510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K260754

B Applicant

Assure Tech., LLC

C Proprietary and Established Names

Fastep COVID-19 Antigen Pen Home Test; Fastep COVID-19 Antigen Pen 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 Fastep COVID-19 Antigen Pen Home Test and Fastep COVID-19 Antigen Pen Test (hereafter referred to as the Fastep COVID-19 Pen Test).

B Measurand:

Nucleocapsid protein antigens 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:

Fastep COVID-19 Antigen Pen Home Test:

The Fastep COVID-19 Antigen Pen Home 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 swab specimens from individuals with signs and symptoms of COVID-19.

This test is for use by individuals aged 14 years and 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 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 appropriate follow-up care from their healthcare provider.

Performance characteristics for SARS-CoV-2 were established from September 2023 to February 2025 when SARS-CoV-2 Omicron variant 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.

Fastep COVID-19 Antigen Pen Test:

The Fastep COVID-19 Antigen Pen 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 swab specimens from individuals with signs and symptoms of COVID-19.

This test is for use by individuals aged 14 years and 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 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 appropriate follow-up care from their healthcare provider.

Performance characteristics for SARS-CoV-2 were established from September 2023 to February 2025 when SARS-CoV-2 Omicron variant 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

IVD – For *in vitro* diagnostic use

D Special Instrument Requirements:

Not applicable (N/A)

IV Device/System Characteristics:

A Device Description:

The Fastep COVID-19 Pen Test is a lateral flow immunochromatographic assay for the qualitative detection of SARS-CoV-2 nucleocapsid antigen from self-collected anterior nasal swab specimens from symptomatic individuals. The single-use device consists of a base with built-in buffer and a test "pen" containing an integrated swab and test strip (Figure 1). The test strip is composed of a sample pad, conjugate pad, nitrocellulose membrane, and absorbent pad, with designated control (C) and test (T) lines for result interpretation.

Figure 1: Fastep COVID-19 Pen Test

Materials provided in test kit box:

- 1, 2, 4, 10, 20, and 25 foil packages each containing a test pen and base with extraction buffer (~0.95 mL)
- 1 Quick Reference Instructions (QRI)

Materials needed but not provided in kit box:

- Timer

B Principle of Operation:

The integrated swab is used to collect samples from both nostrils and then inserted into the base containing a built-in buffer, forming a sample mixture. This mixture migrates along the test strip across a nitrocellulose membrane containing two reagent lines: the test line and the control line.

If SARS-CoV-2 nucleocapsid antigen is present in the sample, it binds to anti-SARS-CoV-2 conjugate particles and is captured at the test line, producing a visible, pink-colored line indicating a positive result. The sample continues to migrate to the control (C) region, which contains goat anti-mouse antibodies. To serve as a procedural control, a pink line will always appear in the control line region (C), which confirms proper sample flow and test performance and must be present before interpreting results.

Results must be interpreted visually between 15 and 30 minutes after inserting the test pen into the base containing the extraction buffer. A positive specimen will present two pink lines, one in the control region (C) and one in the test region (T) of the result window, indicating that SARS-CoV-2 antigen was detected. A negative specimen will present only one pink line next to the "C" region of the test window and indicates that SARS-CoV-2 antigen was not detected. The procedural control line must always appear in the "C" region. If a line in the "C" region is not visible after 15 minutes, then the result is invalid.

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):	Candidate Device K260754	Predicate Device K230828
Device Trade Name	Fastep COVID-19 Antigen Pen Home Test; Fastep COVID-19 Antigen Pen Test	Flowflex COVID-19 Antigen Home Test
Intended Use/Indications For Use	Fastep COVID-19 Antigen Pen Home Test: The Fastep COVID-19 Antigen Pen Home 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 swab specimens from individuals with signs and symptoms of COVID-19. This test is for use by individuals aged 14 years and 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 preclude SARS-CoV-2 infections or other pathogens and should not be used as the sole basis for treatment.	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-CoV-2. Negative results do not preclude SARS-CoV-2 infections or other

Device & Predicate Device(s):	Candidate Device K260754	Predicate Device K230828
	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 appropriate follow-up care from their healthcare provider. Performance characteristics for SARS-CoV-2 were established from September 2023 to February 2025 when SARS-CoV-2 Omicron variant 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. Fastep COVID-19 Antigen Pen Test: The Fastep COVID-19 Antigen Pen 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 swab specimens from individuals with signs and symptoms of COVID-19. This test is for use by individuals aged 14 years and 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-	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 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.

