OptiGene Limited
Instructions For Use

COVID-19_Direct Plus RT-LAMP KIT-500

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

OptiGene Limited
Instructions For Use (IFU)

COVID-19_Direct Plus RT-LAMP KIT-500

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

CE IVD

500 reactions

REF COVID-19_Direct Plus RT-LAMP KIT-500

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1. Intended Use

The OptiGene Limited COVID-19_Direct Plus 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_Direct Plus RT-LAMP KIT-500 kit does not require RNA extraction and can be used directly on (i) oropharyngeal / nasopharyngeal swabs and (ii) saliva samples. **This method is to be utilised as a screening test to identify samples from individuals likely to have current SARS-CoV-2 infection, for the use case of asymptomatic testing.** Validation has been performed using (i) neat saliva samples (collected into CE IVD specimen containers that DO NOT contain preservatives, buffers or inactivating solutions) and (ii) Sigma Virocult® oropharyngeal / nasopharyngeal swab samples (Medical Wire & Equipment, Corsham, UK [MW951S]); other swab types and samples require in-house validation. 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_Direct Plus 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. RapiLyze Sample Buffer is provided (sufficient for 500 samples) for sample preparation. For the RT-LAMP reactions, a mastermix (amber bottle,

white lid) is supplied along with a separate primer mix (clear tube, black lid), both sufficient to run 500 reactions (Table 1, Figure 1).

Table 1. COVID-19_Direct Plus RT-LAMP KIT-500 components

Component	Number of vials	Reactions per vial	Appearance
Direct RT-LAMP Mastermix	1	500	Amber tube White lid
10X COVID-19 Primer Mix	1	500	Clear tube Black lid
RapiLyze Sample Buffer	1	500	Clear/opaque bottle Clear/white lid

Figure 1. Contents of the COVID-19_Direct Plus RT-LAMP KIT-500. Note that the RapiLyze Sample Buffer will be supplied in one of two container types.

4.1. Product Description

The COVID-19_Direct Plus RT-LAMP Kit enables the isothermal amplification of SARS-CoV-2 directly from swabs or saliva samples. The mastermix contains a proprietary fast, novel GspSSD2.0 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, a ds-DNA binding dye (FAM detection channel) and a proprietary enhancing agent. The kit further comprises separate (COVID-19) SARS-CoV-2 specific primers. The supplied RapiLyze Sample Buffer neutralises common sample inhibitors enabling simple and rapid processing of crude swab and saliva samples.

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 (Direct RT-LAMP Mastermix label, 10X COVID-19 Primer Mix tube label and RapiLyze Sample Buffer label).
- Keep Direct RT-LAMP Mastermix away from light.
- Keep RapiLyze Sample Buffer 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_Direct Plus 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)
Equipment for heating step**	2 ml screw cap tubes (suitable for heating to 98°C) and a dry heat block capable of heating to 98°C
Plastic wear	2 ml screw cap tubes or 1.5 ml flip-top tubes to make up the reaction mix
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)
Appropriate PPE	

*We recommend reactions are set up using a pre-cooled block, for example Genie® Strip Holders.
**Validation at Hampshire Hospitals NHS Foundation Trust was performed using a Benchmark Digital Dry Bath heat block (cat. BSH1004-E) using non-skirted 2 ml screw cap tubes for the heat step

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 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 protective gowns, pipettes, tips and other equipment/reagents which should not be shared with other spaces.

Table 4. Workspace requirements

Space	Details
Clean workspace	Preparation of reaction mixes, aliquoting of RapiLyze Sample Buffer 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, positive controls (for RT-LAMP and other assays) or post-amplification material must not be handled or stored in this space.
Sample processing workspace	Addition of samples to RapiLyze Sample Buffer; addition of sample in RapiLyze Sample Buffer to reactions. Suitable for handling COVID-19 samples. Minimal equipment/reagents should be stored in this laboratory. Samples should never be handled on an open bench (e.g. an appropriate microbiological safety cabinet should be used).
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/).

