510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I. Background Information:

A 510(k) Number

K243256

B Applicant

Wondfo USA Co., Ltd.

C Proprietary and Established Names

WELLlife COVID-19 / Influenza A&B Home Test and WELLlife COVID-19 / Influenza A&B AntigenTest

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SCA	Class II	21 CFR 866.3987 - Multi-Analyte Respiratory Virus Antigen Detection Test	MI - Microbiology

II. Submission/Device Overview:

A Purpose of Submission:

To obtain a 510(k) clearance for the WELLlife COVID-19 / Influenza A&B Home Test (for OTC use) and WELLlife COVID-19 / Influenza A&B Antigen Test (Professional Use).

B Measurand

Influenza type A and type B nucleoprotein and SARS-CoV-2 nucleocapsid antigens.

C Type of Test

Qualitative Lateral flow Immunoassay

III. Intended Use/Indications for Use:

A Intended Use(s):

Same as Indications for Use below.

B Indication(s) for Use:

WELLlife COVID-19 / Influenza A&B Home Test

The WELLlife COVID-19 / Influenza A&B Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar. This test is for non-prescription home use by individuals aged 14 years or older testing themselves, or adults testing individuals aged 2 years or older.
All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection with influenza, SARS-CoV-2 or other pathogens.
Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up care from their healthcare provider.
Positive results do not rule out co-infection with other respiratory pathogens, and therefore do not substitute for a visit to a healthcare provider or appropriate follow-up.

WELLlife COVID-19 / Influenza A&B Antigen Test

The WELLlife COVID-19 / Influenza A&B Antigen Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar. This test is for use by individuals aged 14 years or older testing themselves, or adults testing aged 2 years or older.
All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection with influenza, SARS-CoV-2, or other pathogens.
Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up care from their healthcare providers.
Positive results do not rule out co-infection with other respiratory pathogens.
Test results should not be used as the sole basis for treatment or other patient management decisions.

C Special Conditions for Use Statement(s):

OTC - Over The Counter

D Special Instrument Requirements:

Not applicable. There is no associated instrumentation as the test is visually read.

E Device/System Characteristics:

1. Device Description:

The WELLlife COVID-19 / Influenza A&B Home Test and the WELLlife COVID-19 / Influenza A&B Antigen Test is a lateral flow immunoassay intended for the qualitative detection and differentiation of SARS-CoV-2, influenza A, and influenza B protein antigens. The test has two versions, one for over the counter (OTC) use, the (WELLlife COVID-19 / Influenza A&B Home Test), and one for professional use (WELLlife COVID-19 / Influenza Antigen A&B)(generically referred to as WELLlife COVID-19 / Influenza A&B Tests for the remainder of this document). Both versions of the WELLlife COVID-19 / Influenza A&B Tests that have an identical general design and are intended to separately detect antigen from influenza A, influenza B, and SARS-CoV-2 in anterior nares swabs from individuals with signs and symptoms of respiratory infection within the first four (4) days of symptom onset. The WELLlife COVID-19 / Influenza A&B Test is validated for testing direct anterior nares samples (ANS) without transport media. The WELLlife COVID-19 / Influenza A&B Test does not use biotin-streptavidin/avidin chemistry.

The WELLlife COVID-19 / Influenza A&B Test consists of the following components:

- Sealed Test Cassettes
- Buffer Tubes
- Swabs (sterile)
- Test box with a slot in the top right corner that serves as the tube holder
- Quick Reference Instructions (QRI)

The test cassette in the test kit is assembled with a test strip in a plastic housing that contains a nitrocellulose membrane with four lines: three test lines (Flu A line, Flu B line and SARS-CoV-2 line) and a control line (C line).

2. Principle of Operation:

The test procedure requires the solubilization of the nucleoproteins (if present) from an AN sample by mixing the swab in extraction buffer liquid for lysis.

The lysed specimen extract is then deposited into the single sample well of the test cassette. If influenza A, influenza B, and/or SARS-CoV-2 viral antigen(s) are present in the sample, they will form a complex with the test's dye-conjugated primary antibodies specific to influenza A, influenza B, and/or SARS-CoV-2. These antigen-antibody-dye complexes will migrate through the test strip membrane via capillary action and bind to a second antibody that is immobilized at the test line(s) of the membrane. This interaction captures, and thereby concentrates, the color-conjugated virus antigen-antibody complexes at the analyte specific test lines, generating a colored line at the specific position of each immobilized capture antibody. Unbound dye-conjugates primary antibodies will continue to migrate to the control (C) position, where immobilized antibodies will capture the free dye-conjugated primary antibodies and form a colored control line.

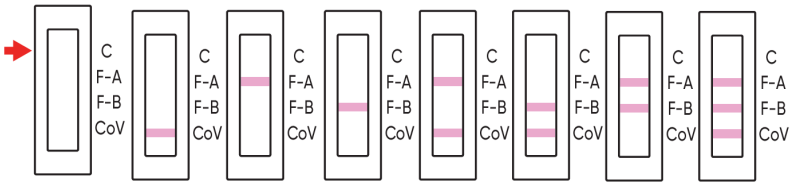
A pink to red line must appear in the control region of the test strip for the results to be considered valid. The appearance of a second and possibly third or fourth light pink to red line in the test line region for influenza A, influenza B, and/or SARS-CoV-2 indicates a positive result for the respective antigen/s. A visible control line with no visible test line in any of the test result windows indicates a negative result. The control line must appear for a result to be valid; tests without a control line cannot be interpreted and must be repeated with a fresh sample and new test device.

3. Interpretation of Results:

The qualitative results of the WELLlife COVID-19 / Influenza A&B Test are visually interpreted by the user. Examples of the positive, negative, and invalid results interpretations are provided within the "Interpretation of Results" section of the QRI.

Results interpretation is described in the figure below.
The C = Control Line, F-A = Flu A Test Line, F-B = Flu B Test Line and CoV = COVID-19 Test Line.

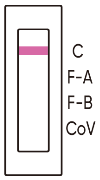
Invalid Result



A red line should always appear at the 'C' position; this is a control line and signals that the test is working properly. A pink to red line should be visible at the control line 'C' in the results window. If a line is not visible at "C", even if any other line is visible in the results window, the result is considered invalid.

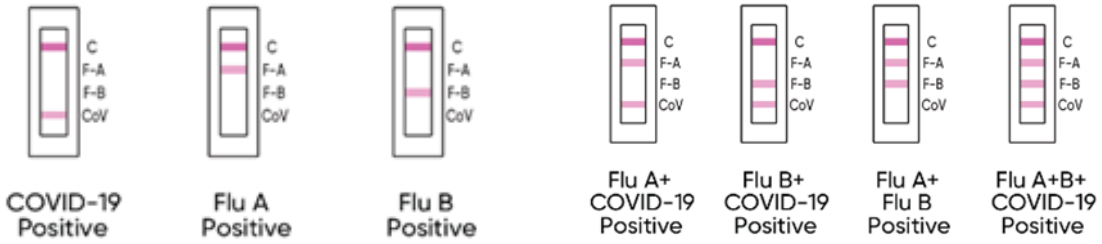
If no band is observed at the C line, the user should NOT CONTINUE reading the results. It means the test is invalid. Testing should be repeated with a new sample and new test kit materials.

