510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I. Background Information:

A. 510(k) Number:

K233842

B. Applicant:

iHealth Labs, Inc

C. Proprietary and Established Names:

iHealth COVID-19 Antigen Rapid Test

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:

To obtain 510(k) clearance for the iHealth COVID-19 Antigen Rapid Test.

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 iHealth COVID-19 Antigen Rapid 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 15 years or older testing themselves, or adults testing individuals aged 2 years or older.

The iHealth COVID-19 Antigen Rapid 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 either 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.

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 October 2022 to June 2023 when the COVID-19 variant 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 iHealth COVID-19 Antigen Rapid Test employs lateral flow immunoassay technology to allow for the rapid detection of nucleocapsid protein from the SARS-CoV-2 virus. This test does not differentiate between SARS-CoV and SARS-CoV-2.

The test package is composed of the following components:

- Test Cassette (test card, individually packaged)
- Extraction Buffer Tube
- Disposable Sterile Nasal Swab
- Package Insert containing Quick Reference Instructions

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

B. Principle of Operation:

To begin the test, a self-collected anterior nares swab sample (ANS) in individuals aged 15 and older or an ANS swab collected by a parent or guardian in individuals between the age of 2 to 14 is inserted into the sample tube. The sample tube contains sample extraction buffer. This buffer lyses any virions contained in the specimen and facilitates exposure of the appropriate viral antigens to the antibodies used in the test. The lysed sample liquid in the sample tube is then added to the Sample Port of the test cassette.

If the extracted specimen contains SARS-CoV-2 antigens, a pink-to-purple T Line from the formation of SARS CoV-2 antigen immune complexes, along with a pink-to-purple C Line will appear on the cassette indicating a positive result. If SARS-CoV-2 antigens are not present, or present at levels below the limit of detection, only a pink-to-purple C Line will appear. The C Line must appear for a sample result to be valid and interpretable.

iHealth Application: The iHealth mobile application constitutes electronic labeling that allows for digitally guided testing of anterior nasal swab samples self-collected from lay users and reporting of results. The software application is intended for use with the iHealth COVID-19 Antigen Rapid Test over the counter (OTC) device as an alternative to the paper copy of the Instructions for Use (IFU) provided in the test kit. The iHealth application is intended only to guide the user in the preparation and execution of the iHealth COVID-19 Antigen Rapid Test, it does not interpret the test result.

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):	**K233842 (Candidate Device)**	**K230828 (Predicate)**
Device Trade Name	iHealth COVID-19 Antigen Rapid Test	ACON Flowflex COVID-19 Antigen Home Test
General Device Characteristic Similarities		

Device & Predicate Device(s):	K233842 (Candidate Device)	K230828 (Predicate)
Intended Use/ Indications For Use	The iHealth COVID-19 Antigen Rapid Test is a visually read lateral flow immunoassay device intended for the rapid, qualitative detection of SARS-CoV-2 nucleocapsid protein antigens directly in anterior nasal (nares) swab specimens from individuals with signs and symptoms of COVID-19 within the first 6 days from symptom onset. This test is for non-prescription home use by individuals aged 15 years or older testing themselves, or adults testing individuals aged 2 years or older. The iHealth COVID-19 Antigen Rapid 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 either 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. 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,	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 either an antigen test or a molecular test for SARS-CoV2. 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 coinfection 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,

Device & Predicate Device(s):	K233842 (Candidate Device)	K230828 (Predicate)
	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 October 2022 to June 2023 when the COVID-19 variant 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.	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 December 2022 to March 2023 when SARSCoV-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.
Regulation Number	21 CFR 866.3984	Same
Product Code	QYT	Same
Analyte	SARS CoV-2 nucleocapsid	Same
Intended Use Setting	OTC	Same
Intended Use Population	Symptomatic	Same
Usage Type	Single use	Same
Specimen Type	Direct Anterior Nasal Swab	Same
Test Result Type	Qualitative	Same
Technology	Lateral flow immunoassay	Same
Detection Format	Visually read	Same
Time to Result	15-30 minutes	Same
Storage Condition	2-30ºC	Same

The differences in the iHealth COVID-19 Antigen Rapid Test (proposed device) and the ACON Flowflex COVID-19 Antigen Home Test (predicate device, K230828) are limited to the minimum age for self-collection and self-testing. This difference does not affect the overall substantial equivalence of the proposed device to the predicate device in terms of the technological similarity, intended use, safety, and effectiveness.