8.2. Analytical precautions

- **Ensure that samples have NOT had prior inactivation (chemical or heat) before entering the assay. This could reduce the analytical sensitivity of the test.**
- **Saliva samples must be collected neat into appropriate laboratory specimen tubes (collection tubes must NOT contain preservatives, buffers [e.g. phosphate buffer saline] or inactivation solutions) as this could reduce the analytical sensitivity of the test.**
- **RapiLyze should be stored at -17°C to -25°C. After thawing, RapiLyze can be stored at 2°C to 8°C for a maximum of one week (if stored at 2°C to 8°C for one week, discard the remaining RapiLyze and do not refreeze).**
- **After thawing, Direct RT-LAMP Mastermix and 10X COVID-19 Primer Mix can be stored at 2°C to 8°C for up to one week.**
- **We do not advise pre-mixing and storage of Direct 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 Direct RT-LAMP Mastermix, RapiLyze 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.
- 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).

8.3. Positive control precautions

- **If using the OptiGene Limited COVID-19 Positive Control, this must be used as a stand-alone reagent and must NOT be mixed with samples (e.g. saliva or swab viral transfer media) as this will shift the reaction anneal temperature and impact result calling.**
- 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_Direct Plus RT-LAMP Procedure

This protocol describes the procedure for using the OptiGene Limited COVID-19_Direct Plus RT-LAMP assay for the detection of SARS-CoV-2 RNA directly from swabs and saliva. This method is to be utilised as a screening test to rapidly identify samples from patients likely to have current SARS-CoV-2 infection. Samples should be collected according to UK Government guidelines.

- Samples **must not** have been inactivated in any way (e.g. chemical or heat treatment) prior to analysis (this could reduce the analytical sensitivity of the test).
- Patient samples **must not** be collected into any sample preservative or buffer as these could inhibit the reaction.
- Samples **must not** be adulterated in any way prior to analysis (e.g. saliva and swab VTM must not be diluted prior to the procedures in this IFU).

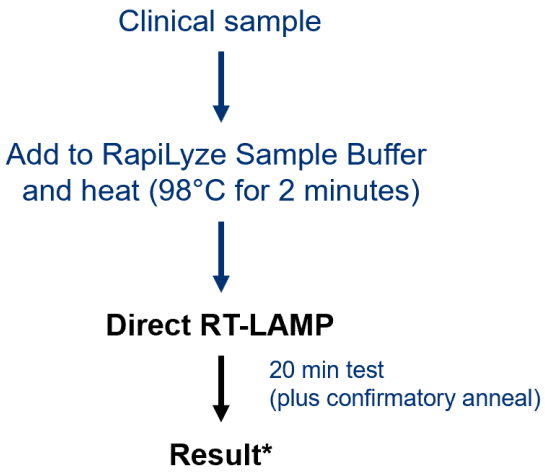

Figure 2. Direct Plus RT-LAMP workflow.
*If higher sensitivity is required, negative samples can be subjected to confirmatory testing (e.g. CE IVD approved qRT-PCR or RT-LAMP following RNA extraction).

9.1. Aliquoting of RapiLyze Sample Buffer for samples and negative sample controls

In the clean workspace:

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

9.1.2. Prepare enough 50 µl aliquots of RapiLyze Sample Buffer in screw cap tubes for the batch of samples to be processed, including one extra as a negative process control (NPC). Seal the tubes for transport into the sample processing room.

9.2. Reaction mix preparation

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

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

9.2.3. Prepare enough reaction mix for the batch of samples. It is recommended that at least one no template control (NTC), one NPC 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 Direct 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)
Direct RT-LAMP Mastermix	17.5
10X COVID-19 Primer Mix	2.5

9.2.4. 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.

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

9.2.6. Loosely close the remaining Genie® tubes (ensuring they do not lock) and transfer the reaction mixes to the sample processing workspace.

9.3. Sample preparation

In the sample processing workspace:

9.3.1. Disinfect work surfaces and pipettes before use.

9.3.2. Label the aliquots of RapiLyze Sample Buffer with the sample numbers to be processed.

9.3.3. Prepare the samples as follows:

- For swabs, vortex the swab tube, then add 50 µl of the virus transport medium (VTM) from the swab container to a 50 µl aliquot of RapiLyze Sample Buffer, resulting in a 1:1 dilution of the VTM.

- For Saliva, add 50 µl of the neat saliva sample to a 50 µl aliquot of RapiLyze Sample Buffer, resulting in a 1:1 dilution of the saliva sample. For viscous samples, use a positive displacement pipette.
- Ensure that a NPC is run alongside, by adding 50 µl NFW to a 50 µl aliquot of RapiLyze Sample Buffer.

