Covid/RSV/Flu Nudge Test

DnaCartridge™ & NudgeBox™

Instructions For Use

Rev 0 - November 2021

dnanudge

CE IVD

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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 Covid/RSV/FluNudge test is a lab-free sample-to-answer RT-PCR test intended for the simultaneous qualitative detection and differentiation of nucleic acid from the SARS-CoV-2, respiratory syncytial virus (RSV), influenza A and influenza B viruses, providing results on the spot, at the point of need. It is designed to be operated by a trained professional and comprises a portable machine (the NudgeBox) and a disposable cartridge (the RVP1 DnaCartridge). The lab-free and user-friendly nature of the product makes it easy to use by following simple instructions. The sample type needs to be nasopharyngeal swab, nasal swab or combined nose and throat swab (see section 16 for instructions on sampling). It is advised that the test is done only by trained nurses/ operators and the sample is collected by a trained nurse or trained healthcare professional. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, RSV and influenza can be similar.

The NudgeBox is classified as IVD medical equipment.
Due to the lab-free nature of the system, Covid/RSV/FluNudge tests are suitable for use by healthcare 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

1. Intended use

The Covid/RSV/FluNudge test is a molecular in-vitro diagnostic test that aids in the detection and diagnosis of RSV, influenza, and COVID-19. The SARS-CoV-2, influenza A, influenza B and RSV viruses are generally detectable in upper respiratory specimens during the acute phase of infection. The RVP1 DnaCartridge contains 4 types of assay for SARS-CoV-2 virus detection, assays for RSV A and RSV B, assays for influenza A and influenza B, 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 for COVID-19 indicates active infection with SARS-CoV-2; a positive result for RSV indicates an active infection with the RSV virus, while a positive result for influenza A and/or influenza B indicates active infection with the influenza A and/or B virus. Positive results do not rule out bacterial infection or co-infection with other viruses.

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

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.

Respiratory Syncytial Virus (RSV), a member of the Pneumoviridae family (formerly Paramyxoviridae), consists of two subgroups: A and B. It is the cause of a contagious disease that affects primarily infants, and the elderly who are immunocompromised (e.g. patients with chronic lung disease or undergoing treatment for conditions that reduce the strength of their immune system).

The virus can cause both upper respiratory infections, such as colds, and lower respiratory infections manifesting as bronchiolitis and pneumonia. Because only weak immunity develops, both children and adults can be re-infected. Symptoms appear four to six days after infection and are usually self-limiting, lasting approximately one to two weeks in infants. In adults, infection lasts about 5 days and presents as symptoms consistent with a cold, such as rhinorrhea, fatigue, headache, and fever.

2. Summary & explanation

Influenza viruses are classified into types A, B, and C, the first two causing the majority of human infections. Influenza A (Flu A) is the most common type of influenza virus in humans and is generally responsible for seasonal flu epidemics and potentially pandemics. Flu A viruses can also infect animals such as birds, pigs, and horses. Infections with influenza B (Flu B) virus are generally restricted to humans and less frequently cause epidemics.

The Covid/RSV/FluNudge test is a molecular in vitro diagnostic test that aids in the detection and differentiation of SARS-CoV-2, RSV, Flu A and Flu B, 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, RSV, Flu A and Flu B RNA in upper respiratory specimens.

Each Cartridge tests for 12 Assays

Assay	#Replicates
Covid - N1	6
Covid - N2	6
Covid - E	6
Covid - IP2	6
RSV - M-gene	6
RSV - N-gene	6
Flu A - M-gene (assay 1)	6
Flu A - M-gene (assay 2)	6
Flu B - HA-gene	6
Flu B - NS1-gene (assay 1)	6
Flu B - NS1-gene (assay 2)	6
RNaseP Control	6

Sampling

Sample

Naospharyngeal, Nasal, or combined nose and throat

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		95	3 seconds
Annealing/Extension	40	60	30 seconds

3. Test specification

Test outcome

SARS-CoV-2 POSITIVE	3 or more COVID gene replicates amplify in any of the assays
SARS-CoV-2 INDETERMINATE	2 COVID gene replicates amplify in any of the assays
RSV POSITIVE	3 or more RSV gene replicates amplify in either of the assays
RSV INDETERMINATE	2 RSV gene replicates amplify in either of the assays
FLU A POSITIVE	3 or more Flu A gene replicates amplify in either of the assays
FLU A INDETERMINATE	2 Flu A gene replicates amplify in either of the assays
FLU B POSITIVE	3 or more Flu B gene replicates amplify in any of the assays
FLU B INDETERMINATE	2 Flu B gene replicates amplify in any of the assays
SARS-CoV-2 NEGATIVE; RSV NEGATIVE; FLU A NEGATIVE; FLU B NEGATIVE	Less than 2 COVID gene replicates amplify; less than 2 RSV gene replicates amplify; less than 2 Flu A replicates amplify; less than 2 Flu B replicates amplify
INVALID	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

