FIND Evaluation of Fujirebio Inc.

Espline SARS-CoV-2

External Report

Version 1.0, 29 March 2021

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Evaluation Process – private sector engagement

FIND is a non-for-profit foundation, whose mission is to find diagnostic solutions to overcome diseases of poverty in lower- and middle-income countries. 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 webpage 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

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 in order 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**
Location of clinical site(s) (city, town)	1. Heidelberg 2. Berlin
Health care level of site(s)	1. Heidelberg: Drive-in testing Center 2. Berlin: Ambulatory testing clinic of Charité – University Hospital
Study period (date to date)	1. Heidelberg: 20 January – 19 February 2021 2. Berlin: 18 January – 22 February 2021
Study cohort inclusion/exclusion	Adults able to ambulate and meeting suspect definition of the Department of public health. Provided informed consent.
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)

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

Sample type, PCR test	Nasopharyngeal swabs (Oropharyngeal if NP contraindicated)

4 Results

4.1 Study Cohort

Country	**Germany**
Total N (valid PCR results)	723
Age [mean (min-max), N]	39.4 (18-80), 723
Gender [%F, (n/N)]	51.6% (371/719)
Symptoms present [%Yes, (n/N)]	62.1% (446/718)
Hospitalized (n, % Yes)	Not applicable
Days from symptom onset [median (Q1-Q3); N]	2 (1-4), 444
Days < 0-3 (n, %)	311, 70%
Days 4-7 (n, %)	106, 24%
Days 8+ (n, %)	27, 6%
Positivity [%, (n/N)]	15% (112/723)
PCR Ct [median (Q1-Q3); N]	20.6 (17.7-26), 112
Ct > 33 (n, %)	14, 12%
Ct > 30 (n, %)	20, 18%
Ct > 25 (n, %)	33, 29%

4.2 Estimation of Clinical Performance

Country	**Germany**
Clinical Sensitivity (95% CI), N	78.6% (70.1, 85.2), 112
Sensitivity days ≤7, N	88.5% (80.1, 93.6), 87
Sensitivity Ct ≤ 33, N	87.8% (79.8, 92.9), 98
Sensitivity Ct ≤ 25, N	92.4% (84.4, 96.5), 79
Clinical Specificity (95% CI), N	100% (99.4, 100), 611

Invalid rate (%, n/N)	0% (0/723)

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