BIOSYNEX EASY DIAGNOSTICS FOR LIFE SWISS	DOC 5A_PRODUCT VERIFICATION AND VALIDATION ANALYTICAL PERFORMANCES		
	Reference : F-QUA-337	Version : 03	Date : 13/07/2020

PROJECT CODE	PRODUCT DESIGNATION	REFERENCE
NA	BIOSYNEX COVID-19 Ag BSS	SW40006
NA	BIOSYNEX® COVID-19 Ag BSX	SW40006_BSX

Content

I. Precision

1. Repeatability (precision intra-assay)

Completion date: 2020.07.13

➢ Objective

Evaluate the precision of BIOSYNEX COVID-19 Ag BSS rapid test within lot.

➢ Material

✓ Description of component

BIOSYNEX COVID-19 Ag BSS rapid test

Lot1: 2006189, Lot2:2006190, Lot3:2006191

✓ Description of samples

Positive Specimen: SARS-CoV-2 cultured virus: Concentration of 1.15 x 10^2 $TCID_{50}$/mL (LoD) and 4.6 x 10^2 $TCID_{50}$/mL (4*LoD)

Negative Specimen: Pooled human nasal matrix obtained from company employees healthy donors and confirmed negative for SARS-CoV-2.

➢ Method

✓ Study protocol

Use the same batch of tests to test the positive and negative specimen and observe the precision in lot. 50µL specimens were added to swabs and then tested in the BIOSYNEX COVID-19 Ag BSS rapid test using the procedure appropriate for patient nasal swab specimens. Three lots were assayed by three operators respectively. Testing at each lot consisted of 10 replicates for each specimen.

Perform the test according to the test procedure in the package insert.

✓ Acceptance criteria

Negative specimen generates negative result and positive specimen generates positive result.

➢ Results

Lot1: 2006189

		Specimens		
		Negative Specimen	1.15 x 10^2 $TCID_{50}$/mL	4.6 x 10^2 $TCID_{50}$/mL
Test	1	-	+	+
	2	-	+	+

	3	-	+	+
	4	-	+	+
	5	-	+	+
	6	-	+	+
	7	-	+	+
	8	-	+	+
	9	-	+	+
	10	-	+	+

Lot2: 2006190

		Specimens		
		Negative Specimen	1.15 x 10^2 $TCID_{50}$/mL	4.6 x 10^2 $TCID_{50}$/mL
Test	1	-	+	+
	2	-	+	+
	3	-	+	+
	4	-	+	+
	5	-	+	+
	6	-	+	+
	7	-	+	+
	8	-	+	+
	9	-	+	+
	10	-	+	+

Lot3: 2006191

		Specimens		
		Negative Specimen	1.15 x 10^2 $TCID_{50}$/mL	4.6 x 10^2 $TCID_{50}$/mL
Test	1	-	+	+
	2	-	+	+
	3	-	+	+
	4	-	+	+
	5	-	+	+
	6	-	+	+
	7	-	+	+
	8	-	+	+
	9	-	+	+
	10	-	+	+

➢ Conclusion

The precision is fine and acceptable for BIOSYNEX COVID-19 Ag BSS rapid test.

2. Reproducibility

1.1 Inter Lots reproducibility (precision inter-assay)

Completion date: 2020.07.13

➢ Objective

To evaluate the precision of BIOSYNEX COVID-19 Ag BSS rapid test between lots.

➢ Material

✓ Description of component

BIOSYNEX COVID-19 Ag BSS rapid test

Lot1: 2006189, Lot2:2006190, Lot3:2006191

✓ Description of samples

Positive Specimen: SARS-CoV-2 cultured virus: Concentration of 1.15 x 10^2 $TCID_{50}$/mL (LoD) and 4.6 x 10^2 $TCID_{50}$/mL (4*LoD)

Negative Specimen: Pooled human nasal matrix obtained from company employees healthy donors and confirmed negative for SARS-CoV-2.

➢ Method

✓ Study protocol

Use different batches of test to test the same negative and positive specimen and observe the precision between lots. 50µL specimens were added to swabs and then tested in the BIOSYNEX COVID-19 Ag BSS rapid test using the procedure appropriate for patient nasal swab specimens.

Perform the test according to the test procedure in the package insert.

✓ Acceptance criteria

Negative specimen generates negative result and positive specimen generates positive result.

➢ Results

		Negative Specimen		
		Lot 1	Lot 2	Lot 3
Test	1	-	-	-
	2	-	-	-
	3	-	-	-

	4	-	-	-
	5	-	-	-
	6	-	-	-
	7	-	-	-
	8	-	-	-
	9	-	-	-
	10	-	-	-

		1.15×10^2 $TCID_{50}$/mL		
		Lot 1	Lot 2	Lot 3
Test	1	+	+	+
	2	+	+	+
	3	+	+	+
	4	+	+	+
	5	+	+	+
	6	+	+	+
	7	+	+	+
	8	+	+	+
	9	+	+	+
	10	+	+	+

		4.6×10^2 $TCID_{50}$/mL		
		Lot 1	Lot 2	Lot 3
Test	1	+	+	+
	2	+	+	+
	3	+	+	+
	4	+	+	+
	5	+	+	+
	6	+	+	+
	7	+	+	+
	8	+	+	+
	9	+	+	+
	10	+	+	+

➢ Conclusion

The precision is fine and acceptable for BIOSYNEX COVID-19 Ag BSS rapid test.