Negative Result



If a control 'C' line is visible and no band is observed at 'F-A', 'F-B' or 'CoV', it means the test is negative. The Flu A, Flu B or COVID-19 virus have not been detected. If respiratory symptoms persist, follow-up care with the user's healthcare provider should be sought.

Positive Result



If the control line at "C" is visible and any other line or multiple lines on 'F-A', 'F-B' and/or 'CoV' are visible, the test is positive for that virus.

NOTE: Any pink to red test line, no matter how faint, should be considered a positive result when the control line is also present.

IV. Substantial Equivalence Information:

A Predicate Device Name:

Healgen Rapid Check COVID-19/Flu A&B Antigen Test

B Predicate 510(k) Numbers:

DEN240029

C Comparison with Predicate(s):

Device & Predicate Device(s):	K243256	DEN240029
Device Trade Name	WELLlife COVID-19 / Influenza A&B Home Test WELLlife COVID-19 / Influenza A&B Antigen Test	Healgen Rapid Check COVID-19/Flu A&B Antigen
General Device Similarities		
Intended Use/ Indications for Use	**WELLlife COVID-19/Influenza A&B Home Test** The WELLlife COVID-19 / Influenza A&B Home Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2	The Healgen Rapid Check COVID-19/Flu A&B Antigen Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab samples

	and influenza can be similar. This test is for non-prescription home use by individuals aged 14 years or older testing themselves, or adults testing individuals aged 2 years or older. All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection with influenza, SARS-CoV-2, or other pathogens. Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up care from their healthcare providers. Positive results do not rule out co-infection with other respiratory pathogens, and therefore do not substitute for a visit to a healthcare provider or appropriate follow-up. **WELLlife COVID-19/Influenza A&B Antigen Test** The WELLlife COVID-19 / Influenza A&B Antigen Test is a lateral flow immunochromatographic assay intended for the qualitative detection and differentiation of influenza A, and influenza B nucleoprotein antigens and SARS-CoV-2 nucleocapsid antigen directly in anterior nasal swab samples from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar. This test is for use by individuals aged 14 years or older testing themselves, or adults testing aged 2 years or older. All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection	from individuals with signs and symptoms of respiratory tract infection. Symptoms of respiratory infections due to SARS-CoV-2 and influenza can be similar. This test is for non-prescription home use by individuals aged 14 years or older testing themselves, or adults testing individuals aged 2 years or older. All negative results are presumptive and should be confirmed with an FDA-cleared molecular assay when determined to be appropriate by a healthcare provider. Negative results do not rule out infection with influenza, SARS-CoV-2 or other pathogens. Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up care from their healthcare provider. Positive results do not rule out co-infection with other respiratory pathogens, and therefore do not substitute for a visit to a healthcare provider or appropriate follow-up.

	with influenza, SARS-CoV-2, or other pathogens. Individuals who test negative and experience continued or worsening respiratory symptoms, such as fever, cough and/or shortness of breath, should seek follow-up care from their healthcare providers. Positive results do not rule out co-infection with other respiratory pathogens. Test results should not be used as the sole basis for treatment or other patient management decisions.	
Test Principle	Lateral flow immunoassay	Same
Sample type	Anterior nasal swab specimens	Same
Viral targets	• SARS-CoV-2 nucleocapsid protein antigens • Nucleoprotein antigen from influenza A virus • Nucleoprotein antigen from influenza B virus	Same
Assay Type	Qualitative	Same
Mode of Results	Visual	Same
Assay Control	Internal procedural control	Same
Storage Temperature	2°C to 30°C	Same
General Device Characteristic Differences		
Time to Result	15 minutes – 20 minutes	10 minutes – 20 minutes

V. Standards/Guidance Documents Referenced:

Document Title	Issued by	Applicable study	Purpose
Special Controls			
Special controls for multi-analyte respiratory virus antigen detection test 21 CFR 866.3987	FDA/CDRH	All Studies	General Use
Guidance Documents			
Guidance: *Submission and review of sterility information in premarket notification (510(k)) submissions for devices labeled as sterile.*	FDA/CDRH	Sterility	General Use
Guidance: *Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process"*		Biocompatibility	General Use

ISO Standards			
Standard: ISO 11135:2014, *Sterilization of health care products - Ethylene oxide - Requirements for development, validation and routine control of a sterilization process for medical devices*	ISO	Sterility	Declaration of Conformity provided
Standard: ISO 10993-7, *Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide Sterilization Residuals*			Declaration of Conformity provided
Standard: ISO 10993-1, *Biological Evaluation of Medical Devices – Part 1: Evaluation and testing within a risk management process*		Biocompatibility	General Use
Standard: ISO 10993-5, *Third edition, Biological evaluation of medical devices – Part 5: Tests for in vitro cytotoxicity*			Declaration of Conformity provided
Standard: ISO 10993-10, *Third Edition, Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization*			Declaration of Conformity provided

VI. Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision:

A precision study was conducted to assess variability between reagent lots, days, runs and operators. Three concentrations of heat inactivated SARS-CoV-2 USA-WA1/2020, live Flu A: H1N1pdm09/A/Victoria/4897/2022, and live Flu B: Yamagata/B/Florida/4/2006 were spiked into pooled negative swab matrix (PNSM) to prepare the following test sample panel members:

1. Negative Sample of each analyte
2. 0.9xLoD of each analyte (individually spiked)
3. Co-spike of SARS-CoV-2 (0.9xLoD) and Flu B (0.8xLoD)
4. Low Positive Sample at 2xLoD for each analyte (individually spiked)
5. Positive Sample at 5xLoD for each analyte (individually spiked)

Samples were blinded and randomized before allotting them to the operators. 50µL of each sample was applied to dry nasal swabs and processed per the IFU of the candidate device. All panel members were tested with three device lots, each in 2 runs per day for each of two operators, and the study was conducted for 10 days (i.e., 1 site x 3 lots x 2 operators x 2 runs per day with 1 replicate each x 10 days). 120 results were obtained for each panel member. All replicates prepared at 2xLoD and 5xLoD, demonstrated 100% agreement across the operators, lots, days and runs tested.

To assess between-lot variability, an additional precision study was conducted with a negative and a lower positive sample (i.e., <1xLoD) that could be expected to only generate positive results approximately 95% of the time instead of 100%. The same sample preparation materials and testing procedure were used as for the original study above. These samples were tested as follows: 1 site x 3 lots x 2 operators x 2 runs per day x 2 sample replicates per run x 3 days and resulted in a total of 72 replicates per panel member. As

expected for a sample with analyte concentrations below the LoD, the precision for the <1xLoD sample was less than 100%.

Together these two precision studies verified acceptable between-lot variability for samples at or above the LoD of the test.

Table 1. Precision study results (greyed cells present positive agreement and non-greyed cells present negative agreement).

