OptiGene Limited
Instructions For Use

COVID-19_RNA RT-LAMP KIT-500

COVID-19_RNA RT-LAMP KIT-500_IFU
Issue 1.5
Publish date 29/11/2021

OptiGene Limited
Instructions For Use (IFU)

COVID-19_RNA RT-LAMP KIT-500

In vitro RT-LAMP assay for SARS-CoV-2 viral RNA

CE IVD

500 reactions

REF COVID-19_RNA RT-LAMP KIT-500

OptiGene Limited, Unit 5 Blatchford Rd, Horsham, West Sussex, RH13 5QR

www.optigene.co.uk
info@optigene.co.uk
+44(0)1403 274980

Contents

1. Intended Use

The OptiGene Limited COVID-19_RNA RT-LAMP KIT-500 is intended to be used for detection of SARS-CoV-2 viral RNA in association with the Genie® platforms (section 6).

The COVID-19_RNA RT-LAMP KIT-500 was developed to be used on extracted RNA (or total nucleic acid) from oropharyngeal / nasopharyngeal swab samples. It is advised that COVID-19_RNA RT-LAMP KIT-500 is used for detection of SARS-CoV-2 RNA from patients with compatible symptoms < 7 days since symptom onset. The nature of the ORF1ab target being associated with replicating virus means > 7 days there is less probability of active virus being present and another diagnostic test should be used.

Validation has been performed using Sigma Virocult® oropharyngeal / nasopharyngeal swab samples (Medical Wire & Equipment, Corsham, UK [MW951S]) and a Maxwell® RSC Viral Total Nucleic Acid Purification Kit and Maxwell® RSC 48 Instrument (Promega UK Ltd., Southampton, UK). Kits are intended for use by laboratory trained personnel in an appropriately equipped facility.

2. Summary

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is a novel Coronavirus that emerged from Wuhan City, Hubei Province of China at the end of 2019 [1]. On 30 January 2020, the World Health Organization declared the outbreak to be a public health emergency of international concern [2].

The causative agent of COVID-19, SARS-CoV-2 is an enveloped, positive sense RNA virus belonging to the *Coronaviridae* family. Regular and reliable detection of SARS-CoV-2 RNA is required to monitor the spread of the virus and for screening of clinical samples from patients displaying relevant symptoms of COVID-19.

3. Principle of the Operation

The OptiGene COVID-19_RNA RT-LAMP KIT-500 is an *in vitro* diagnostic test based on Reverse Transcription Loop-mediated isothermal AMPlification (RT-LAMP) technology for the detection of SARS-CoV-2 viral RNA. The detection is carried out in a one-step, closed tube format where the reverse transcription and subsequent amplification of the specific target sequence occur in the same reaction well. The Genie® II, III & HT devices detect amplified product in real-time using fluorescence detection. The Genie® platforms automatically run an anneal curve at the end of amplification, where the reaction is heated to 98°C and slowly cooled. This acts as a secondary confirmatory check - ensuring LAMP amplicons are specific to SARS-CoV-2. The final result is interpreted and reported automatically from both the amplification and the anneal temperature.

4. Kit Components

The kit is supplied in liquid form. A mastermix (red lid) is supplied along with a separate primer mix (black lid), both sufficient to run 500 reactions (Table 1; Figure 1).

Table 1. COVID-19_RNA RT-LAMP KIT-500 components

Component	Number of vials	Reactions per vial	Lid colour
RNA RT-LAMP Mastermix	1	500	Red
10X COVID-19 Primer Mix	1	500	Black

NOTE: samples should be tested in duplicate, i.e. two reactions per sample.

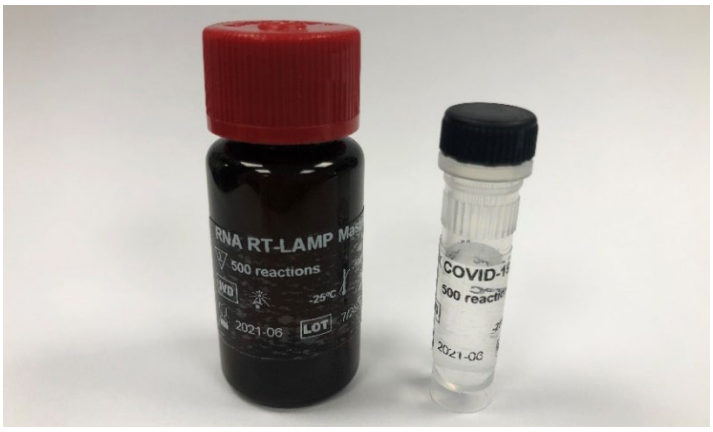

Figure 1. Contents of the COVID-19_RNA RT-LAMP KIT-500.

