



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY**

I Background Information:

A 510(k) Number

K251289

B Applicant

Guangzhou Wondfo Biotech Co., Ltd.

C Proprietary and Established Names

WELLlife COVID-19 Antigen Test Rx

D Regulatory Information

| Product Code(s) | Classification | Regulation Section | Panel |
|-----------------|----------------|--|-------------------|
| QVF | Class II | 21 CFR 866.3982 - Simple Point-Of-Care Device To Directly Detect SARS-Cov-2 Viral Targets From Clinical Specimens In Near-Patient Settings | MI - Microbiology |

II Submission/Device Overview:

A Purpose for Submission:

To obtain substantial equivalence determination for the WELLlife COVID-19 Antigen Test Rx.

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 WELLlife COVID-19 Antigen Test Rx 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 upper respiratory infection. The test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections (COVID-19) in symptomatic individuals when either: tested at least twice over three days with at least 48 hours between tests; or when tested once, and negative by the WELLlife COVID-19 Antigen Test Rx and followed with a molecular test.

A negative test result is presumptive, and does not preclude SARS-CoV-2 infection; it is recommended these results be confirmed by a molecular SARS-CoV-2 assay.

Positive results do not rule out co-infection with other respiratory pathogens and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Performance characteristics for SARS-CoV-2 were established from April 2023 to February 2024 when SARS-CoV-2 Omicron was dominant. When other SARS-CoV-2 virus variants are emerging, performance characteristics may vary.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Not applicable.

IV Device/System Characteristics:

A Device Description:

The WELLlife COVID-19 Antigen Test Rx is a lateral flow immunoassay intended for prescription 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 WELLlife COVID-19 Antigen Test Rx consists of components below:

- Test Cassette
- Tube (pre-filled extraction buffer)
- Swab (sterile)
- Tube Holder (located in kit box)
- Quick Reference Instructions (QRI)
- Instructions for Use (IFU)

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 WELLlife COVID-19 Antigen Test Rx is a sandwich immunochromatographic assay that uses antibodies to detect SARS-CoV-2 nucleocapsid antigen in anterior nasal swab specimen. A nasal swab sample after collection is 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 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 anti-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 is read before 10 minutes or after 20 minutes.

External positive control and negative control swabs are sold separately from the WELLlife COVID-19 Antigen Test Rx and should be processed according to the external controls IFU at a frequency defined by that IFU. The control swabs are intended to be used as quality control samples representative of positive and negative test samples to demonstrate that the reagents are functional, and the assay procedure is performed correctly.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Nano-Check COVID-19 Antigen Test

B Predicate 510(k) Number(s):

K231187

C Comparison with Predicate(s):

| Device & Predicate Device(s): | Nano-Check COVID-19 Antigen Test K231187 (Predicate) | WELLife COVID-19 Antigen Test Rx K251289 |
|--|---|---|
| General Device Characteristic Similarities | | |
| Intended Use/Indications For Use | <p>The Nano-Check COVID-19 Antigen Test is a lateral flow immunochromatographic assay for the rapid, qualitative detection of SARS-CoV-2 nucleoprotein protein antigens directly in anterior nasal swab specimens from individuals with signs and symptoms of upper respiratory infection (i.e., symptomatic) when testing is started within 4 days of symptom onset. The test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections (COVID-19) in symptomatic individuals when either: tested at least twice over three days with at least 48 hours between tests; or when tested once, and negative by the Nano-Check COVID-19 Antigen Test and followed with a molecular test.</p> <p>The test does not differentiate between SARS-CoV or SARS-CoV-2.</p> <p>A negative test result is presumptive, and it is recommended these results be confirmed by a molecular SARS-CoV-2 assay.</p> <p>Positive results do not rule out co-infection with other bacteria or viruses and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.</p> <p>Performance characteristics for SARS-CoV-2 were established</p> | <p>The WELLife COVID-19 Antigen Test Rx 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 upper respiratory infection. The test is intended for use as an aid in the diagnosis of SARS-CoV-2 infections (COVID-19) in symptomatic individuals when either: tested at least twice over three days with at least 48 hours between tests; or when tested once, and negative by the WELLife COVID-19 Antigen Test Rx and followed with a molecular test.</p> <p>A negative test result is presumptive, and does not preclude SARS-CoV-2 infection; it is recommended these results be confirmed by a molecular SARS-CoV-2 assay.</p> <p>Positive results do not rule out co-infection with other respiratory pathogens and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.</p> <p>Performance characteristics for SARS-CoV-2 were established from April 2023 to February 2024 when SARS-CoV-2</p> |

