510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K241317

B Applicant

Guangzhou Wondfo Biotech Co., Ltd.

C Proprietary and Established Names

Wondfo 2019-nCoV Antigen Test (Lateral Flow Method)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QYT	Class II	21 CFR 866.3984 - Over-The-Counter Test To Detect SARS-Cov-2 From Clinical Specimens	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

The purpose of this submission is to request premarket notification 510(k) clearance for the *Wondfo 2019-nCoV Antigen Test (Lateral Flow Method)*.

B Measurand:

Nucleocapsid protein antigen from SARS-Coronavirus 2 (SARS-CoV-2)

C Type of Test:

Qualitative lateral flow immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) is a visually read lateral flow immunoassay test intended for the qualitative detection of SARS-CoV-2 virus nucleocapsid protein antigen directly in anterior nasal swab specimens from individuals with signs and symptoms of COVID-19.

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. Symptomatic individuals with an initial negative test result must be re-tested once between 48 and 72 hours after the first test using either an antigen test or a molecular test for SARS-CoV-2. Negative results do not rule out SARS-CoV-2 infections or other pathogens and should not be used as the sole basis for treatment.

Positive results do not rule out co-infection with other respiratory pathogens.

This test is not a substitute for visits to a healthcare provider or appropriate follow-up and should not be used to determine any treatments without provider supervision. Individuals who test negative and experience continued or worsening COVID-19 like symptoms, such as fever, cough and/or shortness of breath, should seek follow up care from their healthcare provider.

The performance characteristics for SARS-CoV-2 were established from April 2023 to February 2024 when SARS-CoV-2 Omicron was dominant. Test accuracy may change as new SARS-CoV-2 viruses emerge. Additional testing with a lab-based molecular test (e.g., PCR) should be considered in situations where a new virus or variant is suspected.

C Special Conditions for Use Statement(s):

OTC - Over The Counter

D Special Instrument Requirements:

Not applicable.

IV Device/System Characteristics:

A Device Description:

The Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) is a lateral flow immunoassay intended for non-prescription home use for the qualitative detection of nucleocapsid protein antigen directly in anterior nasal swab specimens from individuals with signs and symptoms of COVID-19 within the first five (5) days of symptom onset. Results are for the identification of SARS-CoV-2 nucleocapsid protein antigen.

The Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) is consisted of components below:

- Tube Holder (located in kit box)
- Test Cassette
- Tube (pre-filled extraction buffer)
- Swab
- Instruction for Use

The test cassette is assembled with a test strip in a plastic housing that contains a nitrocellulose membrane with two lines: a test line (T line) and a control line (C line).

B Principle of Operation:

The Wondfo 2019-nCoV Antigen Test is a sandwich immunochromatographic assay that uses antibodies to detect SARS-CoV-2 nucleocapsid antigen in anterior nasal swab specimen. A nasal swab sample is collected by the lay user and then inserted into the extraction buffer that disrupts the virus particles in the specimen to expose internal viral nucleocapsid antigens. The extracted specimen is then added into the sample well of the test cassette. When an adequate volume of the specimen is added to the sample well (S) of the test cassette, the specimen migrates by capillary action from the sample well over the conjugated pad and across the nitrocellulose membrane test strip. During the migration, the reagents contained in the conjugated pad are solubilized. If SARS-CoV-2 nucleocapsid antigens are present in the sample, the antigens bind to the specific annti-SARS-CoV-2 antibody that is conjugated with dye particles. These antigen-antibody complexes are captured by the anti-SARS-CoV-2 antibody immobilized at the test line region (T) to form sandwich complexes that generate a visible colored test line. Unbound conjugate molecules continue to migrate across the nitrocellulose membrane and are captured at the control line region (C) to result in a visible colored control line that indicates adequate operations and sample flow during the test. If no SARS-CoV-2 nucleocapsid antigens are present in the sample, the conjugate will only be captured at the control line of the test.

