Analytical Sensitivity Study

1. Basic information

Basic information				
Test kit	Lot	Manufacture date	Expiry date	Specification
Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold)	W20200401	2020.04.13	2021.10.13	40 T/kit
	W20200402	2020.04.14	2021.10.14	
	W20200403	2020.04.15	2021.10.15	
Manufacturer		Beijing Hotgen Biotech Co., Ltd.		
Product code	HGCG134A0140		IFU version	V. 2020-09.01[Eng.]
Experiment site	Academy of Military Medical Science of the PLA, Beijing.		Operator	Pan Yunyun
Date of initiation and completion	2020.05.08			
Study protocol				
Saliva samples			One negative sample	

2. Sample sources and information

2.1 Sample sources

Virus cultures were sourced from the Academy of Military Medical Science of the PLA, Beijing. The strain and titer of the virus samples are shown in the table below.

No.	Virus Strain	Virus titer ($TCID_{50}$/mL)	Virus conc. (copies/mL)
1	BetaCoV/Beijing/IMEBJ01/2020-01	$2.01\times10^{6.2}$	2.23×10^{6}
2	BetaCoV/Beijing/IMEBJ01/2020-02	$2.03\times10^{6.2}$	2.25×10^{6}
3	BetaCoV/Beijing/IMEBJ01/2020-03	$2.05\times10^{6.2}$	2.27×10^{6}

2.2 Prepration of virus culture

The virus culture is prepared by cell culture to propagate the virus. First, the digested and dispersed cell suspension is divided into a culture flask, sealed, and placed in an incubator for several days to form an adherent monolayer of cells. Then the virus is inoculated, and the virus reproduces in the nourishing tissue cells. Put the prepared virus culture solution into a 60 ℃ constant incubator for 45 minutes inactivation treatment to make the virus inactive.

3. Determination of the titer of virus culture

The 50% tissue culture infection dose method was used to determine the titer of the novel coronavirus culture. The process is as follows:

3.1 Prepare cells

Take out a cell culture plate, and transfer approximately 8000 to 10000 cells in each well (Add 10 mL of culture solution after digestion of cells in a T25 flask.).

3.2 Dilute the virus to be tested

Add 1.8 mL of virus dilution to each test tube. Add 0.2 mL of virus to the No. 1 test tube, serially dilute it to an appropriate concentration by 10 times, and further dilute it to a desired concentration.

3.3 Inoculation

Add the diluted virus solution to a 96-well plate, 100 μL per well, according to the observation habits, generally adding from right to left, from top to bottom, from high dilution to low dilution (10^{-1}, 10^{-2}) and to stock solution. Incubate in a 37°C CO_2 incubator for 1 hour, then aspirate the virus solution, and add 200 μL of maintenance solution to the culture plate and continue culturing in the 37°C CO_2 incubator.

3.4 Determination

Take out the culture plate and observe the cell pathology under the microscope. Use Reed-Muench method to observe CPE, find out the virus dilution factor that can cause half of the cell bottles or tubes infectious, and calculate the $TCID_{50}$ of the virus solution.

4. Experimental protocol

Three lots of Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold) were used to detect saliva samples to determine the limit of detection.

4.1 Saliva sample

4.1.1 Study on the tentative limit of detection of saliva samples

The virus culture of one virus strain was selected, and the virus titer was determined by the 50% tissue culture infectious dose ($TCID_{50}$) method. Use the clinical negative saliva sample matrix to dilute the virus culture solution to $1\times10^{6\,2}$ $TCID_{50}$/mL, and then perform the serial dilution according to the titers listed in the table below. Use the Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold) to repeat the detection of each virus dilution 3 times. The lowest concentration level that is 100%

detectable is determined as the tentative limit of detection. The tentative limit of detection is $1\times10^{3.2}$ $TCID_{50}$/mL.

No.	Sample titer ($TCID_{50}$/mL)
1	$1\times10^{6\ 2}$
2	$1\times10^{5\ 2}$
3	$1\times10^{4\ 2}$
4	$1\times10^{3\ 2}$
5	$1\times10^{2\ 2}$

4.1.2 Verification of the limit of detection of saliva samples

The virus cultures of three virus strains were selected, and the virus titer was determined by the 50% tissue culture infectious dose ($TCID_{50}$) method. Use the clinical negative saliva sample sample matrix to dilute the three virus culture solutions to $1\times10^{3.2}$ $TCID_{50}$/mL. And then perform the serial dilution according to the titers listed in the table below. Prepare three samples for each gradient concentration, and repeat testing each sample 20 times. The lowest concentration level at which the positive detection rate reaches 95% or more is determined as the limit of detection. The limit of detection is $2.5\times10^{2.2}$ $TCID_{50}$/mL.

No.	Sample Concentration ($TCID_{50}$/mL)
1	$1\times10^{3\ 2}$
2	$5\times10^{2\ 2}$
3	$2.5\times10^{2\ 2}$
4	$1.25\times10^{2\ 2}$
5	$6.25\times10^{1\ 2}$

5. Acceptable criteria

The lowest concentration level at which the positive detection rate reaches 95% or above is determined as the limit of detection.

6. Test results

6.1 Saliva sample

Table 1 Results of the tentative LoD of saliva sample

Sample number	Sample conc.	W20200401			W20200402			W20200403			Positive detection rate
		Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	
1	$1\times10^{6\ 2}$	+	+	+	+	+	+	+	+	+	100%
2	$1\times10^{5\ 2}$	+	+	+	+	+	+	+	+	+	100%
3	$1\times10^{4\ 2}$	+	+	+	+	+	+	+	+	+	100%
4	$1\times10^{3\ 2}$	+	+	+	+	+	+	+	+	+	100%

5	1×10^{2} 2	-	-	-	-	-	-	-	-	-	0%

Table 2 Verification results of the LoD for saliva sample prepared with the virus strain 1st
(Lot 1: W20200401)

Test number	1×10^{3} 2			5×10^{2} 2			2.5×10^{2} 2			1.25×10^{2} 2			6.25×10^{1} 2		
	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3
Test 1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 2	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 3	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 6	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 8	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 9	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 10	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-
Test 11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 12	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 13	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 15	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 16	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-
Test 17	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 18	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 19	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Test 20	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Postive detection rate	100%			100%			95%			3%			0%		

Table 3 Verification results of the LoD for saliva sample prepared with the virus strain 2nd
(Lot 1: W20200401)

Test number	1×10^{3} 2			5×10^{2} 2			2.5×10^{2} 2			1.25×10^{2} 2			6.25×10^{1} 2		
	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3
Test 1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 2	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 3	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 6	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 8	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-

Test 9	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 10	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 12	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 13	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Test 14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 15	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Test 16	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 17	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 18	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 19	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 20	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Positive detection rate	100%			100%			95%			3%			0%		

Table 4 Verification results of the LoD for saliva sample prepared with the virus strain 3rd (Lot 1: W20200401)

Test number	1×10^{3} 2			5×10^{2} 2			2.5×10^{2} 2			1.25×10^{2} 2			6.25×10^{1} 2		
	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3
Test 1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 2	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 3	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 6	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 8	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 9	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 10	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 12	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 13	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 15	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 16	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 17	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 18	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 19	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Test 20	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Positive detectio	100%			100%			95%			0%			0%		

n rate					

Table 5 Verification results of the LoD for saliva sample prepared with the virus strain 1st (Lot 2: W20200402)

Test number	1×10^{3} 2			5×10^{2} 2			2.5×10^{2} 2			1.25×10^{2} 2			6.25×10^{1} 2		
	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3
Test 1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 2	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-
Test 3	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 6	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 8	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 9	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 10	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 12	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 13	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 15	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 16	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 17	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 18	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 19	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Test 20	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-
Positive detection rate	100%			100%			95%			3%			0%		