VI. Standards/Guidance Documents Referenced:

Document	Title	Publisher	Applicable Study
Special Controls under 21 CFR 866.3984 (Over-the-counter test to detect SARS-CoV-2)	Reclassification order for DEN220028 and special controls under 21 CFR 866.3984	FDA/CDRH	All Studies

Document	Title	Publisher	Applicable Study
ISO11135:2014	Sterilization of health care products - Ethylene oxide - Requirements for development, validation and routine control of a sterilization process for medical devices	ISO	Sterility
ISO 10993-7	Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide Sterilization Residuals	ISO	Sterility
FDA Guidance	Submission and Review of Sterility Information in Premarket Notification (510(k)) Submissions for Devices Labeled as Sterile | FDA.	FDA/CDRH	Sterility
ISO 10993-5	Biological evaluation of medical devices Part 5: Tests for in vitro cytotoxicity	ISO	Biocompatibility
ISO 10993-10	Biological evaluation of medical devices Part 10: Tests for irritation and skin sensitization	ISO	Biocompatibility

VII. Performance Characteristics

A. Analytical Performance:

1. Multi-Site Reproducibility:

The purpose of the study was to assess between-site and between-lot variability of three different lots of the iHealth COVID-19 Antigen Rapid Test. Three (3) levels of heat-inactivated SARS-CoV-2 were spiked in negative clinical matrix (NCM, Nasal fluid diluted in 1:3 Saline) as follows:

A. Negative, NCM
B. Low positive (1x LoD)
C. Positive (3x LoD)

Sample panels for use on each day of the study were prepared at a central site, randomized and frozen at -70 ºC for transport to the lab sites where they were thawed at room temperature before testing. The fresh frozen stability of the sample panel was confirmed by an appropriately designed fresh frozen study. The sample panel was tested in a blinded manner by three operators at three CLIA waived sites for 5 non-consecutive days.

Each operator applied 50 µL of each coded sample to each dry nasal swab. Then, the operator processed the sample per the IFU of the proposed device. All three lots were tested by each operator on each testing day. Each sample level was tested in triplicate in each run per operator

per day (i.e., 3 lots x 3 operators x 3 replicates/run x 5 days). A total of 405 tests were run for each concentration level in the panel.

The agreement of obtained results with expected results was 100% for all samples across all lots, operators, and days. Variability in results was not observed between the three independently manufactured lots. The results are summarized in Table 1 below:

Table 1: Reproducibility Study Results

Site	True Negative	Low Positive (1x LoD)	Positive (3x LoD)
Site 1 (External, 3 operators)	135/135	135/135	135/135
Site 2 (External, 3 operators)	135/135	135/135	135/135
Site 3 (External, 3 operators)	135/135	135/135	135/135
Total	405/405	405/405	405/405
% Agreement	100%	100%	100%
95% CI	99.1-100%	99.1-100.%	99.1-100%

2. Linearity

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

3. Analytical Specificity/Interference:

   a. Cross Reactivity/Microbial Interference

To demonstrate that the iHealth COVID-19 Antigen Rapid Test does not react with related viruses, high prevalence disease agents, and normal or pathogenic flora that are reasonably likely to be encountered in nasal swab specimens, the analytical specificity of the iHealth COVID-19 Antigen Rapid Test was evaluated by testing various microorganisms (22), viruses (31), and negative clinical matrix (1) in the absence (cross-reactivity) and presence (microbial interference) of SARS-CoV-2 at 2x LoD ($2.66x10^4$ $TCID_{50}$/mL).

Each organism and each virus was tested in five (5) replicates using heat-inactivated SARS-CoV-2 (hCoV-19/South Africa/CERI-KRISP-K040013/2022). The microbial interference and the cross-reactivity study were conducted simultaneously with samples tested in a randomized and blinded manner.

None of the organisms and viruses above showed cross-reactivity and interference in the assay at the concentrations listed in summary Table 2 below.

Table 2: Cross Reactivity and Microbial Interference Results

Microorganism	Concentration Tested	Cross-Reactivity Negative Agreement	Interference Positive Agreement
Adenovirus Type 1	2.82×10^6 $TCID_{50}$/mL	5/5	5/5
Adenovirus Type 4	2.84×10^5 $TCID_{50}$/mL	5/5	5/5