4.1. Product Description

The COVID-19_RNA RT-LAMP Kit enables the isothermal amplification of SARS-CoV-2 from extracted RNA. The mastermix contains a proprietary fast, novel GspSSD DNA polymerase I (8 U per reaction), Opti-RT reverse transcriptase (7.5 U per reaction), proprietary thermostable inorganic pyrophosphatase, optimised reaction buffer, $MgSO_4$, dNTPs and a ds-DNA binding dye (FAM detection channel). The kit further comprises separate (COVID-19) SARS-CoV-2 specific primers.

4.2. Storage requirements

- The OptiGene Limited COVID-19 RT-LAMP assays are shipped cold.
- On arrival, COVID-19 RT-LAMP assays should be stored in the original packaging at -17°C to -25°C (**NOT** using a frost-free freezer).
- The kits should not be used past the expiry date as indicated on the outer packaging label (RNA RT-LAMP Mastermix and 10X COVID-19 Primer Mix tube labels).
- Keep RNA RT-LAMP Mastermix away from light.
- Reagents may be aliquoted into smaller volumes if necessary.

5. Additional Material & Equipment Required but Not Supplied

The following list (Table 2) includes materials and equipment that are required for use but are not included within the COVID-19_RNA RT-LAMP KIT-500.

Table 2. Additional material and equipment required

Material / Equipment	Details
Adjustable calibrated pipettes	
Pipette tips (filter tips)	Barrier tips appropriate for the pipettes selected
Mini vortex	
Mini tube centrifuge	e.g. Genie® Centrifuge (cat. OP-FUGE)
Genie® II, III or HT device	See Section 6
Genie® tube strips	OptiGene Limited Genie® strips (cat. OP-0008 [section 5.1])
Genie® Strip Holder or cool block**	OptiGene Limited Genie® strip holder (cat. GBLOCK)
Plastic wear	2 ml screw cap tubes or 1.5 ml flip-top tubes
Nuclease-free water	
DNA/RNA degradation solution	e.g. DNAZap™ (cat. AM9890) or equivalent
Disinfectant	Approved disinfectant for COVID-19 samples
Positive control	e.g. OptiGene Limited COVID-19 Positive Control (cat. CD-COV19-100 or CD-COV19-500)
RNA extraction kit*	RNA or total nucleic acid
Appropriate PPE	

*Validation has been performed using Maxwell® RSC Viral Total Nucleic Acid Purification Kit and Maxwell® RSC 48 Instrument; other extraction methods require in-house validation.

**We recommend reactions are set up using a pre-cooled block, for example Genie® Strip Holders.

5.1. Genie® strips

All of the Genie® instruments operate with the OptiGene strip-of-8 reaction tubes (catalogue number Genie® strips OP-0008-50 [50 strips] and OP-0008-500 [500 strips]). This proprietary consumable incorporates attached, locking caps which allow for a closed tube assay to prevent cross-contamination.

6. Hardware Requirements

The OptiGene COVID-19 RT-LAMP tests have been validated for use on the OptiGene Limited Genie® platforms (Table 3). Platforms are manufactured by OptiSense Limited of Horsham, West Sussex.

Table 3. Genie® platforms for running the assay

Platform	Specifications
Genie® III 	**Catalogue number: GEN3-01** Number of wells: 8 wells (1 x Genie® strip) Dimensions: 25 (L) x 16 (W) x 8.5 (H) cm Rechargeable lithium battery: plug in or run for over 4 hours between charges
Genie® II 	**Catalogue number: GEN2-01** Number of wells: 16 wells (2 x Genie® strips) Dimensions: 20 (H) X 21 (D) X 30 (W) cm Rechargeable lithium battery: plug in or run for over 4 hours between charges Full random access: use any block, or number of blocks, at anytime
Genie® HT 	**Catalogue number: GEN-HT-01** Number of wells: 96 wells (12 x Genie® strips) Dimensions: 63.5 (L) X 43.4 (W) X 15.3 (H) cm Internal power supply: universal Full random access: use any block, or number of blocks, at anytime

All of the Genie® instruments should be set up and run by following the instruction manual provided.

7. Procedural Requirements

7.1. Facilities and training requirements

Testing for the presence of SARS-CoV-2 RNA should be performed in an appropriately equipped facility by trained staff.

7.2. Health and Safety Requirements

All samples should be handled as if they are infectious, following conventional biosafety precautions. National guidelines on biosafety should be followed in all circumstances. Used materials, kits and samples should be discarded according to national guidelines on biosafety and local waste policy.

