

## Cross-reactivity and Specificity Test Report

### 1. Objective

To test the analytical specificity and interference of the V-CHEK 2019-nCoV Ag Saliva Rapid Test Card (Immunochromatography).

### 2. Reagents and Materials

1) The following three batches of V-CHEK 2019-nCoV Ag Saliva Rapid Test Card (Immunochromatography) were used.

LOT No.: 05820010C,

LOT No.: 05820011C,

LOT No.: 05820012C.

2) Microbial cross-interference reaction substances:

For all materials from BEI, individual COA shows the detailed information about how the cultures for each microorganism were collected, prepared, and stored, and how the TCID<sub>50</sub>/mL was determined for each microorganism.

N7 *Mycoplasma pneumoniae* is provided by Guangzhou Institute of Respiratory Diseases. It was cultured in ATCC® Medium 2611: *Spiroplasma* medium under 37 °C. It was measured by vigor testing to be 1.2\*10<sup>7</sup> CFU / mL.

N11 *Streptococcus pneumoniae* is provided by Guangzhou Institute of Respiratory Diseases. It was cultured in ATCC® Medium 260: Trypticase soy agar/broth with defibrinated sheep blood under 37 °C. It was measured by vigor testing to be 2.4\*10<sup>7</sup> CFU / mL.

N12 *Chlamydia pneumoniae* is provided by Guangzhou Institute of Respiratory Diseases. It was cultured in Hep-2 cell line under 37 °C. It was measured by vigor testing to be 5\*10<sup>6</sup> CFU / mL.

N14 *Bordetella pertussis* is provided by Guangzhou Institute of Respiratory Diseases. It was isolated from the nasopharyngeal swab of a pertussis patient and cultured with nutrient gravy agar under 37 °C. It was measured by vigor testing to be 2\*10<sup>6</sup> CFU / mL.

N15 *Staphylococcus aureus* is provided by Beijing Baiou Bowei Biotechnology Co., Ltd. It was cultured in nutrient agar medium under 37 °C. It was measured by vigor testing to be 7.8\*10<sup>8</sup> CFU / mL.

N23 *Haemophilus influenzae* is provided by Guangzhou Institute of Respiratory Diseases. It was cultured in ATCC® Medium 814: GC Agar/Broth Medium under 37 °C. It was measured by vigor testing to be 4.3\*10<sup>7</sup> CFU / mL.

N24 *Streptococcus pyogenes* is provided by Guangzhou Institute of Respiratory Diseases. It was cultured in defibrinated sheep blood (blood agar plate) medium under 37 °C with 5% CO<sub>2</sub>. It was measured by vigor testing to be 1.9\*10<sup>7</sup> CFU / mL.

N25      *Candida albicans* is provided by Guangzhou Institute of Respiratory Diseases. It was cultured in YM Agar Medium under 35 °C. It was measured by vigor testing to be  $5.2 \times 10^7$  CFU / mL.

SARS-CoV-2 virus culture is from BEI Resource, catalog NR-52286. NR-52286 is a preparation of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020 that has been inactivated by heating to 65°C for 30 minutes. The material was supplied frozen at a concentration  $1.6 \times 10^5$  TCID<sub>50</sub>/ml, of Pre-Inactivation Titer by TCID<sub>50</sub> Assay in Vero E6 Cells (7 days at 37°C and 5% CO<sub>2</sub>) according to BEI Resource. Desired testing solution is mixture of NR-52286 with base solution accordingly the description below.

### 3. Test Methods

Place the kits and microbial cross-interference reaction substances to room temperature. Open the pouch, take out the test kits and place horizontally. Drop appropriate volume of specimens on the sample pad and read the test results within 15 minutes.

### 4. Test Results

Prepare base solution: Select 100 healthy people and collect saliva as if they were COVID 19 patients, in a container.

#### 4.1 Cross-Reactivity Study

Prepare specimen as a sample according to Table 1, without any SARS-CoV-2 virus culture. All samples were tested three times, and the results were recorded, and the data were analyzed. The sample numbers (N1 to N29), specimen source, concentration are listed in Table 1. The detailed volume used to prepare the final testing solution is listed in line data file, Cross-reactivity section. No cross-reactivity is observed.

