Lucira™ COVID-19 All-In-One Test Kit

Instruction for Use

Pfizer

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Lucira™ COVID-19 All-In-One Test Kit

Instructions for Use

For Use under Emergency Use Authorization (EUA) only
For *in vitro* Diagnostic Use
For Use with Self-collected Nasal Swab Specimens in individuals aged 14 and older
For Use with Provider-collected Nasal Swab Specimens in individuals aged 13 and under For Professional, Non-laboratory, and At-home Use
Rx Only

Intended Use

The Lucira COVID-19 All-In-One Test Kit is a single-use test kit intended to detect the novel coronavirus SARS-CoV-2 that causes COVID-19. This test is authorized for prescription home use with self-collected anterior nasal swab samples from individuals aged 14 years and older who are suspected of COVID-19 by their healthcare provider. This test is also authorized for use at the Point of Care (POC), in patient care settings operating under a CLIA Certificate of Waiver, Certificate of Compliance, or Certificate of Accreditation, with self-collected anterior nasal swab samples from individuals aged 14 years and older, and in individuals aged 13 years and under when the sample is collected by a healthcare provider at the POC. This test utilizes a molecular amplification technology for the detection of SARS-CoV-2 RNA in individuals with known or suspected COVID-19.

SARS-CoV-2 viral RNA is generally detectable in anterior nasal swab samples during the acute phase of infection. Positive results indicate the presence of SARS-CoV-2 viral RNA, but clinical correlation with past medical history and other diagnostic information is necessary to determine infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definitive cause of disease. Individuals who test positive should self-isolate and seek additional care from their healthcare provider.

Negative results are presumptive and confirmation with a molecular assay performed in a laboratory, if necessary for patient management, may be performed. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or management decisions for the individual, including infection control decisions. Negative results should be considered in the context of an individual's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19. Individuals who test negative and continue to experience COVID-19 like symptoms of fever, cough, and/or shortness of breath may still have SARS-CoV-2 infection and should seek follow up care from their physician or healthcare provider.

The Lucira COVID-19 All-In-One Test Kit is for use under the Food and Drug Administration's Emergency Use Authorization only.

All prescribing healthcare providers will report all test results they receive from individuals who use the authorized product to relevant public health authorities in accordance with local, state, and federal requirements, using appropriate LOINC and SNOMED codes, as defined by the Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests provided by CDC.

Summary and Explanation of the Test

Coronavirus disease 2019 (COVID-19) is a pandemic respiratory illness caused by a novel human coronavirus, first isolated in China and named SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) by the World Health Organization.[1-3] SARS-CoV-2 is an enveloped, single-stranded RNA virus of the β genus. The virus has spread globally and can cause mild to severe respiratory illness including death.

The Lucira COVID-19 All-In-One Test Kit is a rapid, instrument-free, single use molecular diagnostic test kit for the qualitative detection of SARS-CoV-2 RNA in anterior nasal swab samples from individuals with known or suspected COVID-19. The test kit contains all the components required to perform testing.

Principles of the Procedures

The Lucira COVID-19 All-In-One Test Kit utilizes RT-LAMP technology to detect RNA of the N gene and Orf7b/8 gene for SARS-CoV-2. This technology can create a signal from a few copies of RNA in less than 30 minutes. The RT-LAMP amplification reaction occurs in two phases, a non-cyclic phase followed by a cyclic phase. During the non-cyclic phase, reverse transcriptase, with RNase H activity, converts the RNA target into cDNA. A DNA polymerase with strand displacement activity then amplifies the cDNA. A successful amplification reaction creates a pH change and subsequently a color change of the halochromic agents within the reaction mixture.

The Sample Vial contains an elution buffer that allows the swab contents to be eluted and lysed at room temperature, releasing viral and human RNA for downstream detection. Upon engagement of the Sample Vial and Test Unit, this eluant enters a fluidic module, contained within the Test Unit that has several individual reaction chambers. The eluant resolubilizes lyophilized reagents, contained within these chambers, which are needed to perform the RT-LAMP reaction. An internal electronic heating element detects this chamber filling and automatically turns on, initiating amplification within the reaction chambers. The reactions are confined within the fluidic unit and no other part of the Test Unit has contact with the sample during amplification. The Test Unit contains chambers that target SARS-CoV-2 RNA, a positive internal control (PIC), and a lysis internal control (LIC).

The color change of the reaction mixture is detected in real time using optical and electronic elements contained within the Test Unit. An on-board microprocessor analyzes the color change data to detect the presence of amplification, and hence the target RNA, in each chamber. A diagnostic algorithm, included in the device firmware, is then used to determine patient infectivity status and the results are shown via LED indicators. Results for the test are displayed as either positive, negative, or invalid. A positive result may show in as few as 11 minutes; a negative or invalid result will display in 30 minutes. The result display persists for a minimum of 1 hour after the test has finished running.

PRECAUTIONS - GENERAL

- For FDA Emergency Use Authorization (EUA) only
- For in vitro diagnostic (IVD) use.
- For prescription use only
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of IVDs for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless declaration is terminated or authorization is revoked sooner.
- Leave kit components sealed in foil pouch until just before use.
- Proper sample collection and sample handling are essential for correct results.
- Do not touch swab tip when handling swab sample.
- Do not use any kit components with visible damage.
- Do not use kit components after their expiration date.
- Choose a level location to do this test where you can let the test sit undisturbed for 30 minutes.
- All kit components are single use items. Do not use with multiple specimens.
- Dispose of kit components and patient samples according to all local regulations.
- At low frequency, clinical samples contain inhibitors that may generate invalid results.
- Performance characteristics of this test have been established with specimen types listed in the Intended Use section only. The performance with other specimen types or samples has not been validated.