1.2 Reproducibility study – between/within lots, between/within days and between operators

Completion date: 2020.07.13 - 2020.07.17

➢ Objective

Evaluate the reproducibility of BIOSYNEX COVID-19 Ag BSS rapid test between/within lot, between/within day and from one operator to another.

➢ Material

✓ Description of component

BIOSYNEX COVID-19 Ag BSS rapid test

Lot1#: 2006189, Lot2#:2006190, Lot3#:2006191

✓ Description of samples

Positive Specimen: SARS-CoV-2 cultured virus has been inactivated by heating at 65°C for 30 minutes, the material was supplied at a concentration of 4.6 x 105 $TCID_{50}$/mL.

Negative Specimen: Pooled human nasal matrix obtained from healthy donors our company employee and confirmed negative for SARS-CoV-2.

➢ Method

✓ Study protocol

The study is designed to verify the reproducibility of BIOSYNEX COVID-19 Ag BSS rapid test between/within lot, between/within day and from one operator to another by testing negative specimen, LOD specimen and 4-fold concentration of LoD specimen with the assay. Three operators are required and each operator must perform three batches of the assay every day, over five days. Each batch must be tested at random once in the morning and once in the afternoon separately according o the following steps:

The SARS-CoV-2 cultured virus supplied at a concentration of 4.6 x $10^5$$TCID_{50}$/mL is spiked into a volume of negative specimen in order to get the LoD specimen and the 4-fold concentration of LoD specimen.

Note: If the test result is invalid, test the specimen again. People who prepare the specimen do not participate in the specimen testing. Operators (A, B, C) do not prepare the specimen.

✓ Test Procedure

Perform the test according to the test procedure in the package insert.

✓ Acceptance criteria

Negative specimen: Negative agreement =100% or positive agreement =0%

Limit of detection specimen: Positive agreement ≥95%

4-fold concentration of LOD specimen: Positive agreement =100%

➢ Results

Table 1: Test results from operator A.

Specimen	Time (Day)	NO. Positive/Tested		
		Lot 1#	Lot 2#	Lot 3#
Negative Specimen	Day 1	0/2	0/2	0/2
	Day 2	0/2	0/2	0/2
	Day 3	0/2	0/2	0/2
	Day 4	0/2	0/2	0/2
	Day 5	0/2	0/2	0/2
Limit of Detection (LOD) Specimen 1.15×10^2 $TCID_{50}$/mL	Day 1	2/2	2/2	2/2
	Day 2	2/2	2/2	2/2
	Day 3	2/2	2/2	2/2
	Day 4	2/2	2/2	2/2
	Day 5	2/2	2/2	2/2
4-fold concentration of Limit of Detection (4 x LOD) Specimen 4.6×10^2 $TCID_{50}$/mL	Day 1	2/2	2/2	2/2
	Day 2	2/2	2/2	2/2
	Day 3	2/2	2/2	2/2
	Day 4	2/2	2/2	2/2
	Day 5	2/2	2/2	2/2

Table 2: Test results from operator B.

Specimen	Time (Day)	NO. Positive/Tested		
		Lot 1#	Lot 2#	Lot 3#
Negative Specimen	Day 1	0/2	0/2	0/2
	Day 2	0/2	0/2	0/2

	Day 3	0/2	0/2	0/2
	Day 4	0/2	0/2	0/2
	Day 5	0/2	0/2	0/2
Limit of Detection (LOD) Specimen 1.15 x 10^2 $TCID_{50}$/mL	Day 1	2/2	2/2	2/2
	Day 2	2/2	2/2	2/2
	Day 3	2/2	2/2	2/2
	Day 4	2/2	2/2	2/2
	Day 5	2/2	2/2	2/2
4-fold concentration of Limit of Detection (4 x LOD) Specimen 4.6 x 10^2 $TCID_{50}$/mL	Day 1	2/2	2/2	2/2
	Day 2	2/2	2/2	2/2
	Day 3	2/2	2/2	2/2
	Day 4	2/2	2/2	2/2
	Day 5	2/2	2/2	2/2

Table 3: Test results from operator C

Specimen	Time (Day)	NO. Positive/Tested		
		Lot 1#	Lot 2#	Lot 3#
Negative Specimen	Day 1	0/2	0/2	0/2
	Day 2	0/2	0/2	0/2
	Day 3	0/2	0/2	0/2
	Day 4	0/2	0/2	0/2
	Day 5	0/2	0/2	0/2
Limit of Detection (LOD) Specimen 1.15 x 10^2 $TCID_{50}$/mL	Day 1	2/2	2/2	2/2
	Day 2	2/2	2/2	2/2