CRITICAL: On addition of the sample to RapiLyze Sample Buffer you should proceed onto the next step (the sample must not be left in the RapiLyze Sample Buffer). Delays in this step may negatively impact the sensitivity of the RT-LAMP kit. Ensure that the pipette is decontaminated between samples.

9.3.4. Ensure screw cap tubes are tightly shut. Using a vortex, pulse vortex the tubes to ensure that the sample and RapiLyze Sample Buffer are well mixed.

9.3.5. Place samples in dry heat block pre-heated to 98°C for two-minutes.

CRITICAL: Once the samples have been heated you should proceed onto the next step (do not store or refrigerate the heated samples prior to use). Delays in this step may negatively impact the sensitivity of the RT-LAMP kit.

NOTE: Once heating is completed, if required, samples can be briefly spun in a centrifuge (pulse spin ≤ 2 seconds) to ensure the sample is at the bottom of the tubes.

9.4. Addition of the sample

In the sample processing workspace:

9.4.1. For each reaction, add 5 µl of the heat-treated sample in RapiLyze Sample Buffer to a RT-LAMP reaction in a Genie® strip. Numerically label the end of the Genie® strips.

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.

9.4.2. Add 5 µl NPC to the NPC reaction. Close the lid to the locked position

9.4.3. 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).

9.4.4. Disinfect the Genie® tubes (ensure each tube is fully locked, spray with disinfectant and dry with a paper towel) before removing from the sample processing workspace. Take these tubes to the amplification workspace.

CAUTION: The Genie® tubes must NOT be opened after removal from the sample processing workspace.

9.5. Setting up the Genie®

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

In the amplification workspace:

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

9.5.2. Ensure the Genie® strips are dry and free from disinfectant before loading onto the machine. 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).

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

9.5.4. Select the heat blocks required and either the SINGLES or DUPLICATES COVID-19 Direct Plus profile, depending on whether samples are being run in single or duplicate reactions (Figure 3). See Figure 4 for the RT-LAMP profile.

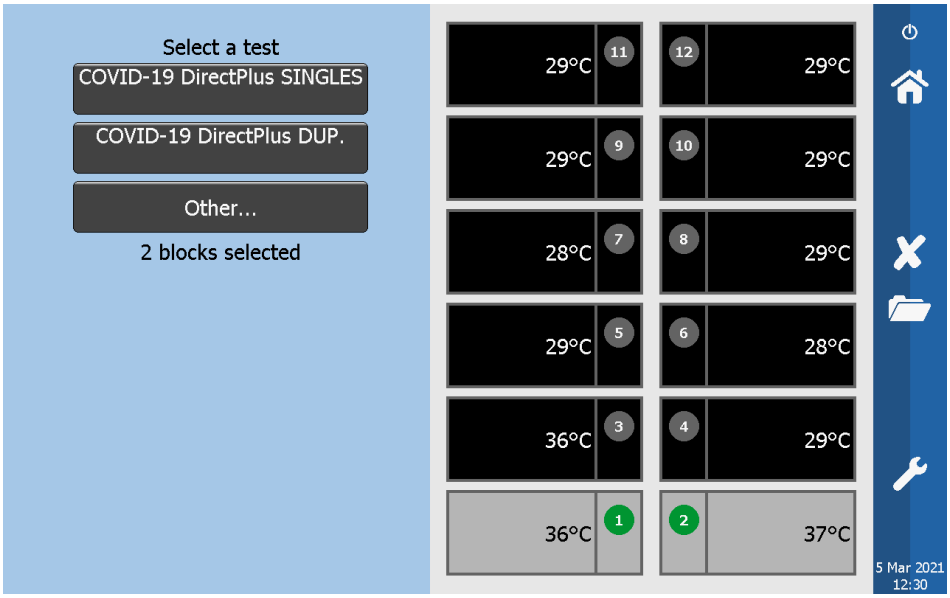

Figure 3. RT-LAMP Direct Plus profiles on a Genie® HT instrument.

