In Vitro Diagnostic (IVD) Reagent Clinical Assessment Report

Assessed Reagent: COVID-19 Coronavirus Real Time PCR Kit

Assessment Period: Dec 2019~Feb 2020

IVD reagent classification & Category of clinical assessment：

Class II □ Class III ■ Homologous product approved in China ■

New IVD reagent ■ Overseas registration □

Clinical study of product change □

Applicant Attn：

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Chapter 1 Overview

1. Abstract

Rapid detection of 2019-nCoV novel coronavirus is the most effective approach to isolate the infection source timely and prevent virus transmission. The COVID-19 Coronavirus Real Time PCR Kit developed by Jiangsu Bioperfectus Technologies Co., Ltd has been widely used in some Centers for Disease Control (CDC) and healthcare units due to the unexpected epidemic outbreak. According to the existing real-life data and verified cases provided by healthcare authorities, the product is undergoing a clinical assessment to validate its effectiveness and safety in clinical practices.

The title of this assessment is COVID-19 Coronavirus Real Time PCR Kit, and the reagent is manufactured by Jiangsu Bioperfectus Technologies Co., Ltd, with Clinical diagnostic criteria as the evaluation reference. Clinical diagnostic criteria, i.e. “Diagnostic and therapeutic methods of 2019-nCoV novel coronavirus infection (4th trial version)” issued by National Health Commission (NHC) of the People’s Republic of China, defines “confirmed cases” as: “suspected cases with at least one of the following etiological evidences - 1) positive result diagnosed by real-time PCR using respiratory tract or blood samples; 2) Highly homologous to 2019-nCoV novel coronavirus analyzed gene sequencing using respiratory tract or blood samples.”

This assessment is carried out in 14 CDCs and clinical units. 2658 novel coronavirus samples were detected using the assessed reagent and results are used to run comparative studies with reference to the clinical diagnostic criteria.

2. Clinical study units

Because of the rapid and wide spread of 2019-nCoV novel coronavirus, all clinical study data were collected by the following units.

No.	Unit	Researcher	No. of Cases	Reference sources	Sampling method
1	CDC, Jiangsu province	Hua Tian	70	BioGerm, GeneoDx, Sequencing results	Positive results confirmed by Bioperfectus
2	CDC, Chongqing city	Hua Ling	14	BioGerm, GeneoDx,	Positive results confirmed by Bioperfectus
3	CDC, Yongchuan, Chongqing city	Jingbo Zou	27	BioGerm, DAAN Gene,	Suspected cases screened by Biperfectus and confirmed eith comparison of issued criteria
4	CDC, Wanzhou, Chongqing city	Zhongkai Lang	29	BioGerm, DAAN Gene,	Suspected cases screened by Biperfectus and confirmed eith comparison

					of issued criteria
5	CDC, Xi'an city	Rui Wu	38	BioGerm,	Positive results confirmed by Bioperfectus
6	CDC, Hunan province	Zhifei Zhan	49	BioGerm, GeneoDx or Huirui Biotech	Positive results confirmed by Bioperfectus
7	CDC, Changde city	Zhaomei Xie	73	BioGerm, GeneoDx or Huirui Biotech	Suspected cases detected by Biperfectus
8	The People's Hospital, Hubei province	Yan Li	1846	Clinical history	Positive results confirmed by Bioperfectus
9	The 2nd People's Hospital, Jingmen city	Ran Tu	54	BioGerm, GeneoDx or Huirui Biotech, confirmed by CDC	Suspected cases detected by Biperfectus
10	The People's Hospital, Dazhi city	Zhonghua Zhu	20	BioGerm	Suspected cases detected by Biperfectus
11	The 4th People's Hospital, Nanning city	Shanqiu Wei	33	BioGerm, GeneoDx or Huirui Biotech, confirmed by CDC	Suspected cases detected by Biperfectus
12	The 2nd People's Hospital, Fuyang city	Tuantuan Li	49	BioGerm, Sequencing results	Suspected cases detected by Biperfectus
13	The People's Hospital, Fuyang city	Jianhua Wang	291	BioGerm, positive results confirmed by CDC	Suspected cases detected by Biperfectus
14	CDC, Tongnan, Chongqing city	Lun Tan	22	BioGerm, GeneoDx	Suspected cases screened by Biperfectus and confirmed eith comparison of issued criteria
	In total		2615		