Sensitivity and Specificity

Trials comparing DnaNudge Covid/RSV/Flu Nudge against laboratory RT-PCR results indicated 97.1% sensitivity and 100% specificity for SARS-CoV-2; 96.4% sensitivity and 100% specificity for RSV; 95.7 % sensitivity and 100 % specificity for Flu A, and 95.6 % sensitivity and 100 % specificity for Flu B. 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 Covid/RSV/Flu Nudge test is an automated in vitro diagnostic test for qualitative detection and differentiation of nucleic acid from SARS-CoV-2, RSV, Flu A and Flu B viruses. The Covid/RSV/FluNudge test is performed using a DnaCartridge (RVP1) 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 Covid/RSV/Flu Nudge test includes reagents for the detection of RNA from SARS-CoV-2, RSV, Flu A and Flu B 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 Covid/RSV/Flu Nudge 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 is 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 platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

5. Covid/RSV/FluNudge test flow

Step 1.
Take a sample
(following instructions in section 16)

Step 2.
The swab is inserted into the DnaCartridge - programmed for Covid-19,RSV and Flu

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 results 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 or nose/throat 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 room temperature, 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 (25°C or less)
- DnaCartridges need to be disposed of based on bio-waste management, using yellow plastic bags

7. Reagents & instruments

Materials provided

Each DnaNudge CovidNudge test kit contains equipment necessary to perform a single test.

Each kit contains:

1 x RVP1 DnaNudge Covid/RSV/Flu Cartridge (Product Code CT-1004)

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

1 x nasal sample kit

- paediatric nasal swab
- disposable scissors

Note: Safety data sheets and training materials are available at: www.dnanudge.com/covid-test-access

Materials required but not provided

DnaNudge NudgeBox hardware v 3.3 or higher

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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 by the NudgeBox during the test for checking the sealing quality of the swab chamber, lysis chamber, AU-SPU connection and AU. 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.
- Positive results for Covid-19 are indicative of SARS-CoV-2-RNA; positive results for RSV are indicative of respiratory syncytial viral RNA; positive results for flu A are indicative of influenza A RNA; positive results for flu B are indicative of influenza B RNA.
- All results positive for COVID-19 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.

Specimens

- Specimens 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 at room temperature (15–30 °C) for up to 8 hours.

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 non-determinate 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.

9. Warnings & precautions

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.
- Do not expose the NudgeBox to rain, moisture, dripping water or splashing water.
- 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, high humidity and excessive vibration.

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 UK-style 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 Nudge Box 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 multiplex 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 an aluminium foil. They should be stored in a cool/room temperature (25°C or less) and dry environment.

Amplification Unit (AU)

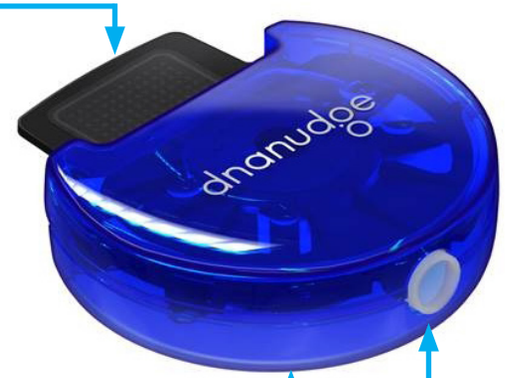
Dried primers and probes for:

SARS-COV-2	- N1 - N3 - E - IP2
RSV	- A - B
FLU	- A - B
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. Do not expose the AU to ambient light if the test cannot start immediately.



Swab Port

Cartridge type: RVP1 (Covid/RSV/FluNudge)
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 pneumatic, thermal, imaging, 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.

The NudgeBox operates at room temperature using the supplied Power Supply Unit.

Nudge Box

WiFi
Bluetooth

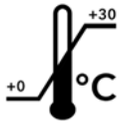
Power Supply Unit

Capsule

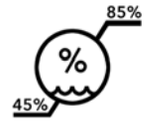
Single-button scanning with LED and miniaturized camera.

13. NudgeBox system transportation

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



Storage and transport temperature



Storage and transport humidity

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.
- Ensure that the room location is no more than 25°C nor will rise in temperature drastically during the day. If the temperature is greater, it may cause the test to take significantly longer.
- 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.

14. NudgeBox installation

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. A maximum of 6 NudgeBoxes should be operated from a single mains power socket.
- 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).

i. Start-up & Calibration

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**

15. Setting up the NudgeBox

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

When the calibration 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 24 for full instructions)

iii. Connect the iPad to the Nudge Box

(When setting up for the first time)

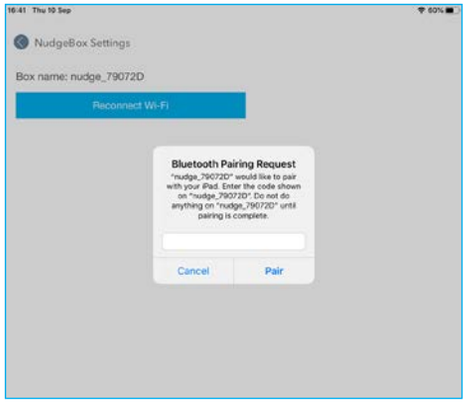
In the Operator App select the Settings icon in the top right corner and choose 'QR Code'.

