Illumina COVIDSeq Test Instructions for Use

FOR IN VITRO DIAGNOSTIC USE

FOR USE UNDER AN EMERGENCY USE AUTHORIZATION (EUA) ONLY

FOR PRESCRIPTION USE ONLY

Intended Use

The Illumina® COVIDSeq™ Test is a Next-Generation Sequencing (NGS) *in vitro* diagnostic test on the Illumina NovaSeq 6000 Sequencing System, NextSeq 500 Sequencing System, NextSeq 550 Sequencing System, or NextSeq 550Dx Instrument intended for the qualitative detection of SARS-CoV-2 RNA from nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal wash/aspirates, nasal aspirates, and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive results to the appropriate health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. The Illumina® COVIDSeq™ Test is intended for use by qualified and trained clinical laboratory personnel specifically trained in the use of the NovaSeq 6000 Sequencing System, the NextSeq 500 Sequencing System, the NextSeq 550 Sequencing System, or the NextSeq 550Dx Instrument, as well as Next-Generation Sequencing workflows and *in vitro* diagnostic procedures. The Illumina® COVIDSeq™ Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation of the Assay

SARS-CoV-2 belongs to a large family of coronaviruses that lead to respiratory tract diseases in humans ranging from seasonal cold to severe infections, including Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS).[1] SARS-CoV-2 leads to the COVID-19 disease, which is associated in the majority of infections with mild respiratory symptoms. However, for patients with underlying medical conditions and advanced age COVID-19 may lead to severe illness. [1] The primary route of SARS-CoV-2 transmission between humans is via respiratory route, including droplets of saliva or discharge from infected patients. [1] Confirmation of COVID-19 relies on detection of SARS-CoV-2 RNA from a patient during an ongoing, current infection. [2]

The Illumina COVIDSeq Test is intended for detection of SARS-CoV-2 virus RNA under FDA Emergency Use Authorization and virus genome analysis for research use. Insight into the SARS-CoV-2 strain present in the sample enables tracking of virus strains. This test has been designed to sequence up to 3072 samples simultaneously using the NovaSeq 6000 system or up to 384 samples using the NextSeq 500/550 systems or NextSeq 550DX instrument to detect and sequence SARS-CoV-2 RNA and internal controls.

Table 4 Illumina COVIDSeqTest Box 4 – 3072 Samples, Part # 20043436

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	114	ELB HT	Elution Buffer	2°C to 8°C, pre PCR environment
1	845	TWB HT	Tagmentation Wash Buffer HT	2°C to 8°C, post PCR environment

Table 5 Illumina COVIDSeq Test Box 5 – 3072 Samples, Part # 20043648

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	45.1	EPH3 HT	Elution Prime Fragment 3HC Mix	-25°C to -15°C pre PCR environment
1	100.6	IPM HT	Illumina PCR Mix	-25°C to -15°C, pre PCR environment
1	78.9	EPM HT	Enhanced PCR Mix	-25°C to -15°C, pre PCR environment

Table 6 Illumina COVIDSeq Test Box 6 – 3072 Samples, Part # 20043647

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	4.6	RVT HT	Reverse Transcriptase HT	-25°C to -15°C, pre PCR environment
1	41.1	FSM HT	First Strand Mix	-25°C to -15°C, pre PCR environment

Table 7 Illumina COVIDSEQ Test Box 7 – 3072 Samples, Part # 20043439

Quantity	Label Volume (ml)	Reagent	Description	Storage
1	14.4	CPP1 HT	COVIDSeq Primer Pool 1	-25°C to -15°C, pre PCR environment
1	14.4	CPP2 HT	COVIDSeq Primer Pool 2	-25°C to -15°C, pre PCR environment
1	37.6	TB1 HT	Tagmentation Buffer 1 HT	-25°C to -15°C, post PCR environment

Table 8 Illumina COVIDSeq Positive Control HT, Part # 20043401

Quantity	Label Volume	Reagent	Description	Storage
1	100 µl	COVIDSeq Positive Control HT	COVIDSeq Positive control HT	-85°C to -65°C, post PCR environment

IDT for Illumina- PCR Indexes , Store at -25°C to -15°C

The Illumina COVIDSeq Test requires 8 IDT for Illumina PCR Indexes Sets 1–4 (384 Indexes) for a total 96 indexes, 96 sample index adapter plates.

*If the positive control fails for a given index set, all patient samples associated with that index set are reported as invalid.

Performance Characteristics

The following data outlined in the clinical performance and analytical performance sections were generated by using the protocols and materials outlined in the Instructions for Use starting with nasopharyngeal (NP) swab samples. All sequencing data for this section were generated on a NovaSeq 6000 Sequencing System.

Analytical Sensitivity

The analytical sensitivity (Limit of Detection (LOD)) of the Illumina COVIDSeq Test using the Zymo extraction method was determined by serial dilution of heat inactived SARS-CoV-2 virus with known concentration (ATCC VR-1986HK) into pooled negative clinical matrix (nasopharyngeal swab specimen) to 1000 copies per ml, 750 copies per ml, 500 copies per ml, and 250 copies per ml. For each serial dilution, 22–24 extraction replicates were used for two library preparations and evaluated using the Illumina COVIDSeq Test. The LOD using Zymo extraction was determined to be 500 copies per ml.

