Multiple Real-Time PCR Kit for Detection of SARS-CoV-2
Clinical Trial Report(Part)

Product name: Multiple Real-Time PCR kit for Detection of SARS-CoV-2

Trial started on: Oct 22, 2021

Trial ended on: Oct 31, 2021

Clinical trial institution: 01 Beijing Youan Hospital, Capital Medical University
Principal investigator (signature):
02 Beijing Center for Diseases Prevention and Control
Principal investigator (signature):
03 Jinan Infectious Disease Hospital Affiliated to Shandong University
Principal investigator (signature):
04 Yantai City Hospital for Infectious Diseases
Principal investigator (signature):

Statistics made by: Beijing Kewei Clinical Diagnostic Reagent Inc.

Person in charge for statistics(signature):

Applicant: Beijing Kewei Clinical Diagnostic Reagent Inc.

Person of contact/Contact method: Wang Qi/010-56315045/18701651263

Reported on: Oct 31, 2021

Source data stored in: 01 Beijing Youan Hospital, Capital Medical University
02 Beijing Center for Diseases Prevention and Control
03 Jinan Infectious Disease Hospital Affiliated to Shandong University
04 Yantai City Hospital for Infectious Diseases

Research Summary

In this clinical trial, the "Multiple Real-Time PCR kit for Detection of SARS-CoV-2" manufactured by Beijing Kewei Clinical Diagnostic Reagent Inc. was assessed (hereinafter referred to as the "assessed kit"), and compared with the "Multiple Real-Time PCR kit for Detection of SARS-CoV-2" manufactured by Shanghai BioGerm Medical Technology Co., Ltd. (hereinafter referred to as the "compared kit") in testing the remaining samples of 840 patients after clinical detection. The 6 samples not fit for this trial, and the same samples from the same patients, and the samples failed to be retested or failed to judge the results according to the instruction manual were rejected. The entire clinical trial of the assessed kit was designed and studied in strict accordance with the clinical trial protocol. The entire clinical trial was carried out under strict control. The tests and analysis were performed by specially trained testing personnel. All the test data were analyzed by IBM SPSS (Ver=23.0). The results are as follows:

(1) This clinical trial was completed in four clinical trial institutions. After comparison with confirmed and excluded cases, a total of 589 patients were included in the statistics (excluding 127 patients in Jinan), of which, 299 (50.76%) were male, 287 (48.73%) were female, and 3 (0.51%) had unknown gender. and their age ranged from 0 to 92 years. There were 252 (42.78%) confirmed cases and 320 (54.33%) excluded cases, and 17 (2.37%) rejected (released from quarantine) cases of SARS-CoV-2. In statistics of sample types, 7 patients provided both throat swab and saliva samples, so there were 407 (68.29%) throat swab samples and 189 (31.71%) saliva samples. A total of 794 samples were included for comparison with the compared kit, of which, 590 were throat swab samples, and 204 were saliva samples.

(2) The positive coincidence rate (sensitivity) between the assessed kit's test results and clinical diagnosis was 91.67%, the negative coincidence rate (specificity) was 100.00%, and the general coincidence rate (accuracy) was 96.43%. The kappa value was 0.926>0.75, indicating good consistence between the assessed kit's test results and clinical diagnosis.

(3) The positive coincidence rate between test results of assessed kit and compared kit was 98.27%, the negative coincidence rate was 93.43%, and the general coincidence rate was 94.84%. The kappa value was 0.880>0.750, indicating good consistence between test results of both kits.

(4) In stratified statistics of throat swab samples, the positive coincidence rate (sensitivity) between the assessed kit's test results and clinical diagnosis was 90.48%, the negative coincidence rate (specificity) was 100.00%, and the general coincidence rate (accuracy) was 96.56%. The kappa value was 0.924>0.75, indicating good consistence between the assessed kit's test results and clinical diagnosis. In saliva samples, the positive coincidence rate (sensitivity) between the assessed kit's test results and clinical diagnosis was 93.75%, the negative coincidence rate (specificity) was 100.00%, and the general coincidence rate (accuracy) was 96.30%. The kappa value was 0.924>0.75, indicating good consistence between the assessed kit's test results and clinical diagnosis.