Microorganism	Concentration Tested	Cross-Reactivity Negative Agreement	Interference Positive Agreement
Adenovirus Type 7A	3.16×10^5 $TCID_{50}$/mL	5/5	5/5
Adenovirus Type 8	1.13×10^5 U/mL	5/5	5/5
Adenovirus Type 31	1.13×10^5 $TCID_{50}$/mL	5/5	5/5
Adenovirus Type 41	3.80×10^5 $TCID_{50}$/mL	5/5	5/5
Bordetella pertussis	1.16×10^9 CFU/mL	5/5	5/5
Candida albicans	1.31×10^7 CFU/mL	5/5	5/5
Chlamydia pneumonia	1.33×10^8 IFU/mL	5/5	5/5
Enterovirus, Type 68	2.84×10^5 $TCID_{50}$/mL	5/5	5/5
Enterovirus, Type 71	1.13×10^5 $TCID_{50}$/mL	5/5	5/5
Haemophilus influenzae	1.42×10^8 CFU/mL	5/5	5/5
Human coronavirus 229E	4.50×10^4 $TCID_{50}$/mL	5/5	5/5
Human coronavirus HKU1 (Np swab)	Ct 33.8	5/5	5/5
Human coronavirus NL63	2.84×10^5 $TCID_{50}$/mL	5/5	5/5
Human coronavirus OC43	1.36×10^5 $TCID_{50}$/mL	5/5	5/5
Human Metapneumovirus 3, type B1	9.4×10^4 $TCID_{50}$/mL	5/5	5/5
Human Metapneumovirus 4, type B2	3.33×10^5 $TCID_{50}$/mL	5/5	5/5
Human Metapneumovirus 9 Type A1	1.13×10^5 $TCID_{50}$/mL	5/5	5/5
Influenza A H3N2 (Wisconsin/67/05	1.13×10^5 $TCID_{50}$/mL	5/5	5/5
Influenza B (Victoria/504/00)	1.26×10^5 $TCID_{50}$/mL	5/5	5/5
Legionella pneumophila	1.42×10^9 CFU/mL	5/5	5/5
MERS-coronavirus (NATrol Stock)	Ct 27.1	5/5	5/5
SARS-coronavirus	1.0×10^5 CFU/mL	5/5	5/5
Mycobacterium tuberculosis	1.21×10^7 CFU/mL	5/5	5/5
Mycoplasma pneumoniae	2.16×10^8 CCU/mL	5/5	5/5
Parainfluenza virus 1	3.80×10^5 $TCID_{50}$/mL	5/5	5/5
Parainfluenza virus 2	3.39×10^6 $TCID_{50}$/mL	5/5	5/5
Parainfluenza virus 3	1.15×10^6 $TCID_{50}$/mL	5/5	5/5
Parainfluenza virus 4A	1.13×10^5 $TCID_{50}$/mL	5/5	5/5
Parainfluenza virus 4B	9.55×10^5 $TCID_{50}$/mL	5/5	5/5
Pooled human nasal cavity wash	n/a	5/5	5/5
Pseudomonas aeruginosa	7.09×10^8 CFU/mL	5/5	5/5
Respiratory syncytial virus A	1.05×10^5 $TCID_{50}$/mL	5/5	5/5
Respiratory syncytial virus B	1.51×10^5 $TCID_{50}$/mL	5/5	5/5
Rhinovirus, Type 1A	1.13×10^5 $TCID_{50}$/mL	5/5	5/5
Staphylococcus aureus	1.42×10^8 CFU/mL	5/5	5/5
Staphylococcus epidermidis	6.84×10^8 CFU/mL	5/5	5/5
Streptococcus salivarius	1.35×10^8 CFU/mL	5/5	5/5
Streptococcus pneumoniae	7.22×10^7 CFU/mL	5/5	5/5
Streptococcus pyogenes	4.60×10^8 CFU/mL	5/5	5/5

Microorganism	Concentration Tested	Cross-Reactivity Negative Agreement	Interference Positive Agreement
Corynebacterium diphtheriae	3.43 x 10^7 CFU/mL	5/5	5/5
Escherichia coli	8.16 x 10^8 CFU/mL	5/5	5/5
Lactobacillus acidophilus	6.58 x 10^7 CFU/mL	5/5	5/5
Moraxella catarrhalis	5.92 x 10^7 CFU/mL	5/5	5/5
Neisseria Elongata	1.07 x 10^9 CFU/mL	5/5	5/5
Neisseria meningitidis serogroup A	2.04 x 10^7 CFU/mL	5/5	5/5
Neisseria meningitidis serogroup B	9.15 x 10^7 CFU/mL	5/5	5/5
Neisseria meningitidis serogroup C	7.90 x 10^7 CFU/mL	5/5	5/5
Epstein-Barr virus	9.70 x 10^6 cp/mL	5/5	5/5
Mumps virus	9.55 x 10^5 $TCID_{50}$/mL	5/5	5/5
Cytomegalovirus (CMV)	1.13 x 10^5 $TCID_{50}$/mL	5/5	5/5
Measles virus	1.23 x 10^7 $TCID_{50}$/mL	5/5	5/5

b. Interfering Substances:

Thirty-one (31) potentially interfering substances were evaluated with the iHealth COVID-19 Antigen Rapid Test. Each substance was tested in three (5) replicates in the absence or presence of heat-inactivated SARS-CoV-2 (hCoV-19/South Africa/CERI-KRISP-K040013/2022) at 2x LoD.

