OptiGene Limited
Instructions For Use

COVID-19 Positive Control

CD-COV19_IFU
Issue 1.0
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OptiGene Limited
Instructions For Use (IFU)

COVID-19 Positive Control

Positive DNA control for

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

CE IVD

Each tube contains 50 reactions

REF CD-COV19-100 (100 reactions)
CD-COV19-500 (500 reactions)

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Contents

1. Intended Use

The COVID-19 Positive Control is a DNA control intended to be used with the following OptiGene Limited *in vitro* diagnostic kits:

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

The COVID-19 Positive Control has been validated for use as a stand-alone reagent. The control is 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. When using molecular diagnostic assays for the detection of SARS-CoV-2 RNA, positive controls can be used to confirm that reactions have been set-up correctly.

3. Principle of the Operation

The COVID-19 Positive Control is a linear double-stranded DNA control developed for use with (i) COVID-19_RNA RT-LAMP KIT-500 and (ii) COVID-19_Direct Plus RT-LAMP KIT-500. COVID-19 RT-LAMP kits are based on Reverse Transcription Loop-mediated isothermal AMPlification (RT-LAMP) for the detection of SARS-CoV-2 RNA. 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 COVID-19 Positive Control contains the LAMP primer-targeting regions of the COVID-19 RT-LAMP kits. When used in combination with the COVID-19 RT-LAMP kits, amplification and detection of the COVID-19 Positive Control indicates that reactions have been set-up correctly.

The Genie® platforms (section 6) automatically run an anneal curve at the end of amplification, where reactions are heated to 98°C and slowly cooled. SARS-CoV-2 and the COVID-19 Positive Control amplicons have different anneal temperatures (Section 11), therefore this stage acts as a secondary confirmatory check - ensuring LAMP amplicons are specific to either SARS-CoV-2 or to the COVID-19 Positive Control. The final result is interpreted and reported automatically from both the amplification plot and the anneal temperature.

4. Components

The COVID-19 Positive Control is supplied in liquid form, with each tube sufficient to run 50 reactions. Two pack sizes are available (Table 1; Figure 1), each provided with a kit data sheet.

Table 1. CD-COV19 components

Catalogue number	Number of vials	Reactions per vial	Lid colour
CD-COV19-100	2	50	White
CD-COV19-500	10	50	White

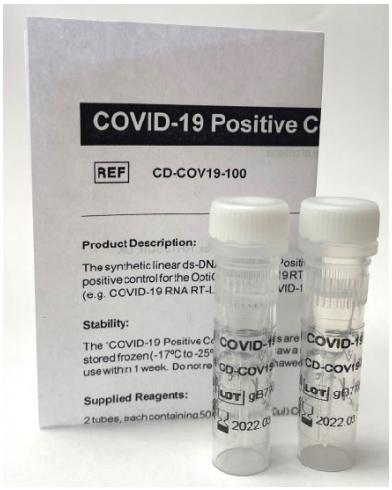

Figure 1. Contents of CD-COV19-100

4.1. Product description

The COVID-19 Positive Control is a synthetic linear double-stranded DNA containing the primer-targeting sequences for the OptiGene COVID-19 RT-LAMP kits. The sequence of the COVID-19 Positive Control differs from that of SARS-CoV-2, allowing differentiation between the annealing temperatures of the COVID-19 Positive Control and SARS-CoV-2 amplicons.

4.2. Storage requirements

- COVID-19 Positive Controls are shipped cold.
- On arrival, COVID-19 Positive Controls should be stored in the original packaging at -17°C to -25°C (**NOT** using a frost-free freezer).
- **Thaw COVID-19 Positive Control between 2°C to 8°C. Continue to store at 2°C to 8°C and use within one week (do not re-freeze).**
- The kits should not be used past the expiry date as indicated on the outer packaging label and individual tube labels.
- Reagents may be aliquoted into smaller volumes if necessary.