In throat swab samples, the positive coincidence rate between the test results of assessed kit and compared kit was 97.69%, the negative coincidence rate was 95.65%, and the general coincidence rate was 96.10%. The kappa value was 0.892>0.75, indicating good consistence between test results of both kits. In saliva samples, the positive coincidence rate between the test results of assessed kit and compared kit was 99.01%, the negative coincidence rate was 83.50%, and the general coincidence rate was 91.18%. The kappa value was 0.824>0.75, indicating good consistence between test results of both kits.

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In suspected cases of novel coronavirus, the positive coincidence rate (sensitivity) between the assessed kit's test results and clinical diagnosis was 91.57%, the negative coincidence rate (specificity) was 100.00%, and the general coincidence rate (accuracy) was 96.31%. The kappa value was 0.924>0.75, indicating good consistence between the assessed kit's test results and clinical diagnosis. The positive coincidence rate between test results of assessed kit and compared kit was 98.21%, the negative coincidence rate was 90.89%, and the general coincidence rate was 93.57%. The kappa value was 0.866>0.75, indicating good consistence between test results of both kits.

In this study, a total of 17 cases were released from quarantine and tested negative by the assessed kit and compared kit, both of which had the same detection rate.

In the negative samples collected confirmed cases of SARS-CoV-2 with nucleic acid tested negative in the early stage, the assessed kit had a detection rate of 66.67%. In the positive samples collected from confirmed cases of SARS-CoV-2 with nucleic acid tested negative in the early stage and positive in the late stage, the assessed kit had a coincidence rate of 100.00%. The assessed kit had an early-stage negative detection rate of 85.71%, which was 57.14% for the compared kit.

Test results of weakly positive samples showed that the assessed kit had a positive detection rate of 83.61% and a negative detection rate of 16.39%, while the compared kit had a positive detection rate of 59.02% and a negative detection rate of 40.98%.

The above test data show that the assessed kit is equivalent to the compared kit and suitable for the detection of SARS-CoV-2 nucleic acid in clinical samples.

Testing personnel

1 Clinical trial institutions

Table 1 Clinical trial institutions and principal investigators

Numbering	Institution	Principal Investigator (PI)
01	Beijing Youan Hospital, Capital Medical University	Yu Yanhua
02	Beijing Center for Diseases Prevention and Control	Chen Lijuan
03	Jinan Infectious Disease Hospital Affiliated to Shandong University	Wang Li
04	Yantai City Hospital for Infectious Diseases	Zou Zhiqiang

Abbreviations

(1) Assessed kit: Multiple Real-Time PCR kit for Detection of SARS-CoV-2 manufactured by Beijing Kewei Clinical Diagnostic Reagent Inc.

(2) Compared kit: Multiple Real-Time PCR kit for Detection of SARS-CoV-2" manufactured by Shanghai BioGerm Medical Technology Co., Ltd.

(3) SARS-CoV-2: Novel coronavirus.

Clinical Trial Report

1 Introduction

1.1 Source, physiological, physical and chemical properties of the analyte

Since December 2019, patients had been diagnosed with pneumonia of unknown origin in succession in some medical institutions in Wuhan. Influenza and related diseases had been monitored in Wuhan. Viral pneumonia was found in 27 cases, all of which were diagnosed with viral pneumonia/pulmonary infection.

The SARS-CoV-2 is infectious during the incubation period, which is generally 3-7 days, but no more than 14 days. Fever, weakness and dry cough are the main manifestations. Nasal obstruction, runny nose, and diarrhea are rare. Severe patients develop dyspnea after one week, and the severe cases develop rapidly into ARDS (Acute Respiratory Distress Syndrome), septic shock, difficult-to-tackle metabolic acidosis and bleeding and coagulation dysfunction. The main transmission route is droplet transmission, plus contact transmission. Digestive tract is also the possible transmission route. People are generally susceptible. The elderly and those with underlying diseases had more severe conditions after being infected. Children and infants patients are also observed. The novel coronavirus has been classified as a Class B infectious disease, and is prevented and controlled with the measures for Class A infectious diseases. According to researches, the novel coronavirus is sensitive to heat and can be effectively inactivated by 56 ℃ for 30 minutes, ethyl ether, 75% ethanol (alcohol), chlorine-containing disinfectant, peracetic acid, and chloroform. But it should be emphasized that the virus can't be effectively inactivated by chlorhexidine (CHX).

