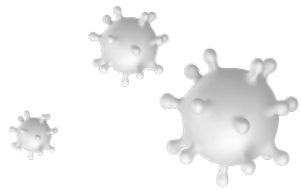
Diagnostic kit Vivid COVID-19 LAMP Direct-G / 200 tests

User Information

Ver. 2.0 (en), 04/2021



GENERAL CAUTIONS!

- This kit is intended for *in vitro* diagnostic purposes.
- Before starting work, read all instructions contained in this document, the outer packaging, and the component labels.
- SARS-CoV-2 is a dangerous pathogen, therefore all relevant regulations and recommendations for handling this type of pathogen should be followed.
- A positive result obtained with the VIVID COVID-19 LAMP Direct-G kit implies the presence of SARS-CoV-2 RNA in the assayed sample and should not be the sole criterion for assessing the infectivity of the person who provided the sample. It is also necessary to consider other factors such as clinical presentation, results of other diagnostic tests and personal/medical history of the person concerned. Similarly, a negative result does not rule out an infection of the tested person, especially in the presence of symptoms consistent with COVID-19. The results should always be interpreted by trained medical personnel. In the event of any discrepancies between the test result and other clinically relevant information, follow the appropriate procedures of the health agency in your jurisdiction.

1. Explanations and abbreviations

BSL: bio-safety level
COVID-19: disease caused by the SARS-CoV-2 virus (COronaVIrus Disease 2019)
DNA: deoxyribonucleic acid
dUTP: deoxyuridine triphosphate
E gene: a gene encoding the small membrane envelope protein of the SARS-CoV-2 virus
LAMP: Loop Mediated Isothermal Amplification
LoD: limit of detection
N gene: a gene encoding the nucleocapsid protein of the SARS-CoV-2 virus
NTC: No Template Control
ORF1ab gene: a gene encoding the Open Reading Frame 1ab of the SARS-CoV-2 virus
PC: Positive Control
qPCR: quantitative Polymerase Chain Reaction
RNA: ribonucleic acid
RNase P gene: a gene encoding human nuclear ribonuclease P
RT-LAMP: reverse transcription LAMP
RT-qPCR: reverse transcription qPCR
SARS-CoV-2: Severe Acute Respiratory Syndrome CoronaVirus 2

2. Intended use

The **Vivid COVID-19 LAMP Direct-G** diagnostic kit is a qualitative in vitro diagnostic test designed to detect the presence of SARS-CoV-2 coronavirus genetic material in samples of biological material obtained from the human upper respiratory tract by gargling using RT-LAMP. The Vivid COVID-19 LAMP Direct-G diagnostic kit is intended for use at properly equipped premises with appropriate safety standards, equipment, and trained personnel.

3. Test principle

The Vivid COVID-19 LAMP Direct-G kit is a diagnostic kit designed to detect the genomic RNA of SARS-CoV-2 in gargle samples without an RNA extraction step.

The Vivid COVID-19 LAMP Direct-G diagnostic kit is an internally developed LAMP test for the detection of SARS-CoV-2 virus suitable for both fluorescent and visual detection of the presence of SARS-CoV-2 RNA. The assay is one-step, i.e., involves RT and LAMP amplification in one reaction (RT-LAMP). In addition, this assay is designed to work with only minimally processed gargle samples with no prior RNA extraction required; samples only have to be heated/incubated with the provided inactivation reagent as per instructions before setting up the reaction. The kit contains a set of primers for the multiplexed detection of SARS-CoV-2 E, ORF1ab and N genes. This primer mix was specifically designed and optimized so that primer interactions do not lead to non-specific amplification products and thus to false positive results that are relatively common to the LAMP method. At the same time, multiplexed LAMP detection significantly increases the sensitivity and reduces the variation in detection time. The second set of primers is intended for the detection of the human RNase P gene, which serves as an internal control to verify correct patient material sampling, sample processing, RNA integrity, and test execution. While sequence-wise this primer set does not distinguish between genomic DNA and messenger RNA it does show significantly increased sensitivity towards RNA substrates in practice. The complete genomic RNA of the SARS-CoV-2 virus enriched with human RNA provided by the Biomedical Center of the Slovak Academy of Sciences is included as an internal positive control. A dry block heater (with or without lid heating) capable of heating samples to at least 95 °C is sufficient for the test. In addition, the kit contains a patented proprietary colorimetric detection system, which is not sensitive to the pH of the input sample. With this system positive samples show a reliable color change from magenta to yellow which is easily visible by the naked eye.