5. Additional Material & Equipment Required but Not Supplied

The COVID-19 Positive Control is intended to be used with OptiGene Limited *in vitro* diagnostic kits (Table 2). All additional materials and equipment required but not supplied, including hardware requirements, can be found within the respective kit's instructions for use (IFU). IFUs are available from the OptiGene website (http://www.optigene.co.uk/human-diagnostics/).

Table 2. COVID-19 RT-LAMP KITS

Kit catalogue code	Details
COVID-19_RNA RT-LAMP KIT-500	Detection of SARS-CoV-2 viral RNA from samples following RNA extraction
COVID-19_Direct Plus RT-LAMP KIT-500	Detection of SARS-CoV-2 viral RNA directly from samples

6. Procedural Requirements

6.1. Facilities and training requirements

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

6.2. Health and safety requirements

The Material Safety Data Sheet (MSDS) for the COVID-19 Positive Control is available from the OptiGene Limited website (http://www.optigene.co.uk/human-diagnostics/).

6.3. Procedural requirements

- **COVID-19 Positive Control must be used as a stand-alone reagent. It should NOT be added to clinical samples, be used as an internal sample control or be put through a nucleic acid extraction procedure.**
- **It is recommended that at least one positive control reaction is set up per batch of samples / reaction mix batches.**
- COVID-19 Positive Control is supplied at the intended working concentration, it should not be diluted.
- COVID-19 Positive Control has a distinct anneal temperature to reduce cross contamination risk.
- COVID-19 Positive Control is a DNA control. Therefore, products used to clean areas where it has been handled must be able to degrade DNA (e.g. DNAZap™ [cat. AM9890] or equivalent).
- Dedicated and separate working areas for RT-LAMP reaction set up, COVID-19 Positive Control addition and amplification are advised. It is good practice to have

these in separate rooms. A unidirectional workflow should be implemented between these areas.

- To reduce the risk of cross-contamination, COVID-19 Positive Control should be handled in a separate workspace, away from the assay set-up.
- Each workspace should have its own dedicated supply of personal protective equipment (PPE) and equipment / reagents, which should not be shared with other spaces.

7. Precautions for Users

7.1. 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 components should be handled using standard laboratory nitrile gloves.

7.2. Analytical precautions

- **Repeated freezing and thawing should be avoided. After thawing (at 2°C to 8°C), COVID-19 Positive Control should be stored at 2°C to 8°C and used within one week.**
- When in use, the time which components are at room temperature should be minimised.
- The RT-LAMP assays are highly sensitive and therefore easily contaminated. Consequently, workspaces must be frequently cleaned following local molecular workspace procedures.
- Before and after each run has been set up, work surfaces and equipment must be cleaned with a DNA degradation solution.
- It is advisable to set up reactions in a PCR preparation hood.
- Ensure mixing of the COVID-19 Positive Control 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.
- Do not use COVID-19 Positive Control past the expiration date.
- If the protective packaging of the COVID-19 Positive Control is damaged upon receipt, please contact OptiGene Limited for instructions.

8. COVID-19 Positive Control Test Procedure

This protocol describes the procedure for using the OptiGene Ltd. COVID-19 Positive Control. The COVID-19 Positive Control is designed for use with either:

(i) COVID-19_RNA RT-LAMP KIT-500
(ii) COVID-19_Direct Plus RT-LAMP KIT-500.

This IFU is intended to be used alongside the IFU for the chosen OptiGene COVID-19 RT-LAMP kit, which are available from the OptiGene website:

http://www.optigene.co.uk/human-diagnostics/

8.1. Reaction mix preparation

Please refer to the IFU for the relevant COVID-19 RT-LAMP kit.

8.2. Addition of COVID-19 Positive Control to the RT-LAMP reactions

8.2.1. Ensure mixing of the COVID-19 Positive Control before use (e.g. pulse vortex)

8.2.2. Add 5 µl COVID-19 Positive Control to the appropriate reaction well(s). Close the lid(s) to the locked position.

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

8.3. Setting up the Genie®

Please refer to the IFU for the relevant COVID-19 RT-LAMP kit.

