Testing management

The clinical trial of the kit is conducted by Private Gelisim Laboratory in accordance with “Technical Guidelines for Clinical Trials of Diagnostic Reagents in-vitro” and “Technical Review Points for Registration of SARS-CoV-2 Antigen/Antibody Detection Reagents” and supervised the whole clinical assessment trial's implementation.

During the trial, the main investigator oversees the overall coordination and management, while the main participants are in charge of the actual trial work. During the clinical trial, the lead researcher monitors the testing laboratory's quality control. Any issues discovered during the test must be reported to the main researcher as soon as possible, and necessary steps must be taken. The person in charge of statistics analyzed the final test results statistically and confirmed by the main investigator then published the report. Finally, a clinical trial report in accordance with the requirements of exempting clinical trials given by Private Gelisim Laboratory.

2.1.4 Research design

2.1.4.1 General design

The synchronous blind test and methodological comparison design uses for this test.

This test uses a blind test to minimize the potential effect of individual prejudices and personal interests of researchers on test results during the clinical trial period. That is, the research staff in this test are unaware of the sample's specific details, and the sample's clinical data will not be published before the end of the test. The samples were coded by the blind editor authorized by the clinical trial after they were enrolled, and the blind editor authorized by the clinical trial was not involved in the clinical trial's test process. The coded sample must be tested according to the reagent test specification by the testing personnel. Clinical test researchers should strictly follow the requirements of the product specification for test procedure and interpretation check during the test phase, and the results obtained during the test process should be accurately reported in the data collection table.

2.1.4.2 Measures to reduce and avoid bias

1) To avoid selection bias, subjects were screened solely according to the clinical trial protocol's inclusion and exclusion requirements.

2) Prior to the start of the experiment, the sponsor must train the participants in the clinical trial protocol and the use of the research reagent, ensure that the clinical trial protocol and the operation of the research reagent are consistent, and encourage contact among clinical trial investigators during the trial.

3) The clinical trial staff must maintain and calibrate or quality check all of the equipment to be used prior to the start of the trial. The applicant shall conduct a clinical trial pretest with the clinical trial researcher in order to familiarize and master the product's operation process, technological results, and other aspects, and to minimize trial operation error.

4) During the test, the clinical test researcher must perform quality control work in compliance with the reagent specification's requirements and operate in strict accordance with the test schedule. The clinical trial supervisor will monitor the work and ensure that the clinical trial researchers follow the test plan to the letter.

5) After the clinical trial is over, the data must be saved and sorted. When issues with the data are discovered, the researcher must double-check and confirm the information to prevent documenting errors.

2.1.4.3 Clinical sample selection

2.1.4.3.1 Inclusion criteria

Since this is an in vitro qualitative test kit, it can only be used in clinic for auxiliary diagnosis of SARS-CoV-2 and Flu A/B pneumonia; it cannot diagnose the clinical disease. As a result, in clinical practice, the positive and negative samples are primarily distinguished, and the samples used are from suspected SARS-CoV-2 and Flu A/B pneumonia cases.

The subjects of this clinical study were divided into two groups, positive and negative, based on the findings of the reference sample. The result of PCR was used as the classification basis in the consistency comparison of experimental reagent and reference group.

1) Inclusion criteria of sample: the sample should be a large enough sample including a well-documented source, with people of varying ages, genders, and other characteristics. Samples are collected and handled in compliance with the reagent specification or applicable regulations. Age, sex, sample collection date, and clinical diagnosis, such as confirmation or exclusion of SARS-CoV-2 and Flu A/B infection, should all be included in the sample details. For RT-PCR test, nasopharyngeal swab must be used.

2) Positive group inclusion criterion: clinically reported cases were obtained, and the samples met the criteria of 1. Positive samples must be taken to patients within the 7 days from symptoms onset.

3) Negative group inclusion criteria: clinically excluded cases were obtained, and the samples met the criteria of 1.

2.1.4.3.2 Exclusion criteria

1) There is no detail about the time of sample collection or the case.

2) The sample size is insufficient for the test to be completed.

