BioVendor Group LAMP

ENG

Product Data Sheet:

SARS-CoV-2 Direct

MULTILAMP 96-KIT

Catalogue number:
RDLAMP0002

European Union:

IVD CE

Rest of the world:

For research use only!

For professional use.

BioVendor R&D®

BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

CONTENTS

1. INTENDED USE

RDLAMP0002 BioVendor SARS-CoV-2 Direct MULTILAMP 96-kit is intended for the detection of SARS-CoV-2 viral nucleic acid causing Covid-19 disease together with detection of human DNA as an internal control of sample collection quality (RNAseP) and is based on the principle of the RT-LAMP method.

Features

- **European Union: For in vitro diagnostic!**
- Rest of the world: For research use only!
- The total assay time is less than **40 min**
- The use of SARS-CoV-2 Direct MULTILAMP 96-kit allows the **isolation free detection** of viral nucleic acid and allows the sample analysis directly after collection
- The method is intended for examination of persons for the presence of SARS-CoV-2 virus
- The kit detects SARS-CoV-2 virus nucleic acid from nasopharyngeal and/or nasal swabs collected into **non-inactivating medium**
- The kit capacity is 96 reactions

Abbreviations

Ct	Cycle threshold
IC	Internal Control
LoD	Limit of Detection
min	minute
N	Not detected
N/A	Not applicable
NFW	Nuclease Free Water
RFU	Relative Fluorescent Unit

2. STORAGE, EXPIRATION

Store the kit at -20 °C. Under these conditions, all components are stable until the expiration date (see label on the box).

- The SARS-CoV-2 Direct MULTILAMP 96-kit is delivered frozen at -20 °C.
- After delivery store the SARS-CoV-2 Direct MULTILAMP 96-kit at -20 °C.
- **Protect kit components from light**.
- Reagents may be aliquoted if necessary.
- Avoid repeated freeze-thaw cycles.
- Do not use the kit after the expiration date.
- For stability of opened reagents see Chapter 9.

3. INTRODUCTION

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is an enveloped RNA virus of the *Coronavirideae* family. It is the causative agent of Covid-19, which spread worldwide from China's Hubei province at the end of 2019. In January 2020, a global health threat was declared by the World Health Organization (WHO) [1].

The virus is spread by contact with each other, primarily through droplets, which the infected person excretes when coughing, sneezing or speaking. Infection can occur by inhaling or transmitting the virus in a droplet from the surface to the face (typically by touching the mouth, nose or eyes). It is not clearly determined how long the virus survives outside the human body [2].

The incubation period is between 1 and 14 days, the usual time from infection to the first symptoms is 5 to 6 days. The main symptoms include dry cough, shortness of breath, fatigue and fever, more severe cases can lead to pneumonia, kidney failure and even death. Symptoms usually come gradually and about 80 % of patients recover without the need for hospitalization. The disease progression and severity also depends on the overall health and physical condition of the patient [3].

Reliable and rapid testing of the population for the presence of the virus is a critical step in stopping the spread of the disease and stabilizing the pandemic situation.

4. TEST PRINCIPLE

The SARS-CoV-2 Direct MULTILAMP 96-kit is an *in vitro* test kit for the detection of SARS-CoV-2 RNA together with human DNA (RNAseP gene) based on the principle of amplification under isothermal conditions. The reaction takes place in a single tube (multiplex reaction) at constant temperature and uses the principle of the Reverse Transcription Loop Mediated Isothermal Amplification (RT-LAMP). The input material for the LAMP reaction can be directly the sample medium from the collection tube without the need of viral RNA isolation. In the LAMP reaction, the target sequence is amplified at constant temperature using a set of primers and a fluorescently labelled displaceable probe, in the presence of a polymerase which, in addition to replication activity, also has the strand dissociation ability. The presence of loop primers increases the rate and dynamics of the reaction, so the amount of nucleic acid generated in LAMP reactions tends to be higher than in classical thermocyclic amplification, and detection of the target sequence may be more than half faster.

Detection of the presence of target sequence is mediated by the fluorescence elevation produced by isothermal amplification on an optional platform detecting the FAM channel for SARS-CoV-2 target (480-530 nm) and Cy5 channel for RNAseP target (650 – 670 nm).

