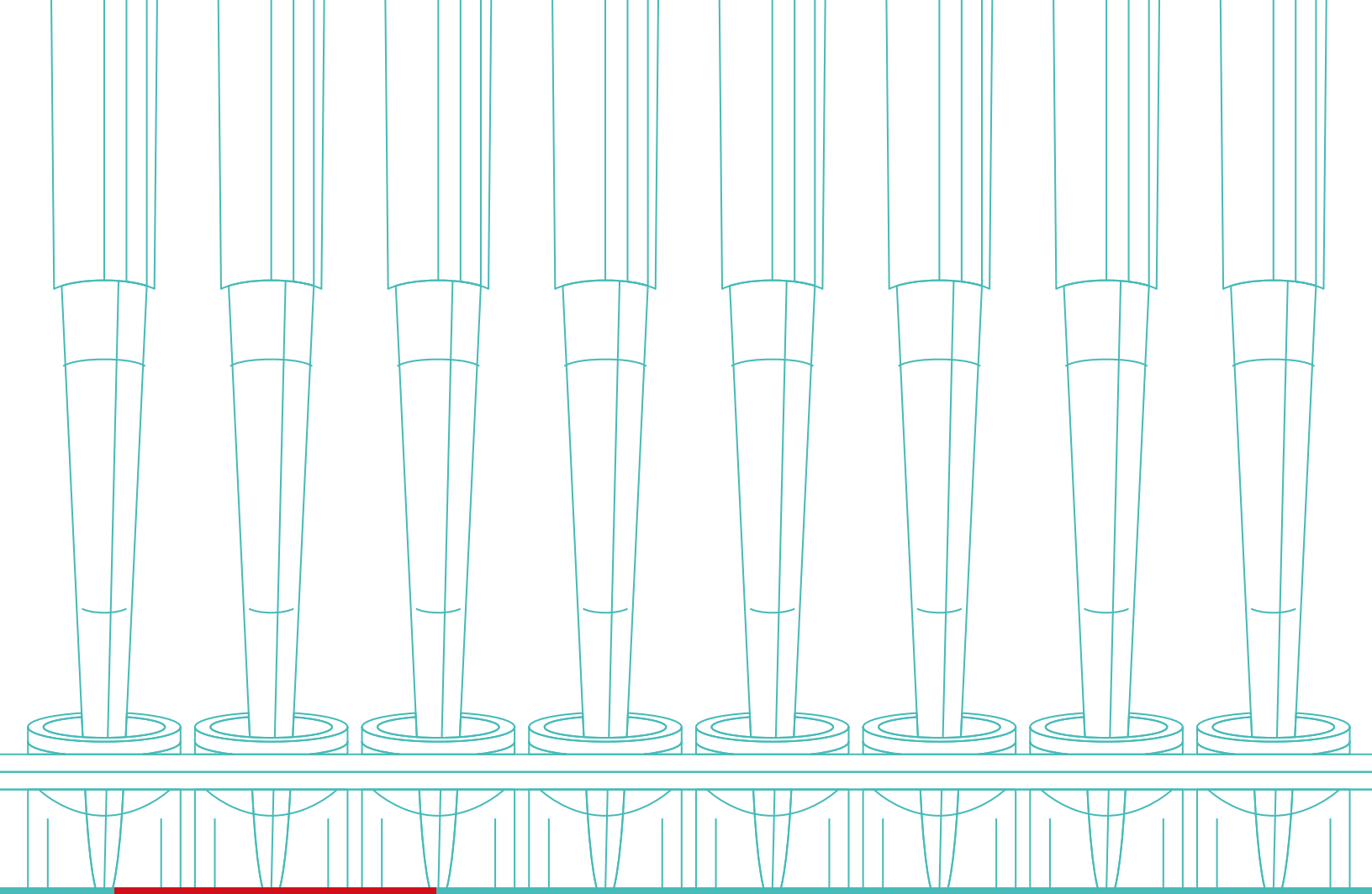


Scientific
Fact Sheet

Allplex™
SARS-CoV-2 fast PCR Assay

Seegene

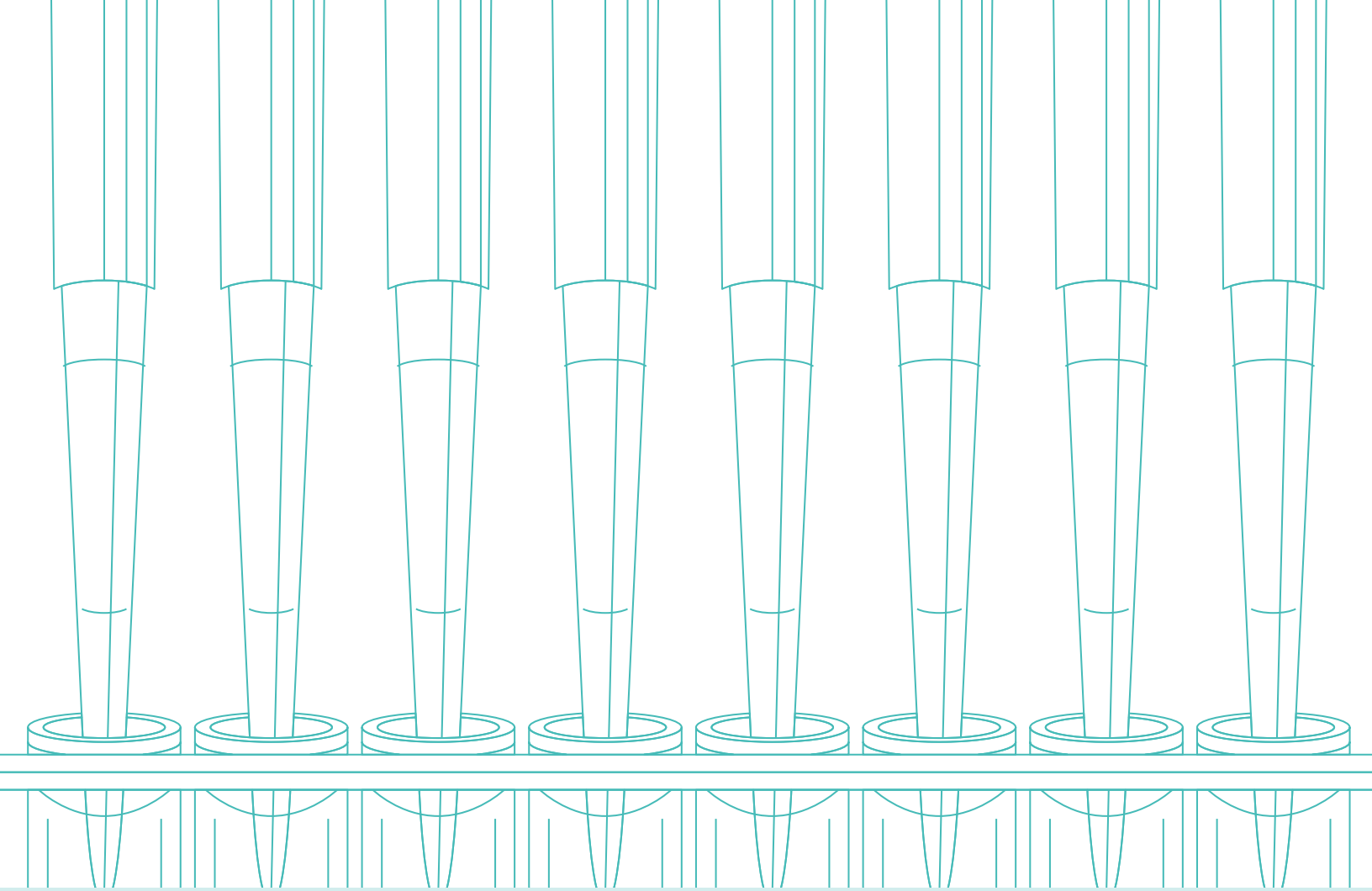
Intended Use

Allplex™ SARS-CoV-2 fast PCR Assay is a qualitative real-time one-step RT-PCR *in vitro* diagnostic test for detection of SARS-CoV-2 target genes (E, RdRP, and N genes) in specimens obtained from individuals exhibiting signs or symptoms of a respiratory infection (SARS-CoV-2, the worldwide pandemic respiratory infection onset in 2019). Allplex™ SARS-CoV-2 fast PCR Assay is intended for professional use only and as an aid in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information.

