CovidNudge®

DnaCartridge® & NudgeBox®

Instructions For Use

Rev 11 - October 2022

NudgeBox Analyser – BX-0004
NudgeBox – 9101004
CovidNudge DnaCartridge – CT-0001

DN-ENG-IFU-001 Rev 11 - 9501002 - 09-2022

dnanudge®

CE

IVD

In this document the terms “NudgeBox Analyser” and “CovidNudge Test” are used interchangeably.

In this document the terms “Capsule” and “DnaBean” are used interchangeably.

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The latest version of this IFU can be downloaded from:
dnanudge.com/covid-test-access

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1. Intended use

The DnaNudge CovidNudge test is a lab-free sample-to-answer RT-PCR test intended for qualitative detection of nucleic acid from the SARS-CoV-2 virus for an individual with clinical symptoms of, or people at risk of exposure to, SARS-CoV-2, providing results on the spot, at the point of need.

For the CovidNudge test, the sample type needs to be nasopharyngeal. It is advised that the sample is collected by a professional trained in nasopharyngeal swabbing.

The CovidNudge Test (including the NudgeBox Analyser and DnaCartridge) is classified as a Class 3 IVD medical device in Australia (Therapeutic Goods (Medical Devices) Regulations 2002).

Due to the lab-free nature of the system, CovidNudge tests are suitable for use by trained professionals in a wide range of settings:

-Clinical environments (hospitals, GP surgeries, dentists, etc.)
-Care homes, nurseries, schools
-Airports, train stations, borders
-Corporate offices, factories, farms

The CovidNudge test is a molecular in-vitro diagnostic test that aids in the detection of SARS-CoV-2 and diagnosis of COVID-19. The SARS-CoV-2 virus is generally detectable in upper respiratory specimens during the acute phase of infection.

The CovidNudge test is intended to be used on persons for whom SARS-CoV-2 is suspected, i.e. those who are experiencing clinical symptoms consistent with Covid-19, or those who have had contact with persons with Covid-19.

The CovidNudge DnaCartridge contains 6 types of assay for SARS-CoV-2 virus detection as well as a control assay for human RNaseP. If the control assay does not sufficiently amplify, the test will be reported as invalid due to insufficient levels of human RNA in the sample. This is usually due to insufficient swabbing.

A positive result indicates the presence of the SARS-CoV-2 virus.

Negative results do not rule out infection with SARS-CoV-2 and should not be used as the sole basis for treatment. No test is 100% accurate, especially at low levels of infection and the CovidNudge test is no exception.

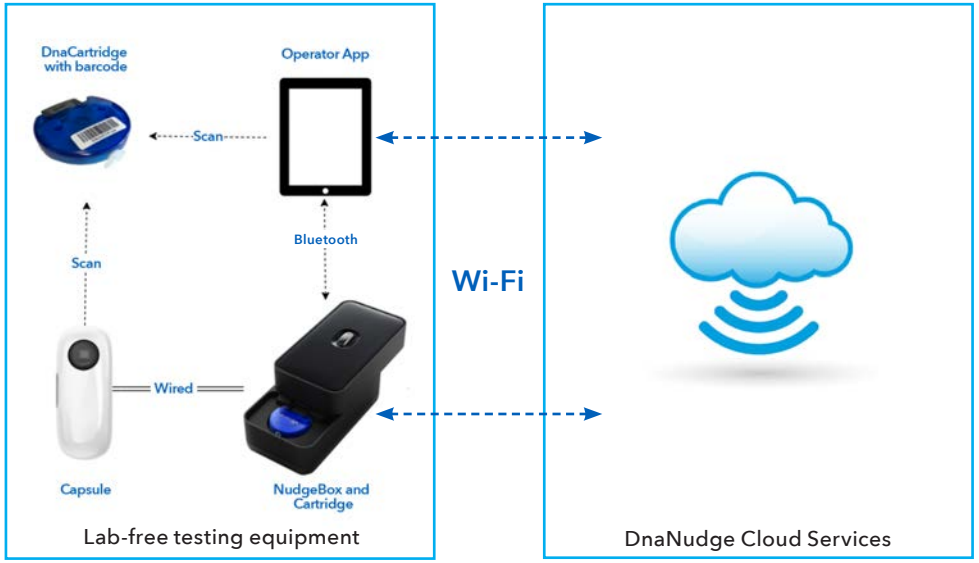
NOTE: As with any molecular test, mutations within the target genes of SARS-CoV-2 could affect primer and/or probe binding resulting in a failure to detect the presence of the virus. However the use of multiple gene targets in the DnaNudge CovidNudge test make this less likely than in some other tests which only detect one or two gene targets.

2. Summary & explanation

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019. The International Committee for Taxonomy of Viruses (ICTV) named the novel coronavirus SARS-CoV-2. It is responsible for the ongoing COVID-19 pandemic and infection can result in severe illness or death.

The CovidNudge test is a molecular in vitro diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 and is based on widely used nucleic acid amplification technology. The CovidNudge test contains primers and internal controls used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.

The DnaCartridge is a disposable, sealed, and integrated lab-on-chip device that enables sample-to-result PCR/RT-PCR. The NudgeBox, provides thermal cycling and mechanics to drive the DnaCartridge and has barcode scanner (Capsule). The user interface (Operator App) is the mobile application on the iPad for WiFi setting of the NudgeBox via Bluetooth and registering samples/ cartridges and monitoring test progress/result. DnaNudge Cloud Services provide all the server-side processing capabilities and data storage for the NudgeBox and the Operator App. An overview of the NudgeBox system is shown in the diagram below.



3. Test specification

Each Cartridge tests for 7 Assays

Assay	#Replicates
CDC - N1	10
CDC - N2	10
CDC - N3	10
Charité Berlin - E	10
Institut Pasteur - RdRP-IP2	9
Institut Pasteur - RdRP-IP4	9
RNaseP Control	6

Sampling

Sample
Nasopharyngeal

Swab
Paediatric nasal swab
(needs to be less than 3mm wide to fit into the DnaCartridge)

RT-PCR

Step	#Cycles	Temperature	Time
RT	1	50	5 minutes
RT inac/Go taq active	1	95	2 minutes
Denaturation	40	95	3 seconds
Annealing/Extension		60	30 seconds

Test outcome

Positive: If 4 or more viral gene replicates amplify in any of the assays

Indeterminate: If 2 or 3 of the viral gene replicates amplify in any of the assays

Negative: If less than 2 of the assays except the control assay amplifies

Invalid: If less than 2 replicates of the control assay amplifies

Error: In the event of any technical error during the sample preparation phase of the test, the NudgeBox will indicate with flashing red LEDs

See Section 20 'Interpretation of Results' for full details and reasons to repeat the test

Sensitivity and Specificity

Trials comparing DnaNudge CovidNudge against NHS laboratory results indicated 97% sensitivity and 100% specificity. The swabbing method and swab type are both important factors in achieving good results. The control assay adds a higher degree of reliability by ensuring that a false negative result is not reported in the case of insufficient sample collection.

4. Principle of the procedure

The CovidNudge test is an automated in vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2.

The CovidNudge test is performed using a DnaCartridge and NudgeBox supplied by DnaNudge Ltd.

The DnaCartridge and NudgeBox automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The system consists of a DnaCartridge, a NudgeBox, remote (cloud) software and an Operator app for running tests and viewing the results. The system requires the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized.

The CovidNudge test includes reagents for the detection of RNA from SARS-CoV-2 in nasal swab specimens. A human RNA control primer is also included in the DnaCartridge. The human RNA control is present to ensure adequate processing of the sample; if the human RNA primer fails to amplify this indicates inadequate swabbing and the result is reported as invalid to minimise false negative reporting.

The CovidNudge test also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. If any deviation from the ideal reaction conditions are detected, the test will abort.

The swab specimen is collected and placed directly into the sample chamber of the DnaCartridge. The DnaCartridge is loaded onto the NudgeBox analyser, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

5. CovidNudge test flow

Step 1.
A quick swab
(Nasopharyngeal)

Step 2.
The swab is inserted into the DnaCartridge - programmed for Covid-19

Step 3.
The barcode on the DnaCartridge is scanned by the Capsule, and the Capsule placed onto the NudgeBox

Step 4.
The NudgeBox runs the test and sends the test data to the secure DnaNudge cloud

Step 5.
The cloud analyses the test data and sends the results to the clinician via the Operator App and/or standard hospital or lab integration systems
Sample to answer :~1.5 hours

6. Requirements from the site

- A reliable WiFi connection allowing access to the secure DnaNudge Cloud server or appropriate location for a mobile hotspot with good reception
- Mains power supply
- Paediatric nasal swabs (nasopharyngeal sample)
- Double plastic bags
  (in case of transferring patient DnaCartridges between sites)
- Dedicated point-of-contacts
- Steady and secure space for the NudgeBoxes to operate at regulated temperature (see Section 30 for detailed operating conditions), to operate away from any physical disturbance and with plenty of free space around both the NudgeBox and Power Supply Unit to allow for adequate ventilation and maintenance. Do not obstruct the NudgeBox ventilation openings
- Dry and ideally cool / room temperature storage for DnaCartridges (see section 30)
- DnaCartridges need to be disposed of based on bio-waste management, using yellow plastic bags
- Approved cleaning materials
  (see section 22)

7. Reagents & instruments

Materials provided

DnaNudge CovidNudge Test Kit

Each kit contains the equipment necessary to perform a single test:

1 x DnaNudge COVID Nudge Cartridge

- Lysis reagent: 450 uL per cartridge
- Wash buffer : 450 uL per cartridge
- Elution reagent: 450uL per cartridge
- 1 Step RT-PCR lyophilised bead

1 x nasopharyngeal sample kit

- Paediatric nasal swab
- Disposable scissors

External Controls

- Positive Control: HE0062-DN
  SARS-CoV-2 Process Control (Pellet) from Microbologics (CE-IVD)
- Negative Control: HE0058-DN
  Process Control (Pellet) from Microbiologics (CE-IVD)

Additional external controls can be obtained through a local Sponsor (see page 2 for their details).

