Department of Health & Social Care

Technical Validation of Primer Design Ltd PROmate™ COVID-19 Direct Workflow Solution IFU version 3.0

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Assay Description and Intended Purpose

1. PROmate™ COVID-19(Primerdesign Ltd) is direct qRT-PCR workflow solution that detects a single ORF1ab region. The solution is inclusive of sample preparation, qPCR amplification and analysis on genesig® q16 and q32 instruments. The assay is designed for use with dry anterior nasal swabs for screening or diagnosis of symptomatic and asymptotic COVID-19 infection. The methodology is extraction free and designed for rapid testing of small batches of 16 or 32 samples (including a minimum to 2 control samples). The time to result for 16-32 reactions is approximately 80-90 minutes.

2. The PROmate lysis buffer has been assessed by HCM, Porton Down PHE laboratory and the report has been reviewed by the technical validation group. Inactivation was demonstrated to produce >6.2 Log^{10} reduction in TCID50/ml following a 5-minute incubation at ambient temperature in PROmate sample lysis buffer. Standard local risk assessment is required for testing laboratories. The IFU (Version3.0) states that the assay can be performed both inside and outside of a biological safety cabinet. Two sample preparation protocols are provided by the manufacturer to support this. All validation work took place in clinical laboratories within biological safety cabinets. During validation, sites attempted both sample preparation methods. Validation sites reported risks using the method that is external to a biological safety cabinet method. Specifically, they note that care is needed to avoid splashing when cutting swab shaft into the sample buffer container. Care is also required when decontaminating with reusable scissors between samples to prevent contamination. Disposable scissors are recommended.

   Recommended additional equipment:

   - Vortex
   - Microcentrifuge (CL2 users)
   - Adjustable 10µl or 20µl micropipette, or a fixed 5µl micropipette
   - Aerosol barrier pipette tips with filters
   - 0.1ml qPCR tubes (for balancing partial runs)
   - Disposable gloves
   - • Scissors (optional)
   - 10% bleach (1:10 dilution of commercial 5.25 to 6.0% hypochlorite bleach)

- 70% Alcohol (either ethanol or isopropanol)

Type of Sample used in Validation

1. The assay is validated for use with anterior nasal samples collected on to dry flocked swabs that have been validated for use with the PROmate assay. Swabs are stored into a sterile dry container. No other sample type or collection media is validated or suitable. Viral transport medium and saline have demonstrated reduced sensitivity (LoD) of direct RT-PCR assays. Laboratories cannot use any sample type other than dry anterior nasal swabs for validation or clinical

2. Only approved swabs or locally validated alternatives can be used with the PROmate assay. For the validation, dry anterior nasal swabs were obtained and compared to nasopharyngeal swabs that were collected into viral transport medium and tested by the laboratory standard of care (SoC) assay. Quantified control material was provided by the national institute of biological standards and controls (NIBSC) to independently evidence the limit of detection. Prepared quantified material was directly added into PROmate lysis buffer. Primerdesign Ltd have validated Twist Bioscience® SARS-CoV-2 RNA for use with the PROmate assay as an independent quality control or to evidence limit of detection.

3. Whole virus control and dry nasal swabs were used in the validation. The use of PROmate with extracted RNA has not been validated. As per the IFU, validation samples were tested without prior extraction. The volume of sample required per test is 5ul prepared in a lysate of PROmate buffer.

4. Dry swabs were collected and tested according to the PROmate IFU and compared to the standard of care (SoC) comparator assays. All comparator SoC samples were collected according to the manufacturers IFU (see table below). Samples for the PROmate and SoC assays were collected at the same time (Dual sampling).

Sample Collection and Storage conditions	**Swabs: Dry Dracon or polyester flocked swabs in a sterile container**
Transport temperature	2 to 8ºC ≤ 72hrs
Short-term storage (pre-extraction)	2 to 8ºC ≤ 72hrs
Long-term storage (pre-extraction)	≤ -70°C for longer periods

Interfering substances

Samples with obvious blood or other particulate matter are not compatible with PROmate™. The assay not been tested against interfering substances as recommended in the MHRA TPP.

