GENOMTEC

SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit

Instructions for use

Real-Time Reverse Transcription Loop-Mediated Isothermal Amplification Test with streamlined sample preparation procedure for qualitative detection of nucleic acid from SARS-CoV-2 virus.

REF GA00C

IVD

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CE

Genomtec SA
ul. Bierutowska 57-59,
51-317, Wrocław, Poland
Tel: +48 793 440 931

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

The information in this guide is subject to change without notice.

Please ensure that you are using a current version of the IFU. Refer to http://genomtec.com/support for latest version.

Document revision	Date	Description
A	27 April 2021	Initial release in English
B	25 May 2021	Second release in English with minor corrections
C	15 July 2021	General data and content update
D	21 September 2021	Update on the interpretation of low-positive results
E	07 January 2022	Manufacturer’s address update

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1. Definitions

Abbreviation	Definition
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
LAMP	Loop-Mediated Isothermal Amplification
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
RNase	Ribonuclease
DNase	Deoxyribonuclease
IFU	Instructions for Use
PI	Product Information
MERS	Middle East Respiratory Syndrome
NAAT	Nucleic Acid Amplification Test
cDNA	Complimentary DNA
FAM	Fluorescein amidite
PCR	Polymerase Chain Reaction
LOD	Limit of detection
RT	Reverse transcription
BLAST	Basic Local Alignment Search Tool
EvaGreen®	A green fluorescent nucleic acid dye

2. References

Tsugunori Notomi, Hiroto Okayama, Harumi Masubuchi, Toshihiro Yonekawa, Keiko Watanabe, Nobuyuki Amino, and Tetsu Hase. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 2000 Jun 15; 28(12): e63.

Hong TC, Mai QL, Cuong DV, et al. Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. J Clin Microbiol. 2004;42(5):1956-1961. doi:10.1128/jcm.42.5.1956-1961.2004

2.1. Patents/Patent Applications

U.S. Patent entitled: "Methods of Using Dyes in Association with Nucleic Acid Staining or Detection and Associated Technology" U.S. Patent No. US7,803,943 B2

U.S. Patent entitled: "Dimeric and Trimeric Nucleic Acid Dyes, and Associated Systems and Methods" U.S. Patent No. US7,776, 567 B2

3. Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit general product information

3.1. Intended purpose

The Genomtec® SARS CoV-2 EvaGreen® Direct-RT-LAMP test is a CE-IVD Laboratory Kit containing controls and reagents intended for a streamlined RNA isolation procedure from biological samples, followed by reverse transcription and amplification of nucleic acid in an isothermal reaction, specifically Loop-medicated Isothermal Amplification (LAMP). It is a qualitative assay detecting specifically SARS-CoV-2 in saliva and throat / nasopharyngeal swab specimens, omitting the standard laboratory RNA purification method, from the individuals suspected of COVID-19.

The results obtained will identify the presence of SARS-CoV-2 RNA in the sample. The positive result obtained with the diagnostic test should only be taken into consideration together with the patient's clinical history and other diagnostic results while concluding the final infection status.

A negative result obtained with the Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit diagnostic test does not exclude infection with the SARS CoV-2, and when interpreting this result and determining the infection status, the results of other diagnostic tests as well as the patient's medical history (clinical condition) should also be taken into account.

Testing with the Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit is intended for molecular diagnostic clinical laboratory use by qualified and trained clinical laboratory personnel.

- Note: - The test is not sterile and does not require a sterile operating environment.
- Note: - The assay is not for self-testing.

3.2. Summary and product description

Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit contains assay enough to prepare 50 streamlined RNA isolations and perform 50 reactions (including controls and assay mixes) required for the RT-LAMP detection of RNA from the SARS-CoV-2. Specifically, Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit confirms presence of *N* and / or *S* genes encoding Nucleocapsid spike protein and the surface protein, respectively, in the analysed human sample.

Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit includes the following reagents:

AmpMix	Vial containing mix of chemical reagents and enzymes in quantity enough to prepare 50 analyte and inhibition control reactions, as well as positive and negative controls
Duo-Primers	Vial containing primers composition recognizing specific fragment of *N* and S genes SARS-CoV-2
C-Primers	Vial containing control primers composition recognising specific fragment of the human genome (controlling appropriateness of biological sample collection and sample processing)
Control+	Vial containing Genomtec® SARS-CoV-2 Positive Control in the form of a synthetic SARS CoV-2 cDNA fragments.
LysBuffer	Vial containing Genomtec® buffer used for lysis of a biological sample and preparation of a supernatant-enriched phase with sample's RNA
Water	Vial containing DNase/RNase-Free Water.

The kit also includes the product information insert (PI00CrA) which provides the instructions and the download link for the Instructions for Use (this document).

3.3. Principles of the procedure

Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit is designed to simplify sample's RNA isolation procedure producing supernatant-enriched RNA fraction that is directly used for reaction amplification set up to detect certain fragments of the N and S genes of the SARS-CoV-2 virus that are specific for SARS-CoV-2, despite the fact that the N genes and S are also present in other coronaviruses (e.g., SARS, MERS).

Primers used in the Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit recognize specific, conserved N and S gene sequences with 100% homology achieved only for the SARS-CoV-2 virus and confirmed variants, even though there is a certain homology N and S genes among other coronaviruses.

Genomtec recognises difficulties in recognition of a proper epidemiological situation and way of SARS-CoV-2 virus transmission in the population, therefore the User is advised to follow the latest guidance provided by World Health Organisation (WHO), or local Healthcare Authorities guidelines.

The appropriate decision on epidemic status and virus transmission should be provided by each Country's appropriate Healthcare and / or Epidemiological Agencies. Genomtec will not provide any advice on the epidemiological status of any country or geographical area.

The diagnostic workflow consists of:

- Sample collection followed by a streamlined sample preparation to obtain supernatant-total RNA enriched fraction that is being used in subsequent assay step.
- Reverse transcription of the nucleic acid present in the supernatant fraction and simultaneous cDNA amplification using the Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit.
- The RT-LAMP process occurs at constant temperature (isothermal condition), where set of five primers recognises seven specific genomic sequences encompassing targeted SARS-CoV-2 gene *N* and set of six primers recognises eight specific genomic sequences encompassing targeted SARS-CoV-2 gene *S*. In the first stage Reverse Transcription (RT) is being performed generating cDNA. Simultaneously with RT in the same constant temperature all eleven primers anneal and amplify the cDNA template. In the presence of targeted genomic fragment increasing cDNA concentration prompts fluorescent dye binding, (EvaGreen®; for details see Section 2, References) simultaneously increasing the fluorescence intensity.
- Fluorescence is monitored by the Real-Time PCR instruments (equipped with FAM / SYBR / green channel detector) and the readout is provided that next undergoes analysis (see Section 8, Interpretation of Results).

