GENOMTEC

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

Instructions for use

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

REF GA00B

IVD

Σ 50

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	30 October 2020	Initial release
B	25 November 2020	Second release
C	26 July 2021	Third release, general update
E	07 January 2022	Manufacturer’s address update

Table of contents

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® RT-LAMP CE-IVD Duo Kit general product information

3.1. Intended purpose

The Genomtec® SARS CoV-2 EvaGreen® RT-LAMP Duo test is a CE-IVD Laboratory Kit containing controls and reagents intended for 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 throat swab, nasopharyngeal swab or saliva specimens from 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.

Similarly, negative results do not exclude entirely COVID-19 disease and should be accompanied by other diagnostic solution ruling out absence of SARS-CoV-2 in patients. The wholistic patient management should carefully consider spectrum of clinical symptoms, patient history, linked to available epidemiological data.

Testing with the Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo 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® RT-LAMP CE-IVD Duo Kit contains assay enough to 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® RT-LAMP CE-IVD Duo Kit confirms presence of *N* and *S* genes encoding Nucleocapsid spike protein and the surface protein, respectively, in the analysed human sample.

Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo 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, and additionally 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 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.
Water	Vial containing DNase/RNase-Free Sterile Water.

The kit also includes the product information insert (PI00BrA) 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® RT-LAMP CE-IVD Duo Kit targets specific genomic regions of SARS-CoV-2 *N* and *S* genes that are unique for SARS-CoV-2, even though the genes *N* and *S* are present in other coronaviruses (e.g. SARS, MERS).

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).

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 and nucleic acid (RNA) purification.
- Reverse transcription of the purified nucleic acid and simultaneous cDNA amplification using the Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo 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 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 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
Roche Cobas z480

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, and additionally 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 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.
Water	Vial containing DNase/RNase-Free Sterile Water.

3.4.2. Materials required but not provided

- 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: -30°C to -10°C.

- Laboratory microcentrifuge (for 48 microtubes size 1.5 to 2 mLs) 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.
- Sterilise aerosol barrier (filtered) pipette tips.

4. Precautions

Testing with the Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo 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.
- The quality of RNA preparation (purification) may influence the quality of the RT-LAMP reaction, therefore it is recommended for laboratories to use previously validated purification method for each sample type (saliva, throat swab, or nasopharyngeal swab). Sample RNA purification for the purpose to be used with this product has been confirmed for the following RNA isolation kits - Roche: MagNA Pure 96 DNA and Viral NA Small Volume Kit, High Pure Viral Nucleic Acid Kit, High Pure Viral RNA Kit; Qiagen: EZ1 Virus Mini Kit v2.0, EZ1 DSP Virus Kit, QIAamp MinElute, QIAamp Viral RNA Mini Kit Virus Spin Kit, QIAamp DSP Virus Kit.

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.

The sediment observed at the bottom of AmpMix vial may occur naturally. In such case it is recommended to leave the vial for 10 min at room temperature and then mixing its content by pipetting.

Table 1.

Reagent	Quantity	Volume	Storage limits	Shipping limits	Shelf life
Genomtec® SARS-CoV-2 AmpMix	1 vial	1350 µl	-22°C to -15°C	Dry or wet ice	Six months
Genomtec® SARS-CoV-2 Duo-Primers	1 vial	100 µl	-22°C to -15°C	Dry or wet ice	Six months
Genomtec® SARS-CoV-2 C-Primers	1 vial	100 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
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.

6. Quality control

6.1. Assay controls

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

Include the below Controls:

Type of Control	Contents and targets	Function
Positive Control	Synthetic SARS-CoV-2 cDNA with amplification mix 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 Control	Amplification mix with detection primers recognising specific sequences on targeted *N* and *S* gene of SARS-CoV-2 combined with DNase/RNase-Free Water	Ensures lack of cross-contamination arising from assay set-up
Inhibition Control	Amplification mix and control primers with added patient's extracted RNA sample. Primers target reference sequence of a human genome.	Controls possible inhibition of the amplification and appropriate sample collection procedure (e.g. 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 throat swab and nasopharyngeal swab specimens.

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

The specimen should be transported on the Universal Transport Medium, 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. RNA samples must be shipped on dry ice.