	Day 3	2/2	2/2	2/2
	Day 4	2/2	2/2	2/2
	Day 5	2/2	2/2	2/2
4-fold concentration of Limit of Detection (4 x LOD) Specimen 4.6 x 10^2 $TCID_{50}$/mL	Day 1	2/2	2/2	2/2
	Day 2	2/2	2/2	2/2
	Day 3	2/2	2/2	2/2
	Day 4	2/2	2/2	2/2
	Day 5	2/2	2/2	2/2

Table 4: Summary of results between/within lot

Specimen	Total NO. Positive/Tested			Total NO. Positive/Tested	Positive Agreement %
	Lot 1#	Lot 2#	Lot 3#		
Negative Specimen	0/30	0/30	0/30	0/90	0%
Limit of Detection (LOD) Specimen 1.15 x 10^2 $TCID_{50}$/mL	30/30	30/30	30/30	90/90	100%
4-fold concentration of Limit of Detection (4 x LOD) Specimen 4.6 x 10^2 $TCID_{50}$/mL	30/30	30/30	30/30	90/90	100%

Table 5: Summary of results between/within day

Specimen	Total NO. Positive/Tested					Total NO. Positive/Tested	Positive Agreement %
	Day1	Day2	Day3	Day4	Day5		
Negative Specimen	0/18	0/18	0/18	0/18	0/18	0/90	0%

Limit of Detection (LOD) Specimen 1.15 x 10^2 $TCID_{50}$/mL	18/18	18/18	18/18	18/18	18/18	90/90	100%
4-fold concentration of Limit of Detection (4 x LOD) Specimen 4.6 x 10^2 $TCID_{50}$/mL	18/18	18/18	18/18	18/18	18/18	90/90	100%

Table 6: Summary of results operator to operator

Specimen	Total NO. Positive/Tested			Total NO. Positive/Tested	Positive Agreement %
	Operator A	Operator B	Operator C		
Negative Specimen	0/30	0/30	0/30	0/90	0%
Limit of Detection (LOD) Specimen 1.15 x 10^2 $TCID_{50}$/mL	30/30	30/30	30/30	90/90	100%
4-fold concentration of Limit of Detection (4 x LOD) Specimen 4.6 x 10^2 $TCID_{50}$/mL	30/30	30/30	30/30	90/90	100%

➢ Conclusion

The reproducibility of BIOSYNEX COVID-19 Ag BSS rapid test between/within lot, between/within day and operator to operator can meet the acceptance criteria.

II. Analytical sensitivity

Date of the report: 2020.07.10

➢ Objective

Evaluate the limit of detection of BIOSYNEX COVID-19 Ag BSS rapid test.

➢ Material

✓ Description of component

BIOSYNEX COVID-19 Ag BSS rapid test

Lot1: 2006189, Lot2:2006190, Lot3:2006191

✓ Description of samples

SARS-CoV-2 cultured virus confirmed by PCR and has been inactivated by heating at 65°C for 30 minutes, The material was supplied at a concentration of 4.6 x 10^5 $TCID_{50}$/mL.

Pooled human nasal matrix obtained from company employees healthy donors and confirmed negative for SARS-CoV-2.

➢ Method

✓ Study protocol

The LOD for the BIOSYNEX COVID-19 Ag BSS rapid test was established using limiting dilutions of a viral sample inactivated by heating at 65°C for 30 minutes. The material was supplied at a concentration of 4.6 x $10^5$$TCID_{50}$/ml. In this study, designed to estimate the LOD of the assay when using a direct nasal swab, the starting material was spiked into a volume of pooled human nasal matrix obtained from healthy donors and confirmed negative for SARS-CoV-2. An initial range-finding study was performed testing devices in triplicate using a 10-fold dilution series. At each dilution, 50µL samples were added to swabs and then tested in the BIOSYNEX COVID-19 Ag BSS rapid test using the procedure appropriate for patient nasal swab specimens. A concentration was chosen between the last dilution to give 9 positive results and the first to give 9 negative results. Using this concentration, the LoD was further refined with a 2-fold dilution series. At each dilution, then tested in 60 replicates tested in the same way using three lots of BIOSYNEX COVID-19 Ag BSS rapid test.

Perform the test according to the test procedure in the package insert.

✓ Acceptance criteria

Acceptable Limit of detection level: Positive agreement ≥ 90%

➢ Results

Table 1: The results of 10-fold dilution series.

Dilution series	Concentration	BIOSYNEX COVID-19 Ag BSS rapid test			NO. Positive/Tested
		Lot 1	Lot 2	Lot 3	
Neat	4.6×10^5 $TCID_{50}$/mL	3/3	3/3	3/3	9/9
1/10	4.6×10^4 $TCID_{50}$/mL	3/3	3/3	3/3	9/9
1/100	4.6×10^3 $TCID_{50}$/mL	3/3	3/3	3/3	9/9
1/1000	4.6×10^2 $TCID_{50}$/mL	3/3	3/3	3/3	9/9
1/10000	4.6×10 $TCID_{50}$/mL	0/3	0/3	0/3	0/9

Table 2: The results of 2-fold dilution series.