Device & Predicate Device(s):	Candidate Device K260754	Predicate Device K230828
	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 appropriate follow-up care from their healthcare provider. Performance characteristics for SARS-CoV-2 were established from September 2023 to February 2025 when SARS-CoV-2 Omicron variant 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.	
General Device Characteristic Similarities		
Intended Use Setting	OTC	Same
Analyte	Nucleocapsid protein antigen from SARS-CoV-2	Same
Technology	Lateral flow immunoassay	Same
Sample Type	Anterior nasal specimen	Same
Assay Type	Qualitative	Same
Interpretation	Visually read	Same
Reading Time	15-30 min	Same
General Device Characteristic Differences		
Swab Format	Integrated sterile swab	Individually packed sterile disposable nasal swab
Tube holder	No	Yes

VI Standards/Guidance Documents Referenced:

Document	Title	Publisher	Applicable study
21 CFR 866.3984	Reclassification order for DEN220028 and special controls under 21 CFR 866.3984	FDA/CDRH	All Studies
11137-1:2025	Sterilization of health care products - Radiation - Part 1: Requirements for development validation and routine control of a sterilization process for medical devices [Including: Amendment 1 (2013) and Amendment 2 (2018)]	ISO	Sterility
11137-2:2013	Sterilization of health care products — Radiation Part 2: Establishing the sterilization dose	ISO	Sterility
11137-3:2017	Sterilization of health care products - Radiation - Part 3: Guidance on dosimetric aspects of development validation and routine control	ISO	Sterility
11737-1:2018/Amd 1:2021	Sterilization of health care products - Microbiological methods - Part 1: Determination of a population of microorganisms on product [Including Amendment 1 (2021)]	ISO	Sterility
11737-2:2019	Sterilization of medical devices - Microbiological methods - Part 2: Tests of sterility performed in the definition validation and maintenance of a sterilization process	ISO	Sterility
10993-5: 2009	Biological Evaluation of Medical Devices - Tests for *in vitro* cytotoxicity	ISO	Biocompatibility
10993-10: 2021	Biological Evaluation of Medical Devices –Tests for irritation and skin sensitization	ISO	Biocompatibility
10993-23:2021	Biological Evaluation of Medical Devices – Tests for irritation	ISO	Biocompatibility

VII Performance Characteristics:

A Analytical Performance:

1. **Precision Study:**

The purpose of the study was to assess lot-to-lot variability of three different lots of the Fastep COVID-19 Pen Test. Gamma-irradiated SARS-CoV-2 USA_WA1/2020 was spiked in negative nasal fluid and aliquoted as follows.

1) Negative sample (neat negative nasal fluid)
2) C5 sample (0.16x LoD)
3) C95 sample (1x LoD)
4) Moderate positive sample (3x LoD)

All sample aliquots tested in the study were randomized and blinded. For each concentration, 5 replicates were tested by 6 operators across 3 different testing sites (2 per site), between three (3) lots, and tested for 5 days (i.e., 3 lots x 6 operators x 5 replicates/run x 5 days). A total of 450 tests were tested for each panel member.

All negative samples and replicates prepared at 3x LoD demonstrated 100% agreement across the operators, lots, days, runs and sites tested. Results with concentrations at 0.16x LoD and 1x LoD yielded less than 100% positive agreement, however, results showed minor variability between lots and were deemed acceptable. The results are summarized below.

