SGTi-flex

COVID-19 & Flu A/B Ag DUO

IVD REF CAGD025E CE

INTENDED USE

SGTi-flex COVID-19 & Flu A/B Ag DUO is an immunoassay for the simultaneous qualitative detection of Nucleocapsid protein antigen from SARS-CoV-2, Influenza virus type A or type B antigens in nasopharyngeal swab specimens directly collected. The test is used as an aid in the rapid diagnosis of SARS-CoV-2 and influenza A and B viral infections.

SUMMARY AND EXPLANATION

The novel coronavirus (SARS-CoV-2) was identified in December 2019, and in February 2020, the World Health Organization (WHO) officially named the disease caused by SARS-CoV-2 as COVID-19 (Coronavirus Disease 2019). Belonging to the family Coronaviridae, it has a positive-sense single-stranded RNA and can be transmitted between people. The coronaviruses identified for human infection include 229E, NL63 belonging to α-Corona viruses and HKU1, OC43, SARS-CoV, MERS-CoV belonging to β-Coronaviruses. The new coronavirus was published under the name of SARS-CoV-2, with 80% of genetic similarity to SARS-CoV by ICTV (International Committee on Taxonomy of Viruses).

COVID-19 spreads mainly through respiratory droplets, which cause lethargy, fever, dry cough, and dyspnea when infected. It can be even led to death with its severe symptoms like sepsis, MOF (Multiple Organ Failure) and ARDS (Acute Respiratory Distress Syndrome). It is more contagious than SARS which caused more than 800 deaths and 8,000 infected patients. Moreover, it has an incubation period of about 3 days to up to 16 days and becomes a big threat as infectivity appears even during the incubation period. There is currently no specific treatment for COVID-19, and rapid and accurate diagnosis is an important issue for isolation of patients with symptoms of suspected COVID-19.

Influenza is an acute respiratory disease caused by influenza virus type A or type B. Influenza is caused by the antigenic drift of the influenza virus, which causes 10 to 20% of the population to be epidemic every winter. The global pandemic of influenza A, which occurs every 10 to 40 years, is a major threat to mankind due to antigenic shifts. As a result of monitoring the national influenza epidemic in Korea, we can confirm that influenza is prevalent every winter (October to April).

Influenza is an acute febrile respiratory disease, which is accompanied by respiratory symptoms such as sore throat and cough together with the symptoms of headache, fever, chills and muscle aches. The symptoms of the patient are so diverse. There are cases of respiratory symptoms that do not have fever similar to a cold. Or there are cases typically accompanied by high fever and respiratory symptoms. Differential diagnosis is difficult because it is very similar to common colds caused by various respiratory viruses, especially in winter. However, influenza and cold are other diseases. Unlike colds, they can cause fatal complications. Differential diagnosis is needed because they can use antiviral drugs and effective vaccines.

COVID-19 and Influenza, which are respiratory diseases, have similar infection routes and some of the symptoms, so rapid and accurate differential diagnosis is very important.

PRINCIPLE

SGTi-flex COVID-19 & Flu A/B Ag DUO is an immunoassay for the simultaneous qualitative detection of Nucleocapsid protein antigen from SARS-CoV-2, Influenza virus type A or type B antigens in nasopharyngeal swab specimens directly collected. The SARS-CoV-2 or influenza A/B antigens are extracted from swab in the extraction buffer and the extracted sample solutions are loaded to the sample well of the Test Cassette. When the sample is loaded, the detection antibody binds to SARS-CoV-2 or influenza A/B antigen and flows through the membrane. The detection antibody-gold conjugate and SARS-CoV-2 or influenza A/B antigen move to the test line area and are accumulated by the capture antibody immobilized on the membrane. This leads to the generation of a reddish colored band. The intensity of the band depends on quantity of SARS-CoV-2 or influenza A/B antigen and the test results are interpreted by user's eye according to the instructions for use.

MATERIALS SUPPLIED

- Test Cassette ····· 25
- Extraction Buffer ····· 25 (0.4 mL/tube)
- Instructions for Use ····· 1
- Dropping cap ····· 25
- Sample collection swab ····· 25

STORAGE AND STABILITY

1) Store SGTi-flex COVID-19 & Flu A/B Ag DUO Test Cassette and Extraction Buffer at 2~30°C (36~86°F).
2) If SGTi-flex COVID-19 & Flu A/B Ag DUO Test Cassette and Extraction Buffer are stored in cold storage, allow them for 30 minutes to return to room temperature before testing.
3) Do not open the pouch of Test Cassette until ready to use.
4) After opening aluminum pouch, Test Cassette should be used immediately.
5) Keep away from direct sunlight.