5. PRECAUTIONS

- **For professional use!**
- The kit components do not contain infectious material.
- All samples for testing with the SARS-CoV-2 Direct MULTILAMP 96-kit should be handled as if they are infectious, following conventional biosafety precautions.
- Use respiratory, hand and eye protection when working (respirator FFP2 and higher, protective disposable gloves, goggles or shield).
- Do not drink, eat or smoke in areas where biological material is handled.

6. TECHNICAL HINTS

- The kit is intended for the detection of SARS-CoV-2 viral nucleic acid together with detection of human RNAseP from samples collected into non-inactivating medium (e.g. Nucleic Acid Release Reagent or VTM).
- The kit is validated on samples of nasopharyngeal swabs and nasal swabs collected into **non-inactivating medium** for stabilization and release of nucleic acids: Nucleic Acid Release Reagent (Jiangsu Mole Bioscience). The use of samples obtained by other sampling procedures requires in-house validation.
- The sample for analysis with the SARS-CoV-2 Direct MULTILAMP 96-kit must not be chemically inactivated.
- Sample intended for analysis with SARS-CoV-2 Direct MULTILAMP 96-kit must be collected according to the recommendations of the collection kit manufacturer (see chap. 8. Recommended material).
- The kit is intended for use by professional users in an adequate laboratory environment.
- Before and after each test, the working environment must be decontaminated with standard RNase, DNase disinfectant. Working in an unsuitable environment can lead to contamination of the kit components.
- SARS-CoV-2 Direct MULTILAMP 96-kit targets the **S region of the viral RNA and specific region of human gene for RNAseP**. For optional using of any positive controls, these must contain target regions. Using controls that do not contain target regions, may lead to a false negative result.
- LAMP Master Mix is supplied in two aliquots, each is sufficient for 48 reactions. We do not recommend repeat more than 2 freeze-thaw cycles, multiple thawing may affect test quality and results.
- Let individual kit components thaw just before use. Minimize the time that the reagents are at room temperature. We recommend the use of cooling racks.
- Vortex gently the reagents before using.
- Avoid cross contamination of samples and reagents. Use disposable pipette tips for each sample and reagent.
- Do not open the tubes after the LAMP reaction and dispose used material in accordance with the legislation.
- Do not mix reagents with different LOT numbers.

7. REAGENT SUPPLIED

The kit is supplied as "ready to use" to perform 96 reactions (Table 1). The kit includes a **LAMP Master Mix** containing all the necessary components of the reaction.

Component	1 tube volume (µl)	Number of Tubes	State
LAMP Master Mix	1320	2	ready to use

Table 1: Components of the SARS-CoV-2 Direct MULTILAMP 96-kit

8. MATERIAL REQUIRED BUT NOT SUPPLIED

For nasopharyngeal swab:

- non-inactivating collection medium e.g.:
  - Nucleic Acid Release Reagent, cat. number P049T06601 (Jiangsu Mole Bioscience)
  - VTM medium
- nasopharyngeal collection swab (flexible flocked swab) e.g. Disposable sampling swab (Jiangsu Mole Bioscience)

For nasal swab:

- non-inactivating collection medium e.g.:
  - Nucleic Acid Release Reagent, cat. number P049T06601 (Jiangsu Mole Bioscience)
  - VTM medium
- nasal collection swab (regular flocked swab) e.g. FLOQSwabs 502CS01 (Copan)

- Thermocycler for measuring fluorescence with the FAM channel (480 - 530 nm) and Cy5 channel (650 – 670nm) with heating to 65 °C or fluorescence thermo-reader Sparsek FISSH 1 with integrated protocol for SARS-CoV-2 Direct MULTILAMP 96-kit
- Thermoblock for heat inactivation at 95 °C
- PCR tubes / strips / plates suitable for fluorescence detection instrument (suitable size, profile and light transmittance) – transparent, colourless plastics marked "suitable for PCR" are recommended
- Automatic pipettes for volumes 5 - 100 µl, or automatic dosing pipes for volumes 5 - 25 µl
- Disposable filter tips
- Vortex, centrifuge for tubes with a volume of ≤ 2 ml
- Test tube racks
- Commercially available solutions for surface decontamination
- Disposable gloves

9. PREPARATION OF REAGENTS

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on the label.