Results are interpreted between 10 and 20 minutes after adding the extracted sample into the sample well. A false negative or false positive result may occur if the test result before 10 minutes or after 20 minutes.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Flowflex COVID-19 Antigen Home Test

B Predicate 510(k) Number(s):

K230828

C Comparison with Predicate(s):

Device & Predicate Device(s):	K241317	K230828
Device Trade Name	Wondfo 2019-nCoV Antigen Test (Lateral	Flowflex COVID-19 Antigen Home Test
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) is a visually read lateral flow immunoassay test intended for the qualitative detection of SARS-CoV-2 virus nucleocapsid protein antigen directly in anterior nasal swab specimens from individuals with signs and symptoms of COVID-19. 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. Wondfo 2019-nCoV Antigen Test (Lateral Flow Method) does not differentiate between SARS-CoV and SARS-CoV-2. All negative results are presumptive. Symptomatic individuals with an initial negative test result must be re-tested once between 48 and 72 hours after the first test using either an antigen test or a molecular test for SARS-CoV-2. Negative results do not rule out SARS-CoV-2 infections or other pathogens and should not be used as the sole basis for treatment. Positive results do not rule out co-infection with other respiratory pathogens. This test is not a substitute for visits to a healthcare provider or appropriate follow-up and should not be used to determine any treatments without provider	The Flowflex COVID-19 Antigen Home Test is a visually read lateral flow immunoassay device intended for the rapid, qualitative detection of SARS-CoV-2 virus nucleocapsid protein antigen directly in anterior nasal swab specimens from individuals with signs and symptoms of COVID-19 within the first 6 days of symptom onset. 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. The Flowflex COVID-19 Antigen Home Test does not differentiate between SARS-CoV and SARS-CoV-2. All negative results are presumptive. Symptomatic individuals with an initial negative test result must be re-tested once between 48 and 72 hours after the first test using an antigen test or a molecular test for SARS-CoV-2. Negative results do not preclude SARS-CoV-2 infections or other pathogens and should not be used as the sole basis for treatment. Positive results do not rule out co-infection with other respiratory pathogens. Performance characteristics for SARS-CoV-2 were established from December 2022 to March 2023 of the SARS-CoV-2 pandemic when SARS-CoV-2 Omicron was the

	supervision. Individuals who test negative and experience continued or worsening COVID-19 like symptoms, such as fever, cough and/or shortness of breath, should seek follow up care from their healthcare provider. The performance characteristics for SARS-CoV-2 were established from April 2023 to February 2024 when SARS-CoV-2 Omicron was dominant. Test accuracy may change as new SARS-CoV-2 viruses emerge. Additional testing with a lab-based molecular test (e.g., PCR) should be considered in situations where a new virus or variant is suspected.	predominant SARS-CoV-2 variant in circulation. When other SARS-CoV-2 virus variant are emerging, performance characteristics may vary. This test is not a substitute for visits to a healthcare provider or appropriate follow-up and should not be used to determine any treatments without provider supervision. Individuals who test negative and experience continued or worsening COVID-19 like symptoms, such as fever, cough and/or shortness of breath, should seek follow up care from their healthcare provider.
Regulation Number	21 CFR 866.3984	Same
Disease Population	COVID-19	Same
Intended Users / Use Environment	OTC Lay User	Same
Test Principle	Lateral flow immunoassay	Same
Sample Type	Anterior nasal swab specimens	Same
Assay Target	SARS-CoV-2 nucleocapsid protein antigens	Same
Assay Type	Qualitative	Same
Mode of Results	Visual	Same
Assay Control	Internal Procedural Control	Same
General Device Characteristic Differences		
Minimum Incubation Time	10 minutes	15 minutes

VI Standards/Guidance Documents Referenced:

Document Title	Issued by	Applicable study
Special controls for Over- the-counter test to detect SARS-CoV-2 from clinical specimens (Reclassification order for DEN220028 and special controls under 21 CFR 866.3984)	FDA/CDRH	All Studies
Guidance: *Submission and review of sterility information in premarket notification (510(k)) submissions for devices labeled as sterile*.	FDA/CDRH	Sterility
Guidance: *Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1:*	FDA/CDRH	Biocompatibility

Document Title	Issued by	Applicable study
Evaluation and testing within a risk management process"		
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
Standard: ISO 10993-7, *Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide Sterilization Residuals*		
Standard: ISO 10993-1, *Biological Evaluation of Medical Devices – Part 1: Evaluation and testing within a risk management process*	ISO	Biocompatibility
Standard: ISO 10993-5, *Third edition, Biological evaluation of medical devices – Part 5: Tests for in vitro cytotoxicity*		
Standard: *ISO 10993-10, Third Edition, Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization*		

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision:

A precision study was conducted to assess variability with respect to days, operators, and device lots. The study included three device lots, each tested every day by three operators for 20 days; testing was conducted in duplicates for each sample concentration (i.e., 3 operators x 20 days x 3 lots x 2 runs per day x 2 replicates per sample per run = 720 results per sample panel member). Three levels of heat inactivated SARS-CoV-2 Omicron Variant lineage BA.5 (Isolate USA/COR-22-063113/2022) were spiked into negative clinical nasal swab matrix (NCM) as follows:

1. Negative Sample
2. Below LoD (0.9xLoD)
3. Low Positive Sample at 1.5xLoD
4. Positive Sample at 3xLoD

50µL of each sample was applied to dry nasal swabs. After blinding and randomizing, samples were processed per the IFU of the candidate device.
Precision was observed to be 100% for all replicates prepared at 1.5xLoD and 3xLoD, demonstrating no variability in the performance of the candidate assay across the conditions, operators, lots, and days tested.