		Replicate Breakdown				Detection Rate		
Sequencing System	ATCC Heat Inactivated Virus (copies per ml)	Zymo Extractions	Library Preparations	Detected	Total	Percent Detected	Lower CI	Upper CI
NextSeq 550 (High output)	250	22	2	38	44	86.4%	73.3%	93.6%
	500	24	2	48	48	100.0%	92.6%	100.0%
	750	24	2	46	48	95.8%	86.0%	98.8%
	1000	24	2	48	48	100.0%	92.6%	100.0%
NovaSeq 6000 (SP flow cell)	250	22	2	38	44	86.4%	73.3%	93.6%
	500	24	2	48	48	100.0%	92.6%	100.0%
	750	24	2	46	48	95.8%	86.0%	98.8%
	1000	24	2	48	48	100.0%	92.6%	100.0%
NovaSeq 6000 (S4 flow cell)	250	22	2	38	44	86.4%	73.3%	93.6%
	500	24	2	48	48	100.0%	92.6%	100.0%
	750	24	2	46	48	95.8%	86.0%	98.8%
	1000	24	2	48	48	100.0%	92.6%	100.0%

The LOD of the Illumina COVIDSeq Test using the Qiagen extraction method was determined by serial dilution of heat inactivated SARS-CoV-2 virus with known concentration (ATCC VR-1986HK) into pooled negative clinical matrix (nasopharyngeal swab specimen) to 1500 copies per ml, 1000 copies per ml, 750 copies per ml, and 500 copies per ml. The LOD using Qiagen extraction was determined to be 1000 copies per ml.

		Replicate Breakdown				Detection Rate		
Sequencing System	ATCC Heat Inactivated Virus (copies per ml)	Qiagen Extractions	Library Preparations	Detected	Total	Percent Detected	Lower CI	Upper CI
NextSeq 550 (High output)	500	22	2	41	44	93.2%	81.8%	97.7%
	750	24	2	32	48	66.7%	52.5%	78.3%
	1000	24	2	48	48	100.0%	92.6%	100.0%
	1500	24	2	46	48	95.8%	86.0%	98.8%

		Replicate Breakdown				Detection Rate		
Sequencing System	ATCC Heat Inactivated Virus (copies per ml)	Qiagen Extractions	Library Preparations	Detected	Total	Percent Detected	Lower CI	Upper CI
NovaSeq 6000 (SP flow cell)	500	22	2	40	44	90.9%	78.8%	96.4%
	750	24	2	31	48	64.6%	50.4%	76.6%
	1000	24	2	48	48	100.0%	92.6%	100.0%
	1500	24	2	46	48	95.8%	86.0%	98.8%
NovaSeq 6000 (S4 flow cell)	500	22	2	42	44	95.5%	84.9%	98.7%
	750	24	2	32	48	66.7%	52.5%	78.3%
	1000	24	2	48	48	100.0%	92.6%	100.0%
	1500	24	2	46	48	95.8%	86.0%	98.8%

Inclusivity

In silico primer analysis and synthetic short read analysis were performed to evaluate the inclusivity of the Illumina COVIDSeq Test. For primer analysis, SARS-CoV-2 sequences available on GISAID (1382 sequences) and NCBI (162 sequences) were evaluated. A BLASTn (NCBI) analysis was performed to quantify the level of primer homology across these sequences by querying each individual SARS-CoV-2 primer sequence against the downloaded SARS-CoV-2 sequences.

The following table below summarizes the homology analysis for all 1544 SARS-CoV-2 sequences.

Percent of Primer Pairs Homology	Mean	Median	5th percentile	95th percentile
100% homology	97.1%	98.0%	93.9%	100%
>80% homology	97.8%	98.0%	94.9%	100%

In summary, the Illumina COVIDSeq Test has excellent coverage across known strains of SARS-CoV-2.

Cross-Reactivity

In silico analyzes were performed to evaluate the cross-reactivity of the Illumina COVIDSeq Test with representative common respiratory pathogens. For the primer analysis, 38 non SARS-CoV-2 consensus genomes were downloaded from NCBI as the negative sample cohort. A BLASTn (NCBI) analysis was then performed to quantify the number of primer pairs with more than 80% homology with each of the genomes in the cohort.

The following table shows the results from the analysis.

Pathogen Name	NCBI Accession Number	Number of Primer Pairs With Homology Above 80%
Adenovirus (e.g. C1 Ad. 71)*	AC 000017 (1) AC 000007 (2) AC_000008 (5) AC 000018 (7) AC 000019 (35) NC_012959(54)	0
Human Metapneumovirus (hMPV)	NC_039199	0
Parainfluenza virus 1	NC_003461	0
Parainfluenza virus 2	NC_003443	0
Parainfluenza virus 3	NC_001796	0