Reagents are supplied as ready to use.

LAMP Master Mix

Let thaw enough LAMP Master Mix for the batch of samples just before preparing the reaction and keep it cool prior to use.

Stability and Storage: LAMP Master Mix is supplied in two aliquots; each is sufficient for 48 reactions. Always store at -20 °C and protect from light.

It is not recommended to repeat more than two freeze-thaw cycles of LAMP Master Mix aliquots, multiple thawing may affect test quality and results. Freeze the reagent immediately after using the aliquot. Opened LAMP Master Mix is stable 6 months stored at -20 °C.

10. PREPARATION OF SAMPLES

The kit detects SARS-CoV-2 viral nucleic acid from nasopharyngeal and nasal swabs collected into non-inactivating medium (e.g. Nucleic Acid Release Reagent or VTM).

Sample collected into non-inactivating VTM requires heat inactivation at 95 °C.

The use of samples obtained by other sampling procedures requires in-house validation.

Respect the collection, handling and storage recommendations according to the collection kit manufacturer.

Sample in non-inactivating detergent-based medium (e.g. Nucleic Acid Release Reagent)

Allow the sample to lyse in the collection tube for at least 10 minutes (or according to the collection kit manufacturer's instructions), vortex thoroughly and briefly centrifuge. The sample is ready for analysis.

Note: To improve the quality of the test, it is possible to inactivate the sample at 95 °C for 5 minutes, then cool briefly to 4 °C. Then proceed according to chapter 11. Assay procedure.

Sample in non-inactivating VTM medium

Before analysis, inactivate the sample at 95 °C for 5 minutes, then cool briefly to 4 °C. Vortex thoroughly and centrifuge briefly. The sample is ready for analysis.

Proceed according to chapter 11. Assay procedure.

Recommendation: Thermal inactivation of the sample improves the quality of the subsequent analysis. The short exposure to high temperature helps to release nucleic acids and is beneficial for all types of non-inactivation media. Thermal inactivation is necessary when using VTM media.

Generally, the use of nasal spray is not recommended before collection of swabs (follow the instructions of the collection kit manufacturer). Before nasal swab is recommend blowing the nose.

11. ASSAY PROCEDURE

1. Prepare reaction tubes/plate for appropriate number of samples.

Precaution: Do not label the tubes at the place of fluorescence reading, it is necessary to allow fluorescence to be read without interference.

2. Pipette **25 µl of LAMP Master Mix** into each tube.

3. Pipette **5 µl of each sample** into the appropriate tube. Centrifuge the reaction tubes briefly (make sure the reaction mixture is at the bottom of the tubes).

4. Place the reaction tubes with the prepared reaction mixture in a suitable detection device (real-time thermocycler or thermo-reader Sparsek FISSH 1)

5. Set the reaction program according to Table 2 and start the reaction.

Instrument type	Step	Temperature	Time	Fluorescence channel
Sparsek FISSH 1	1	Turn on the heating („Measure“)		
	2	Start the measurement („Start“). Program of the reaction is integrated in instrument setting.		
Real-time termocycler (eg. BioRad CFX 96, MIC, Roche 480 Light Cycler)	1	65 °C	1 min (+fluorescence read)	**FAM** (SARS-CoV-2) **+** **Cy5** (RNAseP)
	2	GO TO 1	repeat 30x	

Table 2: Program for reaction

12. RESULTS EVALUATION

12.1 Real-time termocycler

A) Positive signal

A positive result of LAMP amplification is any increase in the fluorescent signal above the normal background level during reaction time (S-shaped curve). An example of positive signal detection from a thermocycler is shown in Figure 1.

The detection of LAMP amplification is **qualitative**.

Interpret the result as positive when detects increase in fluorescence signal above the background level during the reaction.