Note: Safety data sheets and training materials are available at:

www.dnanudge.com/covid-test-access

Materials required but not provided

DnaNudge NudgeBox analyser v 3.3 or higher

8. Internal controls

Internal Controls

There are human control genes on each cartridge for checking the quality of the sample collection and a pressure check by the NudgeBox for checking the quality of the cartridge sealing.

CONTROL

Human control gene (RNaseP)

Ensures a proper swab is taken. If there is not enough human control gene amplified during the test, the result will be invalid which indicates that the sample collection swab has not been done properly. A new swab is required for re-test.

CONTROL

Pressure check

Ensures the amplification unit (AU) and the sample preparation unit (SPU) are well sealed. The cartridge pressure check is carried out during the test for checking the sealing quality of the DnaCartridge. If the check fails at any stage, the test will be aborted and a new swab is required for re-test.

9. Warnings & precautions

General

- For *in vitro* diagnostic use.
- A positive result indicates the presence of the SARS-CoV-2 virus.
- All positive results are required to be reported to the appropriate public health authority.
- Performance characteristics of this test have been established with the specimen types listed in the Intended Use Section only. The performance of this assay with other specimen types or samples has not been evaluated.

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, current clinical guidelines for wearing appropriate PPE when handling potentially infectious samples and disposing of clinical waste must be followed.
- Potential false negatives may be observed due to emerging variants of SARS-CoV-2. however DnaNudge CovidNudge test's performance is not negatively affected by currently identified variants (see section 29 - Analytical Performance ii. Analytical Reactivity (Inclusivity) for the latest variant information.

Specimens

- Specimens (nasopharyngeal swab) should be immediately inserted into the DnaCartridge which should then be sealed. If the DnaCartridge is to be transported to another location for testing, proper storage conditions must be maintained during transport to ensure the integrity of the specimen. Specimen and DnaCartridge stability under shipping conditions other than those recommended has not been evaluated.

Specimen Collection, Transport, and Storage

- Proper specimen collection, storage, and transport (if required) are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. See Section 16 for sample collection procedure. Once collected, the swab should be immediately inserted into the DnaCartridge and sealed. DnaCartridges containing swab specimens can be stored away from light and at 18-25°C, 40-50% RH) for up to 12 hours.

9. Warnings & precautions

Assay/Reagent

- Do not open the DnaCartridge except when inserting the swab.
- Do not use a DnaCartridge that has been dropped after removing it from the packaging.
- Do not shake the DnaCartridge. Shaking or dropping the Cartridge may yield indeterminate results.
- Do not use a DnaCartridge with a damaged barcode label.

- Each single-use DnaCartridge is used to process one test. Do not re-use processed cartridges.

- Each single-use disposable scissors is used to cut one swab. Do not reuse disposable scissors.
- Do not use a DnaCartridge if it appears wet or if the chamber seal appears to have been broken.
- Wear clean lab coats and gloves. Change gloves between the handling of each swab.

- Biological specimens (swabs) and used cartridges should be considered capable of transmitting infectious agents requiring standard precautions. Follow your institution's environmental waste procedures for proper disposal of used cartridges. These materials may exhibit characteristics of chemical hazardous waste requiring specific disposal. If country or regional regulations do not provide clear direction on proper disposal, biological specimens and used cartridges should be disposed per WHO [World Health Organization] medical waste handling and disposal guidelines.

NudgeBox System

- Please follow steps in Section 14: NudgeBox Installation when deploying the NudgeBox in a new environment.
- Before connecting the Power Supply Unit to the mains supply, ensure that the mains supply voltage corresponds to the voltage printed on the Power Supply Unit.
- Do not handle the mains plug of the Power Supply Unit with wet hands.
- Allow time for the Instrument to settle to the operating temperatures if stored in an environment too hot or too cold.
- Do not expose the NudgeBox to rain, moisture, dripping water or splashing water.
- If dew forms due to condensation, the NudgeBox needs to be dried before use.
- Do not place objects filled with liquids on the NudgeBox or the Power Supply Unit.
- Do not place heavy items on the NudgeBox or Power Supply Unit.
- Do not remove the covers from the NudgeBox or Power Supply Unit.
- Do not attempt to repair the NudgeBox or Power Supply Unit yourself.
- Do not place the NudgeBox or Power Supply Unit on anything that may become hot.
- Do not expose the NudgeBox or Power Supply Unit to direct sunlight, excessive humidity and excessive vibration.
- Do not move the NudgeBox when a test is in progress.
- Please discuss the availability and reliability of the internet connection with the responsible organisation. The internet connection to the cloud server is critical to the service. If the internet connection is lost, the NudgeBox will finish the current test but cannot upload the test result or start any new test until the network is restored.

10. Safety notices

Safety may be impaired if the NudgeBox or DNAcartridge is not used as specified, or with accessories which are not approved.

The NudgeBox is powered by a 24V Direct Current (DC) Power Supply Unit which is provided. No other power supply should be connected to the NudgeBox.

The Power Supply Unit must only be connected to a standard AS/NZS 3112 mains socket that includes a protective earth terminal, via the detachable IEC mains supply cord provided. The mains supply cord must be rated at 10A and a 5A fuse must be fitted to the plug. Do not replace the mains supply cord with an inadequately rated cord - doing so could compromise safety.

The NudgeBox should not be disconnected from supply except by disconnecting the mains connector of the Power Supply Unit. The Power Supply Unit should be positioned:

- so as to minimise the risk of the mains cable being accidentally pulled out of it.
- such that it is as easy as possible to quickly disconnect the Power Supply Unit from the mains supply in the event of a serious problem.

Do not attempt to open the NudgeBox while it is running a test.

- Internal surfaces of the NudgeBox reach >90°C during operation.
- The NudgeBox contains motorised parts which could entangle the operator if exposed during operation.

The NudgeBox contains no user-serviceable components. Do not attempt to remove the outer casing of the NudgeBox - doing so could compromise safety.

If the outer casing of the NudgeBox is damaged in any way, turn off the instrument at the mains supply and contact Covid@dnanudge.com. Do not attempt to use the NudgeBox.

The NudgeBox should be mounted on a firm, non-flammable surface. The ventilation holes at the end of the unit must not be obstructed.

11. DnaCartridge overview

The DnaCartridge is a disposable, sealed, and integrated lab-on-chip device that enables sample-to-result PCR.

It consists of two main parts: the amplification unit (AU) and the sample preparation unit (SPU). The AU has dried primers and probes uniquely spotted into each of its 72 reaction wells, providing multi-plex analysis. Each assay is spotted in multiple wells to provide redundancy and reliability. The SPU has the buffers to extract and purify DNA/RNA from a swab sample, as well as lyophilised PCR master-mix to mix with the extracted DNA/RNA before filling the AU for PCR. The PCR happens simultaneously in each of the AU wells.

Each DnaCartridge has a unique barcode and is packaged in a mylar bag. For operating and storage conditions, please see section 30.

Amplification Unit (AU)

Dried primers and probes for:

CDC	- N1 - N2 - N3
Institut Pasteur	- RdRP-IP2 - RdRP-IP4
Charité Berlin	- E
Control	- RNaseP

Sample Preparation Unit (SPU)

Lysis buffer
Wash buffer
Elution buffer
Lyophilised RT-PCR master-mix

Do not touch the AU when handling the DnaCartridge. Touching the AU could lead to an invalid test.

Cartridge type: CovidNudge
Cartridges can be ordered from: Covid@DnaNudge.com

12. NudgeBox system overview

The NudgeBox is a stand-alone device and provides the mechanics to drive the DnaCartridge and run PCR/RT-PCR test lab-free.

It consists of mechanical, pneumatic, thermal, optical, and communication sub-systems.

The NudgeBox is linked to DnaNudge Cloud via Wi-Fi. The Wi-Fi settings can be loaded via Bluetooth using DnaNudge operator App.

Each NudgeBox comes with a scanning module Capsule, to scan the DnaCartridge barcode and initiate the test.

For operating and storage conditions, please see section 30.

NudgeBox

WiFi
Bluetooth

Power Supply Unit

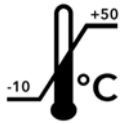
Capsule

Single-button scanning with LED and miniaturized camera.

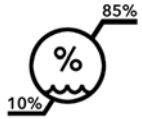
13. NudgeBox system transportation

NudgeBox transportation and storage

The NudgeBox system should only be transported in the supplied shipping case, as shown in the image here.



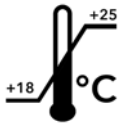
Storage and transport temperature



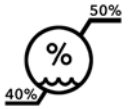
Storage and transport humidity

DnaCartridge transportation and storage

The DnaCartridges should be handled carefully when transported individually. When stored and transported in batches, the DnaCartridges need be to stored in the cardboard boxes provided by DnaNudge or similar double walled cardboard boxes. Do mix a stack of DnaCartridges with other goods.