| | | |
|--|---|--|
| | during the 2022 SARS-CoV-2 pandemic when SARS-CoV-2 Omicron was the predominant SARS-CoV-2 variant in circulation. When other SARS-CoV-2 virus variants are emerging, performance characteristics may vary. | Omicron was dominant. When other SARS-CoV-2 virus variants are emerging, performance characteristics may vary. |
| Regulation number | 21 CFR 866.3982 | Same |
| Intended use population | Individuals with symptoms of COVID-19 | Same |
| Intended use setting | Point-of-care | Same |
| Test principle | Lateral Flow Immunoassay | Same |
| Intended matrix | Anterior nasal swab | 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 | | |
| Reading time | 15 - 20 minutes | 10 - 20 minutes |

VI Standards/Guidance Documents Referenced:

| Document Title | Issued by | Applicable study |
|--|-----------|---------------------------------|
| Special controls for simple point-of-care device to directly detect SARS-CoV-2 viral targets from clinical specimens in near-patient settings (DEN220039 and special controls under 21 CFR 866.3982) | FDA/CDRH | All Studies |
| <i>Submission and review of sterility information in premarket notification (510(k)) submissions for devices labeled as sterile.</i> | FDA/CDRH | Sterility |
| ISO 10993-1, <i>Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process</i> | ISO | Biocompatibility |
| ISO 10993-10, Third Edition, <i>Biological evaluation of medical devices – Part 10: Tests for irritation and skin sensitization</i> | ISO | Biocompatibility |
| ISO 10993-5, Third edition, <i>Biological evaluation of medical devices – Part 5: Tests for in vitro cytotoxicity</i> | ISO | Biocompatibility |
| ISO 11135:2014, <i>Sterilization of health care products - Ethylene oxide - Requirements for development, validation and routine control of a sterilization process for medical devices</i> | ISO | Sterility |
| ISO 10993-7, <i>Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide Sterilization Residuals</i> | ISO | Sterility |
| ISO 10993-1, <i>Biological Evaluation of Medical Devices – Part 1: Evaluation and testing within a risk management process</i> | ISO | All Studies. Risk Management |

VII Performance Characteristics (if/when applicable):

A. Analytical Performance:

1. Precision/Reproducibility:

The precision and reproducibility studies were conducted separately.

a) 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). One (1) negative sample and two (2) samples with inactivated SARS-CoV-2 Omicron Variant lineage BA.5 (Isolate USA/COR-22-063113/2022) were spiked into negative clinical nasal swab matrix (NCM) to prepare a sample panel consisting of:

- Negative sample
- Low positive sample (1.5xLoD)
- Positive sample (3xLoD)

Fifty (50) microliters 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 1.5x LoD and 3x LoD, demonstrating no variability in the performance of the candidate assay across the conditions, operators, lots, and days tested.

Precision study samples were also prepared at 0.9x LoD (below LoD) 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/Total N)* | | | Below LoD (0.9xLoD) (n/Total N)* | | Low Positive (1.5xLoD) (p/Total P)# | | | Positive (3xLoD) (p/Total P)# | | |
|---|--------------------------|------|------|--|-------|---|-------|-------|-------------------------------------|-------|-------|
| 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 | 100% (720/720) | | | 88.89% (64/72) | | 100% (720/720) | | | 100% (720/720) | | |
| 95%CI | 99.47%, 100% | | | 79.58%, 94.26% | | 99.47%, 100% | | | 99.47%, 100% | | |
| * - (n/Total N) = number of negatives/Total number of negatives | | | | | | | | | | | |
| # - (p/Total P) = number of positives/Total number of positives | | | | | | | | | | | |

b) Reproducibility

A reproducibility study was conducted to assess any site-dependent variability in the performance of the candidate device. The study included one (1) device lot that was tested at three CLIA waived sites by three (3) untrained operators per site over 5 days and 3 replicates per sample (i.e., 1 lot x 3 sites x 3 operators x 5 days x 3 replicates = 135 results per sample panel member).

One (1) negative sample and four (4) samples of heat inactivated SARS-CoV-2 XBB (hCoV-19/USA/CA-Stanford-109_S21/2022) were spiked into negative clinical nasal swab matrix (NCM) to prepare a sample panel consisting of:

- Negative Sample
- High negative (0.1x LoD)
- Below LoD (0.8x LoD)
- Low positive (1x LoD)
- Moderate positive (3x LoD)

Fifty (50) microliters of each sample was applied to dry nasal swabs. After blinding and randomizing, samples were processed per the IFU of the candidate device.