Precision study samples were therefore prepared at 0.9xLoD using the same materials for sample preparation as for the original study. These samples were then tested as follows: 2 operators x 3 lots x 3 days x 2 runs per day x 2 replicates per sample per run and resulted in a total of 72 replicates. The precision for the 0.9xLoD sample was less than 100%, which is expected based on the random error for a sample below the LoD. However, the performance was consistent across all three lots tested.

Table 1. Precision Study Summary Results.

	Negative (n/N)			Below LoD (0.9xLoD) (n/N)		Low Positive (1.5xLoD) (n/N)			Positive (3xLoD) (n/N)		
Operator	**1**	**2**	**3**	**1**	**2**	**1**	**2**	**3**	**1**	**2**	**3**
Lot 1	0/80	0/80	0/80	11/12	9/12	80/80	80/80	80/80	80/80	80/80	80/80
Lot 2	0/80	0/80	0/80	10/12	10/12	80/80	80/80	80/80	80/80	80/80	80/80
Lot 3	0/80	0/80	0/80	12/12	12/12	80/80	80/80	80/80	80/80	80/80	80/80
Agreement	**NPA = 100% (720/720)**			**PPA = 88.89% (64/72)**		**PPA = 100% (720/720)**			**PPA = 100% (720/720)**		
95%CI	99.47%, 100%			79.58%, 94.26%		99.47%, 100%			99.47%, 100%		

2. Linearity:

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

3. Analytical Specificity/Interference:

a. Cross-Reactivity and Microbial Interference

A panel of microorganisms commonly found as either pathogens or normal flora in respiratory samples were individually spiked into NCM. 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. The microbial interference testing was conducted in the same manner but in the presence of SARS-CoV-2 Omicron Variant lineage BA.5 co-spiked into the samples at 3xLoD. The testing was performed in triplicates for each microorganism. 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 Testing Results

Microorganism	Concentration Tested	Cross Reactivity Result	Interference Result
Human coronavirus 229E	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Human coronavirus OC43	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Human coronavirus NL63	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
MERS-coronavirus	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Human coronavirus HKU1 (n=2)*	Ct = 20.5 – 22*	0/6	6/6
SARS-CoV Nucleocapsid Protein (His Tag)[#]	0.25 ng/mL	0/3	3/3
Human Adenovirus 1	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Human Metapneumovirus 3 (hMPV-3) Type B1	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus – Type 1	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus – Type 2	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus – Type 3	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Parainfluenza virus – Type 4A	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Influenza A/Darwin/6/21	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Influenza A/Victoria/4897/22	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Influenza B/Washington/02/19	$2x10^5$ $TCID_{50}$/mL	0/3	3/3

Microorganism	Concentration Tested	Cross Reactivity Result	Interference Result
Influenza B/Florida/04/06	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Enterovirus Type 68	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Respiratory syncytial virus	$2x10^5$ $TCID_{50}$/mL	0/3	3/3
Rhinovirus (Isolate: 10/2014 Isolate #1)	$5.62x10^4$ $TCID_{50}$/mL	0/3	3/3
Haemophilus influenzae type b (Eagan)	$2x10^6$ CFU/mL	0/3	3/3
Streptococcus pneumoniae Z022	$2x10^6$ CFU/mL	0/3	3/3
Streptococcus pyogenes Z018	$2x10^6$ CFU/mL	0/3	3/3
Candida albicans Z006	$2x10^6$ CFU/mL	0/3	3/3
Bordetella pertussis A639	$2x10^6$ CFU/mL	0/3	3/3
Mycoplasma pneumoniae M129	$2x10^6$ CCU/mL	0/3	3/3
Mycoplasma tuberculosis H37Ra-1	$2x10^6$ CFU/mL	0/3	3/3
Chlamydia pneumoniae	$2x10^6$ CFU/mL	0/3	3/3
Legionella pneumophila Philadelphia	$2x10^6$ CFU/mL	0/3	3/3
Staphylococcus aureus (strain: COL)	$2x10^6$ CFU/mL	0/3	3/3
Staphylococcus epidermidis MRSE; PR62A	$2x10^6$ CFU/mL	0/3	3/3
Pneumocystis jirovecii (PJP) - S. cerevisiae[#]	$2x10^6$ CFU/mL	0/3	3/3
Pooled human nasal wash	NA	0/3	3/3