PRECAUTIONS – POINT OF CARE SETTINGS

- Only use the kit components provided. Do not use swabs from other tests.
- In point of care settings, it is important to clean the workspace to remove any environmental contamination. Prior to testing, wipe the testing area, with a sanitizing wipe (such as SaniCloth cleaning wipes) or 10% bleach solution.
- Follow universal precautions when handling patient samples. All patient samples should be treated as potentially infectious.
- Use of gloves is recommended when handling patient samples. If gloves are not available, wash and thoroughly dry your hands to prevent any sample contamination.
- External run controls (ERCs) are not required to use this test but are available for purchase from suppliers. See Section D below.

SECTION A: Reagents and Materials

Lucira™ COVID-19 All-In-One Test Kit contents:

- Package Insert (PI);
- Nasal Swab: one sterile flocked nasal swab in a peel-pouch;
- Sample Vial: a single-use, disposable vial containing an elution buffer to release and lyse virions from a nasal swab sample;
- Test Unit: a single-use, disposable unit with lyophilized reagents for multiplexed amplification and electronic readout for detection of SARS-CoV-2 RNA;
- Batteries: two AA batteries for the Test Unit; and
- Plastic disposal bag to dispose of the test kit after use.

NOTE: For optimal performance, use the swabs provided in the kit. Other swabs are not suitable for use with this test.

STORAGE AND HANDLING

- Test kits must always be stored at an ambient temperature (15-30°C / 59-86°F).
- Do not reuse kit components.
- Do not remove the Test Units from the foil pouch until immediately before use.

Section B – Directions for running the Lucira COVID-19 Test

- **Choose a location to do this test where it can sit UNDISTURBED for 30 minutes.**
- **Please read all instructions carefully before you begin.**
- **Do not insert batteries into test unit until ready to perform test.**
- Make sure your test kit contains:
- 2 AA batteries, test unit (pouch 1), sample vial (pouch 2), swab (labeled 3), and plastic disposal bag.
- Wash and dry hands.

1. Set Up Test

- When ready to begin test, open test unit pouch 1.

Open battery door and insert batteries. Check that **Ready light** is on.

- Open sample vial pouch 2.

REMOVE sample vial seal.

Then GENTLY set in test unit but do NOT push the vial down.

- Do not open swab until ready to use.

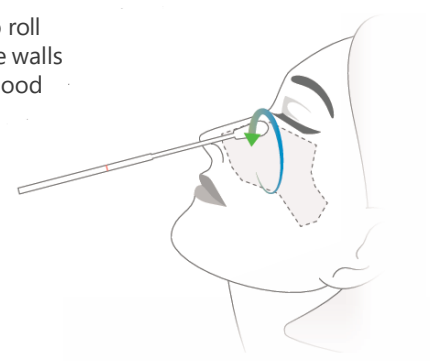
2. Swab Both Nostrils

For this test to work properly, it is important you swab BOTH nostrils.

- Remove swab and hold with handle end. Do not set swab down.
- Tilt head back and **gently insert swab tip until it is fully inside your/patient nostril** and you meet resistance.
- Once swab tip is fully inside nostril, **roll the swab 5 times around the inside walls of your nostril. The swab should be touching the walls of the nostril as you rotate.**
- **Repeat swab step in other nostril.**

Rotate 5x in BOTH nostrils.

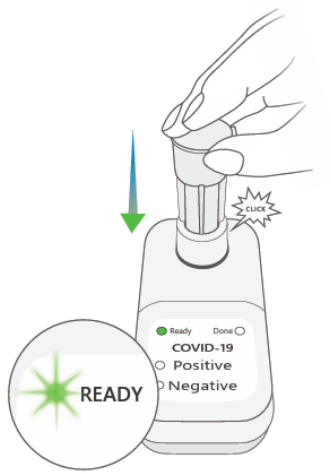
Make sure to roll around inside walls to collect a good sample.



3. Stir Swab and Run Test

- Insert swab into the sample vial until it **touches the bottom.**
- Mix sample by **stirring around the sample vial 15 times.**
- Discard swab.

- Snap cap closed and press vial down into test unit until it clicks.
- Ready light will start **blinking** when test is running.



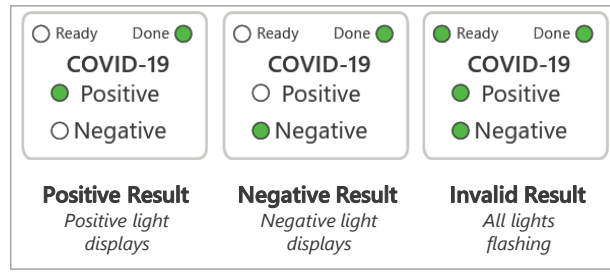
If Ready light is not blinking within 5 seconds, use palm of your hand to press down more firmly to start test.

Do not move test unit once the test has started running.

Wait 30 minutes.

4. Read Result

Test will be ready in 30 minutes.
Done light will display when test is finished.