None of the endogenous and exogenous substances listed in table below interfered with the assay at the concentration listed in Table 3.

Table 3: Interfering Substances Testing Results

Interfering Substances Tested	Without SARS-CoV-2		With 2x LoD SARS-CoV-2	
	Concentration Tested	Positive/ Tested	Concentration Tested	Positive/ Tested
Sore Throat & Cough Lozenges (Benzocaine),	3 mg/mL	0/5	3 mg/mL	5/5
Throat Lozenges (Menthol)	3 mg/mL	0/5	10 mg/mL	5/5
Sore Throat Spray (Phenol)	5% v/v	0/5	5% v/v	5/5
Mucin, bovine submaxillary type I-S	2.5 mg/mL	0/5	2.5 mg/mL	5/5
Whole Blood	2.5% v/v	0/5	2.5%	5/5
Leukocytes	4.8 x 10^6 cells/mL	0/5	4.8 x 10^6 cells/mL	5/5
Zinc	5% v/v	0/5	5% v/v	5/5
Nasal spray (Phenylephrine HCl)	15% v/v	0/5	15% v/v	5/5
Nasal spray (Cromolyn sodium nasal solution)	15% v/v	0/5	15% v/v	5/5
Nasal spray Afrin (Oxymetazoline HCl)	15% v/v	0/5	15% v/v	5/5

Interfering Substances Tested	Without SARS-CoV-2		With 2x LoD SARS-CoV-2	
	Concentration Tested	Positive/ Tested	Concentration Tested	Positive/ Tested
Nasal spray (Sodium Chloride & Preservatives)	15% v/v	0/5	15% v/v	5/5
Nasal corticosteroids (Beclomethasone dipropionate)	15% v/v	0/5	15% v/v	5/5
Nasal corticosteroids (dexamethasone)	15% v/v	0/5	15% v/v	5/5
Nasal corticosteroids (Flunisolide)	15% v/v	0/5	15% v/v	5/5
Nasal corticosteroids Nasocort Allergy 24 hour (Triamcinolone Acetonide)	15% v/v	0/5	15% v/v	5/5
Nasal corticosteroids Rhinocort (Budesonide /Glucocorticoid)	15% v/v	0/5	15% v/v	5/5
Nasal corticosteroids (Mometasone furoate)	15% v/v	0/5	15% v/v	5/5
Nasal corticosteroids (fluticasone propionate)	15% v/v	0/5	15% v/v	5/5
Nasal gel ((Homeopathic Luffa opperculata Galphimia glauce)	1.25%	0/5	1.25%	5/5
Nasal gel ((Luffa opperculata sulfur)	1.25%	0/5	1.25%	5/5
Homeopathic allergy relief (Galphimia glauca)	15% w/v	0/5	15% w/v	5/5
Homeopathic allergy relief (histaminum hydrochloricum)	15% w/v	0/5	15% w/v	5/5
Homeopathic nasal wash (Alkalol)	15% v/v	0/5	15% v/v	5/5
Homeopathic nasal wash (Zicam)	15% v/v	0/5	15% v/v	5/5
Oseltamivir Phosphate (Tamiflu)	5 mg/mL	0/5	5 mg/mL	5/5
Remdesivir	5 mg/mL	0/5	5 mg/mL	5/5
Anti-viral drugs (Molnupiravir)	5 mg/mL	0/5	5 mg/mL	5/5
Antibiotic, nasal ointment (Mupirocin)	10 mg/mL	0/5	10 mg/mL	5/5
Hand sanitizer	15% v/v	0/5	15% v/v	5/5
Hand Soap	15% v/v	0/5	15% v/v	5/5

c. Biotin Interference

The iHealth COVID-19 Antigen Rapid Test uses streptavidin/biotin technology for antibody immobilization. Using the same protocol as for the Endogenous Interference Studies, the iHealth COVID-19 Antigen Rapid Test was tested for biotin interference up to 3500 ng/mL. As shown in Table 4**,** biotin interference was observed at concentrations at or above 350 ng/mL.