WARNING AND PRECAUTIONS

- For *in-vitro* diagnostic use only.
- For use by trained laboratory personnel or healthcare professionals. The result of this test should not be the sole basis for the diagnosis. Confirmatory testing is required.
- Clinical diagnosis through this product should be made through a comprehensive review of the specialist based on other test methods and clinical symptoms.
- Please read the instructions carefully before you begin the test and follow the procedure correctly.
- It is prohibited to reuse Test Cassettes because they are single use only.
- The test result after the expiry date is not reliable.
- Test Cassette is sensitive to moisture and should be stored in a sealed pouch until use. Use Test Cassette immediately after opening the pouch.
- Do not use the Test Cassette if it is broken or the pouch is not stored in sealed.
- Samples and Test Cassette must be at room temperature before testing.
- It is an *in-vitro* diagnostic product and the risk of infection is low because there is no direct contact with the human body. However please be cautious when handling Test Cassette and samples because of the use of clinical samples containing potential infectious sources. Dispose of the used samples and Test Cassettes properly in accordance with the relevant regulations.
- Smoking and eating are prohibited at test site when handing specimens or kit reagents.

TEST PREPARATION

1. Test should be done immediately after sample collecting.

1) If sample swabs are not used immediately after sample collection, specimen is recommended to be stored in deep freezer at -70˚C (or in dry ice or liquid nitrogen). A freezer at -20˚C is NOT recommended.
2) If the specimen is stored at 2-8˚C, it can be stored up to 72 hours.

2. Preparation before Test

1) All samples and reagents should be stored at room temperature and stayed homogenous 15~30 minutes prior to testing.
2) Test cassette is moisture sensitive so should be used **immediately** after opening.

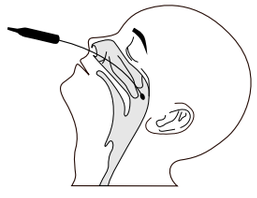
SAMPLE COLLECTION

SGTi-flex COVID-19 & Flu A/B Ag DUO can be performed with nasopharyngeal swab.

1. Remove the sealing foil from the Extraction Buffer Tube and place it in the tube rack.
2. SGTi-flex COVID-19 & Flu A/B Ag DUO uses the sample of nasopharyngeal swab.

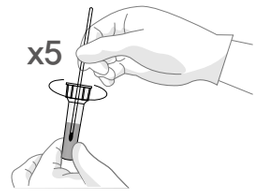
(1) Direct swab

1) Please use single use sample collecting swab.
2) Insert a nasopharyngeal swab into the nostril of the patient, swab over the surface of the posterior nasopharynx. Swab should reach depth equal to distance from nostrils to outer opening of the ear.

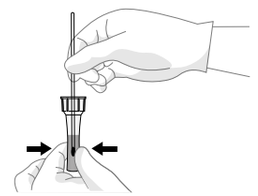


< nasopharyngeal swab >

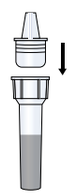
3) Gently rub and roll the swab. Leave swab in place for several seconds to absorb secretions. And slowly remove swab while rotating it.
4) Place the sample collecting swab into the Extraction Buffer Tube containing 400 µL extraction buffer and rotate it more than 5 times to allow extraction.



5) Take the sample collecting swab out by pressing and squeezing the sides of the tube to extract the remaining liquid from the swab. Used swab is classified as infectious waste and dispose of used swab properly in accordance with the relevant regulations.

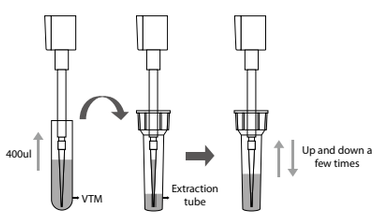


6) Press the Dropping Cap onto the Extraction Buffer Tube containing the processed sample.



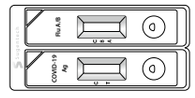
(2) Swab in Viral Transport Media (VTM)

1) Mix the specimen in VTM by vortexing.
2) Using micro pipette, transfer 400 µL of specimen in VTM to the Extraction Buffer Tube.
3) Press the Dropping Cap onto the Extraction Buffer Tube containing the processed sample.