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.

iv. Connecting the iPad to the Nudge Box (cont..)

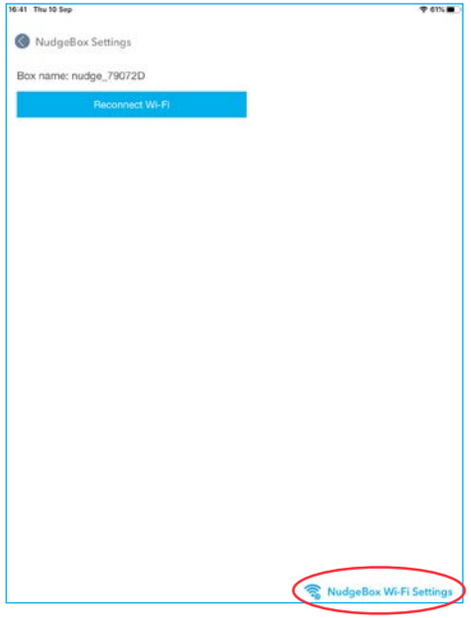
Enter the Bluetooth pairing passcode when prompted to connect the iPad with the NudgeBox.



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 recomended.

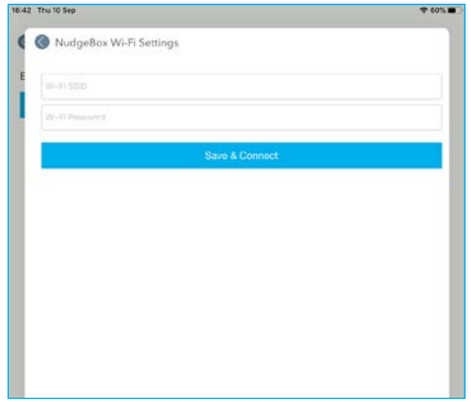
If selecting another WiFi network is required, first select **'NudgeBox Wi-Fi Settings'** in the lower right corner of this screen.



15. Setting up the NudgeBox

v. Changing the Wi-Fi settings

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



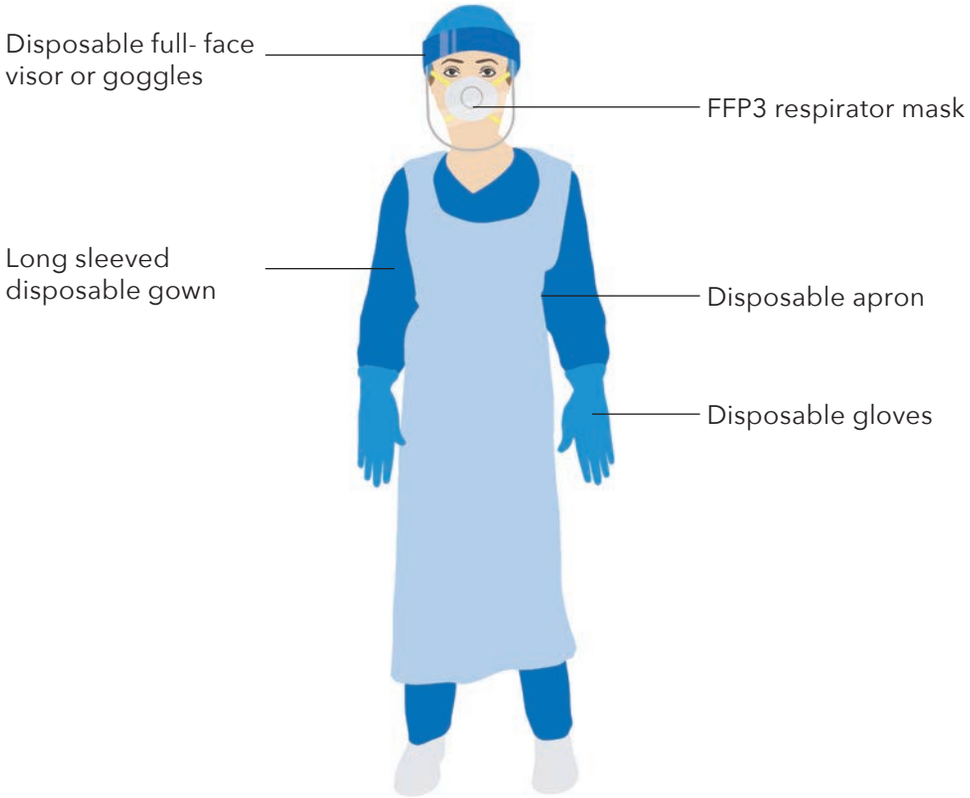
When the NudgeBox is ready to run a test, the left LED will show **steady Green**, the right LED will show **breathing (pulsing) Green**.

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.



16. Taking a sample

Taking a Nasopharyngeal 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).

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.

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).