Table 1: Summary Results of Multi-lot Precision Study

Sample	Number of Positives / Number of Samples Tested (%)			Total number of positives / Total number of samples (%)
	Lot 1	Lot 2	Lot 3	
Negative	0/150 (0.0%)	0/150 (0.0%)	0/150 (0.0%)	0/450 (0.0%)
C_5 sample (0.16x LoD)	5/150 (3.3%)	5/150 (3.3%)	4/150 (2.6%)	14/450 (3.1%)
C_{95} sample (1x LoD)	142/150 (94.6%)	144/150 (96.0%)	145/150 (96.6%)	431/450 (95.8%)
Moderate positive (3x LoD)	150/150 (100.0%)	150/150 (100.0%)	150/150 (100.0%)	450/450 (100.0%)

2. **Linearity:**

N/A

3. **Analytical Specificity/Interference:**

a. **<u>Cross-Reactivity and Microbial Interference:</u>**

Cross-reactivity and microbial interference studies were conducted to determine if other respiratory pathogens/microbial flora that may be present in nasal swab samples could cause a false positive test result or interfere with the detection of a true positive result and cause a false negative result. A panel of viruses, high-prevalence disease agents, and normal or pathogenic flora were used for these studies. The microbial interference and the

cross-reactivity study were conducted simultaneously with samples tested in a randomized and blinded manner.

For the cross-reactivity study, dilutions of the panel organisms were prepared in negative nasal fluid and tested in triplicate in the absence of SARS-CoV-2. No cross-reactivity was observed with the organisms tested (Table 2).

For the microbial interference study, dilutions of the panel organisms were prepared in negative nasal fluid, in the presence of low levels (2x LoD; 1.58×10^3 $TCID_{50}$/mL) of Gamma- irradiated SARS-CoV-2 (USA_WA1/2020) and tested in triplicate. No microbial interference was observed (Table 2).

Table 2: Cross-Reactivity and Microbial Interference Study Results

Virus/Microorganism	Concentration	Units	Number of Positive Results/ Number of Replicates Tested	
			Cross-Reactivity (without analyte)	Interference (2x LoD SARS-CoV-2)
Bordetella pertussis	1.28×10^6	CFU/mL	0/3	3/3
Candida albicans	2.28×10^6	CFU/mL	0/3	3/3
Chlamydia trachomatis	6.25×10^6	CFU/mL	0/3	3/3
Chlamydia pneumoniae	4.50×10^7	IFU/mL	0/3	3/3
Haemophilus influenzae	1.28×10^8	CFU/mL	0/3	3/3
Legionella pneumophila	3.50×10^7	CFU/mL	0/3	3/3
Mycoplasma pneumonia	2.25×10^6	CFU/mL	0/3	3/3
Mycobacterium tuberculosis	3.03×10^7	CFU/mL	0/3	3/3
Pseudomonas aeruginosa	1.73×10^8	CFU/mL	0/3	3/3
Saccharomyces cerevisiae	7.65×10^7	CFU/mL	0/3	3/3
S. aureus MSSA	2.31×10^9	CFU/mL	0/3	3/3
S. aureus MRSA	2.38×10^9	CFU/mL	0/3	3/3
Staphylococcus epidermidis	3.05×10^9	CFU/mL	0/3	3/3
Streptococcus pneumoniae	1.81×10^8	CFU/mL	0/3	3/3
Streptococcus pyogenes	8.00×10^7	CFU/mL	0/3	3/3
Adenovirus	2.88×10^6	$TCID_{50}$/mL	0/3	3/3
Enterovirus 68	2.39×10^6	$TCID_{50}$/mL	0/3	3/3
Human coronavirus 229E	3.15×10^5	$TCID_{50}$/mL	0/3	3/3
Human coronavirus NL63	1.18×10^5	$TCID_{50}$/mL	0/3	3/3
Human coronavirus OC43	7.00×10^6	$TCID_{50}$/mL	0/3	3/3
Human coronavirus HKU1	1.25×10^5	$TCID_{50}$/mL	0/3	3/3
Human Metapneumovirus 9	1.00×10^5	$TCID_{50}$/mL	0/3	3/3
Influenza A Virus	2.63×10^5	$TCID_{50}$/mL	0/3	3/3

Virus/Microorganism	Concentration	Units	Number of Positive Results/ Number of Replicates Tested	
			Cross-Reactivity (without analyte)	Interference (2x LoD SARS-CoV-2)
Influenza B Virus	3.78×10^5	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 1	1.14×10^6	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 2	1.25×10^5	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 3	2.13×10^7	$TCID_{50}$/mL	0/3	3/3
Parainfluenza virus 4b	1.25×10^5	$TCID_{50}$/mL	0/3	3/3
Respiratory syncytial virus	3.78×10^5	$TCID_{50}$/mL	0/3	3/3
Rhinovirus	1.04×10^5	$TCID_{50}$/mL	0/3	3/3
MERS-coronavirus	4.00×10^5	$TCID_{50}$/mL	0/3	3/3
Negative nasal fluid	N/A	N/A	0/3	3/3

b. Endogenous/Exogenous Substances Interference:

The Fastep COVID-19 Pen Test was evaluated for performance in the presence of a panel of common interfering endogenous and exogenous substances. Potentially interfering substances were prepared in negative nasal fluid to the recommended concentration. Virus-negative specimens were evaluated in triplicate on each of the 3 lots of the test device and by three different operators to confirm that the potentially interfering substances were not cross-reactive with the test.

Positive samples were prepared in negative nasal fluid containing Gamma-irradiated SARS-CoV-2 (USA-WA1/2020) at 2x LoD and were evaluated in the presence of interfering substances in triplicate on each of the 3 lots of the test device and three different operators to confirm that the substances did not interfere with the detection of SARS-CoV-2.

No cross-reactivity or interference was observed for any of the substances tested at the concentrations evaluated as shown in the table below.

Table 3: Interfering Substances Study Results

Interfering Substances	Concentration Tested	Number of Positive Results/ Number of Replicates Tested	
		Cross-Reactivity (without analyte)	Interference (2x LoD SARS-CoV-2)
Beclomethasone	15% v/v	0/3	3/3
Biotin	3500 ng/mL	0/3	3/3
Dexamethasone	15% v/v	0/3	3/3
Dyclonine Hydrochloride	2 mg/mL	0/3	3/3
Flunisolide	15% v/v	0/3	3/3
Hand sanitizer	15% v/v	0/3	3/3
Hand Soap	15% v/v	0/3	3/3

Interfering Substances	Concentration Tested	Number of Positive Results/ Number of Replicates Tested	
		Cross-Reactivity (without analyte)	Interference (2x LoD SARS-CoV-2)
Homeopathic allergy relief (Histaminum hydrochloricum)	15% w/v	0/3	3/3
Homeopathic nasal wash (alkalol)	5% v/v	0/3	3/3
Leukocytes	4.8 x 10^6 cells/mL	0/3	3/3
Molnupiravir	10 mg/mL	0/3	3/3
Mucin	2.5 mg/mL	0/3	3/3
Mupirocin	10 mg/mL	0/3	3/3
Nasal corticosteroids (Budesonide)	15% v/v	0/3	3/3
Nasal corticosteroids (fluticasone furate)	5% v/v	0/3	3/3
Nasal corticosteroids (fluticasone propionate)	5% v/v	0/3	3/3
Nasal corticosteroids (Mometasone furoate)	15% v/v	0/3	3/3
Nasal corticosteroids (Triamcinolone Acetonide)	15% v/v	0/3	3/3
Nasal decongestant (Galphimia glauca, Luffa opperculata, sabadilla)	15% v/v	0/3	3/3
Nasal gel	5% v/v	0/3	3/3
Nasal spray (Cromolyn sodium nasal solution)	15% v/v	0/3	3/3
Nasal spray (Oxymetazoline HCl)	15% v/v	0/3	3/3
Nasal spray (Phenylephrine HCl)	15% v/v	0/3	3/3
Nasal spray (Sodium Chloride & Preservatives)	15% v/v	0/3	3/3
Oral Anesthetic Cough Lozenge (Menthol)	3 mg/mL	0/3	3/3
Oseltamivir Phosphate (Tamiflu)	15% w/v	0/3	3/3
Remdesivir	10 mg/mL	0/3	3/3
Sore Throat & Cough Lozenges (Benzocaine, Dextromethorphan HBr)	3 mg/mL	0/3	3/3
Sore Throat Spray (Phenol)	5% v/v	0/3	3/3
Tobramycin	50 ug/mL	0/3	3/3
Whole Blood	2.5% v/v	0/3	3/3

4. **Detection Limit and Assay Reportable Range:**

a. <u>Limit of Detection (LoD):</u>

The Limit of Detection (LoD) for the Fastep COVID-19 Pen Test was established by testing various dilutions of Gamma-irradiated SARS-CoV-2 isolate (USA_WA1/2020) in negative nasal fluid. The LoD was determined as the lowest virus concentration that was detected ≥ 95% of the time (concentration at which at least 19 out of 20 replicates tested positive) for each of 3 lots. The LoD was established in two phases.