Similar to Severe Acute Respiratory Syndrome (SARS), the "SARS-CoV-2" also belongs to the Betacoronavirus Lineage B. It is an enveloped linear positive-sense single-stranded RNA virus, and its RNA sequence is about 300,000 nucleotides long. To cause infection, the virus invades cells through receptors on specific cell surfaces, and uses host cells to produce more of itself. Therefore, it is a direct and effective method to confirm whether the body is infected, by collecting cell samples from specific part of human body for detection of viral RNA nucleic acid.

1.2 Intended use

This kit is used for in vitro qualitative detection of ORF1ab and E genes of SARS-CoV-2 in the throat swab samples of the suspected cases, suspected clustered cases, and other people needing diagnosis or identification of novel coronavirus pneumonia. Definitions of the “suspected cases” and “suspected clustered cases” are subject to the definitions in the documents (active version) such as “Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia” and “Surveillance Programme for Pneumonia Cases of New Coronavirus Infection”, published by the Chinese Centers for Disease Control and Prevention.

This product is only used for the auxiliary diagnosis and emergency reserve for in vitro diagnosis of SARS-CoV-2, during the outbreak of this epidemic since December 2019. However, it cannot be used as the conventional in vitro diagnostic reagent in clinical practice. This product should be used in accordance with the relevant requirements of the “Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia”, “Protocol on Prevention and Control of Novel Coronavirus Pneumonia”, and other documents.

Novel coronavirus nucleic acid should be detected, as required by "CDC Guidance on Laboratory Testing of Novel Coronavirus Pneumonia" and other documents, and the bio-safety should be guaranteed.

1.3 Product principle

This kit processes samples with composite nucleic acid lysis buffer and it integrates the cleavage of nucleic acid, inhibition of RNA enzyme, and protection of RNA to achieve the "one-step" detection of RNA virus sample. RNA reverse transcription reaction, polymerase chain reaction (PCR), and Taqman technology are adopted by this kit. Besides, specific primer is designed to amplify the corresponding nucleic acid fragment, according to the nucleic acid sequence of the virus. Meanwhile, the highly specific TaqMan probe can bond with the corresponding nucleic acid fragment, undergo hydrolysis under the exonuclease activity of reverse transcriptase/Taq polymerase, and generate fluorescent signal. According to the relationship between the fluorescent signal and cycles of amplification, a real-time amplification curve can be obtained. The internal

control is used for the quality control of false negative and PCR interference.

Qualitative detection is performed to genes ORF1ab (passage FAM) and E (passage CY5) of SARS-CoV-2. Meanwhile, the internal control (passage VIC) is also set for the quality control, thereby achieving the detection of SARS-CoV-2 nucleic acid.

1.4 Application status of domestic and foreign similar products on the market

The National Medical Products Administration has launched the emergency approval procedures for medical devices immediately since the epidemic outbreak of SARS-CoV-2. So far, more than ten manufacturers have been approved for marketing of the SARS-CoV-2 nucleic acid detection kits. In all of the above kits, nucleic acids are prepared through traditional purification, followed by PCR amplification. This method is complicated, requires the nucleic acid purifier, is time-consuming and difficult to detect rapidly in the current epidemic situation. Since there are few proteins and other PCR interferents in throat swab samples, Beijing Kewei Clinical Diagnostic Reagent Inc. has developed the "virus lysis buffer", and formulated a new method for direct PCR amplification of samples. This new method involves no special purification step or loss of nucleic acids, and it avoids laboratory contamination. It is easy to operate, with no need of special equipment, and suitable for rapid diagnosis in the current epidemic situation.

1.5 Cooperative relationship of the applicant with clinical trial institutions

Beijing Kewei Clinical Diagnostic Reagent Inc. has entrusted Beijing Youan Hospital, Capital Medical University, Beijing Center for Diseases Prevention and Control, Jinan Infectious Disease Hospital Affiliated to Shandong University, and Yantai City Hospital for Infectious Diseases, to evaluate the clinical trial of its “Multiple Real-Time PCR kit for Detection of SARS-CoV-2".

