OptiGene

OptiGene Limited
Instructions For Use

Human mRNA RT-LAMP Control Primer Mix

Inhibition and Sample Integrity Control (ISIC) Primer Mix for RT-LAMP

P-ISIC-Human_mRNA_IFU
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OptiGene Limited
Instructions For Use (IFU)

Human mRNA RT-LAMP Control Primer Mix

Inhibition and Sample Integrity Control (ISIC) Primer Mix for RT-LAMP

CE IVD

Σ Each tube contains 500 reactions

REF
P-ISIC-Human_mRNA-500 (500 reactions)
P-ISIC-Human_mRNA-2500 (2500 reactions)
P-ISIC-Human_mRNA-5000 (5000 reactions)

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Contents

1. Intended Use

The Human mRNA RT-LAMP Control Primer Mix is intended for use as an inhibition and sample integrity control (ISIC) primer mix for Reverse Transcription Loop-mediated isothermal AMPlification (RT-LAMP). Primers target human ribosomal mRNA. The primer mix is intended to be used with OptiGene RT-LAMP reagents and has been tested for use with the following mastermixes (Table 1):

Table 1. OptiGene Limited *in vitro* diagnostic kits

Mastermix	Sample type	Kit catalogue code
RNA RT-LAMP Mastermix	Extracted RNA	COVID-19_RNA RT-LAMP KIT-500

When used in combination with OptiGene RT-LAMP reagents, amplification and detection of human ribosomal mRNA indicates that sample processing has been performed correctly and that the sample is not inhibitory. The Human mRNA RT-LAMP Control Primer Mix is intended for use as a singleplex primer set. It must not be used in combination with other RT-LAMP primers as an internal control primer set, it must be set-up as a separate RT-LAMP reaction. The kit is intended for use by laboratory trained personnel in an appropriately equipped facility.

2. Summary

False negative results can be achieved in molecular assays for a number of reasons, including failures in sample extraction, sample degradation, the presence of inhibitory substances and incorrect assay set-up.

By confirming the presence of host nucleic acid within a sample, the Human mRNA RT-LAMP Control Primer Mix can be used to ensure that sample processing has been performed correctly and that a sample is not inhibitory.

3. Principle of the Operation

The Human mRNA RT-LAMP Control Primer Mix is an *in vitro* diagnostic primer set for use in RT-LAMP for the detection of human ribosomal mRNA. 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 monitor amplification of product in real-time using fluorescence detection. 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. This acts as a secondary confirmatory check - ensuring LAMP amplicons are specific to human ribosomal mRNA. The final result is interpreted and reported automatically from both the amplification time and the anneal temperature.

4. Components

The Human mRNA RT-LAMP Control Primer Mix is supplied in liquid form, with each tube sufficient to run 500 reactions. Three pack sizes are available (Table 2), each provided with a kit data sheet (Figure 1).

Table 2. Human mRNA RT-LAMP Control Primer Mix components

Catalogue number	Number of vials	Reactions per vial	Lid colour
P-ISIC-Human_mRNA-500	1	500	Blue
P-ISIC-Human_mRNA-2500	5	500	Blue
P-ISIC-Human_mRNA-5000	10	500	Blue

Figure 1. Contents of P-ISIC-Human_mRNA-500

4.1. Product description

The Human mRNA RT-LAMP Control Primer Mix is a 10X primer mix, containing oligonucleotide primers specific to human ribosomal mRNA. The product is designed to be used alongside OptiGene RT-LAMP reagents.

4.2. Storage requirements

- Human mRNA RT-LAMP Control Primer Mix is shipped cold.
- On arrival, store in the original packaging at -17°C to -25°C (**NOT** using a frost-free freezer).
- The reagents 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 following list (Table 3) includes materials and equipment that are required for use but are not included within the Human mRNA RT-LAMP Control Primer Mix.

Table 3. Additional material and equipment required

Material / Equipment	Details
RT-LAMP Mastermix	OptiGene Ltd. *in vitro* diagnostic kits
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
RNA extraction kit**	RNA or total nucleic acid
Appropriate PPE	

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

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 Human mRNA RT-LAMP Control Primer Mix has been validated for use on the OptiGene Limited Genie® platforms (Table 4).

Table 4. 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

The Human mRNA RT-LAMP Control Primer Mix should be used in an appropriately equipped facility by trained staff.

7.2. Health and safety requirements

All samples should be handled 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.

The Material Safety Data Sheet (MSDS) for the Human mRNA RT-LAMP Control Primer Mix is available from the OptiGene Limited website (http://www.optigene.co.uk/human-diagnostics/).