Table 1: Specimen for cross-reactivity table

	Specimen type	Strain	Source	Concentration	Specimen Supplier, part #
N1	Adenovirus 10	J.J.	Seed Virus	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-51435
N2	Parainfluenza Virus 2	Type 2	Seed Virus	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-3229
N3	Influenza B Virus	B/Brisbane/60/2008 (Victoria Lineage)	Pooled allantoic fluid	1*10 <sup>5</sup> CEID <sub>50</sub> /mL	BEI NR-42005
N4	Influenza B Virus	B/Texas/06/2011 (Yamagata Lineage)	Pooled allantoic fluid	1*10 <sup>5</sup> CEID <sub>50</sub> /mL	BEI NR-44024
N5	Influenza A Virus	A/Brownsville/39H/2009 (H1N1)pdm09	Cell Isolate	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-20346
N6	Influenza A Virus	A/Aichi/2/1968 (H3N2)	Seed Virus	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-3177
N7	Mycoplasma pneumoniae	M129	Isolated	1*10 <sup>6</sup> CFU / mL	Guangzhou Institute of Respiratory Diseases
N8	Human Metapneumovirus (hMPV)	TN/96-213	Cell lysate and supernatant	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-22243
N9	Enterovirus D68	US/IL/14-18952	Cell lysate and supernatant	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-49131
N10	Respiratory syncytial virus	B1	Cell lysate and supernatant	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-4052
N11	Streptococcus pneumoniae	SPEC1	Bacterial culture	1*10 <sup>6</sup> CFU / mL	Guangzhou Institute of Respiratory Diseases
N12	Chlamydia pneumoniae	AR-39	Isolate	1*10 <sup>6</sup> CFU / mL	Guangzhou Institute of Respiratory Diseases
N13	Rhinovirus 35	164A	Seed Virus	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-51452
N14	Bordetella pertussis	H973	Isolated	1*10 <sup>6</sup> CFU / mL	Guangzhou Institute of Respiratory Diseases
N15	Staphylococcus aureus	VCU006	Isolated	1*10 <sup>6</sup> CFU / mL	Beijing Baiou Bowei Biotechnology Co., Ltd.bio-52742
N16	Human coronavirus	OC43	Isolated	8.9*10 <sup>4</sup> TCID <sub>50</sub> /mL	BEI NR-52725
N17	Human Coronavirus	229E	Isolated	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-52726
N18	Negative Nasal Matrix			N/A	N/A
N19	Human coronavirus	NL63	Isolated	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-470
N20	Parainfluenza virus 1	HPIV1/FRA/2922/1106/2009	Isolated	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-48680
N21	Parainfluenza virus 3	NIH 47885	Isolated	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-3233
N22	Parainfluenza virus 4	4B, 19503	Isolated	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-3238
N23	Haemophilus influenzae	Type B	Oligosaccharide-Human Serum Albumin Conjugate (HbO-HA Antigen)	1*10 <sup>6</sup> CFU / mL	Guangzhou Institute of Respiratory Diseases
N24	Streptococcus pyogenes	ABC020061659	Isolated	1*10 <sup>6</sup> CFU / mL	Guangzhou Institute of Respiratory Diseases
N25	Candida albicans	CAI4-F2-Neut5L-NAT1-mCherry-GFP	Culture	1*10 <sup>6</sup> CFU / mL	Guangzhou Institute of Respiratory Diseases
N26	SARS-coronavirus	Urbani strain	Gamma-Irradiated	1*10 <sup>6</sup> PFU / mL	BEI NR-9548
N27	MERS-coronavirus	EMC/2012	Cell Lysate	1*10 <sup>5</sup> TCID <sub>50</sub> /mL	BEI NR-50549
N28	Human coronavirus	HKU1	Recombinant	0.5 mg/ml	BEI NR-53713
N29	Mycobacterium tuberculosis	HN878	Culture Filtrate Proteins	0.581 mg/ml	BEI NR-14827

The V-CHEK 2019-nCoV Ag Saliva Rapid Test Card (Immunochromatography) tested negative in the above substances. No cross reactivity was observed.

#### **4.2 Interference Study**

Add the SARS-CoV-2 virus culture to the microorganisms to obtain the final concentration of  $3 \times \text{LoD}$  ( $3.0 \times 10^2$  TCID<sub>50</sub> / mL) and test these samples according to the instructions. Each sample was tested 3 times. The sample numbers (R1 to R28) specimen source, concentration are listed in Table 2. The detailed volume used to prepare the final testing solution is listed in line data file, Cross-reactivity section.