7.3. Procedural requirements

- Dedicated and separate working areas for RT-LAMP reaction set up, sample preparation, nucleic acid extraction and amplification are advised. It is good practice to have these in separate rooms. A unidirectional workflow should be implemented between these areas (Table 4).
- Each workspace should have its own dedicated supply of PPE and equipment/ reagents which should not be shared with other spaces.

Table 4. Workspace requirements

Space	Details
Clean workspace	Preparation of reaction mixes; aliquoting of nuclease free water. Suitable for the preparation of RT-LAMP reaction mixes. Nuclease free water used for controls must be aliquoted and stored in this space. Original swab samples, RNA extracts, or post-amplification material must not be handled or stored in this space.
RNA addition workspace	Addition of RNA to reactions.
Amplification workspace	Genie® amplification.

8. Precautions for Users

8.1. General precautions

- This product is intended for use by trained users only, such as laboratory technicians and laboratory trained health professionals, with molecular biology experience. Individuals should be trained to undertake the procedures stated in this booklet including analysis of results.
- National guidelines on biosafety should be followed in all circumstances.
- All kit components should be handled using standard laboratory nitrile gloves.
- The Material Safety Data Sheet (MSDS) for the COVID-19 RT-LAMP kits are available from the OptiGene Limited website (http://www.optigene.co.uk/human-diagnostics/).
- For the extraction kit, consult the relevant MSDS from the supplier.

8.2. Analytical precautions

- **Ensure that samples have NOT had prior inactivation (chemical or heat) before the RNA extraction process. This could reduce the analytical sensitivity of the test.**
- **After thawing, individual components of the COVID-19_RNA RT-LAMP KIT-500 can be stored at 2°C to 8°C for up to one week.**
- **We do not advise pre-mixing and storage of RNA RT-LAMP Mastermix and 10X COVID-19 Primer Mix for more than 1 hour at 2°C to 8°C prior to starting reactions.**
- When in use, the time which components are at room temperature should be minimised.
- Repeated thawing and freezing of the RNA RT-LAMP Mastermix and 10X COVID-19 Primer Mix should be kept to a minimum and should not exceed 5 freeze-thaw cycles.
- Before and after each run has been set up, work surfaces and equipment must be cleaned with a DNA/RNA degradation solution.
- The RT-LAMP assays are highly sensitive and therefore easily contaminated. Consequently, workspaces must be frequently cleaned following local molecular workspace procedures.

- It is advisable to set up reactions in a PCR preparation hood.
- Ensure mixing of each kit component before use.
- **Post-amplification RT-LAMP reactions must NOT be opened.**
- Pipette tips must be barriered (filter tips) to prevent contamination.
- Use DNase/RNase-free disposable plasticware and pipette tips.
- Care should be taken to avoid contamination of the kit components. Where contamination is suspected, the kit should be discarded as laboratory waste.
- Do not use the kits past the expiration date.
- If the protective packaging of the kit is damaged upon receipt, please contact OptiGene Limited for instructions.
- The RT-LAMP primers target the SARS-CoV-2 *ORF1ab* genomic region. If performing in-house validation, the comparator test should also target a genomic region (any comparison to a sub-genomic region may artificially alter the apparent sensitivity of RT-LAMP).
- It is advised that COVID-19_RNA RT-LAMP KIT-500 is used for detection of SARS-CoV-2 RNA from patients with compatible symptoms < 7 days since symptom onset. The nature of the ORF1ab target being associated with replicating virus means > 7 days there is less probability of active virus being present and another diagnostic test should be used.

8.3. Positive control precautions

- **If using the OptiGene Limited** COVID-19 Positive Control**, this must be used as a stand-alone reagent.**
- The OptiGene Limited COVID-19 Positive Control has a distinct anneal to reduce cross contamination risk.
- RT-LAMP is not always compatible with positive controls traditionally used for other molecular tests including qRT-PCR. **A control that has been heat treated/gamma irradiated is unlikely to be suitable and can give a false lack of sensitivity.**
- Ensure that positive controls have not had prior inactivation (chemical or heat) prior to entering the assay.
- Positive controls should be handled away from the assay set-up to avoid contamination.
- Alternative commercially supplied controls may also contaminate the OptiGene Limited RT-LAMP assays.
- To reduce the risk of cross-contamination, positive controls should be handled in a separate space.

9. COVID-19_RNA RT-LAMP KIT-500 Test Procedure

This protocol describes the procedure for using the OptiGene Ltd. COVID-19_RNA RT-LAMP Kit-500 assay for the detection of SARS-CoV-2 from extracted RNA (Figure 2).

Samples should be collected according to UK Government guidelines. For the RNA extraction, consult the instructions for use of the extraction system / kit.