3) Prior to the examination, it was discovered that the sample preservation process had been contaminated, resulting in turbidity.

4) According to the researchers, the sample does not meet the test criteria.

2.1.4.3.3 Rejection criteria

1) Samples that fail to complete the test due to instrument or human error (sample contamination during operation).

2) The sample test results come from samples that were not processed and evaluated according to the experimental reagent's instructions.

2.1.4.4 Samples distribution

The following are the specific criteria for clinical sample size:

1) There should be a minimum of 50 confirmed cases.

2) It is recommended that at least 50 cases be omitted.

2.1.4.5 Sample collection, storage, and transportation methods

Collection of nasopharyngeal secretion: Insert the sterile swab into the place where the nasopharyngeal secretions are the most and rotate the swab close to the inner wall of the nasal cavity 3 times, remove the swab.

Collection from nasal secretion: Insert the sterile swab into the place where the nasal secretions are the most at the front nose and rotate the swab close to the inner wall of the nasal cavity 3 times, remove the swab.

The collected swab samples are stable in 30 mins if they kept in the sample extraction solution provided with the kit. After the collection, samples must be tested as soon as possible. The nasopharyngeal swab samples were dissolved in Copan Diagnostics Inc.'s 2 ml UTM and sent for PCR testing.

2.1.4.6 Test Procedure

For the Antigen Combo Test, nasal and nasopharyngeal swab samples were mixed with 500µL of extraction buffer separately. Then, the 100 µL of the sample were added onto the sample well of the test cassette. At the end of the 15 minutes, the results were interpreted.

For the PCR procedure, nasopharyngeal swabs, were collected by a healthcare provider in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19. Swabs (Dacron or polyester flocked) were placed immediately into a sterile transfer tube [Bio-Speedy® vNAT® Transfer Tube (BS-NA-513-100)] containing 2 ml of viral transport medium [vNAT®

Viral Nucleic Acid Buffer (BS-NA-510)]. Rest of the specimens were stored at -80oC in accordance with the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19.

PCR Kit

"Bio-Speedy® COVID-19/Flu RT-qPCR" is used for detecting the Influenza A, Influenza B, and pandemic virus SARS-CoV-2 (2019-nCoV) causing Coronavirus Disease 2019 (COVID-19). "Bio-Speedy® COVID-19/Flu RT-qPCR" kit tests and distinguishes SARS-CoV-2, Influenza A, and Influenza B in a single multiplex RT-qPCR reaction in less 30 minutes. The kit is applied by healthcare providers to respiratory tract samples taken from individuals with suspected disease. The "Bio-Speedy® COVID-19/Flu RT-qPCR" kit, besides targeting the Orf1ab and N gene regions which are common in all SARS-CoV-2 variants, targets the Influenza A specific Membrane Protein (M) gene region and the Influenza B specific Nuclear Export Protein (NEP) gene region detection. The human RNase-P oligo set in the kit targets exone-exone junction in the mRNA and does not target the human genome. Hence it is used for controlling the sampling, integrity of RNA, nucleic acid extraction, and inhibition of both reverse transcription and qPCR. The kit also contains negative and positive control templates for testing the contamination and the qPCR reactive stability, respectively.

RINATM-M14 Nucleic Acid Isolation Robot system successfully used in combination with Bio-Speedy® COVID-19/Flu RT-qPCR. Extracted nucleic acids were stored at -800C and (if re-testing is expected) stored in aliquots.

Setting up RT-PCR Reaction Master mix Plate:

1. Make sure that all necessary equipment and devices are suitable, calibrated, and functional before starting the experiments.
2. Decontaminate equipment and workspace and prepare everything needed for the following experiment to keep the workflow short and repeatable.
3. Thaw all components of Bio-Speedy® COVID-19/Flu RT-qPCR on ice and mix gently but thoroughly to ensure even distribution of components. Collect liquid at the bottom of the tube with a quick spin. Make sure the sample is well mixed.
4. Distribute 5µL of the 2X Prime Script Mix and 2.5µL of CVD19/FLU Oligo Mix to each well of your PCR plate.