Note: Data collected from clinical record provided by above units

Chapter 2 Clinical assessment report

1. Introduction

1.1 Background information

1.1.1. Biological characteristics

Coronavirus (CoV) is single-stranded RNA virus from the family Coronaviridae and is divided into three genera: α, β and γ. The α and β genus are only pathogenic to mammals. The γ genus mainly causes bird infection. CoV is mainly transmitted through direct contact with secretions or through aerosols and droplets. There is also evidence that it can be transmitted through the faecal-oral route. CoV infections generally manifest as upper respiratory tract infections and/or gastrointestinal symptoms, and severe cases are more common in infants, the elderly, and people with low immune function. Up to now, there have been six kinds of CoV (CoV-229E, CoV-OC43, CoV-NL63, CoV-HKU1, SARS-CoV and MERS-CoV) causing human respiratory diseases, which are important pathogens of human respiratory infection. On 31 December 2019, WHO was informed of a cluster of cases of pneumonia of unknown cause detected in Wuhan City, Hubei Province of China, and a novel Coronavirus (named 2019-nCoV tentatively) belonging to β genus was found

Coronaviruses were first identified in the 1960s, but we don't know where they come from. They get their name from their crown-like shape. Sometimes, but not often, a coronavirus can infect both animals and humans. Most coronaviruses spread through infected people coughing and sneezing, by touching an infected person's hands or face, or by touching things such as doorknobs that infected people have touched. They are heat sensitive and could be deactivated at 56℃ 30 min or by using ether, 75% ethanol, fluorine-containing sanitizer, peracetic acid, chloroform and etc..

Symptoms caused by 2019-nCoV include fever, cough, limbs weakness, runny nose and rhinobyon. Shortness of breath is found in almost half of patients and serious cases tend to Acute Respiratory Distress Syndrome, Septic shock, metabolic acidosis and Bleeding and coagulation dysfunction. It is noteworthy that some patients have no apparent symptoms after infected and recovered after 1 week.

1.1.2 Significance of clinical diagnostics

The epidemic situation caused by 2019-nCoV novel coronavirus has been spread from Wuhan city to entire territory of China. National Health Commission of PRC

announced No. 1 bulletin in early 2020 and defined the 2019-nCoV caused pneumonia as class II infectious diseases while adopted class I actions for prevention and control. Since 23 January 2020, most provinces and regions including Hubei, Beijing, Shanghai, Anhui, Guangdong, Tianjin, Chongqing and etc. have triggered the highest level responding mechanism for emergent events of public health.

According to "Diagnostic and therapeutic methods of 2019-nCoV novel coronavirus infection (4th trial version)" issued by NHC, the main symptoms of this diseases are fever, cough and limbs weakness, while some severe cases have mid-level fever or no fever. However, from the viewpoint of epidemiology, these recessive severe patients could also be the sources of transmission and should be focused as well. In addition, all respiratory diseases including influenza outbreak frequently in winter. According to 4th trial version instructed, one should identify 2019-nCoV novel coronavirus with known pathogens such as virus of influenza, parainfluenza, adenovirus, respiratory syncytial virus, rhinovirus, human metapneumovirus, and chlamydia pneumonia, mycoplasma pneumonia, streptococcus pneumonia.

Not only helps to confirm suspected 2019-nCoV novel coronavirus cases, real-time PCR technology is also of importance to identification of pathogens causing respiratory diseases.

1.1.3 Current clinical and laboratory diagnostic methods

Typical laboratory virus detection approaches include blood routine examination, biochemical and immunological analysis, isolation and culture of virus, serological test and nucleic acid test. Blood routine examination and/or biochemical and immunological analysis are most commonly used but they have low specificity. Virus isolation and culture is acknowledged as golden standard. However, as a novel coronavirus, isolation and culture of 2019-nCoV takes long period and is not suitable for clinical practice and early diagnostics. Serological test is simple and fast but has low sensitivity and easily interference. More importantly, there's mo existing antigen or antibody test kits available for 2019-nCoV in such a short period.

Compared with above methods, nucleic acid test has many advantages, such as 1) various available sample types including upper respiratory tract samples (throat swab, nasal swab, nasopharyngeal extract), lower respiratory tract samples (sputum, respiratory tract extract, bronchial perfusate, bronchoalveolar lavage fluid, lung

tissue biopsy specimens), blood samples, serum samples; 2) low sample volume required; 3) high sensitivity and specificity, rapid and easy-to-use. Real-time PCR has superior sensitivity capable of identifying 2019-nCoV at very early stages.