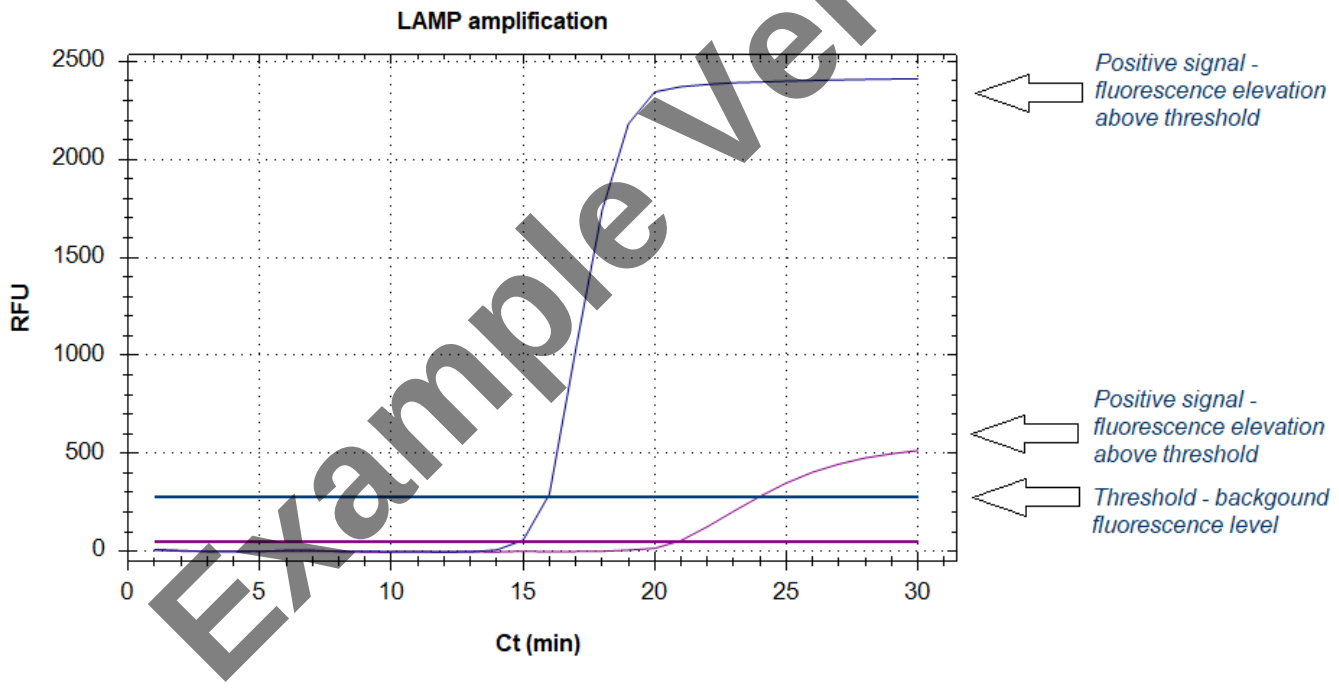


Figure 1: The fluorescence curve record (positive signal).

B) Negative signal

If there is not target sequence (SARS-CoV-2/RNAseP) in the sample at the beginning of the reaction, no change in the fluorescence signal is detected.

Interpret the result as negative when there is no detection of the fluorescent signal elevation during the reaction time. The example of the thermocycler record for negative signal is shown in the Figure 2.