Patients: Please share your test result with your healthcare provider. The bottom panel of the test kit box has a photo guide that can be used to share your results.

Healthcare Providers: All Lucira COVID-19 All-In-One Test Kit results should be reported to relevant public health authorities in accordance with local, state, and federal requirements, using appropriate LOINC and SNOMED codes, as defined by the Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests provided by CDC.

For more information on reporting, please see Pfizer's Healthcare Provider Guide to Reporting.

If the test is POSITIVE

It is very likely you have COVID-19 and it is important to be under the care of a healthcare provider. It is likely you will be asked to isolate yourself at home to avoid spreading the virus to others. There is a very small chance that this test can give a positive result that is wrong (a false positive). Your healthcare provider will work with you to determine how best to care for you based on your test results along with medical history and your symptoms.

If you test NEGATIVE

A negative result means the virus that causes COVID-19 was not found in your sample. If you took this test while you have symptoms, a negative test result usually means that your current illness was not caused by COVID-19.

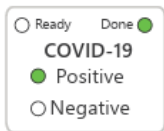
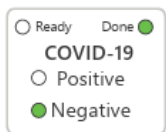
However, it is possible for this test to give a negative result that is incorrect (a false negative) in some people with COVID-19. This means you could possibly still have COVID-19 even though the test is negative. If this is the case, your healthcare provider will consider the test result with all other aspects of your history such as symptoms and possible exposures to decide how to care for you. It is important you work with your healthcare provider to help you understand the next steps you should take.

5. Dispose of Test Kit

After test is completed, place the test unit in plastic disposal bag and dispose all test kit materials in trash.

Section C - Test Unit Display Result

When the test is complete, the results are clearly displayed on the Test Unit.

Display	Interpretation of Results and Follow-up Actions
	COVID-19 Positive SARS-CoV-2 Viral RNA detected.
	COVID-19 Negative SARS-CoV-2 Viral RNA not detected.
 All LEDs flashing	Invalid Result Test should be repeated.

Section D - Quality Control Testing for Point of Care Settings

External run controls (ERCs) are not required to use this test kit.

In certain point of care or CLIA Waiver laboratory settings, ERCs may be tested, regularly or when new test kits are received, in order to train new operators or conform with local regulations, accrediting groups, or the lab's standard Quality Control procedures. Pfizer recommends the use of commercially available positive and negative external run controls from Zeptometrix Inc. (Negative control Cat # NATSARS(COV2)-NEG and Positive control Cat # NATSARS(COV2)-ERC). Instructions to use these controls with the Lucira COVID-19 All-In-One Test Kit are provided below:

1. Always test negative control before positive control in order to avoid any template contamination
2. Ensure control tubes are well mixed by inverting 5 times and then tapping the bottom of the tube against the work surface 3 times
3. Dip swab in ERC tube till swab head is immersed in liquid, hold for 5 seconds
4. Run Swab on test kit per standard PI

If the correct control results are not obtained, repeat the control tests. Results from the repeated assay(s) must be acceptable to proceed. If acceptable results are not obtained, do not perform patient tests or report patient results and contact your distributor for Technical Support before testing new patient specimens.

Keep External Run Control Vials in refrigerated storage until control testing is performed. Do not use beyond manufacturer labeled expiry date. Each ERC vial can be used up to 4 times. It is recommended to mark the used vial and record how many times it has been used.

Section E - Conditions of Authorization for HCP and Point of Care Settings

The Lucira COVID-19 All-In-One Test Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas

However, to assist HCP and Point of Care Settings1 using the Lucira COVID-19 All-In-One Test Kit, the relevant Conditions of Authorization are listed below:

A. All prescribing healthcare providers must collect information on the performance of your product in the ordinary course of business and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and you (1-888-582-4724) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

B. All prescribing healthcare providers must report all test results they receive from individuals who use your product to relevant public health authorities in accordance with local, state, and federal requirements, using appropriate LOINC and SNOMED codes, as defined by the Laboratory In Vitro Diagnostics (LIVD) Test Code Mapping for SARS-CoV-2 Tests provided by the Centers for Disease Control (available at: https://www.cdc.gov/csels/dls/sars-cov-2-livd-codes.html). Healthcare providers will also report to Pfizer Inc., when requested by Pfizer Inc., how many individuals reported test results compared to how many of your product they prescribed.

C. Point-of-care settings using your product must use your product without any deviations from the authorized labeling.

D. Point-of-care settings that receive your product must notify the relevant public health authorities of their intent to use your product prior to initiating testing.

E. Point-of-care settings using your product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

F. Point-of-care settings must collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and you (1-888-582-4724) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

G. Healthcare providers and point-of-care settings using your product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

PERFORMANCE CHARACTERISTICS

1) Limit of Detection (LoD) - Analytical Sensitivity

Quantified heat-inactivated SARS-CoV-2 virus was serially diluted in Natural Nasal Swab Matrix (NNSM), 35 µL was pipetted onto a fresh, unused nasal swab, and run on two device lots. The LoD for the device was determined by testing three (3) target concentrations on each lot of devices. For each lot, each concentration was tested in replicates of seven (7) devices by three (3) unique operators, for a total of 21 replicates per concentration. The LoD for each lot was separately determined as the lowest concentration of genome copy equivalents per swab that yielded greater than 95% positive results. The preliminary LoD for the device was defined as the highest LoD of the two lots.