Analyte in sample	Concentration	Analyte Test Line	Agreement with Expected Results (# with expected results/# total tested			Percent Agreement (n/N) (95% CI)
			Lot 1	Lot 2	Lot 3	
Negative		SARS-CoV-2	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu A	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu B	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
FluB +SCV2	0.8 x LoD 0.9 x LoD	SARS-CoV-2	66.67% (16/24)	91.67% (22/24)	83.34% (20/24)	80.56% (58/72) (69.97 - 88.05%)
		Flu A	100% (24/24)	100% (24/24)	100%* (24/24)	100% (72/72) (94.94 - 100%)
		Flu B	91.67% (22/24)	95.84% (23/24)	75% (18/24)	87.50% (63/72) (77.92 - 93.28%)
Flu A	0.9 x LoD	SARS-CoV-2	100% (24/24)	100% (24/24)	100% (24/24)	100% (72/72) (94.94 - 100%)
		Flu A	83.33% (20/24)	100% (24/24)	79.17% (19/24)	87.50% (63/72) (77.92 - 93.28%)
		Flu B	100% (24/24)	100% (24/24)	100% (24/24)	100% (72/72) (94.94 - 100%)
SCV2	2x LoD	SARS-CoV-2	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu A	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu B	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
Flu A		SARS-CoV-2	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu A	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu B	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
Flu B		SARS-CoV-2	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu A	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu B	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)

Analyte in sample	Concentration	Analyte Test Line	Agreement with Expected Results (# with expected results/# total tested			Percent Agreement (n/N) (95% CI)
			Lot 1	Lot 2	Lot 3	
SCV2	5x LoD	SARS-CoV-2	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu A	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu B	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
Flu A		SARS-CoV-2	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu A	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu B	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
Flu B		SARS-CoV-2	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu A	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)
		Flu B	100% (40/40)	100% (40/40)	100% (40/40)	100% (120/120) (96.9 - 100%)

2. Linearity:

Not applicable; the device is a qualitative assay with binary visually-read results.

3. Analytical Specificity/Interference:

a. Cross Reactivity and Microbial Interference

Cross-reactivity and microbial interference studies were conducted to determine if other respiratory pathogens/microbial flora that may be present in nasal swab samples could cause a false positive test result or interfere with a true positive test result. A panel of microorganisms commonly found as either pathogens or normal flora in respiratory samples were individually spiked into pooled nasal wash (PNW). In the cross-reactivity study, the organisms were evaluated for their ability to cross-react with the test by adding 50µl of each sample directly to the test swab and then processing the sample swabs per the IFU. Each organism was tested in replicates of three (3) without SARS-CoV-2, Influenza A, or Influenza B present in the sample.

The microbial interference testing was conducted in the same manner, but samples were prepared in the presence of UV-inactivated SARS-CoV-2, live influenza A and B co-spiked into the samples at 3x LoD. The testing was performed in triplicates for each microorganism. Results are summarized in Table 2. Neither cross-reactivity nor microbial interference was observed for any of the tested microorganisms at the concentration used in the study.

Table 2. Cross reactivity and microbial interference results

Microorganism	Test Concentration	Cross-reactivity (# pos / total)	Interference (# pos / total)
SARS-CoV-1	1.25 x 10^5 PFU/ml	0/3	3/3
MERS-coronavirus	1.47 x 10^5 $TCID_{50}$/mL	0/3	3/3
Human coronavirus OC43	7.00 x 10^5 $TCID_{50}$/mL	0/3	3/3
Human coronavirus 229E	1.58 x 10^5 $TCID_{50}$/mL	0/3	3/3
Human coronavirus NL63	7.05 x 10^4 $TCID_{50}$/mL	0/3	3/3
Adenovirus, Type 1 (Adenoid 71)	2.23 x 10^5 $TCID_{50}$/mL	0/3	3/3
Adenovirus Type 7	1.58 x 10^5 $TCID_{50}$/mL	0/3	3/3
Cytomegalovirus	7.05 x 10^4 $TCID_{50}$/mL	0/3	3/3
Epstein Barr Virus	1.83 × 10^6 CP/mL	0/3	3/3
Human Metapneumovirus (hMPV)	3.50 x 10^5 $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 1	2.00 x 10^5 $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 2	1.75 x 10^5 $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 3	7.00 x 10^5 $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 4	2.39 x 10^5 $TCID_{50}$/mL	0/3	3/3
Enterovirus Type (e.g. 68), Species D Type 68	2.23 x 10^5 $TCID_{50}$/mL	0/3	3/3
Respiratory syncytial virus A	3.50 x 10^5 $TCID_{50}$/mL	0/3	3/3
Respiratory syncytial virus B	2.29 x 10^5 $TCID_{50}$/mL	0/3	3/3
Rhinovirus 1A	7.05 x 10^4 $TCID_{50}$/mL	0/3	3/3
Bordetella pertussis	2.90 x 10^8 CFU/mL	0/3	3/3
Candida albicans	1.21 × 10^7 CFU/mL	0/3	3/3
Chlamydia pneumoniae	4.33 × 10^6 IFU/mL	0/3	3/3
Corynebacterium xerosis	2.30 × 10^7 CFU/mL	0/3	3/3
Escherichia coli	1.79 x 10^8 CFU/mL	0/3	3/3
Hemophilus influenzae	9.68 × 10^6 CFU/mL	0/3	3/3
Lactobacillus acidophilus	1.21 × 10^7 CFU/mL	0/3	3/3
Legionella spp pneumophila	6.50 × 10^6 CFU/mL	0/3	3/3
Moraxella catarrhalis	2.50 x 10^8 CFU/mL	0/3	3/3
Mycoplasma pneumoniae	2.50 × 10^7 CFU/mL	0/3	3/3
Mycobacterium tuberculosis	3.03 × 10^6 CFU/mL	0/3	3/3
Neisseria meningitidis	3.43 × 10^6 CFU/mL	0/3	3/3
Neisseria elongata	2.68 x 10^8 CFU/mL	0/3	3/3
Pneumocystis jirovecii	1.30 × 10^7 CFU/mL	0/3	3/3
Pseudomonas aeruginosa	3.45 x 10^8 CFU/mL	0/3	3/3
Staphylococcus aureus	2.60 x 10^8 CFU/mL	0/3	3/3
Staphylococcus epidermidis	9.00 × 10^7 CFU/mL	0/3	3/3
Streptococcus salivarius	1.01 × 10^6 CFU/mL	0/3	3/3
Streptococcus pneumoniae	1.81 × 10^7 CFU/mL	0/3	3/3
Streptococcus pyogenes	7.50 × 10^7 CFU/mL	0/3	3/3
Measles	8.48 x 10^5 $TCID_{50}$/mL	0/3	3/3
Mumps	8.48 x 10^5 $TCID_{50}$/mL	0/3	3/3
PNW	N/A	0/3	3/3
Human coronavirus HKU1	1.74 × 10^7 GE/mL	0/3	3/3

b. Exogenous and Endogenous Interference Study

The WELLlife COVID-19 / Influenza A&B Test was evaluated for performance in the presence of potentially interfering substances that might be present in respiratory specimens.