5.2.4 Analysis of confirmed cases with nucleic acid tested negative in early stage

In this study, there were 3 confirmed cases with nucleic acid tested negative in early stage. Case 1 provided three samples, and either of Case 2 and Case 3 provided two samples. The results are shown in Table 30:

Table 32 Analysis of test results of the assessed kit and compared kit in confirmed cases with nucleic acid tested negative in early stage

case	Sample	Test result of compared kit	Test result of assessed kit	Consistent?
1	2002100165	Negative	Positive	No
	2002180142	Positive	Positive	Yes
	2002210087	Positive	Positive	Yes
2	2002120300	Negative	Positive	No
	2002210243	Positive	Positive	Yes
3	2002210041	Negative	Negative	Yes
	2002240066	Positive	Positive	Yes

Table 33 Comparison of the detection rate of assessed kit and compared kit in confirmed cases with nucleic acid tested negative in early stage

Reagent	Early-stage negative samples	Positive samples
Detection rate of assessed kit	66.67%	100.00%
Detection rate of compared kit	0.00%	100.00%

Table 34 Comparison of the detection rate of assessed kit and compared kit in cases with nucleic acid tested

negative in early stage

Reagent source	Number of samples	Negative	Positive	Detection rate
Assessed kit	7	1	6	85.71%
Compared kit	7	3	4	57.14%

The assessed kit had a detection rate of 66.67% in the confirmed cases with nucleic acid tested negative in early stage, and in the samples with negative results in clinical detection in early stage. In the late-stage positive samples, the assessed kit had a coincidence rate of 100.00%. The assessed kit had a detection rate of 85.71% in the cases with negative results in clinical detection in early stage, and this figure was 57.14% for the compared kit.

5.2.5 Detection of weakly positive samples

Table 35 Comparison of the detection rate of assessed kit and compared kit for weakly positive samples

Group	Positive by assessed kit (detection rate, %)	Negative by assessed kit (detection rate, %)	Positive by compared kit (detection rate, %)	Negative by compared kit (detection rate, %)
Confirmed	83.61%	16.39%	59.02%	40.98%
Excluded	0.00%	0.00%	0.00%	0.00%

The 122 weakly positive samples tested by the compared kit, with Ct value greater than 35, were included in the statistics. The assessed kit had a positive detection rate of 83.61% and a negative detection rate of 16.39%, while the compared kit had a positive detection rate of 59.02% and a negative detection rate of 40.98%.

5.2.6 Statistics of positive, negative, and retest by compared kit

Table 36 Statistics of positive, negative, and retest by compared kit

Judgment	Gene	Confirmed	Excluded	Coincidence rate with clinical diagnosis
Positive by assessed kit	ORF1ab+E positive	247	0	100.00%
	ORF1ab single positive, retested, ORF1ab+E positive	5	0	100.00%
	ORF1ab single positive, retested, ORF1ab positive	0	0	-
	ORF1ab single positive, retested, E positive	0	0	100.00%
	E single positive, retested, ORF1ab+E positive	10	0	100.00%
	E single positive, retested, ORF1ab positive	2	0	100.00%
	E single positive, retested, E positive	0	0	-
Negative by assessed kit	ORF1ab+E negative	30	500	94.34%
	ORF1ab single positive, retested, ORF1ab+E negative	0	0	-
	E single positive, retested, ORF1ab+E negative	0	0	-

6 Discussions and Conclusions

In this clinical trial, the "Multiple Real-Time PCR kit for Detection of SARS-CoV-2" manufactured by Shanghai BioGerm Medical Technology Co., Ltd. was selected as the compared kit, and the clinical diagnosis was used for review. The entire clinical trial was carried out under strict control. The tests were performed by specially trained testing personnel.