When used as recommended, one kit package is sufficient to test 200 samples (200 reactions for SARS-CoV-2 and 200 reactions for RNase P). For details on the preparation of assays, see section 7.3. Preparation of the reaction mixture. The human RNase P internal positive control assay serves to verify the quality of sample collection and processing and to determine the presence of human RNA in the extracted sample, thus eliminating false negative results.

4. Kit composition

- **1x mixture of lyophilized primers for detection of SARS-CoV-2, marked SARS-CoV-2 Primer Mix (must be dissolved in 1100 µl PCR water)**
- **1x mixture of lyophilized primers for the detection of human RNase P, marked RNase P Primer Mix (must be dissolved in 550 µl PCR water)**
- **1x lyophilized PC SARS-CoV-2 BMC5 (must be dissolved in 250 µl PCR water)**
- **4x 1.875 ml LAMP 2x Master Mix**
- **2x 1 ml LAMP 10x Lysis Buffer**
- **2x 5 ml PCR Water, nuclease free**
- **1x Instructions for use**

5. Storage and shelf life

CAUTION!
Do not use the kit after the expiry date, which is stated on the outer box.

NOTICE!

- **Wear suitable protective clothing, gloves, and eye/face protection.**
- **Always wash your hands thoroughly when handling specimens and reagents**

All kit components must be transported and stored at –20 °C. LAMP 2x Master Mix contains components that are light-sensitive and should be protected from light whenever possible. The shelf life of the kit is a maximum of 12 months from the manufacturing date. The exact expiration date of the kit is indicated on the outer packaging. Likewise, the expiration date of individual components of the kit is indicated on the inner packaging.

For users who perform fewer reactions in a run, we recommend aliquoting all kit components according to internal procedures and the standard number of reactions per run. Aliquoting the kit components minimizes the need to reopen individual tubes and thus minimizes the risk of contamination of the kit components. Aliquoting the components of the kit will also prevent repeated thawing and freezing of the individual components, which may lead to reduced efficiency. Before aliquoting, it is necessary to completely thaw the individual components of the kit. Thawing is optimally performed gently, by incubation in a refrigerator (at 4 °C) for approximately 2 hours. Immediately before aliquoting, it is necessary to mix the contents of the tubes thoroughly, until the mixture is homogeneous. In the case of tubes containing enzymes (LAMP 2x Master Mix), we recommend mixing by rotating the tube several times or short pulse vortexing.

6. Consumables and equipment not included in the kit

- *(for sample inactivation)* Dry block heater capable of heating samples to at least 95 °C
- *(for amplification)* Heating instrument capable of heating samples to at least 65 °C - the **Vivid COVID-19 LAMP Direct-G** kit has been validated and tested on the following devices:
  - » Real-time PCR instruments: Agilent devices - Mx3005P® and AriaMx®; Thermo Fisher Scientific - QuantStudio™ 5; BioRad - CFX96™
  - » Thermocyclers: SensoQuest – SensoQuest Gradient Labcycler
  - » Dry block heaters: Thermo Fisher Scientific – the Digital Dry Baths range of products and Fisherbrand™ Isotemp Digital Dry Baths/Block Heaters; Eppendorf - ThermoMixer® C
- Vortex mixer
- Mini centrifuge
- PCR plate centrifuge (when working with PCR plates)
- Gargle collection kits
- Personal protective equipment: powder-free disposable laboratory gloves, goggles, protective shield, FFP3 respirator, protective clothing

- Laboratory plasticware certified sterile and free of DNA, RNA, DNases, and RNases: reagent tubes, PCR tubes, PCR strips, PCR plates, PCR foils or other consumables compatible with the chosen method to run the reaction; sterile pipette tips with filter
- Autoclavable adjustable micropipettes
- Bio-waste container
- Autoclavable test tube racks
- Coolers for reagent vessels of choice

7. Workflow

CAUTION!

Work with the kit must be performed by qualified personnel.

NOTICE!