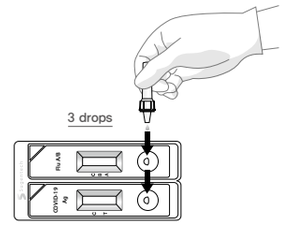


TEST PROCEDURE

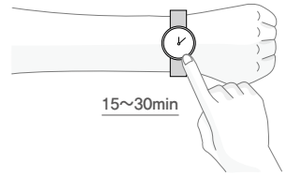
1. Open the pouch and take out the Test Cassette. Place it on a flat, dry and clean surface.



2. Invert the Extraction Buffer Tube and add 3 drops of processed sample into the sample well on the each Test Cassette.
Attention: After the drop is completely absorbed, add the next drop.



3. Read the results in 15~30 minutes after dispensing the sample. Some positive results may appear faster right after the reaction. The result after 30 minutes is invalid.



INTERPRETATION OF TEST RESULTS

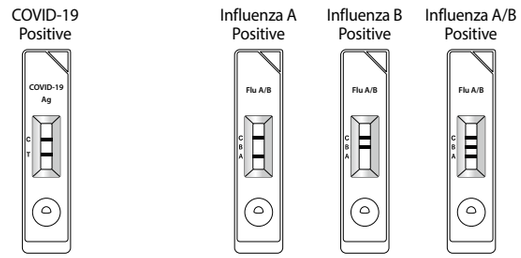
1. Positive

(1) COVID-19 Positive

- Control line (C) and Test line (T) are appeared in the result window: Positive for SARS-CoV-2.

(2) Influenza A/B Positive

- Control line (C) and Test line (A) are appeared in the result window: Positive for Influenza A.
- Control line (C) and Test line (B) are appeared in the result window: Positive for Influenza B.
- Control line (C) and two Test lines [(A) & (B)] are appeared together in the result window: Positive for both Influenza A and B.



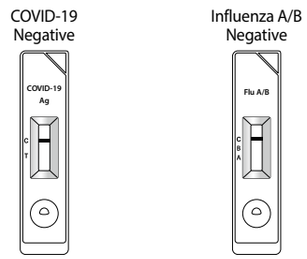
2. Negative

(1) COVID-19 Negative

- If only Control line (C) appears in the result window: Negative for SARS-CoV-2.

(2) Influenza A/B Negative

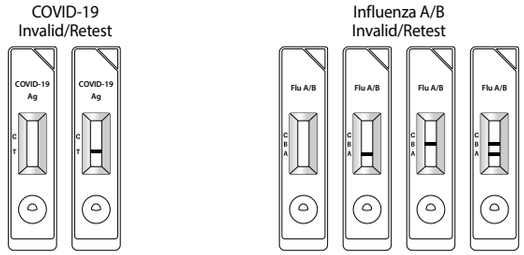
- If only Control line (C) appears in the result window: Negative for both Influenza A and B.



3. Invalid/Retest

• If Control line (C) is not appeared in the result window, it is determined to be invalid test.

• Perform test again using new Test Cassette.



QUALITY CONTROL

A procedural control is included in the test. A colored line appearing in the control line region(C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

LIMITATIONS OF THE SYSTEM

1. The test is for qualitative detection of SARS-CoV-2 and influenza A/B antigen in human nasopharyngeal and it does not indicate the quantification of the virus.
2. The test is for *in-vitro* diagnostic use only.
3. Negative results do not rule out SARS-CoV-2 or influenza A/B infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
4. Test result may vary due to storage and stability of specimen and Extraction Buffer.
5. SARS-CoV may cause positive results. SARS-CoV can be detected as a cross reaction.
6. Specimen with rarely high reactivity for a particular antibody such as anti-mouse antibody can affect the performance of the test results.
7. False positive results might be happened due to the non-specific cross-reaction of some components in the specimen to the antibody.
8. Interfering materials above the limited concentrations, untested interfering substance to be administered to the specimen and other substances that may affect the results may affect the results.

PERFORMANCE CHARACTERISTICS

1. COVID-19

(1) Limit of Detection (LOD)

The sensitivity using direct swab is 3.5 x10^2 $TCID_{50}$/mL based on Gamma Irradiated SARS-CoV-2 (BEI Resources, NR-52287, USA-WA1/2020).

The sensitivity using swab in viral transport media is 2.8 x10^3 $TCID_{50}$/mL based on Gamma Irradiated SARS-CoV-2 (BEI Resources, NR-52287, USA-WA1/2020).

