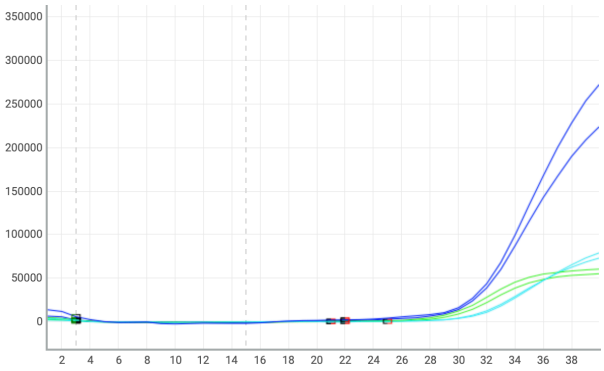
Performance Validation

YOUSEQ

COVID19 Multiplex qPCR kit performance data

E-gene & S-gene



Version 2.0

Introduction

This kit employs a thorough 2 target strategy to detect SARS-CoV-2 (Covid19) and endogenous Human RNA control. The primers and probes have been designed to show specificity to all publicly available SARS-CoV-2 sequences and no other closely related viruses, by *in-silico* analysis.

Below is an in-depth performance validation including real-world positive and negative samples for YouSeq Covid19 multiplex qPCR Kit.

Analytical Sensitivity

Analytical sensitivity of the assay is defined as the lowest concentration of input target material (Analyte), that is reliably detected with 95% Confidence. The below assessment was performance using contrived samples spiked into negative nasopharyngeal samples with known copy number reference RNA template. The samples represent a serially diluted positive samples of known concentration.

The results table below demonstrates the YouSeq Multiplex Covid19 qPCR Kit detects **1.83 copies/µL** of SARS-CoV-2 viral RNA with a confidence ≥95%. This concentration therefore serves as the limit of detection.

Calibrated Qt. (Cp/uL)	Avg. Ct value		Std. Dev.		Percent. Pos.
	E-gene	S-gene	E-gene	S-gene	
366.19	27.42	27.05	0.54	1.59	100%
36.62	30.86	30.36	0.80	0.76	100%
3.66	35.14	34.44	0.87	0.92	100%
1.83	35.55	35.99	1.16	1.35	100%
0.73	35.60	36.29	1.95	0.92	66.6%
0.37	35.88	36.98	0.00	0.00	33.3%
0.18	38.98	37.86	0.00	0.00	33.3%
0.11	37.02	38.00	0.00	0.00	33.3%
0.07	-	-	-	-	0%

Sensitivity results based on SARS-CoV-2 synthetic DNA Template

The results table below demonstrates the YouSeq Multiplex Covid19 qPCR Kit detects **0.8 copies/µL** of SARS-CoV-2 on synthetic DNA template with a confidence ≥95%.

Calibrated Qt. (Cp/uL)	Avg. Ct value		Std. Dev.		Percent. Pos.
	E-gene	S-gene	E-gene	S-gene	
200,000	15.61	16.93	0.16	0.05	100%
20,000	19.22	20.47	0.42	0.03	100%
2000	22.40	23.81	0.73	0.03	100%
20	29.43	30.71	0.31	0.40	100%
2	32.68	34.16	0.75	0.42	100%
1.6	33.20	34.15	0.74	0.35	100%

1.2	33.38	35.03	0.77	0.11	100%
0.8	34.26	34.86	1.23	0.62	100%
0.4	35.83	38.00	0.44	-	33%
0.2	35.50	-	0.22	-	0%

Testing all carried using ThermoFisher Quantstudio 5, however, this LoD should be representative across all other branded Calibrated instrumentations capable to running FAM, HEX and CY5 labelled probes.

Analytical Specificity

Specificity of detection is arguably the most critical factor in design of assays, to prevent false positive and false negatives. Upon in-depth *in silico* analysis, YouSeq ensured that the design is bioinformatically specific to all publicly available sequences of SARS-CoV-2 virus strains. The cross-reactivity with other non-SARS-CoV-2, either closely or sparsely related species was evaluated and proven to exhibit no cross-reactivity.

For testing in-vitro, a panel sourced from Zeptometrix Respiratory Verification Panel (RVP) was tested. These are whole genome samples which required RNA extracting alongside our internal Extraction control.