- **Workspaces should be arranged in such a way that there are separate, dedicated rooms (zones), laboratory equipment, and consumables for each step in the workflow: sample collection, sample inactivation and amplification/detection. The amplified products should never come into contact with space, equipment, and consumables intended for the collection of samples or for the preparation of amplification mixtures.**
- **Maintain separate specialized laboratory equipment and supplies for each step in the procedure: nucleic acid isolation, preparation of amplification reactions, or amplification and detection of amplification products.**
- **Use lab coats, gloves, and all other protective equipment exclusively for either nucleic acid isolation or preparation of amplification reactions or for amplification / detection of amplification products themselves. Never use the same lab coats, gloves or aids in different rooms (zones).**
- **Always handle all biological samples as potentially infectious material and avoid direct contact with biological material. Avoid spilling samples and reagents and generating aerosols.**

To perform RT-LAMP, having at least 2 different sources of heating is recommended:

a) A heating source capable of heating samples to 95 °C – a dry block heater with blocks suitable for 0.5 ml tubes, 0.2 ml PCR tubes/strips or PCR plates. This source should be preheated at all times and can also be used for the optional reaction termination step (see section 7.5. RT-LAMP reaction parameters).

b) A heating source capable of heating samples to 65 °C – a dry block heater, thermocycler, or PCR instrument suitable for 0.2 ml PCR tubes/strips or PCR plates. Large volume reaction vessels (e.g., 0.5 ml) can be used but we recommend against using them without ensuring the lids are heated due to possible water condensation issues.

7.1 Sampling and sample preparation

CAUTION!

Vivid COVID-19 LAMP Direct-G kit is designed to be used with direct gargle samples only. Using sample types other than described below may lead to impaired functional characteristics of the assay!

NOTICE!

- **The gargling procedure described below is only a recommendation and matches the instructions given to patients during sample collection for clinical validation. The optimal method of gargling for the detection of SARS-CoV-2 has not been determined.**
- **It is NOT advisable to eat, drink, brush teeth, rinse mouth, gargle, chew gum or smoke/vape at least one hour before collecting a sample.**
- **Commercial saline solutions are recommended; however, in-house prepared solutions are acceptable as long as the water source used is WFI (Water for Injection) quality or equivalent.**

Sample collection:

- The patient is given a suitable container (e.g., polypropylene 50 ml tube) containing 5 ml of isotonic saline solution (0.9% NaCl by weight in water).
- The patient is then asked to gargle with the provided saline solution 10 times for 5 seconds each taking care not to swallow the solution. This takes around 1 minute in total.
- The patient then slowly and carefully spews out the gargle into a prepared container (for example the same one saline was provided in). Once the container is disinfected from the outside, the
- gargle sample is ready for further processing.
- If inactivation cannot be performed immediately, store the fresh gargle samples at 4 °C for up to a week.

Sample processing:

- Carefully vortex the gargle sample to resuspend any settled material. Aggressive vortexing may lead to foaming and splashing which carries a significant biohazard risk at this point as the sample has not been inactivated yet.
- In a tube with a minimal volume of 200 µl (for example a PCR or 0.5 ml tube) mix gargle (9 parts) with the provided LAMP 10x Lysis Buffer (1 part), specifically:

  90 µl gargle + 10 µl LAMP 10x Lysis Buffer
- Close the tube and gently mix.
- Let incubate at room temperature for 3 minutes. Modestly longer incubation times (up to 10 minutes) are not detrimental.
- Heat the sample at 95 °C for 7 minutes. This step thermally inactivates viral particles and releases viral RNA into solution.
- Let the sample cool down for a few seconds. Then briefly centrifuge the sample on a tabletop mini centrifuge for 1 minute. Precipitated proteins and other debris will form a pellet at the bottom.
- The resulting supernatant is to be used as the input for the RT-LAMP reaction.
- Ideally, inactivated gargle samples should be assayed as soon as possible; however, they are stable for up to a day at –20 °C. For long-term, samples should be stored at –70 °C or below. Avoid repeated thawing and freezing of samples.

CAUTION!

During the workflow, always wear personal protective equipment and work in a designated area. Handle all samples with caution and treat them as potentially infectious material.

7.2. Preparation of reagents

UPOZORNENIA!

Use the UV decontamination cycle before and after to thoroughly decontaminate the working area if possible.

Always prepare reagents for amplification separately, preparing reagents exclusively for one analysis at a time.

NOTICE!

- **Use only reagents contained in this kit and reagents recommended by the manufacturer.**
- **Do not combine or mix reagents from different lots.**

Primer mixtures for the detection of SARS-CoV-2 and human RNase P and the positive control are supplied in lyophilized form to increase stability. Therefore, when using the kit for the first time, it is necessary to dissolve the primers and positive control in PCR water. Because the amount of primers in the SARS-CoV-2 Primer Mix is very high, it may take longer than expected to dissolve the entire pellet. Dilute the lyophilized kit components as follows:

Table 1. **Preparation of lyophilized reagents**

Kit component	PCR Water volume to dissolve	Useable reactions
SARS-CoV-2 Primer Mix	1100 µl	200
RNase P Primer Mix	550 µl	200
PC SARS-CoV-2 BMC5	250 µl	40

CAUTION!

