Department of Health & Social Care

Technical Validation of OptiGene RT LAMP Assay (Direct and RNA Formats)

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Contents

Assay description and intended use 2

Type of sample used in validation 3

Equipment and reagents 4

Performance characteristics 5

Precision and robustness 6

Analytical specificity (Interferences and cross-reactions) 8

Diagnostic sensitivity and specificity (Clinical Validation with confirmed positives and negatives) 10

Additional data 12

Assay description and intended use

- OptiGene’s COVID-19 RT-LAMP assay is a nucleic-acid based amplification test for the qualitative or semi quantitative detection of nucleic acid from the SARS-CoV-2 in human respiratory specimens (nasopharyngeal swabs/oropharyngeal swabs and saliva). The RT-LAMP assay targets the positive sense viral genomic RNA within the Orf1ab region. The assay has two formats, an RNA version for use on extracted RNA and a Direct version for use on crude clinical samples

- The assay is intended for use by professionals and trained in laboratory settings.

- The OptiGene RT-LAMP assay is performed using the OptiGene Genie instruments consisting of the Genie HT (High throughput), Genie II and the Genie II.

Type of sample used in validation

- The validation was performed on nasopharyngeal/oropharyngeal swabs and saliva.
- Swabs were collected in viral transport medium (Virocult). Saliva was collected in plastic tubes. The samples were collected in clinical settings.

Equipment and reagents

All the equipment required to perform the test is supplied by the manufacturer except for laboratory consumables, saliva collection pots and nasopharyngeal/oropharyngeal swab

Performance characteristics

Analytical Sensitivity of SARS COV-2 targets

- The analytical sensitivity (ASe) for the RNA and Direct RT-LAMP assays were evaluated using a blinded panel of NIBSC inactivated virus ranging from 107/ml to 102/ml, noting that inactivated virus can only provide an estimate of limit of detection because the inactivation will have degraded the RNA somewhat (Table 1 and 2). These viral samples were run in both the Direct (bypassing the heat and lysis step) and RNA RT-LAMP mastermixes. The Primer Design Winterplex assay which includes a SARS-CoV-2 gene target which has close proximity to the LAMP assay target was used as a comparator after the RT-LAMP reactions had been performed and the panel unblinded by NIBSC. Both RT-LAMP assay formats detected to 103 copies/ml (1000 copies/ml).

- Linearity and efficiency: Not applicable (RT-LAMP assays are not linear).

Precision and robustness

- Repeatability and inter-operator reproducibility were measured by running eight replicates of four clinical samples with three different operators. Each operator independently mixed the sample 1:1 in RapiLyze, which was used for each of their replicates. Inter-platform reproducibility was measured by running eight replicates of a clinical sample with across two platforms. The same 1:1 sample in RapiLyze Sample Buffer was used across the two platforms.

- Inter-operator precision:

    - Mean time to positivity in minutes (% coefficient of variation)

    - Mean annual temperature [Number of replicates positive]

RT-qPCR CT	**Operator 1**	**Operator 2**	**Operator 3**	**Reproducibility between operators**
19.43	06.45 (0.67) 84.19 (8/8)	06:34 (0.94) 84.04°C [8/8]	07:03 (0.95) 84.11°C [8/8]	06:48 (3.59)
22.59	10:30 (4.95) 84.72°C [8/8]	10:16 (3.64) 84.36°C [8/8]	12:01 (3.39) 84.27°C [8/8]	10:55 (8.68)
20.15	06:55 (1.86) 84.19°C [8/8]	06:43 (1.14) 84.08° [8/8]	08:03 (2.36) 84.06°C [8/8]	07:13 (9.90)
23.33	14:20 (10.14) 84.47°C [8/8]	14:03 (16.79) 84.24°C [6/8]	13:52 (14.62) 84.26°C [7/8]	14:05 (1.64)

VTM: Viral Transport Medium. Criteria for acceptance: (i) mean time to positivity does not vary more than 20% and (ii) the mean anneal temperatures are within +/- 1°C. TP: time to positivity (minutes:seconds). Samples were collected on 24/09/2020; tests were performed on 18/10/2020 [samples stored short term at 4°C and long term at -20°C