- Note: nasopharyngeal aspirate, and bronchoalveolar lavage (BAL) specimens have not been validated for use.
- Note: saliva samples have been validated by spiking SARS-CoV-2 negative human saliva samples with a heat-inactivated SARS-CoV-2 virus of different concentration with subsequent RNA extraction procedure. Saliva samples were not included in the clinical evaluation described in Section 9.4.

7. Operating instructions

7.1. Before starting the test

IMPORTANT

- 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 purification 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 Amplification Mix) from light.
- Each sample requires a concomitant inhibition control run and positive and negative 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 to preserve their potency.

7.1.1. Preparation step 1

The first step in the diagnostic workflow is sample collection (throat swab or nasopharyngeal swab specimens or saliva) and sample RNA purification (adequate for the sample collection type).

7.1.2. Preparation step 2

The quality of RNA purification may influence the quality of the RT-LAMP reaction; therefore, it is recommended for laboratories to use previously validated and commercially available purification method. For the saliva sample the minimum recommended volume at point of RNA purification is 200 µl.

7.1.3. Preparation step 3

All provided and necessary reagents and assay components should be defrosted at ≤4°C, followed by gentle vortexing (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 control 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 20 µl

Table 2. Reaction plate set-up.

Reagent	Analyte	Inhibition Control	Positive Control	Negative Control
Genomtec® SARS-CoV-2 AmpMix	13.5 µl	13.5 µl	13.5 µl	13.5 µl
Genomtec® SARS-CoV-2 Duo-Primers	1.5µl	-	1.5µl	1.5µl
Genomtec® SARS-CoV-2 C-Primers	-	1.5µl	-	-
Sample RNA	5 µl	5 µl	-	-
Genomtec® SARS-CoV-2 Control+	-	-	5 µl	-
DNase/RNase-Free Water	-	-	-	5 µl
Total Volume	20 µl	20 µl	20 µl	20 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.

7.2.4. Test step 4

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

Step	Temperature [°C]	Time [Sec.]	Cycles/ repeats
Amplification 1	64	30	30
Amplification 2*	64	30	

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

Signal detection is performed after every minute of the heating cycle (resulting in 29 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

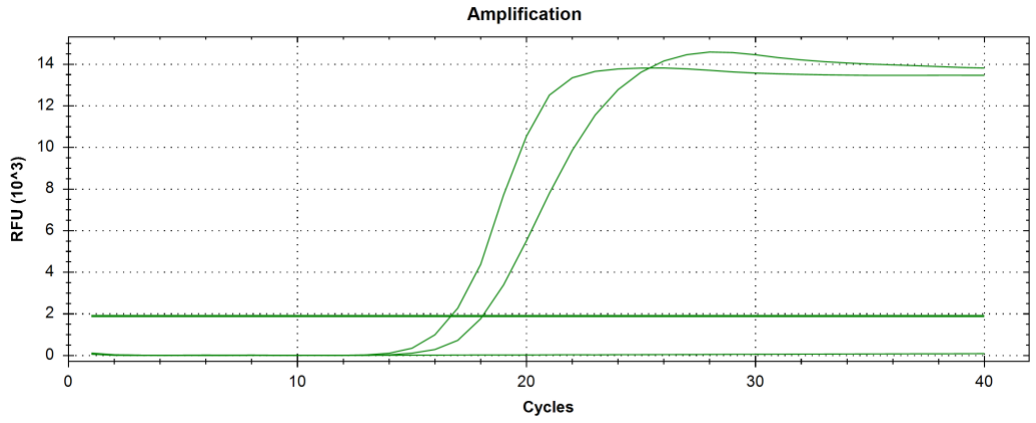
from the *S* gene amplicon. The two targets are identified based on their melting temperature. Real time instrument settings are presented in table below.