The pellets of lyophilized primers and the positive control may get released during transport; therefore, it is necessary to centrifuge each tube briefly before opening. Omission of this step may result in loss of material from the tube, which may affect thefunctional characteristics of the kit.

7.3. Preparation of the reaction mixture

The recommended **total volume per reaction is 50 µl for SARS-CoV-2 detection and 25 µl for RNase P detection**. To prepare the reaction mixture, the individual components of the kit must be mixed in the following ratio:

Table 2. **Reaction mixture preparation**

Kit component	Component volume per test reaction	
	SARS-CoV-2	RNáza P
PCR Water	12 µl	6 µl
LAMP 2x Master Mix	25 µl	12.5 µl
SARS-CoV-2 Primer Mix	5 µl	-
RNase P Primer Mix	-	2.5 µl
Total volume	**42 µl**	**21 µl**

A minimum of 1 negative control must be included in each analysis to verify the presence of contamination. As a negative control, a no-template control (NTC) containing PCR water instead of an unknown sample is used. Each primer combination tested (SARS-CoV-2 and RNase P) must have its own NTC. Similarly, it is necessary to include at least 1 positive control to verify the analysis procedure is correct and the functionality of kit components, where the reaction containing the PC SARS-CoV-2 BMC5 control material serves as a positive control.

Prepare the required number of clean PCR tubes, PCR strips, PCR plates or other suitable reaction vessels and place them in a refrigerated cooling rack, if available. Mix the prepared reaction mixture thoroughly, but at the same time gently, by inverting the tube several times or by short pulse vortexing. Then centrifuge it briefly to remove droplets from inside the cap and to collect the entire contents at the bottom of the tube. Pipette the prepared reaction mixtures into the individual reaction vessels according to the required number of reactions and sample positions.

Table 3. **Sample addition**

Sample type	Tested sample		Positive control		NTC	
	SARS-CoV-2	RNase P	SARS-CoV-2	RNase P	SARS-CoV-2	RNase P
Inactivated sample supernatant	8 µl	4 µl	-	-	-	-
PC SARS-CoV-2 BMC5	-	-	8 µl	4 µl	-	-
PCR Water	-	-	-	-	8 µl	4 µl

After use, return all components of the kit to the freezer (at -20 ° C). Avoid repeated thawing and freezing of kit components. If re-use is planned within less than 2 hours, store the kit components at 4 ° C.

NOTICE!

- **The reaction mixture has limited stability, use it as soon as possible after preparation. If the reaction mixture cannot be used immediately, store it in a refrigerator at 4 °C for at most 30 minutes.**
- **When preparing multiple reactions, it is recommended to make at least 5% extra reaction mixture to account for pipetting errors (we suggest 5% extra rounded up to the nearest whole number of reactions).**

7.4. Final reaction and controls preparation

Add samples to the prepared reaction mixture according to the table below. **The resulting total reaction volume is 50 µl for the SARS-CoV-2 assay and 25 µl for the RNase P assay.**

NOTICE!

- **Using a sample input volume other than proposed may lead to compromised assay performance.**
- **If using a block heater to run the reactions, make sure it is preheated to the operating temperature of 65 °C.**
- **The dry block should be specific to the reaction vessels of choice to ensure good contact between the vessels and the dry block.**

Transfer the reaction vessels with the pipetted reaction mixture from the clean zone (preferably a laminar box) to the zone reserved for handling positive controls and patient samples (e.g., PCR box). Add samples and seal reaction vessels tightly (caps or optical foil). Mix the mixture in the reaction vessels and centrifuge briefly so that all the liquid is at the bottom of the tubes/wells and place them in a pre-prepared heating device.

7.5. RT-LAMP reaction parameters

Follow the instructions below to set test conditions such as reaction temperature, reaction time, and reaction termination.

Reaction parameters:

- **Reaction temperature: 65 °C**
- **Reaction volume: 50/25 µl**
- **Runtime: 40 minutes standard, 50 minutes for ambiguous samples *(see section 8. Interpretation of results)***
- **Reaction termination (optional): 1 minute at 95 °C or quickly chilling down the reaction vessels to below 40 °C**

NOTICE!

