

## **Analytical Specificity – Interference Studies**

Product Name: Rapid SARS-CoV-2 Antigen Test Card

Catalog No.: 1N40C5

File No.	RR1N40018
Date	2020.06.24-2021.01.25
Drafted by / Date	Mengjuan Wu 2021.01.26
Reviewed by / Date	Zhijuan Jia 2021.01.27
Approved by / Date	Haolong Shen 2021.01.27

Xiamen Boson Biotech Co., Ltd.

## Table of Contents

1.	Purpose .....	1
2.	References .....	1
3.	Personnel and Responsibility .....	1
4.	Evaluated Reagents .....	1
5.	Microorganism Interference Studies .....	1
6.	Endogenous and Exogenous Interference Studies .....	6

## 1. Purpose

To analyze the effect of common microorganisms, exogenous and endogenous interfering substances on test results and evaluate the product's anti-interference performance.

## 2. References

	Document No.	Document
1	BS EN 13612:2002	Performance evaluation of in vitro diagnostic medical devices

## 3. Personnel and Responsibility

Name	Position	Education	Responsibility
Haolong Shen	Management Representative	B.S.	Approval of study report
Zhijuan Jia	R&D Manager	M.S.	Review of study report
Kesai Liu	R&D Engineer	M.S.	Study implementation, recording, analysis of results, and report drafting
Mengjuan Wu	R&D Vice Manager	M.S.	Study implementation, recording, analysis of results, and report drafting

## 4. Evaluated Reagents

Rapid SARS-CoV-2 Antigen Test Card (1N40C5)		
	Lot Number	Manufacturer
1	H20061502	Xiamen Boson Biotech Co., Ltd.

## 5. Microorganism Interference Studies

### 5.1. Materials

	Name	Lot No. (Catalog No.)	Notes
1	SARS-CoV-2 viral culture	NR-52281 (USA-WA1/2020)	ZeptoMetrix Corporation
2	Human coronavirus 229E	R20051235	ZeptoMetrix Corporation
3	Human coronavirus OC43	R20051233	ZeptoMetrix Corporation
4	Human coronavirus NL63	R20051234	ZeptoMetrix Corporation
5	Parainfluenza virus 1	R20051715	ZeptoMetrix Corporation
6	Parainfluenza virus 2	R20051716	ZeptoMetrix Corporation
7	Parainfluenza virus 3	R20051717	ZeptoMetrix Corporation
8	Enterovirus EV71	R20051718	ATCC
9	Respiratory syncytial virus	R20051612	ZeptoMetrix Corporation
10	Rhinovirus	R20051714	ATCC
11	Influenza A virus (H1N1)	R19101301	National Institute for the Control of Pharmaceutical and Biological Products (NICPBP)
12	Influenza A virus (H3N2)	R19101302	NICPBP
13	Influenza B virus	R19101303	NICPBP

	(Yamagata)		
14	Influenza B virus (Victoria)	R19101304	NICPBP
15	Adeno virus 71	R20051719	ATCC
16	MERS-coronavirus	R20051232	ZeptoMetrix Corporation
17	Chlamydia pneumoniae	R20062510	ATCC
18	Streptococcus pneumoniae	R20062511	ATCC
19	Streptococcus pyogenes	R20121823	ATCC
20	Bordetella pertussis	R20062513	ZeptoMetrix Corporation
21	Mycobacterium tuberculosis	R20062514	ATCC
22	Legionella pneumophila	R20062515	ATCC
23	Mycoplasma pneumoniae	R20121502	ATCC
24	Haemophilus influenzae	R20062506	ATCC
25	Candida albicans	Q18071804	CMCC(F)98001
26	Staphylococcus aureus	Q19072903	CMCC(B)26003
27	Pseudomonas aeruginosa	Q18071806	CMCC(B)10104
28	Escherichia coli	Q19072901	CMCC(B)44102
29	Human Metapneumovirus (hMPV)	R20082113	ZeptoMetrix Corporation
30	Human coronavirus HKU1	R20121852	BioVector NTCC Inc.
31	Pneumocystis jirovecii (PJP)	R20082115	ATCC
32	Parainfluenza virus 4	R20121850	ZeptoMetrix Corporation
33	Staphylococcus epidermidis	R20121822	ATCC
34	Nasal wash	R20122308	The Alkalol Company

## 5.2. Methods

### 5.2.1. Sample Preparation

#### 1) Negative Control Samples

Use the nasopharyngeal swabs to collect nasopharyngeal swabs from healthy individuals, and add to the extraction tube with sample extraction buffer. Mix well and use as the negative control samples.