The assessed reagent has been developed and used in frontlines of epidemic situation caused by 2019-nCoV novel coronavirus. In order to further evaluate the effectiveness and safety of the kit, clinical assessment is carried out based on existing real-life data collected by healthcare units.

1.2 Product information

1.2.1. Technical principle

The kit adopts real-time PCR and hydrolysis probes within a tube to realize multiplexing detection of pathogens. The entire process is run in closed tubes without introducing contaminations. Amplification curve is plotted by background software showing real-time detections.

The kit was developed based on fluorescent PCR technology. Specific primers and probes are tailored in accordance with specific gene areas of 2019-nCoV Novel Coronavirus. Probes consist of a reporter dye at 5’ and quenching dye at 3’. The fluorescent signals emitted from reporter dye are absorbed by the quencher, so it doesn’t emit signals. During amplification, probes bonded to templates are cut off by Taq enzyme (5’-3’ exonuclease activity), separating reporter dye from the quencher, generating fluorescent signals, the PCR instrument will then automatically draw a real-time amplification curve based on the signal change, finally realizing the qualitative detection of 2019-nCoV Novel Coronavirus at the nucleic acid level. In order to avoid the possibility of false negative result, the kit utilizes human housekeeping gene, labeled with ROX, as internal control to monitor the process of sample collection, transportation and extraction. Sampling, transportation and nucleic acid extraction are monitored by CY5 fluorescein labeled internal control to avoid false negative results.

1.2.2. Composition

Components	**Specification/volume**	
	25T	50T
RT-PCR Buffer	188μL	375μL
RT-PCR Enzyme Mix	125μL	250μL
Reaction Mix	100μL	200μL

Positive Control	500μL	500μL
Blank Control (RNase-free Water)	250μL	250μL

1.2.3. Intended use

The Bioperfectus Technologies Novel Coronavirus (2019-nCoV) Real Time PCR Kit is an in vitro diagnostic test used for the detection of a new type of coronavirus (2019-nCoV) from Wuhan, Hubei Province, China.

2. Purpose of clinical assessment

Assess the effectiveness and safety of the kit in clinical practices through comparison study with the clinical diagnostic criteria (include results obtained by using real-time PCR or sequencing).

3. Study design

3.1 Overall study design

Blind comparison between the results obtained using the assessed kit and confirmed cases that diagnosed according to clinical diagnostic criteria defined by NHC is carried out, and then statistically calculate the consistency or difference between them. These data is used to assess the effectiveness and safety of the Bioperfectus kit. Experimental operations are strictly conducted according to Bioperfectus kit user instruction manual.

3.2 Reference reagents/methods selection and reasons

None of products have been registered by National Medical Products Administration (NMPA) of China when Bioperfectus launched the kit. After the gene sequence of 2019-nCoV Novel Coronavirus was decoded and published by Chinese CDC, Wuhan CDC and Shanghai public health clinical centre, several Chinese IVD companies developed their PCR kits subsequently, including Shanghai GeneoDx Biotech Co., Ltd., Shanghai BioGerm Medical Biotechnology Co.,Ltd., Shanghai Huirui Biotechnology Co.,Ltd., DAAN Gene Co., Ltd., Shanghai ZJ Bio-Tech Co., Ltd., and Shenzhen BGI group. Other methods based on antigen or antibody only announced by two companies in Beijing and Tianjian and they have not registered with NMPA.

Recently, China NHC issued “Diagnostic and therapeutic methods of 2019-nCoV novel coronavirus infection (4th trial version)” and defined defines “confirmed cases” as: “suspected cases with at least one of the following etiological evidences - 1) positive result diagnosed by real-time PCR using respiratory tract or blood samples; 2) Highly

homologous to 2019-nCoV novel coronavirus analyzed gene sequencing using respiratory tract or blood samples". As a result, we select reference kits among above listed products and also in accordance with the clinical diagnostic criteria defined by NHC.

Assessed reagent：

Name：Novel Coronavirus (2019-nCoV) Real Time PCR Kit

Manufacturer：Jiangsu Bioperfectus Technologies Co., Ltd

Specification：25T/kit，50T/kit

Sample type：sputum, throat swab, lower respiratory tract secreta

Applicable instruments：ABI 7500、QuantStudio™ 5、Roche LightCycler®480、Bio-Rad CFX96™、SLAN-96P/S real-time PCR thermal cyclers.

