INSTRUCTIONS FOR USE

FLUXERGY TEST KIT COVID-19

REF **5339-CE / 6177-CE**

For Use with the Fluxergy Analyzer CAT # 5506-CE / 5506-CE-L

***In Vitro* Diagnostic Medical Device**

CE IVD

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Instructions For Use (PN: 5773D03)
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1. Intended Use

Fluxergy Test Kit COVID-19 is a real-time reverse transcriptase (rRT) polymerase chain reaction (PCR) test, intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab samples (NPS) collected by a healthcare worker from patients suspected of COVID-19 by their healthcare provider. Testing should be performed by laboratories or in other qualified professional care settings that meet their local registration or licensing requirements for in-vitro diagnostic testing.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Fluxergy Test Kit COVID-19 is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

2. Explanation of the Test

The Fluxergy Test Kit COVID-19 is a qualitative real-time reverse transcription polymerase chain reaction (rRT- PCR) test. The SARS-CoV-2 primer and probe set is designed to detect RNA from SARS-CoV-2 in nasopharyngeal swab samples (NPS) from patients with signs and symptoms of infection who are suspected of COVID-19. The test performance is monitored by standardized internal controls and provides results within 1 hour from when the test is initiated with a suspected sample.

3. Principles of the Procedure

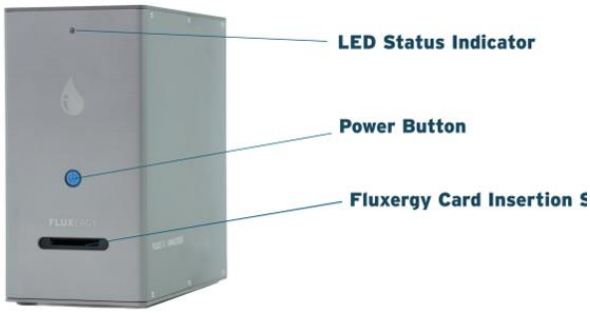
3.1 Overview

The Fluxergy Test Kit COVID-19 enables processing, amplification, and detection of SARS-CoV-2 RNA from infected nasopharyngeal swabs samples. The assay consists of one Fluxergy Reaction Mix COVID-19 and one Fluxergy PCR Card. The assay is performed on the Fluxergy Analyzer instrument which is controlled by an external computer equipped with Fluxergy Works Software.

3.2 Fluxergy PCR Card

The Fluxergy PCR Card is a disposable card into whichthe PCR reagents mixed with test samples are manually pipetted in. Each card contains a single sample/reagent input well and microfluidicchannels that control the flow of liquid, and reaction wells. The Fluxergy PCR Card is self- contained to prevent cross-contamination between samples.

In the Fluxergy Test Kit COVID-19, fluorescent probes are used together with corresponding forward and reverse primers to amplify SARS- CoV-2 RNA and exogenous internal control. Two well-conserved regions of the SARS-CoV-2 genome are targeted to identify SARS-CoV-2 RNA in the specimen. Internal control is used to detect PCR failure and/or inhibition in addition to monitoring adequate sample processing.



3.3 Fluxergy Analyzer

The Fluxergy Analyzer instrument is a rapid RT-PCR thermocycler used for the identification of nucleic acid from biological specimens. The Fluxergy Analyzer performs amplification, detection, and analysis of fluorescent signals generated during PCR.

3.4 Process

In the Fluxergy Test Kit COVID-19, a NPS samplecollected in viral transport media is mixed with ready-to-use Fluxergy Reaction Mix COVID-19 toprepare the complete test master mix. The master mix is then loaded onto the Fluxergy PCR Card. After loading the Fluxergy PCR Card into the Fluxergy Analyzer instrument, the run is initiated. Approximately in 1 hour, Fluxergy Works will complete the thermal cycling and analysis.

3.5 Primer and Probe Sets

Identification of the SARS-CoV-2 virus occurs using target specific primers and fluorescent-labeled probes that hybridize to a conserved region of the non-structural polyprotein (orf1ab) and the N gene of the SARS-CoV-2 virus.

4. Reagents and Instruments

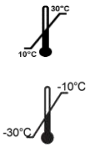
The Fluxergy Test Kit COVID-19 is to be used with the following instrument, reagents, and supplies:

4.1 Materials Provided

The Fluxergy Test Kit COVID-19 (single pack) contains sufficient reagents and consumables to test a single specimen or quality control sample. The Fluxergy Test Kit COVID-19 contains the following and is available in packs of 10 (CAT #5339-CE) or 100 (CAT #6177-CE):

- Fluxergy Reaction Mix COVID-19
- Fluxergy PCR Card

4.1.1 Storage and Handling



- Store the Fluxergy PCR Card at 10 to 30°C
- Do not open individual Fluxergy PCR Card packaging until you are ready to test
- Store the Fluxergy Reaction Mix COVID-19 at -30 to -10°C
- Do not thaw or open Fluxergy Reaction Mix COVID-19 reagent until you are ready to test
- Freeze thaws of Fluxergy Reaction Mix COVID-19 will interfere with test results