Equipment required:

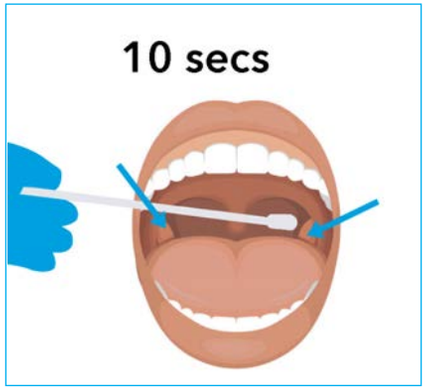
- DnaNudge Nasal Kit

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.



16. Taking a sample

Taking a Nasal 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).

Equipment required:

- DnaNudge Nasal Kit

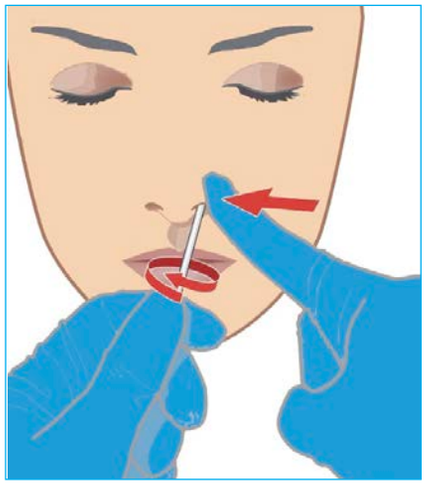
Procedure:

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

ii. Insert the nasal swab 1 to 1.5 cm into a nostril. Rotate the swab against the inside of the nostril for 3 seconds while applying pressure with a finger to the outside of the nostril.

iii. Repeat on the other nostril with the same swab, using external pressure on the outside of the other nostril.

To avoid specimen contamination, do not touch the swab tip to anything other than the inside of the nostril.



- **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, leaving the bud/swab tip in the DnaCartridge (as shown on the dotted line here)

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

iv. Lock the DnaCartrige closed with the cap provided.

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 a swab into the DnaCartridge

Loading a paediatric nasal 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 immediate processing.

If the test cannot be run immediately, the cartridge 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.

i. The healthcare professional should enter the unique patient details into the Operator App - 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 page 24)

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.

19. Running the test

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

If leaked fluid is visible after the Cartridge is removed, please refer to the Disinfection procedure in "Cleaning and Maintenance" - Section 22.

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 Covid/RSV/FluNudge test evaluates for 1 different viral gene targets and one human RNA control gene:

Gene	# Replicates		
Covid-N1	6	RSV (2 targets)	12
Covid-N2	6	Flu-A (2 targets)	12
Covid-E	6	Flu-B (3 targets)	18
Covid-IP2	6	RNaesP Control	6

Table 1

The results are interpreted automatically by the DnaNudge Covid/RSV/Flu Nudge system and are accessed via the Operator App.

The DnaNudge Covid/RSV/FluNudge test provides results based on the detection of the gene targets in **Table 1** as given in **Table 2.**

Result of the test	SARS-CoV-2 gene targets	RSV gene targets	Flu A gene targets	Flu B gene targets	Human control
COVID POSITIVE	++	N/A	N/A	N/A	+
COVID INDETERMINATE	+	N/A	N/A	N/A	+
RSV POSITIVE	N/A	++	N/A	N/A	+
RSV INDETERMINATE	N/A	+	N/A	N/A	+
FLU A POSITIVE	N/A	N/A	++	N/A	+
FLU A INDETERMINATE	N/A	N/A	+	N/A	+
FLU B POSITIVE	N/A	N/A	N/A	++	+
FLU B INDETERMINATE	N/A	N/A	N/A	+	+
COVID NEGATIVE	-	N/A	N/A	N/A	+
RSV NEGATIVE	N/A	-	N/A	N/A	+
FLU A NEGATIVE	N/A	N/A	-	N/A	+
FLU B NEGATIVE	N/A	N/A	N/A	-	+
INVALID	N/A	N/A	N/A	N/A	-
ERROR	N/A	N/A	N/A	N/A	N/A

Table 2 - DnaNudge Covid/RSV/FluNudge Possible Test Results

20. Interpretation of results

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

Result of the test	Interpretation
SARS-CoV-2 POSITIVE	3 or more COVID gene replicates amplify in any of the assays
SARS-CoV-2 INDETERMINATE	2 COVID gene replicates amplify in any of the assays
RSV POSITIVE	3 or more RSV gene replicates amplify in either of the assays
RSV INDETERMINATE	2 RSV gene replicates amplify in either of the assays
FLU A POSITIVE	3 or more FLU A replicates amplify in either of the assays
FLU A INDETERMINATE	2 FLU A replicates amplify in either of the assays
FLU B POSITIVE	3 or more FLU B replicates amplify in any of the assays
FLU B INDETERMINATE	2 FLU B replicates amplify in any of the assays
SARS-CoV-2 NEGATIVE; RSV NEGATIVE; FLU A NEGATIVE; FLU B NEGATIVE	Less than 2 COVID gene replicates amplify; less than 2 RSV gene replicates amplify; less than 2 Flu A replicates amplify; less than 2 Flu B replicates amplify
INVALID	Less than 2 of the control replicates amplify
ERROR	In the event of any technical error during the sample preparation phase of the test, the Nudge-Box will indicate with flashing red LEDs