Comparison method：

Name：clinical diagnostic criteria (include results obtained by using real-time PCR or sequencing)

Sample type：sputum, throat swab, lower respiratory tract secreta

3.3 Sample source

Positive, negative or suspected cases during the 2019-nCoV novel coronavirus epidemic situation collected by 14 CDCs and healthcare units.

3.4 Sample population and reasons

Sample population collected for the clinical assessment is estimated using equation (1)

$$n = \frac{[Z_{1-\alpha/2}]^2 P(1-P)}{\Delta^2} \quad \text{.....Equation (1)}$$

where n is the sample population; $Z1\text{-}\alpha/2$ is the quantile of standard normal distribution; P is expectation of evaluation indicator; Δ is accepted error magnitude of P and normally is between 0.05~0.1.

The study evaluates the assessed reagent through comparison method by both confirmed and excluded cases from the clinical record. The expected positive case ratio is up to 90% according to the pre-clinical assessment results. Therefore minimum quantity of the positive sample population (n1) is estimated using equation (1).

$$n1 = \frac{1.96^2 \times 0.9 \times (1-0.9)}{0.05^2} = 138$$

Similarly, pre-clinical assessment results suggest up to 95% specificity and therefore minimum quantity of the negative sample population (n1) is estimated using equation (1).

$$n2 = \frac{1.96^2 \times 0.95 \times (1-0.95)}{0.05^2} = 73$$

Consequently, qualified assessment is ensured on the basis of minimum positive and negative sample population of 138 and 73 respectively.

3.5 Sample collection and preservation

3.5.1 Sample collection

Sample collection follows instructions from "Laboratory diagnostic technical guidance (2nd version) of pneumonia infected by 2019-nCoV novel coronavirus" issued by China NHC in 22 January 2020.

3.5.2 Sample preservation

Sample collected for virus isolation and nucleic acid detection should be tested as soon as possible. Sample preserved at 4℃ (to be tested within 24 hours) or -70℃ and colder (to be tested later than 24 hours). Sample should be transported to laboratory immediately after collection and refrigerated with dry ice for long-distance transportation.

4. Statistical method

Statistical analysis of the clinical assessment using qualitative analysis method that widely accepted. The typical method is Kappa consistency evaluation as follows.

Assessed reagent	Clinical diagnostic criteria		**Total**
	Positive	**Negative**	
Positive	A	B	A+B
Negative	C	D	C+D
Total	A+C	B+D	N(A+B+C+D)

Statistical analysis：

(1) Sensitivity：A/（A+C）

(2) Specificity：D/（B+D） Sensitivity and specificity are in the range of 0%～100%，and superior consistency suggested by values close to 100%.

(3) Overall coincidence rate：（A+D）/（A+B+C+D）

(4) Confidence interval of 95% overall coincidence rate：

[100%（Q1-Q2）/Q3，100%(Q1+Q2)/Q3]

where：$Q1=2(A+D)+1.96^2$；$Q2=1.96\times sqrt[1.96^2+4(A+D)(B+C)/N]$；$Q3=2(N+1.96^2)$

(5) Kappa=（Pa-Pe）/(1-Pe), where Pa=(A+D)/N，

$Pe=[(A+B)(A+C)+(B+D)(C+D)]/N^2$

Kappa value denotes the consistency of detected results. Kappa≥0.75 suggests good consistency and ＜0.40 suggest unsatisfied consistency.

(6) Hypothesis testing of Kappa value (U testing)

U=K/Se（k），$Se(k)=\sqrt{\frac{Pa(1-Pa)}{N(1-Pe)^2}}$，where U is the quantile of standard normal distribution; Se (K) the standard error of K.

H0：Overall Kappa coefficient ≤0

H1：Overall Kappa coefficient＞0

When U＞1.96，P＜0.05，reject H_0，consider Overall Kappa coefficient＞0，i.e. the consistency of results are valid.

5. Results and analysis

5.1 Sample information analysis

Sample collected from 13 CDCs and healthcare units are illustrated in below table and additional information are noted.