Published : March 2022

Scientific Fact Sheet

Seegene



Seegene

Allplex™ SARS-CoV-2 fast PCR Assay

Boost Up Your Test Efficiency

An easy solution to meet the growing demand for testing with high quality & speed by maximizing the value of existing systems and laboratory space

Summary of Performance

Analytical and clinical performance data show that the Allplex™ SARS-CoV-2 fast PCR Assay is an ideal solution for efficient mass screening, made possible by high accuracy, reliability, and short TAT.

High accuracy

Total 99% and 96% agreement rates when compared to existing RT-PCR assays proven in 11 real-world studies using over 1,700 samples with and without extraction, respectively

Reliability

Coverage of triple target genes (E, RdRP, and N genes) using primers of more than 99.29% *in silico* coverage for sequenced cases ensures less false-negative results and reliable detection, irrespective of variants including Omicron and its Stealth version. Endogenous IC validates the whole process even when applying an extraction-free method.

Fast TAT

With a PCR running time of 52 minutes, the total TAT (1.5 hr) for 94 samples is significantly shorter than conventional PCR assays when using an extraction-free method. This allows high throughput of 1,128 tests in 12 hours using one STARlet and one CFX96.

Snapshot of the Product

Table 1. Specification of Allplex™ SARS-CoV-2 fast PCR Assay

Specification	Allplex™ SARS-CoV-2 fast PCR Assay	
Targets	**Analytes**	**Fluorophore channels**
	SARS-CoV-2 E gene	FAM
	Endogenous Internal Control (IC)	HEX
	SARS-CoV-2 RdRP gene	Cal Red 610
	SARS-CoV-2 N gene	Quasar 670
PCR Running Time	52 minutes	
Validated Specimens	Nasopharyngeal swab*, Saliva**	
Extraction Systems	Seegene STARlet (Seegene), Seegene NIMBUS (Seegene), SEEPREP32™ (Seegene), Microlab STARlet (Hamilton), Microlab NIMBUS (Hamilton), MagNA Pure 96 (Roche Diagnostics), Maelstrom™ 9600 (Taiwan Advanced Nanotech Inc.)	
PCR Systems	CFX96™ Real-time PCR Detection System (CFX Manager™ Software-IVD v1.6), CFX96™ Dx System (CFX Manager™ Dx Software v3.1), Applied Biosystems™ 7500 (Thermo Fisher Scientific)**	
Certification	CE-IVD	

* Extraction-free method applicable
** Upcoming

Analytical Performances

1. Sensitivity

To determine the sensitivity of Allplex™ SARS-CoV-2 fast PCR Assay, the detection limit was verified using genomic RNA (TWIST BIOSCIENCE) and two different kinds of standard materials, inactivated SARS-CoV-2 virus (BEI and NIBSC).
The detection limit was 50 RNA copies/reaction for all targets (E, RdRP, and N genes) of the genomic RNA.
When the nucleic acids were extracted from the standard material using Microlab NIMBUS IVD and analyzed with Allplex™ SARS-CoV-2 fast PCR Assay, the detection limit was 61 GE/mL and 195 IU/mL (BEI and NIBSC, respectively).

Table 2. Limit of Detection (LoD) of Allplex™ SARS-CoV-2 fast PCR Assay

Extraction	Type	Organism	Source	Limit of Detection
Standard extraction	Genomic RNA	Twist Synthetic SARS-CoV-2 RNA Control 2 (MN908947.3)	TWIST BIOSCIENCE (Cat No. 102024)	50 RNA copies / reaction
	Inactivated SARS-CoV-2 virus	SARS-Related Coronavirus 2 (Isolate USA-WA1/2020, Gamma-Irradiated)	BEI (Cat No. NR-52287)	61 GE/mL*
		First WHO International Standard for SARS-CoV-2 RNA (Isolate: England/02/2020)	NIBSC (Cat No. 20/146)	195 IU/mL**

* GE/mL = copies/mL
** SARS-COV-2 is considered as "detected" if the auto-interpretation in Seegene Viewer appears as "SARS-CoV-2" or "SARS-CoV-2 Presumptive positive"