- Inter platform reproducibility:

Sample	**qRT-PCR CT**	**Genie® HT**	**Genie® III**	**Reproducibility between platforms**
Clinical patient sample 1 (Swab VTM)	19.43	06:45 (0.67) 84.19°C [8/8]	06:58 (2.64) 83.90°C [8/8]	06:51 (2.26)

Sample	qRT-PCR CT	Genie® HT	Genie® III	Reproducibility between platforms
Clinical patient sample 2 (Saliva)	22.59	10:30 (4.95) 84.72°C [8/8]	10:30 (5.31) 84.20°C [8/8]	10:30 (0.04)
Clinical patient sample 3 (Swab VTM)	20./15	06:55 (1.86) 84.19°C [8/8]	07:06 (1.53) 83.88°C [8/8]	07:01 (1.91)
Clinical patient sample 4 (Saliva)	23.13	14:20 (10.14) 84.47°C [8/8]	14:11 (12.17) 83.97°C [7/8]	14:15 (0.76)

VTM: Viral Transport Medium. Criteria for acceptance: (i) mean time to positivity does not vary more than 20% and (ii) the mean anneal temperatures are within +/- 1°C. TP: time to positivity (minutes:seconds). Samples were collected on 24/09/2020; tests were performed on 18/10/2020 [samples stored short term at 4°C and long term at -20°C

Analytical specificity (Interferences and cross-reactions)

- Analytical specificity (ASp) was determined using the NATtrol™ Respiratory Verification Panel 2 (ZeptoMetrix Corporation, New York, United States) containing pathogens causing indistinguishable clinical signs to COVID-19 (n=22). No cross reactivity was observed in either assay (Direct RT-LAMP and RNA RT-LAMP).Bullet
    - Table 1: PANEL MEMBERS

Panel Member	**Strain**
Influenza A H 1NT	A/New Caledonia2088
Influenza A H3	A/Brisbane/1007
Influenza A 2009 H1N1pdm	ANY/0209**
Influenza B	B/Florida/0206
Metapneumonia B***	Peru6-2003
Respiratory Syncytial virus	N/A
Rhinovirus 1A	N/A
Parainfluenza virus Type 1	N/A
Parainfluenza virus Type 2	N/A
Parainfluenza virus Type 3	N/A
Parainfluenza virus Type 4	N/A
Adenovirus Type 3	N/A
Coronavirus NL63	N/A
Coronavirus 229E	N/A
Coronavirus OC43	N/A
Coronavirus HKU-1	N/A
M. pneumoniae	M129
C. pneumoniae	CWL-029
B. pertussis	A639

Panel Member	Strain
Adenovirus Type 31	N/A
Adenovirus Type 1	N/A
B. parapertussis	A747
Negative	N/A

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

- Samples selected for the validation were appropriate to the assay. Low medium and high viral load samples were tested d to avoid increasing or lowering diagnostic sensitivity and specificity
    - Table 2. Range of viral loads for validation samples

CT Range RNA Swab LAMP	**CT<25**	**CT <33**	**Ct <45**
Sample number (n)	92	138	186

CT Range RNA Saliva LAMP	**CT<25**	**CT <33**	**Ct <45**
Sample number (n)	28	46	50

CT Range RNA Swab LAMP	**CT<25**	**CT <33**	**Ct <45**
Sample number (n)	98	159	173

CT Range RNA Saliva LAMP	**CT<25**	**CT <33**	**Ct <45**
Sample number (n)	51	86	99

- Diagnostic sensitivity: Confirmed clinical samples from patients (positive RT-qPCR result) were compared. The CT values or equivalent for both the assessed and comparator assays were included in the submitted validation data.
    - 186 positive swabs analysed by RNA RT-LAMP;
    - 50 positive saliva samples analysed by RNA RT-LAMP;

  - 173 positive swabs analysed by Direct RT-LAMP;
  - 158 positive saliva samples analysed by Direct RT-LAMP.