4.2 Materials Required but Not Provided

- Sample Collection Materials:
  - A rayon, polyester, or nylon-flocked swab with 80mm breakoff point (not cotton): Copan SKU #502CS01, orequivalent,
  - 3 mL of viral transport medium (VTM): Copan SKU #330C, Puritan SKU#UT-300, BD UVT SKU #220527/220528/220529/220531, or equivalent.
- Fluxergy Analyzer (CAT #5506-CE) and (CAT #5506-CE-L), sold separately.
- Barcode scanner
  - → Light Source: 650 – 670nm
  - → Scanner Type: Bi-directional
  - → Host System Interfaces: USB
  - → Indicators: LED & Buzzer
  - → Supported Barcode Formats (minimum requirement): 2D Data Matrix, 2D GS1 Data Matrix
  - → Stand with hands-free operation capability.
  - → Laptop to install Fluxergy Works software.
- Laptop Computer recommended requirements
  - → Operating System, 64-bit
  - → Windows 10 (build 1151 or later) with Intel Core i5 2.5GHz processor or equivalent
  - → RAM: 8GB DDR4
  - → HDD: 250GB
  - → Screen: 1080p
  - → USB: 2x2.0 port (for scanner and mouse)
  - → Networking: Ethernet port
- Minivortexer, FisherBrand Part Number 14-955-151 or equivalent
- Microcentrifuge, FisherBrand. Part Number 12-006-902 or equivalent
- Micropipettes (20 μL and 200 μL) and associated pipette tips
- -20° C manual defrost freezer
- Instructions and Documents
  - Instructions for Use, SDS, and additional resource documents can be found at www.fluxergy.com/downloads

5. Warnings and Precautions

5.1 General

- For *in vitro* diagnostic use.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Report all positive results to the appropriate health authorities as required.
- Ensure that you save your sample in case follow up testing is needed.
- Authorized for use only with the equipment, materials, and supplies indicated in Section 4. Use with equipment, materials, and supplies other than those indicated above in Section 4 may cause errors and erroneous results.
- 
All biological specimens, including used Fluxergy PCR Cards, used sample collection materials, used sample transfer materials, and used reagents, should be handled as if infectious, using good laboratory procedures as outlinedby your local or national authorities, for example: Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID 19), https://www.cdc.gov/coronavirus/2019-ncov/lab/lab- biosafety-guidelines.html[1] or Laboratory Biosafety Manual – 4th Edition (WHO).[2]
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Bleach introduced into a sample may damage DNA and RNA in that sample, which may lead to an erroneous result.
- Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) disposable clean powder- free gloves. Protect skin, eyes, and mucus membranes. Change gloves often when handling equipment, reagents, or samples.[3]
- This product may contain components or chemicals that may cause cancer if ingested.
- Dispose of materials used in this assay, including reagents, samples, and used buffer tubes, according to local regulations.

5.2 Test/Reagent

- The Fluxergy Test Kit COVID-19 is not compatible with cotton swabs. Residue found in cotton-tipped and calciumalginate swabs can inhibit PCR assays; therefore, these types of swabs should not be used.
- The Fluxergy Test Kit COVID-19 including Fluxergy PCR Card, and Fluxergy Reaction Mix COVID-19 are onlycompatible and for use only with the Fluxergy Analyzer.
- Do not handle samples or Fluxergy PCR Card in a biosafety cabinet which is used for SARS-CoV-2 culture orimmunofluorescence testing.
- Do not use a test kit or components that are damaged.
- 
Each single-use Fluxergy PCR Card and single-use Fluxergy Reaction Mix are used to process one sample. Do notreuse processed Fluxergy PCR Card and Fluxergy Reaction Mix.
- 
Each pipette tip is used to transfer one sample.
- Do not use reuse pipette tips for separate pipetting steps.
- Prior to processing samples, thoroughly clean both the work area with a suitable cleaner such as freshly prepared10% bleach or a similar disinfectant.
- Fluxergy Reaction Mix, Fluxergy PCR Card, and samples should be handled and tested one-at-a-time.
- Always change gloves and clean the work area between using each Fluxergy PCR Card and Fluxergy Reaction Mix.
- Use clean gloves to remove materials from bulk packaging and reseal bulk-packaging when not in use (e.g.Fluxergy PCR Card bulk packaging).
- **Always check the expiration date on the Fluxergy PCR Cards and Fluxergy Reaction Mixes. Do not use kitcomponents after the expiration date.**

6. Sample Requirements

6.1 Sample Type and Sample Volume

Improperly collected, transported, or handled samples risk the potential for false positive, false negative or erroneous results. The detection of viral nucleic acid is dependent upon proper sample collection, handling, transportation, storage, and preparation. Follow CDC's Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html[4] and Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html:

- Insert a swab into nostril parallel to the palate. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.
- Place swab into tube containing 3 mL of viral transport medium. Immediately break off swab tip at break line andcap sample collection tube tightly. Transport medium must be in 3 mL volume for expected performance.
- Acceptable specimen types include only nasopharyngeal swab (NPS)
- 14μL of sample (nasopharyngeal swab in transport solution) will be used for 1 test.

6.2 Transport and Storage

Samples should be processed and tested with the Fluxergy Test Kit COVID-19 immediately after sample collection.

* Note: Performance is not guaranteed if samples are not tested immediately. Extended heat and fluctuation of temperature will degrade sample and affect detection of nucleic acid. Follow CDC's Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines- clinical-specimens.html and Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.

7. Test Procedure

7.1 Setting up a Fluxergy Analyzer

Refer to the Fluxergy Analyzer IFU for how to:

- Setup a Fluxergy Analyzer
- Managing devices on Fluxergy Works software
- If using multiple Fluxergy Analyzers, ensure that each device is labeled and uniquely named.
  - The Fluxergy Analyzer will not uniquely flash or prompt to identify itself.
- Adding users on Fluxergy Works software

Prior to running a Fluxergy Test Kit COVID-19, make sure the Fluxergy Analyzer is on and connected to the FluxergyWorks software.