2. Reproducibility

The reproducibility test was prepared, including Negative, High Negative (0.1X LoD), Low Positive (1X LoD), and Moderate Positive (3X LoD) samples. The kit was tested for five days at each testing site, two runs per day by two different experimenters and triplicate of each target. The positive rates were observed for each target for the reproducibility study: 100.0% for Moderate Positive samples, ≥95% for Low Positive samples. The reproducibility of the Allplex™ SARS-CoV-2 fast PCR Assay was evaluated between runs, sites, and product lots. Positive rates for all concentrations and coefficient of variation (CV) values met criteria of less than or equal to 5% (≤5%). The results satisfied the criteria set above, thus confirming the reproducible performances of Allplex™ SARS-CoV-2 fast PCR Assay.

Table 3. Results of reproducibility test

Target		LoD	Criteria	Positive rate (%)	Coefficient of Variation (%)		
					Site-to-Site	Lot-to-Lot	Experimenter-to-Experimenter
First WHO International Standard for SARS-CoV-2 RNA	E gene	3X LoD	100% Detected	100.00	0.16	0.03	0.32
		1X LoD	≥ 95% Detected	100.00	0.28	0.14	0.20
		0.1X LoD	Not detected or detected below 1X LoD detection rate	31.33	0.39	0.15	0.59
	RdRP gene	3X LoD	100% Detected	100.00	0.19	0.28	1.07
		1X LoD	≥ 95% Detected	100.00	0.61	0.20	0.70
		0.1X LoD	Not detected or detected below 1X LoD detection rate	13.33	0.39	0.15	0.59
	N gene	3X LoD	100% Detected	100.00	0.43	0.26	1.36
		1X LoD	≥ 95% Detected	100.00	0.47	0.36	2.56
		0.1X LoD	Not detected or detected below 1X LoD detection rate	13.33	0.39	0.15	0.59

3. *In silico* analysis

In silico analysis was performed to confirm that the existence of SARS-CoV-2 variants does not influence the target gene detection of Allplex™ SARS-CoV-2 fast PCR assay. The coverage of each target gene primer was determined using SARS-CoV-2 sequence databases such as GISAID and NCBI. As a result, all the gene coverage was more than 99%, thus confirming extensive coverage of Allplex™ SARS-CoV-2 fast PCR assay to detect all the SARS-CoV-2.

Table 4. Results of *in silico* analysis

Database	Target	Coverage (%)*	Total count**
GISAID (As of Dec 30, 2021)	E gene	99.78	5,816,982
	RdRP gene	99.65	5,790,310
	N gene	99.22	5,812,546
NCBI (As of Jan 4, 2022)	E gene	99.79	2,991,528
	RdRP gene	99.66	2,965,019
	N gene	99.43	2,989,487
Total	E gene	99.78	8,808,510
	RdRP gene	99.65	8,755,329
	N gene	99.29	8,802,033

* Coverage (%) = The number of matched sequences / Total count

** Total count: The number of sequenced cases uploaded to each database

Clinical Performances

1. Comparison with a CE-IVD approved comparator

1-1. Standard extraction

The clinical performance evaluation included 640 nasopharyngeal swab specimens. The clinical performance of the Allplex™ SARS-CoV-2 fast PCR Assay was evaluated through the comparison with other SARS-CoV-2 Real-Time RT-PCR Diagnostic Panel, which was CE-IVD approved before. This comparison test shows more than 95% of agreement rate in the clinical sample, confirming that the quality of Allplex™ SARS-CoV-2 fast PCR Assay is valid.
The performance is summarized in table 5.

Table 5. Results of clinical performance – comparison with a CE-IVD approved comparator

		CE-IVD Approved Comparator		
		Positive	Negative	Total
Allplex™ SARS-CoV-2 fast PCR Assay	Positive	120	1*	121
	Negative	1*	518	519
	Total	121	519	640

- PPA (Positive Percent Agreement): 99.17% (95% CI: 95.48% to 99.98%)
- NPA (Negative Percent Agreement): 99.81% (95% CI: 98.39% to 100.00%)
- OPA (Overall Percent Agreement): 99.69% (95% CI: 98.88% to 99.96%)
- Kappa value: 0.990 (95% CI: 0.976 to 1.000)