- Samples **must not** have been inactivated in any way (e.g. chemical or heat treatment) prior to the RNA extraction process (this could reduce the analytical sensitivity of the test).
- Samples **must not** be adulterated in any way prior to analysis (e.g. swab VTM must not be diluted prior to the procedures in this IFU).

NOTE: When performing the RNA extraction, a 'negative extraction control' (NEC) should be performed alongside the samples (as a separate sample).

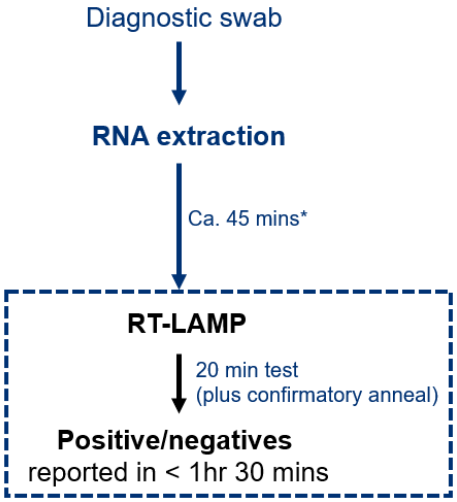

Figure 2. RT-LAMP workflow from extracted RNA. This procedure covers the boxed section. *Dependent upon extraction kit.

9.1. Reaction mix preparation

In the clean workspace:

9.1.1. Wipe surfaces and pipettes with DNA/RNA degradation solution.

9.1.2. Ensure the RNA RT-LAMP Mastermix and Primer Mix are well mixed (vortexed) before use.

9.1.3. Prepare enough reaction mix for the batch of samples. Samples should be tested in duplicate. It is recommended that at least one 'no template control' (NTC), one 'negative extraction control' (NEC) and one 'positive control' is used per batch of samples. Prepare the reaction mix as specified in Table 5. For example. If testing

20 samples, prepare enough reaction mix for these samples, controls, plus extra for pipetting error.

NOTE: A fresh reaction mix should be prepared before each batch of samples is tested. The time between mixing of the RNA RT-LAMP Mastermix and 10X COVID-19 Primer Mix and starting your reactions should be less than one hour. During this time, keep all reagents between 2°C to 8°C.

Table 5. Reaction mix preparation

Reagent	Volume per reaction (µl)
RNA RT-LAMP Mastermix	17.5
10X COVID-19 Primer Mix	2.5

9.1.4. After briefly vortexing (≤ 5 seconds), aliquot 20 µl of the prepared reaction mix into each required Genie® tube.

NOTE: Pipette the reaction mix into Genie® strips in a pre-cooled block (2°C to 8°C), for example Genie® Strip Holders.

9.1.5. For the NTC: Add 5 µl nuclease free water to the NTC reaction and close the lid to the locked position. Ensure the reaction is well mixed.

9.1.6. Loosely close the remaining Genie® tubes (ensuring they do not lock) and transfer to the RNA addition workspace. If necessary, numerically label the end of the Genie® strips.

9.2. Addition of extracted sample RNA to the Genie® strip

In the RNA addition workspace:

9.2.1. Add 5 µl of the extracted RNA sample to two adjacent Genie® tubes. Samples should be tested in duplicate: wells 1&2; wells 3&4; wells 5&6; wells 7&8. **Duplicates must be set up in the configuration shown in Figure 3: Genie® platforms analyse duplicates according to this set up**. Ensure the reactions are well mixed (e.g. pulse vortex).

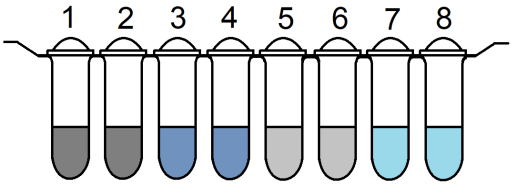

Figure 3. RT-LAMP duplicate layout. Duplicates should be run in wells 1&2; wells 3&4; wells 5&6; wells 7&8.

CAUTION: Only open the lids of Genie® tubes for one sample at a time; keep the others loosely closed until required. Close to the locked position after

addition of each individual sample. Samples should be added to reactions within a pre-cooled block (2°C to 8°C), for example Genie® Strip Holders.

9.3. Addition of negative extraction and positive controls to the Genie® strip

9.3.1. Add 5 µl NEC to the NEC reaction. Close the lid to the locked position.

9.3.2. Add 5 µl 'positive control' to the positive control reactions (e.g. OptiGene Limited COVID-19 Positive Control, following manufacturer's instructions). Close the lid to the locked position.

NOTE: Ensure that reactions are well mixed (e.g. pulse vortex).