Table 6 Verification results of the LoD for saliva sample prepared with the virus strain 2nd (Lot 2: W20200402)

Test number	1×10^{3} 2			5×10^{2} 2			2.5×10^{2} 2			1.25×10^{2} 2			6.25×10^{1} 2		
	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3
Test 1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 2	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 3	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 6	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-

Test 8	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Test 9	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 10	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 12	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 13	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 15	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 16	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 17	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 18	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 19	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 20	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Positive detection rate	100%			100%			95%			0%			0%		

Table 7 Verification results of the LoD for saliva sample prepared with the virus strain 3rd (Lot 2: W20200402)

Test number	1×10^{3} 2			5×10^{2} 2			2.5×10^{2} 2			1.25×10^{2} 2			6.25×10^{1} 2		
	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3
Test 1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 2	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 3	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 6	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 8	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 9	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 10	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 12	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 13	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 15	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 16	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 17	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 18	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 19	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Test 20	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Positive	100%			100%			95%			0%			0%		

detectio n rate					

Table 8 Verification results of the LoD for saliva sample prepared with the virus strain 1st (Lot 3: W20200403)

Test number	1×10^{3} 2			5×10^{2} 2			2.5×10^{2} 2			1.25×10^{2} 2			6.25×10^{1} 2		
	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3
Test 1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 2	+	+	+	+	+	+	+	+	+	-	-	+	-	-	-
Test 3	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 6	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 8	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-
Test 9	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 10	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 12	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 13	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 15	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 16	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 17	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 18	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 19	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Test 20	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Positive detectio n rate	100%			100%			95%			3%			0%		

Table 9 Verification results of the LoD for saliva sample prepared with the virus strain 2nd (Lot 3: W20200403)

Test number	1×10^{3} 2			5×10^{2} 2			2.5×10^{2} 2			1.25×10^{2} 2			6.25×10^{1} 2		
	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3
Test 1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 2	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 3	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 4	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Test 5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-

Test 6	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
Test 7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 8	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 9	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 10	+	+	+	+	+	+	-	+	+	-	-	-	-	-	-
Test 11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 12	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 13	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Test 14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 15	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 16	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 17	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 18	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 19	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 20	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Positive detection rate	100%			100%			95%			3%			0%		

Table 10 Verification results of the LoD for saliva sample prepared with the virus strain 3rd (Lot 3: W20200403)

Test number	1×10^{3} 2			5×10^{2} 2			2.5×10^{2} 2			1.25×10^{2} 2			6.25×10^{1} 2		
	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3	NO 1	NO 2	NO 3
Test 1	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 2	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 3	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 4	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 5	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 6	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 7	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 8	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 9	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 10	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 11	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 12	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 13	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 14	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 15	+	+	+	+	+	+	+	-	+	-	-	-	-	-	-
Test 16	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 17	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 18	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Test 19	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-

Test 20	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Positive detection rate	100%			100%			95%			0%			0%		

7. Conclusions

7.1 Saliva sample

(1) According to the results in Table 1, the limit of detection of the saliva sample was initially determined to be $1\times10^{3.2}$ $TCID_{50}$/mL.

(2) According to the results in Table 2 to Table 10, the limit of detection of saliva sample was determined to be $2.5\times10^{2.2}$ $TCID_{50}$/mL.

Sample Stability

1. Basic information

Basic information				
Test kit	Lot	Date of manufacture	Expiry date	Specification
Novel Coronavirus 2019-nCOV Antigen Test (Colloidal Gold)	W20200401	20200413	20211013	40 T/kit
Manufacturer		Beijing Hotgen Biotech Co., Ltd.		
Product code	HGCG134A0140		IFU version	V. 2020-09.01[Eng.]
Experiment Site	Academy of Military Medical Sciences		Operator	Pan, Yunyun
Date of Initiation and completion		2020.4.13~2020.4.15		
Study protocol				
Saliva sample	Samples		10 positive samples (P1-P10), 10 negative samples (N1-N10)	
	Storage condition and Test interval		2~8℃, 0/8/16/24/30 hours	

2.Sample sources and basic information

2.1 Sample sources

The samples used in this experiment were prepared by researchers from the Academy of Military Medical Sciences using virus culture solution and negative saliva samples. The numbers of 10 positive saliva samples prepared are (P1-P10), and the numbers of 10 negative saliva samples prepared are (N1-N10).

2.2 Sample preparation process

(1) Collect the saliva samples of 10 healthy people respectively, and use the Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probling) produced by Daan Gene Co., Ltd. Of Yat-sen University hereinafter referred to as "Nucleic Acid Testing", the registration number on the registration certificate: certified 20203400749, nucleic acid instrument model ABI7500) for testing. The test results are shown in Table 1.

Table 1 Test Results of saliva Samples of 10 Healthy People

saliva Samples

Sample No.	Instrumental value (Ct)	Result
N1	Not detected	-
N2	Not detected	-
N3	Not detected	-
N4	Not detected	-
N5	Not detected	-
N6	Not detected	-
N7	Not detected	-
N8	Not detected	-
N9	Not detected	-
N10	Not detected	-

(2) Add the virus culture with a concentration of $1*10^6$ $TCID_{50}$/mL to saliva samples identified in Table 1 above respectively for dilution to obtain positive saliva samples with different concentrations (for low concentration positive samples, their concentration is 2~3×LOD, where the LOD is $2.5×10^{2.2}$ $TCID_{50}$/mL), and use the nucleic acid detection reagent for testing. The test results are shown in Table 2.

Table 2 Test Results of Virus Culture of 10 saliva samples

Nasal swabs			
Sample No.	Final concentration of virus culture ($TCID_{50}$/mL)	Instrumental value (Ct)	Results
P1	$7.13×10^{2.2}$	30.47	+
P2	$6.50×10^{2.2}$	33.06	+
P3	$5.25×10^{2.2}$	34.35	+
P4	$5.75×10^{2.2}$	34.12	+
P5	$5.50×10^{2.2}$	34.27	+
P6	$6.25×10^{2.2}$	33.45	+
P7	$6.75×10^{2.2}$	32.90	+
P8	$6.15×10^{2.2}$	33.57	+
P9	$5.55×10^{2.2}$	34.06	+
P10	$6.75×10^{2.2}$	32.92	+

2. Experiment protocol

The sample type applicable to this kit is saliva sample, and the sample transport medium is not involved. This sample stability experiment does not involve the transportation of samples. Each sample type contains 10 low concentration positive samples (2~3×LoD) and 10 negative samples. A lot of novel coronavirus (2019-nCoV) antigen detection kits (colloidal gold method) are used to study the stability of saliva samples. In the experiment, each sample type shall contain 10 low concentration

positive samples (2~3×LoD) and 10 negative samples.

3. Study on stability of samples stored at 2~8℃

Select 10 saliva samples respectively for extraction with the sample extract, store them at 2~8℃ respectively, and perform a test at 0h, 8h, 16h, 24h and 30h after storage.

4.Acceptable criteria

The saliva samples are extracted with the sample extract and stored at 2~8℃ for different periods of time. If the test result is consistent with the negative and positive background of the samples, it means that the samples are stable during the different periods of time when they are stored at 2~8℃. Therefore, you can choose the time for the samples to be stored at 2~8℃.