Dilution series	Concentration	BIOSYNEX COVID-19 Ag BSS rapid test			NO. Positive /Tested	Positive agreement
		Lot 1	Lot 2	Lot 3		
Neat	4.6×10^2 $TCID_{50}$/mL	20/20	20/20	20/20	60/60	100%
1/2	2.3×10^2 $TCID_{50}$/mL	20/20	20/20	20/20	60/60	100%
1/4	1.15×10^2 $TCID_{50}$/mL	20/20	20/20	20/20	60/60	100%
1/8	5.75×10 $TCID_{50}$/mL	6/20	3/20	5/20	14/60	23.3%
1/16	2.88×10 $TCID_{50}$/mL	0/20	0/20	0/20	0/60	0%

➢ Conclusion

From the study above:

The Limit of Detection of the BIOSYNEX COVID-19 Ag BSS rapid test is 1.15×10^2 $TCID_{50}$/mL.

III. Analytical specificity

1. Interference Testing

➢ Objective

Evaluate if the substances found in respiratory specimen interfere with the BIOSYNEX COVID-19 Ag BSS rapid test.

➢ Material

✓ Description of component

BIOSYNEX COVID-19 Ag BSS rapid test
Lot1: 2006189, Lot2:2006190, Lot3:2006191

✓ Description of samples

Limit of detection references: SARS-CoV-2 cultured virus with concentration of 1.15 x 10^2 $TCID_{50}$/mL.
Pooled human nasal matrix obtained from our company employee healthy donors and confirmed negative for SARS-CoV-2.
Potential interfering substances: Human blood (EDTA anticoagulated), Mucin, Antiviral drugs (Oseltamivir phosphate, Ribavirin), Antibiotics/antibacterial drugs (Levofloxacin, Azithromycin, Meropenem, Tobramycin), Nasal sprays or nose drops (Phenylephrine, Oxymetazoline, 0.9% sodium chloride, A natural soothing ALKALOL), Nasal corticosteroids (Beclomethasone, Hexadecadrol, Flunisolide, Triamcinolone, Budesonide, Mometasone, Fluticasone, Fluticasone propionate).

➢ Method

✓ Study protocol

Test simulated specimens using the BIOSYNEX COVID-19 Ag BSS rapid test to evaluate if there is any substance interferences. These potential interfering substances were spiked separately into negative pooled human nasal matrix and SARS-CoV-2 cultured virus as the simulated specimens. 50µL simulated specimens were added to swabs and then tested in the BIOSYNEX COVID-19 Ag BSS rapid test using the procedure appropriate for patient nasal swab specimens.

Perform the test according to the test procedure in the package insert.

➢ Results

Test Results of negative pooled human nasal matrix simulated specimens

Potential interfering substances were spiked into Negative pooled human nasal matrix		Concentration	BIOSYNEX COVID-19 Ag BSS rapid test		
			Lot 1	Lot 2	Lot 3
	Human blood (EDTA anticoagulated)	20% (v/v)	-	-	-

	Mucin	5mg/ml	-	-	-
Antiviral drugs	Oseltamivir phosphate	5mg/ml	-	-	-
	Ribavirin	5mg/ml	-	-	-
Antibiotics /antibacterial drugs	Levofloxacin	5mg/ml	-	-	-
	Azithromycin	5mg/ml	-	-	-
	Meropenem	5mg/ml	-	-	-
	Tobramycin	2mg/ml	-	-	-
Nasal sprays or nose drops	Phenylephrine	20%(v/v)	-	-	-
	oxymetazoline	20%(v/v)	-	-	-
	0.9% sodium chloride	20%(v/v)	-	-	-
	A natural soothing ALKALOL	20%(v/v)	-	-	-
Nasal corticosteroids	Beclomethasone	20%(v/v)	-	-	-
	Hexadecadrol	20%(v/v)	-	-	-
	Flunisolide	20%(v/v)	-	-	-
	Triamcinolone	20%(v/v)	-	-	-
	Budesonide	20%(v/v)	-	-	-
	Mometasone	20%(v/v)	-	-	-
	Fluticasone	20%(v/v)	-	-	-
	Fluticasone propionate	20%(v/v)	-	-	-

Note: "-"=negative result

Test Results of SARS-CoV-2 cultured virus simulated specimens

Potential interfering substances were spiked into Limit of detection references (SARS-CoV-2 cultured virus with concentration of 1.15 x 10^2 $TCID_{50}$/mL)		Concentration	BIOSYNEX COVID-19 Ag BSS rapid test		
			Lot 1	Lot 2	Lot 3
	Human blood(EDTA anticoagulated)	20% (v/v)	+	+	+
	Mucin	5mg/ml	+	+	+
Antiviral drugs	Oseltamivir phosphate	5mg/ml	+	+	+
	Ribavirin	5mg/ml	+	+	+