Table 4: Biotin Interference Study Results

Biotin Concentration (ng/mL)	No SARS CoV-2 (negative agreement)	SARS CoV-2 at 2x LoD (positive agreement)
200	0/5	5/5
350	0/5	3/5
700	0/5	0/5
1750	0/5	0/5
3500	0/5	0/5

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. Internal Controls

The iHealth COVID-19 Antigen Rapid Test contains the control line (C) of the device as an internal procedural control that indicates whether the applied specimen has migrated properly across the device. The sample pad of the device contains control IgG molecules that are captured at the control line by specific anti- IgG antibodies. As sample addition wicks into the membrane and resolves the reagents in the sample pad a red colored line indicating the formation of immune complexes should appear at the control line when sufficient sample is added. If the extracted specimen contains SARS-CoV-2 antigens, a pink-to-purple T Line, along with a pink-to-purple C Line will appear on the COVID-19 Test Card indicating a positive result. If SARS-CoV-2 antigens are not present, or present at very low levels, only the pink-to-purple C Line will appear. Test results without a visible control line cannot be interpreted (i.e., are invalid) and need to be retested with a new sample and a new device.

b. Sample Stability

Samples in the OTC environment will not undergo storage as the IFU instructs the user to immediately proceed from the sample collection to the testing steps.

c. Real-Time Stability

Shelf-life of the iHealth COVID-19 Antigen Rapid Test under the intended storage condition (2-30°C) was assessed in a real-time stability study using three (3) lots of test kit stored at 2-8°C with 41.3% - 45.6% RH, and 30°C with 91.4% - 95.4 RH. The test kits were assessed with negative samples and contrived positive samples prepared using heat inactivated SARS CoV-2 hCoV-19/South Africa/CERI-KRISP-K040013/2022 (Lineage BA.5) at 3x LoD. Five replicates per timepoint per storage condition were analyzed. The data collected in this study supports a shelf life of 13 months.

d. Transportation Stability

The stability of the iHealth COVID-19 Antigen Rapid Test kit under different shipping conditions was determined for three lots of test kits that were stored at two different temperature conditions:

1. *Winter Profile:* Kits were stored at 20°C with 11.2 - 15.4% relative humidity for 7 days, followed by 18°C at the same RH conditions for 4 hours (winter profile)
2. *Summer Profile:* Kits were stored at 45°C at 90.2% - 93.9 % RH for 7 days followed by 22°C at the same RH conditions for 4 hrs. (summer profile).

All negative samples tested negative, all positive samples (3x LoD, 4.0 x104 TCID50/mL) tested positive in all storage conditions at the 7-day time point.

6. Detection Limit:

a. LoD Testing

The LoD of the was determined by evaluating different dilutions of heat-inactivated SARS-CoV-2 (hCoV-19/South Africa/CERI-KRISP-K040013/2022 (Lineage BA.5) in negative nasal matrix (NNM). The LoD was determined as the lowest virus concentration that was detected ≥ 95% of the time (i.e., concentration at which at least 19 out of 20 replicates tested positive). The limit of detection was established in two (2) phases: a range finding and a confirmatory LoD study.

Range Finding LoD Study

Five serial dilutions were made from heat inactivated SARS-CoV-2 virus into negative nasal matrix (NNM). Five (5) replicates were tested on one kit lot of the assay for each of five 1:10 dilutions to determine the preliminary LoD concentration of the device. The lowest concentration with 5/5 positive results from each lot was considered the preliminary LoD. For each replicate 50 µL of virus dilution was applied to a swab and the swab was processed according to the IFU. The results are summarized below in Table 5:

Table 5: Preliminary LoD Study Summary

Concentration of SARS CoV-2 applied to dry swab	Kit Lot 2023014 Positive/ Tested
3.98 x 10^6 $TCID_{50}$/mL	5/5
3.98 x 10^5 $TCID_{50}$/mL	5/5
3.98 x 10^4 $TCID_{50}$/mL	**5/5**
3.98 x 10^3 $TCID_{50}$/mL	3/5
3.98 x 10^2 $TCID_{50}$/mL	0/5

Confirmatory LoD Study

The preliminary LoD test sample concentration and a 3-fold dilution series were tested on each of 3 lots on three consecutive days. The Sponsor tested a total of twenty (n = 20) replicates for the confirmatory dilutions to confirm the LoD for each lot, at least 19 of the 20 replicates should be positive. Per lot, the final confirmation data set included the confirmed LoD level (1.33 x10^4 $TCID_{50}$/mL) with at least one additional level tested above and below this value to demonstrate that levels above the LoD were 100% positive and levels below the LoD were <95% positive. The results are summarized below in Table 6.