Preliminary LoD Study

A series of 1/10, 1/100, 1/1000 and 1/10,000 dilutions were prepared from the Gamma-irradiated SARS-CoV-2 virus diluted into negative nasal fluid. Three replicates of each serial dilution were tested across each lot to determine the preliminary LoD concentration of the device. For each replicate, 50 µl of the sample aliquots were pipetted onto the swab for testing and processed according to the Instructions for Use (IFU). The lowest concentration with 9/9 positive results in the preliminary LoD study was further diluted by a series of 1:2, 1:4, and 1:8 dilutions where each dilution was tested in triplicate for each lot. The lowest concentration, with 9/9 positive results from each lot was considered the preliminary LoD for the virus strain. The results are summarized below.

Table 4: Preliminary LoD Study Summary

Isolate/Lineage	SARS-CoV-2 Concentration		Number of Positive Results/ Number of Replicates Tested
	$TCID_{50}$/mL	$TCID_{50}$/Swab	
SARS-CoV-2 (USA_WA1/2020) (Gamma-irradiated)	7.9×10^4	3.9×10^3	9/9
	7.9×10^3	3.9×10^2	9/9
	7.9×10^2	**3.9×10^1**	**9/9**
	7.9×10^1	3.9×10^0	0/9
	3.9×10^2	1.9×10^1	6/9
	1.9×10^2	9.5×10^0	0/9
	9.9×10^1	4.9×10^0	0/9

Confirmatory LoD Study

The preliminary LoD concentration, as well as concentrations above and below the LoD, were tested in a total of twenty (20) replicates using the same Gamma-irradiated SARS-CoV-2 virus for each of the same lots evaluated in the preliminary LoD study. To confirm the LoD for each lot, at least 19 of the 20 replicates should be positive. The final confirmation data set included the confirmed LoD concentration, and at least one concentration above and below the LoD to demonstrate 100% detection above the LoD and <95% detection below the LoD. The results are summarized below.

Table 5: Confirmatory LoD Study Summary of SARS-CoV-2

Isolate/Lineage	SARS-CoV-2 Concentration		Number of Positive Results/ Number of Replicates Tested
	$TCID_{50}$/mL	$TCID_{50}$/Swab	
SARS-CoV-2	1.1×10^3 (1.5x LoD)	5.9×10^1	60/60
	9.8×10^2 (1.25x LoD)	4.9×10^1	60/60

Isolate/Lineage	SARS-CoV-2 Concentration		Number of Positive Results/ Number of Replicates Tested
	$TCID_{50}$/mL	$TCID_{50}$/Swab	
(USA_WA1/2020) (Gamma-irradiated)	**7.9×10^2 (1x LoD)**	**3.9×10^1**	**59/60**
	5.9×10^2 (0.75x LoD)	2.9×10^1	50/60
	3.9×10^2 (0.5x LoD)	1.9×10^1	41/60

The LoD for the Fastep COVID-19 Pen Test was determined to be 7.9×10^2 $TCID_{50}$/mL which equates to 39.5 $TCID_{50}$/swab across all tested lots.

b. International Standard LoD Study:

The LoD of the Fastep COVID-19 Pen Test was determined by evaluating different dilutions of SARS-CoV-2 antigen (NIBSC code: 21/368) in negative nasal fluid. SARS-CoV-2 Antigen (NIBSC 21/368) was used for testing 2 lots of the Fastep COVID-19 Pen Test. 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 LoD was established in two (2) phases.

Preliminary LoD Study

Serial dilutions of SARS-CoV-2 antigen (1:10, 1:100, and 1:1000) were prepared in negative nasal fluid. Three replicates were tested on 2 lots of the test device. For each replicate 50 µL of virus dilution was applied to the swab and it was processed according to the IFU. The lowest concentration with 6/6 positive results was further diluted in a series of 1:2, 1:4, and 1:8 dilutions. The lowest concentration with 6/6 positive results following this dilution series was considered the preliminary LoD. The results are summarized below.