Storage and transport temperature



Storage humidity when the mylar bag is opened

Use within 12 hours

14. NudgeBox installation

NudgeBox Positioning Selection

- Ensure that the installation surface is level and free of debris (if unsure, place a round pen or pencil on the surface and see if it rolls in any direction.)
- Ensure that the surface can hold up to 10 kilograms for the NudgeBox and other materials.
- Ensure that the surface is dry/not prone to have any moisture collect on it. Moisture inside the NudgeBox may cause serious damage.
- Ensure that the surface is free of dust/ not prone to gather excessive amounts of dust. Excessive dust inside the NudgeBox may cause serious damage.
- Check there are no water sources above the NudgeBox that may potentially drip onto/around the NudgeBox.
- Do not place the NudgeBox on the ground in an area with heavy foot traffic.
- Do not place the NudgeBox on the ground where a swinging door may strike it.
- Ensure that there is a clearance of at least 15cm (the width of a NudgeBox) on the left and right sides of the NudgeBox. This is to ensure proper ventilation.
- Ensure there is a minimum of 15 cm clearance directly behind the NudgeBox so that nothing can prevent the top of the box from sliding open (the Power Supply Unit and cord may sit directly behind the NudgeBox if they sit low enough to not block the sliding movement).
- Ensure that the NudgeBox is no closer than 5cm to an edge on the front side and no closer than 15 cm on the left and right sides.
- Remember that the power cable has a limited length. Ensure that the position is sufficiently close to a power outlet.
- The recommended temperature is below 25°C. Ensure that the room temperature will not rise greater than 45°C.
- Do not install the NudgeBox in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these can interfere with the proper operation.

Condition Inspection

- Remove the NudgeBox from the transportation case, **always handling with two clean hands.**
- Inspect the outside of the Nudgebox for any damage. If found please notify **covid@dnanudge.com**
- Check that the lid of the NudgeBox slides open and closed correctly.
- Place the NudgeBox on a stable surface. Turn the Box onto one side and check that all four rubber-bottomed “feet” are screwed snugly into the bottom of the NudgeBox; if not, hand-tighten.
- Place the NudgeBox upright again on the working surface. Gently push the top and bottom covers to ensure that they are screwed in tightly and do not shift around. If loose, do not use the NudgeBox and contact **covid@dnanudge.com**
- When shifting the NudgeBox onto its side and back again, if any rattling or loose components can be heard do not use the NudgeBox and contact **covid@dnanudge.com**

Power

- Identify a mains power socket that you would like to use that is within 2 metres of the NudgeBox.
- The NudgeBox can draw up to 130W, so if multiple NudgeBoxes are operated from a multi-socket extension lead, please ensure the extension lead is suitably rated. Do not exceed the power limit of the mains power socket when connecting multiple NudgeBoxes to it.
- If area is known to have power interruptions/outages, please use an Uninterrupted Power Supply (UPS). Ensure that it can provide sufficient power of 130 Watts per NudgeBox.
- Remove the Power Supply Unit and the mains cable from the transportation case and connect together.
- Plug the Power Supply Unit into the wall outlet and position accordingly.
- Ensure there is no tension at any point along the power cord going from the wall outlet to the rear of the NudgeBox.
- Plug the power cord into the rear of the NudgeBox. The Standby Button LED should now light up **red** (see image in Section 15).

15. Setting up the NudgeBox

i. Start-up & Initialisation

The NudgeBox should be closed and empty before turning on.
Slide the NudgeBox top open. Check that the inside is clear of any cartridge (if found, please remove and properly dispose in a Clinical waste stream). Slide the NudgeBox top closed.

Connect the Power Supply Unit that is provided with the NudgeBox at the location shown here. ***Note: Only the provided Power Supply Unit must be used. The USB-C connection is for diagnostics and is to be used by service personnel only. Misuse may cause an error in the system.***

Fit the capsule into its recess on the top of the NudgeBox. ***Note: If the capsule has lost its charge during transport, it may need to be charged for 2 minutes on the NudgeBox before proceeding with the setup.***

Before running a new test, please re-start the NudgeBox by pressing the Standby button at the back of the box twice: once to turn the light **red**, and again to turn the light **green**

Standby button red:
Nudgebox is in Standby
Standby button green:
Nudgebox is ON

When the NudgeBox is off, the LED lights on the front panel will be off. Once the NudgeBox is turned on, both LED lights will flash...**Red-Green-Blue**

After switching on, please wait for a few seconds for the NudgeBox to initialise. Do not slide open the NudgeBox until initialisation is complete.

When the initialisation is complete, the left LED will show **steady Blue**,and the right LED will show **breathing (pulsing) Green.**

ii. Run a NudgeBox Health Check

(When setting up in a new location, please first run a Health Check. Refer to Section 25 for full instructions)

iii. Connect the iPad to the NudgeBox

(When setting up for the first time)

In the Operator App select the Settings icon in the top right corner and choose 'QR Code'.

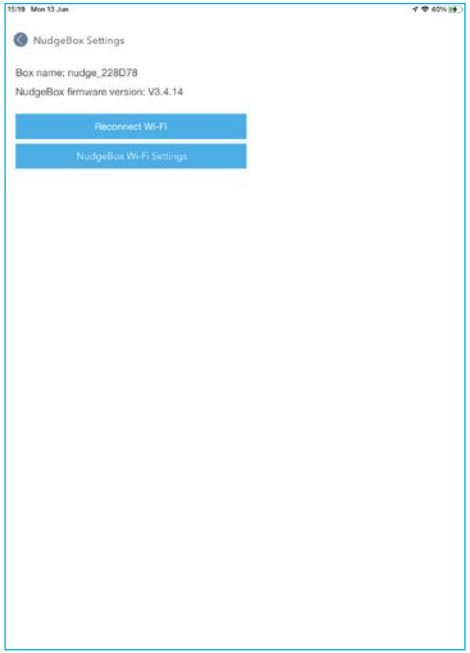
Use the iPad to scan the QR code on the NudgeBox.

Once the QR code is scanned, the iPad will automatically connect to the NudgeBox via Bluetooth.

15. Setting up the NudgeBox

iv. Connecting the iPad to the NudgeBox (cont..)

After scanning the QR code, the operator App will display the details of the NudgeBox (e.g. NudgeBox name, firmware version and regional setting).

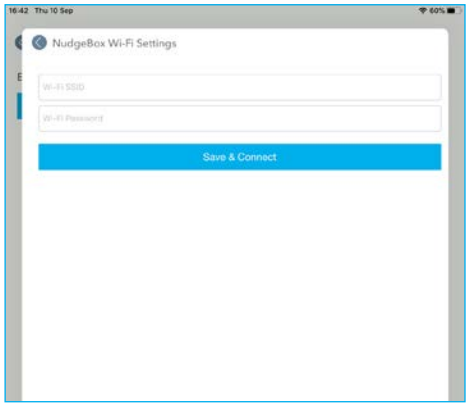


v. Changing the Wi-Fi settings

In most cases, the NudgeBox will be pre-configured to join the correct Wi-Fi network, and changing these settings is not recommended.

If selecting another WiFi network is required, first select **'NudgeBox Wi-Fi Settings'.**

Enter the Wi-Fi SSID, and Wi-Fi Password and select **'Save & Connect'.**



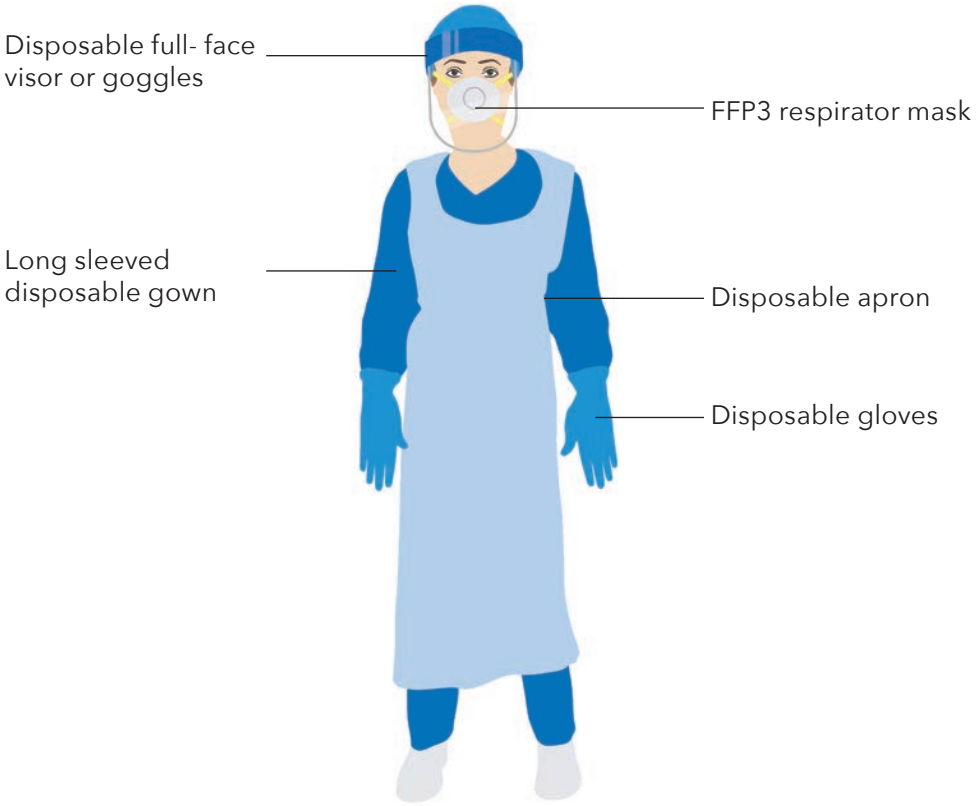
When the NudgeBox is ready to run a test, the left LED will show **steady Green**, the right LED will show **breathing (pulsing) Green**.

PLEASE DO NOT START ANY TEST BEFORE THE CONNECTION STABILISES AND THE GREEN LIGHTS IS OBSERVED.

16. Taking a sample

Samples should be collected by a healthcare professional / experienced nurse who is trained in the technique.

The healthcare professional must wear appropriate PPE. Please refer to the latest recommended guidelines.



Taking a nasopharyngeal sample

The swabs suitable for nasopharyngeal use and tested with the DnaCartridge are paediatric nasal swabs (the swab tip needs to be less than 3mm).
DO NOT USE Isohelix buccal swabs to take a nasopharyngeal sample.