Equipment and reagents

1. The manufacturer states that calibration and maintenance for the Q16 and Q32 is not required but can be provided on request. Ancillary equipment is listed in section Assay Description and Intended Purpose.

2. The manufacturers reagents are provided in two separate packets; an ambient box and a frozen pouch received on dry ice. The PROmate™ pouch on dry ice must be stored frozen (-25°C to -15°C). The PROmate™ box should be stored at ambient temperature. Positive and negative controls are provided within the kit. The positive control contains a high viral load (CT 14 to 22) and caution is required when handling to prevent contamination. Laboratories should also use an independent IQC as per UKAS requirements. The PROmate assay includes an internal control. The purpose of the IC is to monitor the integrity of the PCR run and control for inhibition from patient samples, however it does not confirm the presence of human material. Reagents required but not provided by the manufacturer are listed in section 2, Performance characteristics

Analytical Sensitivity and Linearity of SARS COV-2 targets.

1. Dilution series:

   One validation site performed a standard dilution series of whole SARS-CoV-2 virus (Victoria strain) inactivated by heat and acid was provided by NIBSC. The concentration of the dilution series ranges from 10^7 to 10^1copies per ml, based on comparative quantitation using a CE marked commercial assay that is within the MHRA TPP for laboratory-based assays. Results are presented below.

2. Linearity and efficiency:

   A standard dilution series of whole virus SARS-CoV-2 (Victoria strain) inactivated by heat and acetic acid was analysed are presented below. Lowest Limits of Detection (LLOD):

   The manufacturer states in the IFU that the PROmate™ COVID-19 is defined as 0.96copies/ul, or 960 copies/ml. The limit of detection was calculated using SARS-CoV-2 whole genome RNA provided by Twist Bioscience® (MT007544.1). The full method is described in the IFU and is summarised in the table below. Validation sites verified the limit of detection using Twist DNA material Design.

Sample type	SARS-CoV-2 Viral RNA Concentration (copies/µL)	Positive calls/Total no. results included in analysis	% Replicate Detection	Mean Cq	Cq Standard Deviation
Anterior nasal swab	0.96	58/60	96.67	35.57	0.93
Anterior nasal swab	0.88	50/60	83.33	35.82	0.89
Anterior nasal swab	0.80	18/20	90.00	36.17	0.98

3. Independent Limit of detection:

   One validation site tested whole SARS-CoV-2 virus Victoria strain inactivated by heat and acid added directly to PROmate buffer. The viral load was approximated according to a validated CE marked assay prior. The dilution series ranged from 10^7 to 10^1 copies/ml. Virus was detected to 10^2 and is within the manufacturers stated performance.

Precision and robustness

1. Intra-assay precision, lysate stability:

   The stability of samples prepared in PROmate buffer and reproducibility of the replicates has not been defined in the IFU. Prepared samples are intended for immediate use. The validation laboratories performed replicate testing of prepared sample lysates to evaluate reproducibility and stability over time. 2 samples were tested in triplicate over 5 timepoints over 24-hour period. Cq values for target and inhibition control (IC) in stored samples were used to demonstrate precision and stability.

   Robustness, reproducibly and stability of Cq ORF1ab values for positive samples stored refrigerated in prepared sample lysate.

ORF1ab Cq stability of 2 positive sample triple replicates over time (hours)

Replicate Number	0 hours	2 hours	4 hours	8 hours	24 hours	Mean Cq	SD
1	33.85	33.26	33.46	33.41	32.94	33.38	0.30
1	33.51	33.66	33.76	34.07	33.54	33.70	0.20
1	33.9	32.51	33.78	33.58	33.45	33.44	0.49
2	24.63	24.38	24.53	25	25.77	24.86	0.50
2	24.62	24.26	23.63	24.82	25.7	24.61	0.68
2	24.49	24.26	23.34	25.12	25.49	24.54	0.74

Robustness, reproducibly and stability of Cq internal inhibition control (IC) values for positive samples stored refrigerated in prepared sample lysate over 24 hours.