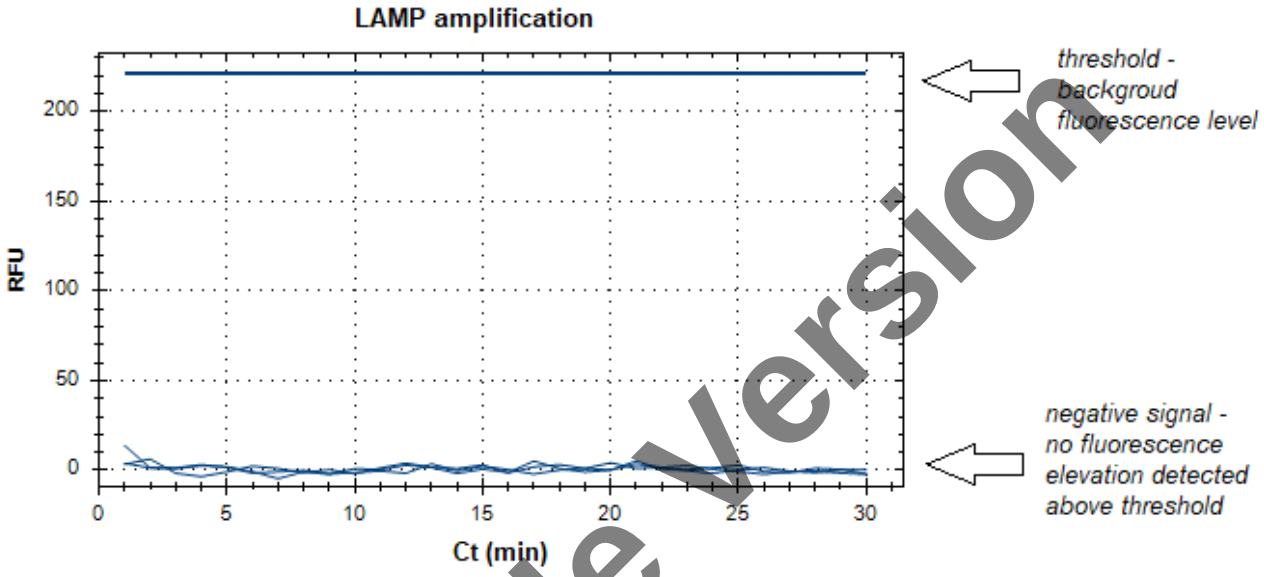


Figure 2: The fluorescence record without the detection of fluorescence signal (negative signal).

12.2 Sparsek FISSH 1

Test result is interpreted automatically by integrated algorithm which evaluates changes is fluoresce levels for both detection channels. The final result is information about positive/negative or invalid test result for analysed sample.

13. RESULTS INTERPRETATION

A summary of the results interpretation is given in Table 3.

In the case of analysis by the Sparsek FISSH 1 reader, the results are interpreted automatically and displayed on the instrument screen.

SARS-CoV-2 detection (FAM)	IC RNAseP detection (Cy5)	Test result	Interpretation
positive	positive	**positive**	test is valid
positive	negative	**positive**	test is valid
negative	positive	**negative**	test is valid
negative	negative	**invalid**	test is not valid, repeat the test with new sample collection

Table 3: Interpretation of results of the SARS-CoV-2 Direct MULTILAMP 96-kit

More information is in chapter 16. Troubleshooting and frequently ask questions.

A) Positive result

The sample is considered **SARS-CoV-2 positive if a positive signal is detected in the FAM channel.**

The internal control signal (Cy5) can be positive or negative. High concentrations of SARS-CoV-2 in the sample may inhibit the reaction of RNAseP detection.

B) Negative result

The sample is considered **SARS-CoV-2 negative if there is a negative signal in the FAM channel and at the same time a positive signal is detected in the Cy5 channel for internal control.**

The internal control signal is detected only if the sample contains sufficient material collected. If both the internal control (Cy5) and SARS-CoV-2 (FAM) signals are negative, the test is invalid and a new sample must be collected.

14. TEST LIMITATIONS

- **The quality of the measurement is affected by the quality of the sample. Proper sample collection, transport and storage procedures are critical for the measurement.**
- Test results should be evaluated by a healthcare professional with respect to the patient's medical history, clinical condition, and other diagnostic results.
- SARS-CoV-2 Direct MULTILAMP 96-kit is designed specifically for the detection of SARS-CoV-2 virus, a negative result does not exclude the infection by other pathogens or weak infection with the amount of SARS-CoV-2 virus below the limit of detection of the method.
- All instructions in this document should be followed when performing measurements. Any deviations may affect the quality and reliability of the results.

15. KIT CHARACTERISTICS

15.1 ANALYTICAL FUNCTION

This chapter presents the characteristic analytical data of the SARS-CoV-2 Direct MULTILAMP 96-kit manufactured by BioVendor.