5. In the RNA samples that have been isolated by RINATM-M14, is added to the wells. Only 2.5uL of sample is added to each well.
6. Use the nuclease-free water provided with the kit instead of the RNA in the negative control, and the plasmid DNA provided with the kit in the positive control.
7. qPCR plate surface is sealed with a seal.
8. Transfer the reactions to the PCR device, then cycle according to these guidelines:

qPCR Program		
Cycle	**Temperature**	**Duration**
1	52°C	5 min
1	95°C	10 sec
40	95°C	1 sec
	55°C	12 sec
	FAM/HEX/ROX/CY5 Read	

** If Cq-FAM is ≤33, conclude as positive, otherwise conclude as negative.

**If Cq-ROX is ≤33, conclude as positive, otherwise conclude as negative.

**If Cq-CY5 is ≤33, conclude as positive, otherwise conclude as negative.

2.1.4.7 Reagents and instruments for clinical research

Table 1: Evaluation Reagent Information

Name of Reagent	RapidFor™ SARS-CoV-2 & Flu A/B Antigen Combo Test
Specification	1 Test/Kit or 25 Tests/Kit
Company	Vitrosens Biyoteknoloji LTD. ŞTİ.
Lot Number	S1022020101
Preservation Condition	2°C ˜ 30°C

Table 2: Information of Reference Reagent

Name of Reagent	Biospeedy COVID-19/Flu RT-qPCR Kit
Specification	100 Reactions/kit
Catalog No	BS-SY-SI-100
Company	Bioeksen R&D Technologies Inc.
Preservation Condition	(-15°C) - (- 25°C)

2.1.4.7 Quality control

1)Definition

The operation of techniques and activities, such as monitoring, under the quality assurance system to verify that the study quality meets the specifications is known as quality control. To ensure that all data is trustworthy and properly located, quality control must be applied at every point of data processing.

2) Monitoring of study

Authorized and qualified inspectors will perform routine remote primary data checks in accordance with the monitoring plan to verify compliance with protocols and regulations and assist investigators during the outbreak.

3) Quality control of laboratory

A unified test index, standard operating procedures, and quality management procedures must be developed by the testing laboratory.

4) Testing process of reagent quality control

The quality control line in each test must have a red stripe (qualified quality control). If the quality control line does not have a red strip (unqualified quality control), the cause must be determined and the quality control result must be retested before the quality control result is qualified, ensuring the system's reliability and stability.

5) Researchers' qualification

Researchers taking part in the clinical trial must meet the clinical trial's specialization, qualification, and skill requirements, as well as pass the qualification test. Personnel specifications should be fairly consistent.

6) Researchers' training before the experiment

Before the trial start, Private Gelisim Laboratory is responsible for the training of researchers to help clinical researchers fully understand the overall situation, scheme, etc. of the trial.

2.1.4.8 Statistical analysis method of clinical trial data

For statistical analysis, use statistical software or the formula below.

Table 3: Data analysis of consistency

Experimental Group	Reference Group		Sum
	Positive	Negative	
Positive	a	b	a + b
Negative	c	d	c + d
Sum	a + c	b + d	a + b + c + d
Sensitivity	a / (a + c)		
Specificity	d / (b + d)		

2.2 Clinical Trial Results and Analysis

2.2.1 Overall distribution of samples

A total of 284 SARS-CoV-2 positive nasal and nasopharyngeal samples, 181 Influenza A positive nasal and nasopharyngeal samples, and 166 positive nasal and nasopharyngeal samples were enrolled in this study for the accuracy comparison of sample detection results by experimental reagent and PCR result, and no duplicate samples were observed. There were 460 negative samples of PCR results among them.