Antibiotics /antibacterial drugs	Levofloxacin	5mg/ml	+	+	+
	Azithromycin	5mg/ml	+	+	+
	Meropenem	5mg/ml	+	+	+
	Tobramycin	2mg/ml	+	+	+
Nasal sprays or nose drops	Phenylephrine	20%(v/v)	+	+	+
	oxymetazoline	20%(v/v)	+	+	+
	0.9% sodium chloride	20%(v/v)	+	+	+
	A natural soothing ALKALOL	20%(v/v)	+	+	+
Nasal corticosteroids	Beclomethasone	20%(v/v)	+	+	+
	Hexadecadrol	20%(v/v)	+	+	+
	Flunisolide	20%(v/v)	+	+	+
	Triamcinolone	20%(v/v)	+	+	+
	Budesonide	20%(v/v)	+	+	+
	Mometasone	20%(v/v)	+	+	+
	Fluticasone	20%(v/v)	+	+	+
	Fluticasone propionate	20%(v/v)	+	+	+

Note: "+"= positive results

➢ Conclusion

According to the results, we can conclude that no mentioned substances interfered with the BIOSYNEX COVID-19 Ag BSS rapid test.

2. Cross reactivity

➢ Objective

Evaluate the cross-reactivity of BIOSYNEX COVID-19 Ag BSS rapid test with potential cross-reactivity pathogens.

➢ Material

✓ Description of component

BIOSYNEX COVID-19 Ag BSS rapid test

Lot1: 2006189, Lot2:2006190, Lot3:2006191

➢ Method

✓ Study protocol

To find out if there is cross-reaction with possible pathogens in the clinical specimens, use the BIOSYNEX COVID-19 Ag BSS rapid test to test these specimens.

Test above specimen with BIOSYNEX COVID-19 Ag BSS rapid test. Add 50µL specimen to the swab and perform the test according to the test procedure in the package insert. Three lots of the tests will be used per specimen, each specimen will be tested once per lot. A total of three operators is needed, each
operator taking care of one lot.

Note: If the test result is valid, test the specimen again. People who prepare the specimen do not participate in the specimen testing. Operators (A, B, C) do not prepare the specimen.

✓ Specimen preparation

Pathogens	
Respiratory syncytial virus Type A	Human coronavirus NL63
Respiratory syncytial virus Type B	Human coronavirus HKU1
Novel influenza A H1N1 virus (2019)	Parainfluenza virus 1
Seasonal influenza A H1N1 virus	Parainfluenza virus 2
Influenza A H3N2 virus	Parainfluenza virus 3
Influenza A H5N1 virus	Parainfluenza virus 4
Influenza B Yamagata	Haemophilus influenzae
Influenza B Victoria	Streptococcus pyogenes
Rhinovirus	Streptococcus pneumoniae
Adenovirus 3	Candida albicans
Adenovirus 7	Bordetella pertussis
EV-A71	Mycoplasma pneumoniae
Mycobacterium tuberculosis	Chlamydia pneumoniae

Mumps virus	Legionella pneumophila
Human coronavirus 229E	Pooled human nasal wash
Human coronavirus OC43	

➢ Results

Pathogens	Concentration	BIOSYNEX COVID-19 Ag BSS rapid test		
		Lot1	Lot2	Lot3
Respiratory syncytial virus Type A	5.5×10^{7}PFU/ml	-	-	-
Respiratory syncytial virus Type B	$2.8\times10^{5}TCID_{50}$/ml	-	-	-
Novel influenza A H1N1 virus (2019)	1×10^{6}PFU/ml	-	-	-
Seasonal influenza A H1N1 virus	1×10^{5}PFU/ml	-	-	-
Influenza A H3N2 virus	1×10^{6}PFU/ml	-	-	-
Influenza A H5N1 virus	1×10^{6}PFU/ml	-	-	-
Influenza B Yamagata	1×10^{5}PFU/ml	-	-	-
Influenza B Victoria	1×10^{6}PFU/ml	-	-	-
Rhinovirus	1×10^{6}PFU/ml	-	-	-
Adenovirus 3	$5\times10^{7.5}$TCID50/ml	-	-	-
Adenovirus 7	2.8×10^{6}TCID50/ml	-	-	-
EV-A71	1×10^{5}PFU/ml	-	-	-
Mycobacterium tuberculosis	1×10^{3} bacteria/ml	-	-	-
Mumps virus	1×10^{5}PFU/ml	-	-	-
Human coronavirus 229E	1×10^{5}PFU/ml	-	-	-
Human coronavirus OC43	1×10^{5}PFU/ml	-	-	-
Human coronavirus NL63	1×10^{6}PFU/ml	-	-	-
Human coronavirus HKU1	1×10^{6}PFU/ml	-	-	-
Parainfluenza virus 1	7.3×10^{6}PFU/ml	-	-	-
Parainfluenza virus 2	1×10^{6}PFU/ml	-	-	-
Parainfluenza virus 3	5.8×10^{6}PFU/ml	-	-	-
Parainfluenza virus 4	2.6×10^{6}PFU/ml	-	-	-
Haemophilus influenzae	5.2×10^{6}CFU/ml	-	-	-
Streptococcus pyogenes	3.6×10^{6}CFU/ml	-	-	-
Streptococcus pneumoniae	4.2×10^{6}CFU/ml	-	-	-
Candida albicans	1×10^{7}CFU/ml	-	-	-
Bordetella pertussis	1×10^{4} bacteria/ml	-	-	-
Mycoplasma pneumoniae	1.2×10^{6}CFU/ml	-	-	-
Chlamydia pneumoniae	2.3×10^{6}IFU/ml	-	-	-
Legionella pneumophila	1×10^{4} bacteria/ml	-	-	-