Analytical sensitivity (LoD)

Limit of detection (LoD) of SARS-CoV-2 Direct MULTILAMP 96-kit was tested using the First WHO International Standard for SARS-CoV-2 RNA (NIBSC, cat. n. 20/146) in serial dilution. Based on the results, the LoD of the kit was determined to be 10^2 copies/µl. A positive signal for this concentration was detected in all replicates.

Detection of SARS-CoV-2 variants

The ability of detection different mutant variants of SARS-CoV-2 was tested using known genetic variants listed in Table 4.

Variant	Genotype	Detection with SARS-CoV-2 Direct MULTILAMP 96-kit (FAM)
wild type	B.1.	positive
Beta	B.1.351 – VOC 5014.V2	positive
Alfa	B.1.1.7 - VOC 202012/01	positive
Delta	B.1.617.2	positive
Omicron	B.1.1.529	positive

Table 4: Determination of the SARS-CoV-2 Direct MULTILAMP 96-kit on different viral variants

Analytical specificity

Verification of cross-reactivity was performed *in silico* by comparing the sequences of related and other possible interacting microorganisms with the sequences of primers and probes providing specific detection of SARS-CoV-2 virus by SARS-CoV-2 Direct MULTILAMP 96-kit technology. It was verified that the only possible cross-reaction could occur with SARS-coronavirus, which, however, is very rare in the population (Table 5).

Pathogens from the *Coronovirideae* family	**Cross-reactivity of primers and probes**
Human coronavirus 229E	excluded *in silico*
Human coronavirus OC43	excluded *in silico*
Human coronavirus HKU1	excluded *in silico*
Human coronavirus NL63	excluded *in silico*
SARS-coronavirus	**≤ 80 %**
MERS-coronavirus	excluded *in silico*
Other pathogens	**Cross-reactivity of primers and probes**
Adenovirus (C1 and 71)	excluded *in silico*
Human Metapneumovirus (hMPV)	excluded *in silico*
Parainfluenza virus 1-4	excluded *in silico*
Influenza A & B	excluded *in silico*
Enterovirus (for ex. EV68)	excluded *in silico*
Respiratory syncytial virus	excluded *in silico*
Rhinovirus	excluded *in silico*
Chlamydia pneumoniae	excluded *in silico*
Haemophilus influenzae	excluded *in silico*
Legionella pneumophila	excluded *in silico*
Mycobacterium tuberculosis	excluded *in silico*
Streptococcus pneumoniae	excluded *in silico*
Streptococcus pyogenes	excluded *in silico*
Bordetella pertussis	excluded *in silico*
Mycoplasma pneumoniae	excluded *in silico*
Pneumocystis jirovecii (PJP)	excluded *in silico*
Candida albicans	excluded *in silico*
Pseudomonas aeruginosa	excluded *in silico*
Staphylococcus epidermis	excluded *in silico*
Staphylococcus salivarius	excluded *in silico*

Table 5: In silico verification of cross-reactivity of primers and probes of SARS CoV-2 Direct MULTILAMP 96-kit technology

Repeatability

The repeatability of the method was tested in separate and independent experiments by measuring the same set of samples under the same conditions. Qualitative detection of the presence of SARS-CoV-2 virus was performed with 100% agreement, detection of internal control with 93% agreement. An overall repeatability of the method is ≥ 98 % (Table 6).

Sample	Concentration (copies/µl)	Number of replicates (n)	SARS-CoV-2: agreement of results with expected (%)	RNAseP: agreement of results with expected (%)
International standard SARS-CoV-2 WHO 20/146	10^4	7	**100%**	**100%**
	10^3	7	**100%**	**100%**
	10^2	7	**100%**	**100%**
Positive control for RNAseP (IC)	10^4	5	**100%**	**100%**
	10^3	5	**100%**	**100%**
	10^2	5	**100%**	**60%**
Positive control for SARS-CoV-2 (PC)	10^4	5	**100%**	**100%**
	10^3	5	**100%**	**100%**
	10^2	5	**100%**	**100%**
NC	collection medium	7	**100%**	**100%**
Overall repeatability (n = 58)			**98%**	