- **While not required, reaction termination is recommended if using colorimetric detection of amplification especially if the results are not evaluated within a few minutes after the amplification has finished. However, if using colorimetric detection, first verify the presence of possible inconclusive samples before proceeding with the reaction termination step.**

CAUTION!

Handle amplification products with extreme care to avoid dispersal into the testing area and possible contamination of new test specimens. Use pipettes designated exclusively for handling amplification products and use disposable filter tips that are certified sterile and free of DNA, RNA, DNases, and RNases.

Do not under any circumstances open the reaction vessels after performing LAMP amplification. LAMP amplification produces orders of magnitude more amplicons than PCR. Even low levels of LAMP amplicon contamination may result in spuriously positive results. While this diagnostic kit does utilize dUTP and thermolabile uracil-DNA glycosylase to reduce the impact of a possible contamination, it may be insufficient to prevent false positive results in areas heavily contaminated with amplicons. If reaction vessels are known to have been opened accidentally, implement amplicon decontamination procedures as soon as possible.

7.6. Analysis of the obtained data

To analyze visual changes, you will need the RT-LAMP color scale, which can be found in section 8. Interpretation of results.

Color change interpretation:

» A reaction is considered **positive** if the final reaction mix color **is NOT group 0 to 1 (any color judged to be more yellow than group 1)** according to the RT-LAMP color chart as judged by the assay operator.

» A reaction is considered **negative** if the final reaction mix color **is NOT group 1 to 3 (any color judged to be more magenta/pink than group 1)** according to the RT-LAMP color chart as judged by the assay operator.

» A reaction is considered **inconclusive** if the final reaction mix color **is group 1** according to the RT-LAMP color chart as judged by the assay operator.

CAUTION!

To achieve consistent results, evaluate colorimetric changes in a well-lit area preferably with a white background!

If color change interpretation is to be performed more than an hour after finishing the reaction, store the reaction vessels in a dark area. The reaction mix is light-sensitive, and its color will slowly fade upon exposure to light (a significant loss of color is observed after 2 days of light exposure).

8. Interpretation of results

8.1. Interpretation of results — controls

CAUTION!

Deviation from the expected performance of the controls suggests improper assay set up and/or execution, or failure/malfunction of reagents and/or equipment could have occurred. Invalidate the run and re-test.

No template control (NTC)
The NTC consists of using nuclease-free water (PCR water) in the RT-LAMP reactions instead of RNA.

The NTC reactions for all primer sets should not display any overt color change (reaction mix stays magenta/pink). If any of the NTC reactions change color, sample contamination may have occurred. Invalidate the run and repeat the assay with strict adherence to the guidelines.

Positive control (PC SARS-CoV-2 BMC5)
The PC SARS-CoV-2 BMC5 consists of lyophilized isolated genomic RNA of SARS-CoV-2 spiked with human RNA and co-precipitant. The PC SARS-CoV-2 BMC5 will yield a positive result with all primer sets (i.e., SARS-CoV-2 and RNase P). Indications of an error or failure in the workflow or analysis of the experiment are indicated by inconclusive color change or its absence. In the case of a negative result in the positive control, it is not possible to unambiguously determine the correctness of other positive/negative results obtained in the given analysis and to distinguish between negative and false negative results. Therefore, the output of such an analysis cannot be evaluated.

Table 4. **Expected performance of controls included in the Vivid COVID-19 LAMP Direct-G kit**

Control type	External control	SARS-CoV-2	RNase P	Expected color change	Possible causes of the unexpected results
Positive	PC SARS-CoV-2 BMC5	+	+	Magenta → yellow	Substantial reagent failure including primer or positive control integrity
Negative	NTC	–	–	No color change (magenta)	Reagent and/ or environmental contamination

8.2. Interpretation of results — test reactions

RNase P assay (sampling control)
All clinical samples should exhibit a positive reaction with the human RNase P primer set. Failure to detect RNase P in any clinical specimens may indicate:
- Absence of sufficient human cellular material due to poor gargling technique, or poor collection, or loss of specimen integrity.
- Improper handling/processing/storage of clinical materials resulting in loss of RNA and/or RNA degradation.
- Improper assay set up and execution.
- Reagent or equipment malfunction.