The kit has been validated on the below Real-Time PCR Instruments:
Bio-Rad CFX96 Dx System
Bio-Rad CFX96 System
Thermofisher QuantStudio 7 Pro

3.4. Reagents and materials

3.4.1. Materials and reagents provided

AmpMix	Vial containing mix of chemical reagents and enzymes in quantity enough to prepare 50 analyte and inhibition control reactions as well as positive and negative controls
Duo-Primers	Vial containing primers composition recognising specific fragment of *N* and *S* SARS-CoV-2 genes
C-Primers	Vial containing control primers composition recognising specific fragment of the human genome (controlling appropriateness of biological sample collection and RNA purification)

Control+	Vial containing Genomtec® SARS-CoV-2 Positive Control in the form of a synthetic SARS CoV-2 cDNA fragments.
LysBuffer	Vial containing Genomtec® buffer used for lysis of a biological sample and preparation of a supernatant-enriched phase with sample's RNA
Water	Vial containing DNase/RNase-Free Water.

3.4.2. Materials required but not provided

- Thermo-shaker that holds 1.5 millilitre Eppendorf tubes and can operate at 95°C or above, with recommended shaking mode (range of 500-900 rpm)
- Thermal Cycler with FAM channel for fluorescence detection and 96 well plate holder or block suitable for strips of PCR-tubes with optically clear caps, maintained and calibrated according to the manufacturer's instructions. The list of validated Real-Time PCR Instruments is provided in Section 3.3, Principles of the procedure.
- Laboratory freezers: -25°C to -10°C.
- Laboratory microcentrifuge (for 48 microtubes size 1.5 to 2 mL) and a centrifuge with a rotor for microplates or PCR strips.
- Laboratory mixer, vortex or equivalent.
- Single and / or multichannel adjustable pipettes working in volume range:
  - 0,5-10µl
  - 10-100µl
  - 100-1000µl
- Cooling block or ice.
- PCR 96-Well Reaction Plate or strips of PCR-tubes with optically clear caps.
- Optical Adhesive Film.
- Sterile, aerosol barrier (filtered) pipette tips.

4. Precautions

Testing with the Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of Real-Time PCR and / or LAMP and in vitro diagnostic procedures. Use separate areas for the preparation of patient samples and controls to prevent cross-contamination.

Samples and reagents must be handled under a biological safety cabinet. PCR workstation should be sterilised with UV light for minimum of 30 minutes before use.

- All specimens should always be treated as potentially infectious and/or biohazardous in accordance with safe laboratory procedures.
- Use personal protective equipment (PPE) according to local guidelines for the handling of potentially infectious samples.
- Always use sterile, nuclease-free pipette tips with aerosol barriers (filtered).
- Do not eat, drink or smoke in the working area.
- Manufacturer does not provide warranty if any modifications to assay reagents, assay protocol, or instrumentation were made, and these are in violation of the In Vitro Diagnostic Directive 98/79/EC.
- Do not use the kit after the expiry date.
- NEVER OPEN THE PCR-PLATE OR PCR-TUBES AFTER THE ANALYSIS PROCESS AS IT MIGHT CAUSE CONTAMINATION BY THE AMPLICONS PRESENT IN POSITIVE REACTIONS.
- Dispose of waste in compliance with the local biohazard regulations. Check safety procedures set by your institution for working with chemicals and handling biological specimens.
- Avoid sample or reagent contact with the skin, eyes, and mucous membranes. If skin, eyes, or mucous membranes come into contact, rinse immediately with water and seek medical advice immediately.
- In case of sample or reagents coming in contact with skin, eyes or mucous membranes, or if swallowed, immediately follow the laboratory post-exposure protocol.
- Clean and disinfect all reagents and / or sample spillage with disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant
- Safety Data Sheets are available upon request with Genomtec or Authorised Distributor.
- Laboratories may be required to report all positive results to the appropriate local Health Authorities.
- Positive results in this test indicate presence of SARS-CoV-2 RNA in a patient sample.
- Reagents must be stored and handled as specified in Table 1.
- For the streamlined RNA preparation step, mix well the Genomtec® SARS-CoV-2 LysBuffer (11ml) provided with the kit prior use. Preferably mix LysBuffer by touch-vortexing (3 seconds) or by pipetting a couple of times in the original bottle every five Eppendorf tubes added.

5. Storage, shipping and stability

IMPORTANT!

POSITIVE CONTROL SHOULD BE ALIQUOTED INTO SMALLER VOLUMES TO PREVENT ITS DE-GRADATION AND PROTECT AGAINST MULTIPLE THAW-FREEZING CYCLES.

Amplification Mix, Detection and Control primers are stable for at least three freeze - thaw cycles.

It is completely normal for a precipitate to appear at the bottom of the AmpMix tube. In this case, it is recommended to leave the tube for about 10 minutes at room temperature and then mix the contents by pipetting.

Upon arrival store LysBuffer in the laboratory refrigerator (5 ± 3ºC).

Table 1.

Reagent	Quantity	Volume	Storage limits	Shipping limits	Shelf life
Genomtec® SARS-CoV-2 AmpMix	1 vial	1780 µl	-22°C to -15°C	Dry or wet ice	Six months
Genomtec® SARS-CoV-2 Duo-Primers	1 vial	137 µl	-22°C to -15°C	Dry or wet ice	Six months
Genomtec® SARS-CoV-2 C-Primers	1 vial	137 µl	-22°C to -15°C	Dry or wet ice	Six months
Genomtec® SARS-CoV-2 Control+	1 vial	40 µl	-22°C to -15°C	Dry or wet ice	Six months
Genomtec® LysBuffer	1 vial	11 ml	5 ± 3ºC	Ambient / wet ice	Six months
DNase/RNase-Free Water	1 vial	1000 µl	-22°C to -15°C	Dry or wet ice	Six months

If the product is delivered within 48 hours since it left the indicated temperature storage condition, wet ice can be used for shipping.