7.2 Test Features

Fluxergy Test Kit COVID-19 Features	
Sample Type	Polyester, rayon, or nylon-flocked nasopharyngeal swab (NPS) collectedin 3 mL of viral transport medium
Minimum amount of sample required	100 µL
Sample Processing Volume	14 µL
Duration of Test	Approximately 1 hour

7.3 Sample Collection

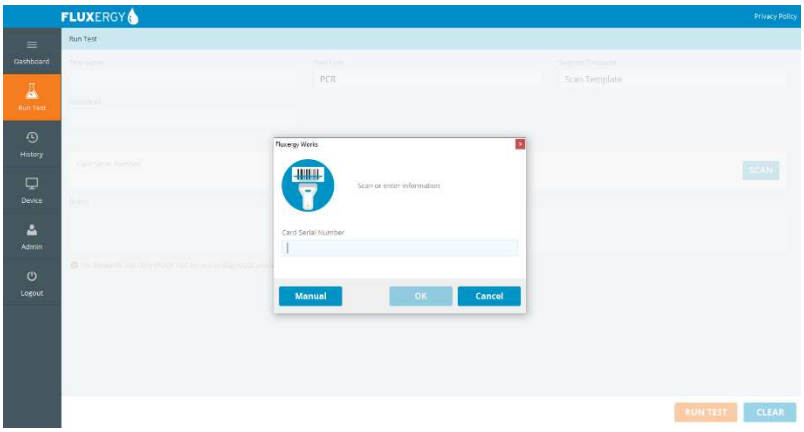
1. Refer to section 6.1 for patient sample collection.
2. **Do not discard sample after use in case follow-up testing is needed.**

IMPORTANT:

- **Thaw Fluxergy Reaction Mix COVID-19 at room temperature before use (5 min). Ensure that the ReactionMix is completely thawed and use immediately. (Do not vortex the Reaction Mixes).**
- **Start the test within 4 minutes of adding the sample to the Fluxergy PCR Card.**

7.4 Set Up and Run Your Test on Fluxergy Works Software

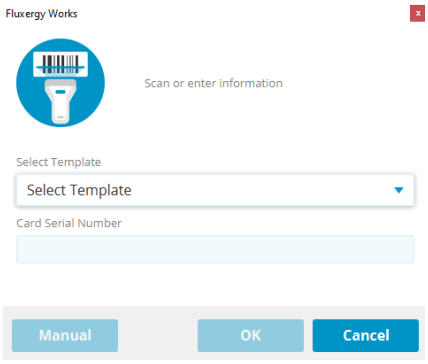
1. Open Fluxergy Works on the laptop and log in using your user ID and password.



2. Click the “Run Test” tab on the side-bar on the left side of your screen.

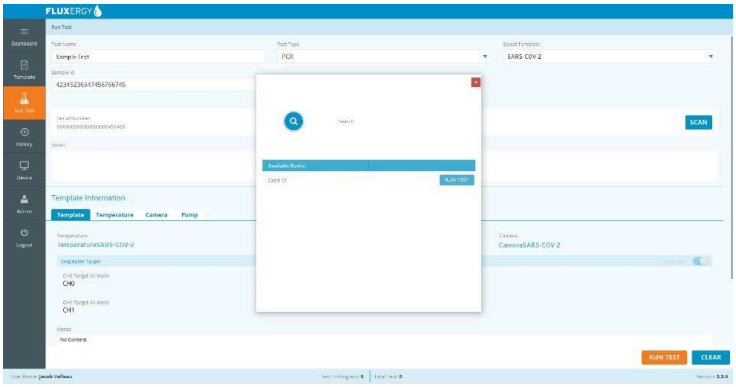
3. Scan the barcode on the Fluxergy PCR Card Package.

   Note: For best results, ensure that the scanning surface is flat, and entire barcode can be captured.



4. If the barcode cannot be scanned a prompt will appear, Click “Manual” and select the correct assay from the dropdown list.

5. Enter the serial number in the “Card Serial Number”, Click “OK”.



6. Type in “Test Name” and Sample ID. Sample specific information can also be included in the Notes section.

7. If a Test is a Retest, append the Test name with “_Retest”.

8. Once the Reaction Mix is fully thawed, briefly spin down the Reaction Mix tube using a mini centrifuge.

9. Vortex the swab sample in VTM for approximately 90 seconds to dislodge genetic material (If the sample sits before use, re-vortex for 3 seconds).

10. Pipette 14 µL of (swab + VTM) sample into the Reaction Mix tube.

11. Mix the sample and Reaction Mix by gently flicking the bottom of the tube 5 times.

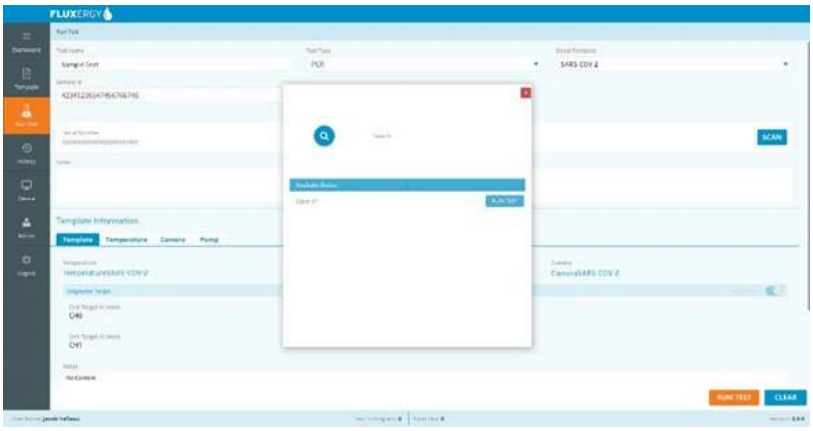
12. Briefly spin down using a mini centrifuge to get residual fluid off cap and walls of the tube.

    Caution: DO NOT use pipette to mix sample and Reaction Mix!

13. Place the Fluxergy PCR Card on a flat surface.
14. Carefully dispense 130 µL into the loading port of the Fluxergy PCR Card.