No.	Unit	Positive cases	Negative cases	Suspected cases	Note
1	CDC, Jiangsu province	70	0	-	Include additional 3 homologous samples
2	CDC, Chongqing city	14	0	-	Include additional 3 homologous samples
3	CDC, Yongchuan, Chongqing city	3	24	-	24 negative samples without patient info
4	CDC, Wanzhou, Chongqing city	14	15	-	
5	CDC, Xi’an city	11	27	-	
6	CDC, Hunan province	49	0	-	
7	CDC, Changde city	21	52	-	52 negative samples without patient info
8	The People's Hospital, Hubei province	690（694）	1156（1185）	33（35）	Some of suspected patient is unwilling to revisit and is not included

9	The 2nd People's Hospital, Jingmen city	21	37（33）	6 to be confirmed	4 cases are confirmed as negative and the rest 2 are excluded
10	The People's Hospital, Dazhi city	9	11	-	
11	The 4th People's Hospital, Nanning city	4	29	-	
12	The 2nd People's Hospital, Fuyang city	5	44	1suspected case, assessed reagents shows week positive	44 negative samples without patient info. 1 suspected case confirmed positive with subsequent detections
13	The People's Hospital, Fuyang city	10	281	3 suspected cases, assessed reagents shows positive	2 of 3 suspected cases confirmed positive and the rest 1 rejects subsequent detections
14	CDC, Tongnan, Chongqing city	1（2）	21	-	2 positive cases and one was deleted by Chongqing CDC
	Total	922	1693	43	

Note: in the table A (B), B is statistical data from clinical history. The data from Hubei People's Hospital is sample population not patient population. Please refer to A as patient population.

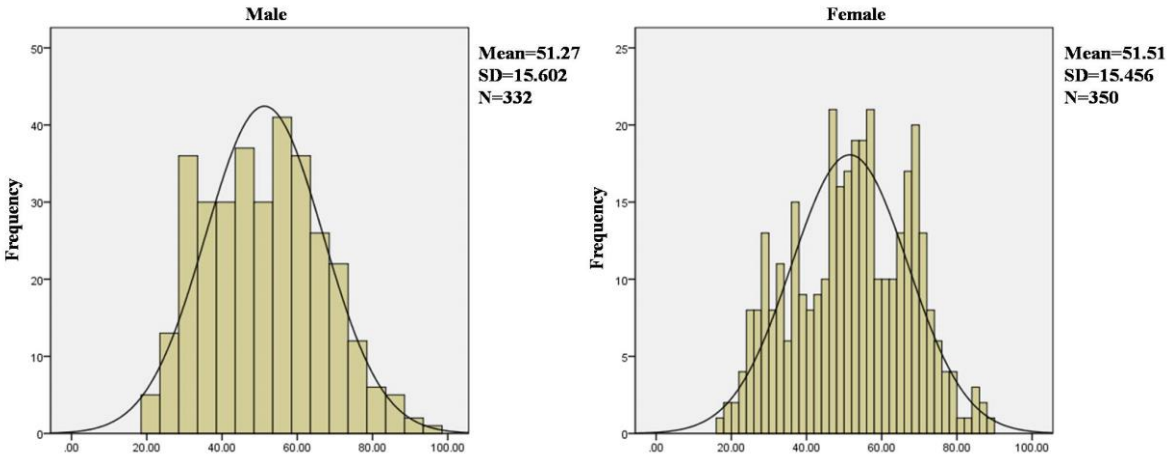
Sample summary.

This assessment initially adopted 2658 samples, including 922 positive cases, 1693 negative cases, 43 suspected cases, and 7 of these were confirmed (4 positive and 3 negative) by subsequent tests. All the rest unconfirmed cases were not included. Finally, assessed samples include 925 positive, 1697 negative and 36 suspected cases and they were tested using sputum, throat swab, nasal swab, nasopharyngeal swab and lower respiratory tract secreta and etc. 120 negative cases among 2622 (925+1697) samples has no clinical record (24 from Chongqing Yongchuan CDC, 44 from Fuyang 2nd People's Hospital, 52 from Changde CDC) and the rest 2502 cases include 46 sputum samples, 630 throat swab samples, 1 lower respiratory tract secreta sample, 3 nasal swab samples, 1838 nasopharyngeal swab samples, 1 bronchoalveolar lavage fluid sample and 1 whole blood sample. Among which there’re 18 homologous

samples (sample collected twice from the same patient).