Reproducibility was observed to be above 99% for all samples except those prepared at 0.8x LoD (which is as expected for samples below the LoD), demonstrating no variability in the performance of the candidate assay across the sites, operators, lots, and days tested.

Table 2. Reproducibility Study Summary Results

| | Negative (n/Total N)* | | | High Negative (0.1x LoD) (n/Total N)* | | | Low Positive (0.8x LoD) (p/Total P)# | | | Weak Positive (1x LoD) (p/Total P)# | | | Moderate Positive (3x LoD) (p/Total P)# | | |
|--|--------------------------|-------|-------|---|-------|-------|--|-------|-------|---|-------|-------|---|-------|-------|
| Operator | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Site A | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 | 13/15 | 13/15 | 14/15 | 15/15 | 15/15 | 14/15 | 15/15 | 15/15 | 15/15 |
| Site B | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 | 13/15 | 14/15 | 13/15 | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 |
| Site C | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 | 12/15 | 14/15 | 13/15 | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 | 15/15 |
| Agreement | 100% (135/135) | | | 100% (135/135) | | | 88.15% (119/135) | | | 99.26% (134/135) | | | 100% (135/135) | | |
| 95% CI | 97.23%, 100% | | | 97.23%, 100% | | | 81.61%, 92.57% | | | 95.92%, 99.87% | | | 97.23%, 100% | | |
| * - (n/Total N) = number of negatives/Total number of negatives # - (p/Total P) = number of positives/Total number of positives | | | | | | | | | | | | | | | |

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 2-3xLoD. 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 3. Cross-Reactivity and Microbial Interference Testing Results

| Microorganism | Concentration Tested | Cross Reactivity Result (no analyte) (number of positives/total) | Interference Result (number of positives/total) |
|--|---|--|---|
| 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)* ⁶ | 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 |
| 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 |
| <i>Haemophilus influenzae</i> type b (Eagan) | 2x10 ⁶ CFU/mL | 0/3 | 3/3 |
| <i>Streptococcus pneumoniae</i> Z022 | 2x10 ⁶ CFU/mL | 0/3 | 3/3 |
| <i>Streptococcus pyogenes</i> Z018 | 2x10 ⁶ CFU/mL | 0/3 | 3/3 |
| <i>Candida albicans</i> Z006 | 2x10 ⁶ CFU/mL | 0/3 | 3/3 |
| <i>Bordetella pertussis</i> A639 | 2x10 ⁶ CFU/mL | 0/3 | 3/3 |
| <i>Mycoplasma pneumoniae</i> | 2x10 ⁶ CCU/mL | 0/3 | 3/3 |

| Microorganism | Concentration Tested | Cross Reactivity Result (no analyte) (number of positives/total) | Interference Result (number of positives/total) |
|--|--------------------------|--|---|
| <i>Mycoplasma tuberculosis</i> | 2x10 ⁶ CFU/mL | 0/3 | 3/3 |
| <i>Chlamydia pneumoniae</i> | 2x10 ⁶ CFU/mL | 0/3 | 3/3 |
| <i>Legionella pneumophila</i> Philadelphia | 2x10 ⁶ CFU/mL | 0/3 | 3/3 |
| <i>Staphylococcus aureus</i> | 2x10 ⁶ CFU/mL | 0/3 | 3/3 |
| <i>Staphylococcus epidermidis</i> | 2x10 ⁶ CFU/mL | 0/3 | 3/3 |
| <i>Pneumocystis jirovecii</i> (PJP) - <i>S. cerevisiae</i> [#] | 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 | | | |
| [€] Tested with 2xLoD of SARS-CoV-2 Omicron Variant lineage BA.5 while the other potential cross-reactants/interferents were tested at 3xLoD. | | | |

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 the table below and testing them in NCM with or without SARS-CoV-2 virus at 2-3x LoD. 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 4. Endogenous/Exogenous Interfering Substances Summary Data

| Interfering Substances | Concentration | Cross-reactivity (no analyte) (# positive/total) | Interference (# positive/total) |
|---|------------------------------|--|---------------------------------|
| Whole Blood | 2.5% | 0/3 | 3/3 |
| Leukocytes | ≥1 x10 ⁶ cells/mL | 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 |
| 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 |