    Caution: Pipette the sample mix directly onto the loading port. Avoid pipetting onto the loading port's wall.

15. Press the plastic cap onto the loading port of the Fluxergy PCR Card.
16. Once the Fluxergy PCR Card is loaded and cap placed onto the loading port do not invert, shake, or drop the Fluxergy PCR Card.



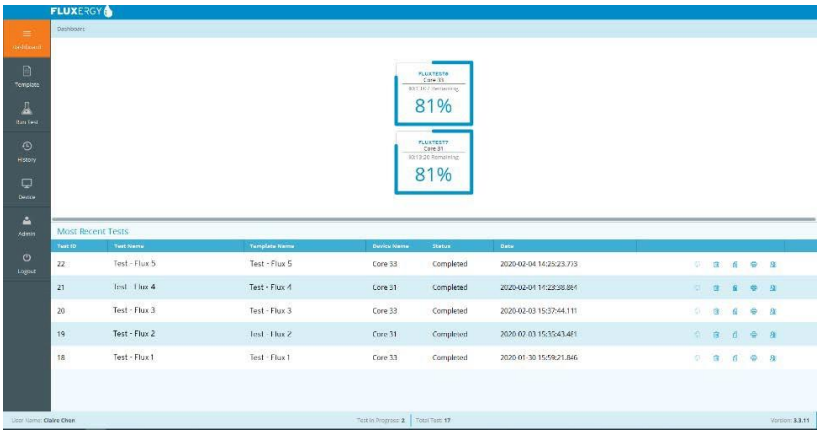
17. Click "RUN TEST" at the right of the Fluxergy Works window. Select a device from the list of available devices.

    Note: Fluxergy Works automatically filters unavailable devices.

18. Fluxergy Works will prompt you to insert a loaded Fluxergy PCR Card.

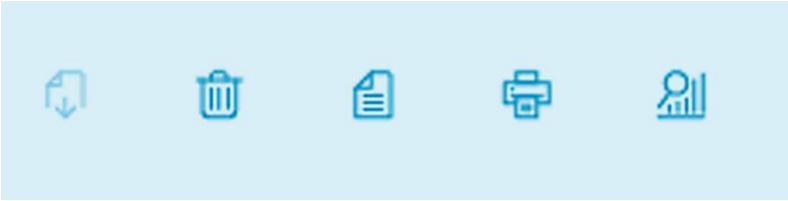
    Note: Immediately proceed to running a test, the user has 4 minutes to insert a Fluxergy PCR Card and run test.

19. Insert the Fluxergy PCR Card with sample and Reaction Mix into Fluxergy Analyzer.
20. Click "OK" on Fluxergy Works to begin the test.

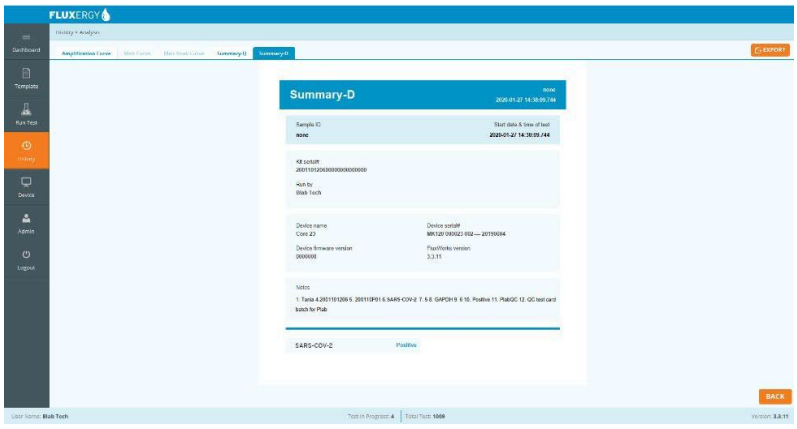


21. The test will be complete in about 1 hour. Tests in progress can be viewed on the Dashboard.

7.5 Accessing your Results



1. Once you select “OK” to run the tes you will be redirected to th dashboard. Here, you can find yo tests in progress, as well as a list your most recent tests.
2. To see all tests, select the “Histor tab. On the right-hand side of th window, you can choose to delet export, print, or view results for eac test.
3. Selecting the trash icon will delete te data permanently. Selecting the fi icon will allow user to view templa information for the test. Selecting th printer icon will enable you to print te summaries. Select the far-right optio the magnification glass and grap icon, to view and export your results
4. Summary D in each test will conta the qualitative test result.



* For detailed instructions on how to view and print the results, see the *Fluxergy Analyzer IFU.*

8. Quality Control

8.1 Internal Control

An internal control, included as part of the Reaction Mix, is used in the background of every test to validate a truenegative. Amplification must occur in the internal control channel for a negative to be qualified as a negative.

8.2 External Control

Fluxergy recommends including one positive and one negative control daily when clinical samples are tested. TheFluxergy Test Kit COVID-19 is not supplied with a Positive or negative control.

8.2.1 Negative Control

- A “no template”, negative control is used when a new system is first set up, as well as for training or proficiencytesting. The routine QC testing frequency will be based on the labs IQCP requirements.
- The negative control is added to Fluxergy Reaction Mix COVID-19 in the same way a patient sample would be(refer to section 7 of IFU for Test Procedure).
- Based on its internal validation, Fluxergy recommends using an approved viral transport medium as the negativecontrol.