Table 6: Confirmatory LoD Study Summary

Concentration of SARS CoV-2 applied to dry swab	Lot 2023104 Positive/ Tested	Lot 2023105 Positive/ Tested	Lot 2023106 Positive/ Tested
3.98 x 10^4 $TCID_{50}$/mL (Preliminary LoD)	20/20	20/20	20/20
1.33 x 10^4 $TCID_{50}$/mL (Confirmed LoD)	20/20	20/20	19/20
4.43 x 10^3 $TCID_{50}$/mL (0.3x LoD)	15/20	17/20	16/20

The limit of detection for the iHealth COVID-19 Antigen Rapid Test using SARS-CoV-2 strain hCoV-19/South Africa/CERI-KRISP-K040013/2022, was confirmed to be 1.33 x 10^4 $TCID_{50}$/mL (6.65 x10^2 $TCID_{50}$/swab) with all three tested lots.

b. WHO Standard for SARS-CoV-2 Antigen (NIBSC 21/368)

A study was performed to also determine the Limit of Detection (LoD) for iHealth COVID-19 Antigen Rapid Test in ANS samples using the WHO International Standard for SARS-CoV-2 Antigen (NIBSC 21/368) as a standardized material.

As per the WHO instructions, the international standard material was reconstituted in 0.25 mL of ultra-pure water. Following reconstitution, the ampule was left at ambient temperature for 20 minutes and then mixed thoroughly, avoiding generation of excess foam. The reconstitution of the material yielded a final stock concentration equal to 2.0 x10^4 IU/mL. This sample was diluted 1:5 to yield a concentration of 4.0 x 10^3 IU/mL.

Range Finding LoD Study

A preliminary LoD concentration was determined by testing two series of 2-fold dilutions of the 4.0 x 10^3 IU/mL solution spiked into negative nasal matrix (NNM) in replicates of three (3), using three test lots. Once the lowest concentration with 3 out of 3 positive replicates was established, it was considered the preliminary LoD. The results of this preliminary LoD study are shown as Table 7:

Table 7: Results LoD Range Finding with WHO SARS-CoV-2 Antigen Standard

NIBSC 21/368 Concentration applied to dry swab	Positive/ Tested
Initial Range Finding	
4.00 x 10^3 IU/mL	9/9
2.00 x 10^3 IU/mL	9/9
1.00 x 10^3 IU/mL	9/9
5.00 x 10^2 IU/mL	**9/9**
2.50 x10^2 IU/mL	**0/9**
2nd Round Range Finding	
8.00 x 10^2 IU/mL	9/9
4.00 x 10^2 IU/mL	**9/9**
2.00 x 10^2 IU/mL	0/9

Confirmatory LoD Study

The preliminary LoD was confirmed by testing an additional twenty (20) replicates per lot at the preliminary LoD. The results of this testing, as shown in Table 8 confirmed the Limit of Detection for the WHO International Standard Antigen to be 4.00 x10^2 IU/mL and 20 IU/swab.

Table 8: WHO SARS-CoV-2 Standard Confirmatory LoD Results

Concentration of WHO International Standard for SARS-CoV-2 antigen applied to dry swab	Positive/ Tested
4.00 x 10^2 IU/mL	**59/60**

7. High Dose Hook Effect

The purpose of this study was to evaluate the effect of a high concentration of SARS antigen on the performance of the iHealth COVID-19 Antigen Rapid Test. Fifty microliters (50 µl) of heat-inactivated SARS-CoV-2 (hCoV-19/South Africa/CERI-KRISP-K040013/2022 Lineage BA.5) at 3.98 x 10^6 $TCID_{50}$/mL concentration (300x LoD) was added to each swab and was tested in five (5) replicates using the iHealth COVID-19 Antigen Rapid Test. The testing was conducted according to the Instructions for Use for the test. The results are summarized in Table 9 below:

Table 9: High Dose Hook Effect Study Results

Concentration of SARS CoV-2 BA.5 applied to dry swab	Kit Lot 2023014 Positive/ Tested
3.98 x 10^6 $TCID_{50}$/mL	5/5

8. Inclusivity (Analytical Reactivity)

Analytical reactivity for iHealth COVID-19 Antigen Rapid Test was demonstrated using 14 additional strains/isolates of SARS CoV-2 virus. Heat-inactivated SARS-CoV-2 isolates were each diluted into NNM at different concentrations. Each concentration was tested with 5 replicates until all 5 dilutions tested negative, or two consecutive dilutions produced one or more negative replicates out of 5. The reactivity of the IHealth COVID-19 Antigen Rapid Test with the variants is summarized in Table 10 below, with the lowest concentration that returned 100% positive replicates (i.e., 5/5).