Table 6: Preliminary LoD for SARS-CoV-2 Antigen (NIBSC code: 21/368)

Concentration (IU/mL)	Concentration on swab (IU/swab)	Number of Positive Results/ Number of Replicates Tested
2.0×10^3	1.0×10^2	6/6
2.0×10^2	1.0×10^1	0/6
2.0×10^1	1.0×10^0	0/6
1.0×10^3	**5.0×10^1**	**6/6**
5.0×10^2	2.5×10^1	0/6
2.5 x 102	1.2 x 101	0/6

The Fastep COVID-19 Pen Test can detect the SARS-CoV-2 Antigen (NIBSC 21/368) at the preliminary LoD of 1.0×10^3 IU/mL in negative nasal fluid.

Confirmatory LoD Study

The preliminary LoD concentration, a concentration above (1.2x LoD), and a concentration below (0.75x LoD) were tested on the same 2 lots. A total of 20 replicates were tested for the confirmatory dilutions for SARS-CoV-2 Antigen (NIBSC 21/368) for each lot. To confirm the LoD for each lot, at least 19 of the 20 replicates should be positive. The results are summarized below.

Table 7: Confirmatory LoD for SARS-CoV-2 Antigen (NIBSC code: 21/368)

Concentration (IU/mL)	Concentration on swab (IU/swab)	Number of Positive Results/ Number of Replicates Tested
1.2×10^3	6.0×10^1	40/40
1.0×10^3	5.0×10^1	40/40
7.5×10^2	**3.7×10^1**	**39/40**

The confirmatory LoD for the International Standard for SARS-CoV-2 antigen (NIBSC code: 21/368) in negative nasal fluid was confirmed to be 750 IU/mL which equates to 37.5 IU/swab.

c. Assay Reportable Range:

N/A

5. **High Dose Hook Effect:**

A high-dose hook effect study was conducted to evaluate whether high concentrations of SARS-CoV-2 produced false negative results. Gamma-irradiated SARS-CoV-2 was serially diluted in negative nasal fluid. Following preparation, 50 µL of each sample was applied to swabs, which were then processed according to the IFU. Each concentration was tested in five replicates across 3 lots of the test device and by three operators. The results demonstrated no evidence of a high-dose hook effect across the concentrations evaluated in this study as shown below.

Table 8: High-Dose Hook Effect Study Results

Concentration ($TCID_{50}$/mL)	Dilution	Positive/ Total	% Positive
7.9×10^5	1000x LoD	15/15	100.0%
7.9×10^4	100x LoD	15/15	100.0%
7.9×10^3	10x LoD	15/15	100.0%
7.9×10^2	1x LoD	15/15	100.0%

6. **Inclusivity:**

Analytical reactivity testing for the Fastep COVID-19 Pen Test was conducted to ensure that the device can adequately detect a variety of SARS-CoV-2 strains. A selection of temporally, geographically, and genetically diverse SARS-CoV-2 strains were diluted in negative nasal fluid and tested at multiple concentrations. Each concentration was tested in 5 replicates per lot across 3 lots (15 replicates). Results are summarized below and demonstrate that the test can detect SARS-CoV-2 across the variants evaluated.

Table 9: Inclusivity Results

SARS-CoV-2 Variants	Lowest Concentration ($TCID_{50}$/mL) with 100% detection
SARS-CoV-2 virus (USA_WA1/2020) (Wild-Type)	7.90×10^{2}
B.1.1.7 (Alpha)	8.88×10^{2}
B.1.351 (Beta)	3.78×10^{3}
P.1 (Gamma)	3.15×10^{3}
B.1.617.2 (Delta)	1.04×10^{3}
B.1.1.529 (Omicron)	6.26×10^{2}
BA.2.3 (Omicron)	5.85×10^{1}
KP.2 (Omicron)	3.30×10^{3}
KP.3 (Omicron)	5.13×10^{3}
BA.2.86 (Omicron)	4.80×10^{3}

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

a. **Internal controls:**

The Fastep COVID-19 Pen Test contains a built-in internal procedural control. The appearance of the pink line in the control line region (C) indicates that sufficient flow of the sample occurred and that the test reagents are functioning properly. If no visible signal appears on the control line, the test result is invalid.