Negative PNW specimens were evaluated in triplicate to confirm that the potentially interfering substances were not cross-reactive with the test. Contrived positive samples containing 3x co-spiked analytes for SARS-CoV-2, Influenza A H1N1, and Influenza B Yamagata in PNSM (same as the strains tested in the co-spiked LoD study) were also evaluated in the presence of the interfering substances in triplicate to confirm these substances to not interfere with the detection of the target analytes. Testing was performed with a panel of endogenous and exogenous substances diluted in PNSM to the recommended concentration. Results are summarized in Table 3.

Table 3. Endogenous/exogenous interfering substances study results

Potential Interferent	**Concentration**	**Without Analytes (# pos / total)***			**With Analytes (3x LoD, co-spiked) (# pos / total)***		
		SCV2	**Flu A**	**Flu B**	**SCV2**	**Flu A**	**Flu B**
Human Whole Blood (EDTA tube)	4% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Leukocytes	1×10^6 cells/mL	0/3	0/3	0/3	3/3	3/3	3/3
	5×10^5 cells/mL	NT	NT	NT	3/3	3/3	3/3
Mucin	0.50%	0/3	0/3	0/3	3/3	3/3	3/3
Mucin (Bovine submaxillary glands Type I-S)	2.5 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Chloraseptic (Menthol/Benzocaine)	1.5 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Zinc (TheraZinc Throat Spray)	15% v/v	0/3	3/3	0/3	3/3	3/3	3/3
	5% v/v	0/3	3/3	0/3	NT	NT	NT
	2.5% v/v	0/3	0/3	0/3	NT	NT	NT
	1.5% v/v	0/3	0/3	0/3	NT	NT	NT
Throat lozenges (Menthol/Benzocaine)	3 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Naso GEL (NeilMed)	5% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal gel (Galphimia glauca, Histanium hydrocloricum, Luffa operculata, Sulfur)	1.25%	0/3	0/3	0/3	3/3	3/3	3/3
Nasal Drops (Phenylephrine)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal Spray (Oxymetazoline)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal Spray (Cromolyn)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal spray (Saline)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal spray (Alkalol)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Zicam	5% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Zicam nasal spray (Galphimia glauca, Luffa operculata)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3

Potential Interferent	Concentration	Without Analytes (# pos / total)*			With Analytes (3x LoD, co-spiked) (# pos / total)*		
		SCV2	Flu A	Flu B	SCV2	Flu A	Flu B
Homeopathic (Alkalol)	10% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Homeopathic allergy relief (Histaminum hydrochloricum)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Sore Throat Phenol Spray	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Fluticasone Propionate	5% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Nasal corticosteroid (Fluticasone)	15% v/v	0/3	0/3	0/3	0/3	0/3	0/3
	5% v/v	NT*	NT	NT	3/3	3/3	3/3
	1.5% v/v	NT	NT	NT	3/3	3/3	3/3
Nasal corticosteroid (Dexamethasone)	1 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Nasal corticosteroid (Triamcinolone)	15% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Tamiflu (Oseltamivir Phosphate)	5 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Tobramycin	4 µg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Mupirocin	10 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
FluMist/ FluMist Quadrivalent Live intranasal influenza virus vaccine	15% v/v	0/3	3/3	3/3	3/3	3/3	3/3
	6% v/v	0/3	3/3	0/3	3/3	3/3	3/3
	3% v/v	0/3	3/3	0/3	3/3	3/3	3/3
	1.5% v/v	0/3	3/3	0/3	3/3	3/3	3/3
	0.75% v/v	0/3	3/3	0/3	3/3	3/3	3/3
	0.375% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Zanamivir	282 ng/mL	0/3	0/3	0/3	3/3	3/3	3/3
Remdesivir	10 mg/mL	0/3	0/3	0/3	3/3	3/3	3/3
Biotin	3,500 ng/mL	0/3	0/3	0/3	3/3	3/3	3/3
Body & Hand Lotion	0.5% w/v	0/3	0/3	0/3	3/3	3/3	3/3
Body Lotion, with 1.2% dimethicone	0.5% w/v	0/3	0/3	0/3	3/3	3/3	3/3
Hand Lotion	5% w/v	0/3	0/3	0/3	3/3	3/3	3/3
Hand Sanitizer with Aloe, 62% ethyl alcohol	5% v/v	0/3	0/3	0/3	3/3	3/3	3/3
Hand Sanitizer cream lotion	15% v/v	0/3	0/3	0/3	3/3	3/3	0/3
	7.5% v/v	-	-	-	3/3	3/3	3/3
Hand Sanitizer, 80% ethanol, fast drying	15% v/v	3/3	0/3	2/3	3/3	3/3	3/3
	7.5% v/v	3/3	0/3	1/3	NT	NT	NT
	3.75% v/v	3/3	0/3	0/3	NT	NT	NT
	1.875% v/v	0/3	0/3	0/3	NT	NT	NT
Hand soap liquid gel	10% w/v	0/3	0/3	0/3	3/3	3/3	0/3
	1% w/v	NT	NT	NT	3/3	3/3	0/3
	0.1% w/v	NT	NT	NT	3/3	3/3	0/3
	0.5% w/v	NT	NT	NT	3/3	3/3	3/3

**NT: Not tested*

c. Competitive Interference:

Competitive interference of the test's analytes was tested with different combinations of low (3x LoD) and high concentrations of Flu A, Flu B and SARS-CoV-2 prepared in PNW were added onto a swab and then tested with one reagent lot. The study used inactivated SARS-CoV-2 but live influenza A and 2 live influenza B virus strains.

The table below summarizes the results of the competitive interference study. For each condition tested all three replicates tested at the low target analyte condition tested positive in the presence of a second target analyte at high concentrations. No false positive results were observed.

Table 4. Competitive interference results

SARS-CoV-2&Influenza A &Influenza B Virus (Yamagata Lineage)					
SARS-CoV-2 (USA-WA1/2020)		**Influenza A Virus (H1N1pdm09) A/Victoria/4897/2022**		**Influenza B Virus (Yamagata Lineage) B/Florida/4/2006**	
Concentration ($TCID_{50}$/mL)	**Percent Agreement**	**Concentration ($TCID_{50}$/mL)**	**Percent Agreement**	**Concentration ($TCID_{50}$/mL)**	**Percent Agreement**
-	100%	**High**	100%	**Low**	100%
Low	100%	**High**	100%	**-**	100%
Low	100%	**High**	100%	**Low**	100%
-	100%	**Low**	0	**High**	100%
-	100%	**Low**	0	**High***	100%
-	100%	**Low**	100%	**High*****	100%
-	100%	**Low**	100%	**High****	100%
Low	100%	**-**	100%	**High**	100%
Low	100%	**Low**	0	**High**	100%
Low	100%	**Low**	0	**High***	100%
Low	100%	**Low**	100%	**High*****	100%
Low	100%	**Low**	100%	**High****	100%
High	100%	**Low**	100%	**-**	100%
High	100%	**-**	100%	**Low**	100%
High	100%	**Low**	100%	**Low**	100%
SARS-CoV-2&Influenza A &Influenza B Virus (Victoria Lineage)					
SARS-CoV-2 (USA-WA1/2020)		**Influenza A Virus (H1N1pdm09) A/Victoria/4897/2022**		**Influenza B Virus (Victoria Lineage) B/Washington/02/19**	
Concentration ($TCID_{50}$/mL)	**Percent Agreement**	**Concentration ($TCID_{50}$/mL)**	**Percent Agreement**	**Concentration ($TCID_{50}$/mL)**	**Percent Agreement**
-	100%	**High**	100%	**Low**	100%
Low	100%	**High**	100%	**-**	100%
Low	100%	**High**	100%	**Low**	100%
-	100%	**Low**	100%	**High**	100%
Low	100%	**-**	100%	**High**	100%
Low	100%	**Low**	100%	**High**	100%
High	100%	**Low**	100%	**-**	100%
High	100%	**-**	100%	**Low**	100%
High	100%	**Low**	100%	**Low**	100%