Table 3

21. Quality control

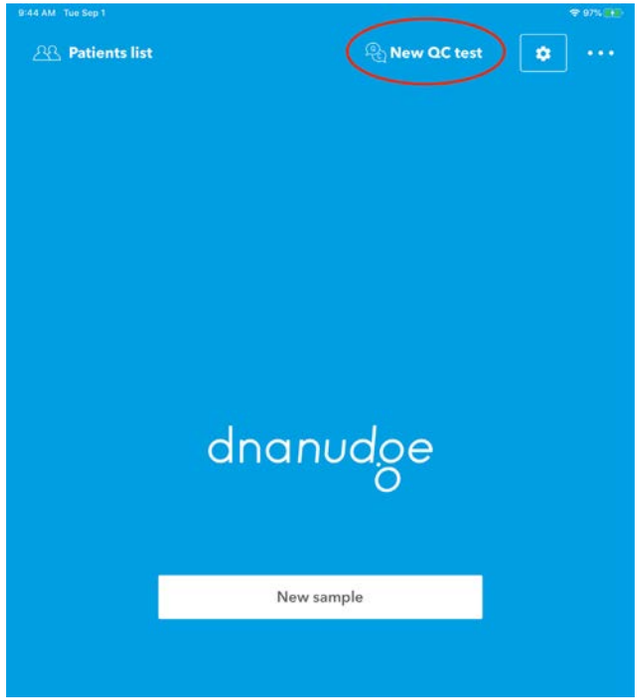
Samples for regular Quality Control should be registered using the 'New QC test' function in the Operator App as outlined below. If a QC sample is registered as if it were a patient sample, there must be sufficient RNaseP (human genetic control) present in the QC sample to avoid the result being reported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

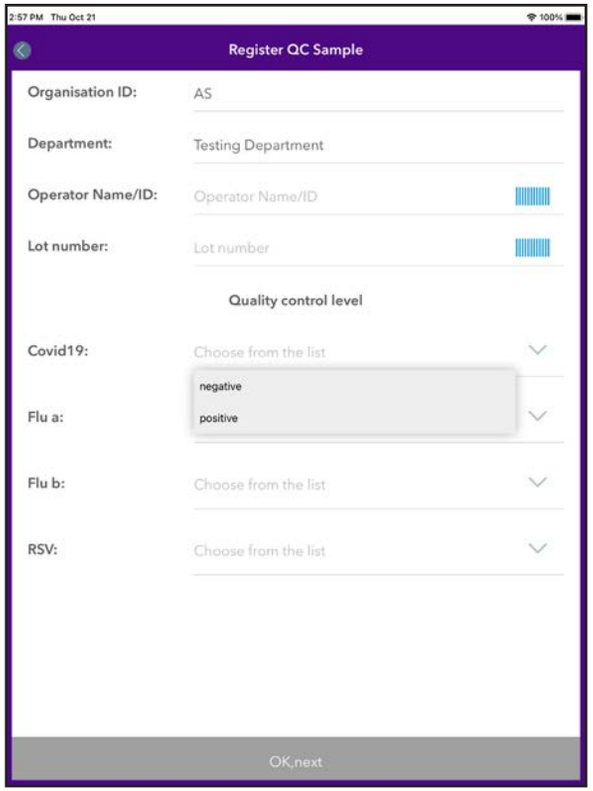
Registering a QC sample

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. Scan the DnaCartridge barcode with the Operator App.



iii. 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)

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

v. Quality control level
The quality control levels for Covid, RSV, Flu A and Flu B can be independently set to Positive or Negative. If the control sample is testing for a single virus only, the level for the other viruses should be set to negative.

vi. 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

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.

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:

- **Positive Control Covid:**
  HE0062S SARS-CoV-2 Process Control (Pellet) from Microbiologics (CE-IVD)
- **Positive Control RSV:**
  Zeptometrix RSV Type A Stock (Quantitative) (1 mL), Catalog# NATRSVA-STQ **or** RSV Type B Stock (Quantitative) (1 mL), Catalog# NATRSVB-STQ
- **Positive Control Flu A:**
  Influenza A H1 Stock (Quantitative) (1 mL), Catalog# NATFLUAH1-STQ
- **Positive Control Flu B:**
  Influenza B Stock (Quantitative) (1mL), Catalog# NATFLUB-STQ
- **Negative Control:**
  HE0058N Process Control (Pellet) from Microbiologics (CE-IVD)

Method for Covid Positive Control and/or Negative Control

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 the Table here.
- Insert swab into the cartridge sample chamber, seal, and run a test following standard procedure.

21. Quality control

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 -80degC for up to one month. Stored material should be shaken well before use.