Table 6: Overall repeatability of the SARS CoV-2 Direct MULTILAMP 96-kit

Robustness

The robustness of the assay was tested using a series of identical samples in 3 independent experiments. A defined change of conditions was made in each independent experiment. According to the results (Table 7) the SARS-CoV-2 Direct MULTILAMP 96-kit was shown to give robustness ≥ 96 **%** with changes in the reaction conditions that were simulated:

1) lower reaction temperature (standard 65 °C, experimental 60 °C)

2) change of the thermocycler (BioRad CFX96 vs. MIC)

3) change of operator

		Agreement with expected results (%)			
Sample	**Concentration (copies/µl)**	**Standard conditions**	**Temperature change**	**Thermocycler change**	**Operator change**
Positive control for SARS-CoV-2 (PC)	10^6	100%	100%	100%	100%
	10^4				
	10^2				
International standard SARS-CoV-2 WHO 20/146	10^4	100%	100%	100%	83%
	10^3				
	10^2				
Nasal swab	x	100%	100%	100%	100%
NC	collection medium	100%	100%	100%	100%
Overall robustness under target conditions (%)		**100%**	**100%**	**100%**	**96%**

Table 7: Robustness of the method with defined changes in experimental conditions compared to the standard protocol

15.2 CLINICAL FUNCTION

The diagnostic parameters of the BioVendor SARS-CoV-2 Direct MULTILAMP 96-kit were evaluated by comparison with a commercially available CE-IVD RT-qPCR method on a set of nasopharyngeal swab samples (n = 92). Clinical function on nasopharyngeal swabs was compared with samples of nasal swabs. The results are shown in Table 8.

		Diagnostic senzitivity	**Diagnostic specificity**	**Positive predictive value**	**Negative predictive value**
	PCR result	SARS-CoV-2 Direct MULTILAMP 96-kit			
Nasopharyngeal swabs	Ct < 35	91 %	100 %	100 %	92 %
	Ct < 32	93 %			94 %
	Ct < 30	100 %			100 %
	Ct < 25	100 %			100 %
nasal swabs: comparison with clinical function of nasopharyngeal swabs	x	92 %	100 %	100 %	83 %

Table 8: Diagnostic accuracy of the SARS-CoV-2 Direct MULTILAMP 96-kit determined on clinical samples of nasopharyngeal and nasal swabs.

16. TROUBLE SHOOTING AND FREQUENTLY ASKED QUESTIONS

No.	Problem	Possible cause	Solution
1	Negative result for internal control (Cy5; RNAseP) in sample, where is negative result for SARS-CoV-2 (FAM).	Sample was collected incorrectly and do not contain enough human material.	Test result is invalid. Repeat the test with new sample collection.
2	Negative result for internal control (Cy5; RNAseP) in sample, where is positive result for SARS-CoV-2 (FAM).	LAMP reaction is multiplexed and reaction for RNAseP detection can be inhibited by higher amount and better dynamics of SARS-CoV-2 reaction.	Test result is valid and sample is interpreted as SARS-CoV-2 positive.
3	Sample is interpreted as negative, but patient has symptoms of Covid-19.	The patient's viral load may be below the limit of detection of the assay. The patient may suffer another respiratory disease.	If there is uncertainty, analyze the patient sample using another confirmatory method (RT-qPCR).

17. REFERENCES

For more references to this product, visit our website www.biovendor.com.

[1] Coronavirus disease (COVID-19) Weekly Epidemiological Update and Weekly Operational Updates (available at https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports)

[2] CDC. Coronavirus Disease 2019 (COVID-19). Centers for Disease Control and Prevention (available at: https://www.cdc.gov/coronavirus/2019-ncov/faq.html)