If expected delivery occurs >48 h since the product left the indicated temperature storage condition, the shipping must be on the dry ice for all reagents, besides Genomtec® LysBuffer that should be shipped in the ambient temperature.

6. Quality control

6.1. Assay controls

Positive, negative SARS-CoV-2, negative human test controls as well as the inhibition control must be included to accurately interpret patient test results. Including the controls minimises occurrence of potential false results.

Include the below Controls:

Type of Control	Contents and targets	Function
Positive Control	Synthetic SARS-CoV-2 cDNA with AmpMIx and detection primers directed against specific genomic sequence of targeted *N* and *S* genes	Ensures the proper reaction conditions as well as stability of the assay reagents
Negative SARS-CoV-2 Control	AmpMix with detection primers recognising specific sequences on targeted *N* and / or *S* gene of SARS-CoV-2 combined with DNase/RNase-Free Water	Ensures lack of human genetic material cross-contamination arising from assay set-up
Negative Human Control	AmpMix with control primers recognising specific fragment of the human genome combined with DNase/RNase-Free Water	Ensures lack of external viral genetic material cross-contamination arising from assay set-up
Inhibition Control (IC)	AmpMix and control primers with added patient's extracted RNA sample. Primers targeting human Ribonuclease P gene.	Controls possible inhibition of the amplification and appropriate sample collection procedure (e.g., saliva or throat swab taken from human), and RNA purification efficiency

6.2. Specimen collection, handling, transport and storage

Patient samples must be collected according to appropriate laboratory guidelines. These include saliva as well as throat swab and nasopharyngeal swab specimens.

Treat all samples and controls as if they are capable of transmitting infectious pathogen.

For procurement of saliva, use approved medical saliva collectors / devices that allow ease of sample donation by the patient. Once saliva is present in the collector / tube, seal it well to prevent extrinsic material contamination and evaporation. Collect saliva to a dry collector / tube without addition of any stabilizing agents. The specimen may be tested immediately after collection and its storage must comply with the Manufacturer's instruction. For prolonged storage it is recommended to store saliva up to one week in the fridge (5 ± 3ºC), or not longer than 4 weeks at -20°C. Avoid repeated freeze-thaw cycles.

For the swab, the specimen should be collected into a dry viral collection system, transported and stored (for the duration and storage temperature) according to manufacturer's instruction, or other recommended equivalent applicable to specimen type. The specimen may be tested immediately after collection and its storage must comply with the Universal Transport Medium Manufacturer's requirements (for the duration and storage temperature). Avoid repeated freeze-thaw cycles.

- Note: nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) specimens have not been validated for use.

- Note: swab samples have been validated by spiking CDC SARS-CoV-2 standard (Heat Inactivated SARS-CoV-2, cat. no. ATCC® VR-1986HK) directly onto the negative human swab samples producing different SARS-CoV-2 viral concentrations with subsequent RNA sample preparation (dry swab mixed in 200 µl LysBuffer and heated) and Direct-RT-LAMP amplification according to Section 7. Swab samples were not included in the clinical evaluation described in Section 9.4.

7. Operating instructions

7.1. Before starting the test

IMPORTANT

- Ensure LysBuffer reagent is well mixed by touch-vortexing (at least 3 seconds) or by pipetting a couple of times in the original bottle every five Eppendorf tubes added (to ensure that in pipetted slurry are present equal amounts of the solid and aqueous phases).
- If working with large number of samples, to minimise degradation of RNA analytes it is advised to keep the plate / PCR microtubes as well as RNA analytes on ice / in cooling block until it is loaded into the Real-Time PCR instrument.
- Use thermocycler to run the plate immediately after preparation. Failure to do so could result in degraded RNA samples.
- Prevent contamination implementing separate areas for RNA isolation and reaction amplification; prepare reagents in a PCR workstation (with dual decontamination action by UV) and use separate pipettes for controls and samples, and always use aerosol barrier pipette tips
- Maintain an RNase-free environment.
- Protect kit components (particularly AmpMix) from light.
- Each sample requires a concomitant inhibition control run and positive and negative human and negative SARS-CoV-2 controls are required to be included for each assay. (see “Quality Control” in Section 6)
- After thawing, please place all reagents and assay components onto the cooling block or ice, besides LysBuffer that can warm to an ambient temperature, to preserve their potency, as long as in the AmpMix tube no precipitation is observed.

7.1.1. Preparation step 1

The first step in the diagnostic workflow is sample collection (saliva or throat swab or nasopharyngeal swab specimens) and sample RNA preparation. Remember to use dry swabs without viral transport medium. Use Eppendorf tubes (1.5 ml) for mixture preparation and the heating stage. Mix well (3 seconds vortexing or by turning tubes a couple of time upside down), place in heat block and incubate for 5 min at 95°C, preferably with shaking 500-900 rpm. Cool down and spin briefly after heating to collect evaporate and liquid from the cap and to produce a clear supernatant (contains isolated total RNA). Follow the below guidance:

Sample type / reagent	Saliva	Dry Swab
Sample (volume)	100 µl	Submerge dry swab in LysBuffer and vigorously shake for several seconds
Genomtec® LysBuffer	200µl	200µl
Heating & shaking	95°C / 500-900 rpm	
Duration	5 minutes	

7.1.2. Preparation step 2

The quality of RNA isolation may influence the quality of the Direct-RT-LAMP reaction; therefore, it is recommended for laboratories to use a thermo-block with a shaking option and with monitored temperature control, whereas when transferring the RNA-containing supernatant avoid disturbing the sediment at the bottom of a tube.

7.1.3. Preparation step 3

All provided and necessary reagents and assay components should be defrosted at ≤4°C, followed by gentle mixing and brief centrifugation (to collect evaporate and liquid from the cap).

7.1.4. Preparation step 4

Set-up the reaction according to Point 7.2 Performing the test.

7.2. Performing the test

7.2.1. Test step 1

Prepare all assay Controls and samples according to the Table 2. Each sample requires simultaneous run with the assay Inhibition Control. Individually performed assay requires additional reaction for positive and negative controls to be incorporated. Pipette Amplification Mix with either Detection or Control Primers to designated wells followed by addition of either DNase/RNase Free Water or Sample or the required Control. The final volume in each well is 25 µl.