Method for RSV, Flu A and Flu B Positive Control

- Pipette 50uL of control material directly into the swab chamber. Seal the cartridge and run as a standard test. The sample should be registered as a QC test to avoid an invalid result.

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.

22. Cleaning and Maintenance

Disinfection procedure

- Always wear a new pair of gloves to disinfect the Instrument.
- Always use DnaNudge approved materials to disinfect the Instrument.
- 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.
- 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 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 NudgeBox 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 NudgeBox 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.

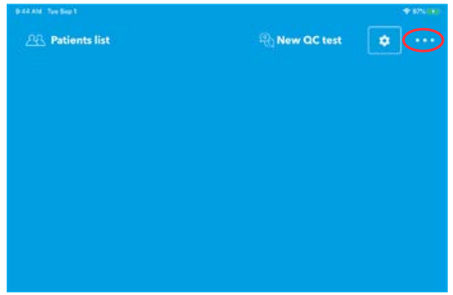
If a build-up of guanidinium chloride salt residue is found inside the cartridge cavity, please refer to the Disinfection procedure in “Cleaning and Maintenance” - Section 22.

If the NudgeBox will not open at the end of the test, turn the NudgeBox off and back on again using the Standby button at the rear. The NudgeBox should re-calibrate (see Section 15.i) 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.

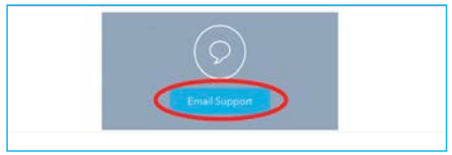
23. Troubleshooting

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. They test the NudgeBox pressure and thermal subsystems. 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 page 44).
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 new feature is disabled by default accounts, so users should contact the DnaNudge system admin (operator.app@dnanudge.com) to enable it.

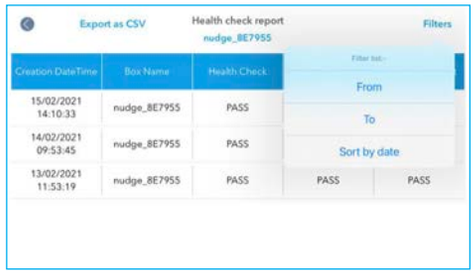
Once this feature is enabled, from the next login, the user can see this additional option from the main screen menu.

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".**

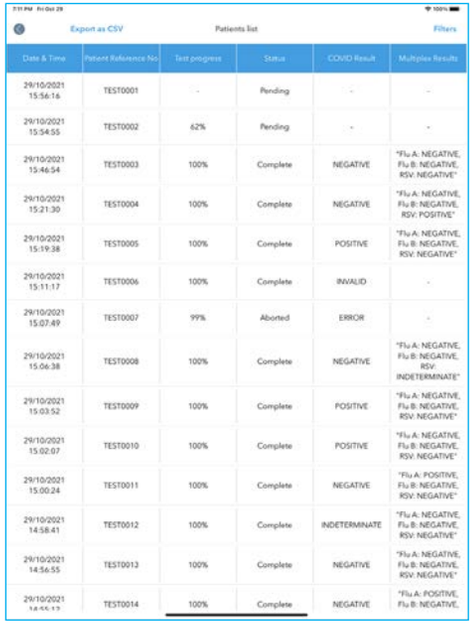


27. Test progress and patient results

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)



'Patients list' page

Selecting a particular test will display test result details

Clinical Evaluation

The performance of the DnaNudge Covid/RSV/FluNudge test was evaluated using archived clinical nasopharyngeal swab specimens in viral transport medium. Archived specimens were selected by previously known analyte result. A total of 1018 samples were tested on the Covid/RSV/FluNudge platform side by side with a laboratory PCR platform in a randomized and blinded fashion.

Clinical validation results for both Covid, RSV and Flu samples are summarised in Table 4.

Table 4

	SARS-CoV-2	Flu A	Flu B	RSV
Number of Specimens	258	247	247	266
TP	101	45	44	108
FP	0	0	0	0
TN	154	200	201	154
FN	3	2	2	4
PPA	97.12	95.74	95.65	96.43
(95% CI)	91.80 - 99.40	85.46 - 99.48	85.16 - 99.47	91.11 - 99.02
NPA	100	100	100	100
(95% CI)	97.63 - 100	98.17 - 100	98.18 - 100	97.63 - 100

28. Performance characteristics

Limitations

- The performance of the DnaNudge Covid/RSV/FluNudge test has only been established in nasopharyngeal and nasal samples taken using a paediatric nasal swab. Use of the DnaNudge Covid/RSV/FluNudge 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.
- The DnaNudge Covid/RSV/FluNudge test cannot rule out infection with other bacteria or viral pathogens.

i. Analytical Sensitivity (Limit of Detection)

Method to verify LOD - COVID-19

Material Required

- HE0062S 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 (DN0062) 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.

Limit of Detection was assessed using CE-marked SARS-Cov-2 process control pellet (HE0062S, Microbiologics Inc). The pellet was dissolved in 100ul 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 50 viral copies per swab or 1k viral copies/ml.