Equipment required:

- DnaNudge Nasal Kit

i. The healthcare professional should enquire of any known nasal obstruction, then ask the patient to blow their nose, and sit upright with their head tilted back.

ii. Using the paediatric nasal swab to reach the nasopharynx, one swab should be taken for 7-15 seconds from one nostril. Count from 1 to 7 (minimum) and gently twist the swab at the same time.

If double-swabbing for Quality Control purposes, the nasal swab must be taken from a separate site to the diagnostic control swab, because subsequent swabs of the same area have been shown to harbour reduced virus in both preliminary COVID-19 studies and influenza studies.

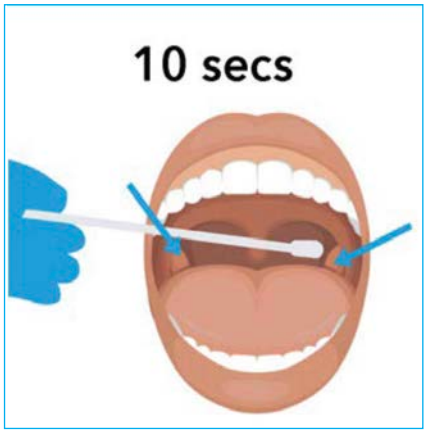
16. Taking a sample

Taking a combined Nose and Throat sample

The swabs suitable for nasopharyngeal use and tested with a DnaCartridge are paediatric nasal swabs (the swab tip needs to be less than 3mm).
DO NOT USE Isohelix buccal swabs to take a nasopharyngeal sample.

Equipment required:

- DnaNudge Nasal Kit

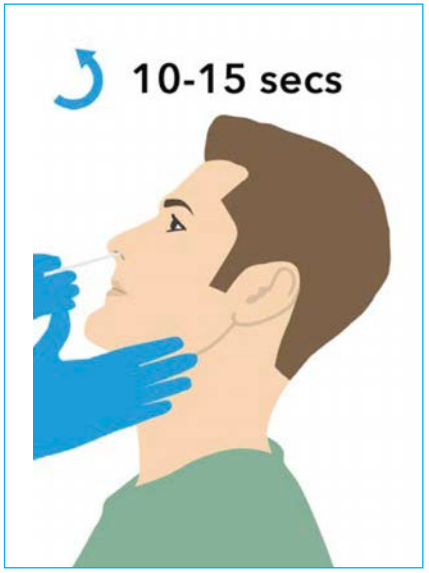


Procedure:

i. The healthcare professional should enquire of any known nasal obstruction, then ask the patient to blow their nose.

ii. Ask the patient to open their mouth wide, and rub the fabric tip of the swab over both tonsils (or where they would have been) with good contact at least 3 times.

iii. Put the same end of the swab gently into one nostril until a slight resistance is felt (about 2.5cm into the nostril). Rub the swab 5 times along the inside of the nostril.

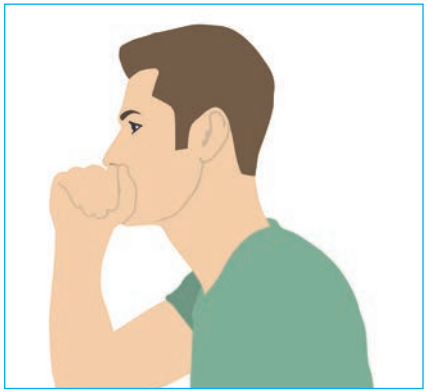


Taking a sputum sample

The swabs most suitable for sputum samples and tested with the DnaCartridge are Isohelix buccal swabs.

Equipment required:

- DnaNudge Mouth Kit
- Oragene500 saliva sample collection tube (not provided)



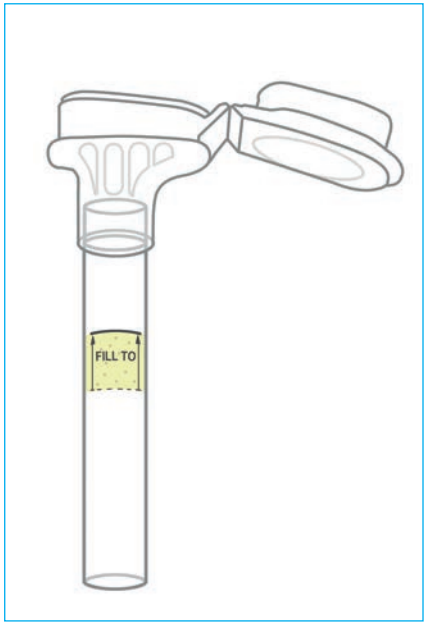
Procedure:

i. The healthcare professional should prepare the patient for the procedure by asking them to sit upright, rinse their mouth with water and spit out prior to sputum collection.

ii. The patient should be asked to take a few deep breaths to help loosen secretions; please note, if patient is on a nebuliser, give the nebuliser first and wait 10 minutes before taking a sample.

iii. The healthcare professional should ask patient to cover their mouth before forcing out a deep cough to release the sputum.

iv. Sputum should be collected in the specimen tube. Ideally the sputum sample should be no less than the size of a small fingernail.



16. Taking a sample

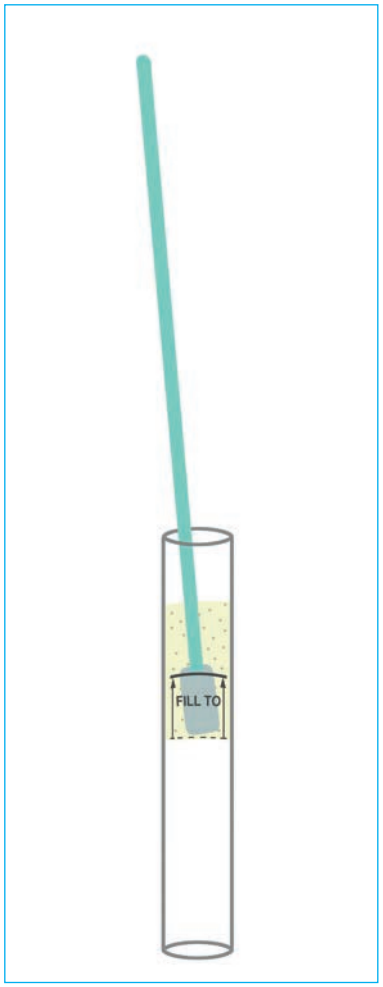
v. Hold the tube upright with one hand, and close the funnel lid with the other hand by firmly pushing the lid until you hear a loud click. The liquid in the lid will be released into the tube to mix with the sputum. Make sure that the lid is closed tightly.

vi. Hold the tube upright. Unscrew the funnel from the tube. Discard the funnel as clinical waste

vii. Use the small screw cap to close the tube tightly.

viii. Shake the capped tube for 5 seconds.

ix. Remove cap from Isohelix Swab while retaining the bung with stopper, and mix the swab in the sputum, rubbing gently for 10 seconds to get a good sputum sample on the swab. When extracting the swab from the specimen pot, remove any excess sputum residue hanging from the swab by wiping the swab gently against the inside edge of the pot.



It is important that the healthcare professional checks the quality of the sputum to ensure it is not simply saliva, but rather sputum (mixture of phlegm and mucous). If the patient is unable to provide any sputum, advise to keep hydrated where possible, and encourage deep breathing to try again in an hour.

17. Loading the swab into a DnaCartridge

- **Before using the DnaCartridge, check the "Use By Date." Do not use if this date has passed.**
- **Do not take the cartridge out of the packaging until you are ready to collect a sample.**
- **Do not touch the black part of the DnaCartridge (AU) when handling it. Touching the AU could lead to an invalid test.**

Loading a paediatric nasal swab

i. The healthcare professional should remove the cap from the DnaCartridge and insert the protruding end of the swab.

ii. Using disposable scissors cut the swab tail off (as shown on the dotted line here) - leave only the swab head in the swab chamber.

iii. Use the plastic stem to push the swab head as far into the swab chamber as possible in direction (1) and to the bottom of the swab chamber in direction (2).

iv. Discard the swab tail and scissors in a "sharps" bin.

v. Reseal the bung tightly.

vi. The DnaCartridge should be labelled with a barcode sticker containing the unique patient identifier (e.g., Medical Record Number). This barcode MUST NOT obscure the existing Cartridge barcode. Note: If such a barcode is not available, a suitable alternative method of sample labelling must be used.

17. Loading the swab into a DnaCartridge

vi. The healthcare professional must wipe down the DnaCartridge with a disinfectant wipe prior to inserting it into a specimen bag and ensuring the bag is properly sealed.

vii. The bagged DnaCartridge should go onto a clean tray, and be taken to the NudgeBox for processing.

If the test cannot be run immediately, the cartridge should be stored away from ambient light.

Please do not force any other types of swabs, as this will damage the DnaCartridge pressure during the test and may result in leakage and NudgeBox damage.

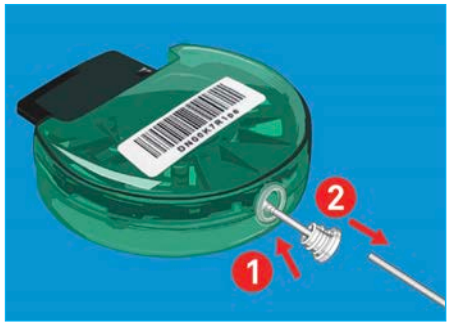
- **Before using the DnaCartridge, check the "Use by" date. Do not use if this date has passed.**
- **Do not take the cartridge out of the packaging until you are ready to collect a sample.**
- **Do not touch the black part of the DnaCartridge (AU) when handling it. Touching the AU could lead to an invalid test.**

Loading an Isohelix buccal swab

i. Push the stopper down on the swab to seal the swab head in the swab chamber (1).

ii. Pull the plastic stem of the swab, leaving the swabhead in the swab chamber (2).

iii. Discard the swab tail in a "sharps" bin.



iv. Replace the stopper with the original bung which came with the Cartridge.

v. The DnaCartridge should be labelled with a barcode sticker containing the unique patient identifier (MRN or NHS number). This barcode MUST NOT obscure the existing Cartridge barcode. Note: If such a barcode is not available, a suitable alternative method of sample labelling must be used.