8.4. Interpretation of results

8.4.1. Genie® software will automatically analyse the results and report the sample as Control Positive if the amplification was successful.

NOTE: SARS-CoV-2 and negative results will be reported according to the profile being used. Please refer to the specific IFU for the COVID-19 RT-LAMP kit.

8.4.2. If a duplicate profile is being used, Genie® software will report the sample as Control Positive if the COVID-19 Positive Control 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 positive and negative reactions. A Control 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.

8.4.3. 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.

8.4.1. 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.

Troubleshooting

- A Control Positive result in a no template control may indicate contamination of the clean workspace.
- A Control Positive result in the negative sample control, negative process control, negative extraction control or for a sample may indicate contamination at sample processing, extraction or sample addition stages.
- If contamination is observed, determine where the contamination has occurred, then thoroughly clean the workspace before repeating the RT-LAMP run(s).
- The COVID-19 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. Limitations of the Test

- 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.
- Interpretation of results should account for the possibility of false negative and false positive results (Table 3).

Table 3. Potential causes of false negative and false positive results

False negative results	False positive results
Improper handling and/or storage of COVID-19 Positive Control	Improper handling of COVID-19 Positive Control
Deviation from handbook protocol	Deviation from handbook protocol
	Contamination of workspaces
	Opening of reactions post-amplification

10. Quality Control

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

11. Performance Evaluation

COVID-19 Positive Control performance validation has been generated by GeneSys Biotech Ltd using Genie® platforms.

11.1. Repeatability and reproducibility

Repeatability and inter-operator reproducibility were measured by running eight replicates of three LOTs of COVID-19 Positive Control for both (i) COVID-19_RNA RT-LAMP KIT-500 and (ii) COVID-19_Direct Plus RT-LAMP KIT-500 (Table 4). For each sample, 100% of the replicates were detected.

Table 4. Repeatability and inter-operator reproducibility

		Mean time to positivity in minutes (% coefficient of variation) [Mean anneal temperature]			
LOT	Kit	Operator 1	Operator 2	Operator 3	Reproducibility between operators
gB77/2/j	COVID-19_Direct Plus RT-LAMP KIT-500	05:11 (0.84) [86.68ºC]	05:35 (0.36) [86.67ºC]	05:27 (0.78) [86.30ºC]	05:25 (3.89) [86.55ºC]
gB77/2/k	COVID-19_Direct Plus RT-LAMP KIT-500	05:34 (3.73) [86.67ºC]	05:21 (1.08) [86.65ºC]	05:39 (1.68) [86.57ºC]	05:31 (2.77) [86.63ºC]
gB77/2/l	COVID-19_Direct Plus RT-LAMP KIT-500	05:33 (0.79) [86.54ºC]	05:24 (0.75) [86.65ºC]	05:29 (0.74) [86.55ºC]	05:29 (1.26) [86.58ºC]
gB77/2/m	COVID-19_RNA RT-LAMP KIT-500	04:56 (0.43) [86.87ºC]	04:47 (0.26) [86.85ºC]	05:19 (0.41) [86.82ºC]	05:00 (5.57) [86.85ºC]
gB77/2/n	COVID-19_RNA RT-LAMP KIT-500	05:11 (0.73) [86.81ºC]	04:56 (0.99) [86.87ºC]	05:16 (0.77) [86.82ºC]	05:08 (3.44) [86.83ºC]
gB77/2/o	COVID-19_RNA RT-LAMP KIT-500	05:18 (0.56) [86.83ºC]	05:02 (0.28) [86.84ºC]	05:20 (1.08) [86.78ºC]	05:13 (3.16) [86.81ºC]

Criteria for acceptance: (i) mean time to positivity does not vary more than 20% and (ii) the mean anneal temperatures are within +/- 1°C. Time to positivity (minutes:seconds)

Inter-platform reproducibility was measured by running eight replicates of three LOTs of COVID-19 Positive Control for both (i) COVID-19_RNA RT-LAMP KIT-500 and (ii) COVID-19_Direct Plus RT-LAMP KIT-500 (Table 5). For each sample, 100% of the replicates were detected.