Table 1. LOD Determination Results

Genome equivalent / Swab (per reaction)	Positive/Total Valid			Percent Positive		
	Preliminary Lot 1	Preliminary Lot 2	Confirmatory	Preliminary Lot 1	Preliminary Lot 2	Confirmatory
2700*	20/21	20/21	20/20	95.2%	95.2%	100%
1350	18/21	20/21	--	85.7%	95.2%	--
675	16/21	15/21	--	76.2%	71.4%	--

*2700 cp/swab is determined as LOD and is equivalent to 900 cp/mL of VTM assuming 100% elution of the swab in 3 mL of VTM.

The LoD for Lot 1 was determined to be 2700 copies per swab, while Lot 2 was 1350 genome equivalents per swab. As such, 2700 copies per swab was reported as the preliminary LoD.

The LoD was confirmed by testing 20 replicates at the preliminary LoD concentration on a single lot. All 20 of 20 devices at this concentration were positive.

2) Inclusivity (analytical sensitivity)

Inclusivity of the Lucira COVID-19 All-In-One Test Kit was demonstrated by in-silico reactivity of the assay against all publicly available SARS-CoV-2 strains using the assay's primers and probes. A total of 28,555 SARS-CoV-2 sequences were downloaded from the Global Initiative on Sharing All Influenza Data (GISAID, https://www.gisaid.org) database, and trimmed from whole genomes to 2.4-3.6kb windows covering the target regions. All 28,555 (100%) of the downloaded valid sequences were found to be reactive to at least one primer set of the assay.

Regarding the number of SNPs, none of the sequences had 3 or more mismatches to either primer set. 95.98% and 98.35% of the 28,555 sequences evaluated had 100% identity to Set 1 and Set 2, respectively. A single nucleotide mismatch in one of the primers for LAMP assays is not expected to impact the limit of detection, unless it is in the leading end of the primer as previously demonstrated by work on MERS-CoV (PMID 25103205).

3) Cross-reactivity (Analytical Specificity)

Analytical specificity of the Lucira COVID-19 All-In-One Test Kit was demonstrated by testing cross-reactivity with and interference from other organisms as well as endogenous substances.

a. Cross-reactivity (organisms tested in the absence of SARS-CoV-2)

The specificity of the assay was evaluated in cross-reactivity testing using 33 commensal organisms, including 13 bacteria/fungi, and 20 viruses. The cross-reactivity testing confirmed that none of the 33 organisms were cross reactive with the Lucira COVID-19 All-In-One Test Kit at the concentrations tested.

Table 2. Summary of Results

Organism Tested	Concentration Tested (per mL of spike)	Number Pos / # Tested	Cross Reactive
Bordetella pertussis	1.34E+10 CFU/mL	0/3	No
Candida albicans	6.27E+08 CFU/mL	0/3	No
Chlamydia pneumoniae	1.25E+07 IFU/mL	0/3	No
Haemophilus influenzae	2.27E+09 CFU/mL	0/3	No
Legionella pneumophila	6.82E+09 CFU/mL	0/3	No
Mycobacterium tuberculosis	6.86E+07 CFU/mL	0/3	No
Mycoplasma pneumoniae	3.98E+07 CCU/mL	0/3	No
Pseudomonas aeruginosa	6.64E+07 CFU/mL	0/3	No
Pneumocystis jirovecii	1.0E+08 nuclei/mL	0/3	No
Staphylococcus epidermidis	1.32E+08 CFU/mL	0/3	No
Streptococcus pneumoniae	4.73E+08 CFU/mL	0/3	No
Streptococcus pyogenes (Group A)	4.30E+09 CFU/mL	0/3	No
Staphylococcus salivarius	1.2E+08 CFU/mL	0/3	No
Adenovirus 1	1.02E+08 TCID50/mL	0/3	No
Adenovirus 7	1.02E+08 TCID50/mL	0/3	No
Coronavirus 229E	1.26E+06 TCID50/mL	0/3	No
Coronavirus NL63	1.1E+06 TCID50/mL	0/3	No

Coronavirus OC43	1E+6.18 TCID50/mL	0/3	No
Enterovirus/Coxsackievirus B4	6.61E+06 TCID50/mL	0/3	No
Enterovirus Type 68	9.55E+06 TCID50/mL	0/3	No
Enterovirus Type 71	4.17E+05 TCID50/mL	0/3	No
Human metapneumovirus	1.0E+06 TCID50/mL	0/3	No
Influenza A (pH1N1)	1.58E+07 EID50/mL	0/3	No
Influenza B (Yamagata)	1.1E+08 CEID50/mL	0/3	No
MERS Coronavirus	8.9E+05 TCID50/mL	0/3	No
Parainfluenza 1	8.9E+06 TCID50/mL	0/3	No
Parainfluenza 2	1.0E+08 TCID50/mL	0/3*	No
Parainfluenza 3	6.6E+06 TCID50/mL	0/3	No
Parainfluenza 4A	4.57E+06 TCID50/mL	0/3	No
Respiratory Syncytial Virus Type A	3.16E+06 TCID50/mL	0/3	No
Respiratory Syncytial Virus Type B	1.26E+06 TCID50/mL	0/3	No
Rhinovirus 1A	1.58E+08 TCID50/mL	0/3	No
SARS Coronavirus (SARS-CoV-1)	1.0E+08 PFU/mL	0/3	No

* One replicate repeated with another device due to an initial Invalid result.

