FIND EVALUATION UPDATE: SARS-COV-2 MOLECULAR DIAGNOSTICS

In February 2020, FIND launched an expression of interest (EOI) for test developers of *in vitro* diagnostics (IVDs) that detect SARS-CoV-2 nucleic acid (molecular tests) to participate in independent evaluation studies. Over 200 submissions were received and applications were reviewed according to the following scoring criteria (Table 1).

Table 1: Scoring criteria	**Max score**
Limit of detection	3
Regulatory status	2
Type of organization	1
Quality management system	1
Other products available in low- and middle-income countries	3
TOTAL	**10**

As of August 2020, FIND is no longer accepting applications to evaluate molecular tests.

FIND conducted independent evaluations at the University Hospitals of Geneva (HUG) to verify the limit of detection (LOD) – as reported by the manufacturers – and the clinical performance of 22 manual molecular test kits in comparison to an in-house PCR protocol that was optimized based on the Tib Mobiol assay. The LOD analysis was performed using cultured viral stocks from a clinical isolate from Switzerland that was quantified using an E gene standard. The clinical performance analysis was conducted on extracted samples from individuals suspected to have COVID-19. 50 of which were reference PCR positive and 100 of which were reference PCR negative. Data are summarized in **Table 2**.

Additionally, a limited clinical performance evaluation of the Cepheid Xpert Xpress SARS-CoV-2 assay was also performed at HUG. A second collaborating site, the Translational Health Science and Technology Institute (THSTI) conducted a similar limited clinical performance evaluation of the Mobio TrueNat SARS-CoV-2 assay. Both studies were performed using frozen, stored respiratory samples from COVID-19 suspects. Results on the performance of these automated near-POC assays are shown in **Table 3**.