Method to verify LOD - RSV

Material Required

- Respiratory Syncytial Virus Type A Stock (Quantitative) (1 mL), Catalog# NATRSVA-STQ
- Respiratory Syncytial Virus Type B Stock (Quantitative) (1 mL), Catalog# NATRSVB-STQ

Limit of detection for RSV was assessed using CE-Marked liquid control material from Zeptometrix (listed above) available at a concentration of 10^6 copies/mL (1,000 copies per uL). The required volume of control material is taken and diluted 1:2 to give 500 copies per uL , 50uL is then pipetted directly into the swab chamber of the RVP-1 DnaCartridge.
LOD was determined to be 25k per test for RSV.

29. Analytical performance

Method to verify LOD - Flu A/Flu B

Material Required

- Flu A: Influenza A H1 Stock (Quantitative) (1 mL), Catalog# NATFLUAH1-STQ
- Influenza B Stock (Quantitative) (1mL), Catalog# NATFLUB-STQ

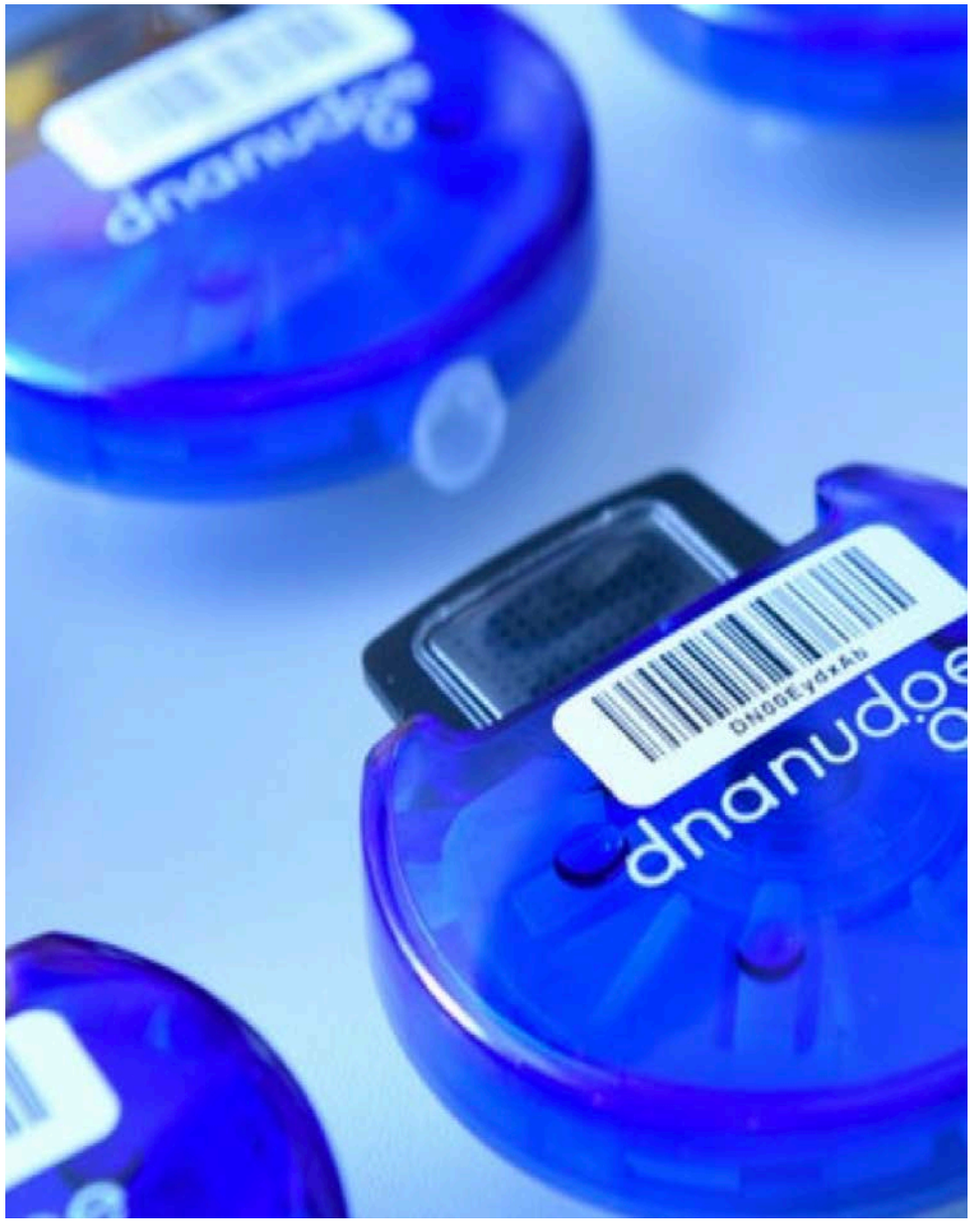
Limits of detection for Flu A and Flu B were assessed using CE-Marked liquid control material from Zeptometrix (listed above) available at a concentration of 10^6 copies/mL (1,000 copies per uL). 50uL of control material is pipetted directly into the swab chamber of the RVP-1 DnaCartridge.
LOD was determined to be 50k per test for Flu A and Flu B.