Gender and age distribution

243 cases without age information (mainly from samples provided by Hubei People's Hospital and original sample sources were indicated from Wuhan 9th People's Hospital, Wuchang CDC and Hongshan CDC respectively) are excluded from the 925 positive cases. The rest 682 positive cases include 332 males and 350 females. The median of age statistics is 51, matching the median age 49 reported by "Progress and risk assessment of 2019-nCoV novel coronavirus epidemic situation" issued by Chinese CDC in 27 January 2020. The median of ages for male and female are 51 and 52 respectively.



5.2 Statistics and analysis of consistency between assessed reagent and clinical criteria

Based on the assessment plan, 2622 samples are tested with the assessed reagent and results are compared with the clinical diagnostic record. The consistency is shown in below table.

Consistency between assessed reagent and clinical record

Assessed reagent	Clinical record		In total
	Positive	Negative	
Positive	925	0	925
Negative	0	1697	1697
In total	925	1697	2622

（1）Positive consistency=925/925=100%

（2）Negative consistency = 1697/1697=100%

（3）Overall coincidence rate =（925+1697）/ 2622=100%

（4）Kappa= 1

（5）Hypothesis testing of Kappa value (U testing)

Se（k）=0, and U=K/ Se（k）>> 95% of the quantile of standard normal distribution 1. 96. Therefore P < 0. 05; Reject H0 and accept H1, i.e. the consistency of results are valid.

5.2.1 **Throat swab samples**

Consistency between assessed reagent and clinical record

Assessed reagent	**Clinical record**		**In total**
	Positive	Negative	
Positive	209	0	209
Negative	0	421	421
In total	209	421	630

630 throat swab samples show 100% positive consistency, 100% negative consistency and 100% overall consistency. Kappa=1> 0.75 in Kappa hypothesis testing and therefore the consistency of results are valid.

5.2.2 **Nasopharyngeal swab samples**

Consistency between assessed reagent and clinical record

Assessed reagent	**Clinical record**		**In total**
	Positive	Negative	
Positive	696	0	696
Negative	0	1142	1142
In total	696	1142	1838

1838 nasopharyngeal swab samples show 100% positive consistency, 100% negative consistency and 100% overall consistency. Kappa=1> 0.75 in Kappa hypothesis testing and therefore the consistency of results are valid.

5.2.3 **Sputum samples**

Consistency between assessed reagent and clinical record

Assessed reagent	Clinical record		In total
	Positive	Negative	
Positive	23	0	23
Negative	0	23	23
In total	23	23	46

46 sputum samples show 100% positive consistency, 100% negative consistency and 100% overall consistency. Kappa=1> 0.75 in Kappa hypothesis testing and therefore the consistency of results are valid.

5.2.4 **Lower respiratory tract secreta**

1 lower respiratory tract secreta sample was detected positive and consistents with the clinical diagnostic record.

5.2.5 **Nasal swab samples**

All 3 nasal swab samples were detected positive and consistent with the clinical diagnostic record.

5.2.6 **Bronchoalveolar lavage fluid sample**

1 bronchoalveolar lavage fluid sample was detected positive and consistents with the clinical diagnostic record.

5.2.7 **Whole blood sample**

1 whole blood sample was detected negative and consistents with the clinical diagnostic record.

5.3 Statistics and analysis of consistency of homologous samples tested by assessed reagent

In total 18 homologous samples including 8 positive and 10 negative were collected by Jiangsu CDC (3 cases), Chongqing CDC (3 cases), Hubei People's Hospital (12 cases). The results show 100% consistency and detail information is illustrated in below table.

Tracing No.	G	A	Clinical history	Sample type	Results by assessed reagent	clinical diagnostic record	Clinical confirmation basis	Source of samples
2020 No.747-1	M	37	viral pneumonia	Sputum/ throat swab	P(+)/ P(+)	P(+)	Real-time PCR/ Sequencing	Jiangsu CDC
2020 No.773-2	M	44	viral pneumonia	Throat/Nasal swab	P(+)/ P(+)	P(+)	Real-time PCR	Jiangsu CDC
2020 No.781	F	47	viral pneumonia	Sputum/throat swab	P(+)/ P(+)	P(+)	Real-time PCR	Jiangsu CDC
FY20001	F	44	viral pneumonia	NP swab/ sputum	P(+)/ P(+)	P(+)	Real-time PCR	Chongqing CDC