MOLECULAR ASSAY EVALUATION PROTOCOL SUMMARY

Table 2: Results for 22 manual (open) molecular tests evaluated at HUG

	Company	Product name	Product number	Gene target	Verified LOD (copies / reaction)	Avg Ct (lowest dilution 19/1)	Clinical sensitivity (50 positives)	Clinical specificity* (100 negatives)	Lot No.	PCR platform**	Supplier recommended Ct cut-off
1.	**altona Diagnostics**	RealStar® SARS-CoV-2 RT-PCR Kit 1.0	821003/ 821005	E	1–10	35.45	92% (95%CI: 81, 97)	100% (95%CI: 96, 100)	020567	BioRad CFX96 deep well	None; any signal could be considered positive
				S	1–10	35.99	92% (95%CI: 81, 97)	100% (95%CI: 96, 100)			
2.	**Atila BioSystems Inc.**	Atila iAMP COVID-19 Detection (isothermal detection)	iAMP-COVID-100-RUO	ORF1ab	50–100	N/A	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	COVID20200320	BioRad CFX96 deep well	Any signal is considered positive (isothermal
				N	1–10	N/A	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
3.	**Beijing Wantai Biological Pharmacy Enterprise Co. Ltd**	Wantai SARS-CoV-2 RT-PCR Kit	WS-1248	ORF1ab	1–10	36.20	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	nCoVP20200305	BioRad CFX96 deep well	≤40
				N	1–10	37.12	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
4.	**BGI Health (HK) Co. Ltd**	Real-time Fluorescent RT-PCR kit for detection 2019-nCoV (DE-IVD)	MFG030010	ORF1	1–10	32.43	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	6202020305	Roche LightCycler 480	≤38
5.	**bioMérieux SA**	ARGENE® SARS-COV-2 R-GENE® **[b]**	423720 (CE-IVD) 423717 (RUO)	N	10–50	36.44	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	1007389610 1007947520	BioRad CFX96 deep well	Any signal considered positive
				RdRP	10–50	32.44	96%**[a]** (95%CI: 87, 99)	100% (95%CI: 96, 100)			
6.	**Bioneer Corporation**	AccuPower® SARS-CoV-2 Real-Time RT-PCR Kit	SCV-2122	E	10–50	35.85	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	200931E	BioRad CFX96 deep well	<38
				RdRP	10–50	36.18	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
7.	**Boditech Med. Inc.**	ExAmplar COVID-19 real-time PCR kit (L)	UFPK-4	E	10–50	34.9	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	WLQCB02L	BioRad CFX96 deep well	≤42
				RdRP	50–100	33.46	90% (95%CI: 79, 96)	100% (95%CI: 96, 100)			
8.	**CerTest Biotec S.L.**	VIASURE SARS-CoV-2 Real Time PCR Detection Kit	VS-NC0112L VS-NC0212L	ORF1ab	10–50	35.16	98% (95%CI: 90, 100)	100% (95%CI: 96, 100)	NCO212L-023	BioRad CFX96 deep well	≤40
				N	1–10	35.46	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
9.	**DAAN Gene Co. Ltd of Sun Yat-Sen University**	Detection Kit for 2019-Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing)	DA0930-DA0932	ORF1	1–10	38.76	100% (95%CI: 93, 100)	96%* (95%CI: 90, 98)	20200107	Roche LightCycler 480	≤40
				N	1–10	36.97	100% (95%CI: 93, 100)	98%* (95%CI: 93, 99)			
10.	**EUROIMMUN AG**	EURORealTime SARS-CoV-**[c]**	MP 2606-0425	ORF1ab/N	1–10	37.88	100% (95%CI: 93, 100)	98%* (95%CI: 93, 99)	0200320AL	Light Cycler 480 II	Any signal considered positive
11.	**GeneFirst Ltd**	The Novel Coronavirus (2019-nCoV) Nucleic Acid Test Kit	MIYA-COVID19	ORF1	1–10	35.45	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	00072	BioRad CFX96 deep well	≤37.0 positive 37-40 indeterminate >40 negative
				N	1–10	36.72	98% (95%CI: 90, 100)	100% (95%CI: 96, 100)			
12.	**KH Medical Co. Ltd**	RADI COVID-19 Detection Kit	RV008	S	1–10	37.94	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	V008.200202	BioRad CFX96 deep well	≤40
				RdRP	10–50	36.74	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
13.	**PerkinElmer Inc.**	PerkinElmer® SARS-CoV-2 Real-time RT-PCR Assay **[c,d]**	SY580	N	1–10	39.43	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)	8202020003	BioRad CFX96 deep well	≤42
				ORF1	1–10	38.99	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
14.	**Primerdesign Ltd**	Coronavirus COVID-19 genesig® Real-Time PCR assay **[c]**	Z-Path-COVID-19-CE	RdRP	1–10	36.7	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	JIN-03780-0009	LightCycler 480	Any signal regarded as positive
15.	**QuantumDx**	QuantuMDx SARS-CoV-2 RT-PCR Detection Assay	Q20003	Orf1, N, S	1–10	36.8	100% (95% CI: 92, 100)	100% (95% CI: 96, 100)	P01100	BioRad CFX96 deep well	≤40
16.	**R-Biopharm AG**	RIDABGENE SARS-CoV-2 RUO	PG6815RUO	E	1–10	37.99	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	21120N	BioRad CFX96 deep well	None; any signal could be considered positive
17.	**Sansure Biotech Inc.**	Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) **[e]**	S3102E	ORF1	10–50	35.16	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	20200072C	Thermofisher Quantstudio 5	≤40
				N	10–50	34.96	100% (95%CI: 93, 100)	95%* (95%CI: 89–98)			
18.	**SD Biosensor Inc.**	STANDARD M nCoV Real-Time Detection Kit	M-NCOV-01	E	1–10	37.43	100% (95%CI: 93, 100)	97%* (95%CI: 92, 99)	MNC00120005	Roche LightCycler 480	≤41
				ORF1	1–10	36.99	100% (95%CI: 93, 100)	99%* (95%CI: 95, 100)			
19.	**Seegene Inc.**	Allplex™ 2019-nCoV Assay	RP10244Y RP10243X	E	1–10	33.3	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	RP4320C24	BioRad CFX96	≤40
				N	1–10	36.74	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
				RdRP	1–10	34.73	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
20.	**Shanghai Kehua Bio-Engineering Co. Ltd**	KHB Diagnostic kit for SARS-CoV-2 Nucleic Acid (Real-time PCR)	KH-G-M-574-48	ORF1	1–10	30.39	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	20037410	BioRad CFX96 deep well	More than two targets detected an curve is of S shape
				N	1–10	32.95	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
				E	1–10	31.72	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
21.	**ThermoFisher Scientific**	TaqPath™ COVID-19 CE-IVD RT-PCR Kit **[f]**	A48067	ORF1ab; S protein; N protein	1–10	NA	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	2225262	Quantstudio 5	Not Applicable (Automated software interpretation)
22.	**Vela Diagnostics**	ViroKey™ SARS-CoV-2 RT-PCR Test **[c]**	300682	RdRP	10–50	30.95	94% (95%CI: 84, 98)	100% (95%CI: 96, 100)	1000003597	BioRad CFX96 deep well	≤40
				ORF1	1–10	35.57	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)			
	Tib Molbiol/Roche Diagnostics	ModuleDx Kit SARS-CoV-2 (COVID19) E-gene (Tib-Molbiol) + LightCycler Multiplex RNA Virus Master (Roche)	53-0776-96 07541105001	E	1–10	33.34	100% (95%CI: 93, 100)	100% (95%CI: 96, 100)	48202019 48274100	Roche LightCycler 480	Define the cut-off 2–4 cycles higher than obtained Cp value for 10 copies