In addition, in silico analysis was conducted to verify the assay does not cross-react with other high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in a clinical specimen.
Whole genome sequences were downloaded from NCBI.

BLAST alignments showed that only SARS-CoV-1 has > 80% homology on individual primers.

Table 3. Cross-Reactivity BLAST Results

Species	Set 1	Set 2
SARS-CoV-1	B1c (100%), F1c (100%)	F2 (100%), F3 (84%)
MERS-CoV	No alignment found	No alignment found
Human coronavirus 229E	No alignment found	No alignment found
Human coronavirus OC43	No alignment found	No alignment found
Human coronavirus HKU1	No alignment found	No alignment found
Human coronavirus NL63	No alignment found	No alignment found
Adenovirus (e.g. C1 Ad. 71)	No alignment found	No alignment found
Human Metapneumovirus (hMPV)	No alignment found	No alignment found
Parainfluenza virus 1-4	No alignment found	No alignment found
Influenza A & B	No alignment found	No alignment found
Enterovirus (e.g. EV68)	No alignment found	No alignment found
Respiratory syncytial virus	No alignment found	No alignment found
Rhinovirus	No alignment found	No alignment found
Chlamydia pneumoniae	No alignment found	No alignment found
Haemophilus influenzae	No alignment found	F1c (65%)
Legionella pneumophila	No alignment found	No alignment found
Mycobacterium tuberculosis	No alignment found	No alignment found
Streptococcus pneumoniae	No alignment found	No alignment found
Streptococcus pyogenes	No alignment found	No alignment found
Bordetella pertussis	No alignment found	No alignment found
Mycoplasma pneumoniae	No alignment found	No alignment found
Pneumocystis jirovecii (PJP)	No alignment found	No alignment found
Candida albicans	No alignment found	No alignment found
Pseudomonas aeruginosa	No alignment found	No alignment found
Staphylococcus epidermis	No alignment found	No alignment found
Staphylococcus salivarius	No alignment found	No alignment found

b. Microbial Interference Studies (organisms tested in the presence of SARS-CoV-2)

Based on cross-reactivity testing and in silico sequence alignment reported above, SARS-CoV-1 was identified as the only microorganism that may potentially interfere with the performance of the assay for SARS-CoV-2. In addition to SARS-CoV-1, Influenza (Flu) A, Flu B, respiratory syncytial virus (RSV) A, and RSV B were tested to verify that these high prevalence pathogenic microorganisms do not interfere with the detection of SARS-CoV-2 in the assay. In order to evaluate potential interference, swabs were co-spiked with 35 µL

of the potentially interfering microorganisms, at the concentrations listed in Table 4 below, with SARS-CoV-2 (at just less than 3 × LOD at 8.08 x 103 genome equivalents / swab) diluted in NNSM. Three positive spikes of only SARS-CoV-2 viral template and three NTC devices were run as controls.

The results showed that no microbial interference was detected for any of the microorganisms tested. Devices spiked with SARS-CoV-2 at 8.08 x 103 genome copies/swab (less than 3 × LOD) were positive, regardless of the presence of SARS-CoV-1, Flu A, Flu B, RSV A, or RSV B. All controls gave the expected results as well. As already shown in Table 2 above, these five microorganisms do not cross-react with the assay. Therefore, these microorganisms tested are unlikely to interfere with the performance of the assay.

Table 4. Microbial Interference Testing Results

Organism Tested	Concentration Tested (35 µL spiked per swab)	Number Positive / Valid Tests	Interferes with LUCIRA COVID-19 All-in-One Test Kit
SARS-CoV-1	1.00E+08 PFU / mL	3 / 3	No
Influenza A pH1N1 A/Michigan/45/2015	1.58E+07 EID50 / mL	3 / 3	No
Influenza B Yamagata B/Phuket/3073/2013	1.10E+08 CEID50 / mL	3 / 3	No
Respiratory Syncytial Virus Type A	3.16E+06 TCID50 / mL	3 / 3	No
Respiratory Syncytial Virus Type B	1.26E+06 TCID50 / mL	3 / 3	No

c. Endogenous Interference Substances Studies

Endogenous interference studies were conducted to assess potential interference effects on the Lucira COVID-19 All-In-One Test Kit from substances that may naturally be present in respiratory specimens or artificially introduced onto the nasal swab, including common household items that may be present in the testing environment.

Fifteen (15) interfering substances were tested, including 12 substances that are naturally present or artificially introduced into the nasal cavity, which were tested at the highest medically relevant concentration, and 3 substances that may be present within the testing environment, which were tested at their highest expected

concentrations in the environment. These potentially interfering substances were tested in NNSM spiked with SARS-CoV-2 virus at less than 3× the LoD.

None of the 15 substances tested showed interference effects with the Lucira COVID-19 All-In-One Test Kit.