9.4. Setting up the Genie®

Please refer to the instrument manual for full details. The most up-to-date profile for the COVID-19_RNA RT-LAMP KIT-500 is available from the OptiGene Limited website (http://www.optigene.co.uk/human-diagnostics/).

In the amplification workspace:

9.4.1. Turn on the Genie® II/III or HT machine at the main switch and wait for the software to initialise.

9.4.2. Ensure that the reagents are at the bottom of the tubes and that there are no large bubbles, e.g. by pulse spinning in a Genie® Centrifuge (≤ 2 seconds).

9.4.3. Load each Genie® strip into the chosen heat block.

9.4.4. Select the heat blocks required and the COVID-19 RNA DUPLICATES profile (Figure 4). See Figure 5 for the RT-LAMP profile.

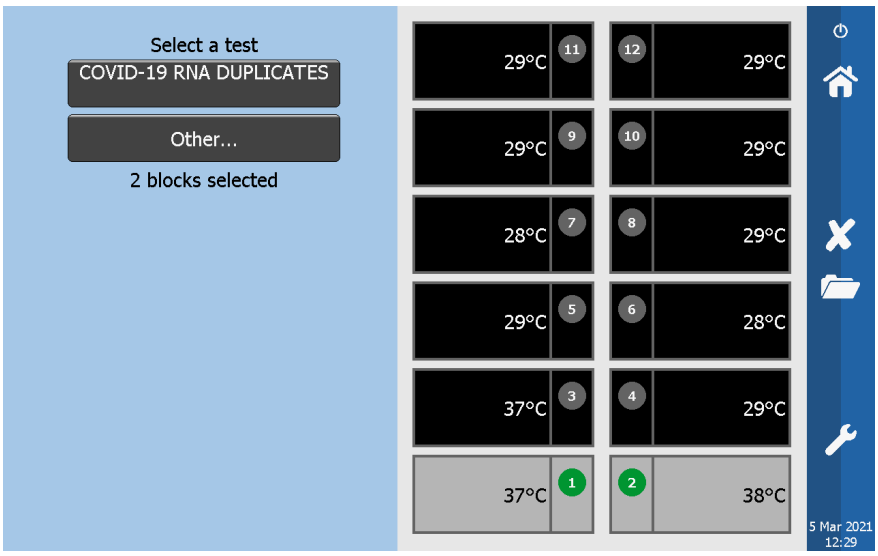

Figure 4. RNA RT-LAMP profile on a Genie® HT instrument.

9.4.5. Follow the screen's instructions, start the test and enter the relevant sample details for each Genie® tube.

NOTE: For the Genie® II and HT, heat blocks are random access and can be used independently of one another.

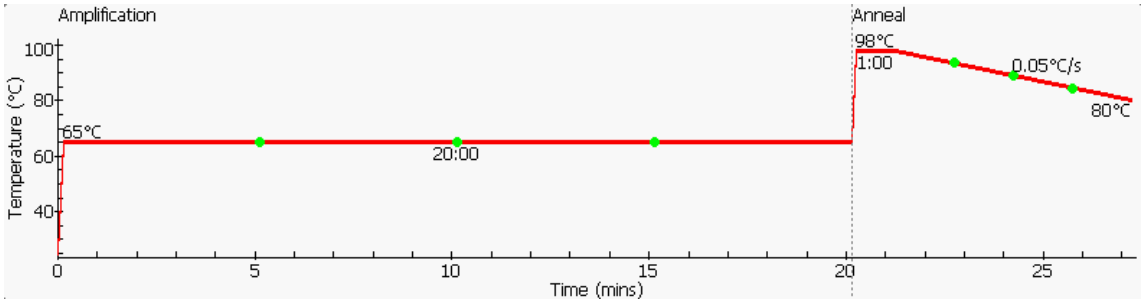

Figure 5. RT-LAMP profile. RT-LAMP is performed at 65°C for 20 minutes. Following amplification, anneal curve analysis is used to confirm that the correct product has amplified (reactions are heated to 98°C for 1 minute, then cooled to 80°C, decreasing the temperature by 0.05°C/s).

Reaction Time

Initial validation of the COVID-19_RNA RT-LAMP KIT-500 was performed using a 20-minute amplification time. However, further evaluation has shown that all RNA samples with qRT-PCR C_T values of ≤ 30 (COVID-19 genesig® Real-Time PCR assay) are detected within 16 minutes [3]. Contact OptiGene Limited for further information.

Single or duplicate samples

Initial validation of the COVID-19_RNA RT-LAMP KIT-500 was performed using duplicate samples [3], with further validation data (12619 samples in total) comprising of 588 samples tested in duplicate and 12031 sampled tested as single replicates [4]. Contact OptiGene Limited for further information and the COVID-19 RNA SINGLES profile (site-specific decision).