Organism	Internal Control Detected	Interpreted Result
Influenza A H1N1	Detected	COVID-19 not Detected
Influenza A H3	Detected	COVID-19 not Detected
Influenza A 2009 H1N1 pdm	Detected	COVID-19 not Detected
Influenza B	Detected	COVID-19 not Detected
Metapneumovirus 8***	Detected	COVID-19 not Detected
Respiratory Syncytial Virus A	Detected	COVID-19 not Detected
Rhinovirus 1A	Detected	COVID-19 not Detected
Parainfluenza virus Type 1	Detected	COVID-19 not Detected
Parainfluenza virus Type 2	Detected	COVID-19 not Detected
Parainfluenza virus Type 3	Detected	COVID-19 not Detected
Parainfluenza virus Type 4	Detected	COVID-19 not Detected
Adenovirus Type 3	Detected	COVID-19 not Detected
Coronavirus NL63	Detected	COVID-19 not Detected
Coronavirus 229E	Detected	COVID-19 not Detected
Coronavirus OC43	Detected	COVID-19 not Detected
Coronavirus HKU-1	Detected	COVID-19 not Detected
M.pneumoniae	Detected	COVID-19 not Detected
C.pneumoniae	Detected	COVID-19 not Detected
B.pertussis	Detected	COVID-19 not Detected
Negative	Detected	COVID-19 not Detected

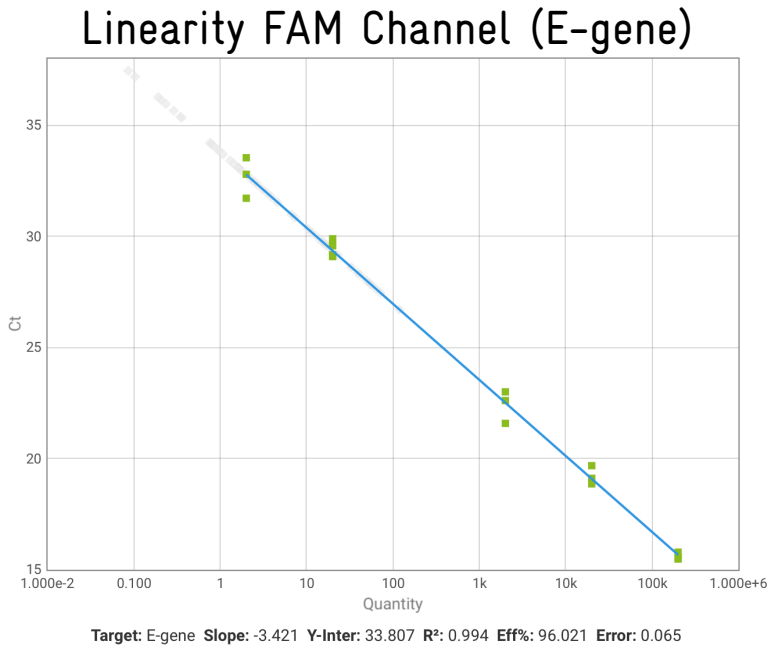
Accuracy

Clinical validation of the YouSeq COVID19 Multiplex qPCR Kit was performed with contrived and anonymised samples with a mixture of 50 positives and 50 negatives. This data can be used to generate a Positive Percentage Agreement (PPA), Negative Percentage Agreement (NPA) and Overall Percentage Agreement (OPA) as a measurement of estimated Diagnostic Accuracy:

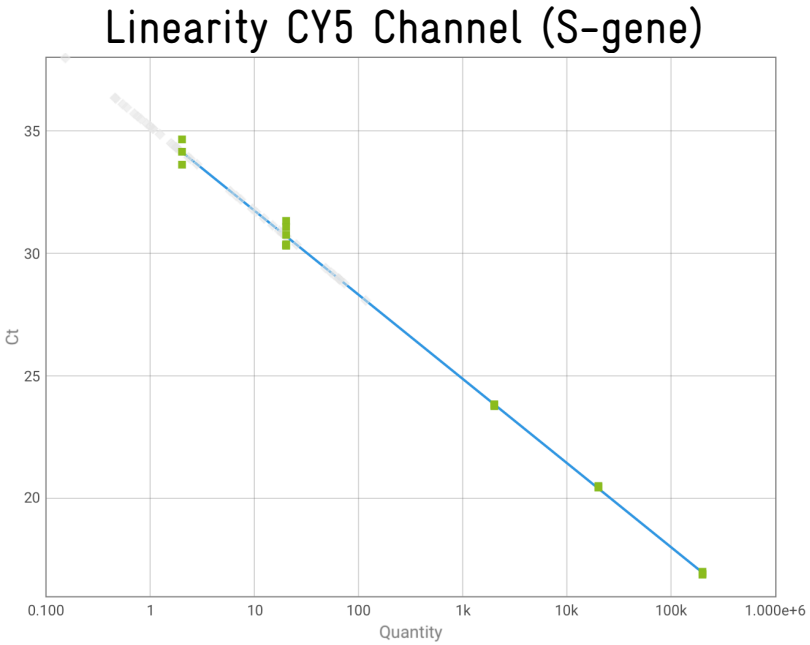
	Contrived Sample Status	
	Positive	Negative
Positive	50	0
Negative	0	50
	Positive Percentage Agreement (PPA)	Negative Percentage Agreement (NPA)
	100%	100%
	Overall Percentage Agreement (OPA)	
	100%	