Table 4: Clinical trials sample distribution

Parameter	**Number of cases**
SARS-CoV-2 Positive	284
Influenza A Positive	181
Influenza B Positive	166
Negative	460

Table 5: PCR results statistical analysis (Nasopharyngeal)

SARS-CoV-2 & Flu A/B Antigen Combo Test	**RT-PCR comparative test result**				
	SARS-CoV-2 Positive	**Influenza A Positive**	**Influenza B Positive**	**Negative**	**Total**
SARS-CoV-2 Positive	281	0	0	1	282
Influenza A Positive	0	179	0	0	179
Influenza B Positive	0	0	164	0	164
Negative	3	2	2	459	466
Total	284	181	166	460	1091

The sensitivity of the test kit for SARS-CoV-2 is 98.94%, for Influenza A is 98.90% and for Influenza B is 98.80%. The specificity of test kit is 99.52%.

Table 5: PCR results statistical analysis (Nasal)

SARS-CoV-2 & Flu A/B Antigen Combo Test	RT-PCR comparative test result				
	SARS-CoV-2 Positive	Influenza A Positive	Influenza B Positive	Negative	Total
SARS-CoV-2 Positive	280	0	0	1	282
Influenza A Positive	0	178	0	1	179
Influenza B Positive	0	0	163	0	163
Negative	4	3	3	458	468
Total	284	181	166	460	1091

The sensitivity of the test kit for SARS-CoV-2 is 98.59%, for Influenza A is 98.34% and for Influenza B is 98.19%. The specificity of test kit is 99.57%.

2.2.3 Analytical Results with correlation to Ct-values of the samples

The sensitivity for samples with a Ct-value of up to 25 is 100%, according to the correlation between the Ct-values of the analyzed samples and the sensitivity. Lower sensitivity values for RapidFor™ SARS-CoV-2 & Flu A/B Antigen Combo Test are associated with samples with a higher Ct value in the RT-PCR and, as a result, less viral RNA copies and viral antigen in the samples. When compared to PCR research, this is line with standards for viral detection by antigen rapid.

2.3 Test Reliability

1)All test sample collection and preservation methods are accurate.

2) During the evaluation phase, the operators received special training to ensure the test results' reliability.

3) When conducting clinical trials, the experiments must be carried out in full compliance with laboratory quality control and clinical trial software standards in clinical hospitals. To ensure the efficacy of clinical trials, the findings were evaluated by experienced researchers.

2.4 Discussion and Conclusion

In this clinical trial with fresh samples, 1091 samples were taken from RT-PCR positive or negative patients. Among them, 284 cases of "SARS-CoV-2 positive group" samples, 181 cases of "Influenza A positive group" samples, 166 cases of "Influenza B positive group" samples and 460 samples of "negative group" were determined by nucleic acid detection.

In nasopharyngeal sample, SARS-CoV-2, 281 positive samples were detected by the assessment reagent, Influenza A samples, 179 positive samples were detected by the assessment reagent and Influenza B positive samples, 164 positive samples were detected by the assessment reagent. The positive coincidence rate of the assessment reagent was 98.94% for SARS-CoV-2, 98.90% for Influenza A and 98.80% for Influenza B. Among negative samples, 459 of the sample detected by the assessment reagent and the negative coincidence rate was 99.52%.

In nasal sample, SARS-CoV-2, 280 positive samples were detected by the assessment reagent, Influenza A samples, 178 positive samples were detected by the assessment reagent and Influenza B positive samples, 163 positive samples were detected by the assessment reagent. The positive coincidence rate of the assessment reagent was 98.59% for SARS-CoV-2, 98.34% for Influenza A and 98.19% for Influenza B. Among negative samples, 458 of the sample detected by the assessment reagent and the negative coincidence rate was 99.57%.

The sensitivity of the RapidFor™ SARS-CoV-2 & Flu A/B Antigen Combo Test calculated from results of all samples was over 98%, with a Ct-value less than 35. As expected, the sensitivity decreases by including samples with higher Ct value.

Conclusion:

In summary, the detection results of diagnostic kit for RapidFor™ SARS-CoV-2 & Flu A/B Antigen Combo Test developed by Vitrosens Biyoteknoloji, and the nucleic acid detection results are in good agreement, and the SARS-CoV-2 antigen detection function can meet the needs of clinical application.

2.5 Explanation of Special Circumstances in Clinical Trial

No

2.6 Appendix

1) Instructions for Use