* **Clinical specificity:** Further investigation is needed to determine if apparent false positives are truly false positives or whether they are due to a false negative reference standard result.
** **PCR platforms:** All products were evaluated on a PCR platform recommended by the supplier, listed in this table. Each test can be performed on other PCR systems, detailed in the product's instructions for use.
[a] The two false negative samples tested positive with the second PCR (PCR 2) that targets E gene of SARS, SARS-COV-2 and/or SARS-like coronaviruses.
[b] Samples for both analytical and clinical analyses were from already-extracted specimen, therefore the methods varied from those recommended by the supplier as the internal control was not included.
[c] Samples for both analytical and clinical analyses were from already-extracted specimen, therefore the methods varied from those recommended by the supplier as the internal control was added to the master mix.
[d] Evaluation procedure varied from recommended protocol. In order to achieve the recommended sample input volume, a 2.5 fold dilution of the samples was used.
[e] Sansure claims a lower LOD of 6.4 cp/ml, which has been independently verified.
[f] Evaluation procedure varied from recommended protocol as source material was already-extracted RNA; extracted MS2 control was added directly to the master mix.

Table 3: Results for evaluation of two near-POC automated tests

Company	Product name	Product number	Gene target	Clinical sensitivity (50 positives)	Clinical specificity* (100 negatives)	Comparator test
Cepheid Inc.	Xpert® Xpress SARS-CoV-2	XPRSARS-COV2-10	N2	100% (95%CI: 92.100)	99% * (95%CI: 95, 100)	Roche Cobas ® SARS-CoV-2
			E	97.7% (95% CI: 88, 100)	100% (95%CI: 96, 100)	N = 44 positive N = 100 negative
Mobio Diagnostics Pvt Ltd	TrueNat SARS-CoV-2 **[1]**	601410020	E+RdRP**[2]**	98% (95% CI: 90.98)	96% * (95% CI:90.98)	altona Diagnostics (n=96) /LabGun™ (n=64) and/or Seegene, Inc. (n=12)
		601420050				N = 51 positive N = 111 negative

** Clinical specificity: Further investigation is needed to determine if apparent false positives are truly false positives or whether they are due to a false negative reference standard result*
[1] Note: evaluation performed at THSTI
[2] RdRP is only used as a reflex test; the results are for combined E+RdRP positives

MORE INFORMATION

For questions relating to the evaluation of molecular tests, please contact our Emerging Threats team.

QUICK LINKS

DIAGNOSIS OF SARS-COV-2 INFECTION AND COVID-19: ACCURACY OF SIGNS AND SYMPTOMS, MOLECULAR, ANTIGEN, AND ANTIBODY TESTS, AND ROUTINE LABORATORY MARKERS
WHO R&D ROADMAP
WHO COVID-19 LABORATORY GUIDANCE