5.Test results

5.1Study on stability of samples stored at 2~8℃

Sample No.	Test results of saliva Sample				
	0h	8h	16h	24h	30h
P1	+	+	+	+	+
P2	+	+	+	+	+
P3	+	+	+	+	+
P4	+	+	+	+	+
P5	+	+	+	+	+
P6	+	+	+	+	+
P7	+	+	+	+	+
P8	+	+	+	+	+
P9	+	+	+	+	+
P10	+	+	+	+	+
N1	−	−	−	−	−
N2	−	−	−	−	−
N3	−	−	−	−	−
N4	−	−	−	−	−
N5	−	−	−	−	−
N6	−	−	−	−	−
N7	−	−	−	−	−
N8	−	−	−	−	−
N9	−	−	−	−	−
N10	−	−	−	−	−

The test results shown in the above table show that among the test results of the saliva samples extracted with the sample extract and stored at 2~8℃ for 30h, the test results of the positive samples are all positive, and the test results of negative samples are all negative, consistent with the background results of the samples. Therefore, it is indicated that the saliva samples shall be kept at 2~8℃ for no more than 24h after being extracted with the sample extract.

6. Conclusion

According to the results of the sample stability experiment, the following conclusions are drawn: saliva samples are extracted with sample extract and stored at 2~8℃ for no more than 24h.

Matrix Equivalence Study

1. Basic information

Basic information				
Test kit	Lot	Date of manufacture	Expiry date	Specification
Novel coronavirus (2019-nCOV) antigen test (colloidal gold)	W20200401	20200413	20211013	40 T/kit
Manufacturer		Beijing Hotgen Biotech Co., Ltd.		
Product code	HGCG134A0140		IFU version	V.2020-09.01[Eng.]
Experiment Sites	Laboratories at Hotgen Biotech		Operator	Pan, Yunyun; Wu, Liping
Date of Initiation and completion	2020.06.05			
Study protocol				
Nasal swabs	Samples		Four positive samples One negative sample	
Throat swabs	Samples		Four positive samples One negative sample	
Saliva samples	Samples		Four positive samples One negative sample	

2. Sample sources and information

2.1 Sample sources

Virus cultures were sourced from the Academy of Military Medical Science of the PLA, Beijing.

2.2 Sample preparation

2.2.1 Preparation of negative samples

Collect specimens of nasal swabs ,throat swabs and saliva samples from healthy people according to the sample collection method in the instructions. As tested by the Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) produced by Daan Gene Co., Ltd. Of Yat-sen University, the registration number on the registration certificate: certified 20203400749, nucleic acid instrument model ABI7500). Test results are negative.

2.2.2 Preparation of low concentration samples

Add virus culture with a concentration of 1×10^{6} TCID50/ml to the negative nasal swabs ,throat swabs and saliva samples, to obtain a final concentration of $6\times10^{2.2}$ TCID50/ml of the virus culture. Test the specimens using the Detection Kit for 2019

Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) produced by Daan Gene Co., Ltd. Of Yat-sen University, the registration number on the registration certificate: certified 20203400749, nucleic acid instrument model ABI7500), and test results are positive. The Ct value is 32.29, 32.17 and 32.45 respectively .

2.2.3 Preparation of medium to high concentration samples

(1) Add virus culture with a concentration of 1×10^{6} TCID50/ml to the negative nasal swabs ,throat swabs and saliva samples to obtain a final concentration of $1.25\times10^{3.2}$ TCID50/ml of the virus culture. As tested by the Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) produced by Daan Gene Co., Ltd. Of Yat-sen University, the registration number on the registration certificate: certified 20203400749, nucleic acid instrument model ABI7500), the test result of the specimen is positive. The Ct value is 28.14, 28.39 and 28.05 respectively .

(2) Add the virus culture with a concentration of 1×10^{6} TCID50/ml to the negative nasal swabs ,throat swabs and saliva samples to obtain a final concentration of $1.5\times10^{3.2}$ TCID50/ml of the virus culture. As tested by Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) produced by Daan Gene Co., Ltd. Of Yat-sen University, the registration number on the registration certificate: certified 20203400749, nucleic acid instrument model ABI7500), the test result of the specimen is positive. The Ct value is 27.87, 27.24 and 27.19 respectively.

(3) Add the virus culture with a concentration of 1×10^{6} TCID50/ml to the negative nasal swabs ,throat swabs and saliva samples to obtain a final concentration of $1.75\times10^{3.2}$ TCID50/ml of the virus culture. As tested by the Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) produced by Daan Gene Co., Ltd. Of Yat-sen University, the registration number on the registration certificate: certified 20203400749, nucleic acid instrument model ABI7500), test results of the specimen are positive. The Ct value is 27.39, 26.37 and 26.35 respectively.

3. Experimental protocol

Test 3 types of sample matrix (nasal swabs ,throat swabs and saliva samples). For

each sample type, prepare 5 concentrations: (1) one negative sample, (2) one low concentration sample, (3) three medium to high concentration samples. Each sample is tested 3 times.

To ensure blind detection, one person is responsible for sample preparation and the other person is responsible for sample testing.

4. Acceptable criteria

After virus cultures at the same concentration level are added to different types of sample matrix (nasal swabs ,throat swabs and saliva samples), the test results are consistent.

5. Test results

Table 1 Test results of samples with 5 concentration levels in 3 sample matrix

Sample No.	Sample Concentration levels	Nasal swabs			Throat swabs			Saliva samples		
1	Negative	-	-	-	-	-	-	-	-	-
2	Low	+	+	+	+	+	+	+	+	+
3	Medium to high	+	+	+	+	+	+	+	+	+
4	Medium to high	+	+	+	+	+	+	+	+	+
5	Medium to high	+	+	+	+	+	+	+	+	+

6. Conclusions

The test results for the nasal swab matrix ,throat swab matrix and saliva matrix after adding virus cultures at the same concentration are consistent.

Interference Study

1. Basic information

Basic information				
Test kit	Lot	Date of manufacture	Expiry date	Specification
Novel Coronavirus 2019-nCOV Antigen Test (Colloidal Gold)	W20200401	20200413	20211013	40 T/kit
Manufacturer		Beijing Hotgen Biotech Co., Ltd		
Product code	HGCG134A0140		IFU version	V. 2020-09.01[Eng.]
Experiment Site	the Academy of Military Medical Science of the PLA, Beijing.		Operator	PanYunyun
Date of Initiation and completion	2020.3.12~2020.3.15			
Study protocol				
Saliva samples	Negative saliva samples (N1-N28), low concentration saliva sample (P1-P28).			

2.Sample sources and basic information

2.1 Sample sources

The samples used in this experiment were prepared by researchers from the Academy of Military Sciences using virus culture fluid and negative saliva samples . Among them, 28 negative saliva samples were numbered N1-N28, and 28 weakly positive saliva samples were numbered P1-P28.

2.2 Sample preparation process

2.2.1 Preparation of negative samples

(1) Collect multiple clinical negative saliva samples, use sample lysate to prepare 28 negative saliva samples. Confirm they are negative samples by testing them using the Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) produced by Daan Gene Co., Ltd. Of Yat-sen University, the registration number on the registration certificate: certified 20203400749, nucleic acid instrument model ABI7500), and the testing results are shown in Table 1.

Table 1 Test results of negative saliva samples

Sample No.	Result	Sample No.	Result
N1	-	N15	-
N2	-	N16	-
N3	-	N17	-
N4	-	N18	-
N5	-	N19	-
N6	-	N20	-
N7	-	N21	-
N8	-	N22	-
N9	-	N23	-
N10	-	N24	-
N11	-	N25	-
N12	-	N26	-
N13	-	N27	-
N14	-	N28	-

2.2.2 Preparation of low-concentration samples

The virus culture at a concentration of 1×10^6 TCID50/mL was added to negative saliva samples and diluted to obtain 28 low-concentration saliva samples with different concentrations (the sample concentration level is 2~3×LoD, where the LoD is 2.5×10^{22} TCID50/mL). Samples were tested using detection kit for nucleic acid testing, and the detection results are shown in Table 2.