Table 10: Inclusivity Study Results

SARS-CoV-2 Variants	Lowest Variant Concentration with 5/5 positive replicates [$TCID_{50}$/mL]
SARS-CoV-2 virus (USA-WA1/2020)	4.57 x 10^3
B.1.1.7 (Alpha)	9.55 x 10^3
B.1.351 (Beta)	3.80 x 10^3
P.1 (Gamma)	1.7 x 10^3
B.1.617.2 (Delta)	1.26 x 10^4
B.1.1.529 (Omicron)	5.01 x 10^2
BA.2. (Omicron)	1.0 x 10^3
BA.2.3 (Omicron)	1.17 x 10^3
BA.4 (Omicron)	6.31 x 10^3
BA.4.6 (Omicron)	1.00 x 10^4

SARS-CoV-2 Variants	Lowest Variant Concentration with 5/5 positive replicates [$TCID_{50}$/mL]
BA.5(Omicron)	3.98×10^3
BQ.1(Omicron)	2.0×10^3
BQ.1.1(Omicron)	7.94×10^4
XBB (Omicron)	1.0×10^3

9. Assay Cut-Off:

Not applicable, the device is a binary qualitative assay.

B. Comparison Studies:

1. Method Comparison with Predicate Device:

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

2. Matrix Comparison:

The iHealth COVID-19 Antigen Rapid Test is only intended for the qualitative detection of the nucleocapsid protein antigen from SARS-CoV-2 in direct anterior nasal swab specimens. As no other specimen or sample type is claimed for this device, a Matrix Comparison study is not applicable.

C. Clinical Studies:

1. Clinical Sensitivity and Specificity:

The performance of the iHealth COVID-19 Antigen Rapid Test was compared to the sample results as generated by a highly sensitive EUA authorized molecular SARS-CoV-2 RT-PCR assay with an extraction step. A prospective clinical study was conducted from December 2022 to February 2023. Twenty-four clinical sites across 4 U.S. states participated in the study and enrolled symptomatic patients within 7 days of symptom onset (DPSO).

Anterior nasal swab samples for the investigational device were either self-collected by a lay user aged ≥15 years or collected by an adult (parent/guardian) from individuals aged 2 - <14 years and were processed by the lay user per the IFU of the proposed device. A nasopharyngeal (NP) swab was collected from patients by a healthcare professional at the clinical study sites who inserted the swab into a tube containing Viral Transport Medium for RT-PCR comparator testing. The order of swab collection for investigational and comparator testing was randomized via a number sequence provided to each study site. A total of 968 eligible subjects were enrolled in this study, 835 subjects (age ≥14 years old) and 133 subjects ages 2 - 13 years. Detailed study subject demographics are given below in Table 11:

Table 11: Study Demographics, Total Eligible Subjects

Age group	Percent total	Total
2-13 years	13.7%	133
14-24 years	15.3%	148
25-64 years	68.2%	660
≥65 years	2.8%	27
Total	**100%**	**968**

Education	Number	Total
High School or lower	483	49.9%
Associate degree	152	15.7%
Bachelors	249	25.7%
Graduate Degree	84	8.7%
Total	**968**	**100%**

The clinical study included about 10% low positive samples as defined by the comparator's mean Ct value at the comparator's LoD. With clinical samples collected within 6 DPSO, the iHealth COVID-19 Antigen Rapid Test had a Positive Percent Agreement (PPA) of 88.9% and Negative Percent Agreement (NPA) of 99.9% when compared to the result of the SARS-CoV-2 RT-PCR comparator assay. The study results are described in Table 12 below. PPA estimates and the number of positives samples stratified by DPSO are provided in Table 13.

Table 12: Clinical Study Results for iHealth COVID-19 Antigen Test:1-6 DPSO

iHealth COVID-19 Antigen Rapid Test	**EUA RT-PCR Comparator**		
	Positive	**Negative**	**Total**
Positive	104	1[b]	105
Negative	13[a]	797	810
Total	117	798	915
Positive Percent Agreement (PPA)	**88.9% (104/117)** (95%CI: 81.9% - 93.4%)		
Negative Percent Agreement (NPA)	**99.9% (797/798)** (95%CI: 99.3% - 100%)		

a) Of the 13 false negatives, 12/13 were positive on a 2nd EUA RT-PCR Assay

b) The 1 false positive was positive on a 2nd EUA RT-PCR Assay

Table 13: PPA Values Stratified by DPSO

Days Post Symptom Onset	iHealth Positives/Total Positives	PPA	95% CI
1	2/4	50.0 %	15.0%-85.0%
2	32/32	100 %	89.0%-100%
3	34/37	91.9 %	78.7%-98.2%
4	22/25	88.0 %	70.0%-95.8%
5	9/12	75.0 %	46.7%-91.1%
6	5/7	71.4 %	35.9%-91.7%
Total	**(104/117)**	**88.9%**	**81.9% -93.4%**

D. Clinical Cut-Off:

There is no clinical cut-off for this device. This section is therefore not applicable.

E. Expected Values/Reference Range:

A patient sample is expected to be negative for SARS-CoV-2.