#### 2) Positive Control Samples

Add SARS-CoV-2 viral cultures into the negative samples, mix well to prepare 3xLoD (limit of detection) viral cultures and use it as the positive control sample.

#### 3) Negative Interference Samples

Add the microorganisms into the negative control samples and prepare negative interference samples with concentrations shown in Table 1.

#### 4) Positive Interference Samples

Add SARS-CoV-2 viral cultures and microorganism interfering substances into the negative

samples and prepare 3xLoD SARS-CoV-2 and microorganism samples with concentrations shown in Table 1.

Table 1. Testing concentrations of microorganism interfering substances

Microorganisms	Concentrations	Microorganisms	Concentrations
Human coronavirus 229E	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	MERS-coronavirus	$1.0 \times 10^5$ PFU/mL
Human coronavirus OC43	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Chlamydia pneumoniae	$2.0 \times 10^6$ IFU/mL
Human coronavirus NL63	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Streptococcus pneumoniae	$2.0 \times 10^6$ CFU/mL
Parainfluenza virus 1	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Streptococcus pyogenes	$2.0 \times 10^6$ CFU/mL
Parainfluenza virus 2	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Bordetella pertussis	$2.0 \times 10^6$ CFU/mL
Parainfluenza virus 3	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Mycobacterium tuberculosis	$2.0 \times 10^6$ CFU/mL
Enterovirus EV71	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Legionella pneumophila	$2.0 \times 10^6$ CFU/mL
Respiratory syncytial virus	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Mycoplasma pneumoniae	$2.0 \times 10^6$ U/mL
Rhinovirus	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Haemophilus influenzae	$2.0 \times 10^6$ CFU/mL
Influenza A virus (H1N1)	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Candida albicans	$2.0 \times 10^6$ CFU/mL
Influenza A virus (H3N2)	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Staphylococcus aureus	$2.0 \times 10^6$ CFU/mL
Influenza B virus (Yamagata)	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Pseudomonas aeruginosa	$2.0 \times 10^6$ CFU/mL
Influenza B virus (Victoria)	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Escherichia coli	$2.0 \times 10^6$ CFU/mL
Adeno virus 71	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Pneumocystis jirovecii (PJP)	$2.0 \times 10^6$ copies/mL
Human Metapneumovirus (hMPV)	$2.0 \times 10^6$ TCID <sub>50</sub> /mL	Human coronavirus HKU1	$2.0 \times 10^6$ TCID <sub>50</sub> /mL
Staphylococcus epidermidis	$2.0 \times 10^6$ CFU/mL	Parainfluenza virus 4	$2.0 \times 10^6$ TCID <sub>50</sub> /mL
Pooled human nasal wash	N/A		

### 5.2.2. Sample Testing

Use the Rapid SARS-CoV-2 Antigen Test Card to test the samples. Perform 3 parallel tests for each sample, and read results 15-20 min after sample addition.

Carry out the test procedures and interpretation of results according to the instructions for use.

### 5.3. Results

Table 2. Test results for microorganism negative interference and negative control samples

Microorganisms	1	2	3
Negative control sample	-	-	-
Human coronavirus 229E negative interference sample	-	-	-
Human coronavirus OC43 negative interference sample	-	-	-
Human coronavirus NL63 negative interference sample	-	-	-
Parainfluenza virus 1 negative interference sample	-	-	-
Parainfluenza virus 2 negative interference sample	-	-	-
Parainfluenza virus 3 negative interference sample	-	-	-
Enterovirus EV71 negative interference sample	-	-	-
Respiratory syncytial virus negative interference sample	-	-	-
Rhinovirus negative interference sample	-	-	-
Influenza A virus (H1N1) negative interference sample	-	-	-
Influenza A virus (H3N2) negative interference sample	-	-	-
Influenza B virus (Yamagata) negative interference sample	-	-	-
Influenza B virus (Victoria) negative interference sample	-	-	-
Adeno virus 71 negative interference sample	-	-	-
MERS-coronavirus negative interference sample	-	-	-
Chlamydia pneumoniae negative interference sample	-	-	-
Streptococcus pneumoniae negative interference sample	-	-	-
Streptococcus pyogenes negative interference sample	-	-	-
Bordetella pertussis negative interference sample	-	-	-
Mycobacterium tuberculosis negative interference sample	-	-	-
Legionella pneumophila negative interference sample	-	-	-
Mycoplasma pneumoniae negative interference sample	-	-	-
Haemophilus influenzae negative interference sample	-	-	-
Candida albicans negative interference sample	-	-	-
Staphylococcus aureus negative interference sample	-	-	-
Pseudomonas aeruginosa negative interference sample	-	-	-