Table 2. Reaction plate set-up.

Reagents	SARS-CoV-2	Inhibition control (IC)	Positive control	Negative SARS-CoV-2 control	Negative human control
Genomtec® SARS-CoV-2 AmpMixD	16,25 µl	16,25 µl	16,25 µl	16,25 µl	16,25 µl
Genomtec® SARS-CoV-2 Duo-Primers	2,5 µl	-	2,5 µl	2,5 µl	-
Genomtec® SARS-CoV-2 C-Primers	-	2,5 µl	-	-	2,5 µl
Lysate - aqueous phase	6.25 µl	6.25 µl	-	-	-
Genomtec® SARS-CoV-2 Control+	-	-	6.25 µl	-	-
DNAse/RNAse free Water	-	-	-	6.25 µl	6.25 µl
Total volume	**25 µl**	**25 µl**	**25 µl**	**25 µl**	**25 µl**

7.2.2. Test step 2

After each addition, pipette up and down ensuring proper mixing.

7.2.3. Test step 3

Seal the plate with Optical Adhesive Film, then centrifuge briefly to collect the liquid at the bottom of the reaction plate / PCR-tube.

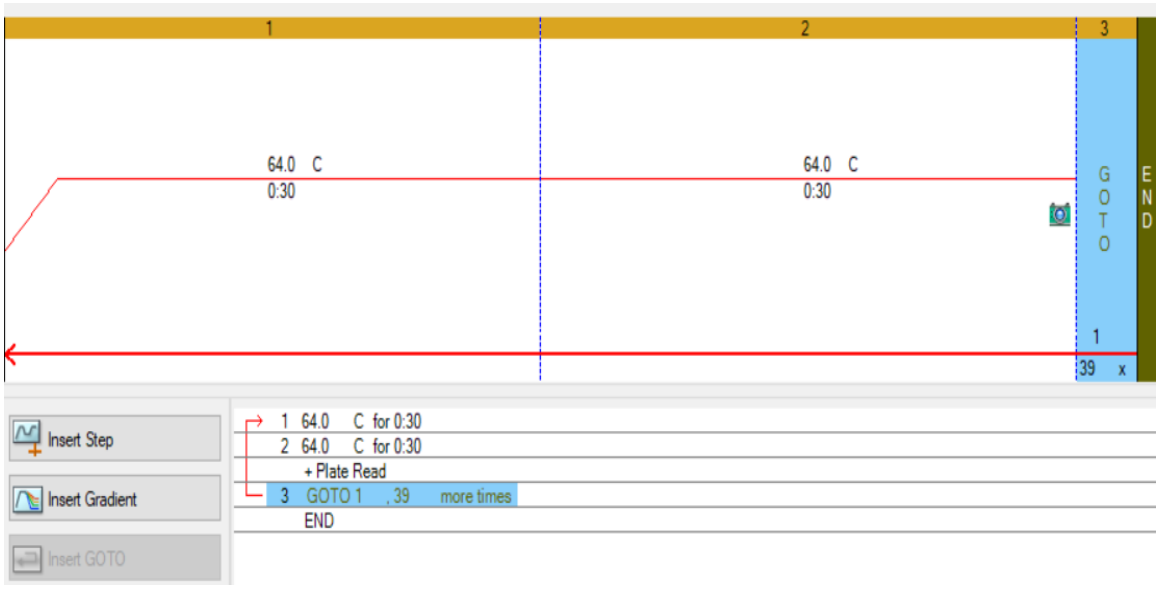
7.2.4. Test step 4

Place PCR-plate or PCR tubes in the Real-Time PCR instrument that is configured as follows:

Step	Temperature [°C]	Time [Sec.]	Fluorescence detection	Cycles/ repeats
Amplification 1	64	30		40
Amplification 2*	64	30	FAM / Green	

* Fluorescence signal readout is performed during step 2 of each repeat.

Signal detection is performed after every minute of the heating cycle (resulting in readings making up the final result of the amplification curve). A sample program for the Bio-Rad CFX96 Dx System is shown in the figure below.



OPTIONAL STEP:

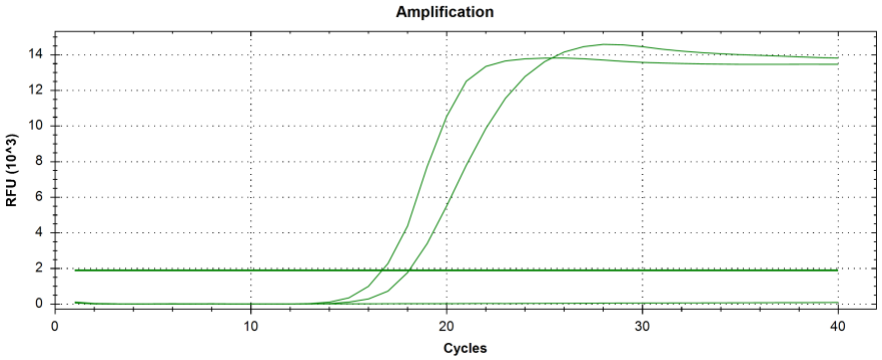
For definite determination of which or both genes (*N* and *S*) were detected in the assay, after the isothermal amplification a melting curve analysis is performed to distinguish the *N* gene amplicon from the *S* gene amplicon. The two targets are identified based on their melting temperature. Real time instrument settings are presented in table below.