Cq IC stability of 2 samples in triple replicates over time (hours)

Replicate Number	0 hours	2 hours	4 hours	8 hours	24 hours	Mean Cq	SD
1	18.71	18.74	18.70	18.72	18.70	18.71	0.01
1	18.70	18.62	18.72	18.65	18.73	18.68	0.04
1	18.73	18.70	18.74	18.67	18.71	18.71	0.02
2	18.71	18.95	19.02	19.10	18.94	18.94	0.13

Replicate Number	0 hours	2 hours	4 hours	8 hours	24 hours	Mean Cq	SD
2	18.93	18.80	19.87	19.09	18.88	19.11	0.39
2	18.87	19.11	18.97	19.03	19.14	19.02	0.10

Cq values for SARS-COV-2 target and IC over a 120-hour time period. CT values increase by 2-3 CTs per 24-hour period and 2 samples with Cq above 30 called negative results by 120 hours.

0 hr	0 hr	0 hr	24 hr	24 hr	24 hr
CT	IC	Result	CT	IC	Result
30.91	18.68	POS	33.46	18.68	POS
24.75	Failed	POS	26.02	18.77	POS
25.37	18.88	POS	28.7	18.64	POS
21.63	18.52	POS	26.97	18.5	POS
32.77	19.88	POS	32.54	19.16	POS

48 hr	48 hr	48 hr	120 hr	120 hr	120 hr
CT	IC	Result	CT	IC	Result
34.7	17.82	POS		17.62	NEG
29.21	17.65	POS	30.91	17.56	POS
31.52	17.63	POS	33.7	17.52	POS
32.65	17.21	POS	35.68	17.63	POS
35.7	18.81	POS		18.88	NEG

Repeatability: The manufacturer analysed 40 replicates at a single concentration and established a Cq standard deviation of 1.02. This is demonstrated in the table below

Sample type	SARS-CoV-2 Viral RNA Concentration (copies/µL in PCR reaction) *	Positive calls/Total no. results included in analysis	% Replicate Detection	Mean Cq	Cq Standard Deviation
Anterior nasal swab	0.88	40/40	100.0	34.76	1.02

Analytical specificity

1. The assay detects a conserved region of the ORF1ab gene. In silico analysis is performed weekly basis by the manufacturer. At the time of validation, the target region of the assay was compatible with the detection of all SARS-COV-2 sequences and variants registered on the GISAID database.

2. Cross-reactivity to non-target samples/organisms. A range of samples either direct clinical samples or spiked samples that are known positives for other diseases. The assay detects the same ORF1ab region. In silico analysis is performed by the manufacturer exhibited no cross-reactivity with non-SARS-CoV-2 species except for two sequences, Bat coronavirus (NCBI Accession No. MN996532.1) and Pangolin coronavirus (NCBI Accession No. MT084071.1) sequences. The primers/probe sequence has 5 mismatches and 7 mismatches respectively, with these viruses and therefore show limited possibility of being detected with the assay.

3. In addition to in silico testing, the IFU includes data for in vitro testing of non-target respiratory target using commercial EQA materials. In total 36 non-target organisms were analysed with no incidences of cross reactivity reported. A full list of organisms is listed in IFU version 3.0

Diagnostic sensitivity and specificity (Clinical validation with confirmed positives and negatives)

1. Samples selected for the validation were appropriate to the assay. Low medium and high viral load samples were included to avoid increasing or lowering DSe and DSp.

   Validation laboratories provided CT values or equivalent for comparator assays. One laboratory performed comparator SoC testing using the non-linear Hologic Panther SARS-COV-2 assay and therefore CT values were not available. For samples tested on this platform, the relative light units (RLU) for positive samples ranged from 1104 to 1149. Details of the comparator and discrepancies assays used in the validation are listed in the appendix.