8.2.2 Positive Control

- A positive template control is used when a new system is first set up, as well as for training or proficiency testing. The routine QC testing frequency will be based on the labs IQCP requirements.
- The positive control is added to Fluxergy Reaction Mix COVID-19 in the same way a patient sample would be (referto section 7 of IFU for Test Procedure).
- Based on its internal validation, Fluxergy recommends using the following positive controls:
  - Heat-inactivated SARS-CoV-2 Culture Fluid (Zeptometrix Catalog # 0810587CFHI-0.5mL) at 2.7 $TCID_{50}$/mL, or
  - SARS-CoV-2 viral particles inactivated by heat treatment and gamma irradiation (SARS-CoV-2 Medium QControl 01, Qnostics Cat # SCV2MQC01-B) at 4000 copies/mL.
  - Fluxergy COVID-19 PCR External Controls (+) available in pack of 1 (CAT #7151) or packs of 10 (CAT #7154), sold separately.

9. Interpretation of Results

9.1 Test Outputs

Test Output	Interpretation	Action
Positive	SARS-CoV-2 RNA present	Report the result to appropriate public health authorities. †
Negative	SARS-CoV-2 RNA not present	Report the result. †

† If the result is not consistent with clinical indications, seek confirmatory testing.

9.2 Error Codes

Error Codes	User Action on First Error	User Action on Second Error
600-625, 627-636	1. Restart Fluxergy Analyzer. 2. Retest sample with new card and mix.	Contact Customer Service
626	1. Dilute sample. 2. Retest diluted sample with new card and mix.	Contact Customer Service
Network Error*	Click to retrieve Test Result.	Follow instructions above for any other errors.

** This error can happen when analyzer loses connectivity with PC.*

9.3 Retests

If an ERROR is shown as the result from the test, there is a strong likelihood that you need to retest the original sample.In cases where sample quality may have played a role, you may need to recollect the sample.

The procedure to retest is as follows:

1. If there is no sample recollection required, use the leftover sample from the original swab and transport media. Ifsample recollection is required, collect according to 7.3 and standard procedure.
2. Use a clean pair of gloves as if starting a new test. Vortex the sample for 90 seconds and follow steps 7.4 – 7.5.
3. Make sure to give a different name to test in Fluxergy Works (e.g. Original Test Name_RETEST).
4. If an Error comes back for a second time, contact customer service, and seek confirmatory testing.

9.4 Restarting the Fluxergy Analyzer Device

For Instructions on how to Restart, Refer to the Fluxergy Analyzer IFU.

10. Limitations

- For use for SARS-CoV-2 testing only.
- For *in vitro* diagnostic use.
- False negatives may occur if the number of viral genome copies in the specimen are below the test limit ofdetection (LoD).
- As with other tests, false positives may occur. Some settings may indicate the need for repeat testing or testing using a different system.
- The test cannot rule out disease or infection caused by other bacterial or viral pathogens. The tests only detect SARS-CoV-2 RNA.
- As with any molecular test, mutations within the target regions of SARS-CoV-2 could affect primer binding,resulting in failure to detect the presence of virus.
- The test was only validated against the following sample type: nasopharyngeal swabs (NPS) immersed in viral transport media.
- PCR only detects RNA and does not determine whether a virus is lytic or whether a patient is infectious. The PCR result must be interpreted by a health care provider along with clinical signs.
- Improperly collected, transported, or handled samples risk the potential for false positive, false negative or erroneous results. The detection of viral nucleic acid is dependent upon proper sample collection, handling, transportation, storage, and preparation. Follow Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html and Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.
- The internal control will not indicate whether nucleic acid has been lost due to inadequate collection, transport, or storage of samples.
- Because the test is a direct PCR, sample dilution into correct volume of transport media is important. Further, correct mixing of the sample is important for test function.

11. Conditions of Use for the Laboratory

Use of the Fluxergy Test Kit COVID-19 must follow procedures outlined in the manufacturer's Instructions for Use and under the conditions set by the health authorities in your country.

- Laboratories using the Fluxergy Test Kit COVID-19 will use the materials and equipment identified in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use the Fluxergy Test Kit COVID-19 are not permitted.
- Authorized laboratories that receive the Fluxergy Test Kit COVID-19 will notify the relevant public health authorities of their intent to run your product prior to initiating testing, as appropriate.
- Authorized laboratories using the Fluxergy Test Kit COVID-19 will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Laboratories will collect information on the performance of the Fluxergy Test Kit COVID-19 and report any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the Fluxergy Test Kit COVID-19 of which they become aware to the appropriate authorities and to Fluxergy (+1 949-305-4201 or customersupport@fluxergy.com).
- All laboratory personnel using the Fluxergy Test Kit COVID-19 must be appropriately trained in in performing and interpreting the results of the Fluxergy Test Kit COVID-19, use appropriate personal protective equipment when handling this kit, and use the Fluxergy Test Kit COVID-19 in accordance with the authorized labeling.
- Fluxergy, authorized distributors, and laboratories using the Fluxergy Test Kit COVID-19 will ensure that records are maintained. Such records will be made available to their national authorities for inspection upon request.

12. Performance Evaluation[5]

12.1 Clinical Performance Evaluation[6]

The clinical performance evaluation of the Fluxergy Test Kit COVID-19 was conducted with archived clinical nasopharyngeal swabs (NPS) in viral transport medium. A total of 95 NPS samples (45 SARS-CoV-2 positive and 50 SARS-CoV-2 negative) were collected from November 2020 to March 2021 during the COVID-19 pandemic in the United States. All samples had been confirmed as positive or negative for SARS-CoV-2 by an authorized RT-PCR test(WHO EUL, US FDA EUA, CE-IVD, Health Canada, Australia TGA, etc.)

The randomized and blinded samples were tested with the Fluxergy Test Kit COVID-19 using Fluxergy Analyzer to generate the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) as an estimate for diagnostic accuracy. PPA and NPA were determined by comparing results of the Fluxergy Test Kit COVID-19 against the expected results. Results of these 95 archived clinical NPS samples are shown in table below.