17. Loading the swab into a DnaCartridge

Loading an Isohelix buccal swab (continued)

vi. The healthcare professional must wipe down the DnaCartridge with a disinfectant wipe prior to inserting it into a specimen bag and ensuring the bag is properly sealed.

vii. The bagged DnaCartridge should go onto a clean tray, and be taken to the NudgeBox for processing.

If the test cannot be run immediately, the cartrigde needs to be stored away from ambient light.

Please do not force any other types of swabs, as this will damage the DnaCartridge pressure during the test and may result in leakage and NudgeBox damage.

18. Registering a patient sample

i. The healthcare professional should enter the unique patient details into the CovidNudge Operator App on the iPad provided - either scanning a barcode (such as an MRN barcode where these are used), or manually entering the patient's details (e.g. MRN Number)

- Make sure the Patient Identifier is correctly filled in

ii Scan the DnaCartridge barcode with the Operator App.

iii. Click the "Submit" button at the bottom of the screen, and **"Successfully submitted"** should show on the screen of the Operator App.

IMPORTANT! PLEASE WAIT TO SEE THE "SUCCESSFULLY SUBMITTED" POP-UP, OTHERWISE THE TEST CANNOT BE STARTED.

18. Registering a patient sample

iv. Please verify that all of the details have been successfully uploaded by going to the **'Patients List'** from the Home Page of the Operator App.

You should see a new record with the Patient Reference, and the Cartridge ID with the time that the registration was done.

07:08 Tue 1 Sep

Export as CSV Patients list

Registration Date & Time	Cartridge ID	Patient Reference No	Box Name	Test progress	Sta
28/08/2020 07:57:56	DN10RLxeAw	12345	nudge_7A5749	100%	Comp
28/08/2020 07:51:59	DN109M479R	1234	nudge_7A5749	100%	Comp
28/08/2020 07:29:49	DN108GvZeY	12345	nudge_7A5749	100%	Comp
28/08/2020 07:18:37	DN10ykeLn6	nonexist1	nudge_7A5749	100%	Comp
28/08/2020 07:10:48	DN106oM99E	1234	nudge_7A5749	100%	Comp
28/08/2020 06:53:47	DN10NOkBvz	53hgh	nudge_7A5749	100%	Comp
28/08/2020 06:35:49	DN10PArwyE	111222333	nudge_7A5749	100%	Comp

19. Running the test

i. Press the button on the Capsule to switch it on. The capsule will flash **Red-Green-Blue**

ii. Press the button on the Capsule again to activate the scanner, and scan the barcode on the DnaCartridge.

When the barcode is scanned successfully, the Capsule will flash **Amber**.

iii. Slide open the NudgeBox and put the loaded DnaCartridge into its location inside the NudgeBox. It should sit onto the locator pins in the NudgeBox cartridge bay. Firmly press down the cartridge into the bay.

iv. Slide the lid of the NudgeBox forwards into the closed position. Check the right LED is **breathing green.**

19. Running the test

v. When the NudgeBox is ready to run a test, the left LED will show **steady green**, the right LED will show **breathing (pulsing) green**. Please do not start any tests if the NudgeBox is not showing these lights. If necessary, please check the Wi-Fi connection (see section 15)

vi. Insert the Capsule into its recess on the top of the NudgeBox (metal contacts side first) and firmly press down. The Capsule should **flash green.**

IMPORTANT! IF THE CAPSULE DOES NOT FLASH GREEN, TAKE IT OUT AND RECONNECT IT UNTIL YOU SEE THE GREEN FLASH.

vii. Press the **green flashing** button on the Capsule to start the test.
The Capsule should **flash yellow**.

The Capsule will show **purple** 3-5 minutes after the test has started.

viii. When the test is running the right LED of the NudgeBox will continue **flashing Green.**

ix. When the test is complete both LEDs will show **steady Green.**

x. When the test is finished, the used DnaCartridge should be removed from the NudgeBox. The NudgeBox needs to be turned off or rebooted before starting the next test.

The used DnaCartridge should be disposed in a Clinical Waste stream and incinerated.

If leaked fluid is visible after the Cartridge is removed, clean the NudgeBox cartridge bay with an approved cleaning wipe. Leaked fluid may contain harmful substances which can irritate the skin. The NudgeBox cannot be assumed to meet the requirements of the EN 61010 Safety standard if there are any traces of leaked fluid remaining.

If the Amplification Unit is visibly damaged or disconnected from the Sample Preparation Unit at the end of the test, additional cleaning precautions may be required. Please send a picture of the damage to **Covid@dnanudge.com**, then disconnect the NudgeBox from the mains supply.

20. Interpretation of results

The DnaNudge CovidNudge test evaluates for 6 different viral gene targets and one human RNA control gene:

Gene	# Replicates
N1 (CDC)	10
N2 (CDC)	10
N3 (CDC)	10
E (Charité Berlin)	10
RdRP-IP2 (Institut Pasteur)	9
RdRP-IP4 (Institut Pasteur)	9
RNaseP Control	6

Table 1

The results are interpreted automatically by the DnaNudge CovidNudge system and are accessed via the Operator App.

The DnaNudge CovidNudge test provides results based on the detection of the gene targets in Table 1 as given Table 2.

Result of test	Viral Gene	Human RNA
INVALID	- / +	-
POSITIVE	+ +	+
INDETERMINATE	+	+
NEGATIVE	-	+
ERROR	N/A	N/A

Table 2 - DnaNudge COVID-19 Possible Test Results

Table 3 provides details of how the test results are defined.

Result of test	Interpretation
INVALID	Less than 2 of the 6 human RNaseP replicates have amplified. Viral gene results are not applicable.
POSITIVE	At least 4 of the viral gene replicates have amplified. This could be 4 from the same gene type, or 4 from distinct genes. 2 or more human RNaseP replicates have amplified.
INDETERMINATE	2 or 3 of the viral gene replicates have amplified. 2 or more human RNaseP replicates have amplified.
NEGATIVE	Less than 2 of the viral gene replicates have amplified. 2 or more human RNaseP replicates have amplified.
ERROR	A technical issue was detected during the test. This is indicated by the right hand LED flashing red and the test is aborted.

Table 3

Reasons to repeat the test

If any of the test results indicated below occur, the test should be repeated:

- An **INVALID** result indicates that insufficient human RNAseP was detected, usually indicating insufficient swabbing. Take a new sample, paying extra attention to the swabbing procedure.
- An **ERROR** indicates a technical failure occurred during the test process. The patient should be retested using a new CovidNudge Cartridge.
- An **INDETERMINATE** result indicates that gene targets of the SARS-CoV-2 virus were identified at the limits of detection. The patient should be retested taking a new sample.

Retest Procedure

A re-test should use a new DnaCartridge and a new swab sample as if this is a new test, following instructions in Sections 16 – 19.

21. Quality control

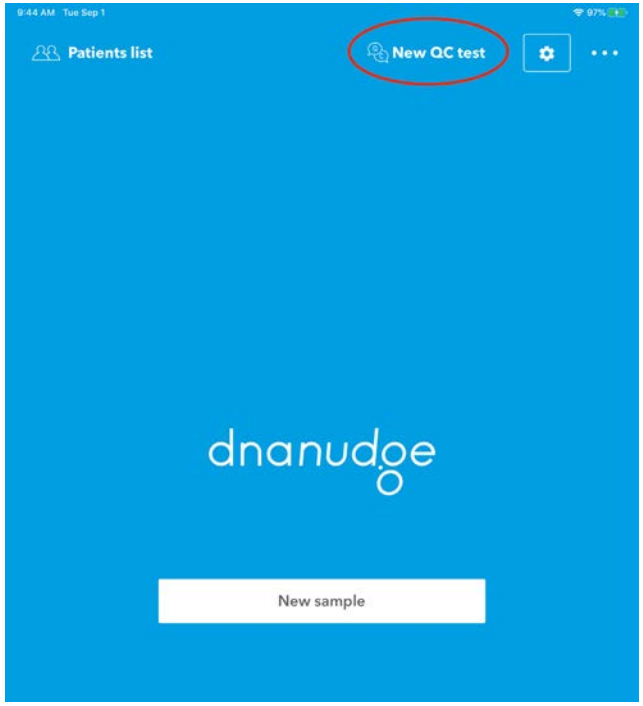
Samples for regular Quality Control should be registered using the 'New QC test' function in the Operator App as outlined below. If a QC sample is registered as if it were a patient sample, there must be sufficient RNaseP (human genetic control) present in the QC sample to avoid the result being reported as 'invalid'.

It is strongly recommended that positive and negative QC controls should be prepared following the methodology given in this section. Using prior (positive) patient samples stored in solution (such as viral transport medium) is not recommended, since the diluted nature of the sample combined with the small volume that can be absorbed by the swab may result in an insufficient input sample concentration.

Registering a QC sample

i. To register a QC sample, select the option "New QC test" in the top right-hand corner of the Covid-Nudge Operator App.

This will take the user to the registration page.