9.5. Interpretation of results

9.5.1. Genie® software will automatically analyse results and report samples as SARS-CoV-2-positive (SARS-CoV-2 RNA detected) or SARS-CoV-2-negative (levels of SARS-CoV-2 RNA below the detection limit of the assay).

9.5.2. For duplicate testing, Genie® software will report a sample as SARS-CoV-2-positive if SARS-CoV-2 RNA is detected in either of the two duplicates.

NOTE: An algorithm on the Genie® platforms analyses both the amplification plot and the anneal temperature to determine SARS-CoV-2-positive and SARS-CoV-2-negative reactions. A SARS-CoV-2-positive is reported automatically if (i) the fluorescence level of the amplification plot rises above a defined threshold and (ii) the peak of the anneal first derivative is above a defined threshold and lies within a specified temperature range.

9.5.3. If using the OptiGene Limited COVID-19 Positive Control, Genie® software will automatically analyse the results and report the sample as "Control Positive" if the amplification was successful.

9.5.4. The results of each run are automatically saved with a unique run number ID and are stored by day and month.

NOTE: For the Genie® HT, the last two digits after the hyphen represent the heat block that the run was performed on.

9.5.5. It is recommended that each batch of samples include at least one NTC, one NSC and a positive control. The run should only be counted as successful if a negative result is achieved in tubes for both negative controls and a positive result achieved for the positive control.

Troubleshooting

- A positive result in the NTC may indicate contamination of the clean workspace.
- A positive result in the NEC may indicate contamination at the sample processing or extraction stages.
- If contamination is observed, determine where the contamination has occurred, then thoroughly clean the workspace before repeating the RT-LAMP run(s).
- The positive control confirms that reactions have been set up correctly. A negative result in the positive control reaction may indicate errors in RT-LAMP reaction set up.

9.5.6. To view the results of a previous run, press the 'file' icon and choose the date that the test was performed. Runs from that date will then be visible to view.

10. Limitations of the Test

- OptiGene Limited COVID-19_RNA RT-LAMP KIT-500 has been validated for use with oropharyngeal / nasopharyngeal swab samples (using Sigma Virocult®, MW951S). In house validation should be performed if using a different sample type.
- OptiGene Limited COVID-19_RNA RT-LAMP KIT-500 has been validated for use with Maxwell® RSC Viral Total Nucleic Acid Purification Kit and Maxwell® RSC 48 Instrument (Promega UK Ltd., Southampton, UK). In house validation should be performed if using a different extraction kit.
- Assay validation has been performed using Genie® platforms only.
- Procedures in this IFU must be followed; any deviations may result in assay failure or cause erroneous results.
- Test quality is dependent on the quality of the sample.
- All results should be interpreted by a health care professional in the context of medical history, clinical symptoms and other diagnostic tests.
- Very low levels of RNA target, below the limit of detection, might be detected but results may not be reproducible.
- COVID-19_RNA RT-LAMP KIT-500 is specific to SARS-CoV-2, as such these RT-LAMP kits cannot rule out diseases caused by other pathogens.
- A negative result for COVID-19_RNA RT-LAMP KIT-500 does not conclusively rule out the possibility of infection.
- Optimum specimen types and timing for peak viral levels during infection caused by SARS-CoV-2 have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- Test performance can be affected because the epidemiology and clinical spectrum of infection caused by SARS-CoV-2 is not fully known (e.g. the optimum samples to collect during the course of infection, and when these specimens are most likely to contain levels of viral RNA that can be readily detected).
- Interpretation of results should account for the possibility of false negative and false positive results (Table 6).

Table 6. Potential causes of false negative and false positive results

False negative results	False positive results
Improper collection, handling and/or storage of samples	Improper handling of samples and/or positive controls
Samples with a viral load below the limit of detection	Contamination of workspaces
Improper sample processing (including unsuitable extraction kit)	Opening of reactions post-amplification
Mutations or polymorphisms in primer or probe binding regions	Deviation from handbook protocol
The presence of RT-LAMP inhibitors or interfering substances	
Deviation from handbook protocol	

11. Quality Control

In accordance with GeneSys Biotech Ltd (ISO9001:2015) Quality Management System, each component of the COVID-19_RNA RT-LAMP KIT-500 is tested against predetermined specifications to ensure consistent product quality. Quality control testing is performed using standard templates with results compared to previous lots.