Escherichia coli negative interference sample	-	-	-
Human Metapneumovirus(hMPV) negative interference sample	-	-	-
Human coronavirus HKU1 negative interference sample	-	-	-
Pneumocystis jirovecii (PJP) negative interference sample	-	-	-
Parainfluenza virus 4 negative interference sample	-	-	-
Staphylococcus epidermidis negative interference sample	-	-	-
Pooled human nasal wash negative interference sample	-	-	-

Table 3. Test results for microorganism positive interference and positive control samples

Microorganisms	1	2	3
Positive control sample	+	+	+
Human coronavirus 229E positive interference sample	+	+	+
Human coronavirus OC43 positive interference sample	+	+	+
Human coronavirus NL63 positive interference sample	+	+	+
Parainfluenza virus 1 positive interference sample	+	+	+
Parainfluenza virus 2 positive interference sample	+	+	+
Parainfluenza virus 3 positive interference sample	+	+	+
Enterovirus EV71 positive interference sample	+	+	+
Respiratory syncytial virus positive interference sample	+	+	+
Rhinovirus positive interference sample	+	+	+
Influenza A virus (H1N1) positive interference sample	+	+	+
Influenza A virus (H3N2) positive interference sample	+	+	+
Influenza B virus (Yamagata) positive interference sample	+	+	+
Influenza B virus (Victoria) positive interference sample	+	+	+
Adeno virus 71 positive interference sample	+	+	+
MERS-coronavirus positive interference sample	+	+	+
Chlamydia pneumoniae positive interference sample	+	+	+
Streptococcus pneumoniae positive interference sample	+	+	+
Streptococcus pyogenes positive interference sample	+	+	+
Bordetella pertussis positive interference sample	+	+	+

Mycobacterium tuberculosis positive interference sample	+	+	+
Legionella pneumophila positive interference sample	+	+	+
Mycoplasma pneumoniae positive interference sample	+	+	+
Haemophilus influenzae positive interference sample	+	+	+
Candida albicans positive interference sample	+	+	+
Staphylococcus aureus positive interference sample	+	+	+
Pseudomonas aeruginosa positive interference sample	+	+	+
Escherichia coli positive interference sample	+	+	+
Human Metapneumovirus (hMPV) positive interference sample	+	+	+
Human coronavirus HKU1 positive interference sample	+	+	+
Pneumocystis jirovecii (PJP) positive interference sample	+	+	+
Parainfluenza virus 4 positive interference sample	+	+	+
Staphylococcus epidermidis positive interference sample	+	+	+
Pooled human nasal wash positive interference sample	+	+	+

#### 5.4. Analysis of Results

The test results for the above microorganism negative interference and negative control samples were all negative. No interference was found.

The test results for the above microorganism positive interference and positive control samples were all positive. No interference was found.

#### 5.5. Conclusion

The above microorganisms did not cause interference on the Rapid SARS-CoV-2 Antigen Test Card at the listed concentrations.

### 6. Endogenous and Exogenous Interference Studies

#### 6.1. Materials

	Name	Lot No. (Catalog No.)	Notes
1	Whole Blood	F20101506	Boson
2	Mucin	R20052019	Sangon Biotech (Shanghai) Co., Ltd.
3	Tobramycin	R20052020	Sangon Biotech (Shanghai) Co., Ltd.
4	Ricola (Menthol)	R20052021	Sangon Biotech (Shanghai) Co., Ltd.
5	Chloraseptic (Benzocaine)	R20052630	Shanghai Yuanye Biotechnology Co., Ltd.



6	Mupirocin	R20052022	Sangon Biotech (Shanghai) Co., Ltd.
7	Tamiflu (Oseltamivir Phosphate)	R20061852	Shanghai Roche Pharmaceuticals Ltd.
8	Allergy Relief Nasal Spray Homeopathic (Alkalol)	R20061853	The Alkalol Company
9	CVS Nasal Drops (Phenylephrine)	R20052631	Shanghai Yuanye Biotechnology Co., Ltd.
10	Afrin (Oxymetazoline)	R20061945	Afrin™
11	CVS Nasal Spray (Cromolyn)	R20061946	CVS Health
12	Fluticasone Propionate	R20052632	Shanghai Yuanye Biotechnology Co., Ltd.
13	Zicam Cold Remedy Nasal Spray	R20061947	ZICAM™
14	Naso GEL (NeilMed)	R21012504	NeilMed
15	Human Anti- mouse Antibody (HAMA)	R20100807	Scantibodies
16	Biotin	R20101501	Sigma

## 6.2. Methods

### 6.2.1. Sample Preparation

#### 1) Negative Control Samples

Use the nasopharyngeal swabs to collect nasopharyngeal swabs from healthy individuals, and add to the extraction tube with sample extraction buffer. Mix well and use as the negative control samples.