(2) Cross-Reactivity

SGTi-flex COVID-19 Ag was evaluated with 20 other virus and 12 bacteria. The results show that the SGTi-flex COVID-19 Ag has no cross-reactivity with samples containing tested viruses and bacteria except on SARS -CoV. The results showed no microbial interference with the organisms at the concentrations tested.

Microorganisms tested for cross reactivity and interference

No	Virus	Bacteria
1	Alpha Coronavirus (229E)	*Hemophilus influenzae*
2	Beta Coronavirus OC43	*Streptococcus Pneumoniae antigen*
3	Human Coronavirus NL63	*Candida albicans*
4	Beta Coronavirus (MERS) NP protein	Pooled human nasal fluid
5	Beta Coronavirus (SARS-CoV) NP protein	*Bordetella pertussis*
6	Adenovirus type 5	*Mycoplasma pneumoniae*
7	Human Metapneumovirus	*Chlamydophila pneumoniae*
8	Parainfluenza Virus serotype 1	*Legionella pneumophila*
9	Parainfluenza Virus serotype 2	*Staphylococcus epidermidis culture*
10	Parainfluenza Virus serotype 3	*Mycobacterium tuberculosis*
11	Parainfluenza Virus serotype 4	*Pneumocystis jirovecii(PJP)*
12	Influenza A/H1N1	*Staphylococcus aureus*
13	Influenza A/H3N2	
14	Influenza A/H5N1	
15	Influenza B	
16	Respiratory Syncytial virus type A	
17	Respiratory Syncytial virus type B	
18	Rhinovirus group A	
19	Human Corona virus HKU1	
20	Enterovirus	

Pneumocystis jirovecii and Human coronavirus HKU1, which were not available for wet testing, were analyzed *in silico* via Basic Local Alignment Search Tool managed by National Center for Biotechnology Information to defined cross-reactivity or interference.

For *Pneumocystis jirovecii*, 45.4% homology was found only a particular part of sequence across 9% of the sequence. It is very unlikely that cross-reaction will occur.

The protein sequences between nucleocapsid protein sequence of human coronavirus HKU1 and nucleocapsid protein of SARS CoV-2 has only 36.7% homology across 82% of sequences. The result of homology between two viruses is relatively very low, but cross-reaction can occur.

(3) Analytical Specificity – Interference test

Various concentrations of potential interfering substances were prepared in negative and positive sample. The results show that the SGTi-flex COVID-19 Ag has no interference by the potential interfering substances below which may exist in specimen, such as drugs, and chemical and biological analytes.

Interfering substances

No.	Substance	Concentration	No.	Substance	Concentration
1	Albumin	50 mg/ml	15	Tamiflu (Oseltamivir)	6 mg/ml
2	Glucose	1.2 mg/ml	16	Acetaminophen	10 mg/ml
3	Hemoglobin	4 mg/ml	17	Ibuprofen	5 mg/ml
4	Bilirubin	5 mg/ml	18	Aspirin	2 mg/mL
5	mucin	1.0 %	19	Naso GEL	5% v/v
6	Whole blood	4.0 %	20	Oxymetazoline	0.1 mg/mL
7	Phenylephrine hydrochloride	10 mg/ml	21	Cromolyn	0.03 mg/mL
8	Dexamethasone	0.6 mg/ml	22	Zicam	5% v/v
9	Flunisolide	2.5 mg/ml	23	Alkalol	10% v/v
10	Budesonide	1 mg/ml	24	Mupirocin	10 mg/mL
11	Benzocaine	5 mg/ml	25	Fluticasone Propionate	5% v/v
12	Menthol	40 mg/ml	26	Sore Throat Phenol Spray	15% v/v
13	Zanamivir	10 mg/ml	27	Heparin sodium salt	3000 U/L
14	Tobramycin	20 mg/ml			

(4) Clinical Agreement Study

Comparison studies between the test device (SGTi-flex COVID-19 Ag) and the predicate device (Reference method, real time RT-PCR) were conducted by lab professionals, using total 377 specimens.

The results for nasopharyngeal swab showed the accuracy (overall percent agreement) was 97.35%. The sensitivity and specificity (positive and negative agreements) were 95.07% and 99.38%, respectively.