FY20002	F		viral pneumonia	NP swab/ sputum	P(+)/ P(+)	P(+)	Real-time PCR	Chongqing CDC
FY20032	F	23	viral pneumonia	NP swab/ sputum	P(+)/ P(+)	P(+)	Real-time PCR	Chongqing CDC
10009937	F	78	Renal dysfunction/ Ureteral tumor	sputum /NP swab	Negative/ Negative	Negative	Real-time PCR	Hubei People's Hospital
10014582	F	65	Lymphadenoma	NP swab/ sputum	Negative / Negative	Negative	Real-time PCR	Hubei People's Hospital
3005939649	M	57	viral pneumonia	sputum /NP swab	P(+)/ P(+)	Negative	Real-time PCR	Hubei People's Hospital
10012432	M	88	Coronary arrhythmia	NP swab/ sputum	Negative/ Negative	Negative	Real-time PCR	Hubei People's Hospital
10003870	F	39	Space-occupying lesions	sputum /NP swab	Negative / Negative	Negative	Real-time PCR	Hubei People's Hospital
10015396	M	90	Pulmonary infection	NP swab/ sputum	Negative/ Negative	Negative	Real-time PCR	Hubei People's Hospital
10016422	M	62	Chronic renal failure	NP swab/ sputum	Negative / Negative	Negative	Real-time PCR	Hubei People's Hospital
1938988	M	91		NP swab/ sputum	Negative / Negative	Negative	Real-time PCR	Hubei People's Hospital
10018189	F	74	Aplastic anemia	NP swab/ sputum	Negative/ Negative	Negative	Real-time PCR	Hubei People's Hospital
10017585	F	86	Hypertension class II	sputum /NP swab	Negative / Negative	Negative	Real-time PCR	Hubei People's Hospital
20003175	F	63	viral pneumonia	sputum /NP swab	P(+)/ P(+)	P(+)	Real-time PCR	Hubei People's Hospital
10017449	M	96	Hypertension	NP swab/ sputum	Negative / Negative	Negative	Real-time PCR	Hubei People's Hospital

5.4 Variation analysis

No variation in this clinical assessment.

5.5 Statistics and analysis affected by various clinical criteria

5.5.1 Clinical study

656 cases from 13 CDCs and healthcare units are used for this study including 235 positive cases and 421 negative cases. References are results tested by using virus gene sequencing and real-time PCR kits (include products from BioGerm, GeneoDx, DAAN gene, Huirui Biotech and etc.). The overall consistency is 100% including 6 positive homologous samples and detail information is illustrated in below table.

Sample type	Assessed reagent positive/ reference positive	Assessed reagent negative/ reference negative	Total	Consistency
Throat swab	209	421	630	100%
nasopharyngeal swab	16	0	16	100%
Sputum	12	0	12	100%
Lower respiratory tract secreta	1	0	1	100%
Nasal swab	3	0	3	100%

Bronchoalveolar lavage fluid	-	-	-	-
Whole blood	-	-	-	-
Total	235	421	656	100%

Note: 6 homologous samples calculated repeated with different sample types.

5.5.1 Analysis using existing clinical data

All clinical data (real-life data) 1846 cases collected in this assessment are from the People's Hospital of Hubei province, including 690 positive and 1156 negative. The positive rate is 37.37%. Median age of positive cases is 56, with 97 the maximum and 18 the minimum. Detail information is illustrated in below table. 12 cases are homologous samples.

Sample type	Assessed reagent positive	Assessed reagent negative	Total	Note
Throat swab	-	-	-	
nasopharyngeal swab	680	1142	1822	
Sputum	11	23	34	
Lower respiratory tract secreta	-	-	-	
Nasal swab	-	-	-	
Bronchoalveolar lavage fluid	1	0	1	
Whole blood	0	1	1	
Total	690	1156	1846	

Note: 12 homologous samples calculated repeated with different sample types.

Samples were collected during 28 January 2020 to 2 February 2002 from 2019-nCoV novel coronavirus clinical diagnosis. The study aim at evaluate the clinical practice application of the assessed reagent. Patient were randomly selected including 2019-nCoV novel coronavirus confirmed cases, 2019-nCoV novel coronavirus suspected cases, other cases to be distinguished from 2019-nCoV novel coronavirus, cases released from

quarantine, discharged cases. Among which, 33 are suspected cases without tracing information and therefore are not included. By reviewing all patient information, 22 patients were diagnosed during 28 January 2020 to 1 February 2002. Some of positive cases turn to negative after quarantine; some of suspected cases have symptoms of pulmonary infection and/or viral pneumonia. Some cases were confirmed positive by subsequent tests after negative result were initially detected at early stage.