F. Other Supportive Performance Characteristics Data

1. Flex Studies

To assess the robustness of the iHealth COVID-19 Antigen Rapid Test, flex studies were conducted that assessed all major aspects of the test procedure (e.g., swab extraction time, drops of sample added to test cassette, development time, delay in sample analysis, swab agitation, sample buffer agitation) and variability of environmental test conditions that the test may be subjected to when in use (e.g., various temperature and humidity stress, cassette disturbance, sample/reagent temperature, lighting). Testing was performed with contrived positive nasal swabs prepared by diluting heat inactivated SARS-CoV-2 virus into negative clinical nasal swab matrix at 2x LoD. The studies support that the test is robust in the intended use condition with an insignificant risk of erroneous result.

2. Usability Study

The design and instructions for use of the iHealth COVID-19 Antigen Rapid Test are very similar to the EUA version of the test authorized in EUA210470, for which a human factors usability study was performed on the critical tasks defined by the major operational steps of the testing procedure per IFU. In this study all users completed all critical tasks with a greater than 95% reliability. The minor changes in design and Instructions for Use do not significantly affect how the user interacts with the test and are not likely to impact usability or performance of this test. Therefore, an additional usability study was not performed in support of this 510(k).

3. Lay-User Readability

The purpose of this study was to determine whether lay users can interpret test results correctly with low positive samples from the iHealth COVID-19 Antigen Rapid Test.

The study was conducted according to an IRB approved Lay User Readability Study Protocol in a simulated home environment. A total of 58 lay users with diverse gender, ages, and educational background, and who met the study inclusion criteria, were enrolled for the Readability Study. Each lay user was asked to interpret four test devices with three different concentrations, prepared with recombinant SARS CoV-2 nucleoprotein that were arranged in a randomized and blinded test panel as listed below, for a total of 85 negative devices, 89 low positive devices, and 58 positive devices:

- Negative, 1.5xLoD, 1.5xLoD, 6xLoD
- Negative, Negative, 1.5xLoD, 6xLoD

50% of the study participants were male and 50% of the study participants were female. 69% of individuals were vision impaired, and about 14% of individuals were either still at high school or

had a high school degree as their highest level of education. The demographics and age distribution of the study are shown in Table 14 and the vison impairment status in Table 15.

The results of the study, stratified by age and overall are shown in Table 16. Negative samples, 1.5x LoD samples with concentrations at 6x LoD were all correctly interpreted by all lay users in the study.

Table 14: Lay User Readability - Demographics and Age Distribution

Age group	Number of lay users	Percentage
15-19 years	5	8.6%
20-30 years	15	25.9%
31-50 years	23	39.7%
51-55 years	2	3.4%
>55 years	13	22.4
Total	**58**	**100%**

Education	Lay user	Total
Some High School	5	8.6%
High School	3	5.2%
Some College	4	6.9%
College	17	29.3%
Some Grad School	1	1.7%
Grad School	28	48.2%
Total	**58**	**100%**

Table 15: Lay User Readability - Vision Impairment of Lay Users

Type of Vision Impairment	Percentage of Total Study Cohort
Near sightedness only	50% (29/58)
Far sightedness only	12% (7/58)
Near sightedness & Far sightedness	3.4% (2/58)
Color Blindness	1.7% (1/58)
Reading	1.7% (1/58)
Total lay users with vision impairment	**69% (40/58)**

Table 16: Readability Study - Results Stratified by Age and Overall

Age group	Sample Concentrations			Correct/ Total*
	Correct/ Negative	Correct/ Low Positive (1.5xLoD)	Correct/ Positive (6xLoD)	
15-19 years	8/8	7/7	5/5	20/20
20-30 years	23/23	22/22	15/15	60/60
31-50 years	32/32	37/37	23/23	92/92
51-55 years	3/3	3/3	2/2	8/8
>55 years	19/19	20/20	13/13	52/52
Total	**85/85**	**89/89**	**58/58**	**232/232**

Age group	Sample Concentrations			Correct/ Total*
	Correct/ Negative	Correct/ Low Positive (1.5xLoD)	Correct/ Positive (6xLoD)	
Negative Agreement	100% (85/85)*	n/a	n/a	**100% (85/85)*** (95%CI: 96%-100%)
Low Positive (1.5xLoD) Agreement	n/a	100% (89/89)*	n/a	**100% (89/89)*** (95%CI: 96%-100%)
Positive (6xLoD) Agreement	n/a	n/a	100% (58/58)	**100% (58/58)*** (95%CI: 94%-100%)

*Based on the randomized panels assigned to study participants, 85 negative devices, 89 low positive devices, and 58 positive devices were tested in the study

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.