**Clinical specimens were evaluated*
[#]Recombinant protein/strains were tested as the live or inactivated strains were hard to obtain

b. Endogenous/Exogenous Interfering Substances Study

A panel of common endogenous and exogenous substances were evaluated for their potential to interfere with the performance of the test device. Samples were contrived by individually adding the substances listed in Table 3 below and testing them in NCM with or without SARS-CoV-2 virus at 2xLoD. 50 µL of each contrived sample was applied to the head of a swab and processed per the proposed IFU of the test. One device lot was used to test the potential interferents in triplicate measurements. No erroneous results were observed.

Table 3. Endogenous / Exogenous Interfering Substances Summary Data

Interfering Substances	Concentration	Cross-reactivity (no analyte) (# positive/total)	Interference (2xLoD) (# positive/total)
Whole Blood	2.5%	0/3	3/3
Mucin	2.5 mg/mL	0/3	3/3
Chloraseptic sore throat lozenges (Benzocaine)	3 mg/mL	0/3	3/3
Chloraseptic sore throat lozenges (Menthol)	3 mg/mL	0/3	3/3
NeilMed (Sodium chloride with preservatives)	15% v/v	0/3	3/3
CVS Nasal Drops (Phenylephrine)	15% v/v	0/3	3/3
Afrin (Oxymetazoline)	15% v/v	0/3	3/3

Interfering Substances	Concentration	Cross-reactivity (no analyte) (# positive/total)	Interference (2xLoD) (# positive/total)
CVS Nasal Spray (Cromolyn)	15% v/v	0/3	3/3
Zicam	15% v/v	0/3	3/3
Homeopathic (Alkalol)	15% v/v	0/3	3/3
Sore Throat Phenol Spray	5% w/v	0/3	3/3
Tobramycin	4 µg/mL	0/3	3/3
Mupirocin	10 mg/mL	0/3	3/3
Fluticasone Propionate	15% v/v	0/3	3/3
Tamiflu (Oseltamivir Phosphate)	5 mg/mL	0/3	3/3
Biotin	3.5 µg/mL	0/3	3/3
Menthol	0.015% w/v	0/3	3/3
Bleach	0.01% v/v	0/3	3/3
Dish Soap	1% v/v	0/3	3/3
Laundry Detergent	1% v/v	0/3	3/3
Multi-Surface Cleaner	1% v/v	0/3	3/3
Hand Soap	1% v/v	0/3	3/3
Laundry Detergent	1% w/v	0/3	3/3
Bar Soap	1% w/v	0/3	3/3
Multipurpose Cleaner	1% v/v	0/3	3/3
Hand Sanitizer	1% v/v	0/3	3/3
Aspirin	15 mg/mL	0/3	3/3
Motrin (Ibuprofen)	50 mg/mL	0/3	3/3
Naproxen	20 mg/mL	0/3	3/3
Budesonide	15% v/v	0/3	3/3
Flunisolide	15% v/v	0/3	3/3
Triamcinolone	15% v/v	0/3	3/3
Dexamethasone	5 mg/mL	0/3	3/3
Beclomethasone	15% v/v	0/3	3/3
Remdesivir	5 mg/mL	0/3	3/3
Molnupiravir	5 mg/mL	0/3	3/3
Leukocytes	≥1 x10^6 cells/mL	0/3	3/3
Zinc	15% v/v	0/3	3/3
Luffa opperculata	1.25%	0/3	3/3
Galphimia glauca	15% v/v	0/3	3/3
Histaminum hydrochloricum	15% v/v	0/3	3/3
Zanamivir	10 mg/mL	0/3	3/3

4. **Assay Reportable Range:**

Not applicable as the device is a qualitative assay that is visually read without numeric output.

