Department of Health & Social Care

# Technical Validation of Abbott

## ID Now

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# Assay description and intended purpose

1. Intended use: ID NOW COVID-19 assay performed on the ID NOW Instrument is a rapid molecular in vitro diagnostic test utilizing an isothermal nucleic acid amplification technology intended for the qualitative detection of nucleic acid from the SARS-CoV-2 viral RNA in direct nasal, nasopharyngeal or throat swabs from individuals who are suspected of COVID-19 by their healthcare provider.

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

   Negative results should be treated as presumptive and, if inconsistent with clinical signs and symptoms or necessary for patient management, should be tested with different authorized or cleared molecular tests. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results should be considered in the context of a patient's recent exposures, history and the presence of clinical signs and symptoms consistent with COVID-19.

2. ID NOW COVID-19 is an automated assay that utilizes isothermal nucleic acid amplification technology for the qualitative detection of SARS-CoV-2 viral nucleic acids. It is comprised of a Sample Receiver, containing elution/lysis buffer, a Test Base, comprising two sealed reaction tubes, each containing a lyophilized pellet, a Transfer Cartridge for transfer of the eluted sample to the Test Base, and the ID NOW Instrument.

   The reaction tubes in the Test Base contain the reagents required for amplification of SARS-CoV-2, as well as an internal control. The templates (similar to primers) designed to target SARS-CoV-2 RNA amplify a unique region of the RdRp segment. Fluorescently-labeled molecular beacons are used to specifically identify each of the amplified RNA targets.

   To perform the assay, the Sample Receiver and Test Base are inserted into the ID NOW Instrument. The sample is added to the Sample Receiver and transferred via the Transfer Cartridge to the Test Base, initiating target amplification. Heating, mixing and detection are provided by the instrument.

   Biosafety: the buffer has been assessed for biosafety by PHE; the report will be published during January at the following website

https://www.gov.uk/government/publications/covid-19-phe-laboratory-assessments-of-inactivation-methods

Report states: Treatment with Abbott ID NOW COVID-19 Elution Buffer reduced mean SARS-CoV-2 titre by 3.6 log10 and 4.0 log10 TCID50/ml after 1 and 5 minute treatment, respectively. While this represents a considerable reduction in virus titre, this product should not be relied upon to completely inactivate infectious samples. Demonstrating complete inactivation is dependent on the starting titre of virus used for testing. Complete inactivation may occur if samples contained lower levels of infectious virus than those tested here, but sample treatments that inactivate virus effectively in our testing may fail to inactivate samples containing higher levels of virus than those evaluated in this study.

This test has been performed using concentrated tissue culture fluid. The effectiveness of this treatment against SARS-CoV-2 may vary when used to inactivate clinical samples or other types of sample matrix. Any results of inactivation testing using other sample matrices will be released as they become available.

Using the test does not create an aerosol risk, and while not observed during validation, there is however a potential splash risk when placing the swab into the test buffer. Careful consideration should therefore be made as to the location and use of this test, including access to appropriate waste streams and use of adequate PPE for the person conducting the test (where they are not the person taking the swab). In all situations, appropriate environmental cleaning after use should be considered.

# Type of sample to be used in validation

1. Samples obtained via nasopharyngeal (NP) or nasal swab in COVID-19 symptomatic patients within 10 days of symptom onset, or fitting the other inclusion criteria below, will be tested on the Abbott ID Now and compared with the reference method or Standard of Care (SOC) used by each laboratory. Two swabs need to be collected; one dry swab for testing immediately (within one hour at room temperature or within 24 hours at 2-8°C) after collection on the Abbot ID Now platform and the other swab placed in viral transport medium (VTM) immediately after collection, for SOC testing at each site.

2. Nasal swabs tested on the Abbott ID Now were compared to NP swabs tested by PCR at each of the three sites, as this was SOC at these sites. Swabs were collected at Leeds, Sheffield and Preston NHS Trusts; control materials for cross reactivity were tested at Basingstoke NHS Trust, control materials to determine LLOD were tested at Leeds NHS Trust.

   Inclusion criteria:

   - Participants who are suspected of having COVID-19

   Nasal Swab Specimen Collection

   - For optimal test performance, use the swabs provided in the test kit. Alternatively, rayon, foam, HydraFlock® Flocked swab (standard tip), HydraFlock® Flocked swab (mini tip), Copan Mini Tip Flocked Swab, or Copan Standard Flocked swabs can be used to collect nasal swab samples.

   - Puritan PurFlock Standard Tip Ultra Flocked Swabs, Puritan PurFlock Mini Tip Ultra Flocked Swabs and Copan Standard Rayon Tip Swabs are not suitable for use in this assay.

   - To collect a nasal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Using gentle rotation, push the swab until resistance is met at the level of the turbinates (less than one inch into the nostril). Rotate the swab several times against the nasal wall then slowly remove from the nostril. Using the same swab, repeat sample collection in the other nostril.