b. **Sample Stability Study:**

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

c. **Shelf-Life Stability Study:**

A real-time stability study was conducted to evaluate stability and determine the shelf-life of the unopened kit. To validate shelf-life, 3 unopened Fastep COVID-19 Pen Test kit lots were stored at 2-8℃ and 30℃. At predefined intervals, three (3) replicates/per lot were evaluated using the following test panel: negative nasal fluid, and two (2) positive samples contrived in negative nasal fluid [2x (1.58×10^{3} $TCID_{50}$/mL), 5x (3.95×10^{3} $TCID_{50}$/mL)] spiked with Gamma-irradiated SARS-CoV-2. At the time of clearance, all study data met the protocol defined acceptance criteria and support storage of the test kits from 2-30°C (36-86°F) for up to 8 months.

d. **Transport Simulation Study**

The transport simulation study assessed the performance of the Fastep COVID-19 Pen Test under simulated worst-case shipping conditions for both summer and winter conditions within the continental United States. The temperature profiles were designed to mimic typical transport scenarios, with temperatures fluctuating between extreme highs and lows. The packages were tested using both a summer and winter thermal challenge profile. Tests were placed into an incubator set to different temperatures, following the order and cycle periods outlined below.

Table 10: Summer Profile

Cycle No.	Temperature	Cycle Period Hours
1	40°C	8
2	22°C	4
3	40°C	6
4	30°C	56
5	40°C	6

Table 11: Winter Profile

Cycle No.	Temperature	Cycle Period Hours
1	-10°C	8
2	18°C	4
3	-10°C	6
4	10°C	56
5	-10°C	6

Baseline performance was assessed before subjecting the test kits to the temperature conditions (T0), followed by post-distribution testing [Td (Td-s= summer, Td-w= winter)] to evaluate any changes. Negative control samples, low positive samples at 2x LoD (1.58×10^3 $TCID_{50}$/ mL), and high positive samples at 5x LoD (3.95×10^3 $TCID_{50}$/ mL) were used for testing. The study involved 3 lots of the test device, with one operator testing each lot using three (3) replicates before and after the final temperature cycle challenge. Test results were recorded as positive, negative, or invalid.

All samples produced expected results following exposure to both simulated summer and winter conditions. The results support the stability of the device under anticipated shipping conditions.

8. **Assay Cut-Off:**

N/A

B Comparison Studies:

1. **Method Comparison with Predicate Device:**

See section C (clinical studies) below.

2. **Matrix Comparison:**

The Fastep COVID-19 Pen Test is only intended for use with 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 Performance Study:

A prospective lay-person clinical study was conducted between September 2023 and February 2025 to assess the performance of the Fastep COVID-19 Pen Test when compared to an FDA cleared RT-PCR assay. The study prospectively enrolled symptomatic subjects at 7 geographically distinct study sites located in the United States. Enrolled subjects were 2 years of age or older and exhibited symptoms of infection consistent with COVID-19.

Testing was performed in a simulated home-use environment. Two anterior nasal swab (AN) specimens were collected from each participant. One swab was collected by study personnel or healthcare professionals, placed into a transport tube containing viral transport media and transported to a central laboratory for testing with a highly sensitive RT-PCR test. The comparator test was performed according to the comparator test IFU. The other swab was collected according to the Fastep COVID-19 Pen Test's QRI: either self-collected by a lay user aged ≥14 years or collected by an adult (parent/guardian) from individuals aged 2 to <14 years and was tested immediately on-site using the Fastep COVID-19 Pen Test. The order of collection for the investigational and comparator AN swab was randomized.

One thousand seventy-seven (1077) samples were deemed evaluable. The demographics of the evaluable subjects are shown below:

Table 12: Patient Demographics

Characteristics of the study population		N=1077	Percent (%)
Sex	Male	447	41.5%
	Female	630	58.5%
Age	≥2 and <14 years of age	219	20.3%
	14-21 years of age	107	9.9%
	22-64 years of age	686	63.7%
	≥65 years of age	65	6.0%
Race	Asian	32	3.0%
	Black or African American	138	12.8%
	White	860	79.9%
	Native Hawaiian or Pacific Islander	1	0.1%
	Mixed race/ Biracial/Other	24	2.2%
	American Indian or Alaska Native	2	0.2%
	Prefer not to say	20	1.9%

The performance of the Fastep COVID-19 Pen Test when compared to FDA-cleared highly sensitive RT-PCR molecular assays are presented in the table below.