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

a. Controls

i. Internal Control:

The Wondfo 2019-nCOV Antigen test has a built-in internal procedural control. A red line should always appear in the control line region (C) indicating that proper volume of sample has been added and that membrane wicking has occurred. The anti-SARS-CoV-2 antibodies are conjugated with colloid gold nanoparticles leading to the coloration of the C line.

ii. External Controls:

This test is for OTC distribution. As such the test kits will not contain external controls as lay users are not required to perform external control testing.

b. Device Stability:

i. Real Time Stability (Shelf life):

The stability of the Wondfo 2019-nCOV Antigen test was determined for the intended storage conditions, 2-30°C (36-86°F), and an intended shelf life of 11 months. Within one month of manufacture three device lots were transferred to three different temperatures (2 – 8°C, 30±2°C and 30±2°C & 95±5% relative humidity), where they were stored for 12 months. Testing of the kit lots was performed every 3 months with 20 replicates/timepoint and lot. Two test samples, corresponding to negative sample and positive sample at 3xLoD, were tested at each time point. All study data were 100% concordant with expected results and thereby supportive of the 11 months shelf life at 2-30°C (36-86°F).

ii. Shipping Stability:

The effects of shipping on the integrity of the test device were evaluated with three device lots that were manufactured within one month of study start. These were exposed to either cycles of temperature and humidity fluctuations or mechanical stress. Temperature cycles included temperatures from -20 to 60°C with relative humidities between 85%, depending on the cycle. All results were concordant with expected results supporting stability during the anticipated shipping conditions for the test.

c. Expected Values:

Patient samples are expected to be negative for SARS-CoV-2.

6. Detection Limit:

a. Limit of Detection:

The limit of detection of the Wondfo 2019-nCOV Antigen test was determined with UV-inactivated SARS-CoV-2 USA-WA1/2020 and BA.5 Omicron isolates. Inactivated SAR-CoV-2 virus was diluted into NCM in 10-fold dilutions. Fifty (50) µl of each dilution was added directly to the test swab and the sample was then processed per the instructions for use. The LoD was assessed with three independent device lots. For the preliminary LoD

study, testing was performed with three replicates and the lowest concentration with >95% detection was then tested with 20 replicates to confirm the LoD.

- For SARS-CoV-2 (USA-WA-1/2020), the LoD was confirmed at $1x10^4$ $TCID_{50}$/mL.
- For SARS-CoV-2 (Lineage BA.5 (Omicron Variant)) the LoD was confirmed at $3.33x10^3$ $TCID_{50}$/mL.

Table 4. LoD Study Summary with UV Inactivated SARS-CoV-2 (USA-WA1/2020)

Concentration		Lot 1		Lot 2		Lot 3	
$TCID_{50}$/mL	**$TCID_{50}$/Swab**	**P**	**C**	**P**	**C**	**P**	**C**
$3.39x10^7$	$1.7x10^6$	100% (3/3)	N	100% (3/3)	N	100% (3/3)	N
$1x10^6$	$5x10^4$	100% (3/3)	N	100% (3/3)	N	100% (3/3)	N
$1x10^5$	$5x10^3$	100% (3/3)	100% (20/20)	100% (3/3)	100% (20/20)	100% (3/3)	100% (20/20)
$1x10^4$	**$5x10^2$**	100% (3/3)	100% (20/20)	100% (3/3)	100% (20/20)	100% (3/3)	100% (20/20)
$3.33x10^3$	$1.67x10^2$	N	95% (19/20)	N	85% (17/20)	N	N
$1x10^3$	$5x10^1$	0% (0/3)	N	0% (0/3)	N	0% (0/3)	N

P: Preliminary Study. C: Confirmatory LoD Study. N: Not tested

Table 5. LoD Study Summary with UV Inactivated SARS-CoV-2 BA.5 (Omicron)

Concentration		Lot 1		Lot 2		Lot 3	
$TCID_{50}$/mL	**$TCID_{50}$/Swab**	**P**	**C**	**P**	**C**	**P**	**C**
$1.98x10^6$	$9.9x10^4$	100% (3/3)	N	100% (3/3)	N	100% (3/3)	N
$1x10^5$	$5x10^3$	100% (3/3)	N	100% (3/3)	N	100% (3/3)	N
$1x10^4$	$5x10^2$	100% (3/3)	100% (20/20)	100% (3/3)	100% (20/20)	100% (3/3)	100% (20/20)
$3.33x10^3$	**$1.67x10^2$**	N	100% (20/20)	N	100% (20/20)	N	100% (20/20)
$1.11x10^3$	$5.55x10^1$	N	65% (13/20)	N	15% (3/20)	N	85% (17/20)
$1x10^3$	$5x10^1$	0% (0/3)	N	0% (0/3)	N	0% (0/3)	N

P: Preliminary Study. C: Confirmatory LoD Study. N: Not tested

b. Limit of Detection with the 1st WHO International Standard (NIBSC 21/368):

Wondfo tested the sensitivity of the test against the 1st WHO International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) spiked into pooled nasal swab sample in saline. The unitage of this material has an assigned value of 5,000 International Units (IU) of SARS-CoV-2 antigen per ampoule when reconstituted per instructions. A 10-fold dilution series was made to determine the preliminary LoD, which was measured using three (3) device lots and in triplicate measurements (n=3). The LoD was confirmed using

20 replicates (n=20) per dilution. The measurements were done by adding 50µl of each dilution directly to the test swab and processing the sample per the test's instructions for use. The lowest concentration of the SARS-CoV-2 antigen at which a minimum of 95% of results were positive was confirmed to be 200 IU/mL or 10 IU/Swab.