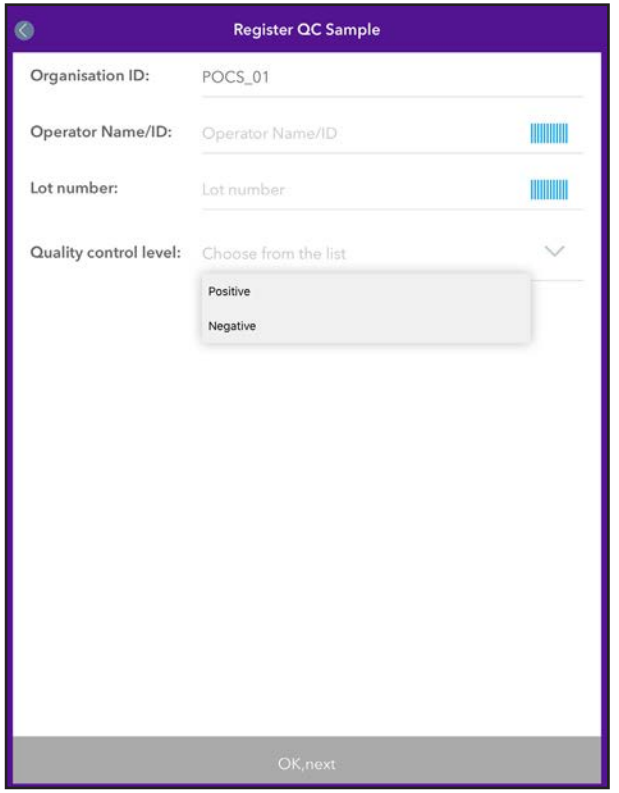
ii. Operator Name/ID
Enter the name or ID of the QC test user. (If DnaNudge is integrated with hospital point of care (POC) middleware, this user should already be registered in the QC)

iii. Lot number
This will be a QC lot number (If DnaNudge is integrated with POC middleware, this QC lot number will already be assigned).

iv Quality control level
Chose an option:

Positive – This will set quality control level for this sample to "positive"

Negative – This will set quality control level for this sample to "negative"



v. When all registration fields are completed, select **"OK,next"** to be taken to the cartridge barcode scan page.

21. Quality control

Completing the QC test

vi. Scan the DnaCartridge barcode with the Operator App.

vii. Run the test as normal, following the steps in **Section 19 "Running a Test"**

viii. The registered QC test progress/result is visible in the CovidNudge "Patients list" section, where the patient ID will be the QC lot number.

Date & Time	Cartridge ID	Patient Reference No	Box Name	Test progress	Status	Result
01/09/2020 14:12:45	DN10WXI29w	0006	nudge_7904A9	76%	Pending	-
01/09/2020 14:03:39	DN10bvnldj	0006	nudge_7904A9	100%	Complete	Negative

ix. Once completed, the QC result can be viewed in the Operator app. (If DnaNudge is integrated with POC middleware, the QC result (PASS/FAIL) will be visible in the relevant hospital QC monitor).

QC Sample Preparation

Material Required:

- External Positive Control: HE0062-DN SARS-CoV-2 Process Control (Pellet) from Microbiologics (CE-IVD) - Provided
- External Negative Control: HE0058-DN Process Control (Pellet) from Microbiologics (CE-IVD) - Provided

Method 1: (single test)

- Tear open pouch at notch. Remove vial from pouch and ensure that the pellet is at the bottom of the vial before opening.
- Tip pellet from vial directly into cartridge sample chamber, seal, and run a test following standard procedure.

Method 2: (multiple tests)

- Tear open pouch at notch. Remove vial from pouch and ensure that the pellet is at the bottom of the vial before opening.
- Add a volume of molecular water into the vial with the pellet according to the table below.
- Recap the vial and shake vigorously until the pellet is completely dissolved.
- Insert a swab into the vial and leave to soak for 10 seconds until the swab becomes fully saturated with liquid. Repeat with further swabs up to the maximum shown in Table below.
- Insert swab into the cartridge sample chamber, seal, and run a test following standard procedure.

Molecular water volume /uL	Max. number of swabs	Comments
500	**5**	
2000	**15-20**	RNaseP concentration will be low – run as a QC test to avoid an Invalid result

- Any remaining hydrated material may be stored at 4°C and used up to 5 days after hydration. Alternatively the liquid may be frozen as aliquots of 50 uL at -80°C for up to one month. Stored material should be shaken well before use.

22. Cleaning and Maintenance

IMPORTANT NOTES

- **Always wear a new pair of gloves before cleaning and disinfecting the Instrument.**
- **Always use DnaNudge approved materials to clean or disinfect the Instrument.**
- **Do NOT spray or pour solution directly onto the Instrument.**
- **The NudgeBox cannot be assumed to meet the requirements of the EN 61010 Safety standard if there are any traces of salt residue or leaked fluid remaining.**
- **Dispose of cleaning and disinfectant materials in accordance with local procedures.**

Daily cleaning procedure

- Always wear a new pair of gloves to clean the Instrument.
- Wipe the external surfaces of the Instrument and the cartridge bay with DnaNudge approved wipes.
- Allow the instrument to air dry before testing the next specimen.
- It is also important to clean the workspace at the same time following local procedure to avoid false results.

Daily cleaning procedure

- If leaked fluid is visible after the Cartridge is removed, clean the NudgeBox cartridge bay with DnaNudge approved wipes. Leaked fluid may contain harmful substances which can irritate the skin.
- If a build-up of guanidinium chloride salt residue is found inside the cartridge cavity, please remove using a dry paper towel to gather and remove the salt, then clean the cavity with DnaNudge approved wipes and allow to dry thoroughly.
- The NudgeBox cannot be assumed to meet the requirements of the EN 61010 Safety standard if there are any traces of salt residue or leaked fluid remaining.
- Dispose of cleaning and disinfectant materials in accordance with local procedures.
- The Instrument is now ready to perform another test.
Before performing a patient test, change gloves and wash hands.
- For technical assistance or questions and information about DnaNudge approved materials, please contact customer support.

DnaNudge approved cleaning materials

CLINELL® - Universal Range
CLINELL® - Alcohol Wipes Range
Lint-free tissue dipped in 70% IMS
(Industrial Methylated Spirit)

23. Troubleshooting

If the right hand LED of the Nudge-Box is **flashing Red** while the test is running, the test will not give results and it will be required to get another swab from the patient and to use a new DnaCartridge.

If the internet link is lost, the box will show **blue/purple** on the left LED. The box will try to reconnect itself. If the test is done (right LED is solid green), it is safe to turn off the box. The Nudge-Box will automatically resend the previous test results before allowing users to start any new test.

If test progress is not updating and the test has run over 2 hours, please reboot the NudgeBox, this is due to an Internet Issue. Once the connection is restored the results will show on the system.

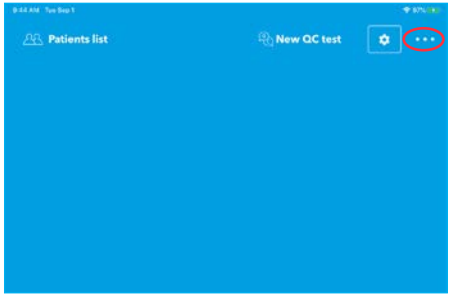
If any large debris falls into the top cover, please disconnect the NudgeBox from the mains supply and contact DnaNudge.

If a build-up of guanidinium chloride salt residue is found inside the cartridge cavity, please remove using a dry paper towel to gather and remove the salt, then clean the cavity with DnaNudge approved wipes and allow to dry thoroughly. Do not use any other cleaning agents without consulting DnaNudge. The NudgeBox cannot be assumed to meet the requirements of the EN 61010 Safety standard if there are any traces of salt residue or leaked fluid remaining.

If the NudgeBox will not open at the end of the test, turn the NudgeBox off and back on again. The NudgeBox should re-initialise (see Section 15) and then release the DnaCartridge. If the NudgeBox still will not open, please disconnect the NudgeBox from the mains supply and contact DnaNudge. Do not attempt to dismantle the NudgeBox.

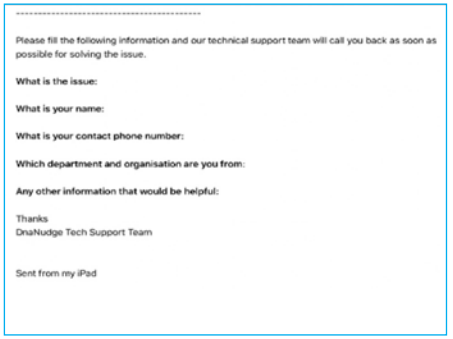
A Technical Support contact request form is available via the Operator App

i. On the main page of the Operator App, select **'...'**



ii. Select **'Email Support'**

iii. Fill out the details on the form and select **'Send'**


Please fill the following information and our technical support team will call you back as soon as possible for solving the issue.

What is the issue:

What is your name:

What is your contact phone number:

Which department and organisation are you from:

Any other information that would be helpful:

Thanks
DnaNudge Tech Support Team

Sent from my iPad

Do not use an internal phone extension as your contact number

Email for technical support: Covid@dnanudge.com

24. Technical self check

If any technical failure happens, the front LEDs on the NudgeBox may **flash red**, indicating test abortion.

In the primary steps of the test, during sample preparation, the NudgeBox checks the pressure profile of the DnaCartridge. If anything causes the DnaCartridge to not properly handle the sample preparation, to the extent detectable by the NudgeBox, the test is aborted. This helps to avoid any potential error from manufacturing or from incorrect DnaCartridge handling.

During the RT-PCR, if the NudgeBox cannot reach the temperature set points, it may abort the test. This avoids running an invalid test. It may indicate a technical failure in the NudgeBox, or an improper environment temperature, or an improper NudgeBox temperature after transmission (for example the NudgeBox might have been transferred in the cold and might have not yet settled at room temperature to operate).

25. Health check routine

In order to ensure a NudgeBox is healthy, routine maintenance health checks are required. The health check test takes about 10 minutes and uses a specific dry and reagent-free DnaCartridge modified for the purpose of instrument checking.

The NudgeBox will flash red green blue on both LEDs to indicate that a Health Check is required. A Health Check will be run:

- **after every 10 tests**
- **after two consecutive failed tests run (flashing red on a 'live' test)**

The box cannot process normal samples until a Health Check has passed.