Table 2 Test results of saliva virus culture

Sample No.	Final concentration of virus culture （TCID50/mL）	Results	Sample No.	Final concentration of virus culture （TCID50/mL）	Results
P1	7.14×10^{22}	+	P15	6.88×10^{22}	+
P2	6.34×10^{22}	+	P16	6.70×10^{22}	+
P3	6.56×10^{22}	+	P17	6.62×10^{22}	+
P4	6.19×10^{22}	+	P18	5.89×10^{22}	+
P5	5.02×10^{22}	+	P19	7.16×10^{22}	+
P6	7.19×10^{22}	+	P20	7.11×10^{22}	+
P7	6.60×10^{22}	+	P21	6.11×10^{22}	+
P8	5.17×10^{22}	+	P22	5.38×10^{22}	+
P9	6.59×10^{22}	+	P23	5.45×10^{22}	+
P10	5.89×10^{22}	+	P24	5.35×10^{22}	+
P11	6.80×10^{22}	+	P25	6.44×10^{22}	+
P12	5.44×10^{22}	+	P26	5.93×10^{22}	+
P13	7.35×10^{22}	+	P27	6.31×10^{22}	+
P14	7.14×10^{22}	+	P28	6.88×10^{22}	+

3. Experimental protocol

3.1 Research on endogenous interfering substances

3.1.1 Study on adding endogenous interfering substances to negative throat swab samples

Two samples of negative saliva sample (N1~N2) that were not added with endogenous interferents were divided into two, one of which was used as the control test; the other was added with a certain concentration of HAMA, Whole Blood (human), EDTA anticoagulated and used as the experimental test. Then three lots of Novel Coronavirus 2019-nCoV Antigen Test kits (Colloidal Gold) were used to detect the samples of the experimental group and the control group. Each sample was repeated three times and the test results were recorded.

3.1.2 Research on adding endogenous interfering substances to low-concentration samples

Two samples of low-concentration saliva samples (P1~P2) that were not added with endogenous interferents were divided into two, one of which was used as the control test; the other was added with a certain concentration of HAMA (800 ng/mL) and blood (EDTA anticoagulation, 10% (w/w)) as the experimental test. Then three lots of Novel Coronavirus 2019-nCoV Antibody Test kits (Colloidal Gold) were used to detect the samples of the experimental group and the control group, and each sample was repeated three times and the test results were recorded.

Table 3 Different concentrations of endogenous interfering substances

Interfering substances	Test conc.
Human anti-mouse antibody, HAMA	800 ng/mL
Whole Blood (human), EDTA anticoagulated	10% (w/w)

3.2 Research on exogenous interfering substances

3.2.1 Study on the addition of exogenous interfering substances to negative saliva samples

Select 26 negative saliva samples (N3~N28) that were not added with endogenous interfering substances and divide into two, one of which was used as the control test; the other was added with a certain concentration of exogenous interfering substances (see Table 4) to be used as the experimental group. Then three lots of Novel

Coronavirus 2019-nCoV Antigen Test kits (Colloidal Gold) were used to detect the samples of the experimental group and the control group, and each sample was repeated three times and the test results were recorded.

3.2.2 Study on the addition of exogenous interfering substances to low-concentration saliva samples

Select 26 low-concentration saliva samples (N3~N28) that were not added with endogenous interfering substances and divide into two, one of which was used as the control test; the other was added with a certain concentration of exogenous interfering substances (see Table 4) to be used as the experimental group. Then three lots of Novel Coronavirus 2019-nCoV Antigen Test kits (Colloidal Gold) were used to detect the samples of the experimental group and the control group, and each sample was repeated three times and the test results were recorded.

Table 4 Different concentrations of exogenous interfering substances

No.	Exogenous factor	Interfering substances	Test conc.
1	Nasal sprays or drops	Phenylephrine	128μg/mL
2		Oxymetazoline	128μg/mL
3		Saline Nasal Spray 10%	10%(v/v)
5	Nasal corticosteroids	Dexamethasone	2μg/mL
6		Flunisolide	0.2μg/mL
7		Triamcinolone acetonide	0.2μg/mL
8		Mometasone	0.5μg/mL
9	Throat lozenges	Strepsils (flurbiprofen 8.75mg)	5% (w/v, 50mg/mL)
10		Thoat candy (mint)	5% (w/v, 50mg/mL)
11	Oral anaesthetic	Anbesol (Benzocaine 20%)	5% (v/v)
12	Anti-viral drugs	α-Interferon-2b	0.01μg/mL
13		Zanamivir (Influenza)	2μg/mL
14		Ribavirin (HCV)	0.2μg/mL
15		Oseltamivir (Influenza)	2μg/mL
16		Peramivir(Influenza)	60μg/mL
17		Lopinavir(HIV)	80μg/mL
18		Ritonavir(HIV)	20μg/mL
19		Arbidol((Influenza)	40μg/mL
20	Antibiotic	Levofloxacin Tablets	40μg/mL
21		Azithromycin	200μg/mL
22		Ceftriaxone	800μg/mL
23		Meropenem	100μg/mL
24	Antibacterial, systemic	Tobramycin	128μg/mL

25	Other	Mucin: bovine submaxillary gland, type I-S	100 μg/mL
26		Biotin	100 μg/mL

4 Acceptable criteria

4.1 Research on endogenous interfering substances

HAMA (800 ng/mL) and blood (EDTA anticoagulation, 10% (W/w)) are considered to have no effect on the test results of this kit if the following criteria are met: In the endogenous interfering substance research experiment, test results of negative saliva sample for the control group and the experimental group were all negative; The test results of low-concentration saliva samples for the control group and the experimental group were all positive.

4.2 Research on exogenous interfering substances

The exogenous interfering substances of certain concentrations described in Table 3 are considered to have no effect on the test results if the following criteria are met: In the endogenous interfering substance research experiment, test results of negative saliva sample for the control group and the experimental group were all negative; The test results of low-concentration saliva samples for the control group and the experimental group were all positive.

5. Test Results

5.1 Test results of study on endogenous interfering substances

Table 5 Test results of negative saliva samples without endogenous interfering substances (control group)

Sample No.	Lot.	Test results of negative samples		
		1	2	3
N1-1	W20200401	—	—	—
	W20200402	—	—	—
	W20200403	—	—	—
N2-1	W20200401	—	—	—
	W20200402	—	—	—
	W20200403	—	—	—

Table 6 Test results of different endogenous interfering substances added to negative saliva samples (experimental group)

Sample No.	Endogenous interfering substances	Concentration	Lot.	Test results of negative samples		
				1	2	3

N1-2	HAMA	800ng/mL	W20200401	—	—	—
			W20200402	—	—	—
			W20200403	—	—	—
N2-2	Whole Blood (human), EDTA anticoagulated	10%(w/w)	W20200401	—	—	—
			W20200402	—	—	—
			W20200403	—	—	—

Table 7 Test results of low-concentration saliva samples without endogenous interfering substances (control group)

Sample No.	Lot.	Test results of low-concentration samples		
		1	2	3
P1-1	W20200401	+	+	+
	W20200402	+	+	+
	W20200403	+	+	+
P2-1	W20200401	+	+	+
	W20200402	+	+	+
	W20200403	+	+	+

Table 8 Test results of different endogenous interfering substances added to low-concentration saliva samples (experimental group)

Sample No.	Endogenous interfering substances	Concentration levels	Lot.	Test results of negative samples		
				1	2	3
P1-2	HAMA	800ng/mL	W20200401	+	+	+
			W20200402	+	+	+
			W20200403	+	+	+
P2-2	Whole Blood (human), EDTA anticoagulated	10%(w/w)	W20200401	+	+	+
			W20200402	+	+	+
			W20200403	+	+	+