		Reference method		
		Positive	Negative	Total
Test device (SGTi-flex COVID-19 Ag)	Positive	135	1	136
	Negative	7	159	166
	Total	142	160	302

(1) Accuracy (Overall percent agreement) : 97.35% (95% CI: 94.86%~98.65%)

(2) Sensitivity (Positive percent agreement) : 95.07% (95% CI: 90.17%~97.59%)

(3) Specificity (Negative percent agreement) : 99.38% (95% CI: 96.55%~99.89%)

2. Influenza A/B

(1) Limit of Detection (LOD)

1) Influenza Antigen A (H1N1): 2.5 ng/mL

2) Influenza Antigen A (H3N2): 5 ng/mL

3) Influenza Antigen B : 5 ng/mL

(2) Cross-Reactivity

Influenza A/B was evaluated with a total of 23 microorganisms. The 14 viruses were evaluated at concentrations for Ct values. The 9 bacteria were tested at a target concentration of approximately 10^8 cells/mL. The results show that the SGTi-flex Influenza A/B has no cross reactivity with added substances such as viruses, bacteria and human influenza viruses.

Virus

1	Rota virus Antigen	8	Human Coxsackie B4
2	Norovirus GII	9	Epstein-Barr Virus
3	Parainfluenza Virus serotype 1	10	Human Norovirus GI
4	Parainfluenza Virus serotype 2	11	Human Meta pneumovirus
5	Parainfluenza Virus serotype 3	12	Human Rhinovirus Genogroup A
6	Parainfluenza Virus serotype 4	13	Human Coronavirus 229E
7	Human Respiratory syncytial virus A2	14	Human Measles Mvi/Mos cow Rus/1988 Genotype A

Bacteria

15	Group A streptococcus antigen	20	Lactobacillus plantarum culture
16	Group B streptococcus antigen	21	Legionella spp culture
17	Streptococcus Pneumoniae antigen	22	Pseudomonas aeruginosa culture
18	Escherichia coli culture	23	Staphylococcus epidermidis culture
19	Corynebacterium glutamicum culture		

(3) Analytical Specificity – Interference test

Various concentrations of potential interfering substances were prepared in negative and positive sample. The results show that the SGTi-flex Influenza A/B has no interferences by the potential interfering substances below which may exist in specimen, such as drugs, chemical and biological analytes.

Interfering substances

No.	Substance	Concentration	No.	Substance	Concentration
1	Albumin	50mg/mL	8	Budesonide	1mg/mL
2	Glucose	1.2mg/mL	9	Benzocaine	200mg/mL
3	Hemoglobin	200mg/mL	10	Menthol	40mg/mL
4	Bilirubin	15mg/mL	11	Zanamivir	10mg/mL
5	Phenylephrine hydrochloride	10mg/mL	12	Tobramycin	40mg/mL
6	Dexamethasone	0.6mg/mL	13	Tamiflu(Oseltamivir)	6mg/mL
7	Flunisolide	2.5mg/mL			

(4) Clinical Agreement Study

Total Clinical Sensitivity and Specificity

Influenza Specimen		SGTi-flex Influenza A/B	
		Positive	Negative
Positive	145	128	17
Negative	136	0	136

• Clinical Sensitivity: 88.27% (95% CI: 83.03%-93.51%)

• Clinical Specificity: 100%

Influenza Positive Specimen

Influenza Positive Specimen		SGTi-flex Influenza A/B	
		Positive	Negative
A type Positive	76	65	11
B type Positive	69	63	6

• A Type Clinical Sensitivity: 85.53% (95% CI: 77.63%-93.43%)

• Type Clinical Sensitivity: 91.30% (95% CI: 84.65%-97.95%)

REFERENCES

1. WHO, Coronavirus disease 2019 (COVID-19) Situation report
2. J.virol. Methods. 2008, 152(1-2): 77-84, A rapid point of care immunoswab assay for SARS-CoV detection
3. WHO Guide for field operations ; Collecting, preserving and shipping specimens for the diagnosis of avian influenza A(H5N1) virus infection.(October 2006)

EXPLANATION OF SYMBOLS USED ON PACKAGE

IVD	*In-vitro* diagnostic medical device	Σ 25	Contains sufficient for 25 tests
	Consult instructions for use.	2°C 30°C	Store between 2°C and 30°C
LOT	Batch code		Use by
	Manufacturer	EC REP	Authorized representative in the European community
	Do not reuse	REF	Catalogue number
	Caution, consult accompanying documents	CE	The device conforms to EU-regulations.

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