COVID-19 Coronavirus Real Time PCR Kit

Clinical Accuracy Test Report

1. Purpose

Analyze the accuracy of the COVID-19 Coronavirus Real Time PCR Kit and verify whether the positive and negative coincidence rates of the comparison test results meet the requirements of the test scheme.

2. Reference standards and regulations

(1) Guidelines for the Technical Review of Registration of Multiple Nucleic Acid Detection Reagents for Respiratory Virus (No.80,2019), issued by the Technical Review Center for Medical Devices of the State Drug Administration of China

(2) Key Points for the Technical Review of the Registration of Novel Coronavirus Nucleic Acid Detection Reagents issued by the Technical Review Center for Medical Devices of the State Drug Administration of China

(3) EN 13612:2002/AC :2002Performance evaluation of in vitro diagnostic medical devices

(4) CLSI EP15- A3: User verification of performance for precision and trueness; Modified Guideline - Second Edition.

(5) ISO 3534-1 2006: Statistics -- Local and Symbols -- Part 1: General statistical terms and terms used in probability.

(6) ISO 17511:2003: In vitro diagnostic medical devices - Measurement of quantities in biological samples - Metrological traceability of values assigned to calibrators and control materials.

(7) EP17-A2: Evaluation of detection capability for clinical laboratory measurement procedures, modified guideline-secondedition.2012

(8) WG/N55 FINAL :2019: Clinical Evidence - Key Definitions and Concepts

3. Product name, batch number, specification

Product Name:	COVID-19 Coronavirus Real Time PCR Kit
Batch number:	Lot 20200104(valid until 2021.01.20)
Specifications:	50 test/kit

Supporting equipment:

Fluorescent quantitative PCR instrument: QuantStudio™ 5

Automatic nucleic acid extraction and separation instrument: SSNP-3000A

4. Sample

No.	Sample type	SARS-CoV-2	Sample number	Remarks
1	Nasopharyngeal swab	Negative	100 cases	Nucleic acid
2	Nasopharyngeal swab	Positive	50 cases	Nucleic acid

3	Swallow swab	Negative	100 cases	Nucleic acid
4	Swallow swab	Positive	50 cases	Nucleic acid
5	Sputum	Negative	20 cases	Nucleic acid
6	Sputum	Positive	10 cases	Nucleic acid
7	Alveolar lavage fluid	Negative	20 cases	Nucleic acid
8	Alveolar lavage fluid	Positive	10 cases	Nucleic acid
9	Whole blood	Negative	20 cases	Nucleic acid
10	Whole blood	Positive	10 cases	Nucleic acid
11	Stimulative sample	Negative	100 cases	Nucleic acid
12	Stimulative sample	Positive	50 cases	Virus-like particles dissolved in negative nasopharyngeal swab samples

The virus-like particles are from Tsinghua University, the negative samples are from Taizhou Centers for Disease Prevention and Control, and the nucleic acid of the positive samples is verified by several research centers such as Centers for Disease Prevention and Control of Jiangsu province, Centers for Disease Prevention and Control of Hunan province and Hubei People's Hospital. These cases and specimens were confirmed to be positive or negative by clinical diagnosis and nucleic acid test.

5. Sample collection

In accordance with Appendix 4 of the National Health Commission's General Office on the Issuance of a Programme for the Diagnosis and Treatment of Pneumonia with SARS-CoV-2 infection (Trial Fourth Edition), the technical guidelines for laboratory testing of pneumonia with SARS-CoV-2 infection (Third Edition) are as follows:

1. Pharynx swab: Use a plastic rod swab of 2 polypropylene fiber heads to wipe both the bilateral pharyngeal tonsils and the posterior wall of the pharynx. The swab head is immersed in a tube containing 3 ml of virus preservation solution (also using isotonic solution, tissue culture solution or phosphate buffer). The tail is discarded and the lid is tightened.

2. Nasal swab: Gently insert a plastic rod swab of a polypropylene fiber head into the nasal palate of the nasal canal and leave for a moment before turning slowly. Take the plastic rod swab of the other polypropylene fiber head and collect the other nostril in the same way. the above two swabs were immersed in a tube containing the same 3 ml sample solution, the tail was discarded and the tube cover was tightened.