Table 6. LoD Study Summary with 1st WHO International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368)

Concentration		Lot 1		Lot 2		Lot 3	
$TCID_{50}$/mL	$TCID_{50}$/Swab	P	C	P	C	P	C
2000	100	100% (3/3)	100% (20/20)	100% (3/3)	100% (20/20)	100% (3/3)	100% (20/20)
200	**10**	**100% (3/3)**	**100% (20/20)**	**100% (3/3)**	**100% (20/20)**	**100% (3/3)**	**100% (20/20)**
20	1	0% (0/3)	N	0% (0/3)	N	0% (0/3)	N
2	0.1	0% (0/3)	N	0% (0/3)	N	0% (0/3)	N
66.7	3.34	0% (0/3)	15% (3/20)	0% (0/3)	10% (2/20)	0% (0/3)	10% (2/20)
0.2	0.01	0% (0/3)	N	0% (0/3)	N	0% (0/3)	N
0.02	0.001	0% (0/3)	N	0% (0/3)	N	0% (0/3)	N

P: Preliminary Study. C: Confirmatory LoD Study.

7. Inclusivity

An evaluation of the sensitivity of the test for the detection of relevant SARS-CoV-2 variants was done in form of an LoD study with eight different SARS-CoV-2 variant strains. Three lots of the Wondfo 2019-nCoV Antigen Test were used. Samples for inclusivity testing were prepared with the same methodology as detailed above for the Limit of Detection study. Viral samples were tested per the IFU in triplicate to first establish the preliminary LoD and then subsequently in replicates of 20 for the confirmatory LoD. The lowest concentration that detected ≥95% of all replicates for each evaluated SARS-CoV-2 strain is shown below.

Beyond the testing described above, Omicron JN.1.1 was independently evaluated with the test showing detection down to 2.28 x104 GE/mL (corresponding to an average Ct of 27.9).

Table 7: LoD of SARS-CoV-2 Variants

Strain/Viral Material	Reactivity (Number positive/ Number tested)			LoD ($TCID_{50}$/mL)
	Lot 1	Lot 2	Lot 3	
SARS-CoV-2 Variant B.1.1.7 (Alpha Variant)	23/23	23/23	23/23	1×10^3
SARS-CoV-2 Lineage B.1.351 (Beta variant)	23/23	23/23	23/23	1×10^3
SARS-CoV-2 Variant Brazil Lineage P.1 (Gamma variant)	23/23	23/23	23/23	1×10^3
SARS-CoV-2 Lineage B.1.617.2 (Delta Variant)	23/23	23/23	23/23	1×10^2
SARS-CoV-2 Lineage B.1.1.529 (Omicron Variant)	23/23	23/23	23/23	1×10^2
SARS-CoV-2 Lineage BA 2.3 (Omicron Variant)	23/23	23/23	23/23	3.33×10^2

Strain/Viral Material	Reactivity (Number positive/ Number tested)	GE/mL*
	Lot 4	
SARS-CoV-2 JN.1.1 (live)	5/5	2.28×10^4

** GE: Genome equivalent/mL*

8. Hook Effect Study

An assessment of whether a high dose hook effect exists for the test was done using a serial dilution of UV-inactivated SARS-CoV-2 virus strains. Multiple virus strains were tested, each spiked into negative NCM. 50µl of sample was added directly to the head of the swabs. Swabs were processed per the test's IFU/QRI. Testing was done across three device lots. Each of the 3 operators performed triplicate measurements for each concentration per lot. No high dose hook effect was observed in the study for any of the strains. Only the data for the most relevant contemporary strain tested in this study (i.e., SARS-CoV-2 Omicron Lineage BA.5) are shown in Table 8 below.