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 MHRA, customers and other regulatory agencies.

The DnaNudge assay has primers/probes targeted at four different gene targets of the SARS-CoV-2 virus (N1, N2, E, IP2). 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.

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



29. Analytical performance

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 of Covid, RSV, FluA and FluB 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	*Haemophilus influenzae*
Enterovirus A/B/C	*Legionella*
Human metapneumovirus	*Leptospira*
Corynebacterium diphtheriae	*Moraxella catarrhalis*
Parainfluenza virus 1-4	*Mycobacterium tuberculosis*
Parechovirus	*Mycoplasma pneumoniae*
Coxiella burnetii	*Neisseria elongate*
Rhinovirus A/B	*Neisseria meningitidis*
Bacillus anthracis	*Pneumocystis jirovecii*
Bordetella pertussis	*Pseudomonas aeruginosa*
Candida albicans	*Staphylococcus aureus*
Chlamydia pneumoniae	*Staphylococcus epidermidis*
Chlamydia psittaci	*Staphylococcus salivarius*
Streptococcus pyogenes	*Streptococcus pneumoniae*

29. Analytical performance

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 or into a saliva matrix, 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	OMNI Saliva 3x LOD (300 copies)	OMNI Saliva NTC	Saliva 3x LOD (150 copies)	Saliva NTC	On Nasal Swab 3x LOD (150 copies)	On Nasal Swab NTC
Listerine mouthwash (no alcohol)	Eucalyptol, Thymol, Methyl Salicilate	90%	Positive	Negative	Positive	Negative	Positive	Negative
Amoxicillin Powder	Amoxycillin	100 mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Blood	N/A	20%	Positive	Negative	Positive	Negative	Positive	Negative
Nasal spray / cough syrup	Phenilephrine	10 mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Mometasone	Mometasone	0.04 mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Cold and flu relief/cough syrup	Guaifenesin	13.3mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Asthma inhaler	Beclometasone	0.068 mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Nasal spray	Triamcinolone	0.04 mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Nasal spray	Flucticasone	0.04 mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Ethanol	Ethanol	40%	Positive	Negative	Positive	Negative	Positive	Negative
Nasal spray	Sodium Chloride	4%	Positive	Negative	Positive	Negative	Positive	Negative
Physiologic saline	Sodium Chloride	0.9%	Positive	Negative	Positive	Negative	Positive	Negative
Antibiotic	Levofloxacin	5 mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Nicotine spray	Nicotine	90%	Positive	Negative	Positive	Negative	Positive	Negative
Steroid inhaler	Budesonide	0.05 mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Nasal spray	Flunisonide	0.04 mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Mucin	N/A	3 mg/ml	Positive	Negative	Positive	Negative	Positive	Negative
Fairy dish-wash liquid		V/V 5%	Positive	Negative	Positive	Negative	Positive	Negative
Glass cleaner		V/V 5%	Positive	Negative	Positive	Negative	Positive	Negative
Toilet cleaner		V/V 5%	Positive	Negative	Positive	Negative	Positive	Negative
Sunscreen		V/V 5%	Positive	Negative	Positive	Negative	Positive	Negative
J&J lotion		V/V 5%	Positive	Negative	Positive	Negative	Positive	Negative
Hand soap		V/V 5%	Positive	Negative	Positive	Negative	Positive	Negative
Body butter		V/V 5%	Positive	Negative	Positive	Negative	Positive	Negative
Toothpaste		V/V 5%	Positive	Negative	Positive	Negative	Positive	Negative

30. Technical specifications

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

Operating temperature range:
16 – 30 °C

Storage temperature range:
0 – 30 °C

Operating humidity range:
Up to 85% RH with air pressure 1 ± 0.1 bar (gauge)

Storage humidity range:
Up to 85% RH 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

30. Technical specifications

EU Declaration of Conformity

DnaNudge Limited hereby declares that this wireless device is in compliance with the Directive 2014/53/EU.

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.

DnaNudge
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Phone: +44 (0)20 8103 9278
dnanudge.com

EC REP

EU Authorised Representative:
Alfredo García de Tuñón
Calle Cervantes, 6
33004 Oviedo
Spain

31. Table of symbols & References

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

IVD — ***In Vitro*** **medical device**

Manufacturer

Caution

Warning: Biological Risk

Warning: Flammable material

Do not re-use

+30 / +0 °C — **Storage and transport temperature**

85% / 45% — **Storage and transport humidity**

Caution: Hot surface

CONTROL — **Control**

EC REP — **EU Authorised Representative**

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

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

Covid/RSV/Flu Nudge Test

DnaCartridge™ & NudgeBox™

Instructions For Use

Rev 0 - November 2021

dnanudge

CE

IVD

DN-ENG-IFU-021 Rev 0
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