   Nasopharyngeal swab sample collection

   - Use sterile rayon, foam, polyester or flocked flexible-shaft NP swabs to collect a nasopharyngeal sample.

- To collect a nasopharyngeal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Pass the swab directly backwards without tipping the swab head up or down. The nasal passage runs parallel to the floor, not parallel to the bridge of the nose. Using gentle rotation, insert the swab into the anterior nare parallel to the palate advancing the swab into the nasopharynx, leave in place for a few seconds, and then slowly rotate the swab as it is being withdrawn.

- To ensure proper collection, the swab should be passed a distance that is halfway of that from the nose to the tip of the ear. This is about half the length of the swab. DO NOT USE FORCE while inserting the swab. The swab should travel smoothly with minimal resistance; if resistance is encountered, withdraw the swab a little bit without taking it out of the nostril. Then elevate the back of the swab and move it forward into the nasopharynx.

# Equipment and reagents

1. Product components supplied:

    - SARS-CoV-2 viral RNA and an internal control.
    - Sample Receivers: Blue plastic components containing 2.5 mL of elution buffer.
    - Transfer Cartridges: White plastic components used to transfer 2 x 100 µL of sample extract from the Sample Receiver to the Test Base.
    - Patient Swabs: Sterile swabs (foam) for use with the ID NOW COVID-19 Test.
    - Positive Control Swab: The positive control swab ensures sample elution/lysis and workflow were performed correctly.
    - Package Insert
    - Quick Reference Instructions

2. Product components required but not supplied:

    - ID NOW Instrument
    - Nasopharyngeal Swabs

3. Assay controls should be tested concurrently with all test samples in each instrumental run. COVIDPC - Positive Control Template with an expected threshold cycle (Ct) value range, serves as a control for amplification and detection of SARS-CoV-2 RNA.

    COVIDNC - Negative Control Template, serves to verify that analyte contamination does not occur during reaction setup.

# Performance characteristics

Sensitivity and Linearity

1. Dilution series: Ideally, this should be calculated using a validated standard dilution series. If not possible (as standard material not available), use 5 clinical positive replicates, with a 5 log dilution, plus 5 negatives. If feasible, repeat over several days, different users/machines (feasibility may be limited due to availability of positive material). Where dry swabs are to be used, known amounts of standard material should be added to the swab, and then tested as per IFU.

   Table 1. results from dilution series; X-ray Inactivated SARS-CoV-2 starting concentration 6.3 105 pfu/ml

| **Machine** | **Replicate** | **63000** | **630** | **6300** | **63** | **6.3** | **0.63** | **0.063** | **0.0063** | **0.00063** | **0.000063** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Negative | Negative | Negative |
| 1 | 2 | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Negative |
| 2 | 1 | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Negative | Negative |
| 2 | 2 | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Positive | Negative | Negative |
| PCR CT value (Alinity) | | 18.63 | 21.34 | 25.63 | 27.63 | 31.44 | 33.28 | 36.31 | 36.31 | 40.45 | 40.74 |

   Table 2. results from additional replicates of X-ray inactivated virus to determine LLOD (10-fold dilution series of pfu/ml)

| **LOD** | **Rep** | **0.063** | **0.0063** | **0.00063** |
|---|---|---|---|---|
| Machine 1 | Rep 1 | Positive | Positive | Negative |
| Machine 1 | Rep 2 | Positive | Positive | Negative |
| Machine 1 | Rep 3 | Positive | Positive | Negative |
| Machine 2 | Rep 1 | Positive | Positive | Negative |

| LOD | Rep | 0.063 | 0.0063 | 0.00063 |
|---|---|---|---|---|
| Machine 2 | Rep 2 | Positive | Positive | Negative |
| Machine 2 | Rep 3 | Positive | Positive | Negative |

Table 3. results from additional replicates of X-ray inactivated virus to determine LLOD (2-fold dilution series); starting concentration 0.0063pfu/ml

| LOD | Rep | 0.00315 | 0.001575 | 0.000788 |
|---|---|---|---|---|
| Machine 1 | Rep 1 | Positive | Positive | Negative |
| Machine 1 | Rep 2 | Negative | Negative | Negative |
| Machine 1 | Rep 3 | Positive | Negative | Negative |
| Machine 2 | Rep 1 | Negative | Negative | Negative |
| Machine 2 | Rep 2 | Positive | Positive | Negative |
| Machine 2 | Rep 3 | Negative | Negative | Negative |

Table 4. dilution series of acetic acid inactivated SARS-CoV-2 in copies/ml.

| Copies/ml | Machine 1 | Machine 2 |
|---|---|---|
| 1000000 | Positive | Positive |
| 100000 | Positive | Positive |
| 10000 | Positive | Positive |
| 1000 | Negative | Positive |
| 0 | Negative | Negative |

2. The IFU states the LLOD to be 125 copies/ml, a similar level, 262copies/ml has been found in a recent publication (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7470790). The LLOD from the acetic acid inactivated material was ~1000copies/ml, but this material may not represent the best to test in this technology.