4. Assay Reportable Range:

Not applicable; the device is a binary qualitative assay that is visually read.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a. Controls

i. Internal Procedural Controls:

A built-in internal procedural control is needed to indicate the test device is functioning properly and to ensure correct use of the device. The internal control is part of the test strip membrane and is therefore automatically run within the development time of each test. The internal procedural control consists of IgG antibodies that are immobilized at the 'C'-Line of the test membrane in the device and captures leftover, unbound IgG complexes to generate a color signal at the 'C-Line'.

ii. External Controls:

External Quality Control materials are not included in the test kit but are available separately for use by professional users.

b. Stability

i. Real Time Stability:

A real-time stability study is being conducted internally at Wondfo to evaluate stability and determine the shelf-life of the unopened test kit. Three lots of unopened WELLlife COVID-19 / Influenza A&B Test kit components (test swabs, test devices, and extraction buffer vials) were stored under two conditions: 30 ± 2°C (which is the upper end of the 15-30º C room temperature range and representative of the 15-30ºC recommended storage conditions) and 2-8°C. At defined intervals, an assessment of each lot with a four-membered sample panel is conducted. Baseline testing was performed within one month of device manufacture.

This study used PNSM as the negative clinical matrix. The four-member sample panel includes the external positive and negative control swabs, negative samples (PNSM only), and co-spiked low positive samples prepared at 3x LoD with inactivated SARS-CoV-2 (USA-WA1/2020), live influenza A (H1N1), or live influenza B (Yamagata). All study data are 100% concordant with expected results and support a shelf-life of up to 9 months when stored between 2-30°C.

ii. Open Kit Stability Study:

An opened test kit stability study was conducted to evaluate the length of time the test device can be left outside of its foil pouch at ambient temperature and humidity. This study used PNW as the negative clinical matrix. Additional details about this matrix are included in Section X.1 below. Negative samples (PNW only) and low positive samples (2x co-spike equivalency LoD of inactivated SARS-CoV-2, live Influenza A and live Influenza B contrived in PNW were prepared and tested in replicates of five (5). Testing was conducted immediately after opening (0 hours), one (1) hour, and two (2) hours after the device foil packaging was opened and allowed to remain in an incubation chamber set to mimic the higher range of room

temperature conditions (30 ± 1ºC). A single lot of test device were analyzed. All results were as expected for all time points.

iii. Transport Stability:

Transport stability under simulated summer and winter shipping conditions was tested to evaluate worst-case shipping and handling conditions over an extended period of time. Performance of unopened test kits was assessed by comparing pre- (T0) and post-distribution (Td) results. Negative samples (PNW only) and low positive samples (3x co-spike equivalency LoD of inactivated SARS-CoV-2, live Influenza A and live Influenza B contrived in PNW) were prepared and tested in replicates of five (5) each. All results were as expected for all time points.

6. Detection Limit:

a. Single Analyte LoD:

A limit of detection (LoD) study was conducted to determine the lowest detectable concentration of SARS-CoV-2 (USA-WA1/2020, UV-inactivated), live influenza A (H1N1 and H3N2), and live influenza B (Victoria and Yamagata lineage) at which at least 95% of all true positive replicates tested positive. Testing was conducted on three lots of test devices.

A preliminary LoD was first determined by testing serial ten-fold dilutions of viral stocks diluted in PNSM in triplicates (n=3). Once the lowest positive ten-fold dilution concentration was established, additional two-fold dilutions were tested in triplicate (n=3).

A 50-µL sample of each virus diluted in PNSM was pipetted onto the dry swab and thereafter processed per the instructions for use. The results of the preliminary LoD testing are summarized in the table below with the preliminary LoD in bold. The preliminary LoD observed were identical for the three reagent lots tested.

Table 5. Preliminary single analyte LoD results

Virus Strain	Virus Concentration		Positive Replicates
	$TCID_{50}$/mL	**$TCID_{50}$/Swab**	
SARS-CoV-2	$3.16x10^5$	15,800	3/3
	$3.16x10^4$	1,580	3/3
	$3.16x10^3$	158	3/3
	$3.16x10^2$	15.8	0/3
	$1.58\ x10^3$	79	3/3
	$7.90\ x10^2$	**39.5**	**3/3**
	$3.95\ x10^2$	19.8	1/3
Influenza A H1N1	$2.02\ x10^4$	1,010	3/3
	$2.02\ x10^3$	101	3/3
	$2.02\ x10^2$	10.1	3/3
	$2.02\ x10^1$	1.01	0/3
	$1.01\ x10^2$	**5.05**	**3/3**
	$5.05\ x10^1$	2.53	1/3
	$2.53\ x10^1$	1.27	0/3

Virus Strain	Virus Concentration		Positive Replicates
	$TCID_{50}$/mL	$TCID_{50}$/Swab	
Influenza A H3N2	4.17×10^4	2,085	3/3
	4.17×10^3	209	3/3
	4.17×10^2	20.9	3/3
	4.17×10^1	2.09	0/3
	2.09×10^2	**10.45**	**3/3**
	1.04×10^2	5.20	0/3
	5.21×10^1	2.61	0/3
Influenza B Victoria	3.16×10^5	15,800	3/3
	3.16×10^4	1,580	3/3
	3.16×10^3	**158**	**3/3**
	3.16×10^2	15.8	0/3
	1.58×10^3	79	0/3
Influenza B Yamagata	1.17×10^4	585	3/3
	1.17×10^3	58.5	3/3
	1.17×10^2	5.85	3/3
	1.17×10^1	0.58	0/3
	5.85×10^1	**2.93**	**3/3**
	2.93×10^1	1.47	0/3

The preliminary LoD of each virus was confirmed by testing an additional twenty replicates for each viral stock. Acceptance criteria for confirmation of the LoD were that at least 95% of the replicates (≥ 19/20) test positive. The confirmed LoDs observed for all strains were identical for the three lots tested. The results of the confirmatory LoD study and the final LoD for each strain are included below.