If the RNase P assay is **negative** for a human clinical specimen, interpret the result as follows:
- If the SARS-CoV-2 assay is **positive** even in the absence of a positive RNase P, the result should be by default considered valid. It is possible that some samples may fail to exhibit RNase P amplification – a negative RNase P signal does not preclude the presence of SARS-CoV-2 RNA in a clinical specimen. This may be due to differences in the stability of human mRNA versus viral RNA in gargle and during sample processing which can be further exacerbated by the higher sensitivity of the SARS-CoV-2 assay compared with RNase P. The observation of an inconclusive color change in the SARS-CoV-2 assay with a negative RNase P suggests that sample contamination may have occurred. In this case, repeating the assay is advisable if possible, see Vivid COVID-19 LAMP Direct-G diagnostic test results interpretation guide.

- If the SARS-CoV-2 assay is **negative**, the result should be considered invalid for the specimen. If residual specimen is available, repeat the test. If both assays remain negative after re-testing, report the results as invalid and collect a new specimen if possible.

SARS-CoV-2 assay
- When all controls exhibit the expected performance, a specimen is considered negative if the SARS-CoV-2 assay is **negative** and human RNase P is **positive**.

- When all controls exhibit the expected performance, a specimen is considered **positive** if the SARS-CoV-2 assay is **positive** and human RNase P assay is either **positive, inconclusive, or negative**. However, under certain conditions the combination of a positive SARS-CoV-2 assay with a negative RNase P assay result may suggest contamination has occurred, see sections RNase P assay (sampling control) and Vivid COVID-19 LAMP Direct-G diagnostic test results interpretation guide above and below, respectively.

- When all controls exhibit the expected performance, a specimen is considered **inconclusive** if the SARS-CoV-2 assay is inconclusive and RNase P assay is either **positive, inconclusive, or negative**. Alternatively, SARS-CoV-2 assay is **negative** and RNase P assay is **inconclusive**.
  - Inconclusive samples should be marked and incubated at 65 °C for 10 more minutes. Afterwards, the operator should again try to classify the color change of the previously inconclusive samples according to the RT-LAMP color chart. If the color change is still judged as inconclusive, invalidate the test result and re-test. If running samples in a multi-assay format (strips, plates, etc.) disregard any negative samples turning positive/inconclusive during this extended incubation time. To minimize operator errors, we highly recommend documenting the final reaction mix color before and after the extended incubation.

- When all controls exhibit the expected performance, a specimen is considered **invalid** if the SARS-CoV-2 assay is **negative** and RNase P assay is **negative**.
  - The specimen should be re-tested if possible. If no specimen is available, collect a new specimen.

Vivid COVID-19 LAMP Direct-G diagnostic test results interpretation guide
The table below lists the expected results for the Vivid COVID-19 LAMP Direct-G diagnostic test.
If a laboratory obtains unexpected results for assay controls or if inconclusive or invalid results are obtained and cannot be resolved through the recommended re-testing, please consult your public health authority.

Table 5. **Test interpretation and corresponding actions**

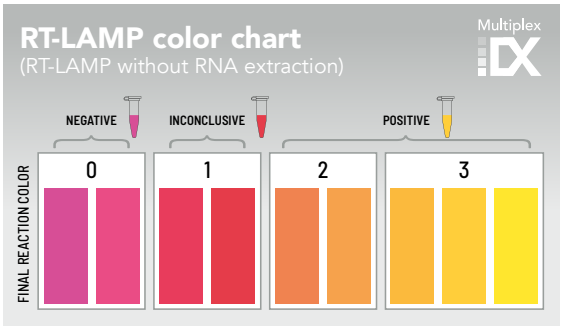
SARS-CoV-2	RNase P	Result interpretation[a]	Report	Actions
+	+	SARS-CoV-2 detected	SARS-CoV-2 positive	Finalize and report the results[a].
+	–/?	SARS-CoV-2 detected	SARS-CoV-2 positive	After consideration[b] finalize and report the results[a].
?	+/–/?	Inconclusive result	Inconclusive	Incubate the inconclusive result for 10 more minutes at 65 °C. If still inconclusive, the result is invalid, and a re-test is necessary.
–	?			
–	+	SARS-CoV-2 not detected	Not detected	Finalize and report the results[a, c].
–	–	Invalid result	Invalid	Re-test. If still invalid, collect a new specimen.