Table 13: SARS-CoV-2 Performance

	Comparator Positive	Comparator Negative	Total
Candidate Positive	109	1	110

	Comparator Positive	Comparator Negative	Total
Candidate Negative	20	947	967
Total	129	948	1077
Positive Percent Agreement (PPA)	84.5% (109/129) (95% C.I.: 77.3%-89.7%)		
Negative Percent Agreement (NPA)	99.9% (947/948) (95% C.I.: 99.4%-100.0%)		

2. **Clinical Sensitivity:**

   Please refer to Section C (Clinical Studies) above for clinical validation. The PPA for the test is as follows:

   SARS-CoV-2: 84.5% (109/129): 95% C.I.: 77.3%-89.7%

3. **Clinical Specificity:**

   Please refer to Section C (Clinical Studies) above for clinical validation. The NPA for the test is as follows:

   SARS-CoV-2: 99.9% (947/948): 95% C.I.: 99.4%-100.0%

4. **Clinical Cut-Off:**

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

5. **Usability and Readability Study:**

   a. **Usability Study:**

   A usability study was conducted to assess the lay users' ability to understand the IFU and to adequately execute the Fastep COVID-19 Pen Test workflow accordingly.

   A total of 113 subjects were enrolled in the human factors assessment studies concurrently with the clinical evaluation at three sites. Out of these subjects, 63 participants self-collected specimens and performed testing using the investigational test, and 50 had their collection performed by another lay user.

   Participants were evaluated for their ability to correctly perform critical and non-critical tasks associated with the testing process. Overall, 99.1% of all critical and 81.9% of non-critical tasks associated with the Fastep COVID-19 Pen Test were performed correctly. Results are summarized below.

Table 14: Critical vs. Non-Critical Tasks Correctly Performed

Steps	Steps performed correctly	Total number of steps	Percentage of steps performed correctly
Critical	1008	1017	99.1%
Non-Critical	185	226	81.9%
Sum	1193	1243	96.0%

Overall, the majority of subjects who participated in the usability evaluation found the instructions clear and easy to follow, and none reported difficulty collecting a sample or

performing the test. Five participants reported difficulty understanding the instructions but were still able to successfully perform the test. Following completion of the usability evaluation, all participants completed a comprehension questionnaire. Results demonstrated a high level of understanding of the test purpose, result interpretation, and appropriate follow-up actions for positive and invalid results.

b. **Readability Study:**

A readability study was conducted to evaluate whether lay users could correctly interpret test results for the Fastep COVID-19 Pen Test in a simulated home-use environment.

A total of 565 lay users interpreted blinded mock test panels representing positive (including low-positive), negative, and invalid results.

Mock test panels included at least one result from each of the following: a negative result, a low positive result at no more than 1.5 - 2x LoD, a positive result at 5x LoD, and an invalid result. Each investigational test with the mock result was blinded and coded with a sample identification number. 96.5% (545/565 tests) of the mock tests were interpreted correctly. Overall agreement for positive, negative, and invalid mock results was 98.9%, 95.3%, and 92.0%, respectively, indicating that most users were able to correctly interpret test results and therefore, the performance was acceptable.

D Other Supportive Study/Device Information:

1. **Flex Studies:**

To assess the robustness of the Fastep COVID-19 Pen Test, flex studies were conducted that assessed all major aspects of the test procedure (e.g., sample volume, reading time, extraction buffer volume, swab elution time and procedure) and variability of environmental test conditions that the test may be subjected to when in use (e.g., disturbance during run, device orientation, lighting, various temperature and humidity stress conditions). Testing was performed by spiking the swab with contrived positive sample generated by diluting Gamma-irradiated SARS-CoV-2 virus into negative nasal fluid at 2x LoD (1.58×10^3 $TCID_{50}$/mL). Flex studies support the robustness of the test under the intended use conditions and demonstrate a low risk of erroneous results.

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). Although the data, when stratified by symptom onset have performance estimates with insufficient statistical confidence, the clinical study data set of this and similar studies for test devices of a similar principle and design, indicate that such mitigation is needed.

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 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

E Expected Values/Reference Range:

Not applicable. An individual sample is expected to be negative for SARS-CoV-2.

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.