12. Performance Evaluation

COVID-19_RNA RT-LAMP KIT-500 performance validation has been led by Hampshire Hospitals NHS Foundation Trust and generated by a number of independent sites [4] using:

- Sigma Virocult® oropharyngeal / nasopharyngeal swab (MW951S) samples (Medical Wire & Equipment, Corsham, UK)
- Maxwell® RSC Viral Total Nucleic Acid Purification Kit (AS1330) and Maxwell® RSC 48 Instrument (Promega UK Ltd., Southampton, UK)
- Genie® platforms (Section 6)

For the comparator real-time RT-PCR (qRT-PCR) assay, RNA was analysed using a range of different methods available at each site [4].

12.1. Evaluation overview

For the full validation report led by Hampshire Hospitals NHS Foundation Trust, see Kidd *et al.* (2021) [4]. The total number of samples which have been collated from the above sites for the purpose of this evaluation are:

- 251 qRT-PCR positive and 12368 qRT-PCR negative naso/oropharyngeal swabs (12619 total)

Diagnostic sensitivity and specificity

The diagnostic sensitive (DSe) and specificity (DSp) derived from the above numbers are as follows (DSe and DSp are calculated as an aggregate across all sites):

Table 7. COVID-19_RNA RT-LAMP KIT-500 summary of validation results

% Detection of swab samples with a viral load of ≥ 10^3 IU/ml	99.53 (95% CI: 97.39 - 99.99)
% Detection of all qRT-PCR swab positives[a]	96.81% (95% CI: 93.84 - 98.38)
Overall Diagnostic Specificity (DSp)[a] (Concordance with RT-qPCR to detect negatives)	99.98% (95% CI: 99.94 - 100.00)

[a] Samples were tested in singles using the qRT-PCR. For the statistics, it was assumed that the qRT-PCR results were correct. Cycle threshold (C_T) is the number of cycles required for the fluorescent signal to cross a defined threshold for the comparator qRT-PCR. For the qRT-PCR, a threshold of ≥38 C_T was set for determining clinically negative samples.
For RNA RT-LAMP, 588 samples were tested in duplicate and 12031 were tested as single replicates. Of those samples tested in duplicate, seven were detected by RNA RT-LAMP in only a single replicate [4]. For duplicate testing, a sample is classed as positive when at least one of the duplicates is RT-LAMP positive.

Analytical sensitivity (ASe) was determined using a ten-fold dilution series of SARS-CoV-2 RNA purified from virus infected tissue culture fluid I (BetaCoV/England/02/2020) obtained from Public Health England (Lot 07.02.2020) and compared to qRT-PCR results. The RT-LAMP was performed in duplicate (Table 8). qRT-PCR was performed using the COVID-19 genesig® Real-Time PCR assay (Primerdesign Ltd, Chandler's Ford, UK) on a Mic qPCR Cycler (Bio Molecular Systems, London, UK). The cycling conditions were adjusted to optimise the assay for the PCR thermocycler: reverse transcription (RT) step of 10 minutes at 55°C, hot-start step of 2 minutes at 95°C, followed by 45 cycles of 95°C for 10 seconds and 60°C for 30 seconds [3].

Table 8. Analytical sensitivity of RNA RT-LAMP

Dilution	qRT-PCR (C_T)	LAMP (T_P)	
		Replicate 1	Replicate 2
Neat	27.5	08:03	08:32
10^{-1}	30.0	10:44	11:45
10^{-2}	33.0	13:03	Negative
10^{-3}	36.0	14:29	Negative
10^{-4}	39.0	Negative	Negative

Samples were tested in duplicate; T_P: time to positivity (minutes:seconds); If SARS-CoV-2 RNA was detected in either of the duplicates, the Genie® calls the sample as positive.

12.2. Repeatability and reproducibility

Repeatability and inter-operator reproducibility were measured by running eight replicates of three samples with three different operators (Table 9). Operators used the same RNA extraction for each sample. For each sample, 100% of the replicates were detected.

Table 9. Repeatability and inter-operator reproducibility

		Mean time to positivity in minutes (% coefficient of variation)			
Sample	qRT-PCR C_T	Operator 1	Operator 2	Operator 3	Reproducibility between operators
Clinical patient sample (RNA diluted 1/10)	21.64	05:16 (0.61)	05:03 (0.51)	04:48 (0.26)	05:02 (4.72)
Viral positive control (diluted 1/10)	24.94	06:05 (1.44)	05:54 (1.22)	05:41 (1.08)	05:54 (3.38)
Viral positive control (diluted 1/100)	29.27	07:08 (4.24)	07:09 (2.72)	06:48 (6.06)	07:02 (2.85)

Criteria for acceptance: (i) mean time to positivity does not vary more than 20% and (ii) the mean anneal temperatures are within +/- 1°C. T_P: time to positivity (minutes:seconds). qRT-PCR was performed using the COVID-19 genesig® Real-Time PCR assay (above).

