Spike Glycoprotein Detection Kit

Pre-Clinical Study Report

Name of in vitro diagnostic reagents used in the test: Spike Glycoprotein Detection Kit

Applicant: Assut Europe SpA

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Summary

The Spike Glycoprotein Detection Kit manufactured by Assut Europe SpA can quickly and qualitatively detect the spike glycoprotein of novel coronavirus (SARS-COV-2) in human saliva samples. It can be used as a supplementary test for COVID-19 diagnosis.

According to the clinical trial plan, the Spike Glycoprotein Detection Kit or "test reagent", is to test saliva samples from healthy subjects and confirmed COVID-19 patients. Test results are compared with another commercial SARS-COV-2 nucleic acid detection kit, which is defined as the "gold standard". The sensitivity, specificity, and total accuracy rate are used to evaluate the feasibility of the test reagent in clinical applications.

Method: A collection of clinical samples are examined by the test reagent and the gold standard in parallel, to calculate the clinical sensitivity, clinical specificity and total accuracy rate of the test reagent.

Standard of criteria for a qualified test reagent: Clinical sensitivity ≥90%, clinical specificity ≥90%, and total accuracy rate ≥90%.

Results: Compared to the gold standard, the clinical sensitivity of test reagent reached 90.0%, the clinical specificity reached 95.0%, and the total coincidence rate reached 93.3%.

Conclusion: The performance of test reagent has a high accuracy rate with the gold standard, proving its good feasibility in diagnosing suspected COVID-19 cases.

Acronyms

Test reagent: The Spike Glycoprotein Detection Kit manufactured by Assut Europe SpA

SARS-COV-2: Novel Corona Virus 2019

Main contents

Introduction

The novel coronavirus SARS-COV-2 is the causative pathogen for the global pandemic of COVID-19. It is contagious in humans, either symptomatically or asymptomatically. Based on current epidemic knowledge, the asymptomatic infection may last for 1 day to 14 days, mainly 3 days to 7 days. Symptoms of COVID-19 include fever, fatigue, and cough. Some patients also complains about nasal obstruction, runny nose, score throat, muscle aches, and diarrhea.

In response to the emergent market needs, Assut Europe SpA has manufactured the Spike Glycoprotein Detection Kit. This product utilizes *in vitro* expressed human angiotensin I converting enzyme 2 (ACE2) protein to capture and visualize viral particles in test samples. Since ACE2 is the cellular receptor of SARS-COV-2 spike glycoprotein, it plays an indispensable role in the disease infection and progression. Through various virus sub-types have emerged during the global pandemic, this product can reliably detect all contagious SARS-COV-2 sub-

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types, as long as they bind to ACE2 as the invasion target.

Production of the Spike Glycoprotein Detection Kit is implemented in Class 100,000 cleanrooms. Quality control processes are included in the manufacture procedures to examine the quality of raw materials, semi-finished products, and finished products.

Trial objective

The objective of current trial is to evaluate the feasibility of test reagent in clinical applications. Data from test reagent are compared to SARS-COV-2 nucleic acid detection reagent, which is referred as the “gold standard”, to estimate the sensitive, specificity, and total accuracy rate of test reagent.

Trail design

Clinical samples for the current trial are collected by the clinical site. Each sample is tested by both the test reagent and gold standard reagent. The clinical sensitivity, clinical specificity and total accuracy rate of test reagent are calculated based on the test results.

Results and analysis

Determining the sample size.

With reference to relevant regulations, and considering the uncertainty of obtaining samples, the number of samples for this clinical trial shall be no less than 60, of which the number of positive samples shall not be less than 30.

Sample collection, storage, and transportation.

Clinical samples are collected from COVID-19 suspects, and preserved in virus preservation solution. Keep the solution frozen at -15°C~-25°C until used.

The “gold standard” reagent

Nucleic acid testing is currently the "gold standard" for COVID-19 diagnosis. The Novel Coronavirus (SARS-COV-2) Real Time Multiplex RT-PCR Kit produced by Shanghai ZJ Bio-Tech Co., Ltd. is chosen as the “gold stardard” reagent. It targets the ORF1ab gene, N gene and E gene of the SARS-COV-2, and is used as an auxiliary diagnosis and emergency reserve reagent for COVID-19.

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Information of test reagent and the "gold standard" reagent.

Test reagent	Spike Glycoprotein Detection Kit		
Specification	25 Tests/Box	Lot:	200401
Storage:	2°C~30°C		
Manufacturer	Assut Europe SpA		

Gold Standard reagent	Novel Coronavirus (SARS-COV-2) Real Time Multiplex RT-PCR Kit
Specification	50 Tests/Box
Storage:	Store at -20±5°C, keep away from light
Manufacturer	Shanghai ZJ Bio-Tech Co., Ltd.

Quality control methods

The clinical trial is strictly implemented in accordance with the corresponding instruction manual.

Statistical analysis method of clinical trial data

		Gold standard reagent		Total
		Positive	Negative	
Test reagent	Positive	a	b	a + b
	Negative	c	d	c + d
Total		a + c	b + d	a + b + c + d

Clinical sensitivity (%) = [a / (a + c)] ×100%

Clinical specificity (%) = [d / (b + d)] ×100%

Total coincidence rate (%) = [(a + d) / (a + b + c + d)] ×100%

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Clinical trial results and analysis

Trail Results

A collection of 120 Saliva samples were tested with test reagents..

Test results are as follows:

		Gold standard reagent		Total
		Positive	Negative	
Test reagent	Positive	36	4	40
	Negative	4	76	80
Total		40	80	120

Result analysis

A total of 120 samples are tested in this trial. Both the test reagent and gold standard reagent find out 40 positive results, of which 36 samples are reported positive by both reagent. Four samples are reported positive only in test reagent, and another 4 samples are reported positive only in gold standard reagent. The other 76 samples are reported negative by both reagents.

Clinical sensitivity (%) = [36 / (36 + 4)] ×100% = 90.0%

Clinical specificity (%) = [76 / (4 + 76)] ×100% = 95.0%

Total accuracy rate (%) = [(36 + 76) / (36 + 4 + 4 + 76)] ×100% = 93.3%

Discussion and conclusion

The Spike Glycoprotein Detection Kit manufactured by Assut Europe SpA can quickly and qualitatively detect SARS-COV-2 in human Saliva samples. It can be used as a supplementary test for COVID-19 diagnosis.

In this clinic trial, the Novel Coronavirus (SARS-COV-2) Real Time Multiplex RT-PCR Kit produced by Shanghai ZJ Bio-Tech Co., Ltd., a commercial SARS-COV-2 kit, is used as the "gold standard" reagent. In a collection of 120 clinical samples examined in this trial, the test reagent have shown clinical sensitivity of 90.0%, clinical specificity of 95.0%, and a total accuracy rate of 93.3%.

In summary, the overall accuracy rate between the test reagent and the gold standard reagent is relatively high, and the test reagent can be used clinically for the diagnosis of suspected cases of SARS-COV-2.

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