5.2 Test results of study on endogenous interfering substances

Table 9 Test results of negative saliva samples without endogenous interfering substances (control group)

Sample No.	Lot.	Test results of negative samples			Sample No.	Lot.	Test results of negative samples		
		1	2	3			1	2	3
N3-1	W20200401	—	—	—	N16-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N4-1	W20200401	—	—	—	N17-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N5-1	W20200401	—	—	—	N18-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N6-1	W20200401	—	—	—	N19-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N7-1	W20200401	—	—	—	N20-1	W20200401	—	—	—

	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N8-1	W20200401	—	—	—	N21-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N9-1	W20200401	—	—	—	N22-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N10-1	W20200401	—	—	—	N23-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N11-1	W20200401	—	—	—	N24-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N12-1	W20200401	—	—	—	N25-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N13-1	W20200401	—	—	—	N26-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N14-1	W20200401	—	—	—	N27-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N15-1	W20200401	—	—	—	N28-1	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—

Table 7.2 Test results of different exogenous interfering substances added to negative saliva samples (experimental group)

Sample No.	Lot.	Test results of negative samples			Sample No.	Lot.	Test results of negative samples		
		1	2	3			1	2	3
N3-2	W20200401	—	—	—	N16-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N4-2	W20200401	—	—	—	N17-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N5-2	W20200401	—	—	—	N18-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N6-2	W20200401	—	—	—	N19-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N7-2	W20200401	—	—	—	N20-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N8-2	W20200401	—	—	—	N21-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—

	W20200403	—	—	—		W20200403	—	—	—
N9-2	W20200401	—	—	—	N22-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N10-2	W20200401	—	—	—	N23-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N11-2	W20200401	—	—	—	N24-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N12-2	W20200401	—	—	—	N25-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N13-2	W20200401	—	—	—	N26-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N14-2	W20200401	—	—	—	N27-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—
N15-2	W20200401	—	—	—	N28-2	W20200401	—	—	—
	W20200402	—	—	—		W20200402	—	—	—
	W20200403	—	—	—		W20200403	—	—	—

Table 8.1 Test results of low-concentration saliva samples without exogenous interfering substances (control group)

Sample No.	Lot.	Test results of low-concentration samples			Sample No.	Lot.	Test results of low-concentration samples		
		1	2	3			1	2	3
P3-1	W20200401	+	+	+	P16-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P4-1	W20200401	+	+	+	P17-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P5-1	W20200401	+	+	+	P18-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P6-1	W20200401	+	+	+	P19-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P7-1	W20200401	+	+	+	P20-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P8-1	W20200401	+	+	+	P21-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P9-1	W20200401	+	+	+	P22-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P10-1	W20200401	+	+	+	P23-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+

	W20200403	+	+	+		W20200403	+	+	+
P11-1	W20200401	+	+	+	P24-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P12-1	W20200401	+	+	+	P25-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P13-1	W20200401	+	+	+	P26-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P14-1	W20200401	+	+	+	P27-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P15-1	W20200401	+	+	+	P28-1	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+

Table 8.2 Test results of different exogenous interfering substances added to low-concentration saliva samples (experimental group)

Sample No.	Lot.	Test results of low-concentration samples			Sample No.	Lot.	Test results of low-concentration samples		
		1	2	3			1	2	3
P3-2	W20200401	+	+	+	P16-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P4-2	W20200401	+	+	+	P17-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P5-2	W20200401	+	+	+	P18-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P6-2	W20200401	+	+	+	P19-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P7-2	W20200401	+	+	+	P20-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P8-2	W20200401	+	+	+	P21-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P9-2	W20200401	+	+	+	P22-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P10-2	W20200401	+	+	+	P23-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P11-2	W20200401	+	+	+	P24-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P12-2	W20200401	+	+	+	P25-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P13-2	W20200401	+	+	+	P26-2	W20200401	+	+	+

	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P14-2	W20200401	+	+	+	P27-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+
P15-2	W20200401	+	+	+	P28-2	W20200401	+	+	+
	W20200402	+	+	+		W20200402	+	+	+
	W20200403	+	+	+		W20200403	+	+	+

6 .Conclusion

Through the above experiments, it is proved that the endogenous interfering substances and exogenous interfering substances studied have no influence on the detection results of this kit.

Cross-reaction Study

1. Basic information

Basic information				
Test kit	Lot	Date of manufacture	Expiry date	Specification
Novel Coronavirus 2019-nCOV) Antigen Test (Colloidal Gold)	W20200401 W20200402 W20200403	20200413 20200414 20200415	20211013 20211014 20211015	40 T/Kit
Manufacturer		Beijing Hotgen Biotech Co., Ltd		
Product code	HGCG134A0140		IFU version	V. 2020-09.01[Eng.]
Experiment Site	Laboratories at Beijing Hotgen Biotech Co., Ltd		Operator	Pan Yunyun; Wu Liping
Date of Initiation and completion	2020.06.05			
Study protocol				
Saliva samples			Four positive samples One negative sample	

2. Sample sources and information

2.1 Sample sources

Virus cultures were sourced from the Academy of Military Medical Science of the PLA, Beijing.

3. Experimental protocol

(1) Prepare the cross-reaction samples listed below:

1) Virus sample: add the virus culture to the negative saliva matrix and dilute to a concentration of 2×10^5 $TCID_{50}$/mL;

2) Bacterial sample: add the bacterial culture to the negative saliva matrix and dilute to a concentration of 5×10^6 CFU/mL.

3) Pneumocystis jirovecii (PJP): Collect saliva samples that are positive for Pneumocystis jirovecii for testing.

4) Pooled human nasal wash: Collect nasal washes from multiple people, combine and mix them, and use them directly for detection.

(2) Use Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold) kits that are from three lots to test the above cross-reactive samples, and repeat the test 3 times for each sample.

4. Acceptable criteria

Use this kit to test samples containing the following interfering substances, and the test results are all negative.

5. Test results

Table 5.1 Test results of cross-substance samples

No.	Crossing reacting substance	Strain	Concentration of cross reacting substance	Test result 1	Test result 2	Test result 3
1	Human Coronavirus	HKU1	2×10^5 $TCID_{50}$/mL	-	-	-
2		229E	2×10^5 $TCID_{50}$/mL	-	-	-
3		OC43	2×10^5 $TCID_{50}$/mL	-	-	-
4		NL63	2×10^5 $TCID_{50}$/mL	-	-	-
5		SARS	2×10^5 $TCID_{50}$/mL	-	-	-
6		MERS	2×10^5 $TCID_{50}$/mL	-	-	-
7	Adenovirus	Type 1	2×10^5 $TCID_{50}$/mL	-	-	-
8		Type 2	2×10^5 $TCID_{50}$/mL	-	-	-
9		Type 3	2×10^5 $TCID_{50}$/mL	-	-	-
10		Type 4	2×10^5 $TCID_{50}$/mL	-	-	-
11		Type 5	2×10^5 $TCID_{50}$/mL	-	-	-
12		Type 7	2×10^5 $TCID_{50}$/mL	-	-	-
13		Type 55	2×10^5 $TCID_{50}$/mL	-	-	-
16	Human Metapneumovirus (hMPV)	hMPV 3 Type B1 / Peru2-2002	2×10^5 $TCID_{50}$/mL	-	-	-
17		hMPV 16 Type A1 / IA10-2003	2×10^5 $TCID_{50}$/mL	-	-	-
18	Parainfluenza virus	Type 1	2×10^5 $TCID_{50}$/mL	-	-	-
19		Type 2	2×10^5 $TCID_{50}$/mL	-	-	-
20		Type 3	2×10^5 $TCID_{50}$/mL	-	-	-
21		Type 4A	2×10^5 $TCID_{50}$/mL	-	-	-
22	Influenza A	H1N1	2×10^5 $TCID_{50}$/mL	-	-	-
23		H3N2	2×10^5 $TCID_{50}$/mL	-	-	-
24		H5N1	2×10^5 $TCID_{50}$/mL	-	-	-
25		H7N9	2×10^5 $TCID_{50}$/mL	-	-	-
27	Influenza B	Yamagata	2×10^5 $TCID_{50}$/mL	-	-	-
28		Victoria	2×10^5 $TCID_{50}$/mL	-	-	-
30	Enterovirus	Type 68	2×10^5 $TCID_{50}$/mL	-	-	-
31		09/2014 isolate 4	2×10^5 $TCID_{50}$/mL	-	-	-
32	Respiratory syncytial virus	Type A	2×10^5 $TCID_{50}$/mL	-	-	-
33		Type B	2×10^5 $TCID_{50}$/mL	-	-	-
34	Rhinovirus	A16	2×10^5 $TCID_{50}$/mL	-	-	-
35		Type B42	2×10^5 $TCID_{50}$/mL	-	-	-