Melt Curve	Temperature	Time [min.]	Fluorescence detection
Step 1	65.0°C to 95.0°C, increment 0.5 °C	0:05	FAM / green
Step 2	80.0°C	5:00	
Step 3	10.0°C	10:00	

7.2.5. Test step 5

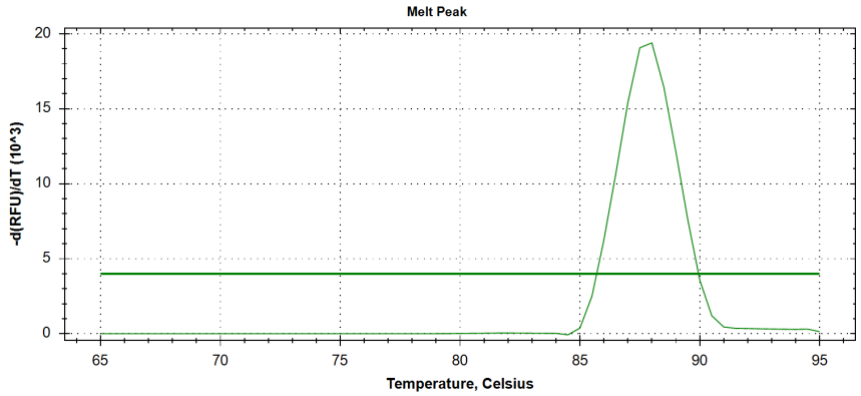
The Real-Time PCR instrument must be able to operate on 25 µl total volume in an individual well of a multi-well PCR plate or individual tube in a strip of PCR-tubes with optically clear caps and must be equipped with optics and filters allowing fluorescence reading at FAM (SYBR / green) channel. The list of validated Real-Time PCR Instruments is provided in Section 3.3, Principles of the procedure. Use provided Instrument's Software to set up the run protocol.

An example of the amplification curves obtained for NTC, positive sample together with IC as well as background threshold is shown below.

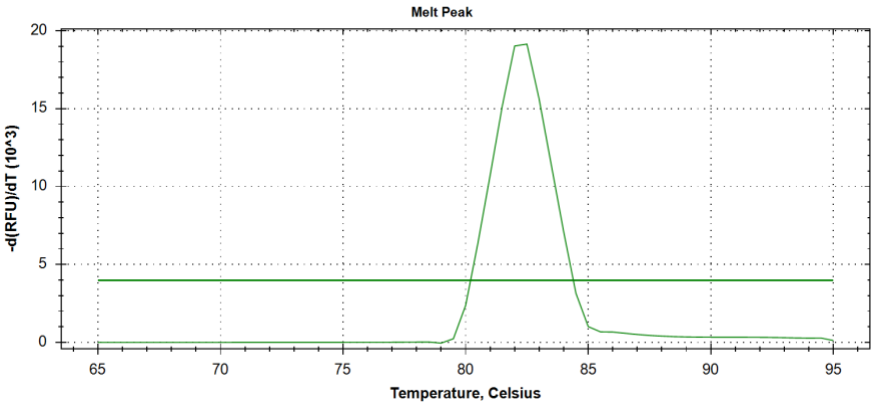


Below is an example of the melting curve obtained for the N gene amplicon, with a T_m of approx. 88.0ºC, for the S gene amplicon, with a T_m of approx. 82.5ºC, as well as the inhibition control, with a T_m of approx. 84.0ºC. Melt analysis results were obtained on a Bio-Rad CFX96 Dx System instrument. On another Real-Time PCR thermocycler, the Tm temperatures may differ slightly from those indicated below but should be within the range of 82.5ºC to 83.5ºC for the SARS-CoV-2 *S* gene, 87.5ºC to 88ºC for the *N* gene SARS-CoV-2 and 83.5.0ºC to 84.5ºC for inhibition control gene.

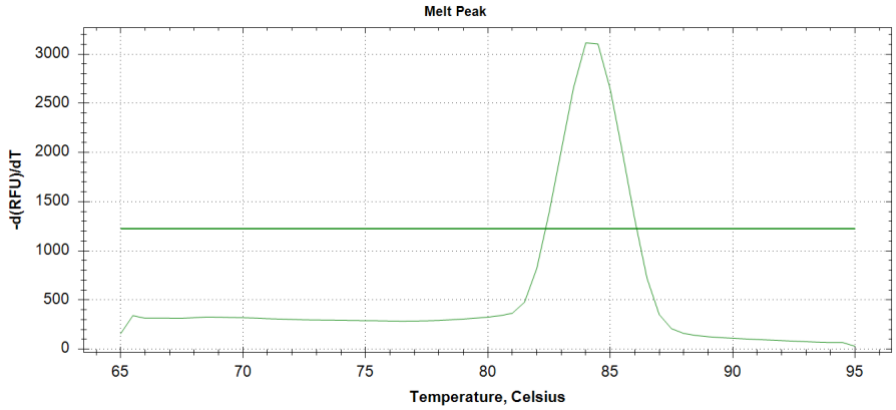
Melting Curve for gene *N* (melting temperature 88ºC)



Melting Curve for gene *S* (melting temperature 82.5º C)



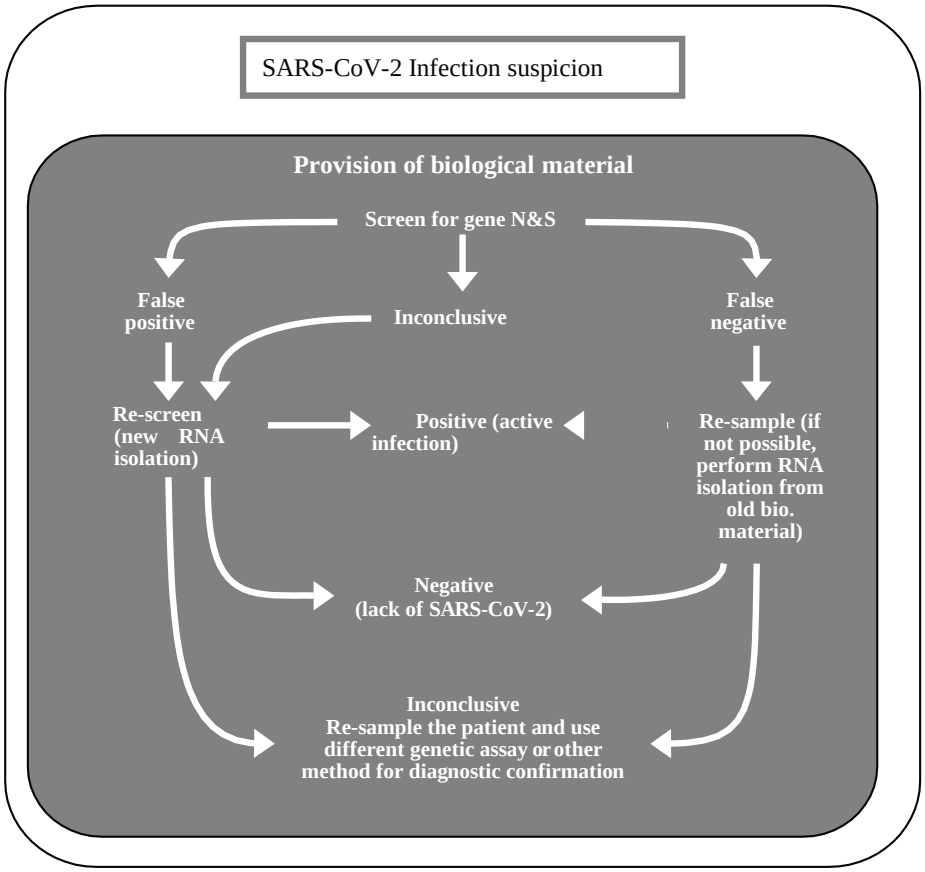
Melting Curve for inhibition control (melting temperature 84.0º C)



8. Results interpretation

8.1. Interpreting patients' results

Please refer to the schematic diagram 'COVID-19 diagnostic pathway.