Clinical Performance Results of Fluxergy Test Kit COVID-19			
	Expected Results		
Fluxergy Test Kit COVID-19	**Positive**	**Negative**	**TOTAL**
Positive	45	0	***45***
Negative	0	50	***50***
TOTAL	***45***	***50***	***95***
PPA	*100.00% (95% CI: 92.31% - 100.00%)**		
NPA	*100.00% (95% CI: 92.89% - 100.00%)**		

** During the study, any samples with errors were retested as instructed in IFU.*

12.2 Analytical Performance Evaluation

12.2.1 Limit of Detection (LoD)[7] – Analytical Sensitivity

The LoD of the Fluxergy Test Kit COVID-19 was determined using heat inactivated particles of SARS-Related Coronavirus 2 (SARS-CoV-2, Isolate: USA-WA1/2020). This virus was originally isolated from a patient with COVID-19 (GenBank MN985325). The cultivated virus titered by an infectious test and subsequently heat inactivated by the vendor (Zeptometrix Catalog # 0810587CFHI-0.5mL). Viral inactivation was verified by the absence of viral growth in tissue culture-based infectivity assays.

A preliminary LoD was determined by testing in triplicate 10-fold serial dilutions of the heat inactivated virus spiked into pooled negative nasopharyngeal swab (NPS) matrix. NPS samples were collected in Puritan UniTranz-RT 3mL Universal Transport Solution (SKU#: UT-300). The collected NPS matrix samples were qualified as negatives by RT- PCR (Fluxergy's COVID-19 TaqMan assay) before using them for virus spiking. The tentative LoD was confirmed by testing an additional 20 replicates. The limit of detection was defined as the lowest concentration at which each target is detected at least 95% of the time. The claimed LoD of the Fluxergy Test Kit COVID-19 is 0.3 $TCID_{50}$/mL.

Confirmatory LoD of the Fluxergy Test Kit COVID-19 in pooled nasopharyngeal swab matrix.

LoD Determination using USA-WA1/2020 Strain	
Concentration Tested	**# Tested / # Detected (%)**
0.3 $TCID_{50}$ / mL.	20/20 (100%)

12.2.2 Inclusivity – Analytical Reactivity

The inclusivity of the Fluxergy's Test Kit COVID-19 was evaluated using *in-silico* analysis of the assay primers and probesagainst 54,652 sequences available in the Global Initiative on Sharing Avian Influenza Data (GISAID) and the National Center for Biotechnology Information (NCBI) databases for two gene targets, N and ORF1ab.

The analysis demonstrated that the regions recognized by the Fluxergy Test Kit COVID-19's primers and probes have >= 98.95% homology with all available SARS-CoV-2 sequences.

The nucleotide mismatches observed were predicted to have no impact on the assay performance. In addition, a two- target assay design mitigates the occurrence of false negative results due to failure to amplify the individual target sequences.

In-Silico Inclusivity Report				
Database	**% Identity to N gene**		**% Identity to ORF1ab**	
	Probe (% Match)	**Primers (% Match)**	**Probe (% Match)**	**Primers (% Match)**
NCBI	5049/5055*(99.88%)	5055/5055(100%)	4497/4541*(99.03%)	4507/4541*(99.25%)
GISAID	49073/49597*(98.95%)	49482/49597*(99.77%)	49512/49597*(99.83%)	49535/49597*(99.87%)

** The nucleotide mismatches have no predicted impact on the assay performance.*

12.2.3 Cross-Reactivity – Analytical Specificity

The Fluxergy Test Kit COVID-19 was tested for its specificity by both direct testing of organisms (wet-lab testing) and by *in-silico* analysis.

a) Wet Lab Testing

Cross-reactivity of Fluxergy Test Kit COVID-19 was evaluated by wet-lab testing using the Fluxergy Analyzer. A panel of 18 micro-organisms presented below, were tested at the indicated concentrations (8.9×10^5 $TCID_{50}$ per mL to 2.8×10^8 $CEID_{50}$ per mL). For some pathogens tested, titer was estimated based on their total nucleic acid concentration of stock material. These organisms are on the high priority list due to the reasonable likelihood they may be present in upper respiratory samples. The pathogen stocks were diluted in pooled NPS matrix (Nasopharyngeal swab samples were collected in Puritan UniTranz-RT 3mL Universal Transport Solution (SKU#: UT-300) and then qualified as negatives by RT-PCR with Fluxergy's COVID-19 TaqMan assay) to reduce the effect microbial growth media and to obtain the desired testing concentrations. 14 microliters of prepared pathogen stocks were then used to prepare master mix and set up cross-reactivity runs.

None of the organisms listed in below table interfered with Fluxergy Test Kit COVID-19 performance by generating false-positive results.

Cross-Reactivity of Fluxergy Test Kit COVID-19 (Wet Lab Testing)			
Organism	**Strain**	**Cat # (BEI Resources)**	**Concentration**
Bordetella holmesii	H785	NR-44175	2.04×10^8 copies/mL*
Candida albicans	23R	NR-29339	2.2×10^7 copies/mL*
Enterovirus D68	US/IL/14-18952	NR-49131	1.6×10^7 $TCID_{50}$/mL
Haemophilus haemolyticus	F0397	HM-469	7.5×10^7 /mL*
Human Coronavirus NL63	NL63	NR-470	5.5×10^5 $TCID_{50}$/mL
Human respiratory syncytial A	A2000/3-4	NR-28530	2.8×10^6 $TCID_{50}$/mL
Human respiratory syncytial B	B1	NR-4052	4.4×10^5 $TCID_{50}$/mL
Influenza A virus (H1N1)	A/Beijing/262/1995 (H1N1)	NR-12277	2.8×10^8 $CEID_{50}$/mL
Influenza A virus (H3N2)	A/Brisbane/10/2007 (H3N2)	NR-12283	2.2×10^8 $CEID_{50}$/mL
Influenza A Virus pdm09	A/NewYork/18/2009 (H1N1)pdm09	NR-49451	1.3×10^{10} copies/ mL*
Influenza B virus (Victoria)	B/Brisbane/60/2008	NR-42005	6.25×10^6 $CEID_{50}$/mL
MERS-Coronavirus	EMC/2012	NR-50549	8.9×10^5 $TCID_{50}$/mL
Pseudomonas aeruginosa	EnvKY1	NR-51329	1.4×10^7 copies/mL*
Rhinovirus 50	A2 #58	NR-51455	2×10^6 $TCID_{50}$/mL
SARS-CoV	Urbani strain	NR-9548	1×10^8 pfu/mL
Staphylococcus epidermidis	VCU013	NR-9548	8.5×10^5 copies/mL
Streptococcus pneumoniae	EMC23F	NR-51859	4.18×10^6 copies/mL*
Streptococcus pyogenes	ABC020063118	NR-48702	6.3×10^6 copies/mL*