36	Chlamydia pneumoniae	TWAR strain TW-183	5×10^6 CFU/mL	-	-	-
37	Haemophilus influenzae	NCTC 4560	5×10^6 CFU/mL	-	-	-
38	Legionella pneumophila	Bloomington-2	5×10^6 CFU/mL	-	-	-
39		Los Angeles-1	5×10^6 CFU/mL	-	-	-
40		82A3105	5×10^6 CFU/mL	-	-	-
41	Mycobacterium tuberculosis	K	5×10^6 CFU/mL	-	-	-
42		Erdman	5×10^6 CFU/mL	-	-	-
43		HN878	5×10^6 CFU/mL	-	-	-
44		CDC1551	5×10^6 CFU/mL	-	-	-
45		H37Rv	5×10^6 CFU/mL	-	-	-
46	Streptococcus pneumonia	4752-98 [Maryland (D1)6B-17]	5×10^6 CFU/mL	-	-	-
47		178 [Poland 23F-16]	5×10^6 CFU/mL	-	-	-
48		262 [CIP 104340]	5×10^6 CFU/mL	-	-	-
49		Slovakia 14-10 [29055]	5×10^6 CFU/mL	-	-	-
50	Streptococcus pyrogens	Typing strain T1 [NCIB 11841, SF 130]	5×10^6 CFU/mL	-	-	-
51	Bordetela pertussis	NCCP 13671	5×10^6 CFU/mL	-	-	-
52	Mycoplasma pneumoniae	Mutant 22	5×10^6 CFU/mL	-	-	-
53		FH strain of Eaton Agent [NCTC 10119]	5×10^6 CFU/mL	-	-	-
54		M129-B7	5×10^6 CFU/mL	-	-	-
55	Pneumocystis jirovecii (PJP)	N/A	N/A	-	-	-
56	Pooled human nasal wash	N/A	N/A	-	-	-
57	Candida albicans	3147	5×10^6 CFU/mL	-	-	-
58	Pseudomonas aeruginosa	R. Hugh 813	5×10^6 CFU/mL	-	-	-
59	Staphylococcus epidermidis	FDA strain PCI 1200	5×10^6 CFU/mL	-	-	-
60	Streptococcus salivarius	S21B [IFO 13956]	5×10^6 CFU/mL	-	-	-

6. Conclusions:

The above-mentioned interfering substances have no cross-interference with this kit.

Novel Coronavirus 2019-nCOVAntigen Test (Colloidal Gold)

High-dose Hook Effect Study

1. Basic information

Basic information				
Test kit	Lot	Date of manufacture	Expiry date	Specification
Novel Coronavirus 2019-nCOV antigen test (Colloidal Gold)	W20200401 W20200402 W20200403	20200413 20200414 20200415	20211013 20211014 20211015	40 T/ kit
Manufacturer		Beijing Hotgen Biotech Co., Ltd		
Experiment Site	Institute of Microbiology and Epidemiology of the Academy of Military Medical Science of the PLA, Beijing.		Operator	Zhao Yong
Date of Initiation and completion		2020.04.20~2020.04.25		
Study protocol				
Saliva sample	Samples		Extraction of the novel coronavirus cultures	
	Test method		50% tissue culture infectious dose method (TCID50)	

2. Sample source and basic information

2.1 Sample source

This experiment commissioned the Institute of Microbiology and Epidemiology of the Academy of Military Medical Sciences to cultivate the novel coronavirus and extract the virus culture

3. Experimental protocol

The Novel Coronavirus 2019-nCoV Antigen Test kit is used to qualitatively detect the novel coronavirus antigen in human saliva samples in vitro. The high-concentration sample selected in this experiment is $1\times10^{6.2}$ $TCID_{50}$/mL virus culture. After the high-concentration sample is serially diluted, Novel Coronavirus 2019-nCoV Antigen

Test kits from three lots are used to detect virus cultures of different concentrations. Each concentration is tested three times.

4. Acceptance criteria

In this experiment, the detection result can be judged according to the color changes. The concentration at which the color of the color band begins to decrease as the concentration increases is taken as the lowest concentration when the hook effect occurs.

5. Test results

The test results are shown in Table 1.

Table 1 Test results of virus cultures at different concentrations

Lot	W20200401	W20200402	W20200403
Virus concentration（$TCID_{50}$/mL）	Test result	Test result	Test result
$1\times10^{3\ 2}$	+	+	+
	+	+	+
	+	+	+
$5\times10^{3\ 2}$	+	+	+
	+	+	+
	+	+	+
$1\times10^{4\ 2}$	++	++	++
	++	++	++
	++	++	++
$1\times10^{5\ 2}$	+++	+++	+++
	+++	+++	+++
	+++	+++	+++
$1\times10^{6\ 2}$	+++	+++	+++
	+++	+++	+++
	+++	+++	+++

6. Conclusions

The above experimental results show that using three lots of test kits to test virus samples with different concentration gradients, the test results are all positive. As the virus concentration increases, the color signal continues to increase, and there is no color decrease. Therefore, no hook effects occur when using this kit to detects a high-titer level ($1\times10^{6.2}$ $TCID_{50}$/mL) of novel coronavirus antigen-positive samples.

Hotgen

Declaration of Conformity

Manufacturer: Beijing Hotgen Biotech Co.,Ltd.
9th building, No.9 Tianfu Street, Biomedical Base,
Daxing District, Beijing,102600, P.R.China

European Representative: [redacted]

Product Name: Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold)

Analyte: Protein Antigen of 2019-nCoV

Brand name: Hotgen Biotech, CORA CHECK-19

Model	Trade name	Contains
Model A	Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold)	1. Novel Coronavirus Antigen Test Cassette 2. Sample extraction buffer 3. Disposable virus sampling swab
Model B	Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold)	1. Novel Coronavirus Antigen Test Cassette 2. Sample extraction buffer 3. Disposable virus sampling swab 4. Novel coronavirus 2019-nCoV Antigen Quality Control
Model C	Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold) - Saliva	1. Novel Coronavirus Antigen Test Cassette 2. Sample extraction buffer 3. Saliva collector 4. Biohazard specimen bag
Model D	Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold) - Saliva	1. Novel Coronavirus Antigen Test Cassette 2. Sample extraction buffer 3. Saliva collector 4. Biohazard specimen bag 5. Novel coronavirus 2019-nCoV Antigen Quality Control

Classification : **Others of ANNEX II of IVDD**

Conformity Assessment Route: **Annex III**

We, Beijing Hotgen Biotech Co., Ltd., herewith declare on our solo responsiblity that the above mentioned products meet the transposition into national law, the provisions of the following EC Council Directives and Standards. All supporting documentations are retained under the premises of the manufacturer.