   CT distribution of comparator samples obtained using SoC assays at three validation sites

   Comparator SoC CT values

CT	Laboratory A	Laboratory B	Laboratory C	Total
<25	55	39	28	122
≥25-<30	20	5	21	46
≥30-35	10	3	27	40
≥35	0	2	5	7

2. Diagnostic specificity and sensitivity:

   Confirmed clinical samples (positive RT-qPCR result) were used for validation. In total 759 samples of which 242 were positive and 517 were negative were tested across 4 validation sites. Clinical sensitivity (95% CI) and positive predictive value (PPV) was calculated in comparison to CE marked reference methods with sensitivity, specificity and limit of detection within the specifications of the laboratory based MHRA TPP. The validation took place during a period of nationally high prevalence rates for infection and this is reflected in the spread of CT values obtained. Overall sensitivity and the sensitivity according to the CT values of the comparator assay was calculated. These performance characteristics are demonstrated in the tables and charts below It was not possible to report sensitivity by individual validation site as there was insufficient spread of data at lower viral loads to provide statistically reliable representation. It

should be noted that CT values vary according from assay to assay and broadly be used to indicate samples with high, medium and low viral loads.

Validation summary results (all sites)

		Comparator Assay	**Comparator Assay**	
		Positive	Negative	Total
Primer Design PROmate	Positive	221	5	226
Primer Design PROmate	Negative	21	512	533
	Total	242	517	759

Performance characteristics (all data)

	%	**95% CI**
Sensitivity	91.3 %	87.1-94.3
Specificity	99.0 %	97.8-99.6
PPV	97.8 %	-
NPV	96.1 %	-

Sensitivity by CT value was calculated from a subset of data provided by 3 laboratories using semi-quantitative qRT-PCR as the standard of care test method.

Sensitivity by CT Value of SoC assays

Comparator assay SoC CT values

CT	**Laboratory A**	**Laboratory B**	**Laboratory C**	**Total**	**Sensitivity**	**95% CI**
<25	55	39	28	122	100%	96.95-100
≥25-<30	20	5	21	46	95.7%	85.5-99.6
≥30-<35	10	3	27	40	70.0%	54.6-81.9

CT	Laboratory A	Laboratory B	Laboratory C	Total	Sensitivity	95% CI
≥35	0	2	5	7	*28.6%	8.2-64.11

**CT of 35 on the comparator assay is outside of the LoD and reproducible range of the PROmate assay.*

3. Diagnostic specificity:

   517 confirmed negative clinical samples (negative by RT-qPCR result) were analysed across 4 validation sites in alignment with the MHRA TPP. Clinical specificity (95% CI) and negative predictive value (PPV) was be calculated in comparison with CE marked reference methods with sensitivity, specificity in line with the MHRA TPP. The CT values or equivalent for both the assessed and comparator assays are demonstrated in the table above.

4. Performance Summary:

   The validation performance of the PROMate assay aligns with the acceptable performance characteristics for sensitivity and specificity for the point of care (rapid testing).

5. Limitations of the validation:

   CT values in the validation data are skewed towards those with high CTs and this can falsely elevate diagnostic sensitivity. The sensitivity characteristics according to CT value is represented in the validation report and must be considered by those implementing the assay.

   TVG uses a wide range of sites in order to validate new technologies/tests. These independent sites use a range of RT-qPCR assays against different genomic regions and it is recognised that for some assay comparisons the sensitivity of RT-qPCR assay(s) may subtly differ from the true sensitivity of the test if compared to the same genomic region.

   Validation took place in NHS diagnostic laboratories. Real-world usability and performance characteristics in other settings were not considered in this validation. Usability reports in a range of settings will be reported separately.

The composition of the PROmate solution includes reagents, reaction conditions and procedural techniques that are distinct and separate to other test solutions offered by the manufacturer PrimerDesign ltd. The performance characteristics in this validation are specific to the PROmate solution. The performance characteristics and usability reports for PrimerDesign EXSIG and PROmate are not interchangeable. The validation was performed with PROmate IFU version 3.0

Appendix

Comparator SoC assays:

- Altona RealStar Dual target SARS-CoV-2
- Hologic Panther TMA SARS-CoV-2
- Novacyt Genesig HT
- Roche Cobas 6800 SARS-CoV-2
- Discrepancy analysis assays:
- Cepheid Xpress SARS-CoV-2
- Novodiag SARS-CoV-2

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