FIND Evaluation of Bionote, Inc.

NowCheck COVID-19 Ag Test

External Report

Version 1.6, 02 June 2022

Evaluation process – private sector engagement

FIND, the global alliance for diagnostics, seeks to ensure equitable access to reliable diagnosis around the world. It works closely with the private and public sectors and receives funding from donors and some of its industry partners. It has internal fire walls, policies and processes to protect it against any undue influence in its work or the publication of its findings.

More information on our policy and guidelines for working with private sector partners can be found here: https://www.finddx.org/policies/

For the COVID-19 response, FIND has commissioned independent evaluations of in vitro diagnostics following an Expression of Interest (EOI) process available on FIND's website by which all test submissions were scored according to their regulatory status and time to market; the manufacturing and distribution capacity of the supplier; and the supplier-reported clinical and analytical performance.

Document history

Document Version	Date	Comment
1.0	18 September 2020	Initial release
1.1	16 October 2020	Corrected PCR sample type
1.2	3 November 2020	Updated Ct value sub analysis
1.3	5 November 2020	Analysis updated with inclusion of missing data

1.4	10 December 2020	Updated LOD methodology and results
1.5	20 April 2021	Updated Ease-of-use results
1.6	02 June 2022	Variants results added

1 Product info:

Manufacturer name	Bionote, Inc.
Test name	NowCheck COVID-19 Ag Test
Product code(s)	RG1901DG
Pack size(s)	25 Tests/Kit
Contents of kit	Test device, Extraction buffer tube, Nozzle cap, Swab, Paper stand, Film, Instructions for use
Equipment and consumables required, but not provided	Equipment: Timer Consumables: PPE
Product storage (temperature range)	2-30°C
Shelf-life (months)	24 months
Manufacturing site (country)	Republic of Korea

2 Study details:

Study design:	Prospective diagnostic evaluation studies across multiple, independent sites to determine the accuracy of COVID-19 antigen RDTs, using consecutive enrolment. Interim analyses are performed at 25% and 50% enrolment, and the evaluation is stopped if tests do not meet 97% specificity. Presence of symptoms, date of symptom onset and hospitalization status is collected for all enrolled participants.
Index assays:	Novel lateral flow format tests that detect recombinant SARS-CoV-2 antigens.
Reference method:	Results of the index test are compared to the routine, diagnostic RT-PCR result, which is used for clinical management
Limit of detection:	Analytical sensitivity, i.e. Limit of detection, was performed at the Liverpool School of Tropical Medicine in which standardized serial dilutions of cultured viral isolate were prepared. Proprietary swab provided in the kit was soaked in viral dilution series. Dilutions were tested in triplicate and the LOD was defined as the last dilution where all repeats were interpreted as positive.

Clinical performance:	Sensitivity was calculated as the proportion of true positive results detected by NowCheck COVID-19 Ag Test among all positives by the reference method and reported as a percentage. Specificity was calculated as the proportion of true negative specimens, identified as negative by NowCheck COVID-19 Ag Test among all negatives by the reference method, and reported as a percentage. The 95% confidence intervals were calculated to assess the level of uncertainty introduced by sample size, using the Wilson's score method.
Ease of use	A System usability survey and ease of use questionnaire assessing the quality of the test, test preparation, ease of test execution, procedure time, ease of result interpretation, storage conditions and perceived settings of use was completed by operators and a final score out of 100 was calculated.

3 Evaluation details:

Country of collaborator	Brazil
Location of clinical site(s) (city, town)	Marica, state of Rio de Janeiro
Health care level of site(s)	Community Testing Clinic
Study period (date to date)	30 July – 21 Aug 2020
Study cohort inclusion/exclusion	Adults in community meeting national suspect definition Provided informed consent
Sample type, antigen test	Nasopharyngeal swabs
Reference PCR method	Lab-developed assay based on the US CDC protocol, which targets two regions (N1 and N2) of the nucleocapsid (N) gene of SARS-CoV-2 (https://www.fda.gov/media/134922/download)
Sample type, PCR test	Nasopharyngeal swabs

4 Results:

4.1 Study cohort

Country	**Brazil**
Total N	400

Age [mean (min-max), N]	40 (4-84), 396
Gender [%F, (n/N)]	54.7% (219/400)
Symptoms present [%Yes, (n/N)]	100%, (392/392)
Hospitalized (n, % Yes)	Not available
Days from symptom onset [median (Q1-Q3); N]	4 (3-6); 400
Days < 0-3 (n, %)	157, 39.3%
Days 4-7 (n, %)	185, 46.3%
Days 8+ (n, %)	58, 14.5%
Positivity [%, (n/N)]	25.5% (102/400)
PCR Ct [median (Q1-Q3); N]	23.1 (20.7-27.5); 102
Ct > 33 (n, %)	9 (8.8%)
Ct > 30 (n, %)	14 (13.7%)
Ct > 25 (n, %)	44 (43.1%)

4.2 Estimation of clinical performance

Estimation of Clinical Performance Country	**Brazil**
Clinical Sensitivity (95% CI), N	89.2% (81.7, 93.9), 102
Sensitivity days ≤7, N	92.2% (84.8, 96.2), 90
Sensitivity Ct ≤ 33, N	91.4% (83.9, 95.6), 93
Sensitivity Ct ≤ 25, N	94.8% (85.9, 98.2), 58
Clinical Specificity (95% CI), N	97.3% (94.8, 98.6), 298
Invalid rate (%, n/N)	0%

** LoD as reported in IFU: 1.25 X $10^{3.2}$ $TCID_{50}$/ml, which is the equivalent of about 1.4 x 10^3 pfu/ml*

4.3 Estimation of analytical performance

- Supplier reported LOD = 3.12 x $10^{2.2}$ TCID50/ml ~ 3.46 x 10^2 pfu/ml (Isolate BetaCoV/korea/KCDC03/2020, NCCP no. 43326)

- Verified LOD

Variant (lineage)	Lowest dilution detected	Verified LOD concentration	Viral Copy equivalent

UK wild type (B1)	**2.5 x10^2 pfu/ml** ~ 3.525 x 10^2 $TCID_{50}$/ml	**2.5 x10^2 pfu/ml**	**5.9x10^5 genome copies/ml** applied to test
Alpha (B.1.1.7)	**1.0 x10^{2}pfu/ml** ~ 1.41 x 10^2 $TCID_{50}$/ml	**1.0 x10^2 pfu/ml**	**6.3 x10^3 genome copies/ml** applied to test
Gamma (P1)	**1.0 x10^{2}pfu/ml** ~ 1.41 x 10^2 $TCID_{50}$/ml	**1.0 x10^2 pfu/ml**	**4.3x10^4 genome copies/ml** applied to test
Delta (B.1617.2)	**5.0 x10^1 pfu/ml** ~ 7.05 x 10^1 $TCID_{50}$/ml	**5.0 x10^1 pfu/ml**	**8.1 x10^4 genome copies/ml** applied to test
Omicron (BA.1)	**5.0 x10^2 pfu/ml** ~ 7.05 x 10^2 $TCID_{50}$/ml	**5.0x10^2 pfu/ml**	**8.8x10^4 genome copies/ml** applied to test

Note: *viral dilution was applied directly to the test cassette, not to the provided swab*

4.4 Ease of use

NowCheck COVID-19 Ag Test	**88** out of 100	3 operators, 1 country