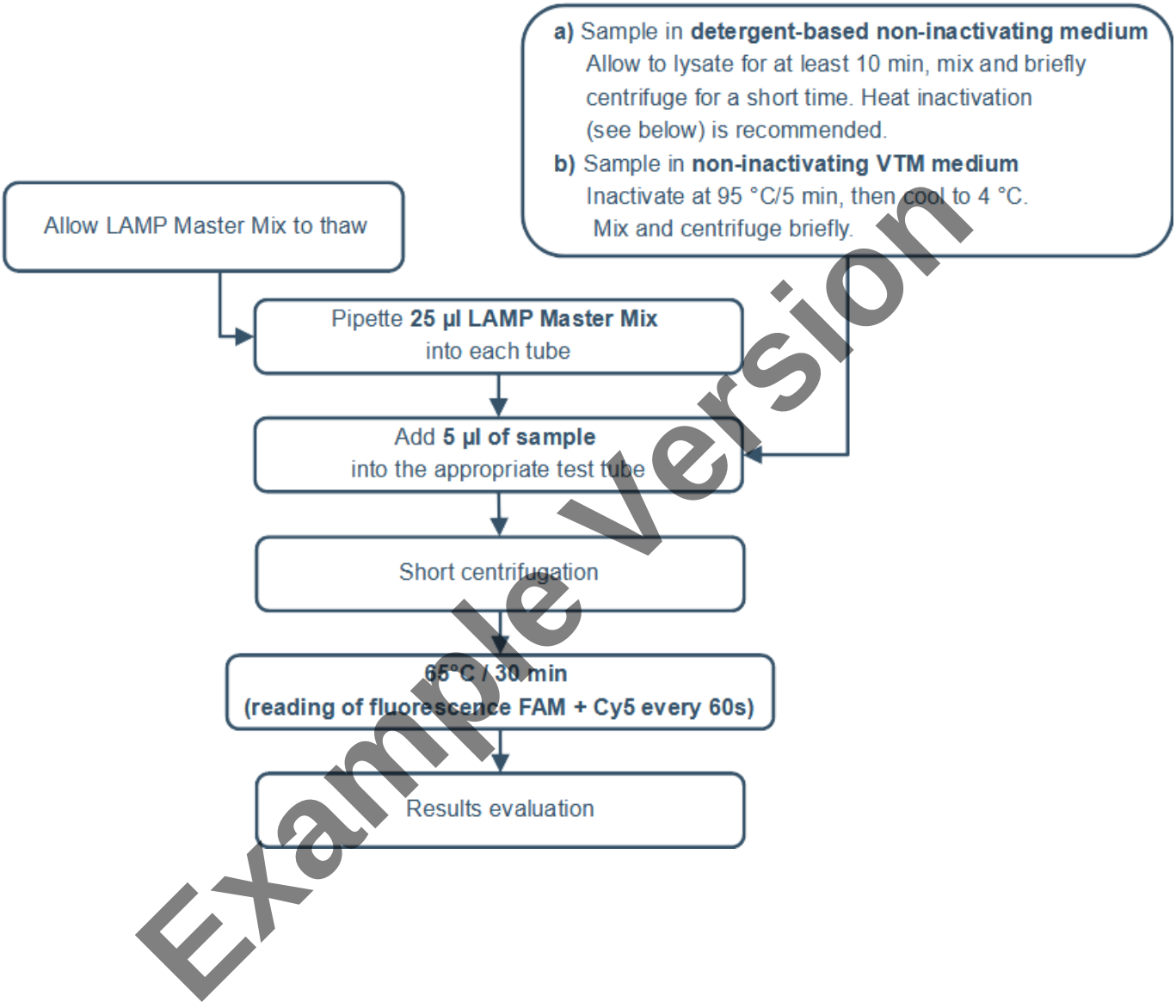
[3] Flu versus coronavirus – similarities and fundamental differences, situation as of 18.3.2020, SZÚ (available at http://www.szu.cz/tema/prevence/chripka-versus-koronavirus-podobnosti-a-zasadni-rozdily-k-18)

18. EXPLANATION OF SYMBOLS

REF	Catalogue number
LOT	Batch code
	Use by date
- 20°C	Temperature limit
	Manufacturer
www.biovendor.com	Read electronic instructions for use - eIFU
Σ 96	The content is sufficient for 96 tests
IVD	*In vitro* diagnostic medical device
CE	CE marking of conformity

19. ATTACHMENT

Quick workflow summary



BioVendor R&D®

BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

Date of last revision: 02.02.2022