Here we used an x-ray inactivated viral preparation from PHE, to determine LLOD, but this is in pfu/ml. The assay detected >90% of replicates containing 0.0063pfu/ml, equivalent to ~CT36.

# Precision and robustness

This information supplied by the company within the IFU

# Analytical specificity (interferences and cross-reactions)

1. Cross-reactivity to non-target samples/organisms. A range of samples either direct clinical samples or spiked samples that are known positives for other diseases, both closely related (i.e., other coronaviruses), syndromic diseases (i.e., other respiratory viruses and bacteria) and common diseases (i.e. HIV, HBV, HCV, VZV, EBV, CMV) should be tested.

   Table 5. cross-reactivity check using Zeptometrix controls

| Pathogen | ID Now Results |
|---|---|
| Coronavirus 229E | negative |
| Coronavirus 229E | negative |
| Coronavirus NL63 | negative |
| Coronavirus NL63 | negative |
| Coronavirus HKU-1 | negative |
| Coronavirus HKU-1 | negative |
| Coronavirus OC43 | negative |
| Coronavirus OC43 | negative |
| Influenza B (Brisbane) | negative |
| Influenza B (Brisbane) | negative |
| Influenza A (H1N1pdm) | negative |
| Influenza A (H1N1pdm) | negative |
| RSV (A2) | negative |
| Rhinovirus (1A) | negative |
| Influenza A (H3) | negative |
| Parainfluenza 2 | negative |
| Parainfluenza 4 | negative |
| Parainfluenza 1 | negative |
| RSV (A2) | negative |
| Rhinovirus (1A) | negative |

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

1. Sensitivity

   Nasal swabs:

   Data clean up; 4 false negative samples were removed from the analysis as they were also negative when tested on another PCR platform. There were 2 samples with false positive results that did have a CT value on the comparator, but it was >40 and so classified as negative, these have also been removed from the analysis. The cleaned dataset is presented in table 6.

   Table 6. Comparison of nasal swabs with reference method (note prevalence of 26%)

| **Abbott Now ID** | **Comparator Assay** | **Comparator assay** | **Total** |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 136 | 7 | 143 |
| Negative | 16 | 425 | 441 |
| Total | 152 | 432 | 584 |

   Sensitivity = 89.5% (95% CI 83.2-93.7%) this meets the acceptable criteria for sensitivity of the POC TPP (desirable>97% = acceptable = >80%).

   Table 7. Comparison of nasal swabs with reference method (note prevalence of 26%)

| **CT Value Range** | **Positive on Abbott ID Now/positive on comparator** | **Sensitivity (%)** |
|---|---|---|
| ≤25 (low) | 22/22 | 100.0 |
| 25-<30 (medium) | 11/11 | 100.0 |
| ≥30 (high) | 12/21 | 57.0 |

| CT Value Range | Positive on Abbott ID Now/positive on comparator | Sensitivity (%) |
|---|---|---|
| 30-35 | 6/7 | 85.7 |
| >35 | 6/14 | 42.8 |

The assay has 100% sensitivity for CT range <30; for >30, there is a sensitivity of 85.7% between 30-35 and 42.8 for CT >35.

Nasopharyngeal swabs:

Table 8. Comparison of nasopharyngeal swabs with reference method (note prevalence of 19%)

| Abbott Now ID | Comparator Assay | Comparator assay | Total |
|---|---|---|---|
| | Positive | Negative | |
| Positive | 69 | 6 | 75 |
| Negative | 5 | 313 | 318 |
| Total | 74 | 319 | 393 |

Sensitivity = 93.2% (95% CI 84.3-97.5%) this meets the acceptable criteria for sensitivity of the POC TPP (desirable>97% = acceptable = >80%).

Table 9. CT range for samples used in the comparison and the sensitivity of the NP swabs at these ranges. CT data were not available for all samples, as some were tested on platforms that did not produce CT values.

| CT Value Range | Positive on Abbott ID Now/positive on comparator | Sensitivity (%) |
|---|---|---|
| ≤25 (low) | 43/44 | 97.7 |
| 25-<30 (medium) | 7/7 | 100.0 |
| ≥30 (high) | 12/16 | 75.0 |
| 30-35 | 10/12 | 83.3 |
| >35 | 2/4 | 50.0 |

2. Specificity

   Nasal swabs:

   Specificity = 98.4% (95% CI 96.5-99.3%) this meets the acceptable criteria for specificity of the POC TPP (desirable>99% = acceptable = >95%).

   Nasopharyngeal swabs:

   Specificity = 98.1% (95% CI 95.7-99.2%) this meets the acceptable criteria for specificity of the POC TPP (desirable>99% = acceptable = >95%).

# Summary

Technologies Validation Group 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.

This test meets the acceptable criteria for sensitivity and specificity designated in the MHRA POC TPP

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