General applicable directives:

In Vitro Diagnostic Medical Devices DIRECTIVE 98/79/EC

Harmonized standards:

EN ISO 13485:2016;
EN ISO 15223-1:2016;
EN ISO 14971:2012;
EN 13975:2003;
EN ISO 18113-1:2011;

Hotgen

EN ISO 18113-2:2011;
EN 13612:2002;
EN ISO 17511:2003;
EN ISO 23640:2015;
EN 13641:2002;
EN 13975:2003;
EN 62366:2008.

Signature:
Name: Lin Changqing
Title: General manager

Place: Beijing, China.
Date of Issue: January 11, 2021

Novel Coronavirus 2019-nCoV Antigen Test

(Colloidal Gold)

Clinical Study Report

Subject Product: Novel Coronavirus 2019-nCoV Antigen Test

(Colloidal Gold)

Test start time: Oct 9th, 2020

Test completion time: Dec 21th, 2020

Model specifications: 40T/kit

Submitted by: Beijing Hotgen Biotech Co., Ltd.

Contact: Ruifeng Xiao

Telephone number: 010-50973600-3627

Beijing Hotgen Biotech Co., Ltd.

CONTENTS

1. Background of the Clinical Study

Coronaviruses are positive-sense single-stranded RNA viruses, with four genera of α, β, γ, and δ. The novel coronavirus is a new type of coronavirus discovered in Wuhan viral pneumonia cases in 2019. On January 12, 2020, the World Health Organization named the virus as 2019-nCoV, which belongs to the β genus. The S protein of 2019-nCoV is located on the viral surface to form a rod-like structure, and it is one of the main antigen proteins of the virus. The S gene is also the main gene for coronavirus typing. The 2019-nCoV can cause viral pneumonia, with main clinical manifestations of fever, fatigue, and respiratory symptoms such as dry cough. Some patients gradually develop breathing difficulties, and in severe cases, acute respiratory distress syndrome, septic shock, irreversible metabolic acidosis, and coagulopathy may occur.

2.Intended Clinical Use and Principle of subject product

This kit is used for in vitro qualitative determination of novel coronavirus antigen in human saliva. It is used as rapid investigation for suspected cases of novel coronavirus, can also be used as a reconfirmation method for nucleic acid detection in discharged cases.

This kit is based on the Colloidal gold immunochromatographic technology, and uses double antibody sandwich method to detect the novel coronavirus antigen in human saliva. The detection line (T line) of the novel coronavirus antigen test cassette was coated with novel coronavirus antibody, and the quality control line (C line) was coated with sheep anti-mouse. During the test, the sample is dropped into the test cassette and the liquid is chromatographed upward under the capillary effect. The novel coronavirus antigen in the sample first binds to the Colloidal gold-labelled novel coronavirus antibody to form a solid phase novel coronavirus antibody-novel coronavirus antigen-labelled novel coronavirus antibody-Colloidal gold complex at the T line position, and form a solid phase sheep anti-mouse-labelled novel coronavirus antibody- Colloidal gold complex was formed at the C line position. After the test is completed, observe the Colloidal gold color reaction of T line and C line to determine results of novel coronavirus antigen in human saliva.

3 Purpose of the Clinical Study

The purpose of this clinical study was to investigate the clinical performance of "Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold)" produced by Beijing Hotgen Biotech Co., Ltd. to detect novel coronavirus (2019-nCoV) antigen in human saliva specimens.

4 Overall Study protocol

4.1 Establishment of clinical trial protocol

The clinical trial protocol was formulated by the applicant in consultation with the clinical trial institution before the clinical trial, and according to the clinical trial protocol, the responsibilities of the applicant, the researcher, and the person in charge of statistics were clearly defined. The applicant organization organizes participation in the trial. All researchers were to be trained in clinical trial protocols and use of in vitro diagnostic reagents for testing.

4.2 Study method introduction

The clinical specimens used in the trial were prospectively taken from the valid specimens (human saliva) from clinical trial institutions. Patients were sequentially enrolled and tested blindly.All collected specimens can be traced back to the corresponding clinical information, including case number, age, gender, type of specimens, collection time, confirmation or exclusion of the novel coronavirus infection, and the nucleic acid test results for disease diagnosis (include the name of nucleic acid detection kit).

The subject product of this study is "Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold)" (*hereinafter referred to as "Antigen Test"*) produced by Beijing Hotgen Biotech Co., Ltd. The product selected for the comparison is RT-PCR Kit.

Results of the Antigen Test and Nucleic Acid Test are compared to evaluate the consistency between the Antigen Test and Nucleic Acid Test. Cases with different test results were comprehensively analyzed by combining the patients' epidemiological background, clinical symptoms, disease outcome, and other information. In this way, the clinical performance of the Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold) (produced by Beijing Hotgen Biotech Co., Ltd) to detect the novel coronavirus (2019-nCoV) antigen in human saliva specimens was evaluated.

4.3 Investigators

The investigators participating in the clinic study were 3 principal investigators, 3 investigators in charge of statistics, 6 operators used to run the assay and several participants used to assist the study.

4.4 Quality control

(1) Select and manage samples strictly in accordance with the requirements of research samples, and samples that do not meet the requirements during the experiment should be excluded;

(2) The clinical trial testing process was strictly performed in accordance with the requirements of the kit instructions;

(3) truthfully, detailed, timely, and carefully record all content to ensure that the content of the test record form is complete, true, reliable, and traceable;

(4) Invalid results due to the kit or other reasons should be re-tested;

(5) When the retesting of the sample occurs due to human error operation, instrument failure, and sample addition failure, the retesting result shall prevail, and the reason for retesting shall be indicated.

5 Clinical Trial Procedures

5.1 Case screening and enrollment

The case enrollment was based on the clinical diagnostic information provided by the clinical trial institutions/centers. All enrolled cases meet the trial requirements for clinical information and specimens.

5.2 Testing of specimens

Specimens of enrolled cases were blindly tested using the Antigen Test and Nucleic Acid Test according to kit instructions, and the results were recorded.

5.3 Statistical analysis of test results

Generate a data table with the information of the case specimen, the corresponding test results of the Antigen Test and Nucleic Acid Test results of the same case at the same period, the confirmation/exclusion results of COVID-19, the disease processes, and the clinical severity of the disease,etc. After verification, the clinical trial database of the project is established.

6. Clinical Trial Specimens

6.1 Specimen types

There is a sample type in this trial: human saliva specimens.

6.2 Collection, processing and storage of specimens

Specimens collection, processing, storage should meet the Instructions for Use of the subject product and the comparator method.

Here are the requirements for subject products.

6.2.1 Collection and treatment of specimens

Saliva samples must be collected through clean and dry saliva collectors.

Unscrew the lid of the sampling tube containing the sample extraction solution, install the saliva collector, collect saliva through the saliva collector to the position of the graduation mark. Remove the saliva collector and screw the lid of the sampling tube, and mix thoroughly.

6.2.2 Storage of specimens

The sample should be used as soon as possible after collection, and cannot be stored for a long time at room temperature. If it cannot be sent for inspection in time, saliva samples can be stored for 24 hours at 2℃~8℃.

6.3 Entry Criteria of Specimens

6.3.1 Inclusion criteria

(1) The total number of enrolled cases is no less than 100, of which no less than 30 positive COVID-19 cases and no less than 30 negative cases.

(2) The enrolled cases should cover a certain number of recovered cases, suspected cases and try to cover patients with various respiratory infectious diseases. The enrolled cases should cover patients with different clinical severity (such as mild, moderate, severe, and critical patients), as well as patients with different disease stages (such as early, middle, and middle-late stage patients).