Genomtec's recommendation for use Product line Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit are as follows:

- Where new or suspected cases of COVID-19 disease arise in the population utilise Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit to aid in diagnosis of SARS-CoV-2. Positive result for SARS-CoV-2 (detected fluorescent signal in the analyte) and all assay controls is enough to indicate active infection.
- If confirmation on detected specific genes' amplicons is required by country specific law /

regulatory system, perform melting curve analysis as described in Sections 7.2.4 and 7.2.5. Based on the resultant melting peaks, the test diagnostic validity is achieved if at least one of the genes' amplicons are detected (N or S). Concomitantly, all assay controls should indicate an adequate valid result.

- It is advised to use threshold between $1,5*10^3$ RFU and $3*10^3$ RFU on BioRad's systems.

In order to assess the analysis results, the positive control for each run should exhibit fluorescence signal, whereas fluorescence signal from all the negative controls should not exceed baseline. For the inhibition control the fluorescent signal must appear the latest in 25th minute of amplification reaction. If the fluorescence signal for the SARS-CoV-2 target in a clinical sample after 38 minutes of amplification exceeds fluorescence signal obtained for the Negative SARS-CoV-2 control, and a typical exponential growth of the fluorescent curve is clearly visible, then such sample is deemed as POSITIVE and indicates the presence of SARS-CoV-2 RNA in the analysed sample.

In cases of a signal being detected in any of the negative controls or no signal detected in positive control the amplification run must be repeated.

To interpret results of the assay please follow the guidance presented in Table 3.

Table 3. Result interpretation for patient samples

The Analyte	Inhibition Control	Results interpretation
+	+	POSITIVE
-	+	NEGATIVE
+	-	INCONCLUSIVE
+	-	FALSE POSITIVE (in case of signal detection only in one or both negative controls)
-	-	FALSE NEGATIVE

ACTIONS:

- POSITIVE – report results to the appropriate Local Health Agency / Provider, as applicable.
- NEGATIVE – report results to the healthcare provider; if the patient in clinical review is symptomatic, consider further diagnostic tests for other pathogens.
- INCONCLUSIVE – re-sample biological material, and if not possible use collected sample, starting from the lysis preparation (Section 7.1.1), and if the repeated result still comes inconclusive, consider patient re-sampling and use of different genetic assay, or other method for diagnostic confirmation.
- FALSE POSITIVE (in case of positive amplification in one of both negative controls) – repeat test with collected sample, starting from the lysis preparation (Section 7.1.1) after decontamination of the biological safety cabined utilised for setting up the reactions.
- FALSE NEGATIVE – repeat genetic test with recommended re-sampling. If the repeated result remains false negative or inconclusive consider using different diagnostic assay or diagnostic procedure.
- If the second diagnostic round confirms presence of both SARS-CoV-2 RNA and human RNA, the result is positive.
- If the second diagnostic round shows no amplification signal from SARS- CoV-2 RNA and the inhibition control is positive, the result is negative.

8.2. Limitations

Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit is intended for the molecular

diagnostic clinical laboratory use by the qualified and trained clinical laboratory personnel. The Laboratory should have implemented Quality System and work in accordance to GLP, and in compliance with the guidelines presented in this Document in order to prevent cross-contamination of RNA clinical samples and other components of the Kit.

- Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit was externally validated on saliva for SARS-CoV-2 detection, whereas internal validation was carried out for swabs as described in Section 6.2. The external clinical evaluation is described in Section 9.4.
- The Kit has been validated on the Real-Time PCR Instruments described in Section 3.3, Principles of the procedure.
- All specimen collection, shipment and storage must be performed according to Section 5 of this Document and country specific guidelines for biological material handling and storage.
- All reagents and assay components must be stored according to conditions described in Table 1. Failure to comply with the guideline may negatively affect the diagnostic procedure, providing false results.
- It is mandatory to correctly implement all processes described in Preparation Steps 7.1.1 – 7.1.2 for each sample type to yield the highest quality of RNA isolate and amplification reaction set up.
- The FALSE NEGATIVE results may be indicative of:
    - Unsuccessful biological material collection.
    - RNA material degradation during storage / transport (lack of guideline compliance).
    - Inefficient lysis and RNA isolation stage (Section 7.1.1.) or combining biological sample with a nucleic acid stabilizing solution present in the collecting tube / device.
    - Presence of RT-LAMP inhibitors in the reaction (working environment and / or laboratory PCR-consumables non-compliance).
    - Mutation in targeted SARS-CoV-2 gene fragments (Genomtec constantly monitors the kit's ability to mis-recognise the targeted sequences in newly appearing SARS-CoV-2 strains).
    - Lack of compliance and execution of the diagnostic stages according to the enclosed Document.
- Positive signal obtained in the negative controls may be indicative of:
    - Cross-contamination of the assay components and / or RNA isolates during reaction set-up.
    - Mix-up of samples.
    - Extrinsic RNA contamination during set-up procedure.
- The negative result obtained with the diagnostic test (Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit) does not disqualify presence of SARS-CoV-2 and any diagnostic recommendation should not be made solely on its basis, instead it is recommended to consider other diagnostic results and patient’s clinical history while concluding the final infection status.

Laboratories may have to report positive results to the appropriate (Public) Health Authorities.

9. Performance characteristics

9.1. Analytical sensitivity (Limit of Detection)

The limit of detection (LOD) is defined as the lowest number of copies of SARS-CoV-2 genes *N* and/or *S* that can be detected by the Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit.