| Interfering Substances | Concentration | Cross-reactivity (no analyte) (# positive/total) | Interference (# positive/total) |
|--|---------------|--|------------------------------------|
| 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 |
| Fluticasone Propionate | 15% v/v | 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 |
| Zanamivir [€] | 10 mg/mL | 0/3 | 3/3 |
| Oseltamivir Phosphate (Tamiflu) | 5 mg/mL | 0/3 | 3/3 |
| Zinc [€] | 15% v/v | 0/3 | 3/3 |
| Sulfur [€] | 1.25% | 0/3 | 3/3 |
| <i>Luffa operculata</i> [€] | 1.25% | 0/3 | 3/3 |
| <i>Galphimia glauca</i> [€] | 15% v/v | 0/3 | 3/3 |
| Histaminum hydrochloricum [€] | 15% v/v | 0/3 | 3/3 |
| [€] Tested with 2xLoD of SARS-CoV-2 Omicron Variant lineage BA.5 while the other potential interferents were tested at 3xLoD. | | | |

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 WELLlife COVID-19 Antigen Test Rx has a built-in internal procedural control. A pink to purple 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:

The external controls (WELLlife COVID-19 Antigen Test Rx Control Kit) will be packaged separately from the test kit. The external control kit consists of a negative control swab composed of negative control buffer dried onto a swab, and a positive control swab that contains dried recombinant SARS-CoV-2 antigen onto a swab.

b) Device Stability:

i. Real Time Stability (Shelf life):

The stability of the WELLlife COVID-19 Antigen Test Rx 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 humidity of 85%. All results were concordant with expected results supporting stability during the anticipated shipping conditions for the test.

c) Sample Stability:

Specimen stability was evaluated using NCM for negative samples and NCM spiked with SARS-CoV-2 Omicron variant lineage BA.5 at 2x LoD for positive samples in replicates of three (3) at 30°C, 4°C, -20°C and -80°C for varying durations in each storage condition. All results were concordant with expected results supporting specimen stability (on the swab) for up to 150 minutes post collection at 30°C, 36 hours at 4°C and 10 days at -20°C and -80°C. However, given that the test will be performed in a near-patient setting, the IFU will indicate to perform the test immediately after collection.

6. Detection Limit:

a) Limit of Detection:

These strains were 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 (3) 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. The last concentrations that produced 20-positive test results/lot from a 1:10 dilution was further evaluated using a 3-fold dilution series, in 20 replicates/lot for each level, to refine the LoD at,

- For SARS-CoV-2 (USA-WA-1/2020), the LoD was confirmed at 1×10^4 TCID₅₀/mL.
- For SARS-CoV-2 (Lineage BA.5 (Omicron Variant)) the LoD was confirmed at 3.33×10^3 TCID₅₀/mL.
- For SARS-CoV-2 (Omicron Variant lineage XBB, hCoV-19/USA/CA-STANFORD-109_S21/2022), the LoD was confirmed at 1×10^4 TCID₅₀/mL.

Table 5. LoD Study Summary

| Concentration | | Lot 1 | | Lot 2 | | Lot 3 | |
|---|--------------------------|------------|--------------|------------|--------------|------------|--------------|
| TCID ₅₀ /mL | TCID ₅₀ /Swab | P | C | P | C | P | C |
| Inactivated SARS-CoV-2 (USA-WA-1/2020) | | | | | | | |
| 3.39×10^7 | 1.7×10^6 | 100% (3/3) | N | 100% (3/3) | N | 100% (3/3) | N |
| 1×10^6 | 5×10^4 | 100% (3/3) | N | 100% (3/3) | N | 100% (3/3) | N |
| 1×10^5 | 5×10^3 | 100% (3/3) | 100% (20/20) | 100% (3/3) | 100% (20/20) | 100% (3/3) | 100% (20/20) |
| 1×10^4 | 5×10^2 | 100% (3/3) | 100% (20/20) | 100% (3/3) | 100% (20/20) | 100% (3/3) | 100% (20/20) |
| 3.33×10^3 | 1.67×10^2 | N | 95% (19/20) | N | 85% (17/20) | N | N |
| 1×10^3 | 5×10^1 | 0% (0/3) | N | 0% (0/3) | N | 0% (0/3) | N |
| Inactivated SARS-CoV-2 BA.5 (Omicron) | | | | | | | |
| 1.98×10^6 | 9.9×10^4 | 100% (3/3) | N | 100% (3/3) | N | 100% (3/3) | N |
| 1×10^5 | 5×10^3 | 100% (3/3) | N | 100% (3/3) | N | 100% (3/3) | N |
| 1×10^4 | 5×10^2 | 100% (3/3) | 100% (20/20) | 100% (3/3) | 100% (20/20) | 100% (3/3) | 100% (20/20) |
| 3.33×10^3 | 1.67×10^2 | N | 100% (20/20) | N | 100% (20/20) | N | 100% (20/20) |
| 1.11×10^3 | 5.55×10^1 | N | 65% (13/20) | N | 15% (3/20) | N | 85% (17/20) |
| 1×10^3 | 5×10^1 | 0% (0/3) | N | 0% (0/3) | N | 0% (0/3) | N |
| Inactivated SARS-CoV-2 (Omicron Variant lineage XBB) | | | | | | | |
| 5.95×10^6 | 2.98×10^5 | 100% (3/3) | N | 100% (3/3) | N | 100% (3/3) | N |
| 1×10^5 | 5.00×10^3 | 100% (3/3) | 100% (20/20) | 100% (3/3) | 100% (20/20) | 100% (3/3) | 100% (20/20) |
| 1×10^4 | 5.00×10^2 | 100% (3/3) | 100% (20/20) | 100% (3/3) | 100% (20/20) | 100% (3/3) | 100% (20/20) |
| 3.33×10^3 | 1.67×10^2 | 0% (0/3) | 15% (3/20) | 0% (0/3) | 20% (4/20) | 0% (0/3) | 50% (10/20) |
| 1.11×10^3 | 5.55×10^1 | 0% (0/3) | | 0% (0/3) | | 0% (0/3) | |
| <i>P: Preliminary Study. C: Confirmatory LoD Study. N: Not tested</i> | | | | | | | |