Table 8. High Dose Hook Effect Data Summary

Virus Concentration ($TCID_{50}$/mL)	Test Results (Agreement #positive /# Total)		
	Lot 1	Lot 2	Lot 3
1.98×10^6	3/3	3/3	3/3
1×10^5	3/3	3/3	3/3
1×10^4	3/3	3/3	3/3
1×10^3	0/3	0/3	0/3
1×10^2	0/3	0/3	0/3
1×10^1	0/3	0/3	0/3

9. Assay Cut-Off:

Not applicable as the device is a qualitative assay that yields visually read binary results.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable. Please refer to Section VII. C. Clinical Studies for performance comparison with a clinical comparator.

2. Matrix Comparison:

This device 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.

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

A prospective lay person clinical study was conducted to assess the performance of the candidate test in a simulated at-home setting when compared to a highly sensitive 510(k)-

cleared SARS-CoV-2 RT-PCR assay with an extraction step. The study enrolled symptomatic subjects at nine (9) clinical study sites between April 2023, and February 2024, when Omicron was the most prevalent SARS-CoV-2 strain in the U.S.

Both the comparator and the candidate test used anterior nasal swab samples, and the sample collection order was alternated (randomized) for each study subject. Comparator test samples were collected by health care professionals at the clinical study site and inserted into Universal Transport Media per the IFU of the comparator test. Samples for the candidate antigen test were collected per the 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.

This study enrolled a total of 1,053 individuals. Of the 1,053 results obtained, 21 were excluded and 1,032 were considered evaluable. The clinical performance estimates are based on these 1,032 study subjects between 0 and 5 DPSO. The 1,032 results consisted of 128 positive and 904 negative study subjects as defined by the comparator result. The Wondfo 2019-nCoV Antigen Test demonstrated the following performance, when compared to the result of the SARS-CoV-2 RT-PCR comparator assay:

- Positive Percent Agreement (PPA) of 84.38% (108/128) (95% CI: 77.10%, 89.65%)
- Negative Percent Agreement (NPA) of 99.67% (901/904) (95% CI: 99.03%, 99.89%).

Table 9: Demographics - Clinical Study Participants

Characteristic	Number of Evaluable Subjects	% of Total
Age		
2-13 years of age	117	11.34%
14-21 years of age	86	8.33%
22-64 years of age	698	67.64%
> 64years of age	131	12.69%
Total	**1,032**	**100%**
Gender		
Male	414	40.12%
Female	618	59.88%
Total	**1,032**	**100%**
Sample Collector		
Self-collected sample	900	87.21%
Sample collected by other	132	12.79%
Total	**1,032**	**100%**

Table 10: Clinical Performance Estimates

Candidate Test	Comparator Test		
	Positive	**Negative**	**Total**
Positive	108	3	111
Negative	20	901	921
Total	**128**	**904**	**1,032**
Positive Percent Agreement (PPA) = 84.38% (108/128) *95% CI: (77.10%, 89.65%)*			
Negative Percent Agreement (NPA) = 99.67% (901/904) *(95% CI: 99.03%, 99.89%)*			

Table 11. Clinical Performance Stratified by DPSO

Days Post Symptom Onset	PPA
0	100% (5/5)
1	90.91% (20/22)
2	82.35% (28/34)
3	83.33% (25/30)
4	86.36% (19/22)
5	73.33% (11/15)
Total	**84.38% (108/128)**

2. Usability Study:

The usability of the test was assessed with a sub cohort of the clinical study. During this study, representative test kit users (self-testers aged 14+, caregiver-child pairs, and caregiver-adult pairs) were observed performing the COVID-19 test while using the IFU/QRI. The observers recorded the proper execution of each task when the enrolled lay user study subject (n=30) conducted the test. The observers did not otherwise interfere with the study subject's sample collection and testing.

Table 12. Usability Study Results

Tasks	Critical (C) or Non-critical (NC)	Task Performed Correctly	Acceptability
Wash hands	NC	86.7% (26/30)	Yes
Check expiration date	NC	73.3% (22/30)	Yes
Unpack kit	NC	100% (26/30)	Yes
Open Tube Cap	NC	100% (30/30)	Yes
Place Tube in tube holder	NC	100% (30/30)	Yes
Remove swab from pouch without contamination/touching tip	C	100% (30/30)	Yes
Swab first nostril 5x/15sec	C	100% (30/30)	Yes
Swab second nostril 5x/15 sec	C	96.7% (29/30)	Yes
Insert swab into tube/touch bottom	C	100% (30/30)	Yes
Stir swab (15x or more)	C	100% (30/30)	Yes
Place tube in holder/Keep swab in tube for 1 minute	C	93.3% (28/30)	Yes
Remove swab while squeezing sides of tube	C	90% (27/30)	Yes