- Diagnostic specificity: Confirmed clinical samples from patients (negative RT-qPCR result) were used. The CT values or equivalent for both the assessed and comparator assays were included in the submitted validation data.
  - 12361 negative swabs analysed by RNA RT-LAMP;
  - 12359 negative saliva samples analysed by RNA RT-LAMP;
  - 356 negative swabs analysed by Direct RT-LAMP;
  - 7693 negative saliva samples analysed by Direct RT-LAMP
- Diagnostic Sensitivity and Specificity

  All samples (Clinical and spikes):
  - RNA RT-LAMP on Swabs: DSe 97% (CI 0.93-0.99); DSp 99% (CI 0.99-1.00)
  - RNA RT-LAMP on Saliva: DSe 82% (CI 0.68-0.91); DSp 100% (CI 0.99-1.00)
  - Direct RT-LAMP on Swabs: DSe 72% (CI 0.64-0.78); DSp 100% (CI 0.98-1.00)
  - Direct RT-LAMP on Saliva: DSe 80%% (CI 0.72-0.85) DSp 100% (CI 0.99-1.00)
- Because Direct RT-LAMP assay use case is to identify medium to high viral loads it is informative to also report the DSe and DSp with a RT-qPCR cut off. When using a cut off <25 and <33.
  - <25 Direct RT-LAMP on Swabs: DSe 100% (CI 0.96-1.00); DSp 100% (CI 0.98-1.00)
  - <25 Direct RT-LAMP on Saliva: DSe 97% (CI 0.88-0.99) DSp 100% (CI 0.99-1.00)
  - <33 Direct RT-LAMP on Swabs: DSe 78% (CI 0.70-0.84); DSp 100% (CI 0.98-1.00)
  - <33 Direct RT-LAMP on Saliva: DSe 83% (CI 0.74-0.89) DSp 100% (CI 0.99-1.00)

Additional data

- Local Verification reports
    - The OptiGene RT-LAMP assay has been locally verified in 9 sites, with the activities of each site listed below.

Site	RT-LAMP Evaluations
Basingstoke and North Hampshire Hospital	-Optimisation and original validation of RNA and Direct RT-LAMP protocols; -Limit of detection and analytical sensitivity (ASe) and specificity (ASp). -RNA RT-LAMP as routine screening tool; -Direct RT-LAMP in decentralised settings (Lab Van); -Direct and RNA Saliva and Swab RT-LAMP Asymptomatic Staff Pilot. -Direct and RNA Saliva and Swab RT-LAMP pair saliva/swab lighthouse lab evaluation.
University Hospital Southampton	-RNA RT-LAMP Asymptomatic Staff Pilot. -RNA RT-LAMP Lighthouse laboratory sample evaluation. -Analytical specificity.
Animal and Plant Health Agency/ MRC Lifecourse Epidemiology Unit (University of Southampton	-Direct Saliva RT-LAMP Mass Population Screening.
Public Health Lab Manchester/ CMFT	-Direct and RNA Swab and Saliva RT-LAMP Asymptomatic Staff Pilot.
Leeds Teaching Hospital NHS Trust	-Direct Swab RT-LAMP Evaluation under CONDOR.
Institute of Cancer & Genomic Science University of Birmingham	-Direct and RNA Saliva and Swab RT-LAMP Asymptomatic Staff Pilot. -Direct and RNA Saliva and Swab RT-LAMP pair saliva/swab lighthouse lab evaluation.
Division of Virology at Porton Down	-Direct and RNA Swab RT-LAMP as a surrogate for infectious virus recovery.
Public Health University Laboratory Gibraltar Health Authority	-Direct and RNA Swab and Saliva RT-LAMP in overseas territories with limited RT-qPCR capability.
Royal Hampshire County Hospital	-Direct and RNA Swab RT-LAMP in hospitals without microbiology labs.

Site	RT-LAMP Evaluations
	-Limit of detection and analytical sensitivity and specificity.

References

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