Table 6. Confirmatory single analyte LoD results

Strain	Virus Concentration		Positive Replicates
	$TCID_{50}$/mL	$TCID_{50}$/Swab	
SARS-CoV-2	7.90×10^2	39.5	20/20
Influenza A H1N1	1.01×10^2	5.05	20/20
Influenza A H3N2	2.09×10^2	10.5	20/20
Influenza B Victoria	3.16×10^3	158	20/20
Influenza B Yamagata	5.85×10^1	2.93	20/20

b. Co-spiked LoD:

After single analyte LoDs were determined, co-spike equivalency testing was conducted to characterize the performance of samples that contained all analytes at their respective 1x LoD concentrations. Based on the individual analyte LoDs, 1x LoD co-spiked samples were prepared by mixing the three virus analytes (inactivated SARS-CoV-2 USA-WA1/2020, live Flu A H1N1, and live Flu B Yamagata lineage) into the same sample using PNSM as clinical matrix. The influenza strains with the lowest confirmatory LoD in the single analyte study were selected for testing in the co-spiked LoD study. The viral strains and stocks/lots used in the co-spike study were the same as those used in the single-analyte LoD study.

Twenty (20) replicates were prepared by pipetting 50 µL of co-spiked sample onto the test swab and testing swabs with the device according to the instructions for use. Equivalency is confirmed separately for each analyte if ≥ 19/20 replicates are positive within 2x LoD of the individually tested analyte. The WELLlife COVID-19 / Influenza A&B Test demonstrated co-spike equivalency for SARS- CoV-2, Influenza A and Influenza B at 1x single analyte LoD. This study supports the use of co-spiked samples in subsequent analytical studies.

Table 7. Confirmatory co-spike analyte LoD results

Viral Strain Co-Spiked in Sample	Final Concentration		Positive Replicates
	$TCID_{50}$/mL	**$TCID_{50}$/Swab**	
SARS-CoV-2 + Influenza A H1N1 + Influenza B Yamagata	7.90×10^2	39.5	20/20
	1.01×10^2	5.05	20/20
	5.85×10^1	2.93	20/20

c. Detection Limit with the NIBSC 21/368 - WHO International Standard:

The LoD of the candidate device was determined with the 1st WHO International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368). The preliminary LoD concentration was determined by testing a series of 2-fold dilutions in PNSM with one device lot, starting with a 5-fold dilution from the stock concentration (20,000 IU/mL) of the WHO International Standard for SARS-CoV-2 Antigen. A preliminary LoD was first determined by testing serial ten-fold dilutions of virus stocks diluted in PNSM in triplicate (n=3). Once the lowest positive ten-fold dilution concentration was established, additional two-fold dilutions were tested in triplicate (n=3).

A 50-µL sample of each virus diluted in PNSM was pipetted onto the dry swab. The swab was then transferred to a pre-filled vial of buffer and mixed per the instructions for use. Following addition and mixing of the swab sample, the extracted sample was added to the sample well of the test device as described in the instructions for use. Test results were read visually at 10 minutes. The results of the preliminary LoD testing are summarized in Table 10 with the preliminary LoD in bold.

Table 8. Preliminary LoD determination for WHO International Standard

Concentration (IU/mL) in PNSM	# of Positivity/ # of Test Replicates		
	SARS-CoV-2	**Flu A**	**Flu B**
4.00E+03	3/3	0/3	0/3
2.00E+03	3/3	0/3	0/3
1.00E+03	3/3	0/3	0/3
5.00E+02	**3/3**	**0/3**	**0/3**
2.50E+02	0/3	0/3	0/3

The preliminary LoD of each virus was confirmed by testing an additional twenty samples for each viral stock. Acceptance criteria for confirmation of the LoD were that at least 95% of the replicates (≥ 19/20) test positive. Based on the test results, the LoD of WHO International Standard for SARS-CoV-2 Antigen was confirmed at 500 IU/mL in PNSM (with 20/20 positive results).

7. High-Dose Hook Effect Study:

The candidate device was tested to determine if it was affected by a high dose hook effect at high concentrations of the three analytes. A high dose of UV inactivated SARS-CoV-2, and live influenza A and B were tested in this study. Fifty (50) microliters of each sample was added directly to the head of the swabs. Swabs were processed per the test's IFU/QRI with one device lot. No high dose hook effect was observed for the high concentration tested.

Table 9. High-dose hook effect study results

Sample	Concentration ($TCID_{50}$/mL)	Positive Results / Total Replicates			
		SARS-CoV-2	Influenza A	Influenza B	Control
SARS-CoV-2	3.16×10^6	3/3	0/3	0/3	3/3
Influenza A (H1N1)	2.02×10^5	0/3	3/3	0/3	3/3
Influenza A (H3N2)	4.17×10^5	0/3	3/3	0/3	3/3
Influenza B (Victoria)	3.16×10^6	0/3	0/3	3/3	3/3
Influenza B (Yamagata)	1.17×10^5	0/3	0/3	3/3	3/3

8. Inclusivity Study:

Analytical reactivity was performed for the WELLlife COVID-19 / Influenza A&B Test to determine if the device can detect the target analytes across a variety of strains. A selection of temporally, geographically, and genetically diverse influenza strains were tested for inclusivity. A detection study was conducted with recent Omicron variants, Influenza A strains and Influenza B strains. A series of ten-fold dilutions of each virus was spiked into PNSM and tested. Once the ten-fold LoD range was established for each strain, an additional three two-fold dilution series of the lowest positive ten-fold dilution for each virus was tested in triplicate to demonstrate inclusivity. Based on this dilution series, the minimum detectable concentration was defined as the lowest concentration for which all three replicates were detected. Results are summarized in Table 10. Data demonstrate that the WELLlife COVID-19 / Influenza A&B Test is inclusive for the SARS-COV-2 and influenza target analytes across a range of strains.

Table 10. Analytical reactivity with relevant variants

Viral Target and Subtype		Strain	Minimum Detectable Concentration (with 3/3 positive results)
SARS-CoV-2	Omicron subvariant	hCoV-19/USA/MD-HP40900/2022 (XBB.1.5)	7.80×10^1 $TCID_{50}$/mL*
		JN.1 Clinical sample pool	9.18×10^4 (Ct 26.4) #
Influenza A	H1N1 pdm2009	A/California/04/2009	2.80×10^3 $TCID_{50}$/mL
		A/Brisbane/02/18	1.51×10^2 $TCID_{50}$/mL
		A/Michigan/45/15	1.86×10^1 $TCID_{50}$/mL
		A/Guangdong-Maonan/SWL 1536/19	2.09×10^2 $TCID_{50}$/mL
		A/NY/03/09	2.29×10^4 $TCID_{50}$/mL
		A/Indiana/02/2020	9.70×10^6 $CEID_{50}$/mL
		A/Wisconsin/588/2019	7.00×10^3 FFU/mL
		A/Sydney/5/2021	4.80×10^3 $TCID_{50}$/mL
		A/Hawaii/66/2019	1.85×10^7 $CEID_{50}$/mL
		A/Wisconsin/67/22	4.21×10^2 $TCID_{50}$/mL