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

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

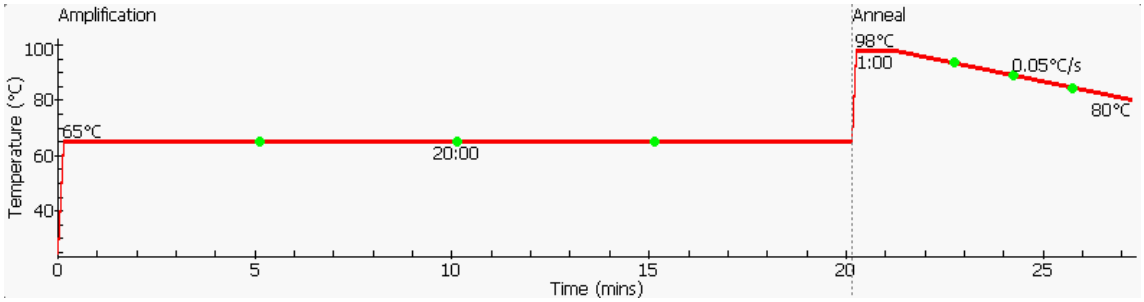

Figure 4. RT-LAMP profile. RT-LAMP is performed at 65°C for 20 minutes. Following amplification, anneal curve analysis is used to confirm that the correct product has amplified (reactions are heated to 98°C for 1 minute, then cooled to 80°C, decreasing the temperature by 0.05°C/s).

Single or duplicate samples

When running the assay in duplicates (site-specific decision), the duplicates must be set up as in Figure 5. The Genie will call the sample POSITIVE if SARS-CoV-2 RNA is detected in either of the duplicate reactions.

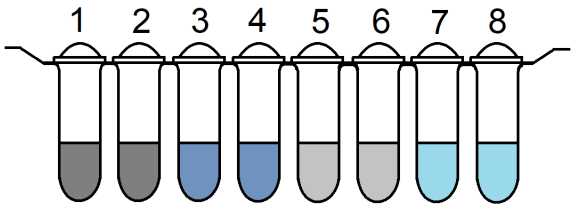

Figure 5. RT-LAMP duplicate layout. Duplicates should be run in wells 1&2; wells 3&4; wells 5&6; wells 7&8.

Reaction Time

Initial validation of the COVID-19_Direct Plus RT-LAMP KIT-500 was performed using a 20-minute amplification time. This time has been reduced to 17-minutes at Hampshire Hospitals NHS Foundation Trust through independent verification and validation. Contact OptiGene Limited for additional information regarding the 17-minute Direct Plus LAMP Genie® profile.

9.6. Interpretation of Results

9.6.1. Genie® software will automatically analyse results and report samples as POSITIVE (SARS-CoV-2 RNA detected) or NEGATIVE (levels of SARS-CoV-2 RNA below the detection limit of the assay [Section 12]).

NOTE: An algorithm on the Genie® platforms analyses both the amplification plot and the anneal temperature to determine POSITIVE and 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.

9.6.2. 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.

9.6.3. 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.

9.6.4. It is recommended that each batch of samples include at least one NTC, one NPC 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 NPC may indicate contamination at the sample processing stage.
- 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.

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.

NOTE: If higher sensitivity is required, negative samples can be subjected to confirmatory testing (e.g. CE IVD approved qRT-PCR or RT-LAMP following RNA extraction).

10. Limitations of the Test

- OptiGene Limited COVID-19_Direct Plus RT-LAMP KIT-500 has been validated for use with saliva (neat) or oropharyngeal / nasopharyngeal swab samples (using Sigma Virocult®, MW951S). In house validation should be performed if using a different sample type.
- 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_Direct Plus RT-LAMP KIT-500 is specific to SARS-CoV-2, as such these RT-LAMP kits cannot rule out diseases caused by other pathogens.
- COVID-19_Direct Plus RT-LAMP KIT-500 is intended as a screening tool identify samples from individuals likely to have current SARS-CoV-2 infection; a negative result does not 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_Direct Plus 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 RT-LAMP KIT-500 performance validation has been led by Hampshire Hospitals NHS Foundation Trust and generated by a number of independent sites [3] using:

- Neat Saliva samples collected into sterile tubes (collected into CE IVD sterile empty tubes that DO NOT contain preservatives, buffers or inactivating solutions).
- Sigma Virocult® oropharyngeal / nasopharyngeal swab (MW951S) samples (Medical Wire & Equipment, Corsham, 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 [3].

12.1. Evaluation overview

For the full validation report led by Hampshire Hospitals NHS Foundation Trust, see Kidd *et al*. (2021) [3]. The total number of samples which have been collated from the above sites for the purpose of this evaluation are:

- 199 qRT-PCR positive and 360 qRT-PCR negative naso/oropharyngeal swabs analysed by Direct RT-LAMP (559 total);
- 247 qRT-PCR positive and 7195 qRT-PCR negative saliva samples analysed by Direct RT-LAMP (7442 total)

This study tried to carefully balance the inclusion of low, medium and high viral load samples.