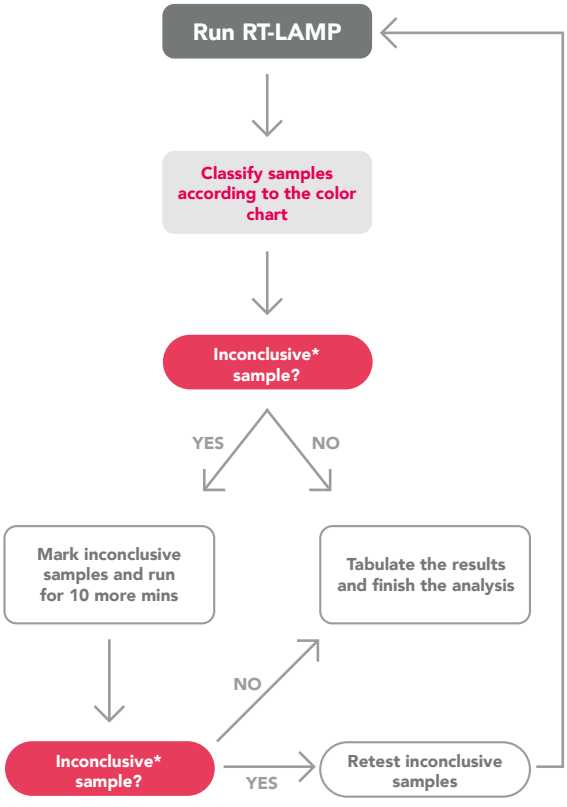
+ = positive result | – = negative result | ? = inconclusive result

[a] Laboratories should report their diagnostic results as appropriate and in compliance with their specific reporting system.

[b] A negative RNase P assay in combination with an inconclusive SARS-CoV-2 assay may suggests that sample contamination has occurred. Depending on the circumstances it is advisable to re-test the sample.

[c] Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation suggest COVID-19 disease while diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If SARS-CoV-2 infection is still suspected, re-testing should be considered in consultation with public health authorities.





* Color classified by the operator as group 1

9. Functional characteristics

9.1. Limit of detection

Evaluation of analytical sensitivity (detection limit) was performed on the SARS-CoV-2 Primer Mix used for the detection of SARS-CoV-2. The test was performed using the positive control „SARS-CoV-2 Standard" (Exact Diagnostics), which in the undiluted state contains 200 copies of synthetic template per 1 µl.

Serial dilutions of the stock standard were prepared, resulting in samples with concentrations of 16.67 copies/µl, 8.33 copies/µl, 5 copies/µl, 3.33 copies/µl, 1.67 copies/µl, 1 copy/µl and 0.67 copies/µl that were used in the analytical sensitivity test. The assay was performed in 8 replicates for each prepared dilution (8 µl of diluted positive control for response corresponding to the recommended 8 µl sample volume for SARS-CoV-2 detection).

The test confirmed the high sensitivity of the **Vivid COVID-19 LAMP Direct-G** kit. Reliable template detection of the SARS-CoV-2 assay was demonstrated down to 1 copy/µl, which was also confirmed in an extensive LoD experiment where 23 out of 24 replicates were positive (over 95% detection success).

Table 6. **Limit of detection of the SARS-CoV-2 assay**

Kópie/µl vzorky	Total replicates	Positive replicates	Inconclusive replicates	Detection success (%)
16,67	8	8/8	0/8	100%
8,33	8	8/8	0/8	100%
5,00	8	8/8	0/8	100%
3,33	8	8/8	0/8	100%
1,67	8	8/8	0/8	100%
1,00	8	7/8	0/8	87.5%
0,67	8	4/8	0/8	50%
1,00*	24	23/24	0/24	95.83%
0,67*	24	18/24	1/24	75%

*Extensive LoD experiment for 1.00 and 0.67 copies/µ

9.2. Test specificity

Evaluation of pathogen specificity (cross-reactivity to other coronaviruses and respiratory viruses) was performed using the control material „Coronavirus RNA specificity panel" (EVAg, European Virus Archive - Global), which contains RNA viruses HCoV-229E, HCoV-OC43, HCoV-NI63, MERS-CoV and SARS-CoV-2, each in a separate tube. A set of respiratory viruses (Vircell) containing RNA of Influenza A H1N1, Novel Influenza A H1N1, Influenza A H3N2, Influenza A H5N1, Novel Influenza B, Human parainfluenza, Respiratory syncytial virus and Human rhinovirus, each provided in a separate tube, were used to assess cross-reactivity to respiratory viruses. The assay was performed in 3 replicates for each of the indicated viruses in an amount of 3 µl for EVAg standards and 1000 copies per reaction for Vircell standards.

