

Cross-reactivity Study Report

1. Purpose

To evaluate potential cross-reactivity and interference of other viruses that may cause symptoms similar with SARS-CoV-2 infection, to other organisms that may cause infectious diseases, as well as to other conditions that may result in atypical immune system activity.

2. Scope

The .Coronavirus Ag rapid saliva test device produced by Innovation Biotech (Beijing) Co., Ltd

3. Materials and Equipment

- (1) The ..Coronavirus Ag rapid saliva test device (Lot: 20201010).
- (2) The inactivated SARS-CoV-2 virus was acquired from Vero E6 cell culture, which titers is 6×10^5 TCID50/ml and the strains is BetaCoV/Wuhan/IPBCAMS-WH-01/2019;
- (3) The negative saliva swabs samples were collected from healthy person following the product instruction.
- (4) The negative matrix samples were acquired by mixture of the negative saliva swabs samples, use for dilute the inactivated SARS-CoV-2 virus.
- (5) Various types of virus and bacterial cultures come from the Chinese Academy of Military Medical Sciences, and the colony count, TCID50 determination and other methods are abused to confirm the concentration of the bacterial solution, detailed information listed in Table 1.

Table 1: Information of Virus/Bacteria/Parasite

No.	Virus/Bacteria/Parasite	Strain	Source/Specimen type	Concentration
1	SARS-coronavirus	N/A	SINO/recombinant protein	25ug/mL
2	MERS-coronavirus	N/A	SINO/recombinant protein	72 ug/mL
3	Adenovirus	Type 1	AMMS / Inactivated culturevirus	1.5E+06TCID50/mL
4		Type 3	AMMS / Inactivated culture virus	7.5E+06TCID50/mL
5		Type 5	AMMS / Inactivated culture virus	4.5E+06TCID50/mL
6		Type 7	AMMS / Inactivated culture virus	1.0E+06TCID50/mL
7		Type 8	AMMS / Inactivated culture virus	1.0E+06TCID50/mL
8		Type 11	AMMS / Inactivated culture virus	2.5E+06TCID50/mL
9		Type 18	AMMS / Inactivated culture virus	2.5E+06TCID50/mL
10		Type 23	AMMS / Inactivated culture virus	6.0E+06TCID50/mL
11		Type 55	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
12		Influenza A	H1N1 Denver	AMMS / Inactivated culture virus
13	H1N1 WS/33		AMMS / Inactivated culture virus	2.0E+08TCID50/mL
14	H1N1 A/Mal/302/54		AMMS / Inactivated culture virus	1.5E+08TCID50/mL
15	H1N1 New Caledonia		AMMS / Inactivated culture virus	7.6E+08TCID50/mL
16	H3N2A/Hong Kong/8/68		AMMS / Inactivated culture virus	4.6E+08TCID50/mL
17	Influenza B	Nevada/03/2011	AMMS / Inactivated culture virus	1.5E+08TCID50/mL
18		B/Lee/40	AMMS / Inactivated culture virus	8.5E+08TCID50/mL

19		B/Taiwan/2/62	AMMS / Inactivated culture virus	4.0E+08TCID50/mL
20	Respiratory syncytial virus	N/A	AMMS / Inactivated culture virus	2.5E+06TCID50/mL
21	Legionella pneumophila	Bloomington-2	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
22		Los Angeles-1	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
23		82A3105	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
24	Mycobacterium tuberculosis	K	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
25		Erdman		1 × 10 ⁵ PFU/mL
26		HN878		1 × 10 ⁵ PFU/mL
27		CDC1551		1 × 10 ⁵ PFU/mL
28		H37Rv		1 × 10 ⁵ PFU/mL
29	Streptococcus pneumonia	4752-98 [Maryland (D1)6B-17]	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
30		178 [Poland 23F-16]	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
31		262 [CIP 104340]	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
32		Slovakia 14-10 [29055]	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
33	Streptococcus pyogens	Typing strain T1[NCIB 11841, SF 130]	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
34	Mycoplasma pneumoniae	Mutant 22	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
35		FH strain of E aton Agent [NCTC10119]	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
36		36M129-B7	AMMS / Inactivated culture virus	1 × 10 ⁵ PFU/mL
37	Coronavirus	229E	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
38		OC43	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
39		NL63	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
40		HKU1	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
41	Human etapneumovirus (hMPV) 3 Type B1	Peru2-2002	AMMS / Inactivated culturvirus	1.5E+06TCID50/mL
42	Human Metapneumovirus (hMPV) 16 Type A1	IA10-2003	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
43	Parainfluenza virus	Type 1	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
44		Type 2	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
45		Type 3	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
46		Type 4A	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
47	RhinoVIRUS A16	N/A	AMMS / Inactivated culture virus	1.5E+06TCID50/mL
48	SARS-CoV-2	BetaCoV/Wuhan/IPB CAMS-WH-01/2019	WIOV / Inactivated culture virus	6E+05TCID50/mL