3. Nasopharyngeal extract or respiratory extract: Use a collector connected to a negative pressure pump to extract mucus from the nasopharynx or extract respiratory secretions from the trachea. Insert the collector head into the nasal cavity or trachea, connect the negative pressure, rotate the collector head and exit slowly, collect the extracted mucus, and rinse the collector with 3 ml sampling fluid once (also can be connected to the 50 ml syringe with a pediatric catheter instead of the collector).

4. Deep cough sputum: After the patient is required to cough deeply, collect the sputum from the cough in a 50ml screw plastic tube containing 3ml of the sample solution.

5. Alveolar lavage fluid: after local anesthesia, the fiberoptic bronchoscope is inserted into the branch tube of the middle lobe of the right lung or the left tongue section through the mouth or nose through the pharynx, and the tip of the tube is jointed into the branch opening of the bronchus.

6. Blood samples: it is recommended to use vacuum blood vessels containing EDTA anticoagulant to collect 5 ml of blood samples, rest at room temperature for 30 minutes, centrifuge at 1500 to 2000 rpm for 10 minutes, collect plasma and blood cells in sterile snail plastic tubes, respectively.

6. Assessment methodology

After the development of this reagent, the following experiments were carried out according to Guidelines for the Technical Review of Registration of Multiple Nucleic Acid Detection Reagents for Respiratory Virus (No.80,2019) and the Key Points for the Technical Review of the Registration of Novel Coronavirus Nucleic Acid Detection Reagents issued by the Medical Devices Technical Review Center of the State Drug Administration.

The clinical accuracy study of the COVID-19 Coronavirus Real Time PCR Kitwas achieved by collecting clinical positive and negative samples and verifying simulated samples.

Compared the clinical sample results and the known sample results, and judged by the positive coincidence rate and the negative coincidence rate.

Positive coincidence rate = (known positive samples/clinical samples) ✕ 100%

Negative coincidence rate =(known negative samples/clinical samples ✕ 100%

7. Pass or not criteria

Positive coincidence rate greater than 98%

Negative coincidence rate greater than 98%

This kit is used to control the transmission of SARS-CoV-2 epidemic, with high sensitivity requirements to avoid false negative in a large area of transmission in the population.

8. Quality control and safety

In the course of each experiment, positive quality control products and negative quality control products should be added to ensure that the experimental results are effective.

9. Experimental results and analysis

The results of this study are shown in the table below. All specimens were tested negative by the kit.

Sample type	Number of samples	Positive results	Specific
Nasopharyngeal swab	100	0(0%)	100%[96.38-100]
Sputum	20	0(0%)	100%[83.16-100]
Alveolar lavage fluid	20	0(0%)	100%[83.16-100]
Swallow swab	100	0(0%)	100%[96.38-100]

Whole blood	20	0(0%)	100%[83.16-100]
Simulated negative samples	100	0(0%)	100%[96.38-100]

All specimens were tested positive by the kit.

Sample type	Number of samples	Positive results	Sensitivity
Nasopharyngeal swab	50	50(100%)	100%[92.89-100]
Sputum	10	10(100%)	100%[69.15-100]
Alveolar lavage fluid	10	10(100%)	100%[69.15-100]
Swallow swab	50	50(100%)	100%[92.89-100]
Whole blood	10	10(100%)	100%[69.15-100]
Simulated positive samples	50	50(100%)	100%[92.89-100]

Conclusion:

The specificity of the COVID-19 Coronavirus Real Time PCR Kitwas verified by nasopharyngeal swab, sputum, alveolar lavage fluid, pharyngeal swab, whole blood. the specificity of all the above sample types was 100%, and the 95% confidence interval of nasopharyngeal swab and pharyngeal swab was 96.38%-100%, while the sputum, alveolar lavage fluid and whole blood samples were only 83.16%-100% because of the smaller samples. Specific validation of all sample types met the acceptable criteria.

The sensitivity of the COVID-19 Coronavirus Real Time PCR Kit was verified by nasopharyngeal swabs, sputum, alveolar lavage fluid, pharyngeal swabs, whole blood, and simulated positive samples. the sensitivity of all the above sample types was 100%, and the 95% confidence interval of nasopharyngeal swabs, pharyngeal swabs, and simulated positive samples was 92.89%-100%, among which the sputum, alveolar lavage fluid, and whole blood samples were only 69.15%-100% because of the smaller samples. Specific validation of all sample types met the acceptable criteria.