Table 5. Inter-platform reproducibility

		Mean time to positivity in minutes (% coefficient of variation) [Mean anneal temperature]			
LOT	Kit	Genie® HT	Genie® II	Genie® II	Reproducibility between platforms
gB77/2/j	COVID-19_Direct Plus RT-LAMP KIT-500	05:11 (0.84) [86.68ºC]	05:28 (3.29) [86.41ºC]	05:14 (1.20) [86.32ºC]	05:18 (2.91) [86.47ºC]
gB77/2/k	COVID-19_Direct Plus RT-LAMP KIT-500	05:34 (3.73) [86.67ºC]	05:46 (0.89) [86.25ºC]	05:20 (1.24) [86.22ºC]	05:33 (3.86) [86.38ºC]
gB77/2/l	COVID-19_Direct Plus RT-LAMP KIT-500	05:33 (0.79) [86.54ºC]	05:41 (2.62) [86.41ºC]	05:32 (0.48) [86.33ºC]	05:35 (1.48) [86.43ºC]
gB77/2/m	COVID-19_RNA RT-LAMP KIT-500	04:56 (0.43) [86.87ºC]	05:21 (2.24) [86.60ºC]	05:10 (3.14) [86.51ºC]	05:09 (4.12) [86.66ºC]
gB77/2/n	COVID-19_RNA RT-LAMP KIT-500	05:11 (0.73) [86.61ºC]	05:27 (3.58) [86.61ºC]	05:10 (1.66) [86.44ºC]	05:16 (3.02) [86.62ºC]
gB77/2/o	COVID-19_RNA RT-LAMP KIT-500	05:18 (0.56) [86.83ºC]	05:31 (0.50) [86.58ºC]	05:20 (0.39) [86.41ºC]	05:23 (2.26) [86.61ºC]

Criteria for acceptance: (i) mean time to positivity does not vary more than 20% and (ii) the mean anneal temperatures are within +/- 1°C. Time to positivity (minutes:seconds)

11.2. Anneal temperature

To ensure that the anneal temperature of the COVID-19 Positive Control differed from that of SARS-CoV-2, eight replicates of three LOTs of COVID-19 Positive Control for both (i) COVID-19_RNA RT-LAMP KIT-500 and (ii) COVID-19_Direct Plus RT-LAMP KIT-500 (Table 4) were run on a Genie® II. Results were compared to a linear double-stranded DNA (LOT gB69/a), containing the SARS-CoV-2 primer-targeting region for the OptiGene COVID-19 RT-LAMP kits.

Table 6. Anneal temperature evaluation

	Mean anneal temperature (LOT number)			
	COVID-19 Positive Control			Control
COVID-19_Direct Plus RT-LAMP KIT-500	86.41ºC (gB77/2/j)	86.25ºC (gB77/2/k)	86.41ºC (gB77/2/l)	83.95ºC (gB69/a)
COVID-19_RNA RT-LAMP KIT-500	86.60ºC (gB77/2/m)	86.61ºC (gB77/2/n)	86.58ºC (gB77/2/o)	83.16ºC (gB69/a)

Criteria for acceptance: COVID-19 Positive Control anneal temperatures are ≥ 86ºC.

12. 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

13. 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

14. Trademarks

Genie® is a registered trademark of OptiGene Limited.

15. 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

16. 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 6 (http://www.optigene.co.uk/human-diagnostics/).

Table 6. COVID-19 Positive Control Version Changes

Version Number	Publication Date	Summary of Changes
V1.0	15/06/2021	N/A

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