Tracing No.	G	A	Clinical history	Sample type	Sampling date	Results by assessed reagent	clinical diagnostic record	Note
10009937	F	78	Renal dysfunction/ Ureteral tumor	Sputum/NP swab	28 Jan 2020/ 31 Jan 2020	Negative/Negative	N	
3005863699	F	41		NP swab/ NP swab	28 Jan 2020/ 30 Jan 2020	Negative/Negative	N	
10015432	M	69	Intracranial space-occupying lesions	NP swab/ NP swab	28 Jan 2020/ 30 Jan 2020	Negative/Negative	N	
10011623	F	57	Pulmonary infection	NP swab/ NP swab/ NP swab	20200128/20200129/20200130	P(+)/Negative/Negative	P(+)	quarantine
10014582	F	65	Lymphadenoma	NP swab/ sputum	28 Jan 2020/ 29 Jan 2020	Negative/Negative	N	
10012432	M	88	Coronary arrhythmia	NP swab/ sputum	28 Jan 2020/ 29 Jan 2020	Negative/Negative	N	
10015871	F	62	Nausea	NP swab/ NP swab/ NP swab/ NP swab	28 Jan 2020/ 30 Jan 2020/31 Jan 2020/ 1 Feb 2020	N/suspected/N/P(+)	P(+)	Quarantine after re-tested
3005945939	M	47		NP swab/ NP swab	28 Jan 2020/ 31 Jan 2020	Negative/P(+)	P(+)	quarantine
3005858799	F	81		NP swab/ NP swab	28 Jan 2020/ 31 Jan 2020	Negative/Negative	N	
3005889255	F	69		NP swab/ NP swab	28 Jan 2020/ 30 Jan 2020	Negative/Negative	N	
10016796	M	27	Pulmonary infection	NP swab/ NP swab	20200129/20200201	Negative/Negative	N	
3001987632	F	34		NP swab/ NP swab	29 Jan 2020/ 30 Jan 2020	P(+)/P(+)	P(+)	quarantine
10008960	M	61	Fracture of femoral neck bone	NP swab/ NP swab	29 Jan 2020/ 31 Jan 2020	Negative/Negative	N	
20003082	M	66	Pulmonary infection/ viral pneumonia	NP swab/ NP swab	30 Jan 2020/ 1 Feb 2020	可疑/阳性(+)	P(+)	Quarantine after re-tested
10015396	M	90	Pulmonary infection	NP swab/sputum	30 Jan 2020/ 31 Jan 2020	Negative/Negative	N	
10018156	M	54	Subarachnoid hemorrhage	NP swab/ NP swab	30 Jan 2020/ 1 Feb 2020	Negative/Negative	N	
10016422	M	62	Chronic renal failure	NP swab/ sputum	31 Jan 2020/ 1 Feb 2020	Negative/Negative	N	
10018174	M	57	Pulmonary infection	NP swab/ NP swab	31 Jan 2020/ 1 Feb 2020	P(+)/P(+)	P(+)	quarantine
3005871580	M	58		NP swab/ NP swab	31 Jan 2020/ 1 Feb 2020	Negative /P(+)	P(+)	quarantine
3005947787	M	51		NP swab/ NP swab	29 Jan 2020/ 31 Jan 2020	Negative/Negative	N	
10015627	M	82	Heart dysfunction	NP swab/ NP swab	30 Jan 2020/ 31 Jan 2020	Negative/Negative	N	
3005886156	M	42		NP swab/ NP swab	30 Jan 2020/ 31 Jan 2020	Negative/Negative	N	

6. Conclusion

Through the clinical assessment of COVID-19 Coronavirus Real Time PCR Kit manufactured by Jiangsu Bioperfectus Technologies Co., Ltd, the following conclusions are obtained.

Comparison of results diagnosed by assessed reagent with clinical diagnostic criteria show 100% positive consistency, 100% negative consistency and 100% overall consistency. Kappa=1> 0.75 in Kappa hypothesis testing and therefore the consistency of results are valid.

Comparison of results diagnosed by assessed reagent with homologous products show 100% positive consistency, 100% negative consistency and 100% overall consistency. Kappa=1> 0.75 in Kappa hypothesis testing and therefore the consistency of results are valid.

7. Reference

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