Note: 1. AMMS: Academy of Military Medical Sciences; 2. WIOV: Wuhan Institute of Virology

4. Procedure

4.1 Samples Preparation

4.1.1 Negative Samples Preparation

- (1) The negative matrix samples was acquired by mixture of the negative saliva swabs samples, use for dilute the Virus/Bacteria/Parasite listed in Table1.
- (2) For No.1 listed in Table1: 100ul SARS-coronavirus (25ug/ml) mix with 400ul negative matrix samples to the final concentration is 5ug/ml.
- (3) For No.2 listed in Table1: 250ul MERS-coronavirus (72ug/ml) mix with 250ul negative matrix samples to the final concentration is 36ug/ml.
- (4) For No.3-No.47 listed in Table1: Adenovirus, Influenza A, Influenza B, Respiratory syncytial virus, Legionella pneumophila, Mycobacterium tuberculosis, Streptococcus pneumonia, Streptococcus pyrogens, Mycoplasma pneumonia, Coronavirus, Human etapneumovirus (hMPV) 3 Type B1, 16Type A1, Parainfluenza virus, RhinoVIRUS A16, take respectively 50ul the above Virus/Bacteria/Parasite mix with 450ul negative matrix samples to the 1/10 dilution.

4.1.2 Positive Samples Preparation

- (1) The positive matrix samples acquired from dilute the SARS-CoV-2 (No.48 listed in Table1) into the negative matrix samples in 1:3000 dilution, the final concentration of SARS-CoV-2 in positive matrix samples is $3 \times \text{LoD}$, define the $3 \times \text{LoD}$ samples as the positive matrix samples.
- (2) For No.1 listed in Table 1: 100ul SARS-coronavirus (25ug/ml) mix with 400ul $3 \times \text{LoD}$ samples to the final concentration is 5ug/ml.
- (3) For No.2 listed in Table 1: 250ul MERS-coronavirus (72ug/ml) mix with 250ul $3 \times \text{LoD}$ samples to the final concentration is 36ug/ml.
- (4) For No.3-No.47 listed in Table 1: Adenovirus, Influenza A, Influenza B, Respiratory syncytial virus, Legionella pneumophila, Mycobacterium tuberculosis, Streptococcus pneumonia, Streptococcus pyrogens, Mycoplasma pneumonia, Coronavirus, Human etapneumovirus (hMPV) 3 Type B1, 16Type A1, Parainfluenza virus, RhinoVIRUS A16: Take respectively 50ul the above Virus/Bacteria/Parasite mix with 450ul $3 \times \text{LoD}$ samples to the 1/10 dilution.

4.2 Test procedure

Use the . .Coronavirus Ag rapid saliva test device test the prepared samples. Each sample test 3 times.

5. Acceptance Criteria

The test results of negative samples of the . .Coronavirus Ag rapid saliva test device should be negative result, the test results of positive samples of the . .Coronavirus Ag rapid saliva test device should be positive result.

6. Results

The . .Coronavirus Ag rapid saliva test device test results were summarized in Table 2.

Table 2: The Test Result for Cross-reactivity

No.	Virus/Bacteria /Parasite	Strain	Concentration	Results					
				Positive Sample			Negative Sample		
				1	2	3	1	2	3