b) Limit of Detection with the International Standard (NIBSC 21/368):

Wondfo tested the sensitivity of the test against the 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 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 the form of an LoD study with seven different SARS-CoV-2 variant strains. Three lots of the WELLife COVID-19 Antigen Test Rx 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 $\geq 95\%$ of all replicates for each evaluated SARS-CoV-2 strain is shown below.

Beyond the testing described above, Omicron JN.1 was independently evaluated with the test showing detection down to 2.28×10^4 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 x 10 ³ |
| SARS-CoV-2 Lineage B.1.351 (Beta variant) | 23/23 | 23/23 | 23/23 | 1 x 10 ³ |
| SARS-CoV-2 Variant Brazil Lineage P.1 (Gamma variant) | 23/23 | 23/23 | 23/23 | 1 x 10 ³ |
| SARS-CoV-2 Lineage B.1.617.2 (Delta Variant) | 23/23 | 23/23 | 23/23 | 1 x 10 ² |
| SARS-CoV-2 Lineage B.1.1.529 (Omicron Variant) | 23/23 | 23/23 | 23/23 | 1 x 10 ² |
| SARS-CoV-2 Lineage BA 2.3 (Omicron Variant) | 23/23 | 23/23 | 23/23 | 3.33 x 10 ² |
| Strain/Viral Material | Reactivity (Number positive/ Number tested) | | | GE/mL* |
| | Lot 4 | | | |
| SARS-CoV-2 JN.1 (live) | 5/5 | | | 2.28 x10 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. Fifty (50) μ l 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 |
| 1.98x10 ⁶ | 3/3 | 3/3 | 3/3 |
| 1x10 ⁵ | 3/3 | 3/3 | 3/3 |
| 1x10 ⁴ | 3/3 | 3/3 | 3/3 |
| 1x10 ³ | 0/3 | 0/3 | 0/3 |
| 1x10 ² | 0/3 | 0/3 | 0/3 |
| 1x10 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 with symptoms upto 5 days post symptom onset (DPSO). The 1,032 results consisted of 128 positive and 904 negative study subjects as defined by the

comparator result. The WELLlife COVID-19 Antigen Test Rx 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 of Clinical Study Participants – Age, Sex and Self-Collection Distribution

| 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% |
| > 64 years of age | 131 | 12.69% |
| Total | 1,032 | 100% |
| Sex | | |
| 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. Serial Testing:

This clinical data set verifies the known lower sensitivity for samples collected on the day of symptom onset (i.e., Day 0) that was observed for test devices of similar technology and design across a multitude of clinical studies. As a mitigation, the Intended Use for this test device (and associated Instructions for Use) include recommendations for repeat testing (i.e., test at least twice over three days with at least 48 hours between tests). This mitigation is 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 K250273 - Page 17 of 17 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>.

D. Clinical Cut-Off:

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

E. Other Supportive Performance Characteristics Data:

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 (e.g., 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 (e.g., lighting, disturbance during use, temperature, and humidity stress conditions). Testing was performed with contrived positive nasal swabs generated by diluting SARS-CoV-2 virus (SARS-CoV-2 Omicron Variant lineage BA.5 viral stock) 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.

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.