#### 2) Positive Control Samples

Add SARS-CoV-2 viral cultures into the negative samples, mix well to prepare 3xLoD (limit of detection) viral cultures and use it as the positive control sample.

#### 3) Negative Interference Samples

Add the endogenous or exogenous interfering substances into the negative control samples and prepare negative interference samples with concentrations shown in Table 4.

#### 4) Positive Interference Samples

Add SARS-CoV-2 viral cultures and interfering substances into the negative samples and prepare 3xLoD SARS-CoV-2 and interference samples with concentrations shown in Table 4.

Table 4. Testing concentrations of interfering substances

Substances	Concentrations	Substances	Concentrations
Whole Blood	1% v/v	Allergy Relief Nasal Spray Homeopathic (Alkalol)	10% v/v
Mucin	2% w/v	CVS Nasal Drops (Phenylephrine)	15% v/v
Tobramycin	0.0004% w/v	Afrin (Oxymetazoline)	15% v/v
Ricola (Menthol)	0.15% w/v	CVS Nasal Spray (Cromolyn)	15% v/v

Chloraseptic (Benzocaine)	0.15% w/v	Fluticasone Propionate	5% v/v
Mupirocin	0.25% w/v	Zicam Cold Remedy Nasal Spray	5% w/v
Tamiflu (Oseltamivir Phosphate)	0.5% w/v	Naso GEL (NeilMed)	5% v/v
Human Anti- mouse Antibody (HAMA)	60 ng/mL	Biotin	1200 ng/mL

### 6.2.2. Sample Testing

Use the Rapid SARS-CoV-2 Antigen Test Card to test the samples. Perform 3 parallel tests for each sample, and read results 15-20 min after sample addition.

Carry out the test procedures and interpretation of results according to the instructions for use.

### 6.3. Results

Table 5. Test results for negative interference and negative control samples

Samples	1	2	3
Negative control sample	-	-	-
Whole Blood negative interference sample	-	-	-
Mucin negative interference sample	-	-	-
Tobramycin negative interference sample	-	-	-
Ricola (Menthol) negative interference sample	-	-	-
Chloraseptic (Benzocaine) negative interference sample	-	-	-
Mupirocin negative interference sample	-	-	-
Tamiflu (Oseltamivir Phosphate) negative interference sample	-	-	-
Allergy Relief Nasal Spray Homeopathic (Alkalol) negative interference sample	-	-	-
CVS Nasal Drops (Phenylephrine) negative interference sample	-	-	-
Afrin (Oxymetazoline) negative interference sample	-	-	-
CVS Nasal Spray (Cromolyn) negative interference sample	-	-	-
Fluticasone Propionate negative interference sample	-	-	-
Zicam Cold Remedy Nasal Spray negative interference sample	-	-	-
Naso GEL (NeilMed) negative interference sample	-	-	-
HAMA negative interference sample	-	-	-
Biotin negative interference sample	-	-	-

Table 6. Test results for positive interference and positive control samples

Microorganisms	1	2	3
Positive control sample	+	+	+
Whole Blood positive interference sample	+	+	+
Mucin positive interference sample	+	+	+
Tobramycin positive interference sample	+	+	+
Ricola (Menthol) positive interference sample	+	+	+
Chloraseptic (Benzocaine) positive interference sample	+	+	+
Mupirocin positive interference sample	+	+	+
Tamiflu (Oseltamivir Phosphate) positive interference sample	+	+	+
Allergy Relief Nasal Spray Homeopathic (Alkalol) positive interference sample	+	+	+
CVS Nasal Drops (Phenylephrine) positive interference sample	+	+	+
Afrin (Oxymetazoline) positive interference sample	+	+	+
CVS Nasal Spray (Cromolyn) positive interference sample	+	+	+
Fluticasone Propionate positive interference sample	+	+	+
Zicam Cold Remedy Nasal Spray positive interference sample	+	+	+
Naso GEL (NeilMed) positive interference sample	+	+	+
HAMA positive interference sample	+	+	+
Biotin positive interference sample	+	+	+

#### 6.4. Analysis of Results

The test results for the above endogenous and exogenous negative interference and negative control samples were all negative. No interference was found.

The test results for the above endogenous and exogenous positive interference and positive control samples were all positive. No interference was found.

#### 6.5. Conclusion

The above endogenous and exogenous substances did not cause interference on the Rapid SARS-CoV-2 Antigen Test Card at the listed concentrations.