1	SARS-coronavirus	N/A	5 ug/ml	4	4	4	-	-	-
2	MERS-coronavirus	N/A	36 ug/mL	4	4	4	-	-	-
3	Adenovirus	Type 1	1.5E+05TCID50/mL	4	4	4	-	-	-
4		Type 3	7.5E+05TCID50/mL	4	4	4	-	-	-
5		Type 5	4.5E+05TCID50/mL	4	4	4	-	-	-
6		Type 7	1.0E+05TCID50/mL	4	4	4	-	-	-
7		Type 8	1.0E+05TCID50/mL	4	4	4	-	-	-
8		Type 11	2.5E+05TCID50/mL	4	4	4	-	-	-
9		Type 18	2.5E+05TCID50/mL	4	4	4	-	-	-
10		Type 23	6.0E+05TCID50/mL	4	4	4	-	-	-
11		Type 55	1.5E+05TCID50/mL	4	4	4	-	-	-
12	Influenza A	H1N1 Denver	3.0E+07TCID50/mL	4	4	4	-	-	-
13		H1N1 WS/33	2.0E+07TCID50/mL	4	4	4	-	-	-
14		H1N1 A/Mal/302/54	1.5E+07TCID50/mL	4	4	4	-	-	-
15		H1N1 New Caledonia	7.6E+07TCID50/mL	4	4	4	-	-	-
16		H3N2A/Hong Kong/8/68	4.6E+07TCID50/mL	4	4	4	-	-	-
17	Influenza B	Nevada/03/2011	1.5E+07TCID50/mL	4	4	4	-	-	-
18		B/Lee/40	8.5E+07TCID50/mL	4	4	4	-	-	-
19		B/Taiwan/2/62	4.0E+07TCID50/mL	4	4	4	-	-	-
20	Respiratory syncytial virus	N/A	2.5E+05TCID50/mL	4	4	4	-	-	-
21	Legionella pneumophila	Bloomington-2	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
22		Los Angeles-1	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
23		82A3105	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
24	Mycobacterium tuberculosis	K	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
25		Erdman	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
26		HN878	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
27		CDC1551	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
28		H37Rv	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
29	Streptococcus pneumonia	4752-98 [Maryland (D1)6B-17]	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
30		178 [Poland 23F-16]	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
31		262 [CIP 104340]	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
32		Slovakia 14-10 [29055]	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-

33	Streptococcus pyrogens	Typing strain T1[NCIB 11841, SF 130]	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
34	Mycoplasma pneumoniae	Mutant 22	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
35		FH strain of Eaton Agent [NCTC10119]	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
36		36M129-B7	1 × 10 ⁵ PFU/mL	4	4	4	-	-	-
37	Coronavirus	229E	1.5E+05TCID50/mL	4	4	4	-	-	-
38		OC43	1.5E+05TCID50/mL	4	4	4	-	-	-
39		NL63	1.5E+05TCID50/mL	4	4	4	-	-	-
40		HKU1	1.5E+05TCID50/mL	4	4	4	-	-	-
41	Human metapneumovirus (hMPV) 3 Type B1	Peru2-2002	1.5E+05TCID50/mL	4	4	4	-	-	-
42	Human Metapneumovirus (hMPV) 16 Type A1	IA10-2003	1.5E+05TCID50/mL	4	4	4	-	-	-
43	Parainfluenza virus	Type 1	1.5E+05TCID50/mL	4	4	4	-	-	-
44		Type 2	1.5E+05TCID50/mL	4	4	4	-	-	-
45		Type 3	1.5E+05TCID50/mL	4	4	4	-	-	-
46		Type 4A	1.5E+05TCID50/mL	4	4	4	-	-	-
47	RhinoVIRUS A16	N/A	1.5E+05TCID50/mL	4	4	4	-	-	-

Note: “-” indicate negative; “Number” indicate positive, the bigger the number, the darker the color.

The results show that Virus/Bacteria/Parasite listed in Table 1 has no inference effect on the negative and positive test results, and these substances do not cross-react with . .Coronavirus Ag rapid saliva test device.

7. Conclusion

The substances which listed in the following table have no cross-reaction with the . .Coronavirus Ag rapid saliva test device.

No.	Virus/Bacteria/Parasite	Strain	Concentration
1	SARS-coronavirus	N/A	5 ug/ml
2	MERS-coronavirus	N/A	36 ug/mL
3	Adenovirus	Type 1	1.5E+05TCID50/mL
4		Type 3	7.5E+05TCID50/mL
5		Type 5	4.5E+05TCID50/mL
6		Type 7	1.0E+05TCID50/mL
7		Type 8	1.0E+05TCID50/mL
8		Type 11	2.5E+05TCID50/mL

9		Type 18	2.5E+05TCID50/mL
10		Type 23	6.0E+05TCID50/mL
11		Type 55	1.5E+05TCID50/mL
12	Influenza A	H1N1 Denver	3.0E+07TCID50/mL
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26		HN878	1 × 10 ⁵ PFU/mL
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28		H37Rv	1 × 10 ⁵ PFU/mL
29	Streptococcus pneumonia	4752-98 [Maryland (D1)6B-17]	1 × 10 ⁵ PFU/mL
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43	Parainfluenza virus	Type 1	1.5E+05TCID50/mL
44		Type 2	1.5E+05TCID50/mL
45		Type 3	1.5E+05TCID50/mL
46		Type 4A	1.5E+05TCID50/mL
47	RhinoVIRUS A16	N/A	1.5E+05TCID50/mL