Running a Health Check

i. Scan the Health Check cartridge with the Capsule.

ii. Slide open the NudgeBox and put the Health Check cartridge into its location inside the NudgeBox. It should sit onto the locator pins in the NudgeBox cartridge bay. Firmly press down the cartridge into the bay.

iii. Insert the Capsule into its recess on the top of the NudgeBox (metal contacts side first) and firmly press down. The Capsule should **flash green. Press the button to start the Health Check.**

iv. If the NudgeBox fails to reach set temperatures (the upper and lower temperatures per test specification) or fails to hold pressure, the right LED on the front of the NudgeBox **flashes red**, indicating a health check failure. When the NudgeBox passes a health check, the right LED on the front of the NudgeBox shows a **steady green.**

NOTE: In the event of a malfunction, please disconnect the NudgeBox from the mains supply and contact DnaNudge (see section 23). Please do not attempt to dismantle the NudgeBox or Power Supply Unit - there are no user-serviceable parts inside.

26. Health check reports

The CovidNudge operator app incorporates a feature allowing users to view and export Health check records and results from a NudgeBox. This feature is disabled by default on new accounts, so users should contact the DnaNudge support covid@dnanudge.com) to enable it.

Once this feature is enabled, from the next login, the user can see this additional option from the main screen menu.

Selecting the '**Health check reports'** option will open up the camera scanner to scan the QR Code on the NudgeBox.

The Health check report page will then be displayed with the complete health check history of the NudgeBox.

This list also contains a filtering option, whereby the health check history for a specific time period can be selected by choosing **"From"** and **"To"** dates:

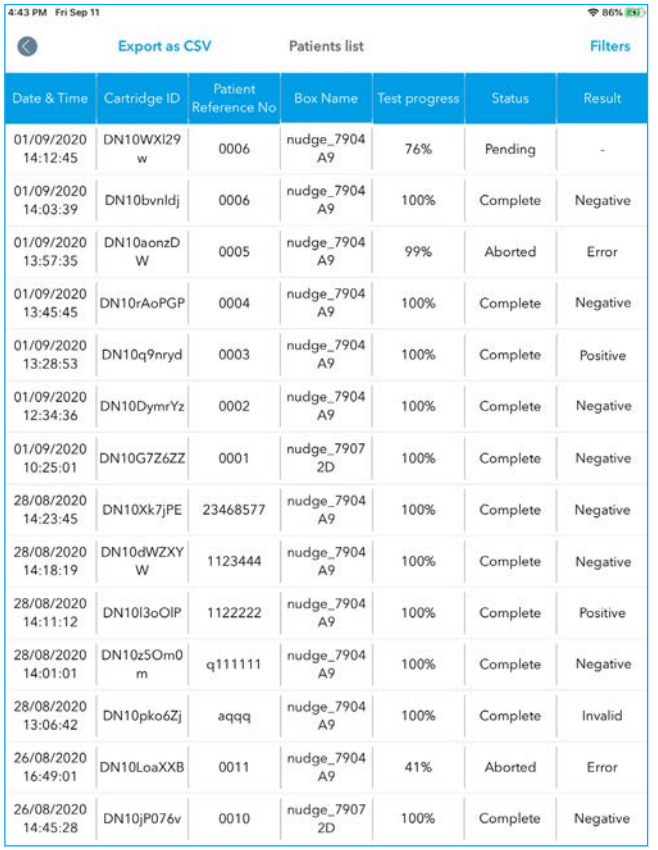
The resulting Health check record can be exported as a CSV file by selecting **"Export as CSV".**

27. Test progress and patient results

The **'Patients list'** page shows the progress and status of tests being run in your location.

Additionally, the DnaNudge Cloud using AWS can be configured to transfer patient results securely to authorised organisations.

- Integration with PointOfCare (POC) middleware solutions
- Bespoke hospital integrations (e.g. through TIE)
- Third-party notification service (e.g. Email, SMS)



Date & Time	Cartridge ID	Patient Reference No	Box Name	Test progress	Status	Result
01/09/2020 14:12:45	DN10WXl29w	0006	nudge_7904A9	76%	Pending	-
01/09/2020 14:03:39	DN10bvnldj	0006	nudge_7904A9	100%	Complete	Negative
01/09/2020 13:57:35	DN10aonzDW	0005	nudge_7904A9	99%	Aborted	Error
01/09/2020 13:45:45	DN10rAoPGP	0004	nudge_7904A9	100%	Complete	Negative
01/09/2020 13:28:53	DN10q9nryd	0003	nudge_7904A9	100%	Complete	Positive
01/09/2020 12:34:36	DN10DymrYz	0002	nudge_7904A9	100%	Complete	Negative
01/09/2020 10:25:01	DN10G7Z6ZZ	0001	nudge_79072D	100%	Complete	Negative
28/08/2020 14:23:45	DN10Xk7jPE	23468577	nudge_7904A9	100%	Complete	Negative
28/08/2020 14:18:19	DN10dWZXYW	1123444	nudge_7904A9	100%	Complete	Negative
28/08/2020 14:11:12	DN10l3oOlP	1122222	nudge_7904A9	100%	Complete	Positive
28/08/2020 14:01:01	DN10z5Om0m	q111111	nudge_7904A9	100%	Complete	Negative
28/08/2020 13:06:42	DN10pko6Zj	aqqq	nudge_7904A9	100%	Complete	Invalid
26/08/2020 16:49:01	DN10LoaXXB	0011	nudge_7904A9	41%	Aborted	Error
26/08/2020 14:45:28	DN10jP076v	0010	nudge_79072D	100%	Complete	Negative

28. Performance characteristics

Clinical Evaluation

Clinical validation took place at three hospital sites in the United Kingdom between April 2020 and December 2021. Paired samples were tested in parallel using CovidNudge and NHS laboratory platforms, with results from CovidNudge testing reported before laboratory results were available. Smaller calibre (paediatric) swabs were used to insert into the DnaNudge cartridge, most commonly a flexible minitip FLOQswabTM (COPAN Diagnostics Inc., Italy), whilst a second parallel nasopharyngeal swab was collected using standard swabs and placed in viral transport medium for processing in a central laboratory as per local protocols.

A total of 934 samples were tested and achieved a sensitivity of 96.9% and specificity of 99.9% (see Tables 4 and 5).

Table 4 - Paired Results from Clinical Evaluation

Nasopharyngeal Samples		Covid Nudge	
		Positive	Negative
Comparator	Positive	123	4
	Negative	1	764

Table 5: Clinical Performance

	Value	95% CI*
Sensitivity	96.85%	92.13% - 99.14%
Specificity	99.87%	99.27% - 100.00%
Positive Likelihood Ratio	740.91	104.47 - 5254.51
Negative Likelihood Ratio	0.03	0.01 - 0.08

*Confidence intervals are "exact" Clopper-Pearson confidence intervals calculated using Medcalc® Statistical Software

Combined Nose and Throat

To validate the use of combined nose and throat samples samples, a clinical validation took place across two hospital sites in London in April and May 2020. Paired nose and throat samples were collected and tested on Covid-Nudge and comparator NHS lab-PCR platforms following the procedures described in Sections 16-17.

For paired testing of 71 positive and 315 negative patients, DnaNudge demonstrated 94.4% sensitivity (95% CI = 86.2 – 98.4%) and 100% specificity (95% CI = 98.8 - 100%) against the comparator lab PCR platform.

Sputum samples

To validate the use of sputum samples, a clinical validation took place across three hospital sites in London in September and October 2020.

Paired nasopharyngeal and sputum samples were collected and tested following the procedures described in Sections 16-17.

For paired testing of 71 positive and 203 negative patients, sputum samples demonstrated 98.6% sensitivity (95% CI = 92.4 - 99.96%) and 100% specificity (95% CI = 96.9 - 100%) against nasopharyngeal samples.

28. Performance characteristics

Limitations

- Performance of the DnaNudge CovidNudge test has only been established in nasopharyngeal samples taken using a paediatric nasal swab and sputum samples using an Isohelix buccal swab. Use of the DnaNudge CovidNudge test with any other specimen and swab types has not been assessed and performance characteristics are unknown.
- A false negative result may occur if a swab sample is improperly collected or handled. False negative results may also occur if only very low levels of the virus are present.
- As with any molecular test, mutations within the target genes of SARS-CoV-2 could affect primer and/or probe binding resulting in a failure to detect the presence of the virus. However the use of multiple gene targets in the DnaNudge CovidNudge test make this less likely than in some other tests which only detect one or two gene targets.
- The DnaNudge CovidNudge test cannot rule out infection with other bacteria or viral pathogens.
- The DnaNudge CovidNudge test may produce an Invalid result. In this event, take a new sample, paying extra attention to the swabbing procedure (see Section 20 - Interpretation of results).

Batch to Batch Precision

Sample Type	Test Results (# of Positives/#of replicates)		
	Cartridge Lot 1	Cartridge Lot 2	Cartridge Lot 3
LP	3/3	3/3	3/3
MP	3/3	3/3	3/3
TN	3/3	3/3	3/3
TNC	3/3	3/3	3/3

The detection of all LP (9/9) and MP (9/9) were detected with 100% success. All TN and TNC were also identified correctly. The agreement of the observed results with actual results were 100% matched. No variabilities were observed between reagents lots. Hence the study suggests batch to batch variabilities are minimal and robust across different manufacturing batches.

29. Analytical performance

i. Analytical Sensitivity (Limit of Detection)

Limit of Detection was assessed using CE-marked SARS-Cov-2 process control pellet (HE0062S, Microbiologics Inc.). The pellet was dissolved in 100 uL molecular water and serially diluted, with 50uL aliquots being absorbed onto a swab and inserted into the Cartridge for standard processing. Limit of detection is 1k viral copies/ml.

