FIND Evaluation of Fujirebio Inc.

ESPLINE SARS-CoV-2

External Report

Version 2.0, 6 October 2021

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	29 March 2021	Initial release
2.0	6 October 2021	Data for South Africa added

1 Product info:

Manufacturer name	Fujirebio Inc.
Test name	Espline® SARS-CoV-2
Product code(s)	231906
Pack size(s)	100 tests/kit
Contents of kit	Reaction Cassette, Sample Extraction Solution (Squeeze Tube), Applicator Tip, instructions for use
Equipment and consumables required, but not provided	Equipment: Timer, refrigerator - optional (for storage of specimens prior to testing, if applicable). Consumables: Sterile swab, PPE
Product Storage (temperature range)	1-30°C
Shelf-life (months)	12 months
Manufacturing site (country)	Japan

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 95% 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:	Verification of 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 Espline SARS-CoV-2 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 Espline SARS-CoV-2 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	**Germany**	**South Africa**
Location of clinical site(s) (city, town)	1. Heidelberg 2. Berlin	Hilcrest and Durban central
Health care level of site(s)	1. Heidelberg: Drive-in testing Center 2. Berlin: Ambulatory testing clinic of Charité – University Hospital	Drive-through testing centers
Study period (date to date)	1. Heidelberg: 20 January – 19 February 2021 2. Berlin: 18 January – 22 February 2021	21 July 2021 – 20 August 2021
Study cohort inclusion/exclusion	Adults able to ambulate and meeting suspect definition of the Department of public health. Provided informed consent.	Inclusion criteria •Residents of the selected communities falling under any of the following criteria: tested COVID-19 positive <7 days; presence of COVID-19 symptoms <7 days; Exposed to COVID-19 5-10 days ago; Heath care worker; Doctor referral for testing; Exclusion criteria •Anyone in the selected communities not willing to participate or unable to provide consent to participate.

Sample type, antigen test	Nasopharyngeal swabs (Oropharyngeal if NP contraindicated, matched to PCR sample type) Swab used: iAMP-COVID19-SCD (synthetic fiber swabs with plastic shafts, and 1.5mL collection tubes were supplied by Fujirebio)	Nasopharyngeal swabs Swab used: iAMP-COVID19-SCD (synthetic fiber swabs with plastic shafts, and 1.5mL collection tubes were supplied by Fujirebio)
Reference PCR method	• Cobas SARS-CoV-2 (Roche Diagnostics Inc) ○ N = 299 • Abbott *RealTime* SARS-CoV-2 (Abbott Molecular, Inc) ○ N = 1 • LightMix® Modular SARS-CoV (COVID19) E-gene (Tib Molbiol) ○ N = 423	Abbott RealTime SARS-CoV-2 (Abbott Molecular, Inc)
Sample type, PCR test	Nasopharyngeal swabs (Oropharyngeal if NP contraindicated)	Nasopharyngeal swabs

4 Results

4.1 Study cohort

Country	**Germany**	**South Africa**
Total N (valid PCR results)	723	494
Age [mean (min-max), N]	39.4 (18-80), 723	36.0 (2-92), 494
Gender [%F, (n/N)]	51.6% (371/719)	57.3% (283/494)
Symptoms present [%Yes, (n/N)]	62.1% (446/718)	73.9% (365/494)
Hospitalized (n, % Yes)	Not applicable	Not applicable
Days from symptom onset [median (Q1-Q3); N]	2 (1-4), 444	3 (1-5), 494
Days < 0-3 (n, %)	311, 70%	169, 66%
Days 4-7 (n, %)	106, 24%	67, 26%

Days 8+ (n, %)	27, 6%	22, 9%
Positivity [%, (n/N)]	15% (112/723)	31% (153/494)
PCR Ct [median (Q1-Q3); N]	20.6 (17.7-26), 112	19.1[1] (15.9-26.8), 153
Ct > 33 (n, %)	14, 12%	17, 3%
Ct > 30 (n, %)	20, 18%	27, 5%
Ct > 25 (n, %)	33, 29%	49, 10%

[1] Adjusted to account for unread cycles (n=10) by the Abbott platform

4.2 Estimation of clinical performance

Country	**Germany**	**South Africa**
Clinical Sensitivity (95% CI), N	78.6% (70.1, 85.2), 112	72% (64.3, 78.6), 150
Sensitivity days ≤7, N	88.5% (80.1, 93.6), 87	75% (65.3, 82.7), 92
Sensitivity Ct ≤ 33, N	87.8% (79.8, 92.9), 98	78.9% (71.3, 85), 133
Sensitivity Ct ≤ 25, N	92.4% (84.4, 96.5), 79	90.1% (82.7, 94.5), 101
Clinical Specificity (95% CI), N	100% (99.4, 100), 611	99.7 (98.4, 99.9), 340
Invalid rate (%, n/N)	0% (0/723)	0.8% (4/494)

4.2.1 Estimation of analytical performance

	Lowest dilution detected	Verified LOD concentration	Viral Copy equivalent	Supplier-reported LOD
Analytical Sensitivity	**5 x 10^1 pfu/ml** ~ 7.1 x 10^1 $TCID_{50}$/ml	**5 x 10^1 pfu/ml**	**1.09 x 10^5 copies/ml** applied to test	25 pg/ml

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

4.3 Ease of use

Espline	75 out of 100	6 operators, Germany