Pooled human nasal wash	100%	-	-	-

Note: "-"=Negative Result

➢ Conclusion

The tested pathogens above did not show any cross-reaction with the BIOSYNEX COVID-19 Ag BSS rapid test.

IV. Robustness

1. Temperature flex study

Completion date: 2020.07.16

➢ Objective

Evaluate the impact of storage at different temperatures (4°C, 25°C, 37°C) on test results.

➢ Material

✓ Description of component

BIOSYNEX COVID-19 Ag BSS rapid test

Cassette Lot1: 2006189, Lot2:2006190, Lot3:2006191

✓ Description of samples

Limit of detection standards (L1, L2, L3)

Positive standard (P1)

Negative standard (N1)

➢ Method

✓ Study protocol

Positive and negative control samples and test devices were divided equally into 3 groups. Each group is placed at a different temperature 4°C, 25°C or 37°C for 15 minutes. After 15 minutes, the test is carried out (according to the test procedure of the package insert) at temperature 4°C, 25°C or 37°C depending on the group. Each control sample was tested in 3 replicates using 3 lots of BIOSYNEX COVID-19 Ag BSS rapid test.

✓ Acceptance criteria

The positive samples (L2, L3, P1) should generate positive results, the negative samples (N1, L1) should generate negative results.

➢ Results

Control Samples	Product Lot#	Testing Temperature		
		4°C	25°C	37°C
N1	Lot 1	3-	3-	3-
	Lot 2	3-	3-	3-
	Lot 3	3-	3-	3-
L1	Lot 1	3-	3-	3-
	Lot 2	3-	3-	3-

	Lot 3	3-	3-	3-
L2	Lot 1	3+	3+	3+
	Lot 2	3+	3+	3+
	Lot 3	3+	3+	3+
L3	Lot 1	3+	3+	3+
	Lot 2	3+	3+	3+
	Lot 3	3+	3+	3+
P1	Lot 1	3+	3+	3+
	Lot 2	3+	3+	3+
	Lot 3	3+	3+	3+

Note: "3+"= 3 positive results, "3-"= 3 negative results.

➢ Conclusion

The product could yield correct results when tested with the samples stored from 4°C to 37°C. Therefore, it can accommodate a wide fluctuation of sample temperatures.

2. Reading time flex study

Completion date: 2020.07.15

➢ Objective

Investigate the read time of the BIOSYNEX COVID-19 Ag BSS rapid testvia interpreting the result at different time
points.

➢ Material

✓ Description of component

BIOSYNEX COVID-19 Ag BSS rapid test
Lot1: 2006189, Lot2:2006190, Lot3:2006191

✓ Description of samples

Internal References:
Limit of Detection References (L1, L2, L3)
Positive Reference (P1)
Negative Reference (N1)

➢ Method

✓ Study protocol

Positive and negative control samples were tested in triplicate on three different lots of products. And the results will be read visually as positive or negative at 5, 10, 15, 20 and 30 minutes. Each control sample was tested in three replicates using three lots of BIOSYNEX COVID-19 Ag BSS rapid test.

✓ Acceptance criteria

The positive samples (L2, L3,P1)should obtain positive results, the negative samples (N1, L1) should obtain negative results. Background is clear.

➢ Results

Control samples	Product Lot#	Read time (Minutes)				
		5	10	15	20	30
N1	Lot 1	3-	3-	3-	3-	3-
	Lot 2	3-	3-	3-	3-	3-
	Lot 3	3-	3-	3-	3-	3-
L1	Lot 1	3-	3-	3-	3-	3-
	Lot 2	3-	3-	3-	3-	3-
	Lot 3	3-	3-	3-	3-	3-
	Lot 1	3-	3-	3+	3+	3+

L2	Lot 2	3-	3-	3+	3+	3+
	Lot 3	3-	3-	3+	3+	3+
L3	Lot 1	3-	3+	3+	3+	3+
	Lot 2	3-	3+	3+	3+	3+
	Lot 3	3-	3+	3+	3+	3+
P1	Lot 1	3+	3+	3+	3+	3+
	Lot 2	3+	3+	3+	3+	3+
	Lot 3	3+	3+	3+	3+	3+

Note: "3+"= 3 positive results, "3-"= 3 negative results.