7.3. Procedural requirements

- **Human mRNA RT-LAMP Control Primer Mix must be used as a stand-alone primer mix in an RT-LAMP reaction. It should NOT be added to other primer mixes or RT-LAMP reactions as an internal sample control.**
- Human mRNA RT-LAMP Control Primer Mix is supplied at the intended working concentration, it should not be diluted.
- 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 5).
- Each workspace should have its own dedicated supply of personal protective equipment (PPE) and equipment / reagents, which should not be shared with other spaces.

Table 5. Workspace requirements

Space	Details
Clean workspace	Preparation of reaction mixes. Suitable for the preparation of RT-LAMP reaction mixes. Original 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 components should be handled using standard laboratory nitrile gloves.
- For the extraction kit, consult the relevant MSDS from the supplier.

8.2. Analytical precautions

- **We do not advise pre-mixing and storage of RT-LAMP Mastermix and Human mRNA RT-LAMP Control Primer Mix for more than 1 hour at 2°C to 8°C prior to starting reactions.**
- **Human mRNA RT-LAMP Control Primer Mix targets human ribosomal mRNA. Care should be taken when setting up assays to prevent self-contamination.**
- When in use, the time which components are at room temperature should be minimised.
- Repeated thawing and freezing of the Human mRNA RT-LAMP Control Primer Mix should be kept to a minimum and should not exceed 5 freeze-thaw cycles.
- After thawing (at 2°C to 8°C), Human mRNA RT-LAMP Control Primer Mix can be stored at 2°C to 8°C for up to one week.
- Before and after each run has been set up, work surfaces and equipment must be cleaned with a DNA/RNA degradation solution.
- 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 the Human mRNA RT-LAMP Control Primer Mix 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 plastic ware 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 Human mRNA RT-LAMP Control Primer Mix past the expiration date.
- If the protective packaging of the Human mRNA RT-LAMP Control Primer Mix is damaged upon receipt, please contact OptiGene Limited for instructions.

9. Human mRNA RT-LAMP Control Primer Mix Test Procedure

This protocol describes the procedure for the Human mRNA RT-LAMP Control Primer Mix as an inhibition and sample integrity control (ISIC) for RT-LAMP.

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 Human mRNA RT-LAMP Control Primer Mix and chosen RT-LAMP mastermix are well mixed (vortexed) before use.

9.1.3. Prepare enough reaction mix for the batch of samples. Prepare the reaction mix as specified in Table 6. For example, if testing 20 samples, prepare enough reaction mix for these samples, 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 Human mRNA RT-LAMP Control Primer Mix and RT-LAMP Mastermix and starting your reactions should be less than one hour. During this time, keep all reagents between 2°C to 8°C.

Table 6. Reaction mix preparation

Reagent	Volume per reaction (µl)
RT-LAMP Mastermix	See IFU for chosen kit*
10X Human mRNA RT-LAMP Control Primer Mix	2.5
	20 µl

*Note that depending on the RT-LAMP mastermix chosen, molecular grade water may be required to achieve the total reaction volume.

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. Loosely close the Genie® tubes (ensuring they do not lock) and transfer to the sample 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 the appropriate reaction well(s) (to give 25 µl final volume). Close the lid(s) to the locked position.

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

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. Setting up the Genie®

Please refer to the instrument manual for full details. The Human mRNA RT-LAMP Control Primer Mix Genie® profile is available from the OptiGene Limited website (http://www.optigene.co.uk/human-diagnostics/).

In the amplification workspace:

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

9.3.2. 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).