(3) The specimen meets the requirements for specimen collection, processing and storage.

(4) The relevant information of the specimen is complete, including the case number, age, gender, type of species, collection time, the confirmation or exclusion of the novel coronavirus infection, etc., and the nucleic acid testing results used for the diagnosis of the disease (including nucleic acid test kit name).

6.3.2 Exclusion criteria of specimens

Cases that do not meet the inclusion criteria, such as

(1) Specimens type does not meet the test requirements;

(2) Does not meet the requirements for collection, processing and storage;

(3) Cases with incomplete clinical information;

(4) Specimens whose quantity does not meet the requirements for testing.

6.3.3 Removal criteria of specimens

(1) Specimens deteriorated;

(2) Specimens that do not meet the entry criteria, or meet the criteria for exclusion but are still tested.

(3)Re-tested specimens due to operational error, instrument failure, and/or sample addition failure. If a retest occurs, remove the earlier result and record the retest result (reasons for a retest should be indicated).

7 Blind Design

The study was designed as a blind study. In the trial, one special researcher de-identifies the test specimens and then hand them over to another test operator for testing, so that the testing operator is completely unclear about the background information of the specimens. After loading the samples and the testing finished, a special data collator merges the background information of the specimens with the test results of the investigational kit to ensure the objectivity of the trial and reduce the bias caused by the expectations of the researchers who are clear about the background of the specimens.

8 Statistical and Analytical Plans

8.1 Data collection

1) Establish a database in Excel, and enter the traceable information of all specimens, background clinical diagnosis, epidemiological data, onset/visit time, sampling time, and diagnosis/exclusion results, etc.

2) Check the data. In principle, no data shall be deleted. Any dropouts shall be explained and recorded. The final statistical data shall be locked and backed up.

8.2 Data statistics

Summarize and compare the Antigen Test and Nucleic Acid Test results in a crosstab (Table 1.), and evaluate the positive consistency rate (sensitivity), negative consistency rate (specificity), and other indicators of Antigen Test and Nucleic Acid Test results. All inconsistent results shall be fully analyzed based on the confirmation/exclusion results, patient's epidemiological background, clinical symptoms, disease outcome and other information.

Table 1. Statistics of Antigen Test and Nucleic Acid Test Results

		Nucleic Acid Test results		Total
		Positive (+)	Negative (-)	
Antigen testing	Positive (+)	A	B	A+B
	Negative (-)	C	D	C+D
Total		A+C	B+D	A+B+C+D

Notes: If there are specimens results of the same case in different periods in the above evaluation, any positive Antigen Test result should be taken into analysis. The same analysis method should apply to the statistics of Nucleic Acid Test results.

(1) Calculation of positive consistency rate (sensitivity), negative consistency rate (specificity) and overall consistency rate (accuracy)

Positive consistency rate (sensitivity) =A/(A+C)×100.00% (95% confidence interval)

Negative consistency rate (specificity) =D/(B+D)×100.00% (95% confidence interval)

Overall consistency rate (accuracy) =(A+D)/(A+B+C+D)×100.00% (95% confidence interval)

The 95% confidence interval is directly calculated using statistical software MedCalc v19.0.7.

(2) Kappa agreement analysis

Calculate the Kappa value of the Antigen Test and Nucleic Acid Test results by the following formula, compare the Kappa value grading in Table 2. to evaluate the consistency of the Antigen Test and Nucleic Acid Test results.

Kappa (K)=[N(A+D)--(R1C1+R2C2)] /[N^2--(R1C1+R2C2)]

Table 2. Consistency Judgment

No.	Kappa Value	Consistency Grading
1	<0	Very poor
2	0~0.2	Poor
3	0.21~0.40	Fair
4	0.41~0.60	Good
5	0.61~0.80	Very good
6	0.81~1.00	Excellent

9. Clinical Trial Results and Analysis

This clinical trial was led by Beijing Hotgen Biotech Co., Ltd., with specimens from 3 clinical trial institutions. The confirmed patient specimens from each clinical trial institution have traceable disease onset dates and Nucleic Acid Test results.

9.1 Composition and number of trial specimens

This clinical trial enrolled a total of 582 clinical cases, including 457 nucleic acid positive cases and 125 nucleic acid negative cases. A total of 582 human saliva speci mens were tested in this trial. The distribution of enrolled cases and numbers in each c linical trial institution are as follows:

Table 4. The distribution of enrolled cases

Sample Collection and Testing sites	PCR Negative	PCR Positive	Total
CPL	153	41	194
SPH	147	39	186
AMM	157	45	202
Total	457	125	582

In addition, the enrolled cases cover recovered cases, suspected cases, and multiple respiratory infections, as well as patients with different severity of disease (such as mild, common, severe, and critical COVID-19 patients), as well as patients with different disease processes (such as early, middle, middle-late stage patients).

The enrolled population covers children, adults, and the elderly, and cover males and females evenly.

9.2 Statistical analysis of test results

This trial enrolled a total of 582 human saliva specimens, of which 125 were positives for Nucleic Acid Test, 457 negatives for Nucleic Acid Test; As for data collection of the corresponding Nucleic Acid Test results.

Summarize the Antigen Test and Nucleic Acid Test results (see Table 5.), and evaluate the positive consistency rate, negative consistency rate, and overall consistency rate of Antigen Test and Nucleic Acid Test.

Table 5. Statistics of Antigen Test and Nucleic Acid Test Results
(human saliva specimens)

		Nucleic Acid Test results		Total
		Positive (+)	Negative (-)	
Antigen Test	Positive (+)	120	1	121
	Negative (-)	5	456	461
Total		125	457	582

The sensitivity, specificity, overall consistency rate, and Kappa value are calculated as follows:

Statistics	Ratio	Percentage (95% confidence interval)
Positive consistency rate (sensitivity)	120/125	96.00% (90.91%～98.69%)
Negative consistency rate (specificity)	456/457	99.78% (98.79%～99.99%)
Overall consistency rate (accuracy)	576/582	98.97% (97.77%～99.62%)
Kappa value	0.9691, excellent agreement	

The above statistical results show that results between Antigen Test and Nucleic Acid Test (human saliva specimens) are highly consistent. 5 human saliva specimens that were positive for the Nucleic Acid Test were negative for the Antigen Test. The disagreement may because that viral load was below the lower detection limit of the Antigen Test and resulted in a false negative.

10.Discussion and Conclusions

10.1 Clinical trial implementation centers

This clinical trial was conducted by Beijing Hotgen Biotech Co., Ltd., with specimens from 3 clinical trial institutions.

10.2 Amounts of specimens in the trial

582 human saliva specimens were tested in this trial, 125 nucleic positive and 457 negatives for nucleic acid test. The enrollment cases cover discharged cases, suspected cases, and cases with other respiratory infections. The enrollment cases cover different severities of disease (i.e. mild, moderate, severe, and critical), different disease stages (i.e. early, middle, middle-late stages), and also cover different ages (children, adults, and elders).

10.3 Analysis of test results

Statistical analysis of the results of the Antigen Test of human saliva specimens and the results of Nucleic Acid Test, positive consistency rate (sensitivity), negative consistency rate (specificity): ,overall consistency rate (accuracy),Kappa value were:96.00%, 99.78%, and 98.97%; Kappa（K）=0.9691.

In summary, using the Antigen Test kit, the Novel Coronavirus 2019-nCoV Antigen Test (Colloidal Gold) produced by Beijing Hotgen Biotech Co., Ltd. to detect human saliva specimens, the results showed excellent agreement with clinical diagnosis results and the Nucleic Acid Test results. The comparison test results of human saliva specimens are highly consistent. Therefore, the Antigen Test kit has good clinical performance.