Viral Target and Subtype		Strain	Minimum Detectable Concentration (with 3/3 positive results)
	H3N2	A/Tasmania/503/2020	1.30 x 10^5 FFU/mL
		A/New York/21/2020	2.60 x 10^5 FFU/mL
		A/Alaska/01/2021	3.75 x 10^4 FFU/mL
		A/Hong Kong/45/2019	1.50 x 10^4 FFU/mL
		A/Hong Kong/2671/19	1.05 x 10^3 $TCID_{50}$/mL
	H3N2v	A/Indiana/08/2011	8.10 x 10^2 $TCID_{50}$/mL
	H1N2v	A/Minnesota/19/2011	4.00 x 10^6 $CEID_{50}$/mL
	H1N1v (Swine Flu)	A/Ohio/09/2015	7.00 x 10^5 $CEID_{50}$/mL
	H5N1	A/mallard/Wisconsin/2576/2009	4.00 x 10^5 $CEID_{50}$/mL
		A/duck/Guangxi/S11002/2024	3.38 x 10^5 EID_{50}/mL
	H5N6	A/duck/Guangxi/S10888/2024	6.76 x 10^5 EID_{50}/mL
	H5N8	A/goose/Liaoning/S1266/2021	6.76 x 10^5 EID_{50}/mL
	H7N3 (Bird Flu)	A/northern pintail/Illinois/ 10OS3959/2010	7.00 x 10^5 $CEID_{50}$/mL
Influenza B	Non-Victoria/ Yamagata	B/Maryland/1/59	3.38 x 10^3 $CEID_{50}$/mL
	Victoria	B/Brisbane/60/2008	1.29 $TCID_{50}$/mL
		B/Colorado/06/17	5.85 x 10^1 $TCID_{50}$/mL
		B/Texas/02/2013	2.45 x 10^1 $TCID_{50}$/mL
		B/Michigan/01/2021	1.43 x 10^4 $TCID_{50}$/mL
	Yamagata	B/Texas/06/2011	7.55 x 10^2 $TCID_{50}$/mL
		B/Utah/09/2014	1.26 x 10^3 $TCID_{50}$/mL
		B/Wisconsin/01/2010	1.78 x 10^2 $TCID_{50}$/mL
* - 10/10 replicates yielded positive results. # - 5/5 replicates yielded positive results.			

9. Assay Cut-Off:

Not applicable as this is a qualitative visually read assay without numeric raw data.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable. See "C. Clinical Studies." for performance comparison with a clinical comparator.

2. Matrix Equivalency:

The WELLlife COVID-19 / Influenza A&B Test is only intended for use with direct anterior nasal swab specimens. As no other specimen or sample type is used with this device, a matrix comparison study to support other sample types for clinical testing with this device was not performed.

However, the sponsor performed the matrix equivalency study between pooled negative nasal swab matrix (PNSM) and the surrogate pooled negative nasal wash (PNW) that was used in

multiple analytical studies. The data demonstrated equivalent performance of the test with both matrices.

C Clinical Studies:

1. Clinical Performance Assessment:

A prospective lay person clinical study was conducted to assess the performance of the candidate test when compared to a 510(k)-cleared SARS-CoV-2 RT-PCR assay with an extraction step. The study prospectively enrolled symptomatic subjects at nine clinical study sites between December 2023, and March 2024.

Two anterior nasal swab samples were collected from each subject (one for the candidate test and one for the comparator test) and the collection order was alternated (randomized by study subject). Comparator test samples were collected by health care professionals at the clinical study site and inserted into viral transport media per the IFU of the comparator test. Samples for the candidate antigen test were collected per the candidate test's QRI and were either self-collected by a lay user aged ≥14 years, or collected by an adult (parent/guardian) from individuals aged 2 to <14 years. 787 study subjects were enrolled in total, of which 705 subjects between 0 and 4 DPSO had valid comparator test results and were evaluable per the study protocol. Detailed study subject demographics are listed below.

Table 11. Clinical study cohort demographics

Characteristic	Number (%) [N=705]
Age (years)	
<14	139 (19.7%)
14-24	99 (14.0%)
25-64	352 (49.9%)
>64	115 (16.3%)
Gender	
Female	433 (61.4%)
Male	272 (38.6%)
Ethnicity	
Hispanic/Latino	313 (44.4%)
Not Hispanic/Latino	377(53.5%)
Unknown/Prefer not to answer	15 (2.1%)

The WELLlife COVID-19 / Influenza A&B Test detected the analytes with the following percent agreements when compared to the result of the SARS-CoV-2 RT-PCR comparator assay:

Table 12. Clinical performance estimates – SARS-CoV-2

	Comparator Positives	Comparator Negatives	Total
Candidate Positives	101	1	102
Candidate Negatives	14	589	603
Total	**115**	**590**	**705**
Positive Percent Agreement (PPA) = 87.8% (101/115), 95% CI (80.6%, 92.6%)			
Negative Percent Agreement (NPA) = 99.8% (589/590), 95% CI (99.1%, 100%)			

Table 13. Clinical performance with SARS-CoV-2 positive subjects stratified by days post symptom onset (DPSO)

DPSO	PPA (n/N)	95% CI
0	60.0% (3/5)	(23.1%, 88.2%)
1	92.9% (26/28)	(77.4%, 98.0%)
2	83.3% (30/36)	(68.1%, 92.1%)
3	96.3% (26/27)	(81.7%, 99.3%)
4	84.2% (16/19)	(62.4%, 94.5%)

Table 14. Clinical performance estimates – Flu A

	Comparator Positives	*Comparator Negatives*	*Total*
Candidate Positives	75	2	77
Candidate Negatives	11	617	628
Total	**86**	**619**	**705**
Positive Percent Agreement (PPA) = 87.2% (75/86), 95% CI (78.5%, 92.7%)			
Negative Percent Agreement (NPA) = 99.7% (617/619), 95% CI (98.8%, 99.9%)			

Table 15. Clinical performance estimates – Flu B

	Comparator Positives	Comparator Negatives	Total
Candidate Positives	29	2	31
Candidate Negatives	4	670	674
Total	**33**	**672**	**705**
Positive Percent Agreement (PPA) = 87.9% (29/33), 95% CI (72.7%, 95.2%)			
Negative Percent Agreement (NPA) = 99.7% (670/672), 95% CI (98.9%, 99.9%)			

2. Usability Study:

The sponsor evaluated the usability of the WELLlife COVID-19 / Influenza A&B Test and the labeling and comprehension of the investigational test QRI when performed by lay users in a simulated home environment. The observers did not otherwise interfere with the study subject's sample collection and testing.

a. User Comprehension

A total of 37 evaluable subjects participated in the human factors assessment portion of the usability study. Of these 37 subjects, 19 performed self-collection and testing and 18 collected and tested a sample from another individual.

Table 16. Demographics of subjects

Factor	Subjects from which samples were collected and tested by another individual (N=18)
Age Group	
≥2 – <14 years of age	12 (66.7%)
14 – 24 years of age	6 (33.3%)
>24 – 64 years of age	0 (0%)
≥65 years of age	0 (0%)
Sex at Birth	
Male	6 (33.3%)
Female	12 (66.7%)

Overall, 92.5% of all critical tasks associated with sample collection and testing with the WELLlife COVID-19 / Influenza A&B Test were performed correctly. 85.1% of all non-critical tasks were performed correctly.