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

9.3.4. Select the heat blocks required and the ISIC-Human mRNA profile (Figure 2). See Figure 3 for the RT-LAMP profile.

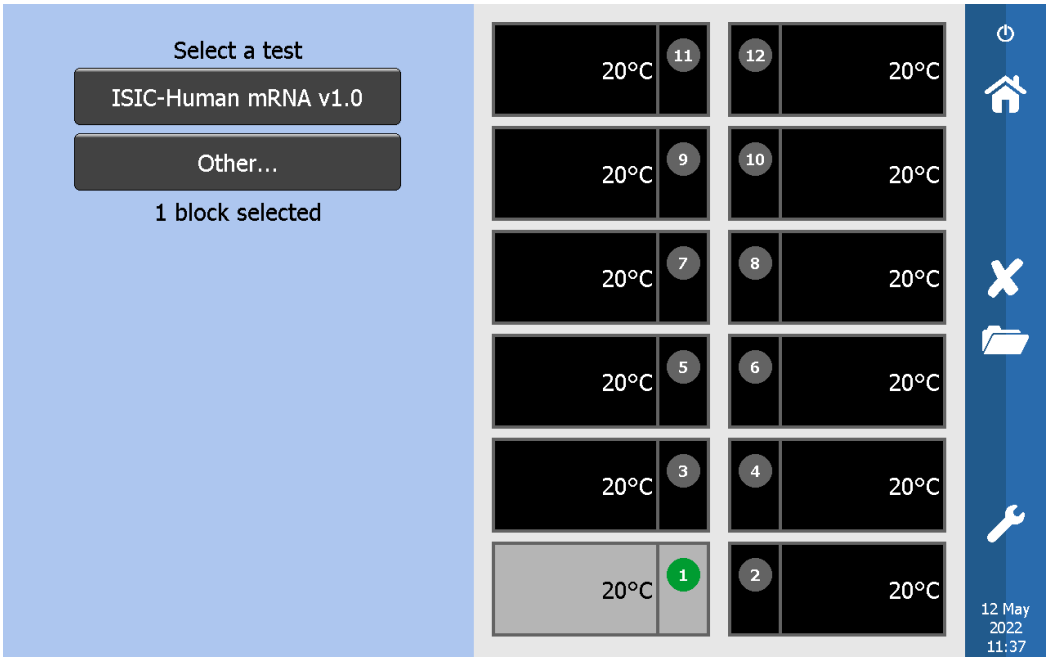

Figure 2. ISIC-Human mRNA profile on a Genie® HT instrument.

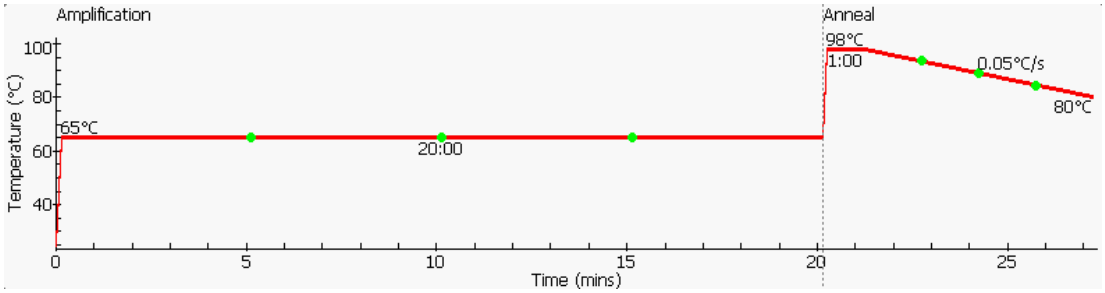

Figure 3. RT-LAMP profile. RT-LAMP is performed at 65°C for 20 minutes. Following amplification, anneal curve analysis is used to confirm that the correct product has amplified (reactions are heated to 98°C for 1 minute, then cooled to 80°C, decreasing the temperature by 0.05°C/s).

9.3.5. 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.

9.4. Interpretation of results

9.4.1. Genie® software will automatically analyse the results and report the sample(s) as positive (Human ribosomal mRNA detected) or negative.

NOTE: An algorithm on the Genie® platforms analyses both the amplification plot and the anneal temperature to determine positive and negative reactions. A 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.

9.4.2. 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.

9.4.3. 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

- If contamination is observed, determine where the contamination has occurred, then thoroughly clean the workspace before repeating the RT-LAMP run(s).
- The Human mRNA RT-LAMP Control Primer Mix indicates that sample processing has been performed correctly and that the sample is not inhibitory. A negative result may indicate errors in sample collection, sample processing, the presence of inhibitors or poor sample integrity.

10. Limitations of the Test

- Human mRNA RT-LAMP Control Primer Mix 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.
- Human mRNA RT-LAMP Control Primer Mix has been validated for use with nucleic acid extracted using the 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.
- Very low levels of mRNA target, below the limit of detection, might be detected but results may not be reproducible.
- Interpretation of results should account for the possibility of false negative and false positive results (Table 7).

Table 7. Potential causes of false negative and false positive results

False negative results	False positive results
Improper handling and/or storage of Human mRNA RT-LAMP Control Primer Mix	Improper handling of Human mRNA RT-LAMP Control Primer Mix
Deviation from handbook protocol	Deviation from handbook protocol
	Contamination of workspaces
	Opening of reactions post-amplification

11. Quality Control

In accordance with GeneSys Biotech Ltd (ISO 9001:2015) Quality Management System, the Human mRNA RT-LAMP Control Primer Mix 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

Performance validation data for the Human mRNA RT-LAMP Control Primer Mix was generated at Hampshire Hospitals NHS Foundation Trust (HHFT) using Genie® platforms. Analysis was conducted using:

- OptiGene Ltd. RNA RT-LAMP Mastermix
- 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
- 20-minute RT-LAMP amplification profile, with amplification time called at 10,000 fluorescence points

12.1. Diagnostic sensitivity

Diagnostic sensitivity was calculated across a total of 183 oropharyngeal / nasopharyngeal swab samples (Table 8). For RT-LAMP, samples were performed in duplicate. Both RT-LAMP duplicate results were required to be positive for a sample to be considered positive. RT-LAMP results were compared to real-time RT-PCR (qRT-PCR) using the Primerdesign Precision® qPCR endogenous control (Cat no. EC-Human, Primer Design Ltd., UK).