In this clinical trial, a total of 840 samples were tested in Beijing Youan Hospital, Capital Medical University, Beijing Center for Diseases Prevention and Control, Jinan Infectious Disease Hospital Affiliated to Shandong

University, and Yantai City Hospital for Infectious Diseases. After comparison with the confirmed and excluded cases, only 589 cases were actually included in the statistics (excluding 127 cases in Jinan), of which, 299 (50.76%) were male, 287 (48.73%) were female, and 3 (0.51%) had unknown gender, and their age ranged from 0 to 92 years. There were 252 (42.78%) confirmed cases and 320 (54.33%) excluded cases, and 17 (2.37%) rejected (released from quarantine) cases of SARS-CoV-2. In statistics of sample types, 7 patients provided both throat swab and saliva samples, so there were 407 (68.29%) throat swab samples and 189 (31.71%) saliva samples. A total of 794 samples were included for comparison with the compared kit, of which, 590 were throat swab samples, and 204 were saliva samples.

Results of the clinical trial showed that the positive coincidence rate (sensitivity) between the assessed kit's test results and clinical diagnosis of all cases was 91.67%, the negative coincidence rate (specificity) was 100.00%, and the general coincidence rate (accuracy) was 96.43%. The kappa value was 0.926>0.75, indicating good consistence between the assessed kit's test results and clinical diagnosis. The positive coincidence rate between the test results of assessed kit and compared kit was 98.27%, the negative coincidence rate was 93.43%, and the general coincidence rate was 94.84%. The kappa value was 0.880>0.750, indicating good consistence between both kits.

In stratified statistics of throat swab samples, the positive coincidence rate (sensitivity) between the assessed kit's test results and clinical diagnosis was 90.48%, the negative coincidence rate (specificity) was 100.00%, and the general coincidence rate (accuracy) was 96.56%. The kappa value was 0.924>0.75, indicating good consistence between the assessed kit's test results and clinical diagnosis. In saliva samples, the positive coincidence rate (sensitivity) between the assessed kit's test results and clinical diagnosis was 93.75%, the negative coincidence rate (specificity) was 100.00%, and the general coincidence rate (accuracy) was 96.30%. The kappa value was 0.924>0.75, indicating good consistence between the assessed kit's test results and clinical diagnosis. In throat swab samples, the positive coincidence rate between the test results of assessed kit and compared kit was 97.69%, the negative coincidence rate was 95.65%, and the general coincidence rate was 96.10%. The kappa value was 0.892>0.75, indicating good consistence between test results of both kits. In saliva samples, the positive coincidence rate between the test results of assessed kit and compared kit was 99.01%, the negative coincidence rate was 83.50%, and the general coincidence rate was 91.18%. The kappa value was 0.824>0.75, indicating good consistence between test results of both kits.

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In the negative samples collected confirmed cases of SARS-CoV-2 with nucleic acid tested negative in the early stage, the assessed kit had a detection rate of 66.67%. In the positive samples collected from confirmed cases of SARS-CoV-2 with nucleic acid tested negative in the early stage and positive in the late stage, the assessed kit had a coincidence rate of 100.00%. The assessed kit had an early-stage negative detection rate of 85.71%, which was 57.14% for the compared kit.

Test results of weakly positive samples showed that the assessed kit had a positive detection rate of 83.61% and a negative detection rate of 16.39%, while the compared kit had a positive detection rate of 59.02% and a negative detection rate of 40.98%.

7 Description of Special Circumstances in the Clinical Trial

All the effective samples meeting the requirements of the clinical trial protocol should included in the statistics, while the samples applicable to neither kit, and the samples with ineffective internal control should be rejected. For samples collected from different parts of the same patient, the lower respiratory tract samples should be retained. For samples of the same type and collected from the same patient, test results of the samples collected in early stage should be included in the statistics. For the samples tested in early stage and requiring retest

according to instruction manual of the kit but not retested, such samples from this patient should be rejected. Samples collected from the cases released for quarantine should be retained. For samples collected from the same patient and tested negative in the early stage and positive in the late stage, results of the samples collected in the late stage should be included in the statistics.

8 Appendix

8.1 Instruction manual of the compared kit

8.2 Comparison table of test results of assessed kit and compared kit in clinical samples

8.3 Main references

(1) "Technical Guideline for Clinical Trial of In Vitro Diagnostic Reagent"

(2) "Key Points of Technical Review for Registration of SARS-CoV-2 Nucleic Acid Detection Kit"

8.4 List of key personnel participating in the clinical trial