Table 17. Critical vs. non-critical tasks performed correctly

Steps	Tasks performed correctly	Total number of tasks	Percentage of tasks performed correctly
Critical	308	333	92.5%
Non-critical	126	148	85.1%
Total	434	481	90.2%

The human factors assessment met the acceptance criteria for performing critical (≥90%) and non-critical (≥80%) tasks and supports the user comprehension and usability of the test device in the OTC environment. This is also supported by the results of the usability/user comprehension questionnaire (shown below).

A total of 36 out of 37 participants (97.3%) found the instructions clear and easy to follow, 34 (91.89%) found sample collection easy to perform, and 36 (97.3%) found the results clear and easy to see.

b. Readability and Accuracy in Results Interpretation

All 37 subjects who participated in the human factors assessment (Usability study) also interpreted a panel of mock tests with results that reflected sample concentrations of 1.9x and 5x LOD. Mock devices were blinded and randomized. About half of the study subjects were vision impaired (see table below). The study did not include individuals with any of the following conditions: macular degeneration, color blindness, diabetic retinopathy, cataracts, or amblyopia/strabismus.

Table 18. Vision impairment of readability study subjects

Type of vision impairment	Number of subjects	Percentage of total human factors participants with vision impairment
Near/Far Sightedness	18	90.00%
Glaucoma	1	5.00%
Other	1	5.00%
Total lay users with vision impairment	**20**	**100.00%**

Each panel of mock tests included 16 different investigational tests with various negative and positive results for each analyte in different concentrations. The overall accuracy of the test interpretation by the lay users is 94.09% (557/592).

Comparative results of the readability study between lay users with and without visual impairment are summarized below.

Table 19. Comparison of correct test interpretation stratified by vision impairment

Panel	Concentration	Subjects without visual impairment (N=17)	Subjects with visual impairment (N=20)
		Correct	Correct
Negative	NA	100.00% (17/17)	100.00% (19/19)*
COVID-19 positive only	1.9xLoD	94.12% (16/17)	100% (20/20)
	5xLoD	100.00% (17/17)	95.00% (19/20)
Flu A positive only	1.9xLoD	100.00% (17/17)	95.00% (19/20)
	5xLoD	82.35% (14/17)	80.00% (16/20)
Flu B positive only	1.9xLoD	94.12% (16/17)	95.00% (19/20)
	5xLoD	100.00% (17/17)	95.00% (19/20)
Flu A and COVID-19 positive	1.9xLoD	100.00% (17/17)	95.00% (19/20)
	5xLoD	100.00% (17/17)	100.00% (20/20)
Flu B and COVID-19 positive	1.9xLoD	82.35% (14/17)	85.00% (17/20)
	5xLoD	100.00% (17/17)	100.00% (20/20)
Flu A and Flu B positive	1.9xLoD	82.35% (14/17)	80.00% (16/20)
	5xLoD	100.00% (17/17)	95.00% (19/20)
Positive on all analytes	1.9xLoD	100.00% (17/17)	100.00% (20/20)
	5xLoD	100.00% (17/17)	100.00% (20/20)
Invalid	NA	86.67% (13/15)*	90.00% (18/20)
*One subject didn't provide answers. **Two subjects didn't provide answers.			

Overall, the readability studies support the use of the WELLlife COVID-19 / Influenza A&B Test by lay users in a home/OTC environment.

c. User Accuracy/Near cutoff Study

A user accuracy/near cutoff study was conducted to assess the performance of the candidate test with samples around the LoD of the device when used by untrained operators at point of care clinical sites. A total of 10 untrained operators across three sites (with at least three operators per site) completed the study. Contrived positive samples were prepared by spiking 50 µL of inactivated SARS- CoV-2, Influenza A, and Influenza B virus in negative clinical matrix (PNSM) at 1.9x LoD. Spiked swabs were frozen at ≤ -20°C and shipped to the study sites for testing. Samples were blinded and randomly split among untrained operators for testing at each site. Results of this study are presented below:

Table 20. Near-LoD user accuracy study results

Sample Type	Percent Accuracy (n/N)			Combined Percent Accuracy (n/N)
	Site 1	Site 2	Site 3	
Negative	100% (4/4)	100% (3/3)	100% (3/3)	100% (10/10)
SCV2 Positive	100% (4/4)	100% (3/3)	100% (3/3)	100% (10/10)
Flu A Positive	100% (4/4)	100% (3/3)	100% (3/3)	100% (10/10)
Flu B Positive	100% (4/4)	66.67% (2/3)	100% (3/3)	90.0% (9/10)
SCV2 Positive	100% (4/4)	100% (3/3)	100% (3/3)	100% (10/10)

Sample Type	Percent Accuracy (n/N)			Combined Percent Accuracy (n/N)
	Site 1	Site 2	Site 3	
Flu A/Flu B Positive	100% (4/4)	100% (3/3)	100% (3/3)	100% (10/10)
Flu A/SCV2 Positive	100% (4/4)	100% (3/3)	100% (3/3)	100% (10/10)
Flu B/SCV2 Positive	100% (4/4)	100% (3/3)	100% (3/3)	100% (10/10)
Flu A/Flu B/SCV2 Positive	100% (4/4)	100% (3/3)	100% (3/3)	100% (10/10)

Note: SCV2 = SARS-CoV-2

D Clinical Cut-Off:

Not Applicable. The candidate device is a qualitative assay with a visually read binary result without numeric raw data.

E Expected Values/Reference Range:

When the test is valid, it produces binary values, positive or negative for SARS-CoV-2, influenza A and influenza B antigens.

F Other Supportive Information:

1. Flex Studies

To assess the robustness and risk for false results of the test when deviating from the IFU/QRI test steps, flex studies were conducted that assessed all major aspects of the test procedure (sample volume, reading time, other deviations from the procedure [delay in mixing, delay in addition of sample to the well, incubation time] and variability of environmental test conditions that the test may be subjected to when in use (lighting, disturbance during use, temperature and humidity stress conditions). Testing was performed with contrived positive nasal swabs generated by diluting SARS-CoV-2 virus into negative NWM at 2xLoD. False results are observed with too little sample volume and insufficient incubation time, specifically with less than two drops of sample and with less than eight minutes incubation. The studies support that the test is robust in the intended use condition with an insignificant risk of erroneous result.

2. Variant Monitoring Plan:

Wondfo plans to conduct ongoing monitoring for new and emerging SARS-CoV-2 viral mutations and variants and assess the impact of the mutations and variants that have been identified as prevalent and/or clinically significant to the performance of the WELLlife COVID-19 / Influenza A&B Test. Wondfo will evaluate on a monthly basis the impact of all new SARS-CoV-2 virus variants that WHO and/or CDC designate as variants of concern (VOC), address FDA requests for any specific monitoring data on new SARS-CoV-2 and influenza variants, and will evaluate any post-market signals related to new virus variants.

The evaluation method will consist of in-silico analysis to determine the potential impact of mutations on the epitope detected by the test's antibodies and the resulting test performance.

When indicated by the results of the in-silico analysis (i.e., when mutations are determined to impact the structural integrity of the analytes' epitopes recognized by the test), wet testing of either virus culture fluid of the new variant or clinical specimens will be performed as availability of these materials allow.

VII. Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

VIII. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.