Tasks	Critical (C) or Non-critical (NC)	Task Performed Correctly	Acceptability
Cap tube	NC	100% (30/30)	Yes
Remove cassette from pouch & use within 1 hour	NC	100% (30/30)	Yes
Open Tube Cap	NC	96.7% (29/30)	Yes
Add 4 drops of sample to sample well	C	96.7% (29/30)	Yes
Set timer to wait 10 minutes (no more than 20 minutes) to read results	C	96.7% (29/30)	Yes

3. Readability and Comprehension:

A readability and comprehension study with lay persons was conducted to evaluate the ability of the intended lay user to correctly read and interpret their test results. Fifty (50) participants were enrolled in the readability and comprehension study. Each study subject was provided with eight randomized mock tests (2 panels with 4 mock devices each) of different concentrations, which they were asked to interpret per the IFU. After interpreting the mock tests, the study subjects were provided with a questionnaire to assess their comprehension of the test results. Each lay user was asked to interpret two panels of 5 test devices with three different concentrations that were arranged in a randomized and blinded manner, with the following sample results:

- Panel 1: Negative, 1.5xLoD, 1.5xLoD, 5xLoD, Invalid
- Panel 2: Negative, Negative, 1.5xLoD, 5xLoD, Invalid

Table 13. Mock Test Interpretation Summary Results

Sample Level	Number of Test Results Across all Participants	Number of Correct Interpretation	Observed Performance (%)
Negative	150	146	97.33%
1.5xLoD	150	140	93.33%
5xLoD	100	100	100%
Invalid	100	100	100%

D Clinical Cut-Off:

The test is a qualitative test with a binary positive/negative signal and has no clinical cut-off.

E Expected Values/Reference Range:

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

F Other Supportive Performance Characteristics Data:

1. <u>Flex Studies:</u>

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, swab extraction time, swab rotation, and tube squeezing) 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 NCM 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. However, these failures are mitigated in the labeling with warning statements in the procedural steps. The studies support that the test is robust in the intended use condition with an insignificant risk of erroneous result.

Lead Reviewer Comment for Internal Discussion Only

A detailed description of the flex studies is available in the internal section of the memo below in section X.7.

2. Serial Testing:

As a mitigation for the low performance of antigen tests very early and at the tail end of infection, the Intended Use for this test device (and associated Instructions for Use) states that negative results are presumptive, and it includes the need for repeat testing (i.e., test at least twice over three days with at least 48 hours between tests). These mitigations are supported by data generated by the National Institutes for Health (NIH) and the University of Massachusetts Chan Medical School (in collaboration with the FDA) demonstrating that repeat testing over multiple days improves test performance and increases the likelihood that a COVID-19 antigen test will accurately detect an infection. These results have informed the FDA's general understanding that repeat testing after a negative result from a COVID-19 antigen test reduces the risk of a false negative result. Please refer to the following studies for additional details:

- Finding a Needle in the Haystack: Design and Implementation of a Digital Site-less Clinical Study of Serial Rapid Antigen Testing to Identify Asymptomatic SARS-CoV-2 Infection - https://www.medrxiv.org/content/10.1101/2022.08.04.22278274v1
- Performance of Screening for SARS-CoV-2 using Rapid Antigen Tests to Detect Incidence of Symptomatic and Asymptomatic SARS-CoV-2 Infection: findings from the Test Us at Home prospective cohort study - https://www.medrxiv.org/content/10.1101/2022.08.05.22278466v1

3. Variant Monitoring Plan:

The sponsor established a variant monitoring plan for K241317 device to confirm the continued performance of the test device with future SARS-CoV-2 variants. Specifically, Wondfo plans a monthly surveillance of emerging SARS-CoV-2 variants by monitoring CDC and WHO websites along with their post-market surveillance reports and other regulatory sources of information. According to the available information, when a new emerging variant of SARS-CoV-2 is found to have high prevalence and causes public health concern (VOC or VOHC), one or more of the following actions will be taken for evaluating the impact of the variant:

i. In-silico analysis evaluation shall be performed for the impact of certain mutations on the target proteins.

ii. Wet-testing evaluation shall be conducted to verify the clinical performance and LoD of the device once the culture of the variant is available.

An evaluation report shall be prepared. The review date and the result of analysis will be maintained in a variant monitoring log. If the aggregate impact of the mutation reduces the

clinical performance of the test by 5% or more or decreases the clinical performance estimates for the test below 80% PPA or the established LoD decreases, these changes will be reported to FDA within 7 days of completing evaluation. In addition, the corrective and preventative action shall be implemented according to the company's standard operating procedures.

VIII Proposed Labeling:

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

IX Conclusion:

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