Inter-platform reproducibility was measured by running eight replicates of three samples with across two platforms (Table 10). The same RNA extraction was used for each sample across the two platforms. For each sample, 100% of the replicates were detected.

Table 10. Inter-platform reproducibility

		Mean time to positivity in minutes (% coefficient of variation)		
Sample	qRT-PCR C_T	Genie® HT	Genie® III	Reproducibility between platforms
Clinical patient sample (RNA diluted 1/10)	21.64	05:16 (0.61)	04:49 (0.61)	05:02 (6.37)
Viral positive control (diluted 1/10)	24.94	06:05 (1.44)	05:39 (1.33)	05:52 (5.35)
Viral positive control (diluted 1/100)	29.27	07:08 (4.24)	06:46 (4.54)	06:57 (3.71)

Criteria for acceptance: (i) mean time to positivity does not vary more than 20% and (ii) the mean anneal temperatures are within +/- 1°C. T_P: time to positivity (minutes:seconds). qRT-PCR was performed using the COVID-19 genesig® Real-Time PCR assay (above).

13. Technical Support

For technical support, please contact OptiGene Limited at:

Address: OptiGene Limited, Unit 5 Blatchford Rd, Horsham, West Sussex, RH13 5QR
Phone: +44(0)1403 274980
Email: info@optigene.co.uk

14. References

1) World Health Organization (21 January 2020). Situation report – 1: Novel Coronavirus (2019-nCoV). Available at https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200121-sitrep-1-2019-ncov.pdf?sfvrsn=20a99c10_4
2) World Health Organization (31 January 2020). Situation report – 11: Novel Coronavirus (2019-nCoV). Available at https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200131-sitrep-11-ncov.pdf?sfvrsn=de7c0f7_4
3) Fowler *et al*. (2020). A highly effective reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the rapid detection of SARS-CoV-2 infection. *Journal of Infection*, doi: 10.1016/j.jinf.2020.10.039.
4) Kidd, S.P. *et al*. (2021). RT-LAMP has high accuracy for detecting SARS-CoV-2 in saliva and naso/oropharyngeal swabs from asymptomatic and symptomatic individuals. medRxiv preprint doi: https://doi.org/10.1101/2021.06.28.21259398

15. Trademarks

16. Explanation of Symbols

Symbol	Explanation
	*In vitro* diagnostics
	Suffices for
	Batch code
	Catalogue number
	Manufacturer
	Use by date
	Consult electronic instructions for use
	Store at (temperature range)
	Keep away from sunlight

17. Changes to the Instructions For Use

The Instructions for Use may be subject to small changes. Any new revisions of the IFU will be published on the OptiGene Limited Website, under a new version number with any changes highlighted in Table 11 (http://www.optigene.co.uk/human-diagnostics/).

Table 11. COVID-19_RNA RT-LAMP KIT-500_IFU Version Changes

Version Number	Publication Date	Summary of Changes
V1.0	10/07/2020	N/A
V1.1	18/12/2020	• Requirement for a positive control • Information regarding caution around inactivated samples • Updated reference for Fowler *et al*. (2020)
V1.2	14/01/2021	• Typographic error in section 8.2 • Clarity over the positive control used (section 12.2) • Clarity added to selection of the duplicates LAMP profile (section 9.4)
V1.3	12/03/2021	• Genie® II screen shots removed. Genie HT® screen shots added in (section 9) • Clarity over the names of profile on Genie® (section 9.4) • Details added of where to find latest Genie® profiles (section 9.4) • Clarity added on how to handle mixed reagents (section 9.1 and 8.2)
V1.4	09/06/2021	• Clarity added over the amount of time to vortex and centrifuge reactions (section 9.1, 9.2, 9.3 & 9.4) • Name of COVID-19 Positive Control given with two catalogue codes (section 5) • Information amended for DNAZap™ (section 5) • Changes to wording in sections 4.2, 7.1, 7.2, 7.3, 8.1, 8.2, 9.1, 9.2 and Table 2
V1.5	29/11/2021	• Statement added to the intended use (section 1) and analytical precautions (section 8.2) regarding use case of RNA test • Statement added on sample collection (section 9) • Clarity added to name of profiles (section 9.4) • Clarity added to statement on reaction time (section 9.4) • Added information regarding singles vs duplicate samples based on publication of new data (section 9.4) • Update to wording in section 12 and 12.1. • Updated performance evaluation based on publication of new data (section 12 and 12.1) • PCR comparator details updated (section 9.4, 12, 12.1, 12.2) • Updated references based on publication of new data (section 14)

COVID-19_RNA RT-LAMP KIT-500_IFU
Issue 1.5
Publish date 29/11/2021