Table 5. Summary of Testing Results

Substance	Concentration Tested	NTC COVID-19 Positive Results / Valid Devices	POS COVID-19 Positive Results / Valid Devices	Interfering Substance
PBS (control)	undiluted	0/3	3/3	No
Afrin	undiluted	0/3	3/3	No
Blood	1.0 % v/v	0/3	3/3	No
Chloroseptic Max	20% w/v	0/3	3/3	No
Flonase Allergy Relief	undiluted	0/3	3/3	No
Mucin	0.5% w/v	0/3	3/3	No
Method All-Purpose Cleaner	5% v/v	0/3*	3/3	No
Mupirocin	4.3 mg/ mL	0/3	3/3	No
NeoSynephrine Cold and Sinus Extra Strength Spray	20% v/v	0/3	3/3	No
Relenza	10 mg/mL	0/3	3/3	No
Saline	undiluted	0/3	3/3	No
Seventh Generation Disinfectant	5% v/v	0/3	3/3	No
Softsoap Moisturizing Hand Soap	5% v/v	0/3**	3/3	No
Tamiflu	6 mg/mL	0/3	3/3	No
Tobramycin	2.5 mg/ mL	0/3	3/3	No
Zicam Allergy Relief	undiluted	0/3	3/3	No

* One replicate repeated with another device due to an initial Invalid result.
**Two replicates repeated with additional devices due to initial Invalid results.

4) Near the Cutoff Study

To demonstrate the reproducibility of the Lucira COVID-19 All-In-One Test Kit with analyte concentrations near the cutoff, a study was conducted with 20 contrived nasal swabs run by untrained, intended users.

The test included 20 well-characterized contrived nasal swab samples: 10 positive contrived samples at 2×LOD for SARS-CoV-2 virus in NNSM and 10 negative contrived samples with NNSM only. This study design tested blinded contrived swabs prepared by Pfizer (Lucira) employees and run by 10 untrained, intended users with various level of experience.

All results in the study were valid and matched the expected results. Overall agreement with expected results was 100% for both SARS-CoV-2 Positive and SARS-CoV-2 Negative samples. The results demonstrate that untrained, intended users are able to use the Lucira COVID-19 All-In-One Test Kit and obtain the expected results.

5) Flex Studies

To assess the robustness of the Lucira COVID-19 All-In-One Test Kit, the company has conducted the following flex studies to test the assay's performance under conditions that may be evaluated as potential user errors and anticipated environmental stresses.

a. Sample Mixing

The study was conducted to evaluate the impact of incomplete or excessive swab mixing in the sample vial on performance of the Lucira COVID-19 All-In-One Test Kit. For each condition, 3 devices were run using the positive samples. All devices tested with positive samples returned a positive result, demonstrating that the assay is robust to incomplete or excessive swab mixing conditions.

Table 6. Summary of Sample Mixing Flex Test Results

Flex Condition	Positive Spike (#Positive/ # Valid
0 Swirls (12 Second Dip)	3/3
5 Swirls	3/3
10 Swirls	3/3
40 Swirls	3/3

b. Contaminating Substance

The study was conducted to evaluate the impact on device performance from hand sanitizer or hand lotion that might be transferred to the swab head from a user's hand. All devices displayed expected results.

Table 7. Summary of Contaminating Substance Flex Test Results

Flex Condition	Positive Spike (# Positive / # Valid)	NTC Condition (# Positive / # Valid)
Bare Hands	3/3	0/3
Hand Lotion	3/3	0/3
Hand Sanitizer	3/3	0/3

c. Battery Removal

The study was conducted to evaluate the impact of removal of a battery, as well as sequential removal and re-insertion of a battery after a device has started running, on the Lucira COVID-19 All-In-One Test Kit performance. The results showed that when battery was removed, the device turned off; and that if the battery was re-inserted, the device displayed an "Invalid" result, as designed.

d. Device Movement

The study was conducted to evaluate the impact of vertical and horizontal device movement and tilting of the device while the device is running on the Lucira COVID-19 All-In-One Test Kit performance. Each device underwent vertical movement at 0 minute to simulate someone picking up the device immediately after starting the device, vertical and horizontal movements at 12 minutes to simulate picking up and moving the test to another room during a test run, and tilting at 12 minutes to simulate the user picking up and tilting the device to observe the display.

Table 8. Movement Condition

Condition	Action	Distance / Angle	Timepoints
Movement	Vertical Movement	1 foot	0 minutes / 12 minutes *
	Horizontal Movement	20 feet	12 minutes *
	Tilting	90° perpendicular to ground (device tilted from long axis horizontal to long axis vertical)	12 minutes *

* The movements at 12 minutes were performed in the following order: Vertical Movement, Horizontal Movement, Tilting.

All devices displayed expected results following the movement conditions.

e. Unlevel Surface

The study was conducted to evaluate the impact of device placement on an angled work surface on the Lucira COVID-19 All-In-One Test Kit performance. Devices were run on a custom fixture with slope. Each sample and orientation were tested in triplicate, with devices kept in their position for the full duration of the run.

All devices run following this test protocol displayed expected results, satisfying the acceptance criteria.

f. Storage of Packaged Devices

The study was conducted to evaluate the tolerance of the Lucira COVID-19 All-In-One Test Kit to short-term storage at low and high temperatures outside the recommended temperature storage range. Device performance was assessed within a storage temperature range of 5°C to 45°C for up to 3 days. For each condition (temperature and duration combination), 3 devices were tested per sample type.

All tested devices displayed expected results following the low and high temperatures.