The test confirmed the high specificity of the **Vivid COVID-19 LAMP Direct-G** kit. A positive result was recorded exclusively in reactions containing SARS-CoV-2 RNA in the presence

of primers for SARS-CoV-2.
In addition, it is well known that the LAMP method is prone to non-specific amplification even in the absence of true template. To demonstrate that the **Vivid COVID-19 LAMP Direct-G** kit does not produce spurious non-specific amplification products if used as instructed, 48 NTC reactions were performed with the SARS-CoV-2 and RNase P primer sets.
The results are summarized in the table below and they demonstrate that both SARS-CoV-2 or RNase P primer sets failed to display any sort of non-specific amplification whether judged by colorimetric change or fluorescence TTR.

Table 7. **Evaluation of non-specific LAMP amplification**

Primer set	Total replicates	Positive replicates	Non-specific amplification rate (%)
SARS-CoV-2	48	0/48	0%
RNáza P	48	0/48	0%

9.3. Clinical performance evaluation

The clinical performance of the **Vivid COVID-19 LAMP Direct-G** diagnostic kit (tested method) was performed on a cohort of 72 patients who did not have a confirmed SARS-CoV-2 virus status at the time of analysis. All analyzed samples were obtained by gargling according to the instructions in this document. At the same time, in samples taken from patients, the presence of viral RNA was detected in samples obtained by gargling with a reference RT-qPCR method used for routine testing by the regional public health authorities of the Slovak Republic. The evaluation of **Vivid COVID-19 LAMP Direct-G** was based on the comparison of the results of the tested method against the reference method. Standard RNA extraction was performed only for the reference method, while for **Vivid COVID-19 LAMP Direct-G** the samples were processed as recommended in this document. The results of the individual assays (E gene and RNase P for the reference method and SARS-CoV-2 and RNase P for the tested method) were evaluated according to the recommended instructions for the given methods.

Testing of this selected set of samples was performed with blinded samples, in an external laboratory involved in international round quality control tests (EQA) organized by institutions such as the European Center for Disease Prevention and Control (ECDC), Institute of Virology, Charité (Berlin, Germany) and the National Institute for Public Health and the Environment (RIVM, Bilthoven, The Netherlands).

The results of the clinical performance evaluation are summarized below. 8 samples were excluded from the analysis due to ambiguous or invalid results (5 for the reference method, 3 for the tested method). The data showed that the **Vivid COVID-19 LAMP Direct-G** kit showed an overall diagnostic specificity of 100% and an overall diagnostic sensitivity of 65.6% compared to the reference method using either the colorimetric or fluorescent detection mode. However, the sensitivity was 100% for samples with Ct < 32 according to the reference method. The results clearly confirm the ability of the Vivid COVID-19 LAMP Direct-G kit to detect SARS-CoV-2 genomic RNA in samples obtained by gargling with minimal processing without RNA extraction and concentration.

Table 8: **Clinical performance evaluation of the Vivid COVID-19 LAMP Direct-G diagnostic kit***

		Reference method (E gene)		
		Positive	Negative	Total
Vivid COVID-19 LAMP Direct-G	Positive	21	0	21
	Negative	11	32	43
	Total	32	32	
		Sensitivity: 65.6%	Specificity: 100%	

Reference method Ct values	Sensitivity
Full set (Ct ≤ 40) (32 RT-qPCR positive samples in total)	65.6%
Ct < 35 (25 RT-qPCR positive samples in total)	80%
Ct < 32 (16 RT-qPCR positive samples in total)	100%
Ct < 28 (7 RT-qPCR positive samples in total)	100%

*A 20 µl reaction format was used for clinical validation.

10. Disposal

NOTICE!

- **Decontaminate any material that has come into contact with biological samples with 3% sodium hypochlorite for a minimum of 30 minutes or autoclave at 121 °C for a minimum of 60 minutes before disposing.**
- **All used equipment, tips, tubes, work materials, and protective clothing should be considered potentially contaminated and disposed of in accordance with applicable infectious waste disposal regulations.**
- **Dispose of remaining reagents and material in accordance with applicable safety regulations.**

11. Troubleshooting and safety reporting (medical device vigilance)

In case of any problems contact:

Manufacturer: **MultiplexDX, s. r. o.**

Address: Ilkovičova 8
841 04 Bratislava, Slovenská republika

Tel.: +421 2 902 68 310
Email: vigilance@multiplexdx.com

12. Symbols

	Manufacturer
LOT	Batch number
-20°C	Recommended storage temperature
Σ	Package size
CE	This product complies with the requirements of European Directive 98/79/EC on *in vitro* diagnostic medical devices
	Date of manufacturing
IVD	*In vitro* diagnostic medical device
	Attention, follow the safety instructions and operating instructions that come with this product

CE IVD Registration code: P 2138A

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