➢ Conclusion

The results showed the ability of the assay to give correct results within 15-30 minutes. Therefore, the product can accommodate a fluctuation of reading times. However, sample imprinting can appear on the membrane if the reading process occur after 20 minutes, and hence, it is recommended to read the results within 15-20 minutes.

3. Sample volume flex

Completion date: 2020.07.15

➢ Objective

Investigate the influence of the used sample volume on the test result.

➢ Material

✓ Description of component

BIOSYNEX COVID-19 Ag BSS rapid test

Lot1: 2006189, Lot2:2006190, Lot3:2006191

✓ Description of samples

Limit of Detection References (L1, L2, L3)

Positive Reference (P1)

Negative Reference (N1)

➢ Method

✓ Study protocol

Positive samples and negative samples were tested in triplicate on three lots of product using different sample volumes of 1 drop, 2 drops, 3 drops, 4 drops and 5 drops.

Perform the test according to the test procedure in the package insert.

✓ Acceptance criteria

The positive samples (L2, L3, P1) should obtain positive results, the negative samples (N1, L1) should obtain negative results. Background is clear.

➢ Results

Control samples	Product Lot#	Sample volume for testing				
		1 drop	2 drops	3 drops	4 drops	5 drops
N1	Lot 1	No flow	-	-	-	-
	Lot 2	No flow	-	-	-	-
	Lot 3	No flow	-	-	-	-
L1	Lot 1	No flow	-	-	-	-
	Lot 2	No flow	-	-	-	-
	Lot 3	No flow	-	-	-	-
	Lot 1	No flow	-	+	+	+

L2	Lot 2	No flow	-	+	+	+
	Lot 3	No flow	-	+	+	+
L3	Lot 1	No flow	+	+	+	+
	Lot 2	No flow	+	+	+	+
	Lot 3	No flow	+	+	+	+
P1	Lot 1	No flow	+	+	+	+
	Lot 2	No flow	+	+	+	+
	Lot 3	No flow	+	+	+	+

Note: "+"= positive result, "-"= negative result.

➢ Conclusion

The results showed that three, four and five drops of sample volume are the right volumes for testing (with clear background), demonstrating the test ability to yield correct results using different volumes of samples. Therefore, the product can accommodate a fluctuation of sample volumes.

V. Equivalence study between nasopharyngeal and nasal swabs

Completion date: 2020.08.05

➢ Objective

The purpose of this study was to compare the performance between nasal and nasopharyngeal swabs. And observe whether the swab breaks during nasal sampling.

➢ Material

✓ Description of component

BIOSYNEX COVID-19 Ag BSS rapid test
Lot: 2006189

✓ Description of samples

Positive specimen:
SARS-CoV-2 cultured virus, the material was supplied at a concentration of 4.6×10^2 $TCID_{50}$/mL.

Nasal matrix:
Human nasal swabs obtained from healthy donors confirmed negative for SARS-CoV-2 were eluted into the saline and pooled together as a negative specimen.

Nasopharyngeal matrix:
Human nasopharyngeal swabs obtained from healthy donors confirmed negative for SARS-CoV-2 were eluted into the saline and pooled together as a negative specimen.

Clinical samples:
30 healthy volunteers were recruited from the company; a nasal sample and a nasopharyngeal sample was collected from each volunteer.

➢ Method

✓ Sample preparation

Simulated nasal swab sample:
The SARS-CoV-2 cultured virus supplied at a concentration of $4.6 \times 10^2 TCID_{50}$/mL was spiked into nasal matrix in order to get the Limit of Detection (LOD) specimen (1.15×10^2 $TCID_{50}$/mL) and the 2-fold concentration of LOD specimen (2.3×10^2 $TCID_{50}$/mL). For each specimen concentration, 50µL of specimen were added to swabs to get simulated nasal swab samples and were tested in the BIOSYNEX COVID-19 Ag BSS rapid test Cassette (Swab) using the procedure appropriate for patient nasal swab specimens.

Simulated nasopharyngeal swab sample:
The SARS-CoV-2 cultured virus supplied at a concentration of $4.6 \times 10^2 TCID_{50}$/mL was spiked into nasopharyngeal matrix in order to get the Limit of Detection (LOD) specimen (1.15×10^2 $TCID_{50}$/mL) and the 2-fold concentration of LOD specimen (2.3×10^2 $TCID_{50}$/mL). For each specimen concentration, 50µL of specimen were added to swabs to get simulated nasopharyngeal swab samples and were

tested in the BIOSYNEX COVID-19 Ag BSS rapid test Cassette (Swab) using the procedure appropriate for patient nasopharyngeal swab specimens.

20 simulated nasal and nasopharyngeal swab samples for each concentration were tested.

Clinical samples:
An equivalence test was also conducted on nasal and nasopharyngeal swab samples collected from 30 healthy volunteers.