Limit of detection of the assay was determined by performing 20 reactions for series of dilutions on contrived positive samples, either for saliva or for swab, spiked with a Heat Inactivated SARS-CoV-2 virus control (cat. no. ATCC® VR-1986HK) and determined at the lowest genes’ copies per reaction at 95% CI. The LOD of the SARS-CoV-2 Direct-RT-LAMP assay for saliva is **2 virus copies / reaction**,

whereas for swab **10 virus copies / reaction** (of either *S* or *N* gene fragments targeted in the assay).

9.2. Analytical reactivity (Inclusivity)

The assay primers were mapped to SARS-CoV-2 reference genome sequence NC_045512.2:28274-29533 Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome. LAMP primers (five) recognising seven sequences for SARS-CoV-2 *N* gene showed 100% homology to the SARS-CoV-2 isolate analysed. LAMP primers (six) recognising eight sequences for SARS-CoV-2 *S* gene showed 100% homology to the SARS-CoV-2 isolate analysed. Primers are designed to amplify highly conservative sequence of *N* and *S* genes of SARS-CoV-2.

In order to confirm coverage by the primers an alignment of 45 sequences of the *N* gene, and 44 sequences of the *S* gene from the whole genome sequencing analysis of SARS-CoV-2 has been performed. 100% inclusivity was confirmed for 6 out of 7 regions of the amplified fragment of *N* gene (mutation site was detected in the 5'end of one primer, which due to the nature of the amplification technology does not influence the reaction efficiency), and for 8 regions of the amplified fragment of *S* gene. Additional analysis of the specificity for mutant strains of the virus carried out *in-silico* and by adding to the mixture consisting of AmpMix and primers recognizing N and S gene fragments cDNA fragments of S and N genes derived from the mutant strains of SARS-CoV-2 virus confirmed that the Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit successfully detects identified below virus strains:

Accession number	Variant	WHO classification
EPI_ISL_723044	B.1.1.7	Alfa
EPI_ISL_825139	B. 1.351	Beta
EPI_ISL_792680	P.1	Gamma
EPI_ISL_2650470	B.1.617.2	Delta
EPI_ISL_2631197	B.1.427/B.1.429	Epsilon
EPI_ISL_2614193	P.2	Zeta
EPI_ISL_1563854	B.1.525	Eta
EPI_ISL_1122452	P.3 (version: 2021-04-01)	Theta
EPI_ISL_2647531	B.1.526	Iota
EPI_ISL_1415353	B.1.617.1	Kappa
EPI_ISL_2536799	C.37	Lambda
EPI_ISL_1259297	Breton (hCoV-19/France/BRE-IPP04233/2021)	N/A

In addition, *in silico* analysis was conducted to verify the assay does not cross-react with other high prevalence pathogens and normal microbiome that are reasonably likely to be encountered in a respiratory tract clinical specimen. For this purpose, BLAST algorithm was utilised. BLAST alignments showed >85% homology for SARS-CoV in two (F3 and B3) out of five individual primers on the set targeting N gene. The S gene primers set showed no known BLAST homology.

9.3. Analytical specificity (Cross Reactivity)

Specificity of Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit was confirmed in a study where assay mix (containing AmpMix and Detection Primers) was spiked with genetic material of the below-mentioned potentially cross-reacting pathogens and the sample was subjected to RT-LAMP run. Furthermore, additional in-silico analysis of designed primers utilising BLAST alignment tool was conducted and show no possible full sequence similarity.

The thirty (30) pathogens utilised in this study included:

Mycoplasma genitalium	Escherichia coli
Streptococcus pyogenes	Candida albicans
Enterococcus faecalis	Mycoplasma pneumoniae
Moraxella catarrhalis	Klebsiella pneumoniae
Legionella pneumophila	Staphylococcus aureus methicilin sensitive (MSSA)
Enterococcus faecium	Acinetobacter baumannii
Mycoplasma hominis	Ureoplasma urealyticum
Haemophilus influenzae	Human genomic DNA
Bordetella pertussis	Staphylococcus aureus methicilin resistant (MRSA)
Pseudomonas aeruginosa	Listeria monocytogenes
Haemophilus ducreyi	Campylobacter jejuni

Chlamydiophila pneumoniae	Mobiluncus mulieris
Influenza A (H1N1, H3N2)	Influenza B
HCoV-OC43	HCoV-229E
SARS-CoV	MERS

None of the above listed pathogens had any effect on RT-LAMP assay performance and cross-reacted.

9.4. Interference Substances Studies

Interference study was conducted on substances of a suspected negative impact on the assay and present in the nasal or throat cavity during patients' sample donation. The interfering substances were spiked with contrived positive saliva samples (with added SARS-CoV-2 standard; see Section 9.1.), collected from confirmed SARS-CoV-2 negative healthy individuals, at concentration at x25 LOD. Relevant pharmacological concentrations of interfering substances were used for each drug investigated, additionally to commonly used oral and mouth hygienic products, whole blood and saliva major component, i.e., mucin. It has been confirmed that at given concentrations summarised in Table 4 Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit retained all diagnostic properties against SARS-CoV-2 virus identification in contrived positive saliva samples.

Drug (active substance) / modality	Concentration (in lysate - saliva and LysBuffer)	Result (N conducted / N agreed)
Whole blood	2.5% v/v	100% (4/4)
Mucin (Bovine submaxillary galnd, type 1-S)	2.5 mg/ml	100% (4/4)
Dentospet A (benzocaine)	5% v/v	100% (4/4)
Tamiflu (oseltamivir)	2.2 µg/ml	100% (4/4)
Bactroban (mupirocin)	5.4 mg/ml	100% (4/4)
Gentamicin (eye solution)	3 ug/ml	100% (4/4)
Acatar Nasal Spray (oxymetazoline)	15% v/v	100% (4/4)
Otrivin Nasal Spray (xylometazoline)	0.05% v/v	100% (4/4)
Beclonasal Aqua Nasal Spray (Glucocorticoid)	5% v/v	100% (4/4)
Momester (steroid)	5% v/v	100% (4/4)
Toothpaste (Colgate)	5% v/v	100% (4/4)
Mouthwash (Listerine)	5% v/v	100% (4/4)

Patients should not use the above listed substances and also drink any beverages or eat within 30 minutes of saliva donation.