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_Direct Plus RT-LAMP KIT-500 summary of validation results

		Clinical samples
Diagnostic sensitivity for swabs	**% Detection of swab samples with a viral load of ≥ 10^6 IU/ml**	100.00 % (95% CI: 95.20 - 100.00)
	% Detection of swab samples with a viral load of ≥ 10^3 IU/ml	78.74 % (95% CI: 71.90 - 84.56)
	% Detection of all qRT-PCR swab positives	70.35% (95% CI 63.67 - 76.26)
Overall Diagnostic Specificity for swabs (DSp)[a] (Concordance with RT-qPCR to detect negatives)		100.00% (95% CI 98.94 - 100.00)
Diagnostic sensitivity for saliva	**% Detection of saliva samples with a viral load of ≥ 10^6 IU/ml**	100.00 (95% CI: 95.20 - 100.00)
	% Detection of saliva samples with a viral load of ≥ 10^3 IU/ml	86.92 (95% CI: 81.95 - 90.94)
	% Detection of all qRT-PCR saliva positives	84.62% (95% CI: 79.59 - 88.58)
Overall Diagnostic Specificity for saliva (DSp)[a] (Concordance with RT-qPCR to detect negatives)		100.00% (95% CI 99.95 - 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, thresholds were as per the Instructions for Use.
For the swab samples, 195 samples were tested in duplicate and 364 tested as single replicates using RT-LAMP. Seven of 195 samples tested in duplicate were positive by Direct RT-LAMP in only one replicate [3]. For the saliva samples, 83 samples were tested in duplicate and 7359 were tested as single replicates using RT-LAMP. Nine of the 83 samples tested in duplicate were negative in one of the duplicates [3]. For duplicate testing, a sample is classed as positive when at least one of the duplicates is RT-LAMP positive.

Analytical sensitivity

The limit of detection (LOD) for the Direct Plus RT-LAMP assay was evaluated using inactivated virus. Standardised and quantified inactivated virus was provided as blinded samples by NIBSC (National Institute for Biological Standards and Control, Potters Bar, UK). 5 µl of these controls were added to the Direct RT-LAMP Mastermix with 10X COVID-19 Primer Mix (the lysis and heat step was not required as the sample was inactivated). Direct RT-LAMP was able to detect 10^3 copies/ml in one of two duplicates (Table 8).

Table 8. Analytical sensitivity of Direct RT-LAMP

NIBSC Control	Concentration	Mean time to positivity in minutes
Inactivated virus	10^6/ml	08:12
Inactivated virus	10^5/ml	09:35
Inactivated virus	10^4/ml	10:14
Inactivated virus	10^3/ml	11:07*
Inactivated virus	10^2/ml	Negative
Inactivated virus	10^1/ml	Negative

T_P: time to positivity (minutes:seconds). Reactions performed in duplicate. *Detected in one of two duplicates.

Analytical specificity

Analytical specificity was determined using a panel of respiratory pathogens. In all cases there was no cross reactivity observed including against and respiratory pathogen including other common coronaviruses (Table 9).

Table 9. COVID-19_Direct Plus RT-LAMP KIT-500 specificity

Respiratory Pathogens	COVID-19_Direct RT-LAMP KIT-500 result
Coronavirus OC43	Not Detected
Adenovirus 31	Not Detected
Parainfluenza 4	Not Detected
Influenza B	Not Detected
Influenza A H3	Not Detected
Parainfluenza 3	Not Detected
Rhinovirus 1A	Not Detected
Coronavirus 229E	Not Detected
Parainfluenza 2	Not Detected
Adenovirus 1	Not Detected
Coronavirus NL63	Not Detected
Respiratory Syncytial virus A	Not Detected
Influenza AH1N1	Not Detected
Parainfluenza 1	Not Detected
M Pneumoniae	Not Detected
Adenovirus 3	Not Detected
Bordetella pertussi	Not Detected
Chlamydia pneumoniae	Not Detected
Bordetella parapertussis	Not Detected
Coronavirus HKU-1	Not Detected
Human metapneumovirus 8	Not Detected
Influenza AH1	Not Detected
Other Pathogens	
Neisseria meningitidis	Not Detected
Streptococcus agalactiae	Not Detected
Haemophilus influenzae	Not Detected
Herpes simplex virus 2	Not Detected
Listeria monocytogenes	Not Detected
Parechovirus type 3	Not Detected
Varicella zoster virus	Not Detected