* *copies/mL, calculated based on the total nucleic acid concentration of extracted stock material.*

b) *In silico Analysis*

To complete the analysis for cross-reactivity, the forward primer, reverse primer and probe sequences (both N and orf1ab)were blasted individually against the following 34 taxids. Results of the *in silic*o analysis are summarized in table below.

Cross-Reactivity of Fluxergy Test Kit COVID-19 (*in silico* analysis)		
Organism	**In-Silico Analysis for % Identity***	
	nCoV- N gene	**nCoV- orf1ab gene**
Adenoviridae (taxid:10508)	No significant similarity found	No significant similarity found
Human metapneumovirus (taxid:162145)	No significant similarity found	No significant similarity found
Human parainfluenza virus 1 (taxid:12730)	No significant similarity found	No significant similarity found
Human parainfluenza virus 2 (taxid:1979160)	No significant similarity found	No significant similarity found
Human parainfluenza virus 3 (taxid:11216)	No significant similarity found	No significant similarity found
Human parainfluenza virus 4 (taxid:1979161)	No significant similarity found	No significant similarity found
Chlamydia pneumoniae (taxid:83558)	No significant similarity found	No significant similarity found
Haemophilus (taxid:724)	No significant similarity found	No significant similarity found
Streptococcus pneumoniae (taxid:1313)	No significant similarity found	No significant similarity found
Streptococcus pyogenes (taxid:1314)	No significant similarity found	No significant similarity found
Bordetella pertussis (taxid:520)	No significant similarity found	No significant similarity found
Bordetella holmesii (taxid:35814)	No significant similarity found	No significant similarity found
Mycoplasma pneumoniae (taxid:2104)	No significant similarity found	No significant similarity found
Pneumocystis jirovecii (taxid:42068)	No significant similarity found	No significant similarity found
Parechovirus (taxid:138954)	No significant similarity found	No significant similarity found
Candida albicans (taxid:5476)	No significant similarity found	No significant similarity found
Corynebacterium diphtheriae (taxid:1717)	No significant similarity found	No significant similarity found
Legionella (taxid:445)	No significant similarity found	No significant similarity found
Bacillus anthracis (taxid:1392)	No significant similarity found	No significant similarity found
Moraxella catarrhalis (taxid:480)	No significant similarity found	No significant similarity found
Neisseria elongata (taxid:495)	No significant similarity found	No significant similarity found
Neisseria meningitidis (taxid:487)	No significant similarity found	No significant similarity found
Pseudomonas aeruginosa group (taxid:136841)	No significant similarity found	No significant similarity found
Staphylococcus epidermidis (taxid:1282)	No significant similarity found	No significant similarity found
Leptospiraceae (taxid:170)	No significant similarity found	No significant similarity found
Chlamydia psittaci (taxid:83554)	No significant similarity found	No significant similarity found
Coxiella burnetii (taxid:777)	No significant similarity found	No significant similarity found
Staphylococcus aureus (taxid:1280)	No significant similarity found	No significant similarity found
Adenoviridae (taxid:10508)	No significant similarity found	No significant similarity found
Mycobacterium tuberculosis (taxid:1773)	No significant similarity found	No significant similarity found
Streptococcus salivarius (taxid:1304)	No significant similarity found	No significant similarity found
Human coronavirus 229E (taxid:11137)	No significant similarity found	No significant similarity found
Human coronavirus OC43 (taxid:31631)	No significant similarity found	No significant similarity found
Human coronavirus HKU1 (taxid:290028)	No significant similarity found	No significant similarity found

* *The amplicon sequences were blasted against low stringency filter [Somewhat similar sequences (blastn)].No alignment showed >70% homology and hence no potential for cross-reactivity.*

Based on the combined wet lab testing and in silico analysis, there is no potential unintended cross-reactivity with other organisms listed in both tables above.

12.2.4 Interference Study[8]

Potential interfering substances from upper respiratory specimens were tested using samples containing the heat inactivated virus at 3x LoD in nuclease free water. Testing was performed with the Fluxergy Test Kit COVID-19 using the Fluxergy Analyzer and included triplicate testing per substance at the indicated levels.