Linear Range

The linear range of YouSeq Coronavirus E-S Multiplex real-time RT-qPCR kit was evaluated by analysing logarithmic dilution series of in vitro transcripts and synthetic DNA fragments. Primering efficiency is a very good indicator for the performance of an assay, which is indicative of sensitivity and quantification capability. Achieving an efficiency between 90 – 110% is optimal and would infer the target amplicon level would double after each cycle. When in a multiplex, assays can be comprised with the presence of another assay amplifying.



Target: E-gene **Slope:** -3.421 **Y-Inter:** 33.807 **R²:** 0.994 **Eff%:** 96.021 **Error:** 0.065



Target: S-gene **Slope:** -3.437 **Y-Inter:** 35.183 **R²:** 0.998 **Eff%:** 95.420 **Error:** 0.040

Precision

The precision of the YouSeq Coronavirus E-S Multiplex real-time RT-qPCR kit was analysed for its intra-assay, inter-assay and inter-lot variability. Variability data are expressed by standard deviation and coefficient of variation.

E-gene (FAM)	Copies/uL	Standard Deviation	Coefficient of Variation (%)
Intra-variability	30	0.56	0.0152
	100	0.61	0.0137
Inter-variability	30	0.60	0.0207
	100	0.75	0.026
Inter-Lot-Variability	30	0.51	0.018
	100	0.41	0.014

S-gene (CY5)	Copies/uL	Standard Deviation	Coefficient of Variation (%)
Intra-variability	30	0.27	0.009
	100	0.19	0.006
Inter-variability	30	0.93	0.03
	100	1.05	0.035
Inter-Lot-Variability	30	0.63	0.021
	100	0.21	0.008

Clinical Sample Validation

Running the assay on clinical samples is the ultimate test to confirm effectiveness of the assay in the real-world and critical for confidence in its performance. The samples were true positives confirmed in a third-party testing facility on an in-house testing kit. Below are example traces from the true clinical samples and a collection of reference positive material used.

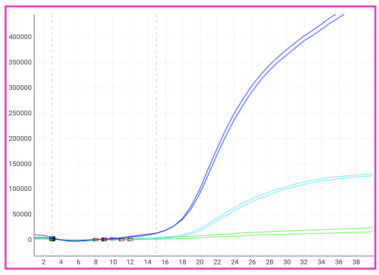
The study details are listed below with total number of samples and those called positive.

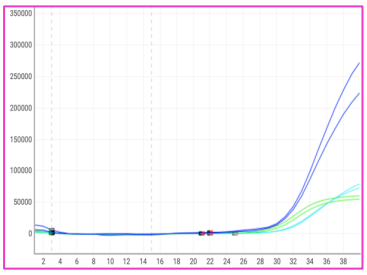
Type of sample	Positive Samples	Positive reference materials	Negative Clinical samples
COVID19 Positive	2/2	2/2	0/7

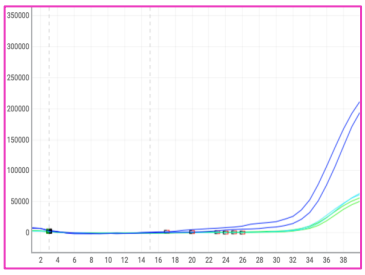
KEY

S-gene
E-gene
RNaseP

Positive Control	Positive sample 1	Positive sample 2







Twist Bioscience Reference material	EVAg reference material	Negative Samples