9.5. Clinical evaluation

A study was performed to evaluate the performance of the Genomtec® SARS-CoV-2 EvaGeen® Direct-RT-LAMP CE-IVD Kit on clinical saliva samples obtained from patients participating in the medical experiment carried out in one independent clinical site (large Teaching Clinical Hospitals with molecular

diagnostic laboratory in Warsaw) and also on contrived positive and negative saliva samples collected among Manufacturer's employees (after obtained informed consent) on the territory of Republic of Poland. Prior study execution the medical experiment was positively opiniated by the Bioethics Committee attached to the Teaching Clinical Hospital in Warsaw, Poland. The study reference samples used in the investigation included swab specimens collected for the routine clinical diagnosis informing treatment pathway, or donated by Manufacturer employees, and were paired with the study saliva samples. The pre-released version of the test was evaluated by the clinical site totalling at 25 clinical specimens tested since Q2/3 2021 during the course of the study, whereas contrived positive and negative samples accounted for 44 specimens. Five clinical samples and one contrived positive sample were removed from the data analysis due to nonconformance with the study protocol (inability to repeat inconclusive RT-PCR result for the patient or breach of interference substances study protocol).

The method of the swab and saliva collection (type and the manufacturer) included standardised viral transport system or dry 5 ml sterile tubes with a screw cap or in some cases a saliva collecting device, respectively, and complied with the general clinical laboratory regulations enforced by the relevant Polish Healthcare Authority (Państwowy Zakład Higieny), and also with the WHO recommendations.

Patients that underwent swab collection were referred to such diagnostic procedure based on either their employer's recommendation for screening (private testing), private individual testing, clinical symptoms, suspicion of contracting the disease due to contact with infected individual(s) or travel and were subjected to quarantine. All patients were referred to conduct the genetic diagnostic test against SARS-CoV-2 by a healthcare professional, the employer, or in a private individual capacity.

There was one swab collected from each patient, that had priority to undergo testing utilizing the standard care molecular diagnostic test based on a Real-Time RT-PCR technology, and also small volume of paired saliva sample around 500 µl). The swab samples underwent established in each clinical site collection process and diagnostic laboratory workflow involving samples' RNA purification and subsequent RT-PCR reaction amplification. The nucleic acid purification was carried with one of the commercially available kits. The Real-Time RT-PCR CE-IVD diagnostic kits (detecting at least two SARS-CoV-2 genes and human internal control gene) were used as reference method for Direct-RT-LAMP assay and the protocol was compliant with the manufacturer's instructions.

The paired saliva sample, collected at time of patients' presentation for swab collection, was analysed using the (pre-released version of) Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit, according to instruction enclosed in this document. Both fresh and stored (up to one week in the fridge, 5 ± 3°C, or not longer than 4 weeks at -20°C) saliva samples were used for the study. For saliva samples a streamlined RNA processing stage was applied according to Section 7.1.1. followed by Direct-RT-LAMP reaction amplification described in Section 7.2.

The validation performed by Genomtec on 44 contrived positive and negative saliva samples was based on the same protocol as described above. In this case CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel was used to evaluate infectious status of the patient referenced to nasopharyngeal swab. To produce contrived positive samples negative saliva samples were spiked with SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat Inactivated (cat. no. BEI Resources NR-52286) to a final concentration 6000 copies/ml.

A total of 63 specimens were included in the analysis of clinical performance. There were 2 unresolved cases by Real-Time RT-PCR, of which one was positive and one negative with Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit, whereas in 3 cases saliva sample did not contain human reference genetic material or produced incorrect fluorescent signal for IC (sample exceeded allowed time in a fridge), and one contrived positive sample breached interference substances guideline. These were excluded from the analysis. Table 4 depicts the overall assay clinical performance vs standard practice Real-Time RT-PCR method.

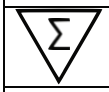
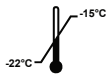
Table 4.

		Reference standard practice Real-Time RT-PCR assay result		
		Positive	Negative	Total
Genomtec® SARS-CoV-2 Direct-RT-LAMP CE-IVD Kit	Positive	30	-	**30**
	Negative	2	31	**33**
	Total	**32**	**31**	**63**
Sensitivity (SE)		30/32 = 93.75% (95% CI: 79.19%-99.23%)		
Specificity (SP)		31/31 = 100% (95% CI: 88.78%-100.00%)		
Positive Predictive Value (PPV)		30/30 = 100% (95% CI: 85.87%-100.00%)		
Negative Predictive Value (NPP)		31/33 = 93.94% (95% CI: 80.20%-98.34%)		

9.5.1. Conclusion

It has been confirmed that Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit test exhibits 100% specificity and 93.75% sensitivity compared with a standard (CE-IVD) laboratory Real-Time RT- PCR diagnostic test performed on swabs when detecting presence or absence of the SARS-CoV-2 virus in mixed clinical and contrived specimens. The PPV and NPP were 100% and 93.94%, respectively, whereas the test accuracy (probability that a patient is correctly classified) was obtained at 96.83% (95% CI: 89.00%-99.61%).

10. Symbols

Symbol (IEC 15223-1 2016)	Description
	Indicates the need for the user to consult the instructions for use.
	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Indicates the total number of IVD tests that can be performed with the IVD.
	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.
	Indicates a medical device that is compliant to the latest EU directive.
	Indicates the date after which the medical device is not to be used.
	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
	Indicates the temperature limits to which the medical device can be safely exposed.
	Indicates a medical device that should not be used if the package has been damaged or opened.
	Indicates a medical device that needs protection from light sources.
AmpMix	Indicates the Genomtec® SARS-CoV-2 AmpMix.
Duo-Primers	Indicates the Genomtec® SARS-CoV-2 Duo-Primers Mix.
C-Primers	Indicates the Genomtec® SARS-CoV-2 C-Primer Mix.
LysBuffer	Indicates the Genomtec® SARS-CoV-2 C-LysBuffer
Control+	Indicates the Genomtec® SARS-CoV-2 Positive Control.
Water	Indicates DNase/RNase-Free Sterile Water.

11. Ordering and contact information

Product	Order number
Genomtec® SARS-CoV-2 EvaGreen® Direct-RT-LAMP CE-IVD Kit - UK language	GA00CUK

For ordering:

https://genomtec.com/en/contact

For technical support:

Gehttps://genomtec.com/en/support/