Assay Interference Verification				
Potential Interfering Substance	**Active Ingredient**	**Conc Tested**	**SARS-CoV-2 Detection (#Detected / #Tested)**	**IC % Detection (#Detected / #Tested)**
Decongestant	Afrin Nasal Spray-Oxymetazoline	15% (v/v)	100% (3/ 3)	100% (3/ 3)
Antibacterial	Tobramycin	4 µg/mL	100% (3/ 3)	100% (3/ 3)
Antibiotic	Amoxicillin	0.5 mg/mL	100% (3/ 3)	100% (3/ 3)
Antibiotic	Cephalexin	0.04 mg/mL	100% (3/ 3)	100% (3/ 3)
Antibiotic	Clindamycin	0.03 mg/mL	100% (3/ 3)	100% (3/ 3)
Antibiotic	Erythromycin	1 mg/mL	100% (3/ 3)	100% (3/ 3)
Antibiotic	Mupirocin	6.6 mg/mL	100% (3/ 3)	100% (3/ 3)
Antibiotic	Penicillin	1200 U/mL	100% (3/ 3)	100% (3/ 3)
Antiviral Drug	Zanamivir	3.3 mg/mL	100% (3/ 3)	100% (3/ 3)
Aspirin	Aspirin	0.62 mg/mL	100% (3/ 3)	100% (3/ 3)
Benadryl	Diphenhydramine	10 µL/Rxn	100% (3/ 3)	100% (3/ 3)
Blood	N/A	2% (v/v)	100% (3/ 3)	100% (3/ 3)
Corticosterone	Corticosterone	4 mg/swab	100% (3/ 3)	100% (3/ 3)
Corticosterone	Fluticasone	5% (v/v)	100% (3/ 3)	100% (3/ 3)
Mucin Protein	Bovine	60 µg/mL	100% (3/ 3)	100% (3/ 3)
Neo-Synephrine	Phenylephrine HCl	0.16 mg/mL	100% (3/ 3)	100% (3/ 3)
Nyquil	Dextromethorphan; Hydrobromide; Doxylamine Succinate	1/200 Dilution	100% (3/ 3)	100% (3/ 3)
Pain Medication	Acetaminophen	1 mg/mL	100% (3/ 3)	100% (3/ 3)
Pain Medication	Nonsteroidal anti-inflammatory drug	1 mg/mL	100% (3/ 3)	100% (3/ 3)
Robitussin Cough	Dextromethorphan HBr; Guaifenesin	2.0 mg/mL	100% (3/ 3)	100% (3/ 3)
Tamiflu Antiviral Drug	Oseltamivir	1 µM	100% (3/ 3)	100% (3/ 3)
Nasal Spray	Saline	15% (v/v)	100% (3/ 3)	100% (3/ 3)
Sore Throat Lozenge	Menthol	1 mg/mL	100% (3/ 3)	100% (3/ 3)
Sore ThroatLozenge	Zinc GluconateGlycine	1 mg/mL	100% (3/ 3)	100% (3/ 3)
Zicam Nasal Gel	Oxymetazoline hydrochloride	5% (v/v)	100% (3/ 3)	100% (3/ 3)
NA	Non-spike Control	3x LoD	100% (3/ 3)	100% (3/ 3)

13. Symbols and Marking

13.1 Symbols on Packaging

Symbol	Meaning
CE	The Fluxergy product conforms to DIRECTIVE 98/79/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 October 1998 on in vitro diagnostic medical devices.
IVD	This symbol indicates that the product is for In Vitro Diagnostic use.
www.fluxergy.com/downloads	The instructions for use are either included or available for download electronically from the website shown.
10	This symbol indicates the number of pieces in the package (10).
100	This symbol indicates the number of pieces in the package (100).
	Indicates that the transport package shall be kept away from rain and in dry conditions. ISO 15223-1:2016 (5.3.4)
YYYY-MM-DD	Indicates the date after which the product is not to be used. The date format is YYYY- MM-DD where YYYY represents the four (4) digit year, MM is the two (2) digit month and DD is the two (2) digit day.
UDI	Indicates the unique device identification data.
	Indicates methods to contact customer support.
REF	Indicates the Fluxergy catalogue number so that the medical device can be identified.
SN	Indicates the Fluxergy serial number so that a specific medical device can be identified.
LOT	Identifies the manufacturer's batch or lot code.
126 µl	Indicates the container's net volume in specific unit of measure.
	Indicates that the item is for single use only and must not be used more than once.
	Indicates the temperature limits to which the product can be safely exposed and gives the maximum and minimum storage temperatures.
	Indicates that the user should not use the product without inspecting the contents of the package if the package is badly damaged.

Symbol	Meaning
	Test Card image.
	Reaction Mix image.
	Indicates the medical device manufacturer, as defined in EU Directive 98/79/EC. This symbol is used to identify the name and address of company that manufactured the product.
EC REP	Indicates the authorized representative in the European Community.
CH REP	Indicates the authorized representative in Switzerland.

13.2 Symbols used in this IFU

Symbol	Meaning
	Indicates that there are potential biological risks associated with the medical device after use. This symbol is used to remind the user that the Fluxergy Test Kit COVID-19 is considered hazardous waste after it has been used to perform a test. The used Fluxergy PCR Card should be disposed of as required by national authorities.

14. Contact and Legal Information

14.1 Fluxergy Headquarter's Location

FLUXERGY
30 Fairbanks, Suite 110 Irvine, CA 92618
USA

14.2 Customer and Technical Support

14.2.1 Contact us by Mail

Attn: Fluxergy Customer Support
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Irvine, CA 92618
USA

14.2.2 Contact us by Email

customersupport@fluxergy.com

14.2.3 Contact us by Phone

+1 (949) 305-4201 US & International

14.3 Authorized Representative

EC REP

CMC Medical Devices & Drugs S.L.
C/ Horacio Lengo N° 18
CP 29006 Málaga, Spain

CH REP

CMC Medical Devices GmbH
Bahnhofstrasse 32
CH- 6300 Zug, Switzerland

15. References

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[3] Clinical And Laboratory Standards Institute. Protection Of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline - Fourth Edition. CLSI M29-A4.

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[8] CLSI. Interference Testing In Clinical Chemistry - 3rd Edition. CLSI EP07-Ed3.