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

7.2.5. Test step 5

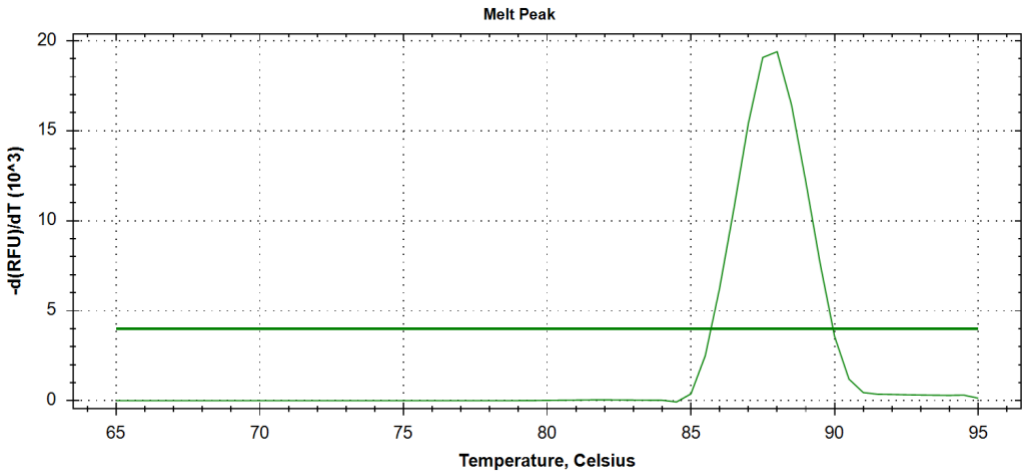
The Real-Time PCR instrument must be able to operate on 20 µl total volume in an individual well of a multi-well PCR plate or individual tube in a strip of PCR-tubes with optically clear caps and must be equipped with optics and filters allowing fluorescence reading at FAM (Green) channel. The list of 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.

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

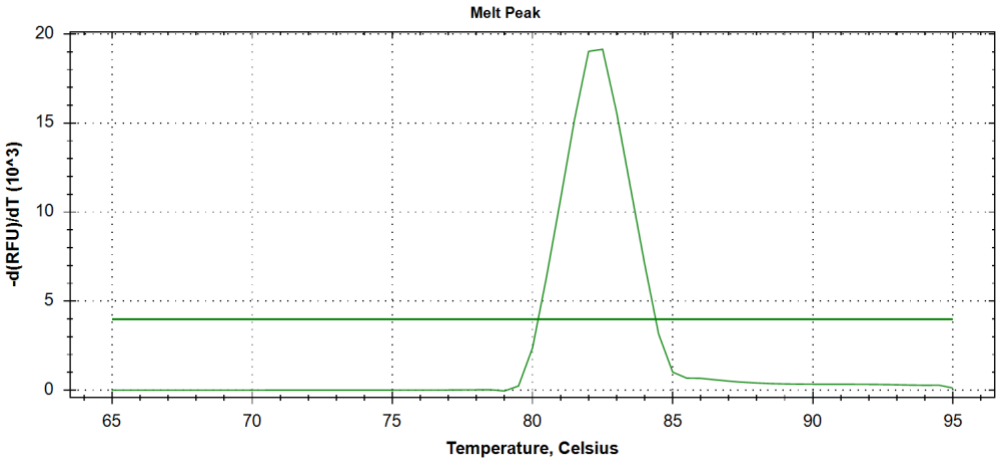


Below you can find an examples of melting peaks corresponding to the *N* gene amplicon, at T_m around 88.0°C, and also to the *S* gene amplicon, at T_m around 82.5°C. These were generated on Bio-Rad CFX96 Dx System. On different Real-Time PCR thermocycler, the Tm might slightly vary but should stay within range of 82.5°C to 83.5°C (gene *S*) and 87.5°C to 88°C (gene *N*).

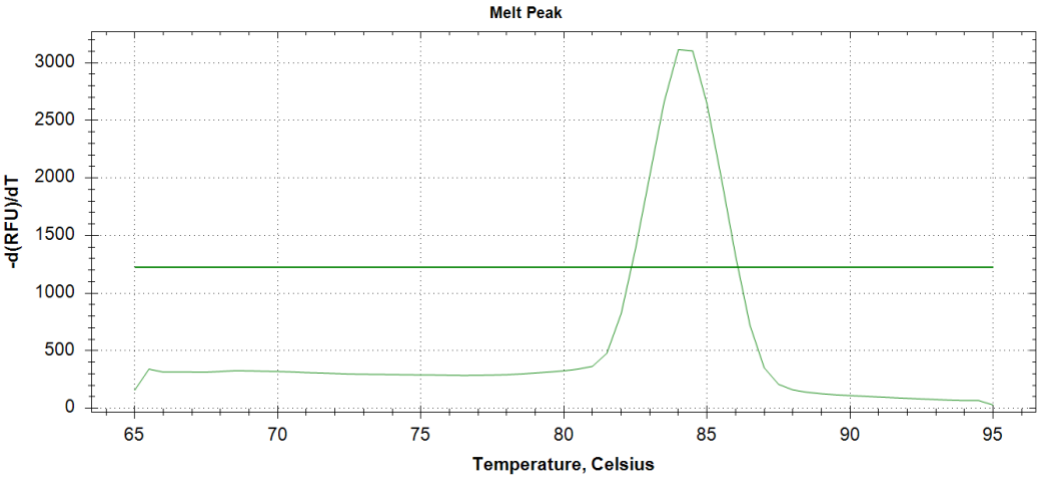
Melting Curve for gene *N* (melting temperature 88° C)