Table 9. Summary of Storage Flex Test Results

Condition	Description	Positive Spike (# Positive / # Valid)	NTC Condition (# Positive / # Valid)
Storage Temperature and Time	5°C – 24 hours	3/3	0/3
Storage Temperature and Time	5°C – 72 hours	3/3	0/3
Storage Temperature and Time	45°C – 24 hours	3/3	0/3
Storage Temperature and Time	45°C – 72 hours	3/3	0/3

g. Shipping and/or storage of kits in extreme conditions

The Lucira COVID-19 All-In-One Test Kits, in a shipper without temperature-control assistance, underwent a climatic simulation per ASTM D4332-14 and a shipping simulation per ASTM D4169-16 Distribution Cycle 13 to demonstrate the stability of packaged test kits under multiple temperature and humidity conditions expected during shipments. Shipping testing was done with four packages containing 24 test kits each packaged in a shipper (single-corrugated ECT>44 cardboard box from Packaging Corporation of America) with no additional temperature-control assistance. These four packages were subjected to testing outlined in the ASTM D4332-14 Standard Practice for Conditioning Containers, Packages, or Packaging Components for Testing as shown in Table 10 below.

Table 10: Temperature and Humidity Conditioning per ASTM D4332-14

Shipper	Temperature/Humidity Conditions			Total Conditioning Time
	+60ºC/15%	-30ºC	+40ºC/90%	
S1	X			24 hours
S2		X		24 hours
S3			X	24 hours
S4	X	X	X	72 hours

Kits from these shippers were tested with known positive and negative samples to evaluate any functional impacts of the climactic and shipping testing. Eight (8) kits from the corners of each shipper were tested and the kits passed the acceptance criteria of successfully classifying four known positive samples, at 3 × LoD in NNSM, and four known negative samples (PBS in NNSM matrix) per shipper. One kit from Shipper S4 generated an invalid result and was successfully retested with an additional kit from the shipper. These functional results demonstrate that there was no impact to the performance of the kits when exposed to several temperature and humidity conditions, including below freezing.

h. Operating Temperature and Humidity Extremes

The study was conducted to evaluate the tolerance of the Lucira COVID-19 All-In-One Test Kit to both temperature and humidity extremes which may be encountered during normal operation of the device by end users. Device performance was assessed within extreme temperature and humidity combinations, with temperature ranging from of 5°C to 45°C and humidity ranging from 5% to 95%.

All devices displayed expected results under the tested environment conditions. The 5°C and 45°C conditions resulted in "Invalid" results for all devices tested as they were out of the temperature range upon the device waking up at battery insertion.

Table 11. Operating Environment Results

Condition	Positive Spike (# Positive / # Valid)	NTC Condition (# Positive / # Valid)
10°C, Low Humidity	3/3	0/3
35°C, Low Humidity	3/3	0/3
45°C, Ambient Humidity	0/0	0/0*
20°C, Ambient Humidity	3/3	0/3
35°C, High Humidity	3/3	0/3
10°C, High Humidity	3/3	0/3
5°C, Ambient Humidity	0/0	0/0*

*All test units tested were invalid by design.

6) Human Usability Study

Human Usability testing was conducted among a total sample of 398 healthy, non-symptomatic users in two locations to evaluate the ability of various ages, ethnicities and education levels to successfully run the Lucira COVID-19 All-In-One Test Kit.

For all of the Usability studies, participants were recruited to come to a research facility and try the Lucira COVID-19 All-In-One Test Kit on their own using only the Package Insert (PI) provided in the test kit box. Subjects were observed through a one-way mirror and videoed so that complete records of their test kit use experience, including nasal self-collection, could be documented and verified. Post-use interviews to assess test kit ease of use and instructions clarity were entered electronically in real time.

All users of all ages were able to successfully start the Lucira Test Kit running. Notably, 96% of all users aged 14 and above were able to successfully start the test running and ready light blinking on the first try and without having to look back at the PI instructions. The remaining were all able to successfully start the test running with one look back at the instructions. 97% of users rating their experience as "Easier or About What They Expected," with 59% of users rated their test experience as "Easier Than What They Expected." Nearly 100% of users rated the overall test kit instructions as sufficient to be able to understand how to perform the test. After using the Lucira COVID-19 All-In-One Test Kit, 100% of users across all age cohorts expressed confidence they could run the test at home on their own. All users were able to interpret the test results correctly.

99% of adults aged 18 and older and 95% of teens aged 14-17 collected sample in both nostrils. 92% of adults aged 18 and older had a swabbing time of at least 11 seconds and a median swabbing time of 18 seconds. 92% of teens aged 14-17 had a swabbing time of 9 seconds and a median swabbing time of 17 seconds.

7) Clinical Evaluation:

A community testing study was conducted to establish the clinical performance of the Lucira COVID-19 All-In-One Test Kit under the intended use conditions. In this study, symptomatic subjects suspected of COVID-19 were tested outside their residence. The subjects independently collected nasal swab samples and ran the test. The results were compared to a high sensitivity molecular FDA Authorized SARS-CoV-2 assay, with the samples collected concurrently with the Lucira Test Kit and run in clinical laboratories.

A total of 101 subjects were enrolled in the study. When compared to a high sensitivity molecular FDA Authorized SARS-CoV-2 assay, the total Positive Percent Agreement (PPA) across all samples was 94.1% (48/51), or 96.0% (48/50) with discrepancy analysis using the Roche cobas SARS-CoV-2 test. All three of the discrepant samples had high Ct values (>37.5) when tested by the comparator assay. Excluding these samples with very low levels of virus, 100% (45/45) positive percent agreement was achieved.