12.2. Repeatability and inter-operator reproducibility

Repeatability and inter-operator reproducibility were measured by running eight replicates of four clinical samples with three different operators (Table 10). Each operator independently mixed the sample 1:1 in RapiLyze, which was used for each of their replicates. Data was generated by Hampshire Hospitals NHS Foundation Trust. RT-LAMP results were compared to qRT-PCR using the Certest VIASURE SARS-CoV-2 Real Time PCR Detection Kit on a Mic qPCR Cycler (Bio Molecular Systems, London, UK). RNA extraction for the qRT-PCR was performed using Maxwell® RSC Viral Total Nucleic Acid Purification Kit and Maxwell® RSC 48 Instrument (Promega UK Ltd., Southampton, UK).

Table 10. Inter-operator reproducibility

		Mean time to positivity in minutes (% coefficient of variation) Mean anneal temperature [Number of replicates positive]			
Sample	qRT-PCR C_T	Operator 1	Operator 2	Operator 3	Reproducibility between operators
Clinical patient sample 1 (Swab VTM)	19.43	06:45 (0.67) 84.19°C [8/8]	06:34 (0.94) 84.04°C [8/8]	07:03 (0.95) 84.11°C [8/8]	06:48 (3.59)
Clinical patient sample 2 (Saliva)	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)
Clinical patient sample 3 (Swab VTM)	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)
Clinical patient sample 4 (Saliva)	23.13	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. T_P: time to positivity (minutes:seconds). Samples were collected on 24/09/2020; tests were performed on 18/10/2020.

12.3. Repeatability and inter-platform reproducibility

Inter-platform reproducibility was measured by running eight replicates of a clinical sample with across two platforms (Table 11). The same 1:1 sample in RapiLyze Sample Buffer was used across the two platforms. Data was generated by Hampshire Hospitals NHS Foundation Trust.

Table 11. Inter-platform reproducibility

		Mean time to positivity in minutes (% coefficient of variation) [Number of replicates positive]		
Sample	qRT-PCR C_T	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)
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. T_P: time to positivity (minutes:seconds). Samples were collected on 24/09/2020; tests were performed on 18/10/2020.

13. Technical Support

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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) 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 12 (http://www.optigene.co.uk/human-diagnostics/).

Table 12. COVID-19_Direct Plus RT-LAMP KIT-500_IFU Version Changes

Version Number	Publication Date	Summary of Changes
V1.0	30/10/2020	N/A
V1.1	18/12/2020	• Date of sampling added to repeatability • Catalogue number change for positive control
V1.2	14/01/2021	• Typographic error in sections 8.2 and 12.1 • Updated clinical statement from Hampshire Hospitals NHS Foundation suggesting a 17-minute amplification time (section 9.5)
V1.3	12/03/2021	• Typographic error in section 1 • Genie® II screen shots replaced with Genie HT screen shots (section 9.5) • Clarity over the names of profiles on Genie® (section 9.5) • Details added of where to find latest Genie® profiles (section 9.5) • Clarity added on how to handle mixed reagents (section 9.2 and 8.2)
V1.4	07/07/2021	• Clarity added to heat block stage (section 9.3) • Clarity added over the amount of time to vortex and centrifuge reactions (section 9.2, 9.3, 9.4 & 9.5) • Clarity added to storage of RapiLyze (section 8.2) • Clarity added over handling of samples once mixed with RapiLyze (section 9.3) • Name of COVID-19 Positive Control given with two catalogue codes (throughout) • Typographic error in section 9.4 • Information amended for DNAZap™ (section 5) • Negative sample control renamed as negative process control (throughout) • Changes to wording (clarifications) in sections 1, 7, 8, 9.2, 9.4, 10, 12.1 • Clarity added to Tables 2 and 9
V1.5	29/11/2021	• Statement added to the intended use (section 1) • Statement added on sample collection (section 9) • Clarity added to name of profiles (section 9.5) • Clarity added to statement on reaction time (section 9.5)

		• Update to wording in section 12 and 12.1. • Updated clinical performance evaluation based on publication of new data (section 12 and 12.1) • PCR comparator details updated (section 12, 12.1, 12.2) • Updated references based on publication of new data (section 14)

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Issue 1.5
Publish date 29/11/2021