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



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



8. Result interpretation

8.1. Interpreting patients' results

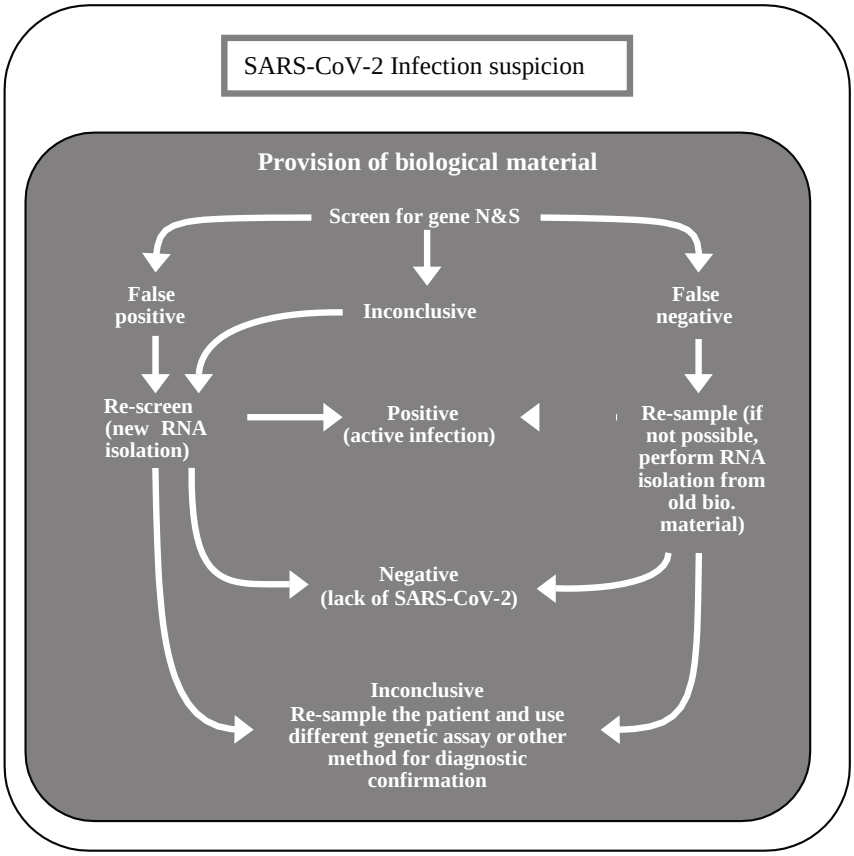
Genomtec's recommendation for use Product line Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo Kit are as follows:

- Where new or suspected cases of COVID-19 disease arise in the population utilise Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo 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.

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.

In cases of a signal being detected in negative control or no signal detected in positive control the amplification run has to be repeated.

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



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	Expected Result
+	+	POSITIVE
-	+	NEGATIVE
+	-	FALSE POSITIVE (in case of positive amplification in negative control)
-	-	FALSE NEGATIVE
+	-	INCONCLUSIVE

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 – repeat test on freshly isolated RNA from the current biological material (use only old RNA sample if purification is not possible) and if the repeat 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 negative control) – repeat test with previously utilised RNA sample 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® RT-LAMP CE-IVD Duo 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® RT-LAMP CE-IVD Duo Kit was externally validated on throat and nasopharangeal swabs for SARS-CoV-2 detection. In the external clinical evaluation as 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 implement previously laboratory-validated RNA purification method for each sample type used to provide the highest quality of RNA isolate.

- The FALSE NEGATIVE results may be indicative of:
    - Unsuccessful biological material collection.
    - RNA material degradation during storage / transport (lack of guideline compliance).
    - Inefficient RNA purification.
    - 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.