* Samples were confirmed as true positive by sequencing

1-2. Extraction-free for swab

A total of 110 nasopharyngeal swab specimens were included in this clinical performance. Clinical performance equivalence of Seegene's Allplex™ SARS-CoV-2 fast PCR Assay in the extraction-free method for the NPS specimens was assessed. The results were compared with results obtained from the samples prepared by extraction method, which has already been CE marked. This comparison test is shown a more than 90% rate of agreement in clinical samples. Therefore, it is confirmed that the quality of Allplex™ SARS-CoV-2 fast PCR Assay in the extraction-free method for NPS specimens is valid.
The performance is summarized in table 6.

Table 6. Results of clinical performance – comparison of extraction and extraction-free method

Allplex™ SARS-CoV-2 fast PCR Assay		Extraction		
		Positive	Negative	Total
Extraction-free	Positive	70	0	70
	Negative	6	34	40
	Total	76	34	110

- PPA (Positive Percent Agreement): 92.11% (95% CI: 83.60% to 97.05%)
- NPA (Negative Percent Agreement): 100.00% (95% CI: 89.72% to 100.00%)
- OPA (Overall Percent Agreement): 94.55% (95% CI: 88.51% to 97.97%)
- Kappa value: 0.878 (95% CI: 0.784 to 0.972)

2. Evaluation from international multicenter studies

2-1. Standard extraction

The Clinical performance of the Allplex™ SARS-CoV-2 fast PCR Assay was evaluated by comparing it with either other real-time PCR-based competitor's or Seegene SARS-CoV-2 assays. Nine laboratories from six countries assessed the clinical performance, and 1,650 clinical samples were analyzed using the extraction-included method. The tests resulted in 99% concordance of Allplex™ SARS-CoV-2 fast PCR Assay with other tests.
The performance of each test is summarized in table 7.

Table 7. Results of clinical performance – comparison with other RT-PCR based assays in extraction method

Evaluation sites		Allplex™ fast PCR Assay (Reference Assay)			Agreement with reference assay (%)	References
Countries	Lab	Positive	Negative	Total		
UAE	A	19 (19)	12 (12)	31	**100.00**	RT-PCR based competitor assays
	B	12 (12)	13 (13)	25	**100.00**	
	C	9 (8)	21 (22)	30	**96.67**	
South Korea	D	320 (319)	720 (721)	1,040	**99.71**	Seegene SARS-CoV-2 assays
Kazakhstan	E	99 (95)	90 (94)	189	**97.88**	
Belgium	F	31 (31)	1 (1)	32	**100.00**	
Israel	G	78 (76)	13 (15)	91	**97.80**	
	H	22 (21)	160 (161)	182	**99.45**	
Latvia	I	15 (15)	15 (15)	30	**100.00**	
Total		**605 (596)**	**1,045 (1,054)**	**1,650**	**99.33**	

- PPA (Positive Percent Agreement): 99.83%
- NPA (Negative Percent Agreement): 99.05%
- OPA (Overall Percent Agreement): 99.33%

2-2. Extraction-free for swab

The clinical performance of the Allplex™ SARS-CoV-2 fast PCR Assay was evaluated by comparing it with Seegene SARS-CoV-2 Assay using the extraction-free method. Two laboratories assessed the clinical performance, and 131 clinical samples were analyzed. The tests resulted in 96% concordance of both assays, validating the Allplex™ SARS-CoV-2 fast PCR Assay with the extraction-free method, enabling its usage in fast mass screening.
The performance of each test is summarized in table 8.

Table 8. Results of clinical performance – comparison with other RT-PCR based assays in extraction-free method

Evaluation sites		Allplex™ fast PCR Assay (Reference Assay)			Agreement with reference assay (%)	References
Countries	Lab	Positive	Negative	Total		
South Korea	J	72 (70)	38 (40)	110	**96.36**	Seegene SARS-CoV-2 assay
Malaysia	K	16 (16)	5 (5)	21	**100.00**	
Total		**88 (86)**	**43 (45)**	**131**	**96.95**	

- PPA (Positive Percent Agreement): 98.84%
- NPA (Negative Percent Agreement): 93.33%
- OPA (Overall Percent Agreement): 96.95%