✓ Study procedure

1) The same cotton swab as in the kit was used (swabs manufactured by Jiangsu Changfeng). About 60 nasal swabs and 60 nasopharyngeal swabs were randomly selected from 200 commercial swabs.
2) Performed tests at room temperature (15-30˚C).
3) 20 simulated nasal and nasopharyngeal swab samples for each concentration were tested according to the procedure appropriate for patient swab specimens in package insert. Refer to the testing information in Table 1 below.

Table-1 Testing information of simulated swabs

Swab type	LOD specimen (1.15×10^2 $TCID_{50}$/mL)	2×LOD specimen (2.3×10^2 $TCID_{50}$/mL)	Blank swab
Nasal swab	20 Replicates	20 Replicates	20 Replicates
Nasopharyngeal swab	20 Replicates	20 Replicates	20 Replicates

1) An equivalence test was also conducted on nasal and nasopharyngeal swab samples collected from 30 healthy volunteers. A nasal swab sample and a nasopharyngeal swab sample was collected at the same time for each volunteer. These nasal and nasopharyngeal swab samples were tested according to the procedure appropriate for patient swab specimens described in package insert. Refer to the testing information in table 2 below.

Table-2 Testing information of people's nasal and nasopharyngeal swab samples

Sampling	Nasal swab sampling	Nasopharyngeal swab sampling
Person 1#	1 test	1 test
Person 2#	1 test	1 test
Person 3#	1 test	1 test
Person 4#	1 test	1 test
Person 5#	1 test	1 test
Person 6#	1 test	1 test
Person 7#	1 test	1 test
Person 8#	1 test	1 test
Person 9#	1 test	1 test
Person 10#	1 test	1 test
Person 11#	1 test	1 test

Person 12#	1 test	1 test
Person 13#	1 test	1 test
Person 14#	1 test	1 test
Person 15#	1 test	1 test
Person 16#	1 test	1 test
Person 17#	1 test	1 test
Person 18#	1 test	1 test
Person 19#	1 test	1 test
Person 20#	1 test	1 test
Person 21#	1 test	1 test
Person 22#	1 test	1 test
Person 23#	1 test	1 test
Person 24#	1 test	1 test
Person 25#	1 test	1 test
Person 26#	1 test	1 test
Person 27#	1 test	1 test
Person 28#	1 test	1 test
Person 29#	1 test	1 test
Person 30#	1 test	1 test

➢ Acceptance criteria

1) The agreement rate of nasal swab samples and nasopharyngeal swab samples was 100%.
2) Blank swabs have no interference with the test results.
3) The swab did not break during the sampling process.

➢ Results

Table 3: The results of swab type

Swab type	**LOD specimen (1.15×10^2 $TCID_{50}$/mL)**		**2×LOD specimen (2.3×10^2 $TCID_{50}$/mL)**		**Blank swab**	
	Replicates	Positive#	Replicates	Positive#	Replicates	Positive#
Nasal swab	20	20	20	20	20	0
Nasopharyngeal swab	20	20	20	20	20	0
Agreement rate %	/	100%	/	100%	/	100%

Table 2: The results of people's nasal and nasopharyngeal swab samples

Sampling	Nasal swab sampling	Nasopharyngeal swab sampling
Person 1#	-	-
Person 2#	-	-
Person 3#	-	-
Person 4#	-	-
Person 5#	-	-
Person 6#	-	-
Person 7#	-	-
Person 8#	-	-
Person 9#	-	-
Person 10#	-	-
Person 11#	-	-
Person 12#	-	-
Person 13#	-	-
Person 14#	-	-
Person 15#	-	-
Person 16#	-	-
Person 17#	-	-
Person 18#	-	-
Person 19#	-	-
Person 20#	-	-
Person 21#	-	-
Person 22#	-	-
Person 23#	-	-
Person 24#	-	-
Person 25#	-	-
Person 26#	-	-
Person 27#	-	-
Person 28#	-	-
Person 29#	-	-
Person 30#	-	-

Note: "-" means negative result.

➢ Conclusion

According to the above results, the agreement rate of nasal swab samples and nasopharyngeal swab samples was 100%. The blank swabs have no interference with the test results. During the sampling process, the swab did not break.

VI. History (changes)

Revision	Date	Part	Reason/Changes
01	2020-09-07	NA	Creation of the document
02	2020-09-16	I.2. II. 2.2 V.1.	Addition of information on the following studies: temperature flex, reading time flex, sample volume flex, precision intra-assay, precision inter-assay. Addition of the Reproducibility study – between/within lots, between/within days and between operators. Reformulation (clarification) of the study protocol of the temperature flex study.
03	2020-11-02	I.2. and III.	Addition of information on analytical sensitivity study: date of the report, place and evaluator
04	2020-11-04	Heading	Addition of the new variant BIOSYNEX® COVID-19 Ag BSX REF. SW40006_BSX
05	2021-02-11	VI.	Following new claim on the BIOSYNEX COVID-19 Ag BSS/ BIOSYNEX COVID-19 Ag BSX (utilisation with either nasopharyngeal or nasal swab), DOC 5A is updated with an equivalence study between nasal and nasopharyngeal swabs (See §VI.)