- The FALSE POSITIVE Positive signal obtained in the negative control may be indicative of:
    - Cross-contamination of the assay components and / or samples (RNA) 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® RT-LAMP CE-IVD Duo 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 all positive results to the appropriate Competent 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® RT-LAMP CE-IVD Duo Kit.

Limit of detection of the assay was determined by performing 20 reactions for series of dilutions of SARS-CoV-2 whole genome RNA from Quantitative Heat-Inactivated SARS-Related Coronavirus 2, Isolate USA-WA1/2020 control and determined at the lowest genes' copies per reaction at 95% CI. The LOD of the SARS-CoV- 2 Duo assay is 20 genome equivalents / 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® RT-LAMP CE-IVD Duo 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® RT-LAMP CE-IVD Duo 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. Clinical evaluation

A clinical study was performed to evaluate the performance of the Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo Kit on mixture of throat and nasopharyngeal swabs obtained from patients suspected of contracting / developing COVID-19 disease on the territory of Republic of Poland. The study samples used in the investigation included leftover specimens collected for the routine clinical care and analysis that would otherwise had been discarded. The pre-released version of the test was evaluated by one independent medical laboratories in Poland, and a total of 40 clinical specimens were tested in Q4 2020 during the course of the study.

The method of sample collection and transport medium used (type and the manufacturer) complied with the general clinical laboratory regulations enforced by the relevant Polish Healthcare Authority (Panstwowy Zakład Higieny), and also with the WHO recommendations.

Patients that underwent swab collection were referred to such diagnostic procedure based on either clinical symptoms, suspicion of contracting the disease due to contact with infected individual(s) or travelled from abroad and were subjected quarantine. All patients were referred to conduct the genetic diagnostic test against SARS-CoV-2 by a healthcare professional.

There was one swab collected from each patient that had priority to undergo testing utilising the standard care molecular diagnostic test based on a Real-Time RT-PCR technology. If the remaining leftover RNA sample contained enough genetic material to set-up an assay using the pre-released version of Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo Kit, it was processed and executed on a standard Real-Time PCR instrument.

The Real-Time RT-PCR CE-IVD diagnostic kits (detecting at least two genes) were used as reference method for RT-LAMP assay and the protocol was compliant with the manufacturer's instructions. Investigated samples included in the study were also processed with the pre-released version of Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo Kit according to instruction enclosed in this document. The nucleic acid purification was carried with one of the commercially available kits. A total of 39 specimens were included in the analysis of clinical performance. In 1 case the specimen that produced unresolved results by Real-Time RT-PCR and positive result with Genomtec® SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo Kit was excluded from the analysis (lack of diagnostic confirmation according to manufacturer). Table 4 depicts the overall assay clinical performance vs standard practice Real-Time RT- PCR method.

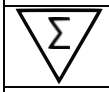
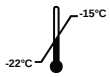
Table 4.

		Reference standard practice RT-qPCR assay result		
		Positive	Negative	Total
Genomtec® SARS-CoV-2 RT-LAMP CE-IVD Duo Kit	Positive	16	-	**16**
	Negative	-	23	**23**
	Total	**16**	**23**	**39**
Sensitivity (SE)		16/16 = 100% (95% CI: 79.41%-100.00%)		
Specificity (SP)		23/23 = 100% (95% CI: 85.18%-100.00%)		
Positive Predictive Value (PPV)		16/16 = 100% (95% CI: 75.93%-100.00%)		
Negative Predictive Value (NPP)		23/23 = 100% (95% CI: 82.20%-100.00%)		

9.4.1. Conclusion

It has been confirmed that Genomtec(R) SARS-CoV-2 EvaGreen® RT-LAMP CE-IVD Duo Kit test exhibits 100% sensitivity and specificity compared with a standard (CE-IVD) laboratory Real-Time RT-PCR diagnostic test when detecting presence or absence of the SARS-CoV-2 virus in clinical specimens. Both, the PPV and NPP were also obtained at 100% as well as the test accuracy (probability that a patient is correctly classified) was obtained at 100% (95% CI: 90.97%-100.00%).

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.
Control+	Indicates the Genomtec® SARS-CoV-2 Positive Control.
Water	Indicates DNase/RNase-Free Sterile Water.

1. Ordering and contact information

Product	Order number
Genomtec® SARS-CoV-2 EvaGreen® RT- LAMP CE-IVD Duo Kit - UK language	GA00BUK

For ordering:

https://genomtec.com/en/contact

For technical support:

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