Table 8. Overall sensitivity of Human mRNA RT-LAMP Control Primer Mix

Diagnostic sensitivity for swabs	
Overall Diagnostic Sensitivity for swabs (DSe)	98.91 % (95% CI: 96.11 – 99.87)

Samples were tested in singles using the qRT-PCR. For the statistics, it was assumed that the qRT-PCR results were correct. For RT-LAMP, samples were tested in duplicate. A sample is classed as positive when both of the RT-LAMP duplicates are positive.

The percentage of samples positive within certain RT-LAMP amplification time cut-offs was also calculated (Table 9).

Table 9. Sensitivity of Human mRNA RT-LAMP Control Primer Mix across different RT-LAMP cut-offs

% Detection of samples within RT-LAMP amplification time cut-offs (minutes:seconds)	
≤ 10:00 minutes	95.63 % (95% CI: 91.57 – 98.09)
≤ 15:00 minutes	97.81 % (95% CI: 94.50 – 99.40)
≤ 20:00 minutes	98.91 % (95% CI: 96.11 – 99.87)

Samples were tested in singles using the qRT-PCR. For the statistics, it was assumed that the qRT-PCR results were correct. For RT-LAMP, samples were tested in duplicate. A sample is classed as positive when both of the RT-LAMP duplicates are positive. To be classed within an amplification time bracket, both duplicates had to be within the given time.

12.2. Diagnostic specificity

Diagnostic specificity was calculated across 12 negative (un-used) oropharyngeal / nasopharyngeal swabs (Table 10). Viral transport medium was extracted from each sample and eight RT-LAMP replicates performed (for sample one, only six replicates were performed due to insufficient nucleic acid).

Table 10. Overall specificity of Human mRNA RT-LAMP Control Primer Mix

Diagnostic sensitivity for swabs	
Overall Diagnostic Specificity for swabs (DSp)	98.94 % (95% CI: 94.21 – 99.97)*

*Of the 94 reactions performed in total, amplification was evident in one reaction.

12.3. Repeatability and reproducibility

Repeatability and inter-operator reproducibility were measured by running eight replicates of three samples with three different operators (Table 11). Operators used the same RNA extraction for each sample. For each sample, 100% of the replicates were detected.

Table 11. Repeatability and inter-operator reproducibility

	Mean time to positivity in minutes (% coefficient of variation) [Mean anneal temperature ºC]			
Sample	Operator 1	Operator 2	Operator 3	Reproducibility between operators
Swab 1	07:43 (1.75) [87.10]	07:34 (2.86) [87.11]	07:23 (3.05) [87.04]	07:33 (2.25) [87.08]
Swab 2	07:14 (0.62) [87.08]	07:11 (1.16) [86.87]	07:02 (0.96) [87.07]	07:09 (1.42) [87.01]
Swab 3	07:12 (3.83) [86.87]	07:32 (3.51) [87.08]	07:17 (2.04) [87.04]	07:20 (2.42) [87.00]

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 samples across three platforms (Table 12). The same RNA extraction was used for each sample across the three platforms. For each sample, 100% of the replicates were detected.

Table 12. Inter-platform reproducibility

	Mean time to positivity in minutes (% coefficient of variation) [Mean anneal temperature]			
Sample	Genie® HT	Genie® II	Genie® III	Reproducibility between platforms
Swab 1	07:43 (1.75) [87.10]	07:49 (1.46) [86.72]	07:37 (3.02) [86.78]	07:43 (1.23) [86.87]
Swab 2	07:14 (0.62) [87.08]	07:23 (1.82) [86.78]	07:13 (1.10) [86.80]	07:17 (1.34) [86.89]
Swab 3	07:12 (3.83) [86.87]	07:31 (2.65) [86.70]	07:33 (1.64) [86.75]	07:25 (2.53) [86.77]

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).

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
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14. Trademarks

Genie® is a registered trademark of OptiGene Limited.

Viroculti® is a registered trademark of Medical Wire & Equipment.

Maxwell® is a registered trademark of Promega.

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)

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

Table 13. Version Changes

Version Number	Publication Date	Summary of Changes
v1.0	13/05/2022	N/A

P-ISIC-Human_mRNA_IFU
Issue 1.0
Publish date 13/05/2022