Table 2 Specimen for interference table

	Specimen type	Strain	Source	Cross reactant final concentration (same as in table 1)
R1	Adenovirus 10	J.J.	Seed Virus	$1 \times 10^5$ TCID <sub>50</sub> /mL
R2	Parainfluenza Virus 2	Type 2	Seed Virus	$1 \times 10^5$ TCID <sub>50</sub> /mL
R3	Influenza B Virus	B/Brisbane/60/2008 (Victoria Lineage)	Pooled allantoic fluid	$1 \times 10^5$ CEID <sub>50</sub> /mL
R4	Influenza B Virus	B/Texas/06/2011 (Yamagata Lineage)	Pooled allantoic fluid	$1 \times 10^5$ CEID <sub>50</sub> /mL
R5	Influenza A Virus	A/Brownsville/39H/2009 (H1N1)pdm09	Cell Isolate	$1 \times 10^5$ TCID <sub>50</sub> /mL
R6	Influenza A Virus	A/Aichi/2/1968 (H3N2)	Seed Virus	$1 \times 10^5$ TCID <sub>50</sub> /mL
R7	Mycoplasma pneumoniae	M129	Isolated	$1 \times 10^6$ CFU / mL
R8	Human Metapneumovirus (hMPV)	TN/96-213	Cell lysate and supernatant	$1 \times 10^5$ TCID <sub>50</sub> /mL
R9	Enterovirus D68	US/IL/14-18952	Cell lysate and supernatant	$1 \times 10^5$ TCID <sub>50</sub> /mL
R10	Respiratory syncytial virus	B1	Cell lysate and supernatant	$1 \times 10^5$ TCID <sub>50</sub> /mL
R11	Streptococcus pneumoniae	SPEC1	Bacterial culture	$1 \times 10^6$ CFU / mL
R12	Chlamydia pneumoniae	AR-39	Isolate	$1 \times 10^6$ CFU / mL
R13	Rhinovirus 35	164A	Seed Virus	$1 \times 10^5$ TCID <sub>50</sub> /mL
R14	Bordetella pertussis	H973	Isolated	$1 \times 10^6$ CFU / mL
R15	Staphylococcus aureus	VCU006	Isolated	$1 \times 10^6$ CFU / mL
R16	Human coronavirus	OC43	Isolated	$8.9 \times 10^4$ TCID <sub>50</sub> /mL
R17	Human Coronavirus	229E	Isolated	$1 \times 10^5$ TCID <sub>50</sub> /mL
R18	Human coronavirus	NL63	Isolated	$1 \times 10^5$ TCID <sub>50</sub> /mL
R19	Parainfluenza virus 1	HPIV1/FRA/29221106/2009	Isolated	$1 \times 10^5$ TCID <sub>50</sub> /mL
R20	Parainfluenza virus 3	NIH 47885	Isolated	$1 \times 10^5$ TCID <sub>50</sub> /mL
R21	Parainfluenza virus 4	4B, 19503	Isolated	$1 \times 10^5$ TCID <sub>50</sub> /mL
R22	Haemophilus influenzae	Type B	Oligosaccharide-Human Serum Albumin Conjugate (HbO-HA Antigen)	$1 \times 10^6$ CFU / mL
R23	Streptococcus pyogenes	ABC020061659	Isolated	$1 \times 10^6$ CFU / mL
R24	Candida albicans	CAI4-F2-Neut5L-NAT1-mCherry-GFP	Culture	$1 \times 10^6$ CFU / mL
R25	SARS-coronavirus	Urbani strain	Gamma-Irradiated	$1 \times 10^6$ PFU / mL
R26	MERS-coronavirus	EMC/2012	Cell Lysate	$1 \times 10^5$ TCID <sub>50</sub> /mL
R27	Human coronavirus	HKU1	Recombinant	0.5 mg/ml
R28	Mycobacterium tuberculosis	HN878	Culture Filtrate Proteins	0.581 mg/ml

### 4.3 Testing result

For a number of microorganism(s), the stock concentration is lower than the recommended

testing concentration. In these cases, it was only possible to test these microorganisms at the stock concentration.

Detailed data are shown in in line data file, Cross-reactivity section. Result from samples N1 to N29 are all negative from the three lots of kits. Result from samples R1 to R28 are all positive from the three batches of kits.

Samples N28 and N29 only showed protein concentration from COA and it was decided to test 50% of the initial concentration as 1mg/mL is a relative high concentration for protein.

In addition, the comparison between SARS-CoV-2 nucleocapsid protein and human coronavirus HKU1 revealed that the only potential for homology is with the HKU1 nucleocapsid phosphoprotein. Homology is relatively low, at 36.7% across 82% of sequences, cross-reactivity cannot be ruled out theoretically but not cross-reactivity observed experimentally. Furthermore, no protein sequence homology was found between SARS-CoV-2 and M. tuberculosis, therefore, homology-based cross-reactivity can be ruled out theoretically in the first place.

## 5 Experimental conclusion

The cross reactivity experiment and interference study were carried out with the three batches of kits. According to the results, the V-CHEK 2019-nCoV Ag Saliva Rapid Test Cards (Immunochromatography) were used and there are no false negatives and false positives in specimens that coexist with other microorganisms. Therefore, the above microorganisms have no cross reactivity or interference from the antigen test kits.

  
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