Method to verify LOD

Material Required:

- HE0062-DN - SARS-CoV-2 Process Control (Pellet) Microbiologics Inc. – CE (IVD) Certified
- RNase/DNAse Free water
- Pipettes, tips and tubes
- Paediatric nasal swab (as provided in Cartridge sample kit)

i. Dissolve 1 pellet (DN-02) in 2ml of water. This gives the stock concentration as 50 copies/uL*. (At this stage can be aliquoted and frozen)

ii. Take 1 aliquot of the 50 copies/uL

iii. Dilute at 1:50 to get 1 copies/uL. Take 50 uL of the above dissolved pellet aliquot and 2450 uL of water in a tube. Mix well.

iv. Take 200 uL of the above tube and pipette to swab chamber directly. Close the lid securely.

v. Run the test on the NudgeBox following the standard process.

*Concentration based on nominal value of 10^5 copies per pellet. Since lot-to-lot variation is possible, please contact DnaNudge for exact concentration of selected lot.

29. Analytical performance

ii. Analytical Reactivity (Inclusivity)

In-silico inclusivity analysis is carried out at regular intervals to assess the ongoing performance of the DnaNudge CovidNudge test as new variants of the SARS-CoV-2 virus emerge. Public databases including COG-UK are downloaded at regular intervals to investigate and review the impact of newly identified variants. Any identified variants that may have an impact on the detection ability of the CovidNudge test are reported to customers and the appropriate regulatory agencies. The CovidNudge assay has primers/probes targeted at five different gene targets of the SARS-CoV-2 virus (n1, n2, n3, e, IP2, IP4). Thus, the assay's performance is robust in the presence of a mutation of one or more viral genetic regions. Analysis to date has concluded that our assay's performance is not negatively affected by currently identified variants of the SARS-CoV-2 virus, as none of the variants showed significant mutations in the genetic regions of the virus targeted by the DnaNudge CovidNudge assay.

iii. Analytical Specificity (Exclusivity)

Exclusivity of the assays with respect to the Coronaviridae family was evaluated in silico by mapping the primer and probe sequences to homologous sequences downloaded from the NCBI database. The Charité Berlin E-gene assay is predicted to detect human SARS-CoV-1 and bat SARS-like coronaviruses of the subgenus Sarbecovirus. No cross-reactivity with human coronaviruses OC43, HKU1, NL63, 229E or MERS-CoV was detected for any assays. NCBI primer-BLAST tool was used to assess potential cross-reactivity with other respiratory pathogens, possible contaminating organisms and human DNA. No unintended cross reactivity was detected for any organisms listed in Table 6.

Table 6 - Assay panel exclusivity

Organism	
Adenovirus A/B/C/D/E	*Corynebacterium diphtheriae*
CMV	*Coxiella burnetii*
EBV	*Haemophilus influenzae*
Enterovirus A/B/C	*Legionella*
Human metapneumovirus	*Leptospira*
HSV1/2	*Moraxella catarrhalis*
Influenza A/B/C	*Mycobacterium tuberculosis*
Parainfluenza virus 1-4	*Mycoplasma pneumoniae*
Parechovirus	*Neisseria elongate*
Respiratory syncytial virus	*Neisseria meningitidis*
Rhinovirus A/B	*Pneumocystis jirovecii*
Bacillus anthracis	*Pseudomonas aeruginosa*
Bordetella parapertussis	*Staphylococcus aureus*
Bordetella pertussis	*Staphylococcus epidermidis*
Candida albicans	*Staphylococcus salivarius*
Chlamydia pneumoniae	*Streptococcus pneumoniae*
Chlamydia psittaci	*Streptococcus pyogenes*

Full details of the in-silico analysis can be obtained by contacting DnaNudge: **covid@dnanudge.com**

29. Analytical performance

iv. Interfering Substances

Potentially interfering substances that may be found in the upper respiratory tract were tested at their highest clinically relevant concentrations to determine the effect on the performance of the assay. Substances tested were chemical substances that can be either naturally present, or that can be artificially introduced into the mouth or nose. Samples were spiked onto nasal swabs, either positive for Covid-19 at 3X the Limit of Detection (LOD), or else negative for Covid-19 (NTC). All tests were repeated in triplicate.

Interference	Active Ingredients	Final Concentration	On Nasal Swab 3x LOD (150 copies)	On Nasal Swab NTC
Listerine mouthwash (no alcohol)	Eucalyptol, Thymol, Methyl Salicilate	90%	Positive	Negative
Amoxicillin Powder	Amoxycillin	100 mg/ml	Positive	Negative
Blood	N/A	20%	Positive	Negative
Nasal spray / cough syrup	Phenilephrine	10 mg/ml	Positive	Negative
Mometasone	Mometasone	0.04 mg/ml	Positive	Negative
Cold and flu relief/cough syrup	Guaifenesin	13.3mg/ml	Positive	Negative
Asthma inhaler	Beclometasone	0.068 mg/ml	Positive	Negative
Nasal spray	Triamcinolone	0.04 mg/ml	Positive	Negative
Nasal spray	Fluticasone	0.04 mg/ml	Positive	Negative
Ethanol	Ethanol	40%	Positive	Negative
Nasal spray	Sodium Chloride	4%	Positive	Negative
Physiologic saline	Sodium Chloride	0.9%	Positive	Negative
Antibiotic	Levofloxacin	5 mg/ml	Positive	Negative
Nicotine spray	Nicotine	90%	Positive	Negative
Steroid inhaler	Budesonide	0.05 mg/ml	Positive	Negative
Nasal spray	Flunisonide	0.04 mg/ml	Positive	Negative
Mucin	N/A	3 mg/ml	Positive	Negative
Fairy dish-wash liquid		V/V 5%	Positive	Negative
Glass cleaner		V/V 5%	Positive	Negative
Toilet cleaner		V/V 5%	Positive	Negative
Sunscreen		V/V 5%	Positive	Negative
J&J lotion		V/V 5%	Positive	Negative
Hand soap		V/V 5%	Positive	Negative
Body butter		V/V 5%	Positive	Negative
Toothpaste		V/V 5%	Positive	Negative

30. Technical specifications

NudgeBox Analyser

Dimensions:
NudgeBox: 280 (L) x 155 (w) x 145 (h) mm (L increases to 385mm when open)

Power Supply Unit:
- XP Power model: 209 (L) x 82 (w) x 43 (h) mm
- EDAC model: 182 (L) x 84.5 (w) x 46 (h) mm

Mass:
NudgeBox: 5kg

Power Supply Unit:
- XP Power model: 0.91kg
- EDAC model: 1.05kg

NudgeBox Analyser Operating temperature range: 15 – 40 °C

NudgeBox Analyser Storage temperature range: -10 – 50 °C

Operating humidity range:
10% to 85% RH (non-condensing) with air pressure 1 ± 0.1 bar (gauge)

Storage humidity range:
10% to 85% RH (non-condensing) with air pressure 1 ± 0.1 bar (gauge)

Maximum altitude of operation: 5000m

Indoor/outdoor:
The NudgeBox and Power Supply Unit are intended for indoor operation only.

Pollution degree: 2

Maximum sound level: 40dBA at 40cm distance

Maximum power consumption: 130W

Power Supply Unit: Models:
- XP Power ALM200PS24 (24V @ 8.4A)
- EDAC EA12501E-2400 (24V @ 8.33A)

Required mains power supply: 100 – 240 V~, 50/60 Hz
Overvoltage CAT specification of NudgeBox: CAT 2

Lifespan of NudgeBox Analyser: 3 years

DnaCartridge

Shelf-life: 9 months from date of manufacture
Storage temperature range: 18 – 25 °C
Storage humidity when mylar bag is open: Use within 12 hours:40 to 50% RH

EU Declaration of Conformity

DnaNudge Limited hereby declares that this wireless device is in compliance with the Directives 2014/53/EU and 98/79/EC.

A copy of the EU Declaration of Conformity is available at **dnanudge.com/covid-test-access**

The Bluetooth and WiFi operating frequency ranges, and corresponding radiated powers, of the NudgeBox are given in the table here.

Type of Wireless	Frequency Band	Maximum radiated power
Bluetooth	2402-2480 MHz	7.0 dBm EIRP
WLAN	2412-2472 MHz	15.0 dBm EIRP
	5180-5825 MHz	15.0 dBm EIRP

FCC Compliance Statement

This device contains:
FCC ID: PVH0965 IC: 5325A-0965

This device complies with Part 15 of the FCC Rules.

Operation is subject to the following two conditions:
(1) This device may not cause harmful interference, and
(2) This device must accept any interference received, including interference that may cause undesired operation.

Caution: Any changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

30. Technical specifications

Disposal of Electronic Devices:

The NudgeBox is an electronic device and carries the symbol shown here. Devices should be disposed of in accordance with the local electronic waste procedures. In compliance with the EU WEEE directive 2012-19-EU, the symbol shown here on the product and/or accompanying documents means that used electrical and electronic products should not be mixed with general waste. If you wish to discard electrical and electronic equipment and do not have an authorised process, please contact DnaNudge for further information.

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Pantonic Pty Ltd
36 Main Street,
Mornington, Victoria,
3931 Australia

31. Table of symbols & References

The table below should be consulted whenever a warning symbol is encountered on the NudgeBox or Cartridge.

***In Vitro* medical device**

Do not re-use

Manufacturer

Temperature Limitation

Caution

Relative Humidity Limitation

Warning: Biological Risk

Caution: Hot surface

Warning: Flammable material

Control

The latest version of this manual can be downloaded from:
dnanudge.com/covid-test-access

For further technical support please email:
covid@dnanudge.com

CovidNudge®

DnaCartridge® & NudgeBox®

Instructions For Use

Rev 11 - October 2022

NudgeBox Analyser – BX-0004
NudgeBox – 9101004
CovidNudge DnaCartridge – CT-0001

DN-ENG-IFU-001 Rev 11 - 9501002 - 09-2022

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