Department of Health & Social Care

# Technical validation report for DNANudge

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# Assay description

The principle and method of the assay (description of the assay according to the manufacturer's instructions for use (IFU))

1. The DNANudge Covid Nudge test is a lab-free sample-to-answer RT-PCR test intended for qualitative screening for COVID-19, 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 DNA Cartridge). The lab-free and user-friendly nature of the product makes it easy to use by following simple instructions. For the Covid Nudge test, the sample type needs to be nasopharyngeal using paediatric nasal swab. It is advised that the test is done only by trained nurses/operators and the sample is collected by a trained nurse or operator.

2. Intended use:

   The results of the Covid Nudge test are for the detection of the SARS-CoV-2 virus which is generally detectable in upper respiratory specimens during the acute phase of infection. The Covid Nudge DNA Cartridge contains 7 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 active infection with SARS-CoV-2. 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 and should not be used as the sole basis for treatment.

   The Covid Nudge test is for screening. For any diagnostic decisions, it is recommended to re-test any patient whose sample is reported as invalid or negative. However, this is at the discretion of the clinical team who may also consider a variety of other parameters for diagnosis.

3. Principal of the procedure:

   The Covid Nudge test is an automated in vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Covid Nudge test is performed using a DNA Cartridge and NudgeBox supplied by DNANudge Ltd.

   The DNA Cartridge 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

DNA Cartridge, 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 Nudge 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 DNA Cartridge. 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 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 are detected, the test will abort.

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

4. The assay is run on the DNANudge system as described above.

**Table 1: the gene targets and number of replicates detected in the DNANudge**

| Genes detected | Number of replicates |
|---|---|
| **Charite Berlin - E-gene** | 9 |
| **Institute Pasteur – RdRP-IP2** | 9 |
| **Institute Pasteur – RdRP-IP4** | 10 |
| **CDC - N1** | 10 |
| **CDC - N2** | 10 |
| **CDC - N3** | 10 |
| **RNAseP Control** | 6 |

**Table 2: results given on the DNANudge system**

| Result | Explanation |
|---|---|
| **Positive** | At least 3 of the COVID-19 assays, as well as 2 or more replicates of the control assay amplify |
| **Negative** | None of the COVID-19 assays amplifies, and 2 or more replicates of the control assay amplify |
| **Indeterminate (previously called weak positive)** | One or two of the COVID-19 assays amplifies, and 2 or more replicates of the control assay amplify |
| **Invalid** | Less than 2 replicates of the control assay amplifies. Swab |

| | |
|---|---|
| | did not gather enough human cell material. |
| **Abort** | In the event of any technical error during the sample preparation phase of the test, the NudgeBox will stop, indicating with flashing red LEDs |

Aborted tests may be due to issues on the system itself or user error;

Nudge system issues;

- pressure check failure
- temperature check failure

User issues;

- pressure check failure caused by swab port failure
- power supply interruption
- initialisation Interruption

# Type of sample to be used in validation

The DNANudge uses nasopharyngeal swabs, however these must be of paediatric size as full-size NP swabs will not fit into the cartridge of the Nudge.  For this in-service validation, samples were collected at nine NHS sites; paediatric size NP swabs and standard NP swabs were collected in duplicate from patients and tested as per the IFU for the Nudge (dry paediatric NP) and the standard of care RT-qPCR assay at each site (NP swab in VTM).

# Equipment and reagents

Product component per kit;

- 1 x DnaNudge COVID Nudge Cartridge
- lysis reagent: 450 uL per cartridge
- wash buffer: 450 uL per cartridge
- elution reagent: 1mL per cartridge
- 1 Step RT-PCR lyophilised cake: 125 ul 4x per cake

Reagents required that are not provided by the manufacturer;

- DnaNudge NudgeBox hardware v 3.3 or higher.
- 1 x disposable scissors
- 1 x paediatric nasal swab

Site requirements;

- 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
- 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
- iPad for the operator app

# Performance characteristics

### Sensitivity and linearity

**Table 3: results from dilution series to determine LLOD from one site using microbiologics standard material.**

| Dilution | Viral copies per swab (50µl) | Number of tests run | Number of positive results |
|---|---|---|---|
| **Undiluted** | 50000 | 3 | 3 |
| **2x dilution** | 25000 | 3 | 3 |
| **5x dilution** | 10000 | 3 | 3 |
| **10x dilution** | 5000 | 3 | 3 |
| **20x dilution** | 2500 | 3 | 3 |
| **50x dilution** | 1000 | 3 | 3 |
| **100x dilution** | 500 | 3 | 3 |
| **200x dilution** | 250 | 20 | 20 |
| **320x dilution** | 156 | 3 | 2 |

### Linearity and efficiency

This assay gives a qualitative result, so this section is not applicable.

### Lowest limits of detection (LLOD)

LLOD according to manufacturer equates to 5000copies/ml. At an independent site, LLOD was determined using standard material provided by Microbiologics; detection of 250copies/50uL (on a swab) equates to LLOD of 5000copies/ml. It should be noted that other external control materials may not work on the DNANudge, as they are not sufficiently concentrated to test on the swab, and control material in VTM cannot be used.

# Precision and robustness

### Intra-assay precision

This assay gives a qualitative result, so this section is not applicable.

### Repeatability

Data for this section is not available.