Table 12. Clinical Study Summary Results

Community Testing Study	A High Sensitivity Molecular FDA Authorized SARS-CoV-2 Assay		
Lucira 07A-CLI-006	Positive	Negative	Total
Positive	48	1	49
Negative	3*	49	49
Total	51	50	101

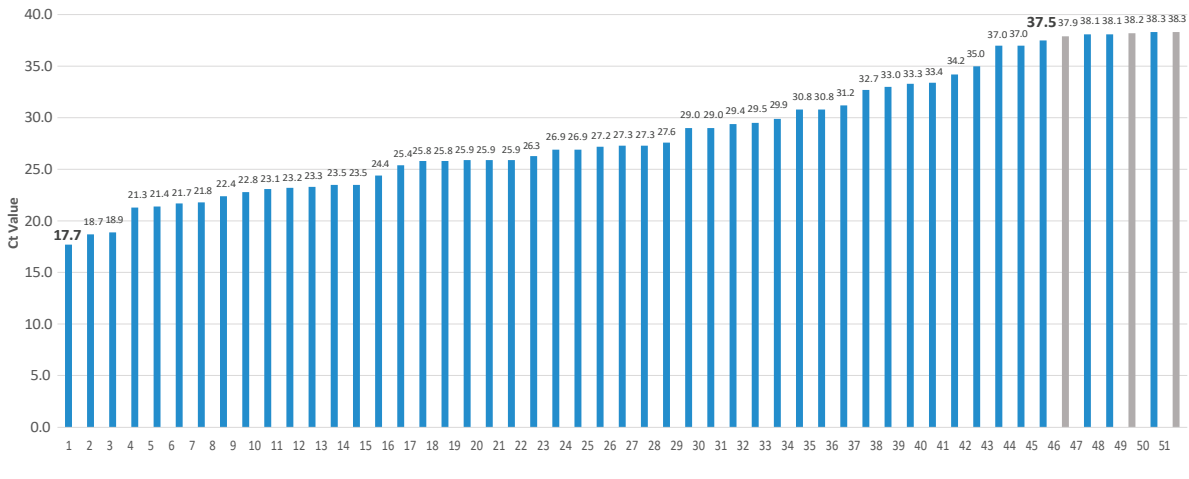
Positive Percent Agreement (PPA)	94.1% (95%CI: 85.5%-98.4%)
Negative Percent Agreement (NPA)	98.0% (95%CI: 89.4%-99.9%)

*All 3 False Negatives occurred in patients with reference test Ct values of >37.5 1 invalid result (0.99% invalid rate); 2 retests (1.98% retest rate)

In the graph below of Ct values of reference positives samples from the Community Testing study, **BLUE** color is used to show that the Lucira test result matched the comparator positive result. **GREY** color is used to show that the Lucira test result did not match the comparator reference test result. Discrepant analysis on a high sensitivity molecular FDA Authorized SARS-CoV-2 assay showed 2/3 of the discrepant Lucira results to be positive matching the comparator, and 1/3 (Ct 38.2) to be negative matching Lucira. Ct values are shown in ascending order.

Table 13. Lucira vs. a High Sensitivity Molecular FDA Authorized SARS-CoV-2 Assay Positive Percent Agreement Summary

PPA= 94.1% across all data (48/51)
PPA= 100% on all Ct Values ≤ 37.5 (45/45)



The # of cycles (Cts) required to detect virus increases when the amount of virus in the sample is low

Note: All 3 Lucira discrepant (GREY) results were among samples with very low levels of virus (Ct values >37.5).

LIMITATIONS

- Performance was evaluated with swab specimens only, using the procedures provided in this package insert. Failure to follow these procedures may alter test performance.
- False negative results may occur if a specimen is improperly collected or handled.
- False negative results may occur if inadequate levels of viruses are present in the specimen.
- False negative results may occur if the virus mutates in the regions targeted by the test.
- This test has not been evaluated for patients without signs and symptoms of COVID-19 infection.
- The test is a qualitative test and does not provide the quantitative value of detected organism present.
- Cross-reactivity with respiratory tract organisms other than those tested in the Analytical Specificity Study may lead to erroneous results.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Analyte targets (viral sequences) may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(s) are infectious, nor are the causative agents for clinical symptoms.
- Positive and negative predictive values are dependent upon prevalence. False negative results are more likely during peak activity when disease prevalence is high and false positive results are more likely during periods of low activity.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

TECHNICAL ASSISTANCE

Contact Pfizer at 888-LUCIRA-4 (582-4724).

REFERENCES

1. Zhu N, Zhang D, Wang W, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382:727-33. PMID: 31978945.
2. https://www.who.int/emergencies/diseases/novel-coronavirus-2019
3. https://www.cdc.gov/coronavirus/2019-ncov/index.html

TABLE OF SYMBOLS

	This symbol indicates that the product is for single use only. It is not to be re- used.
	This symbol indicates that you should consult the instructions for use.
	This symbol indicates that the product has a temperature limitation.
	This symbol indicates the use-by date.
	This symbol indicates the product batch code.
	This symbol indicates the name and location of the product manufacturer.
	This symbol indicates the product's catalog number.
	For Prescription Use Only.
	For In Vitro Diagnostic Use.

Covered by one or more of US Patents 10,146,909, 10,253,357 and other pending US and International Patents