# Analytical specificity (interferences and cross-reactions)

**Table 4: organisms checked for in silico cross reactivity in Gibani et al.** Assessing a novel, lab-free, point-of-care test for SARS-CoV-2 (CovidNudge): a diagnostic accuracy study**. The Lancet Microbe. Volume 1, ISSUE 7, e300-e307, November 01, 2020. DOI: https://doi.org/10.1016/S2666-5247(20)30121-X**

| | |
|---|---|
| Adenovirus A/B/C/D/E | Haemophilus influenzae |
| Enterovirus A/B/C | Legionella |
| Human metapneumovirus | Leptospira |
| Influenza A/B/C | Moraxella catarrhalis |
| Parainfluenza virus 1-4 | Mycobacterium tuberculosis |
| Parechovirus | Mycoplasma pneumoniae |
| Respiratory syncytial virus | 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 |
| Corynebacterium diphtheriae | Streptococcus pneumoniae |
| Coxiella burnetii | Streptococcus pyogenes |

# Diagnostic sensitivity and specificity (clinical validation with confirmed positives and negatives)

**Table 5: comparison with reference method (note prevalence 20.9%) from data from all eleven sites**

| | | **Comparator RT-PCR** | **Comparator RT-PCR** | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| *DNANudge* | Positive | 311 | 13 | 324 |
| *DNANudge* | Negative | 68 | 1422 | 1490 |
| | Total | 379 | 1435 | 1814 |

Sensitivity = 82.1% (95% CI 77.7-85.7) this meets the acceptable criteria for sensitivity of the POC TPP (desirable>97% = acceptable = >80%).

**Table 6: CT range for samples in validation**

| **CT value range** | **Positive on DNANudge/positive on comparator** | **Sensitivity (%)** |
|---|---|---|
| **≤25 (low)** | 122/151 | 80.8 |
| **25-<30 (medium)** | 59/76 | 77.6 |
| **30-35 (high)** | 26/39 | 66.7 |
| **>35** | 3/7 | 42.9 |
| **undefined** | 54 | na |

Sensitivity below CT 25 is only 80%, however, there were issues with cartridges that needed to be recalled during November, which may have affected the data collected for this in-service validation. The data post December was therefore analysed to look at the performance for CT ≤25. The sensitivity improved, with only three with CT<25 being missed, one each of CT 15.32, 22.36 and 24.13; those ~>22 may represent LLOD for this assay.

**Table 7: sensitivity for all cartridges and for those post-Dec 2020**

| | **Data for all 11 sites** | **Data post 1 December** |
|---|---|---|
| **CT value range** | Sensitivity n/N (%) | Sensitivity n/N (%) |
| **≤25 (low)** | 122/151 (80.8) | (20/23) 87.0 |

## Diagnostic specificity

Specificity = 99.1% (95% CI 98.4-99.5%), this meets the desirable criteria for specificity of the POC TPP (desirable>99% = acceptable = >95%).

# Summary

TVG uses a wide range of sites in order to validate new technologies/tests. These independent sites use a range of RT-qPCR assays against different genomic regions and it is recognised that for some assay comparisons the sensitivity of RT-qPCR assay(s) may subtly differ from the true sensitivity of the test if compared to the same genomic region.

Overall, this assay meets the sensitivity and specificity for MHRA TPP for POC assays, but not the LLOD (in line with other POC non-extracted PCR assays that have been assessed). However, there was marked variation in performance across testing sites. Care should, therefore, be taken to understand local performance during local verification.

# Appendix

**Table 8: CT range and sensitivity for each site**

**Positive on DNANudge/Positive on comparator**

**(n/N)**

**Analysis of comparator CT values**

| CT Value Range | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | Site 9 | Site 10 | Site 11 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **<25** | 19/27 | 12/15 | 13/18 | 13/17 | 37/40 | 12/18 | 4/4 | 6/6 | 1/1 | 1/1 | 4/4 | 122/151 |
| **25-30** | 3/11 | 10/10 | 5/13 | 1/4 | 1/1 | 12/20 | 0/2 | 4/5 | 0/2 | 2/2 | 3/6 | 59/76 |
| **30-35** | 0/3 | 11/12 | 0/8 | 1/4 | 0 | 12/16 | 0 | 1/2 | 0 | 1/1 | 0/3 | 26/39 |
| **>35** | 0/1 | 1/1 | 0/1 | 0 | 0 | 2/3 | 0 | 0 | 0 | 0 | 0/1 | 3/7 |
| **Undefined** | 0 | 22 | 0 | 0 | 30 | 0 | 1 | 1 | 0 | 0 | 0 | 54 |

**Sensitivity%**

| CT Value Range | Site 1 | Site 2 | Site 3 | Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | Site 9 | Site 10 | Site 11 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **<25** | 70.37 | 80.00 | 72.22 | 76.47 | 92.50 | 66.67 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 80.8 |
| **25-30** | 27.27 | 100.00 | 38.46 | 25.00 | 100.00 | 60.00 | 0.00 | 80.00 | 0.00 | 100.00 | 50.00 | 77.6 |
| **30-35** | 0.00 | 91.67 | 0.00 | 25.00 | n/a | 75.00 | n/a | 50.00 | n/a | 100.00 | 0.00 | 66.7 |
| **>35** | 0.00 | 100.00 | 0.00 | n/a | n/a | 66.67 | n/a | n/a | n/a | 0.00 | 0.00 | 42.9 |

**Table 9: test failures, either due to lack of internal control or issues with the instrument (combination of instrument error and user error)**

| Total number of samples tested | Number of IC failures | Percentage of IC failures (%) | Number of technical failures | Percentage of Technical failures (%) |
|---|---|---|---|---|
| **2056** | 129 | 6% | 63 | 3% |

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